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An elusive ectomycorrhizal fungus reveals itself: a new species of *Geopora* (Pyronemataceae) associated with *Pinus edulis*

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Abstract: Species of the genus *Geopora* are important ectomycorrhizal associates that can dominate the communities of some plant taxa, such as pinyon pine (*Pinus edulis*), a widespread tree of the western United States. Several members of the genus *Geopora* are known only from ectomycorrhizal root tips and thus have not been described formally. The sporocarps of some *Geopora* species occur infrequently because they depend on wet years for sporulation. In addition, *Geopora* sporocarps can be small and may be hypogeous at some developmental stage, limiting the opportunities for describing their morphology. Using molecular and morphological data, we have described a new species of fungus, *Geopora pinyonensis*, which produced ascocarps after unusually high precipitation at a northern Arizona site in summer 2012. Based on analysis of the ITS and nuLSU regions of the rDNA, *G. pinyonensis* is a new species of *Geopora*. It has small sporocarps and ascospores relative to other members of the genus; however, these morphological features overlap with other species. Using rDNA data from sporocarps and ectomycorrhizal root tips, we show that the sporocarps correspond to an abundant species of ectomycorrhizal fungus associated with pinyon pines that is increasing in abundance in drought-affected landscapes and may promote drought tolerance.

Key words: earth pore, ectomycorrhiza, hypogeous fungi, Pyronemataceae, Sunset Crater

Introduction

Ectomycorrhizal (EcM) fungi are root symbionts that promote host plant growth by increasing plant acquisition of soil nutrients and improving host tolerance to environmental stresses (Smith and Read 1997). They are important both economically and ecologically because they form symbiotic interactions with a range of plant species, particularly trees that dominate woodland and forest communities around the world. Many EcM fungi reproduce sexually through the production of macroscopic sporocarps. At least 6000 species of fungi form ectomycorrhizal associations (Rinaldi et al. 2008, Brundrett 2009), which is ~ 0.5% of the estimated ~ 1 500 000 fungal species (Hawksworth 2001). That number is likely much higher (Tedersoo et al. 2010) due to infrequent production of sporocarps or the production of hypogeous or small nondescript fruiting bodies by some EcM fungi.

Despite the apparently well documented hypogeous habit of some EcM-forming fungi, new species descriptions are still common from either sporocarps or from mycorrhiza (e.g. Guevara et al. 2008, 2013; Guevara-Guerrero et al. 2012; Lantieri et al. 2012). The advantage of characterizing undescribed species directly from the mycorrhiza allows the assessment of their symbiotic interaction. However, there are a substantial number of ectomycorrhizal fungal species characterized by molecular data, which do not match sporocarp collections from the same site or any vouchered specimen in GenBank (Southworth and Frank 2011). These EcM may represent taxa that sporulate rarely, or not at all, and taxa for which the sporocarp is difficult to find or to describe. For example, the mycobionts of EcMs of *Geopora* spp., (Pyronemataceae), identified with molecular methods, are not often reported associated with hypogeous fruiting bodies and are rarely classified to species (Gehring et al. 1998, Fujimura et al. 2005, Tedersoo et al. 2006, Hrynkiewicz et al. 2009, Ishida et al. 2009, Sthultz et al. 2009, Wei et al. 2010, Gordon and Gehring 2011). Moreover, species delimitation in *Geopora* is complicated because of the small number of differentiating morphological characters, values of which tend to overlap among species. Classification of *Geopora* species has relied mainly on the size and shape of the ascospores, position of the apothecia in the ground and the length of excipular hairs (filamentous hyphae on the outside surface of the cup). However, molecular analyses have shown that well supported clades do not correspond to species concepts based on morphological characters (Tamm et al. 2010). Therefore using molecular data in combination with morphological information is considered a more reliable approach to link the EcM to sporocarps and define species (Tamm et al. 2010, Southworth and Frank 2011).

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The difficulties in correctly identifying species of *Geopora* are particularly evident in southwestern USA where arid conditions may not only reduce sporocarp production but also favor the evolution of hypogeous sporocarps that reduce the risk of desiccation (Thiers 1984). For example, in Sunset Crater, Arizona, in a drought-affected pinyon-juniper woodland where a long-term study (more than 20 y) has been conducted, sporocarps have been detected infrequently (Gehring et al. 1998). The study of the effect of EcM on trees in this area is relevant because they have experienced more than 10 y of drought including 2 y of exceptionally dry conditions (1996, 2002) that resulted in widespread mature pinyon pine (*Pinus edulis*) mortality (Breshears et al. 2005, Mueller et al. 2005). However, the EcM fungi associated with *P. edulis*, which is a dominant species of this region, have been well documented (Gehring et al. 1998, Mueller and Gehring 2006, McHugh and Gehring 2006, Hubert and Gehring 2008, Sthultz et al. 2009). Unidentified members of the Pezizales have been observed in almost all studies of pinyon EcM (Gehring et al. 1998, Mueller and Gehring 2006, Sthultz et al. 2009, Gordon and Gehring 2011, Gehring et al. 2013) and in two studies of *P. ponderosa* EcM (Fujimura et al. 2005, Hubert and Gehring 2008). Many of these EcM have been identified as *Geopora*, but only the sporocarps of *G. cooperi*, an infrequent member of the *P. edulis* community, have been observed (Gordon and Gehring 2011). Repeated sampling of varying sites and individual trees within a site before and during drought have shown dramatic increases in the relative abundance of *Geopora* mycorrhizas with drought (Gordon and Gehring 2011, Gehring et al. 2014). Gordon and Gehring (2011) used molecular data to identify several unknown, genetically distinct *Geopora* EcM types, which did not match any vouchered specimens in GenBank. These EcM types were morphologically similar when observed as EcM root tips, but they had different restriction fragment length polymorphism (RFLP) patterns and ITS sequences, representing potential novel species. Sporocarps of these *Geopora* taxa had never been observed, limiting their morphological characterization to indistinguishable EcM root tips. Further characterization of these taxa is particularly important because *Geopora* spp. and other Pezizales species, which are drought-adapted, dominate the EcM community of pinyon pine (Gehring et al. 1998, McHugh and Gehring 2006, Mueller and Gehring 2006, Hubert and Gehring 2008, Sthultz et al. 2009, Gordon and Gehring 2011). Moreover, the EcM communities dominated by *Geopora* spp. substantially enhance the performance of pinyon pine seedlings and trees, compared to communities formed by other taxonomic groups (Gehring et al. 2013, Gehring et al. 2014).

After unusually high precipitation at Sunset Crater, northern Arizona, during the summer monsoon in 2012, we detected sporocarps whose morphology was consistent with that of *Geopora* but distinct from *G. cooperi*, the only member of this genus recorded at the study site. In this study we evaluated whether these sporocarps were related to the mycobionts reported in *P. edulis* by using both DNA sequences and morphological characters of ectomycorrhiza and sporocarps. Herein we describe this new species of *Geopora* on the basis of morphological and molecular characteristics of both mycorrhiza and sporocarps. Furthermore we evaluated the phylogenetic relationships of this new *Geopora* species in relation to the currently accepted species for the genus based on the recent phylogenetic inferences using ITS (e.g. Tamm et al. 2010, Southworth and Frank 2011, Guevara-Guerrero et al. 2012) and nuLSU (Perry et al. 2007).

MATERIALS AND METHODS

Study sites and collection material.—Ten sporocarp specimens were collected 13 Sep 2012 at Sunset Crater, Arizona (35.397689 N, −111.425058 W, 1900 m). This area has a mean annual air temperature of 12 C, mean annual precipitation of 328 mm; the soil is a coarse cinder, classified as a Typic Ustorthent (Selmants and Hart 2008). Dominant vegetation consists of trees and shrubs and is characterized by *Pinus edulis*, *Juniperus monosperma*, *Rhus trilobata*, *Fallugia paradoxa,* and *Ephedra viridis*. Only two sporocarps were exposed on the surface while the rest were buried under the basaltic soil at different developmental stages including maturity. The sporocarps were located in mostly open space between trees on exposed soil without litter. The mosses *Ceratadon purpurea* and *Bryum argentum* occurred near the sporocarps. Sporocarp collections were deposited in the Sam Mitchel Herbarium of Fungi (Denver Botanic Garden) (DBG), voucher catalog DGB 27586.

DNA extraction and sequencing.—DNA was extracted from two sporocarps with a DNA easy mini plant kit (QIAGEN, Valencia, California), according to the manufacturer's instructions. Molecular data were obtained by amplifying the internal transcribed spacer (ITS) region, including ITS1, the 5.8S ribosomal DNA gene and ITS2, with forward primers ITS1F and reverse primer ITS4 (Gardes and Bruns 1993, Tamm et al. 2010) and part of the nuLSU 28S ribosomal DNA gene with forward primer LROR and reverse primer LR5 (Tedersoo et al. 2006, Gordon and Gehring 2011). The nuLSU region was chosen in addition to the ITS because it provides deeper phylogenetic resolution of fungal relationships (Taylor and Bruns 1999, Hansen and Pfister 2006, Smith et al. 2006) because it is less variable than the ITS. Tedersoo et al. (2006) reported that

TABLE I. Maximum identity match of *Geopora* species to the ITS sequence (600 base pairs) of *G. piñonensis*

Geopora species	Query cover	Max identity	Accession no.	Reference
Geopora RFLP type E	100.00%	100.00%	HQ630377.1	Gordon and Gehring 2011
Geopora RFLP type Z	92.00%	97.00%	HQ630380.1	Gordon and Gehring 2011
Pyronemataceae sp.	100.00%	96.00%	GQ281481.1	Wei et al. 2010
Pezizales sp.	100.00%	91.00%	AF266709.1	Bidartondo et al. 2011
Geopora RFLP type K	100.00%	91.00%	HQ630379.1	Gordon and Gehring 2011
Geopora RFLP type J	100.00%	90.00%	HQ630378.1	Gordon and Gehring 2011
Geopora arenicola	100.00%	91.00%	FM206450.1	Tamm et al. 2010
Geopora arenicola	100.00%	91.00%	FM206449.1	Tamm et al. 2010
Geopora arenicola	100.00%	91.00%	FM206444.1	Tamm et al. 2010
Geopora arenicola	100.00%	91.00%	FM206443.1	Tamm et al. 2010

phylogenetic analysis with the nuLSU data resolved the identity of most pezizalean EM fungal sequences to genus or species, and Perry et al. (2007) used it to resolve the relationships within the family Pyronemataceae. Both regions were amplified under these conditions: initial denaturation of 5 min at 95 C was followed by 40 cycles of 30 s at 95 C, 45 s at 53 C and 1:15 min at 65 C, with a final cycle for 10 min at 72 C, using JumpStart™ REDTaq® ReadyMix™ (Sigma-Aldrich, Missouri).

PCR products were purified with Exonuclease I (EXO I) and shrimp alkaline phosphatase (SAP) (Affymetrix|USB, Ohio) following manufacturer's instructions. Sequencing reactions were prepared with BigDye Terminator Ready Reaction Mix 3.1 and sequenced on an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, California) at NAU's Environmental Genetics and Genomics Facility (ENGGEN).

Consensus sequences of ITS and nuLSU were assembled with SeqMan (DNASTAR, Inc., Wisconsin). Sequences were edited with Bioedit 7 (http://www.mbio.ncsu.edu/bioedit/bioedit.html). These sequences were matched to fungal sequences in GenBank (www.ncbi.nlm.nih.gov) with BLAST (Altschul et al. 1990, Thompson et al. 1997). DNA sequences from the sporocarps were deposited in GenBank (accession numbers KF768650–KF7686503). Sequences of *Geopora* species were obtained from GenBank based on Tamm et al. (2010), Southworth and Frank (2011) and Guevara-Guerrero et al. (2012) for ITS and based on Perry et al. (2007) for nuLSU. A total of two ITS new sequences from sporocarps along with 117 ITS sequences of *Geopora* species were aligned with MUSCLE (Edgar 2004). Two nuLSU sequences from the sporocarp were aligned against 19 nuLSU from *Geopora* specimens with BioEdit 7.1.9 (Hall 1993). In both alignments *Pyronema domesticum* and *Wilcoxina mikolae* were included as outgroups. jModeltest2 (Guindon and Gascuel 2003, Darriba et al. 2012) was used with a SUBSTITUTION SCHEME set to 3 to select the evolutionary model to be employed in the Bayesian inference (BI). A GTR + G model was used for the nuLSU region and a SYM + G model for ITS. BI was performed with MrBayes (Bayesian Inference of phylogeny, 3.1; Ronquist and Huelsenbeck 2003). Uniform, prior probabilities and a random starting tree were used. The Markov chain Monte Carlo (MCMC) procedure was run simultaneously and sampled phylogenetic trees every 1000 generations for a total of 3 000 000 and 1 500 000 generations for ITS and nuLSU regions respectively. Each analysis consisted of three hot and one cold chain. We evaluated stationarity by graphing −lnL of trees across all generations and by requiring the standard deviation of the two runs to be less than 0.01. We used a relative burn-in of 25%. A majority rule consensus tree was calculated, collapsing branches whose support was < 50%. The tree was visualized in FigTree (http://tree.bio.ed.ac.uk/software/figtree/) and edited in Mesquite 2.75 (Maddison and Maddison 2011). Sequence alignments and phylogenetic trees were deposited in TreeBASE http://purl.org/phylo/treebase/phylows/study/TB2:S15011.

Morphological observations.—A 15 cm^2 soil patch containing *Geopora* sporocarps was excavated and taken to the lab where macroscopic images were captured with a digital camera (Optronics, California) attached to a dissecting microscope (Leica model MZ6). In the lab sporocarps were examined with a Leica MZ6 dissecting microscope and with a Leica DMLB compound microscope. Microscopic characters were described from either freehand sections of fresh specimens or cross sections of material embedded in Paraplast. Freehand sections were stained with Melzer's reagent (Brundrett et al. 1996) and mounted in 5% KOH. Sporocarps were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer. Samples were dehydrated in an ascending series of ethanol baths and embedded in Paraplast (Fisherbrand, Washington), sectioned at 5–7 μm with a rotatory microtome Leica RM2245 (Leica Microsystems Nussloch GmbH, Nussloch, Germany) and stained with safranin and fast green (Ruzin 1999).

Ectomycorrhiza of pinyon pine from the same area as the sporocarps were collected in 2010 and stored at −20 C for molecular analysis as described above. Ectomycorrhizas with ITS sequences matching up to 99–100% similarity to the sporocarps were histologically screened and morphologically described according to Goodman et al. (1996). For morphological descriptions non-standardized color names are given followed by parenthesized alphanumeric color references (Munsell 1998).

TABLE II. Maximum identity matches of *Geopora* species to the nuLSU sequence (778 base pairs) of *G. piñonensis*

Geopora species	Query cover	Max identity	Accession no.	Reference
Geopora RFLP type E	100.00%	99.00%	HQ630382.1	Gordon and Gehring 2011
Geopora RFLP type Z	91.00%	99.00%	HQ630385.1	Gordon and Gehring 2011
Pyronemataceae sp.	98.00%	99.00%	GQ281477.1	Wei et al. 2010
Geopora RFLP type K	100.00%	98.00%	HQ630384.1	Gordon and Gehring 2011
Geopora sp.	100.00%	98.00%	DQ220345.1	Perry et al. 2007
Geopora RFLP type J	100.00%	97.00%	HQ630383.1	Gordon and Gehring 2011
Geopora cf. cervina	100.00%	97.00%	DQ220344.1	Perry et al. 2007
Geopora sp.	100.00%	97.00%	DQ223973.1	Perry et al. 2007
Geopora sp.	100.00%	97.00%	DQ220338.1	Perry et al. 2007
Geopora arenicola	100.00%	97.00%	DQ220337.1	Perry et al. 2007

RESULTS

Phylogenetic analysis.—The ITS rDNA alignment comprised 108 sequences and were 554 bp long with 268 variable and 201 informative sites. The nuLSU alignment had 23 sequences with a total length of 800 bp, 177 variable sites of which 96 were informative. A total of 3 000 000 and 1 500 000 generations were run in the Bayesian analysis of ITS and nuLSU respectively to reach an average split deviation frequencies of less than 0.01. The sequences from the sporocarps matched the *Geopora* EcM ''type E'' identified with restriction fragment length polymorphism (RFLP) (Gordon and Gehring 2011) with 100% identity for ITS and 99% identity for nuLSU (TABLES I, II). The ITS and nuLSU sequences of *Geopora* ''RFLP type E'' and the new sporocarps (labeled *Geopora pinyonensis*) formed a clade with posterior probability values of 100 for the ITS and 99 for the nuLSU (FIGS. 1, 2). *Geopora* ''RFLP type E'' dominates the ectomycorrhizal community of the pinyon pine in northern Arizona (Gordon and Gehring 2011). Based on the ITS region, the next closest taxon was an unidentified species of Pyronemataceae (accession number GQ281481.1), which establishes ectomycorrhizal associations with *P. tabulaeformis* in China (Wei et al. 2010), the next most closely related taxa was *Geopora* ''RFLP type Z'' (Gordon and Gehring 2011), which is also a pinyon EcM fungus from northern Arizona. These two samples had the highest match to our sample of interest using nuLSU (TABLE II). However, in the Bayesian phylogeny with nuLSU the proximity is inverted in comparison with the ITS phylogenetic inference; the sister group of *G. pinyonensis* is *Geopora* RFLP type Z followed by the unidentified Pyronemataceae. The closer relationship with *Geopora* RFLP type Z was supported with high posterior probabilities in the nuLSU phylogeny.

At least 19 well resolved clades were found with ITS for the genus *Geopora*, with some non-monophyletic species such as *G. cervina*, *G. cooperi*, *G. foliaceae* and *G. arenicola* as well as other undescribed taxa. However, no ITS sequences were available from other species such as *G. pellita*, which according to the nuLSU phylogeny is the basal group of the genus.

TAXONOMY

Geopora pinyonensis L. Flores-Rentería & C.A. Gehring, sp. nov. FIG. 3A–K

MycoBank MB805275

Apothecia are initially hypogeous and closed, later opening to expose the hymenium at ground level, apothecia vary 2.5–13 mm diam in fresh specimens (FIGS. 3A, B) and 1–5 mm diam in dried specimens, regularly symmetrical, globose or with cup shape, 2–10 mm high, lobated, convoluted, fragile, dark brown, furrows filled with mycelia and debris, edge folded or rolled back, tomentose with hyaline or more often brown hairs. Paraphyses simple and not exceeding asci, narrow, about 0.6 μm diam, filiform, septate, straight, unbranched, rounded at apex (FIG. 3C). Asci eight-spored, uniseriate, up to 200 × 14.4–21 μm, operculate, cylindrical, thin-walled, hyaline (FIG. 3D), non-amyloid, hyaline in KOH, no bluish reaction to Melzer's reagent. Ascospores (16.8–)20(–22.3) × 8.5–10.2 μm, the ratio of length and width was 1.9, broadly ellipsoid, smooth, hyaline, slightly thick-walled, hyaline in 5% KOH, with an intracellular granular or oily content, containing one central guttule and sometimes two (FIG. 3E). Abhymenium pale rusty tan (7.5YR 3/4–4/4), hairy, basal tuft, binding soil particles. Mycelial tuft present at the base, hairs rusty brown with debris and soil attached. No KOH reaction on the peridium or mycelium. Hymenium 124–200 μm thick, light brown (to yellow gray [5Y 8/1], smooth; FIG. 3C). Medullary excipulum about 230 μm, composed of dense textura intricata, cells 9–18 μm diam, hyaline (FIG. 3F). Ectal excipulum about 48–53 μm thick, cells generally rounded, non-interlocking, 26–30 × 20–

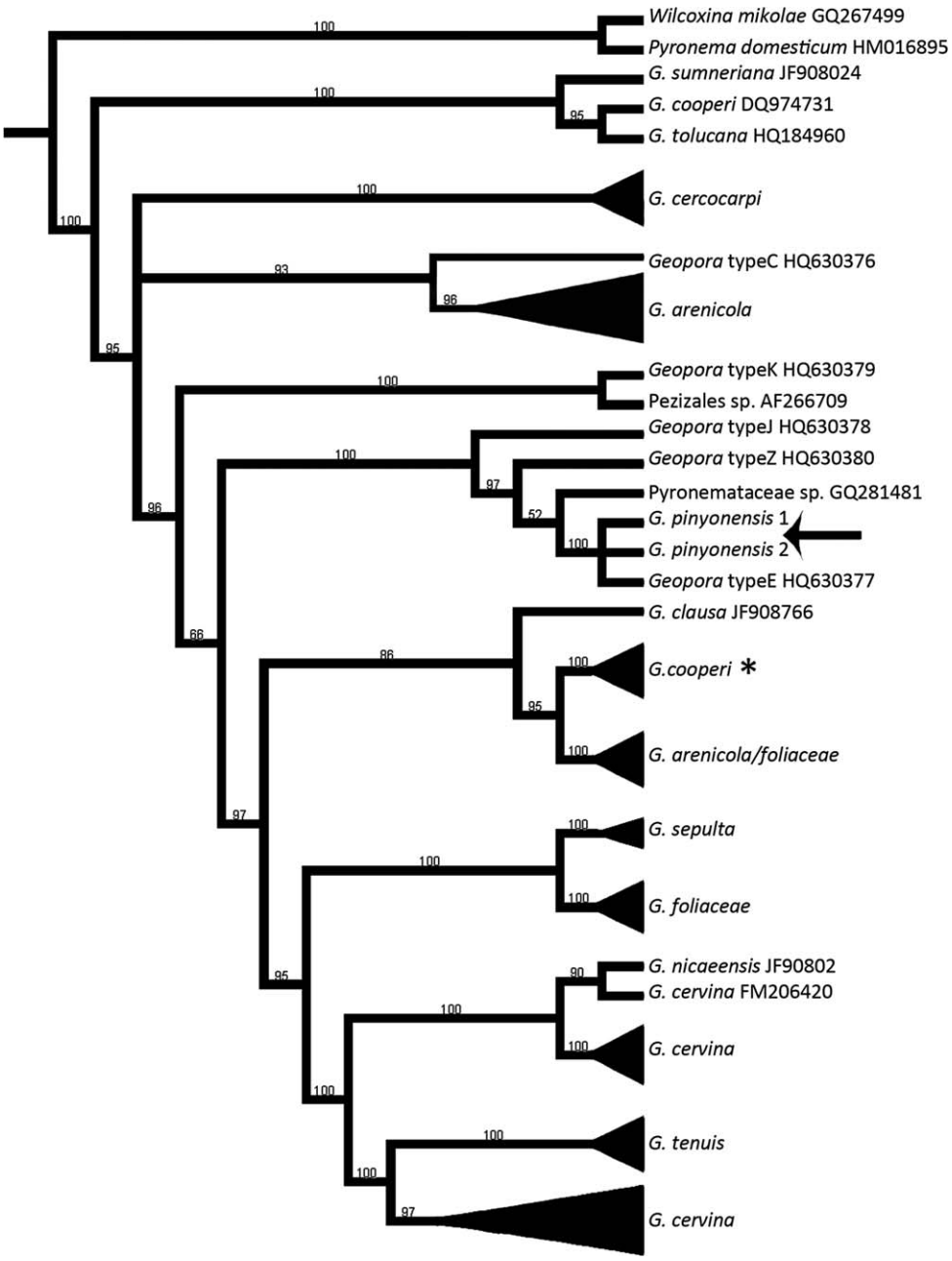

FIG. 1. Bayesian phylogenetic tree inferred from the ITS1, ITS2 and 5.8S. Numbers above branches indicate posterior probability values in percentage. Compressed clades are represented by triangles or by asterisks. Samples of *G. pinyonensis* are indicated by an arrow. Misidentified *G. cooperi* (see Guevara et al. 2012) samples are indicated by an asterisk.

22 μm, hyaline (FIG. 3F). Excipular hairs up to 1500 × 8.8–11.7 mm, rusty brown to hyaline (FIG. 3G), cell wall surface rough (FIG. 3H), wall 1.5 μm thick, branched; septate, septa thicker at margin, thinner (0.7 μm) centrally; apically rounded (FIG. 3H). Gleba hollow with semi-labyrinthoid chambers (FIG. 3I).

Ectomycorrhiza brown mostly bipodial, rarely monopodial, generally 2–3 mm long × 0.5 mm (FIG. 3J); surface texture shiny in places with a covering of short multicellular emanating hyphae 4.5–6 mm wide; mycelial strands (rhizomorphs) rarely present. Hartig net simple; specialized cells not seen (FIG. 3K). Mantle pseudoparenchymatous of non-interlocking irregular synenchyma lacking intercellular spaces; cells 13(10–16) × 9(7–11) μm with rounded to straight sides (FIG. 3L).

Holotype: USA. Arizona: Flagstaff. Sunset Crater, (N35.397689, W-111.425058, 1900 m), in basaltic soil

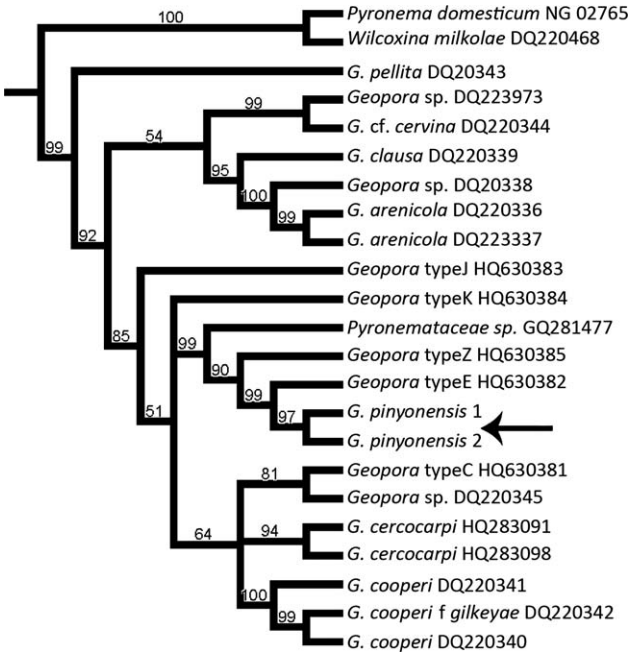

FIG. 2. Bayesian phylogenetic consensus tree inferred from nuLSU sequence data of *Geopora* species and two outgroups. Numbers above branches indicate posterior probability values in percentage. Samples of *G. pinyonensis* are indicated by an arrow.

on pinyon-juniper woodland, 15 Sep 2012. Specimens were deposited in Sam Mitchel Herbarium of Fungi (Denver Botanic Garden) (DBG) Voucher DGB 27586.

Habit and habitat.—Scattered or in clusters, hypogeous, buried deeper in soil, partially emergent at the soil surface, ectomycorrhizal mutualist with *Pinus edulis*. Collection site was pinyon-juniper woodland. Dominant species were pinyon pine (*P. edulis*) and one seed juniper (*Juniperus monosperma*). Subdominants were primarily *Rhus triolobata*, *Ephedra* sp. and *Fallugia paradoxa*. Sporocarps were erumpent on basaltic cinders in the open (i.e. not under any canopy) and near moss (*Ceratodon* sp. and *Bryum* sp.). No woody debris was present near (0.5 m) the fruiting bodies. Sporocarps were gregarious but rare. Only a single clump was found in an area of more than 100 square m and none have been observed in more than 20 y of monitoring fungi at this location. The sporocarps were brown to dark brown. The texture was brittle, and because the flesh of the fruiting bodies was thin they were easily broken. No scent was detected.

Etymology: pinyonensis pertaining to the host, *Pinus edulis*, common name pinyon pine.

Known distribution: Northern Arizona, USA.

DISCUSSION

On the basis of molecular and morphological data we determined that a group of sporocarps collected at Sunset Crater, northern Arizona, correspond to an undescribed *Geopora* species, here named *Geopora pinyonensis*. Furthermore the ITS and nuLSU sequences of these sporocarps had 100% homologies to an abundant member of the EcM community associated with pinyon pines, previously denominated as *Geopora* RFLP type E (Gordon and Gehring 2011). Similarly, Southworth and Frank (2011) linked the sporocarps of a new species, *G. cercocarpi*, to its previously described EcM type (McDonald et al. 2010). Based on morphology and rDNA sequences, the sporocarps collected from Sunset Crater, Arizona, all belong to the same species. This was supported by phylogenetic analyses, which grouped, with high bootstrap values, the consensus sequences from the sporocarps in one clade along with the sequence from EcM *Geopora* RFLP type E. Based in the nuLSU the next closest relative, among the sampled taxa, is another undescribed *Geopora* sp. found in sympatry, *Geopora* RFLP type Z, which forms an EcM association with *P. edulis* (Gordon and Gehring 2011) and has a 97% homology to *G. pinyonensis*. Further molecular and morphological studies should be done to confirm that *Geopora* RFLP type Z represents a different species of *Geopora*. However in the ITS phylogenetic analysis the closest relative is an undescribed species that forms EcM with *P. tabulaeformis* distributed in China and has 96% similarity to *G. pinyonensis*, followed by *Geopora*

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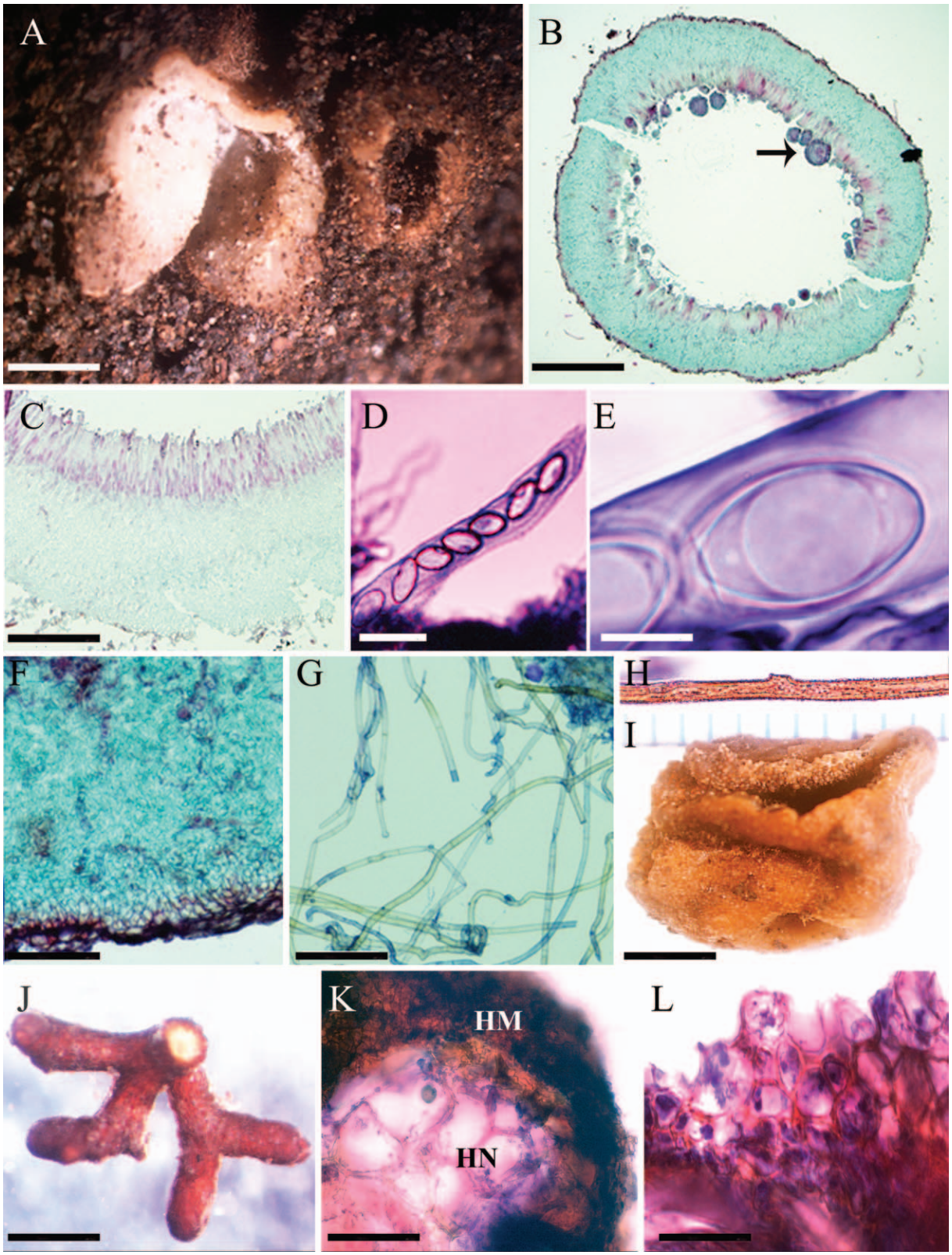
FIG. 3. Morphological description of *Geopora pinyonensis*. A. Hypogeous sporocarps on basaltic soil; bar = 4 mm. B. Cross section of a developing sporocarp parasitized by unknown fungicolous fungi (arrow); bar = 650 μm. C. Cross section of an immature non-parasitized sporocarp showing the hymenium, subhymenium and excipulum; bar = 250 μm. D. Ascus with eight spores, one is not evident, bar = 30 μm. E. Ascospore with central guttule; bar = 8 μm. F. Medullary and ectal excipullum; bar = 100 μm. G. Septate excipular hair; bar = 100 μm. H. Close-up of ornamentations; bar = 80 μm. I. Mature sporocarp showing



perithecium of the parasitic fungiculous fungi; bar = 3 mm. J. Brown ectomycorrhiza from *Pinus edulis* genetically matching the sporocarp; bar = 800 µm. K. Cross section of the ectomycorrhiza showing the hyphal mantle (HM) and the intercellular hyphae of the Hartig net (HN); bar = 90 µm. L. Close-up of the hyphal mantle showing the non-interlocking irregular synenchyma; bar = 20 µm.

RFLP type Z, suggesting sufficient divergence to be recognized as a separate taxon. *G. pinyonensis* and its closest relatives, which are undescribed species, grouped out of the well supported genetic lineages recognized in Tamm et al. (2010).

Of interest, of the unidentified EcM taxa from northern Arizona (see Gordon and Gehring 2011), *Geopora* ''RFLP type E, Z and J'' are grouped together whereas *Geopora* ''RFLP type K'' grouped with an unidentified Pyronemataceae species from the White Mountains of California (accession number AF266709), which is a dominant species of the EcM community of bristlecone pine (*P. longaeva*; Bidartondo et al. 2001). *Geopora* ''RFLP type C'' is closely related to *G. arenicola*.

The total number of species belonging to genus *Geopora* is unclear (Tamm et al. 2010); although at least 31 taxa names are currently recorded in the MycoBank database (Crous et al. 2004), only 10 lineages were genetically recognized in the most comprehensive analysis of the genus to date (Tamm et al. 2010). *Geopora* has been recorded mainly in Europe (Tamm et al. 2010), however recent studies using morphological and genetic information have led to the description of new species in North America, *G. cercocarpi* (Southworth and Frank 2011) and *G. tolucana*, the latter being grouped with species having hypogeous apothecia such as *G. cooperi* (Guevara-Guerrero et al. 2012). However some species shown to belong to *Geopora* on the basis of sequence data previously were classified in other genera such as *Humaria*, *Lachnea*, *Scutellinia* and *Sepultaria* (Burdsall 1968, Tamm et al. 2010 and references therein). The use of ITS sequences of ''*Geopora*'' specimens, incorrectly identified by morphological characters, has resulted in unresolved clades in some analyses due to the fact that morphological features highly overlap among species (Tamm et al. 2010). This is notable (FIG. 1) for i. samples potentially misidentified as *G. cooperi* (asterisk), ii. the clade with high support having samples identified as *G. foliaceae* and *G. arenicola*, and iii. the paraphyly of *G. cervina*. Although other processes such as homoplasy or incomplete lineage sorting could explain the low internal resolution of the genus, the lack of morphological differentiation among species leading to erroneous identifications could be the reason for unresolved clades and paraphyly in the analysis (see Flores-Renteria et al. 2013).

Morphological features, such as sporocarp size, hymenium color, ascospore dimensions and excipular hair length, widely overlap in *Geopora* (Tamm et al. 2010), including *G. pinyonensis* and the other two recently described species, *G. cercocarpi* and *G. tolucana* (Southworth and Frank 2011, Guevara-Guerrero et al. 2012). For example, *G. pinyonensis* has relatively small hypogeous sporocarps (0.25–1.3 cm in fresh samples and 0.1–0.5 cm in dried samples), however the sizes overlap mainly with dried specimens of members of clade VI (see FIG. 1 in Tamm et al. 2010), which also has small sporocarps (0.2–0.3 cm). The size of sporocarps is distinctive in *G. cercocarpi*, which can be up to 5.5 cm diam in fresh samples, according to Southworth and Frank (2011), however *G. sepulta* is 5–6 cm diam (clade VII, Tamm et al. 2010), suggesting that the sporocarp size widely overlaps in *Geopora*. *G. pinyonensis* has a light brown to yellow-gray hymenium; hymenium in other species is white (*G. cercocarpi*, *G. tolucana*), gray (*G. tenuis*), whitish or yellowish brownish (*G. foliaceae*), however some species have intraspecific variation in the hymenium (e.g. *G. arenicola* can be pale gray to lilac-gray, whitish or yellowish brownish, *G. sepulta* has pale gray to lilac-gray or gray hymenium and *G. cervina* light brown to gray (Tamm et al. 2010, Southworth and Frank 2011, Guevara-Guerrero et al. 2012). The intraspecific color variation in the hymenium is due to the amount of light available during growth, with white hymenia growing in shade and darker hymenia growing under light exposure, according to Southworth and Frank (2011). The size and shape of the ascospores was widely used in the past to characterize members of *Geopora* (see Yao and Sponner 1996); *G. pinyonensis* has the smallest ascospores in the genus whereas *G. tolucana* has the largest (Guevara-Guerrero et al. 2012). However, the ascospore size in these species widely overlaps with other members of the genus. Recent studies have shown that the excipular hair length is not an informative taxonomic feature because it has substantial intraspecific variation and broadly overlaps among monophyletic groups detected in the ITS phylogeny (Tamm et al. 2010). The sporocarp shape in the genus is mainly cupulate, but it can be ptychothecial in species of *G. cooperi* and *G. tolucana*, a feature that led to the suggestion that these species be placed into a new genus (discussed in Guevara-Guerrero et al. 2012, Stielow et al. 2013). Therefore, because no obvious morphological feature can be used in isolation to identify to *G. pinyonensis*, morphological analyses should be accompanied by molecular data. Geographic distribution, substrate and ectomycorrhizal host species are some features that also might help identify species of *Geopora*.

Surveys and identification of sporocarps are not only useful to describe the current biodiversity of *Geopora* species but also to understand more about their biology and relationship with host plants (Gehring et al. 1998, Fujimura et al. 2005, Tedersoo et al. 2006, Hrynkiewicz et al. 2009, Ishida et al. 2009, Sthultz et al.

2009, Wei et al. 2010). A large percentage of the few *G. pinyonensis* samples we observed were associated with a small ascomycete fungus that formed perithecia in association with the *Geopora* sporocarp (FIG. 3B). The presence of such fungicolous fungi has not been described for other members of *Geopora*; further investigations should be undertaken to describe the nature of this interaction and whether this feature is exclusive of *G. pinyonensis*. In addition, soil inoculum dominated by *G. pinyonensis* has been shown to strongly increase the growth of *P. edulis,* which recently has suffered large scale mortality associated with drought (Breshears et al. 2005, Mueller et al. 2005, Garrity et al. 2013, Gehring et al. in review). Therefore, sporocarps of *G. pinyonensis* may offer a tool for direct inoculation on *P. edulis* for restoration. *Geopora* as a genus seems to occur in sites prone to drought (Ishida et al. 2009, Gordon and Gehring 2011) and ectomycorrhizal associations with *Geopora* species are being observed in increasing frequency (Southworth and Frank 2011, Guevara-Guerrero et al. 2012, Gehring et al. 2013). Further studies are necessary to determine whether other *Geopora* species also promote host plant growth in stressful environments.

Our observation that *G. pinyonensis* sporocarps formed in association with a wet summer is in agreement with Southworth's and Frank's (2011) hypothesis that fruiting in *Geopora* species occurs during unusually wet conditions. In cases like this where sporocarp production is associated with a rare event the molecular and morphological identification of mycorrhiza would not only complement sporocarp descriptions in studies of biodiversity but could be the primary approach for new species description. Even when sporocarps were observed, their features overlapped with those of closely related species, a finding consistent with previous work showing that morphological and molecular approaches are necessary to resolve phylogenetic relationships in the genus *Geopora*. Understanding more about the phylogenetic relationships of species of genus *Geopora* would help us understand the distribution and ecology of this poorly known but important genus.

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LITERATURE CITED

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410.

Bidartondo MI, Baar J, Bruns TD. 2001. Low ectomycorrhizal inoculum potential and diversity from soils in and near ancient forests of bristlecone pine (*Pinus longaeva*). Can J Bot 79:293–299.

Breshears DD, Cobb NS, Rich PM, Price KP, Allen CD, Balice RG, Romme WH, Kastens JH, Floyd ML, Belnap J, Anderson JJ, Myers OB, Meyer CW. 2005. Regional vegetation die-off in response to global change type. Proc Natl Acad Sci (USA) 102:15144–15148, doi:10.1073/pnas.0505734102

Brundrett M. 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320:37–77, doi:10.1007/s11104-008-9877-9

———, Bougher N, Dell B, Grove T, Malajczuk N. 1996. Working with Mycorrhizas in forestry and agriculture. Canberra: Australian Centre for International Agricultural Research. 374 p.

Burdsall HH Jr. 1968. A revision of the genus *Hydnocystis* (Tuberales) and of the hypogeous species of *Geopora* (Pezizales). Mycologia 60:496–525, doi:10.2307/3757418

Crous PW, Gams W, Stalpers JA, Robert V, Stegehuis G. 2004. MycoBank: an online initiative to launch mycology into the 21st century. Stud Mycol 50:19–22.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat Meth 9:772, doi:10.1038/nmeth.2109

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput, Nucleic Acids Res 32:1792–97.

Flores-Rentería L, Wegier A, Ortega Del Vecchyo D, Ortíz-Medrano A, Piñero D, Whipple AV, Molina-Freaner F, Domínguez CA. 2013. Genetic, morphological, geographical and ecological approaches reveal phylogenetic relationships in complex groups, an example of recently diverged pinyon pine species (subsection Cembroides). Mol Phylogenet Evol 69:940–949, doi:10.1016/j.ympev.2013.06.010

Fujimura KE, Smith JE, Horton TR, Weber NS, Spatafora JW. 2005. Pezizalean mycorrhizas and sporocarps in ponderosa pine (*Pinus ponderosa*) after prescribed fires in eastern Oregon, USA. Mycorrhiza 15:79–86, doi:10.1007/s00572-004-0303-8

Garrity SR, Allen CD, Brumby SP, Gangodagamage C, McDowell NG, Cai DM. 2013. Quantifying tree mortality in a mixed species woodland using multitemporal high spatial resolution satellite imagery. Remote Sens Environ 129:54–65, doi:10.1016/j.rse.2012.10.029

Gehring CA, Flores-Rentería D, Sthultz C, Leonard T, Flores-Rentería L, Whipple A, Whitham T. 2014. Plant genetics and interspecific competitive interactions determine ectomycorrhizal fungal community responses to climate change. Mol Ecol 23:1379–1391, doi:10.1111/mec.12503

———, Theimer TC, Whitham TG, Keim P. 1998. Ectomycorrhizal fungal community structure of pinyon pines growing in two environmental extremes. Ecology 79: 1562–1572, doi:10.1890/0012-9658(1998)079[1562:EFCSOP]2.0.CO;2

Gordon GJ, Gehring CA. 2011. Molecular characterization of pezizalean ectomycorrhizas associated with pinyon pine during drought. Mycorrhiza 21:431–41, doi:10.1007/s00572-010-0349-8

Guevara G, Bonito G, Cázares E, Rodriguez JA, Vilgalys R. 2008. *Tuber regimontanum*, new species of truffle from México. Rev Mex Micol 26:17–20.

———, ———, Trappe JM, Cázares E, Williams G, Healy RA, Schadt C, Vilgalys R. 2013. New North American truffles (*Tuber* spp.) and their ectomycorrhizal associations. Mycologia 105:194–209, doi:10.3852/12-087

Guevara-Guerrero G, Stielow B, Tamm H Cázares-Gonzalez E, Göker M. 2012. *Genea mexicana*, sp. nov. and *Geopora tolucana*, sp. nov., new hypogeous Pyronemataceae from Mexico, and the taxonomy of *Geopora* reevaluated. Mycol Prog 11:711–724, doi:10.1007/s11557-011-0781-y

Guindon S, Gascuel O. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Sys Biol 52:696–704, doi:10.1080/10635150390235520

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98.

Hansen K, Pfister DH. 2006. Systematics of the Pezizomycetes—the operculate discomycetes. Mycologia 98: 1029–1040, doi:10.3852/mycologia.98.6.1029

Hawksworth DL. 2001. The magnitude of fungal diversity: the 15 million species estimate revisited. Mycol Res 105: 1422–1432, doi:10.1017/S0953756201004725

Hrynkiewicz K, Baum C, Niedojadło J, Dahm H. 2009. Promotion of mycorrhiza formation and growth of willows by the bacterial strain Sphingomonas sp. 23L on fly ash. Biol Fertile Soils 45:385–394, doi:10.1007/s00374-008-0346-7

Hubert NA, Gehring CA. 2008. Neighboring trees affect ectomycorrhizal fungal community composition in a woodland-forest ecotone. Mycorrhiza 18:363–374, doi:10.1007/s00572-008-0185-2

Ishida TA, Nara K, Ma SR, Takano T, Liu SK. 2009. Ectomycorrhizal fungal community in alkaline-saline soil in northeastern China. Mycorrhiza 19:329–335, doi:10.1007/s00572-008-0219-9

Lantieri A, Smith ME, Pfister DH. 2012. A new species of *Ruhlandiella* (Pezizaceae) from Italy. Mycol Prog 11: 509–513, doi:10.1007/s11557-011-0766-x

Maddison WP, Maddison DR. 2011. Mesquite 2.75: a modular system for evolutionary analysis. http://mesquiteproject.org

McDonald KR, Pennell J, Frank JL, Southworth D. 2010. Ectomycorrhizas of *Cercocarpus ledifolius* (Rosaceae). Am J Bot 97:1867–1872, doi:10.3732/ajb.0900357

McHugh TA, Gehring CA. 2006. Belowground interactions with arbuscular mycorrhizal shrubs decrease the performance of pinyon pine and the abundance of its ectomycorrhizas. New Phytol 171:171–178, doi:10.1111/j.1469-8137.2006.01735.x

Mueller RC, Gehring CA. 2006. Interactions between an above-ground plant parasite and below-ground ectomycorrhizal fungal communities on pinyon pine. J Ecol 94:276–284, doi:10.1111/j.1365-2745.2006.01105.x

———, Scudder CM, Porter ME, Trotter RT, Gehring CA, Whitham TG. 2005. Differential tree mortality in response to severe drought: evidence for long-term vegetation shifts. J Ecol 93:1085–1093, doi:10.1111/j.1365-2745.2005.01042.x

Munsell color. 1998, Munsell soil color charts. New Windsor, New York: Macbeth. 10 p. 11 plates.

Perry BA, Hansen K, Pfister DH. 2007. A phylogenetic overview of the family Pyronemataceae (Ascomycota, Pezizales). Mycol Res 3:549–571, doi:10.1016/j.mycres.2007.03.014

Rinaldi AC, Comadini O, Kuyper TW. 2008. Ectomycorrhizal fungal diversity: separating the wheat from the chaff. Fungal Divers 33:1–45.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 19:1572–4, doi:10.1093/bioinformatics/btg180

Ruzin SE. 1999. Plant microtechnique and microscopy. New York: Oxford Univ. Press. p 93–95.

Selmants CS, Hart SC. 2008. Substrate age and tree islands influence carbon and nitrogen dynamics across a retrogressive semiarid chronosequence. Global Biogeochem Cy 22:GB1021, doi:10.1029/2007GB003062

Smith ME, Trappe JM, Rizzo DM. 2006. *Genea*, *Genabea* and *Gilkeya* gen. nov.: ascomata and ectomycorrhiza formation in a Quercus woodland. Mycologia 98:699–716, doi:10.3852/mycologia.98.5.699

Smith SE, Read DJ. 1997. Mycorrhizal symbiosis. San Diego: Academic Press. 787 p.

Southworth D, Frank JL. 2011. Linking mycorrhizas to sporocarps: a new species, *Geopora cercocarpi*, on *Cercocarpus ledifolius* (Rosaceae). Mycologia 103: 1194–1200, doi:10.3852/11-053

Sthultz CM, Whitham TG, Kennedy KJ, Deckert R, Gehring CA. 2009. Genetically based susceptibility to herbivory influences the ectomycorrhizal fungal communities of a foundation tree species. New Phytol 184:657–667, doi:10.1111/j.1469-8137.2009.03016.x

Stielow B, Hensel G, Strobelt D, Makonde HM, Rohde M, Dijksterhuis J, Klenk H-P, Göker M. 2013. Hoffmannoscypha, a novel genus of brightly colored, cupulate Pyronemataceae closely related to *Tricharina* and *Geopora*. Mycol Prog 12:675–686, doi:10.1007/s11557-012-0875-1

Tamm H, Poldmaa K, Kullman B. 2010. Phylogenetic relationships in genus *Geopora* (Pyronemataceae, Pezizales). Mycol Prog 9:509–522, doi:10.1007/s11557-010-0659-4

Taylor DL, Bruns TD. 1999. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. Mol Ecol 8:1837–1850, doi:10.1046/j.1365-294x.1999.00773.x

Tedersoo L, Hansen K, Perry BA, Rasmus K. 2006. Molecular and morphological diversity of Pezizalean ectomycorrhiza. New Phytol 170:581–596, doi:10.1111/j.1469-8137.2006.01678.x

———, May TW, Smith ME. 2010. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20:217–263, doi:10.1007/s00572-009-0274-x

Thiers HD. 1984. The secotioid syndrome. Mycologia 76:1–8, doi:10.2307/3792830

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The Clustal X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24:4876–4882, doi:10.1093/nar/25.24.4876

Wei J, Peršoh D, Agerer R. 2010. Four ectomycorrhizae of Pyronemataceae (Pezizomycetes) on Chinese pine (*Pinus tabulaeformis*): morpho-anatomical and molecular-phylogenetic analyses. Mycol Prog 9:267–280, doi:10.1007/s11557-009-0637-x

Yao YJ, Spooner BM. 1996. *Geopora sepulta* (Pezizales) in Britain, with a key to British species of the genus. Kew Bull 51:381–383, doi:10.2307/4119336