Mycorrhiza (2012) 22:403–408
DOI 10.1007/s00572-011-0411-1

ORIGINAL PAPER

# Two species of the Asian endemic genus *Keteleeria* form ectomycorrhizas with diverse fungal symbionts in southwestern China

**Zai-Wei Ge · Matthew E. Smith · Qing-Ying Zhang · Zhu L. Yang**

Received: 19 June 2011 / Accepted: 5 September 2011 / Published online: 14 October 2011


**Abstract** The ectomycorrhizal status of *Keteleeria* species is reported for the first time based on morphological and molecular analyses of root tips from southwestern China. Based on internal transcribed spacer rDNA sequences, we detected 26 ectomycorrhizal (ECM) fungal species on roots of *Keteleeria evelyniana* and *Keteleeria davidiana* collected from natural sites and a botanical garden in Kunming, China. These ECM symbionts represent six fungal lineages, including */russula–lactarius*, */inocybe*, */sebacina*, */tomentella–thelephora*, */wilcoxina*, and */cenococcum*. Our results provide the first evidence of ECM formation by *Keteleeria* and also supply ecologically important information for conservation and restoration efforts to recover populations of *Keteleeria*.

**Keywords** Ectomycorrhizal fungi · *Keteleeria evelyniana* Mast. · *Keteleeria davidiana* (Bertrand) Beissner · Pinaceae

Z.-W. Ge (✉) · Z. L. Yang
Key Laboratory of Biodiversity and Biogeography,
Kunming Institute of Botany, Chinese Academy of Sciences,
Kunming 650204, China
e-mail: zaiweige@gmail.com

M. E. Smith
Biology Department, Duke University,
Durham 27705 NC, USA

M. E. Smith (✉)
Department of Plant Pathology, University of Florida,
Gainesville 32611-0680 FL, USA
e-mail: trufflesmith@gmail.com

Q.-Y. Zhang
Industrial Crops Research Institute,
Yunnan Academy of Agricultural Sciences,
Kunming 650205, China

## Introduction

The coniferous family Pinaceae consists of approximately 235 species in 11 genera that are distributed across the Northern Hemisphere. China is a global hotspot of conifer diversity, and four genera of Pinaceae are considered endemic to China and the adjoining countries in southeast Asia, namely *Keteleeria* Carrière, *Pseudolarix* Gordon, *Nothotsuga* Hu ex C. N. Page, and *Cathaya* Chun & Kuang (Fu et al. 1999). *Keteleeria* contains three to five species and several varieties that are known only from southern China, northern Laos, and Vietnam (Fu et al. 1999), and they all have been considered "living fossils" (Manchester et al. 2009). Based on phylogenetic analysis of chloroplast DNA, *Keteleeria* is resolved as sister to the genus *Abies* within Pinaceae (Lin et al. 2010). Among the *Keteleeria* species, *Keteleeria davidiana* var. *calcarea* (W. C. Cheng & L. K. Fu) Silba, *Keteleeria fortunei* (A. Murray bis) Carrière and *Keteleeria hainanensis* Chun & Tsiang (regarded as the juvenile growth of *Keteleeria evelyniana* by some authors), and *Keteleeria xerophila* Hsueh et S. H. Huo are listed in the China Plant Red List (Fu and Chin 1992). Besides being important components of *Pinus* forests and forests dominated by *Castanopsis delavayi* Franch, *Keteleeria* species can also form pure stands throughout China's southern provinces. Species of *Keteleeria* provide high-quality wood and are excellent sources of lumber for construction, furniture, and wood fiber. *Keteleeria* species are also cultivated for reforestation of degraded habitats and as ornamental plants.

Mycorrhizas constitute an important root symbiosis for approximately 92% of plant families, but the ectomycorrhizal (ECM) symbiosis is phylogenetically restricted and has evolved separately in several lineages of land plants (Wang and Qiu 2006). All of the genera in the family

Pinaceae that have been surveyed form ECM symbioses, or sometimes they form both ectomycorrhizas and arbuscular mycorrhizas (Wang and Qiu 2006). Indeed, most Pinaceae are considered obligate ECM symbionts (Wang and Qiu 2006). The ECM symbiosis has been confirmed in several genera of Pinaceae, including *Abies*, *Cathaya*, *Cedrus*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga*, and *Tsuga* (e.g., Vaario et al. 2006; Wang and Qiu 2006). Studies of ECM fungal communities associated with *Pineaceae* in natural forest habitats usually reveal high fungal diversity (Yamada and Katsuya 2001; Lian et al. 2006; Matsuda and Hijii 2004; Yu et al. 2007). *Keteleeria* has often been assumed to form ECM symbioses because it belongs to the Pinaceae (e.g., Brundrett 2009; Smith and Read 2008), although some researchers (e.g., Tao and Cheng 2009) have stated that *Keteleeria* is probably not ectomycorrhizal. The mycorrhizal status of the genus *Keteleeria* has never been fully documented. In the present study, we confirm the ectomycorrhizal status of two species of *Keteleeria*—*K. evelyniana* Mast. and *K. davidiana* (Bertrand) Beissner—and show that these plants associate with a wide diversity of ECM fungi.

## Materials and methods

### Sampling sites and sample processing

The sampling areas are all located in the vicinity of the Kunming in Yunnan Province, southwestern China (24°23′ N, 102°10′ E). Roots of *K. evelyniana* were sampled at two relatively undisturbed forest sites, Golden Temple Park (GT) and Xishan Park (XS). Both sites are dominated by *Keteleeria* with interspersed individuals of *Pinus armandii* Franch. Soil cores of approximately 30×20×20 cm were taken from the *K. evelyniana* rhizosphere of four trees at each site in September 2010 (GT) and February 2011 (XS). Root samples of *K. davidiana* and *K. davidiana* var. *calcarea* were sampled in a disturbed garden environment at the Kunming Botanical Garden (KBG) in June 2010. We collected eight samples from each tree species or variety, resulting in a total of 16 root samples. Excavated roots were placed in polypropylene bags, transported back to laboratory within 1.5 h, and then stored at 4°C and processed within 2 days.

The ECM roots were soaked in water and then washed over a 1-mm sieve. Root tips were further cleaned in a Petri dish using a brush and a spray bottle. Cleaned sections of ECM roots were observed under a dissecting microscope (Zeiss Stemi 1000) and macroscopically sorted into gross morphotypes based on color, surface texture, type of branching, and emanating hyphae (Agerer 2006). We selected 16 individual ECM root tips representing several different morphotypes from each tree and transferred them to Eppendorf tubes with sterile forceps for molecular analysis. An ACT-2U Auto Camera Tame (2004 Nikon Corporation) was used to photograph ECM roots.

### Molecular protocols and informatics

For *K. davidiana*, ECM root tips were ground in 1.5-ml Eppendorf tubes using a plastic pestle, and DNA was extracted using a modified CTAB extraction protocol (Gardes and Bruns 1993). For *K. evelyniana* and *K. davidiana* var. *calcarea*, DNA was extracted from ECM roots with the Extract-N-Amp kit (Sigma) following the manufacturer's protocol, except that 50% of the suggested volume was used per root tip.

For the host plant identification, two root tips for each host plant species were sampled. The chloroplast trnL region (trnC–trnD) was amplified using the primers trnC (5′-cgaaatcggtagacgctacg-3′) and trnD (5′-ggggatagagggacttgaac-3′) (Taberlet et al. 1991). For the molecular identification of ECM fungi, primers ITS1f, ITS4, and ITS4B were used to amplify the internal transcribed spacer (ITS) region of nuclear rDNA (Gardes and Bruns 1993; White et al. 1990). The PCR reactions were performed on an ABI 2720 thermocycler (Applied Biosystems, Foster City, CA, USA), using 25 μl reaction volumes each containing: 2 μl DNA template, 2.5 μl of 10× amplification buffer (Biomed, Beijing 100097, China) containing 1.5 mM $MgCl_2$, 0.5 μl dNTPs mix (10 mM), 1.0 μl of each primer (5 μM), 0.3 μl Taq DNA polymerase (5 $U\,\mu l^{-1}$; Biomed). Cycling parameters were 94°C for 3 min, followed by 35 cycles of 94°C for 40 s, 50°C for 40 s, and 72°C for 70 s, with a final extension at 72°C for 8 min. Amplification products were electrophoresed in 1% agarose gels stained with gel view (BioTeke Corporation, Beijing, China) and visualized under UV light. A 1 kb DNA ladder (Invitrogen, Carlsbad, CA, USA) was used as marker. Controls with $ddH_2O$ instead of DNA were included in every set of amplifications. In most cases, PCR products were directly sent for sequencing after purification. For samples that failed in direct sequencing, the PCR products were inserted to TakaRa pMD18-T Vector after gel purification. Cloned inserts were checked by PCR using a primer combination of M13 (+)×M13 (−). Colonies were sent to Shanghai Sangon Biological Engineering Technology and Service Co., Ltd., for sequencing after incubation in liquid culture medium.

Root tip sequences were queried against the Genbank database to infer the identity of the host plants and the ECM fungal phylotypes. Molecular operational taxonomic units (MOTUs) were defined as sequences sharing 97% or greater similarity within the ITS region (Smith et al. 2007). Each MOTU was assigned to one of the ectomycorrhizal lineages defined by Tedersoo et al. (2010). The sequences

produced in this study have been deposited in GenBank with accession numbers (JN129390–JN129422).

## Results

The trnL sequences (JN129422, JN129421) obtained from the host plant roots of *K. evelyniana* from XS and GT were either 99% (380/384) or 100% (336/336) similar to *K. evelyniana* sequences retrieved from GenBank (EF395417, AY013741). Similarly, the sequences (JN129420, JN129419) obtained from root tips of *K. davidiana* and *K. davidiana* var. *calcarea* in the Kunming Botanical Garden were 99% (305/308) and 99% (384/386) similar to *K. davidiana* sequences (AP010820, AP010820). These results confirm that the ECM roots we sampled belonged to the target *Keteleeria* species.

Ectomycorrhizal colonization was evident in all *Keteleeria* roots we collected, and we observed several different morphotypes (Fig. 1b–d). Microscopic examination of cross-sectioned ECM roots showed distinct hyphal mantles and well-developed Hartig's nets (Fig. 1a) both hallmark traits of classic ECM colonization. One non-ECM fungus, a species of *Termitomyces*, was found on a single ECM root at the GT site.

Out from the 256 root tips, we obtained 188 successful sequences in total, and the subsequent molecular analysis revealed 26 fungal MOTUs. Among these, five phylotypes were detected on ECM roots of *K. davidiana* in a managed landscape and 24 from ECM roots of *K. evelyniana* growing in seminatural forest stands (Table 1). The ECM fungi we detected represent six lineages: */russula–lactarius*, */inocybe*, */sebacina*, */tomentella–thelephora*, */wilcoxina*, and */cenococcum*. Ascomycete genera detected with *Keteleeria* included *Trichophaea* and *Cenococcum*, and basidiomycete genera included *Russula*, *Lactarius*, *Inocybe*, *Sebacina*, and *Tomentella* (Table 1). Among these, *Russula* spp. were the most diverse and frequently detected (13 MOTUs) followed by *Inocybe* (4 MOTUs), *Tomentella* (3 MOTUs), *Lactarius* (2 MOTUs), and *Cenococcum* (2 MOTUs). Most of the ECM fungi were restricted to a single site, but *Trichopahaea* sp. 1 was found in both of the forest sites (XS and GT) and also in the botanical garden (KMG). Three out of the five ECM MOTUs that were detected in the garden were also found in nearby forest habitats. Only two unique sequences of *Inocybe* spp. were detected in the garden.

## Discussion

In this study, we confirmed that species of *Keteleeria* consistently form symbiotic root associations with ECM fungi. All of the samples from beneath *Keteleeria* contained ECM roots with classic macroscopic and microscopic morphological features (Fig. 1). Using ITS rDNA sequencing, we detected diverse fungal symionts, including 26 MOTUs associated with the roots of

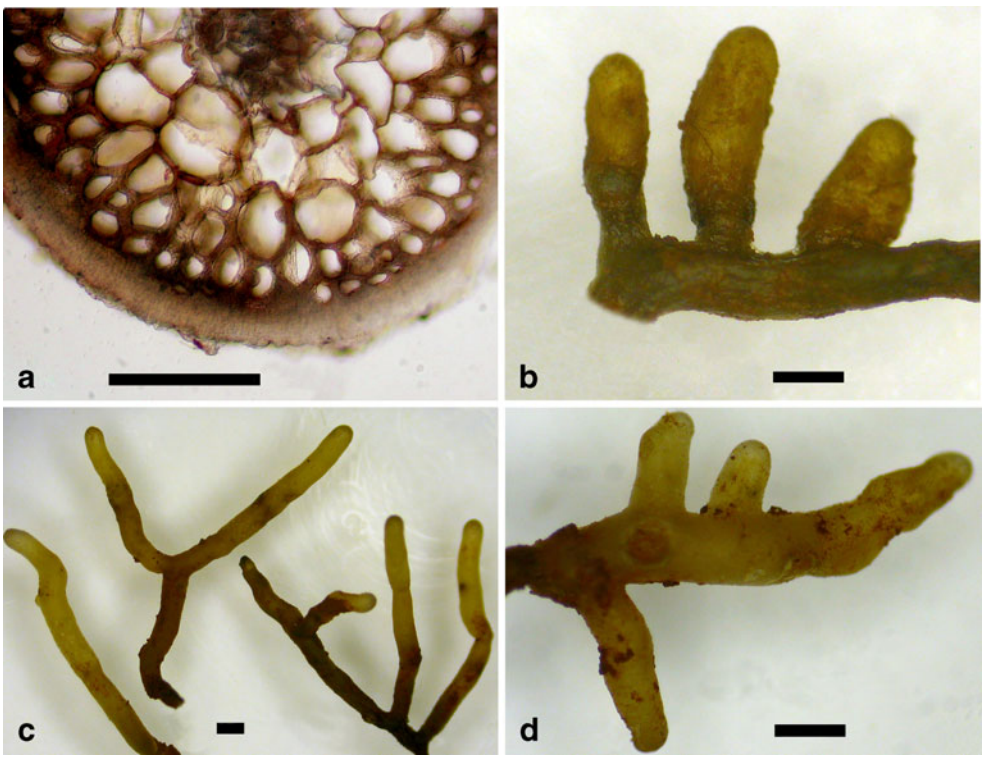


**Fig. 1** Morphological–anatomical characteristics of *Keteleeria* ectomycorrhizae. **a** Transverse section of ectomycorrhiza showing the mantle and Hartig net. **b**–**d** Simple and irregularly pinnate ectomycorrhizal roots. **a** *bar*=0.3 mm; **b**–**d** *bar*=1 mm

**Table 1** List of the 26 species of ectomycorrhizal fungi from six fungal lineages that were detected on roots of two and a variety *Keteleeria* species (Pinaceae) in Yunnan Province, China

| ECM species | ECM lineage | *Keteleeria davidiana* KBG | *Keteleeria davidiana* var. *calcerea* KBG[a] | *Keteleeria evelyniana* GT[b] | *Keteleeria evelyniana* XS | ECM voucher | BLAST match | Similarity (%) |
|---|---|---|---|---|---|---|---|---|
| *Cenococcum geophilum* 1 | /*cenococcum* | | | JN129390 | | G2-3 | *Cenococcum geophilum* DQ179119 | 965/994 (97) |
| *Cenococcum geophilum* 2 | /*cenococcum* | | | JN129391 | | G1-1 | *Cenococcum geophilum* AY394919 | 526/533 (99) |
| *Inocybe* sp. 1 | /*inocybe* | JN129392 | | | | K1-1 | *Inocybe umbrinella* FJ904165 | 585/684 (86) |
| *Inocybe* sp. 2 | /*inocybe* | JN129393 | | | | K4-1 | *Inocybe rimosa* FJ904155 | 510/613 (83) |
| *Inocybe* sp. 3 | /*inocybe* | | | JN129394 | | G2-12 | *Inocybe* cf. *pseudoreducta* FN550906 | 651/736 (88) |
| *Inocybe* sp. 4 | /*inocybe* | | | | JN129395 | X3-12 | *Inocybe* cf. *assimilata* FN550881 | 497/600 (83) |
| *Lactarius* sp. 1 | /*russula–lactarius* | | | JN129396 | | G4-13 | *Lactarius* sp. EF141550 | 702/705 (99) |
| *Lactarius hygrophoroides* | /*russula–lactarius* | | | | JN129397 | X3-4 | *Lactarius hygrophoroides*. HQ318285 | 606/625 (97) |
| *Russula livescens* | /*russula–lactarius* | | JN129398 | | | H3-10 | *Russula livescens* UDB000894 | 700/704 (99) |
| *Russula* sp. 1 | /*russula–lactarius* | | | JN129399 | | G2-13 | *Russula cerolens* HQ604834 | 780/825 (95) |
| *Russula* sp. 2 | /*russula–lactarius* | | | JN129400 | | G2-10 | *Russula pectinatoides* AY880930 | 584/660 (88) |
| *Russula* sp. 3 | /*russula–lactarius* | | | JN129401 | | G2-14 | *Russula* cf. *pectinata* HQ604835 | 678/779 (87) |
| *Russula* sp. 4 | /*russula–lactarius* | | | JN129402 | | G1-16 | *Russula brevipes* FJ845429 | 770/825 (93) |
| *Russula* sp. 5 | /*russula–lactarius* | | | JN129403 | | G4-2 | *Russula puellaris* HQ604852 | 718/821 (87) |
| *Russula* sp. 6 | /*russula–lactarius* | | | JN129404 | | G3-6 | *Russula* sp. AJ534905 | 814/866 (94) |
| *Russula* sp. 7 | /*russula–lactarius* | | | JN129405 | | G3-2 | *Russula integra* HM189840 | 652/683 (95) |
| *Russula* sp. 8 | /*russula–lactarius* | | | JN129406 | | G2-7 | *Russula cerolens* HQ604834 | 798/813 (98) |
| *Russula* sp. 9 | /*russula–lactarius* | | | JN129407 | | G1-6 | *Russula* cf. *maculata* DQ422015 | 820/865 (95) |
| *Russula* sp. 10 | /*russula–lactarius* | | | | JN129408 | X2-2 | *Russula brevipes* FJ845429 | 782/831 (94) |
| *Russula* sp. 11 | /*russula–lactarius* | | | | JN129409 | X3-1 | *Russula bicolor* FJ845435 | 7555/812 (93) |
| *Russula* sp. 12 | /*russula–lactarius* | | | | JN129410 | X1-6 | *Russula* cf. *fuscorubroides* HQ604842 | 767/804 (95) |
| *Sebacina* sp. 1 | /*sebacina* | | | | JN129411 | X3-15 | *Sebacina* sp. DQ974768 | 695/716 (97) |
| *Tomentella* sp. 1 | /*tomentella–thelephora* | | JN129412 | | JN129412 | H1-7 | *Tomentella* ECM of Fagus FM999497 | 628/651 (96) |
| *Tomentella* sp. 2 | /*tomentella–thelephora* | | | JN129413 | | G2-8 | *Tomentella orchid mycorrhiza* EU625857 | 804/833 (97) |
| *Tomentella* sp. 3 | /*tomentella–thelephora* | | | JN129414 | | G1-7 | *Tomentella* ECM of Betula EF218835 | 758/833 (91) |
| *Trichophaea* sp. 1 | /*wilcoxina* | | JN129415 | JN129415 | JN129415 | X4-1 | *Trichophaea* cf. *hybrida* DQ200834 | 522/572 (91) |

[a] Two non-ectomycorrhizal fungi, *Neonectria* cf. *radicicola* (JN129416) and *Tetracladium* cf. *maxilliforme* (JN129417), were also detected on the roots (H4-4 and H4-2, respectively) of *Keteleeria calcerea* at this site

[b] An unknown species of *Agaricales* (JN129418) detected on ectomycorrhizal roots (G1-10) of *Keteleeria evelyniana* was 83% similar (516/620 bp) to *Termitomyces* sp. (AB051890). This fungus may represent a previously unrecognized group or ectomycorrhizal fungi or could represent a contaminant sequence

*Keteleeria*. Half of the fungal phylotypes were members of the */russula–lactarius* lineage, but others belonged to the */inocybe*, */sebacina*, */tomentella–thelephora*, */wilcoxina*, and */cenococcum* lineages. *Russula* spp. and *Lactarius* spp. are well-known ECM genera that are often abundant both above- and belowground in Pinaceae-dominated ecosystems (Matsuda and Hijii 1998, 2004; Yamada and Katsuya 2001).

We recovered far fewer ECM fungi from roots of *K. davidiana* in the botanical garden than from roots of *K. evelyniana* growing in seminatural forests (Table 1). Ectomycorrhizal diversity is often inversely related to time since site disturbance (Boerner et al. 1996) and fertilization often has strong impacts on ECM fungal diversity (Cox et al. 2010; Lilleskov et al. 2002), so it is not surprising that the garden habitat had lower ECM diversity than the forest habitats. The reduced ECM diversity on roots of *K. davidiana* is probably due to a combination of factors, including a highly disturbed soil layer, long-term fertilization, and the fact that the *K. davidiana* plants were initially transplanted into the garden with only a small founder population of ca. 20 individuals. We also cannot rule out that the particular host species, *K. davidiana*, could also be responsible in some way for the lower ECM diversity at the KBG site. However, the low sample size makes definitive statements impossible and more thorough studies will be needed to understand this phenomenon.

Despite the seminatural state of the XS and GT forests and the moderate ECM diversity we found on roots, we expect that all of our sites are influenced by anthropogenic factors. Many taxa in the */russula–lactarius* lineage respond positively to increased levels of nitrogen (e.g., Cox et al. 2010; Lilleskov et al. 2002), and it is possible that elevated nitrogen levels at our sites could have increased the proportion of *Russula* and *Lactarius* species that were recovered. Our sites are located in the Kunming metropolitan area, which has a long history of human habitation and agriculture as well as a significant population of at least six million people. Several economically important ECM fungi, including *Boletus edulis* Bull.: Fr. sensu lato (Wang et al. 1995) and *Tuber indicum* (García-Montero et al. 2010), are harvested from nearby Chinese *Keteleeria* forests and are assumed to form ectomycorrhizas with *Keteleeria*. Clearly, further studies with more intensive sampling over a wider geographic area are needed to fully assess the ECM fungal diversity and community structure with *Keteleeria* species.

## Implications for *Keteleeria* propagation

*Keteleeria* species are important in forestry and in ornamental horticulture so there have been a number of research studies aimed at enhancing the success of *Keteleeria* nursery production and tree transplantation (e.g., Tao and Cheng 2009; Zhang 1999; Zhu et al. 1993). Despite these efforts, *Keteleeria* nursery production remains difficult because seedlings often become highly stressed during transplantation. Since the ECM symbiosis is known to improve plant nutrient status and decrease plant stress (Smith and Read 2008), assessing ECM fungal diversity with *Keteleeria* may be important for ecological restoration, preservation, and maintenance of these endangered species. The finding that *Keteleeria* species consistently form ECM associations suggests that inoculation with ECM fungi may enhance *Keteleeria* nursery efforts and increase survival of seedlings during production and transplantation.

**Acknowledgments** Support for this research was provided by the National Natural Science Foundation of China (no. 30800004) and the Knowledge Innovation Program of the Chinese Academy of Sciences (no. 2010KIBA01).

## References

Agerer R (2006) Fungal relationships and structural identity of their ectomycorrhizae. Mycol Prog 5:67–107

Boerner REJ, DeMars BG, Leicht PN (1996) Spatial patterns of mycorrhizal infectiveness of soils long a successional chronosequence. Mycorrhiza 6:79–90

Brundrett M (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant soil 320:37–77

Cox F, Barsoum N, Lilleskov EA, Bidartondo MI (2010) Nitrogen availability is a primary determinant of conifer mycorrhizas across complex environmental gradients. Ecol Lett 13(9):1103–1113

Fu LK, Chin CM (1992) China plant red data book—rare and endangered plants, vol 1. Science, Beijing, 741 pp

Fu LK, Li N, Mill RR (1999) *Pinaceae*. In: Wu CY, Raven H (eds) Flora of China 4. Science, Beijing, pp 11–52

García-Montero LG, Diaz P, Massimo GD, Garcia-Abril A (2010) A review of research on Chinese *Tuber* species. Mycol Prog 9:315–335

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Lian C, Narimatsu M, Nara K, Hogetsu T (2006) *Tricholoma matsutake* in a natural *Pinus densiflora* forest: correspondence between above- and below-ground genets, association with multiple host trees and alteration of existing ectomycorrhizal communities. New Phytol 171(4):825–836

Lilleskov EA, Fahey TJ, Horton TR, Lovett GM (2002) Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. Ecology 83:104–115

Lin CP, Huang JP, Wu CS, Hsu CY, Chaw SM (2010) Comparative chloroplast genomics reveals the evolution of Pinaceae genera and subfamilies. Genome Biol Evol 2:504–517

Manchester SR, Chen Z-D, Lu A-M, Uemura K (2009) Eastern Asian endemic seed plant genera and their paleogeographic history throughout the Northern Hemisphere. J Syst Evol 47:1–42

Matsuda Y, Hijii N (1998) Spatiotemporal distribution of fruitbodies of ectomycorrhizal fungi in an *Abies firma* forest. Mycorrhiza 8:131–138

Matsuda Y, Hijii N (2004) Ectomycorrhizal fungal communities in an *Abies firma* forest, with special reference to ectomycorrhizal associations between seedlings and mature trees. Can J Botany 82:822–829

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Elsevier, London

Smith ME, Douhan GD, Rizzo DM (2007) Intra-specific and intra-sporocarp ITS variation of ectomycorrhizal fungi as assessed by rDNA sequencing of sporocarps and pooled ectomycorrhizal roots from a *Quercus* woodland. Mycorrhiza 18:15–22

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol Biol 17:1105–1109

Tao LY, Cheng MD (2009) Primary report on the transplantation and domestication cultivation of wild *Keteleeria davidiana* saplings. Guizhou For Sci Technol 37(1):55–57, 26

Tedersoo L, May TW, Smith ME (2010) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20:217–263

Vaario L-M, Xing ST, Xie ZQ, Lun ZM, Sun X, Li YH (2006) In situ and in vitro colonization of *Cathaya argyrophylla* (*Pinaceae*) by ectomycorrhizal fungi. Mycorrhiza 16:137–142

Wang B, Qiu Y-L (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363

Wang Y, Sinclair L, Hall IR, Cole ALJ (1995) *Boletus edulis* sensu lato: a new record for New Zealand. New Zeal J Crop Hort 23:227–231

White TM, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, San Diego, pp 315–321

Yamada A, Katsuya K (2001) The disparity between the number of ectomycorrhizal fungi and those producing fruit bodies in a *Pinus densiflora* stand. Mycol Res 105:957–965

Yu FQ, Xiao YQ, Liu PG (2007) Spatiotemporal distribution of ectomycorrhizal fungi in *Pinus yunnanensis* forests. Acta Ecol Sinica 27(6):2325–2333

Zhang JM (1999) A preliminary study on the effect of silviculture with different seedlings of *Keteleeria fortunei*. J Fujian Coll Forest 19(1):73–76

Zhu JJ, Wei ZJ, Qiu XJ (1993) The experimental study on artificial reproduction of *Keteleeria* tree species. Sci silvae sinicae 29 (1):67–71