# Root-associated fungi of *Pinus wallichiana* A. B. Jackson in Kashmir Himalaya

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Root-associated fungi of *Pinus wallichiana* A. B. Jackson in Kashmir Himalaya

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Abstract

An important factor in the performance of out-planted conifers is the association of plant roots with ectomycorrhizal (EcM) fungi. However, limited information is available about the diversity of root associated EcM fungi of *Pinus wallichiana* A. B. Jackson, a coniferous species endemic to Himalayan forests, which has hampered the reforestation programs in the area. The study was carried out at three major forest areas of the Kashmir Himalaya believed to be pure stands of *Pinus wallichiana*. Fine root-tips harbouring EcM were collected and processed for extraction of fungal DNA which were subsequently subjected to ITS rDNA targeted PCR/ RFLP profiling. DNA sequencing analysis of the overlapping ITS amplifications followed by global nucleotide-blast analyses of the assembled nuclear ribosomal DNA (rDNA) revealed a total of 33 fungal taxa associated with *P. wallichiana*, out of which 23 species were EcM fungi. Out of the 10 non-EcM fungi, we found a peculiar saprophytic wood decaying fungus, *Chalara microchona* associated with *P. wallichiana* for the first time. The study not only reveals the species richness of fungi associated with this conifer but also documents new fungal associations with it, which have not been reported so far. The results in the study set a baseline for the broad association of ectomycorrhizal fungi with *P. wallichiana* which may serve as guiding cue to design reforestation programs in the Kashmir Himalaya.

Key words: Ectomycorrhizae, Kashmir Himalaya; PCR/PFLP; ITS region; *Pinus wallichiana*

Introduction

Forests provide ecological, economic, social, and aesthetic services to natural systems and mankind (Hassan et al. 2005). Even though many Himalayan areas have registered a net positive change in total forest area during the past few decades, some parts of the north-western Himalaya have been constantly undergoing deforestation as well as degradation (Wani et al. 2016). Afforestation and reforestation programs are a widely-used policy instrument for reversing the environmental and livelihood problems...
created by this deforestation (Lamb et al. 2005; Chazdon 2008; Hua et al. 2016). However, a factor restricting the successful establishment of new plantations in degraded lands is the lack of enough nutrients in the soil (van der Heijden et al. 1998; Johnson et al. 2005). Plant species included in the active restoration programs must overcome this nutrient deficiency to achieve satisfactory growth and development (Sousa et al. 2009). An alternative consists of using ectomycorrhizal fungi (EcM), particularly at an early stage of seedling development (Dunabeitia et al. 2004; Alguacil et al. 2005; Caravaca et al. 2005; Teste et al. 2009). The formation of EcM roots increases seedling vigour when resources are limited and enhances the competitive ability of seedlings during establishment (Perry et al. 1989; Nara 2005; Livne-Luzon et al. 2017). It has been estimated that about 90% of the terrestrial plant species undergo an improvement in mineral nutrient uptake due to root symbiosis with mycorrhizal fungi (Brundrett 2009), which in turn, provide the fungus with carbon compounds.

In the north-western Himalaya, coniferous forests are the dominant ecosystems and conifers such as *Pinus wallichiana*, *Cedrus deodara* (Roxb. Ex D. Don) G. Don, *Abies* spp. (*A. pindrow* (Royle ex D. Don) Royle and *A. spectabilis* (D. Don) Mirb. and *Picea smithiana* (Wall.) Bioss. are the most widespread and abundant species in these forests (Dar, 2004). Among these, *P. wallichiana*, commonly called Himalayan White Pine is an endemic species that grows in the valleys and foothills of the Himalaya. Due to its good timber properties and quality, with tall straight trees producing straight-grained wood of good strength, the species is under severe anthropogenic pressure. In a recent study, the EcM colonization was found to increase the growth and survival of *P. wallichiana* seedlings, and significant differences in survival percentage, plant height and plant biomass were observed between the EcM inoculated and control seedlings (Itoo and Reshi 2014); however, the study was carried out on only four EcM species. Based on sporocarp studies, Saba et al. (2015) reported two species of *Inocybe* namely *I. amicta* and *I. mimica* from *Pinus wallichiana* stands of Pakistan. Recently, Sarwar et al. (2018) found *Suillus himalayensis* in symbiotic association with the roots of *P. wallichiana* in the coniferous forests of Pakistan. In another study Saba et al. (2017) and Jabeen et al. (2017) reported EcM fungi namely *Cortinarius longistipitatus* and *Russula ahmadii* respectively, from sporocarps associated with *Cedrus deodara*. Because pines are highly dependent on ectomycorrhizal fungi for establishment (Hasselquist et al. 2005) and growth (Smith and Read 1997), identifying more EcM fungi associated with *P. wallichiana* in natural areas across sites may help the land managers in the successful establishment of *P. wallichiana* seedlings during forest restoration programs.

Previous attempts to document EcM fungal species diversity associated with *P. wallichiana* and other conifer plantations were limited to sporocarp surveys (Pande et al. 2004; Ragonezi et al. 2013), but due to poor correspondence between above and below ground fungal diversity, these are no more
considered to represent the actual EcM taxa associated with root tips (Egger 1995; Gardes and Bruns 1996; Toth and Barta 2010; Jarvis et al. 2013; Cox et al. 2010). Similarly, culturing of EcM and morphotyping of EcM root tips have provided only poor representation of actual EcM diversity as ectomycorrhizae abundantly obtained from root tips were never isolated as pure cultures (Menkis and Vasaitis 2010). Moreover, the EcM morphology of a fungal taxon can change with different hosts and environments (Egger 1995). Because of these limitations, it has become incumbent to employ molecular techniques for characterization of ectomycorrhizal fungi directly from EcM roots (Ding et al. 2011; Douglas et al. 2005). Pertinently, several studies documenting the diversity of ectomycorrhizal fungi in the Kashmir Himalaya are also based upon above-ground sporocarp surveys (Itoo and Reshi 2014) and none of the studies has been carried out so far to explore root-associated ectomycorrhizal fungi of these conifers, which comprise the fungal niche to support the growth of these species. The present study is the first of its kind to explore the below ground EcM fungi associated with P. wallichiana growing in the Kashmir Himalayan forests i.e. the Tangmarg, Pahalgam and Mammar forests, which may help in understanding the fungal associations with this conifer.

**Materials and methods**

**Habitat and host plant**

Kashmir Himalaya is in the extreme northwest of the Himalayan biodiversity hotspot, and harbours a rich floristic diversity of immense scientific interest and supports about 12% of total Indian angiosperm flora and 3% of its endemics, while the region represents only 0.4% of the total geographical area of the country (Dar et al. 2002). The region is marked by well-defined seasonality and resembles that of mountainous and continental parts of the temperate latitudes. The temperature ranges from an average daily maximum of 31ºC and minimum of 15ºC during summer to an average daily maximum of 4ºC and minimum of -4ºC during winter. It receives annual precipitation of about 1,050 mm mostly in the form of snow during the winter months (Khuroo et al. 2007). The forests are predominately coniferous, with P. wallichiana growing naturally along the entire length ranging in altitude from ca. 1800 to 2700 m above mean sea level. Pinus wallichiana A.B. Jackson commonly called Blue Pine or Himalayan White Pine is up to 50 m tall symmetric pyramidal tree. The bark is slate-grey which becomes rough and shallowly fissured on old trees. Leaves are 15-20 cm long, needle-like. Cones are 15-25 cm long, in clusters of 2-3. It is an economically important conifer in the Himalaya with highly resinous wood, usually used for local construction, carpentry and making tea-chests. The species is under threat and the major pressures have been attributed mainly to illicit felling and fuelwood extraction (Dar and Dar 2006).

**Study sites and root sampling**
To examine the EcM fungal species associated with *P. wallichiana*, three prominent forest zones in Kashmir Himalaya viz. Tangmarg, Pahalgam, and Mammar representing three altitudes were selected as study sites (Table 1). The sites comprised of pure stands of *P. wallichiana* constituted of both young and mature trees. At each study site, five 90 m$^2$ plots consisting of about 150 young and mature trees in total were randomly selected as sampling plots for the collection of EcM root tips. Sampling was performed at 2 m away from the main stem of the tree to collect only the fine roots which harbour EcM. The samples were collected at a depth of 15-25 cm from the ground level by removing the top soil using a spade. A total of 600 samples were collected from each site (4 samples from each young or mature tree). Separate labeled sample bags were used for each site and samples from each site were pooled before placing in the specific bag. All the samples were stored at 4°C and were processed within 10 days of sampling. The sampling was done for a period of 2 years and the study sites were visited after every 4 months during 2012 to 2014.

**Molecular analysis of root tips**

Roots were carefully washed to remove attached soil and EcM root tips were sorted from the main roots and processed for isolation of fungal DNA. The genomic DNA was isolated and purified using HiPurA™ Fungal DNA isolation Kit (HiMedia Inc.) following manufacturer’s instructions. For EcM characterization, Internal Transcribed Spacer region (ITS)-rDNA region was targeted using three pairs of gene specific primers (White et al. 1990) to specifically amplify ITS1-, ITS2- and full length ITS- rDNA region (White et al. 1990) respectively. The aim of analyzing all the 3 ITS-rDNA regions was to resolve any possible polymorphism associated with ITS-rDNA region which shows differential distribution among closely related species with respect to length, presence / absence of ITS1 or ITS2 regions. PCR was performed in a 50 µL reaction volumes using 3.0 mM MgCl$_2$, 0.2 µM of each primer, 0.2 mM dNTP, 1 U Taq DNA polymerase (Sigma Inc) with 1µg fungal DNA. PCR products were purified prior to further downstream analysis using HiPurA gel purification kit (Himedia, India) following manufacturer’s instructions. To analyze genetic heterogeneity in the full length ITS-rDNA region of the fungal DNA isolated from ectomycorrhizal root tips associated with *P. wallichiana*, Restriction fragment Length Polymorphism (RFLP) assay of all the three purified PCR amplicons (ITS-1, ITS-2 and full length ITS-rDNA) of all the samples was performed individually using commercially available restriction enzymes (Merck Specialties, Genei) viz. *Hinf I, Alu I, Hae III* (Cullings et al. 2000, 2001). The results were catalogued according to geographic location, ITS region amplified and type of restriction enzyme used. Based upon RFLP patterns, samples were grouped as a) Unique, which differ to a greater extent; b) Polymorphic, which showed marginally different RFLP patterns; or c) Non-polymorphic, which showed
identical RFLP patterns. The non-polymorphic samples were pooled together while other samples were sequenced as such. Supplementary Figure-S1 shows the representative RFLP gels depicting cataloguing of samples according to the pattern of digestion observed. RFLP data from all the samples using 3 enzymes viz HinfI, HaeIII and AluI were analyzed using PAST 2.17C and cladograms were generated by Neighbour Joining Method using Spearman’s correlation. Samples with Jaccard’s similarity coefficient of >0.98 were treated as genotypically identical and thus pooled together for further downstream characterization using Sanger sequencing.

**Identification of fungi**

The amplified PCR products catalogued after RFLP profiling were sequenced with respective primers and the sequences were assembled in BioEdit Sequence Alignment Editor v 7.2.3 (Hall 1999). Each of the sequences was then separately used to perform individual nucleotide-nucleotide searches using nBLAST algorithm at the NCBI website [http://blast.ncbi.nlm.nih.gov/Blast.cgi](http://blast.ncbi.nlm.nih.gov/Blast.cgi) against fungi taxon (Altschul et al. 1997). The outputs from the BLAST searches were sorted on the basis of the maximum identity and were recorded according to their coverage. Sequence similarity with a cutoff of 90% or greater was considered significant, and the best hit was defined as the sequence with the highest maximum identity to the query sequence. All the variant nucleotide sequences were deposited in GenBank (Table 2). Taxa were considered to be ectomycorrhizal (EcM) on the basis of the literature available (Tedersoo 2010; Belfiori et al. 2012; Rinaldi et al. 2008; Pietras et al. 2012; Buscardo et al. 2011; Henkel et al. 2011).

**Results**

**PCR amplification and RFLP analysis:** The fungal genomic DNA templates of samples from all the three study sites were used for amplification of ITS1, ITS2 and full length ITS region using gene specific primer sets (White et al. 1990). Fig. 1 shows the successful PCR amplification of specified products ITS1, ITS2 and Full Length ITS-rDNA in representative samples from each site. The percentage of successful genotyping was 99%. Supplementary Figure-S1 shows representative RFLP patterns of samples with specified restriction enzyme and PCR templates used. Among the 3 restriction enzymes used, HinfI showed extensive digestion of amplicons, followed by AluI and HaeIII respectively. Restriction digestion was used for cataloguing of samples based on the RFLP patterns, to minimize the process of extensive sample sequencing. For instance, Supplementary Figure-S1 depicts the cataloguing of samples with identical RFLP patterns as non-polymorphic, those with marginally similar pattern as polymorphic while the samples which didn’t show any match with the rest of samples were designated as unique. Each of the grouped samples was sequenced using respective forward / reverse primers.

**Identification of Fungal Associations:**
The data obtained from sequencing of samples were assembled and aligned using ClustalW application in BioEdit (v4.1). The consensus sequences so obtained were used for genome-wide search for identification of sequences based upon homology, span and percentage identity of reported sequences. A total of 33 fungal taxa were identified based on sequence scores and BLAST similarity, out of which 23 were EcM fungi (Table 2). From the data, it was observed that among the 3 surveyed sites, the site at lowest altitude (2145 m) was most diverse and reported 19 species, followed by site 2 (17 species) at an altitude of 2388 m and site 3 (altitude 2400 m) with the lowest number of species (12 species). Out of the 33 taxa of root-associated fungi reported in this study, 22 belong to the division Ascomycota and 11 taxa belong to the division Basidiomycota. 24 species were found to be present at a single site only and can be considered as site-specific species, out of which 17 were EcM (Fig. 2). Six species were found to have a broader altitudinal range and were reported at all the three sites. Out of these six species five were EcM in nature. All the other species were found at least on two sites and thus are not specific to a particular altitude. Russulaceae was the dominant family reported in the study represented by two genera and comprised of five species namely Russula brevipes, R. chlorides, R. delica, R. firmula and Lactarius acicularis. The details of percentage identity, query cover and the reference GenBank accession of the taxa are given in Table 2.

**Discussion**

In the present study, we used three tier ITS-analyses based upon ITS-1, ITS-2 and full length ITS-rDNA for genotyping of root-tip associated fungi. A total of 33 fungal species was found to be associated with root tips of *Pinus wallichiana*, out of which 23 were reported EcM fungi. Classical niche theory (Vandermeer 1972) predicts that species assemblages will be structured by the ability of individual species to persist in each set of abiotic conditions, while successfully competing with other species for resources and space. Neutral theory (Hubbell 2001) predicts that assemblages will be structured by stochastic processes such as initial dispersal into an area, or founder effects (where the first species to colonize a resource remains there). Our communities are most likely structured by both theoretical models to a greater or lesser degree. Nestedness of interspecific interactions (Thompson 2005) in which host distribution is the strongest structuring factor at the landscape scale (Gilbert et al. 2007) has been suggested for obligate symbionts like mycorrhizal fungi.

Altitude plays a key role in EcM fungal community by affecting EcM fungal species richness (Bahram et al. 2012) and by influencing fungal community composition (Jarvis et al. 2015). For EcM fungal richness along altitudinal gradients, the mid-domain effect has been shown to exist (Miyamoto et al. 2015). However, the present study revealed a decreasing trend in the number of fungal species with increasing elevation which is in consistence with Bahram et al (2012). The maximum number of EcM
species (16) was present at the lowest altitudes. Changes in species richness and distribution of EcM fungal communities along altitudinal gradients have been attributed to changes in both host distributions and abiotic variables (Kernaghan and Harper 2001; Bahram et al. 2012). Climatic variation has been attributed as an important driver of EcM species richness and community composition at both local and global scales (Tedersoo et al. 2012; Jarvis et al. 2015), suggesting that changes in climate along altitudinal gradients could affect EcM communities. Decline in species richness of EcM fungi with altitude has been attributed to harsher climatic conditions, decreased energy availability and/or less favourable soil conditions at high altitude, while the mid-domain effect has been hypothesized to cause a mid-altitude peak in richness (Kernaghan and Harper 2001; Bahram et al. 2012; Miyamoto et al. 2015).

By focusing on a single host species, it is possible to investigate abiotic drivers of community composition independently of host vegetation change. Two recent studies have used this approach to observe changes in EcM communities associated with holm oak (Quercus ilex) and beech (Fagus sylvatica) along altitudinal gradients (Coince et al. 2014; Scattolin et al. 2014). Scattolin et al. (2014) found that altitude was a driver of EcM community change in Q. ilex forests in the absence of host vegetation change.

The fungal taxa whose ectomycorrhizal status is not yet established are mostly saprophytic or parasitic. In the present study, we found eight non EcM fungi viz., Chalara spp., Chalara holubovae, Chalara hyalocuspica, Hamatocanthoscypha laricionis, Helicodendron websteri, Infundichalara microchona, Microscypha sp and Xenopolyscytalum pinea, associated with the ectomycorrhizal root tips of P. wallichiana. Although Chalara microchona has been isolated from the root tips of Pinus sylvestris seedlings (Menkis and Vasaitis 2010) and Chalara holubovae, Chalara hyalocuspica, Chalara spp. Infundichalara microchona have been reported from coniferous needles (Koukol 2011), we reported these species along with Chalara microchona were obtained from root tips of P. wallichiana for the first time. Other non-mycorrhizal fungi reported in the present study have been associated with the litter decomposition of pines or ferns e.g., Hamatocanthoscypha laricionis is a common fungus found on debris (cones, needles, leaves, etc.) of several different species of conifers including Pinus and Picea, and Microscypha sp. has been collected from dead leaves of Pteridium aquilinum (Bresinsky, 2006). Decayed woody deposits in and on the soil are important to ectomycorrhizal associations, particularly during dry seasons and on dry sites (Harvey et al. 1978, 1979). Walker et al. (2013) concluded from their study that EcM fungal OTUs identified as Tylospora fibrillosa and Russula curtipes were more frequent in decayed wood as compared with control mineral soil. In addition to the saprophytic fungi, two pathogenic fungi were found in the present research, which include Xenopolyscytalum pinea and Helicodendron websteri. These pathogenic fungi species have been reported in previous studies as well (Crous and Groenewald.
In fact, occurrence of saprophytic and parasitic fungi in association with root tips of ectomycorrhizal fungi are supported by studies of Tedersoo et al. (2009) and Menkis et al. (2006).

Five EcM species namely *Russula delica*, *R. brevipes*, *R. cholorides*, *Cadophora* sp. and *Helotiales* sp., out of the total species pool were present at all the three sites. The most common species are believed to withstand the harsh climate conditions as compared to more sensitive species which are present only on a few sites (Pickles et al. 2012; Mohan et al. 2014). Climate change is projected to be a dominant stressor on terrestrial ecosystems in the second half of the 21st century, and will lead to major changes in species distributions and ecosystem function (IPCC 2014). The vulnerability of forest ecosystems to climate change, i.e. their propensity to be adversely affected, is determined by the sensitivity of ecosystem processes to the elements of climate undergoing change and the degree to which the system (including its coupled social elements) can maintain its structure, composition and function in the presence of such change, either by enduring or adapting to it (IPCC 2014). In this backdrop, the widely represented EcM are expected to withstand the changing climate and help in overcoming the harsh climatic conditions by helping the host species to adapt particularly in the seedling stage. In conclusion, the present study reveals the species richness of fungi associated with *Pinus wallichiana* of Kashmir Himalaya and scope of future research in the given region to further explore fungal diversity of other conifers. The restricted fungal diversity of EcM fungi associated with *P. wallichiana* at higher altitudes not only points towards the age of the forest ecosystem which may have adapted itself to climate change for hundreds of years but also points towards vulnerability of forest ecosystems to extinction due to climate change which may prove catastrophic. The presence of wood decaying fungi associated with *P. wallichiana* at lower altitudes also proves that such a disaster may have happened in the past which have lead to persistence of these saprophytic and parasitic fungi in the region. Thus, there is an immediate need to document the fungal diversity associated with conifers of Kashmir Himalaya to withstand any probability of species extinction which may occur due to climatic variations in the region.

**Conflict of interest:** The authors declare that they have no conflict of interest.

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**References**


https://mc06.manuscriptcentral.com/cjfr-pubs


Annexe 1


**Fig. 1.** PCR amplification of specified products ITS1, ITS2 and Full Length ITS-rDNA in representative samples from each site

![PCR amplification image](image)

**Fig. 2.** Venn diagram showing the common and exclusive taxa (E= EcM, N= Non-EcM) associated with *P. wallichiana* roots across the three study sites.

![Venn diagram image](image)
Table 1. Study sites and their geographic location

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<th>Altitude</th>
<th>Latitude</th>
<th>Longitude</th>
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<tr>
<td>Site 1</td>
<td>Tangmarg</td>
<td>2145 m</td>
<td>34º03’ N</td>
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<tr>
<td>Site 2</td>
<td>Pahalgam</td>
<td>2388 m</td>
<td>34º05’ N</td>
<td>75º15’ E</td>
</tr>
<tr>
<td>Site 3</td>
<td>Mammar</td>
<td>2400 m</td>
<td>34º14’ N</td>
<td>75º01’ E</td>
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## Table 2. Fungal associations of *Pinus wallichiana* reported in the study and their classification.

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<th>S. No</th>
<th>Description of Species</th>
<th>Accession no.</th>
<th>Query Cover</th>
<th>Identity percentage</th>
<th>Ref. accession no.</th>
<th>Phylum*</th>
<th>Status ECM / Non ECM</th>
<th>Site**</th>
<th>Reference***</th>
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<td>1.</td>
<td>Cadophora sp.</td>
<td>KM289192</td>
<td>94%</td>
<td>92%</td>
<td>DQ317330.1</td>
<td>A</td>
<td>ECM</td>
<td>T/M/P</td>
<td>Pietras et al. 2012; Stark et al. 2009</td>
</tr>
<tr>
<td>2.</td>
<td>Cenococcum geophilum</td>
<td>KP109910</td>
<td>97%</td>
<td>96%</td>
<td>KC967402.1</td>
<td>A</td>
<td>ECM</td>
<td>M</td>
<td>Fernandez et al. 2013; Pietras et al. 2012</td>
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<tr>
<td>3.</td>
<td>Chalara holubovae</td>
<td>KP109911</td>
<td>91%</td>
<td>92%</td>
<td>KC768074.1</td>
<td>A</td>
<td>Non ECM</td>
<td>T/M/P</td>
<td>Koukol, 2011</td>
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<tr>
<td>4.</td>
<td>Chalara hyalocuspica</td>
<td>MG388301</td>
<td>100%</td>
<td>83%</td>
<td>FR667221.1</td>
<td>A</td>
<td>Non ECM</td>
<td>P</td>
<td>Koukol, 2011</td>
</tr>
<tr>
<td>5.</td>
<td>Chalara microchona</td>
<td>MG434775</td>
<td>100%</td>
<td>88%</td>
<td>HM036588.1</td>
<td>A</td>
<td>Non ECM</td>
<td>P</td>
<td>Koukol, 2011; Menkis and Vasaitis, 2010</td>
</tr>
<tr>
<td>6.</td>
<td>Chalara sp.</td>
<td>KM236230</td>
<td>91%</td>
<td>92%</td>
<td>KC768074.1</td>
<td>A</td>
<td>Non ECM</td>
<td>T</td>
<td>Hewitt et al. 2013</td>
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<td>7.</td>
<td>Geopora cervina</td>
<td>MG434773</td>
<td>100%</td>
<td>84%</td>
<td>JF908021.1</td>
<td>A</td>
<td>ECM</td>
<td>P</td>
<td>Pietras et al. 2012; Tedersoo et al. 2006a</td>
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<td>8.</td>
<td>Hamatocanthoscypha laricionis</td>
<td>KP109912</td>
<td>97%</td>
<td>95%</td>
<td>JN033441.1</td>
<td>A</td>
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<td>M</td>
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<td>Hebeloma sp</td>
<td>MG434780</td>
<td>100%</td>
<td>87%</td>
<td>JN859274.1</td>
<td>B</td>
<td>ECM</td>
<td>M</td>
<td>Jakucs et al. 1999; Pietras et al. 2012; Grogan et al. 2000</td>
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<td>10.</td>
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<td>KM236232</td>
<td>100%</td>
<td>85%</td>
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<td>P</td>
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<td>Helotiales sp.</td>
<td>KM289186</td>
<td>100%</td>
<td>87%</td>
<td>JN859268.1</td>
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<td>ECM</td>
<td>T/M/P</td>
<td>Hrynkiewicz et al. 2015</td>
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<td>12.</td>
<td>Hymenoscyphus sp.</td>
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<td>EU940176.1</td>
<td>A</td>
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<td>T</td>
<td>Brundrett, 2004</td>
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<td>13.</td>
<td>Hypocreales sp.</td>
<td>MG434776</td>
<td>100%</td>
<td>100%</td>
<td>KC119571.1</td>
<td>A</td>
<td>Non ECM</td>
<td>P</td>
<td>Belfiori et al. 2012</td>
</tr>
<tr>
<td>14.</td>
<td>Infundichalara microchona</td>
<td>KP195075</td>
<td>100%</td>
<td>97%</td>
<td>KF359590.1</td>
<td>A</td>
<td>Non ECM</td>
<td>M/P</td>
<td>Koukol, 2011</td>
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<tr>
<td>15.</td>
<td>Lachnum lushanense</td>
<td>MG434782</td>
<td>100%</td>
<td>89%</td>
<td>JF937582.1</td>
<td>A</td>
<td>ECM</td>
<td>T</td>
<td>Buscardo et al. 2011</td>
</tr>
<tr>
<td>16.</td>
<td>Lactarius acicularis</td>
<td>KP109913</td>
<td>70%</td>
<td>99%</td>
<td>HQ318224.1</td>
<td>B</td>
<td>ECM</td>
<td>M</td>
<td>Nuytinck et al. 2004; Pietras et al. 2012</td>
</tr>
<tr>
<td>No.</td>
<td>Species</td>
<td>Accession No.</td>
<td>Match (%)</td>
<td>GenBank No.</td>
<td>Classification</td>
<td>Taxonomy</td>
<td>Source and Reference</td>
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<td>17.</td>
<td><em>Microsrypha</em> <em>sp.</em></td>
<td>MG434781</td>
<td>100%</td>
<td>JN033425.1</td>
<td>A</td>
<td>Non ECM</td>
<td>Thorman and Rice, 2007; Bresinsky, 2006</td>
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<td>18.</td>
<td><em>Phialocephala</em> <em>sp.</em></td>
<td>MG434779</td>
<td>97%</td>
<td>JQ088276.1</td>
<td>A</td>
<td>ECM T</td>
<td>Menkis et al. 2005</td>
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<tr>
<td>19.</td>
<td><em>Phialocephala fortinii</em></td>
<td>KM289188</td>
<td>97%</td>
<td>KJ817278.1</td>
<td>A</td>
<td>ECM T</td>
<td>Menkis et al. 2005</td>
<td></td>
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<tr>
<td>20.</td>
<td><em>Phialophora maitri</em></td>
<td>KM236231</td>
<td>99%</td>
<td>EU314708.1</td>
<td>A</td>
<td>ECM T/P</td>
<td>Rinaldi et al. 2008</td>
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<tr>
<td>21.</td>
<td><em>Phialophora</em> <em>sp.</em></td>
<td>MG434778</td>
<td>77%</td>
<td>HQ207692.1</td>
<td>A</td>
<td>ECM P</td>
<td>Vra˚lstad et al. 2002; Douglas et al. 2005</td>
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<td>23.</td>
<td><em>Russula chlorides</em></td>
<td>KM289190</td>
<td>100%</td>
<td>AY061663.1</td>
<td>B</td>
<td>ECM T/M/P Pietras et al. 2012; Itoo and Reshi, 2014</td>
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<td>24.</td>
<td><em>Russula delica</em></td>
<td>KM289189</td>
<td>100%</td>
<td>KF432955.1</td>
<td>B</td>
<td>ECM T/M/P Pietras et al. 2012; Itoo and Reshi, 2014</td>
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<td>26.</td>
<td><em>Sebacina epigaea</em></td>
<td>KM289191</td>
<td>86%</td>
<td>KF000417.1</td>
<td>B</td>
<td>ECM T</td>
<td>Urban et al. 2003</td>
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<td>27.</td>
<td><em>Sebacina</em> <em>sp.</em></td>
<td>MG434774</td>
<td>64%</td>
<td>KM576578.1</td>
<td>B</td>
<td>ECM M</td>
<td>Urban et al. 2003; Henkel et al. 2011</td>
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<td>28.</td>
<td><em>Thelephoraceae</em> <em>sp.</em></td>
<td>MG434784</td>
<td>99%</td>
<td>FN669279.1</td>
<td>B</td>
<td>ECM T</td>
<td>Mahmood et al. 1999;</td>
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<td>30.</td>
<td><em>Tuber aestivum</em></td>
<td>KP222539</td>
<td>99%</td>
<td>JN975880.1</td>
<td>A</td>
<td>ECM T</td>
<td>Giomaro et al. 2002</td>
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<td>31.</td>
<td><em>Tuber maculatum</em></td>
<td>MG434785</td>
<td>100%</td>
<td>EU784428.1</td>
<td>A</td>
<td>ECM P</td>
<td>Pietras et al. 2012</td>
<td></td>
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<tr>
<td>32.</td>
<td><em>Xenopolyscytalum pinea</em></td>
<td>KM236229</td>
<td>97%</td>
<td>HQ599581.1</td>
<td>A</td>
<td>Non ECM T/P Crous and Groenewald, 2010</td>
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<td>33.</td>
<td><em>Xerocomus</em> <em>sp.</em></td>
<td>MG434783</td>
<td>100%</td>
<td>MF098664.1</td>
<td>A</td>
<td>ECM T</td>
<td>Henkel et al. 2011</td>
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</table>

* A: Ascomycota, B: Basidiomycota;** Site indicates the presence of the taxa at study sites, T= Tangmarg, P= Pahalgam, M= Mammar

***Source for classification of ECM fungi and References are given as Annexe 1; ECM: Ectomycorrhizae*