AN ECTOMYCORRHIZAL THELEPHOROID FUNGUS OF MALAYSIAN DIPTEROCARP SEEDLINGS

Lee SS*, Thi BK & Patahayah M

Forest Research Institute Malaysia, 52109 Kepong, Selangor Darul Ehsan, Malaysia

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LEE SS, THI BK & PATAHAYAH M. 2010. An ectomycorrhizal thelephoroid fungus of Malaysian dipterocarp seedlings. The ectomycorrhizal Dipterocarpaceae are among the most well-known trees in the tropics and this is the most important family of timber trees in Malaysia and South-East Asia. Recent studies and molecular data reveal that members of the Thelephoraceae are common ectomycorrhizal fungi associated with the Dipterocarpaceae. The suspected thelephoroid fungus FP160 was isolated from ectomycorrhizal roots of a *Shorea parvifolia* (Dipterocarpaceae) seedling and kept in the Forest Research Institute Malaysia (FRIM) culture collection. In subsequent inoculation experiments it was able to form morphologically similar ectomycorrhizas with seedlings of two other dipterocarps, namely, *Hopea odorata* and *S. leprosula*, and the exotic fast-growing legume, *Acacia mangium*. A taxonomic identity of this fungus would benefit its possible use in inoculation and planting programmes. This information is also important to expand our limited knowledge of Malaysian mycodiversity. In this paper the morphological characteristics of the ectomycorrhizas formed by FP160 with *H. odorata* and *A. mangium* are described and the fungus identified using molecular methods as a member of the family Thelephoraceae, most likely a *Tomentella* sp. It was not possible to identify the fungus more precisely due to the limited number of sequences available for tropical Thelephoraceae in the public databases.

Keywords: *Acacia mangium*, Dipterocarpaceae, ectomycorrhizas, ITS, Thelephoraceae

LEE SS, THI BK & PATAHAYAH M. 2010. Kulat ektomikoriza Thelephoroid daripada anak benih dipterokarp Malaysia. Pokok Dipterocarpaceae yang mempunyai ektomikoriza merupakan antara pokok yang terkenal di kawasan tropika dan ialah famili pokok kayu-kayan yang terpenting di Malaysia serta Asia Tenggara. Kajian terkini dan data molekul menunjukkan bahawa ahli daripada famili Thelephoraceae ialah kulat ektomikoriza yang biasa berasosiasi dengan Dipterocarpaceae. Kulat FP160 yang disyaki tergolong dalam kumpulan thelephoroid telah dipencilkan dari akar ektomikoriza anak benih *Shorea parvifolia* (Dipterocarpaceae) dan disimpan dalam koleksi kultur Institut Penyelidikan Perhutanan Malaysia (FRIM). Dalam kajian inokulasi yang dijalankan selepas itu, ia didapati mampu membentuk ektomikoriza yang bermorfologi serupa dengan anak benih dua jenis dipterokarpa yang lain iaitu *Hopea odorata* dan *S. leprosula* serta pokok kekacang cepat tumbuh yang eksotik iaitu *Acacia mangium*. Pengecaman taksonomi kulat ini memberi faedah bagi penggunaannya dalam program inokulasi dan penanaman. Maklumat ini juga penting untuk meluaskan pengetahuan kita yang terhad tentang kepelbagaian kulat di Malaysia. Dalam artikel ini, ciri-ciri morfologi ektomikoriza yang terbentuk daripada FP160 dengan *H. odorata* dan *A. mangium* dihuraikan. Dengan menggunakan pendekatan molekul, kulat tersebut dikenal pasti sebagai ahli daripada famili Thelephoraceae, besar kemungkinan *Tomentella* sp. Kulat ini tidak dapat dikenal pasti dengan lebih tepat kerana kekurangan bilangan jujukan dalam pangkalan data umum bagi Thelephoraceae kawasan tropika.

INTRODUCTION

Members of the ectomycorrhizal Dipterocarpaceae are among the most well-known trees in the tropics and this is the most important family of timber trees in Malaysia and South-East Asia. Studies based on observations of basidiomata have shown that the main groups of ectomycorrhizal fungi involved in the symbiotic association with dipterocarps are members of the Russulales, Amanitales, Boletales, Cantharellales and several hypogeous taxa, all members of the Basidiomycota (Watling & Lee 1995, Watling *et al.* 1998, Lee *et al.* 2002, Sangwanit & Suwanarit 2002, Lee *et al.* 2003). Similar results have also been obtained through morphological studies of ectomycorrhizal root tips of dipterocarps (Becker 1983, Pampolina *et al.* 1995, Lee *et al.* 1997). More recent studies using molecular data have revealed that members of the Thelephoraceae

**E-mail: leess@frim.gov.my*

are also common ectomycorrhizal associates of the Dipterocarpaceae (Sirikantaramas *et al.* 2003, Yuwa-Amornpitak *et al.* 2006, Brearley *et al.* 2007, Peay *et al.* 2009).

During a visit to the Forestry Department nursery at Lentang in the state of Pahang, Malaysia in August 2000, seedling roots of the dipterocarps *Neobalanocarpus heimii*, *Shorea leprosula* and *Shorea parvifolia* were observed to be heavily colonised by a bright brownish fungus which formed similarly coloured ectomycorrhizas. More than 10 seedlings of each species were examined and although well infected by the ectomycorrhizas, no fungal fruiting bodies were found. The seedlings were between 9 and 12 months old and were planted in root trainers containing naturally composted oil palm mesocarp fibre (fibre and empty kernels remaining after extraction of oil from the mesocarp of the oil palm fruit which had been exposed to the elements for six months). The composted oil palm mesocarp fibre was obtained from a nearby local palm oil processing factory. Examination of the roots of these seedlings under stereo- and compound microscopes revealed that they were ectomycorrhizal and that the ectomycorrhizas closely resembled *Tomentella* or *Thelephora* ectomycorrhizas morphologically, but their actual identity could not be confirmed due to lack of published descriptions of morphotypes.

In a subsequent experiment, the isolated fungus, named FP160, was able to form morphologically similar ectomycorrhizas with the exotic fast-growing legume, *Acacia mangium* (Lee & Patahayah 2003). Ectomycorrhizas similar to those observed on seedlings of *N. heimii*, *S. leprosula* and *S. parvifolia* in the Lentang nursery were also formed in subsequent inoculation experiments with the dipterocarps, namely, *Hopea odorata* and *S. leprosula* (Lee *et al.* 2008). The fungus also showed good potential in enhancing the growth of dipterocarp seedlings and cuttings in the nursery (Lee *et al.* 2008). A taxonomic identity of this fungus would benefit its possible use in inoculation and planting programmes. This information is also important to expand our limited knowledge of Malaysian mycodiversity (Lee & Chang 2006, Jones 2007). In this paper the morphological characteristics of the ectomycorrhizas formed by FP160 with *H. odorata* (Lee *et al.* 2008) and *A. mangium* (Lee & Patahayah 2003) are described. The fungus FP160 was identified using molecular methods.

MATERIALS AND METHODS

Morphological description of ectomycorrhizas

Ectomycorrhizal roots were obtained from *A. mangium* and *H. odorata* seedlings which had been inoculated with FP160 (Lee & Patahayah 2003, Lee *et al.* 2008). Washed and cleaned roots were examined under stereo- and compound microscopes and the presence of ectomycorrhizas confirmed by examining free-hand sections for the presence of the mantle and Hartig net. The surface view of the mycorrhizas was studied using peeling and squashing techniques and ectomycorrhizal types differentiated using the methods of Agerer (1987–1995) and Ingleby *et al.* (1990).

Isolation of fungus and DNA extraction

The ectomycorrhizal fungus FP160 was isolated from roots of a *S. parvifolia* seedling as described in Lee *et al.* (2008) and stored on Pachlewski's agar (Pachlewski & Pachlewska 1974) slants and as plugs in distilled water in an incubator at 25 $\pm$ 2 °C in the Forest Research Institute Malaysia (FRIM) culture collection. For subsequent experiments, plugs of FP160 were grown in a conical flask containing Pachlewski's liquid media at 25 $\pm$ 2 °C for three weeks in an incubator and the mycelia harvested by filtration through three layers of muslin cloth. The harvested mycelia were washed twice with sterile distilled water and ground in a pestle and mortar with liquid nitrogen, and 0.1 g of fine mycelia powder was used for DNA extraction. DNA was extracted using DNEASY Plant Mini Kit (QIAGEN, USA) according to the manufacturer's instructions. DNA was then eluted in 200 µl of sterile distilled water and quantified using a spectrophotometer at wavelengths of 260 and 280 nm.

Polymerase chain reaction (PCR) amplification and nucleotide sequencing

The ITS regions were amplified by the universal primers ITS1/ITS4 and ITS4/ITS5 (White *et al.* 1990) and the basidiomycete specific primers ITS1-F/ITS-4B (Gardes & Bruns 1993). Amplification was carried out in 25 µl of reaction, containing 1 ng of fungal DNA, 0.725 pmol/µl each of ITS primers, 0.2 mM of dNTP, 1 × PCR

buffer (2.5 mM $MgCl_2$) and 1 U of *Taq* DNA polymerase. For the negative control reaction mixture, the fungal DNA was replaced with sterile distilled water. PCR amplification was performed in a Mastercycler Gradient (Eppendorf) with initial denaturation at 94 °C for 1 min, 30 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, and a final extension step of 72 °C for 10 min, followed by incubation at 4 °C until used. PCR products were separated in 1% agarose gel and visualised by staining with ethidium bromide. The gel was photographed under UV light using Gel Doc™ XR (Bio-Rad).

The amplified fragments were purified with QIAquick PCR Purification Kit (QIAGEN) and then sequenced using ITS1, ITS4 and ITS5 (White *et al.* 1990) and ITS-1F and ITS-4B (Gardes & Bruns 1993). The fragments were sequenced using Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer) following the manufacturer's instructions and analysed in an ABI Prism 377 DNA Sequencer. Raw sequence data were viewed with Chromas Lite Version 2.01 and analysed using CodonCode Aligner software (CodonCode Corporation http://www.codoncode.com/) to make manual corrections and to delete ambiguous regions at the beginning and end of sequences. The resulting DNA sequences corresponding to both the forward and reverse reads were then assembled using the software. BlastN searches were performed against public sequence databases NCBI, EMBL and UNITE (Kõljalg *et al.* 2005) to provide at least tentative identification for FP160. The edited sequences of FP160 and sequences of blast results were aligned using ClustalW 2.0 and subsequently modified by eye. The alignment was deposited in TreeBase (http://www.treebase.org/treebase/) and can be viewed at http://purl.org/phylo/treebase/phylows/study/TB2:S10665?x-access-code=679432e55094dd4b7702badc57abac41&format=html.

Data analysis

Parsimony analysis was performed in PAUP version 4.0b10 (Swofford 2002) using 1000 heuristic searches each with 10 random taxon addition sequences, MAXTREES unlimited, MULPARS in effect and tree bisection and reconnection (TBR) branch swapping. Bootstrap analysis with 1000 replications was also performed under TBR branch swapping to test the significance of the strict consensus parsimonious trees of nucleotide sequences. The tree was rooted with *Trametes versicolor* (Polyporaceae) as outgroup. The resulting phylogenetic tree was visualised with the TreeView programme (Page 1996).

RESULTS AND DISCUSSION

In culture, FP160 isolates grew fairly rapidly, covering the surface of a 90-mm diameter Pachlewski's agar plate in about 14 days. The texture of the mycelial mat was woolly and coloured terra cotta to Titian red (Kornerup & Wanscher 1978). Aerial hyphae were long, 3–4 µm in diameter, thick walled, pale brownish in colour and possessed clamp connections. Hyphae embedded in the agar possessed clamps, were hyaline, 3–4 µm in diameter, had slightly thinner walls than the aerial hyphae and had many short branches. Some interlocking hyphae were also present.

Ectomycorrhizas formed between FP160 and seedlings of the dipterocarps *H. odorata* and *A. mangium* appeared morphologically similar to each other and also to the original ectomycorrhizas observed on the *S. parvifolia* seedlings obtained from the Forest Department nursery at Lentang. The ectomycorrhizas were monopodial-pinnate and infrequently ramified; the unramified ends were straight and cylindrical, bright brown, with thin rhizomorphs bearing distinctive bright yellowish-orange powdery ornamentation on the surface of young members (Figure 1a). They disappeared upon immersion of the roots in water or when the roots were mounted on slides indicating that they were water soluble. These structures were most likely crystals of calcium oxalate such as those reported from the mantle of some ectomycorrhizal root tips (Malajczuk & Cromack 1982). In water the roots appeared smooth and shiny. Older ectomycorrhizas were much darker brown in colour and the surface ornamentation much less obvious.

Hyphae emanating from the well-developed mantle were pale brown to hyaline and differentiated into straight to slightly sinuous hyphae with blunt tips, sometimes with simple septa, or more frequently, with club-shaped to rounded tips bearing clamp connections (Figure 1b). Clamp connections were present on the thick-walled, long ramifying hyphae and rhizomorphs.

The outer surface of the mantle was composed of a layer of irregular plectenchyma while the inner mantle was composed of interlocking large diameter net-like plectenchyma. It was often difficult to distinguish the elements of the mantle in prepared slides due to flattening of the dense surface hyphae and ornamentation. In the dipterocarp roots, a well-developed Hartig net was present between the radially elongated epidermal cells (Figure 1c). In contrast, there was no radial elongation of the epidermal cells in ectomycorrhizal *A. mangium* roots (Figure 1d).

The mantle features and hyphal characteristics of FP160 generally resemble those of ectomycorrhizas formed by species of *Tomentella* (Agerer *et al.* 2001) or *Thelephora* (Agerer 1987–1995, Ingleby *et al.* 1990) but with some differences. Basal clamp connections are reported to be present on the straight to slightly sinuous differentiated hyphae in *T. terrestris* ectomycorrhizas (Agerer 1987–1995, Ingleby *et al.* 1990) but we could not be sure whether such septa were present in our specimens due to the presence of dense surface hyphae. FP160 ectomycorrhizas possess abundant swollen, club-shaped to round-tipped cystidia on the mantle surface which were not reported on *Tomentella* and *Thelephora* ectomycorrhizas previously (Agerer 1987–1995, Ingleby *et al.* 1990, Agerer *et al.* 2001). These cystidia resemble

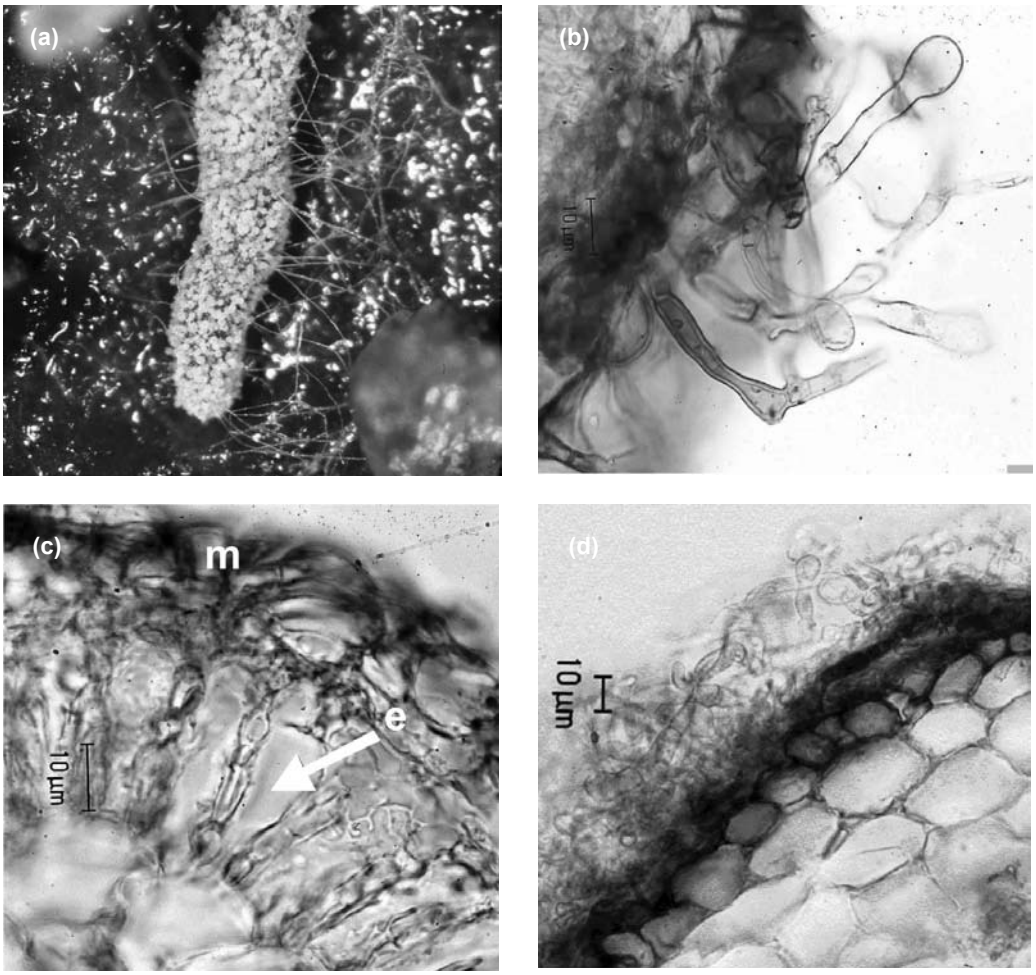

Figure 1 Ectomycorrhizas formed by FP160 on roots of *Hopea odorata* and *Acacia mangium*: (a) external morphology of *H. odorata* root tip showing the unramified tip and ectomycorrhiza root surface covered with bright yellowish-orange powdery ornamentation and abundant emanating hyphae and rhizomorphs; (b) emanating hyphae with clamp connections and club-shaped tips; (c) transverse section of a FP160 ectomycorrhizal *H. odorata* root showing the well developed mantle (m) and Hartig net in between the radially elongated epidermal cells (e and arrow); (d) transverse section of a FP160 ectomycorrhizal *A. mangium* root showing the well developed mantle and emanating hyphae but absence of the Hartig net

the 'conidiophores' found on the mantle of the commonly encountered *Riessiella* which forms ectomycorrhizas with dipterocarps (Julich 1985, Smits 1994, Lee *et al.* 1997). Recent molecular data show that *Riessiella* is placed in the genus *Tomentella* (Tedersoo *et al.* 2007).

DNA extraction and phylogenetic analysis

DNA from mycelial cultures of FP160 was successfully extracted using DNEASY Plant Mini Kit (QIAGEN). Fungus FP160 DNA was faintly amplified by ITS1/ITS4 and although sequence data were obtained, these were not used in subsequent analyses as the results could be unreliable. Although primer pairs ITS1/ITS4 can amplify DNA from most fungi, weak and negative amplification with ITS1/ITS4 has also been reported if base mismatches occur at the primer-binding site under stringent PCR conditions (Wu *et al.* 2002). A strong band was obtained when the fungus FP160 DNA was amplified with primer pairs ITS4/ITS5 and ITS-1F/ITS-4B. The sequence amplified with ITS-1F/ITS-4B showed 91% similarity with blast results in NCBI and 89% similarity in UNITE databases while the sequence amplified with ITS4/ITS5 showed 97 and 91% similarities respectively. Overall sequence similarity and sequence match length were higher using ITS4/ITS5 when compared with ITS-1F/ITS-4B. Sequence similarity and sequence match length of FP160 with species of *Pseudotomentella* were much lower with both sets of primers (data not shown). Since highest sequence similarity and sequence match length were obtained with the sequence amplified using ITS4/ITS5 and most of the sequences available for comparison use universal primer pair ITS4/ITS5, we therefore based our comparisons on this sequence. The total length of the ITS sequence amplified with ITS4/ITS5 was 600 bp with 1–10 bp partial sequence of 18S rRNA, 11–216 bp of ITS1, 217–370 bp of 5.8S rRNA, 371–550 bp of ITS2 and 551–600 bp partial sequence of 28S rRNA. The ITS sequence of FP160 was deposited in GenBank as FJ79479.

FP160 was aligned with the sequences of *Tomentella*, *Thelephora* and *Pseudotomentella*, all taxa in the family Thelephoraceae. The sequences selected from GenBank also included three sequences from Thailand (Table 1), namely, DQ146381 and DQ146384 (uncultured ectomycorrhizas associated with *Hopea ferrea*), and DQ146368 (uncultured ectomycorrhiza associated with *Shorea farinosa*) (Yuwa-Amornpitak *et al.* 2006).

The species with the highest blast similarity of 97% in the NCBI database was an uncultured Thelephoraceae. However, we did not include this fungus in our subsequent analysis because we wanted to compare FP160 with species which had been identified more precisely. From the selected fungi, FP160 showed high similarity of 91% with *Tomentella badia* UDB001656 (corrected to 89% after editing with ClustalW) (Table 1). When compared with isolates from Thailand, FP160 had 83% similarity with DQ146384, 82% with DQ146381 and 81% with DQ146368 (Table 1). DQ146381 had been reported to be very similar to *Tomentella* sp. J54 and DQ146368 very close to *Thelephoraceae* sp. (*sic*) EC117 A52 while DQ146384 was very similar to *Tomentella ellisii* (Yuwa-Amornpitak *et al.* 2006). FP160 showed low similarity with *Pseudotomentella* (Table 1) which appeared as a separate clade with a high bootstrap value (Figure 2). Moreover, *Pseudotomentella* hitherto have never been collected in the tropics of South-East Asia (Tedersoo *et al.* 2010).

Phylogenetic analysis of the ITS region resulted in 81 equally parsimonious trees of 2310 steps, a consistency index (CI) of 0.431 and a retention index (RI) of 0.506. The alignment of the ITS region comprised 44 taxa, including *Trametes versicolor* as outgroup, and was 1383 nucleotides long. Of these, 799 nucleotides were constant sites, 127 were variable sites of parsimony-uninformative and 457 of parsimony-informative nucleotides.

The phylogram shows the most parsimonious tree generated from PAUP 4.0b10 (Figure 2). Bootstrap values below 50% are not shown in the tree. From the phylogram, FP160 forms a clade within the taxa of *Tomentella*. FP160 is placed closest to *Tomentella* sp., family Thelephoraceae (accession number AM412294) with high sequence match of 547 bp. The sequence AM412294 originated from a *Tomentella* sp. fruit body as well as ectomycorrhizal roots collected at Anse Major, Mahe, Seychelles (Tedersoo *et al.* 2007). An isolate of FP160 has been confirmed as an undescribed species of *Tomentella* which clusters together with species from the Seychelles and Australia (T Kõljalg, personal communication). With the limited number of fungal ITS sequences from the tropics available in public databases (Ryberg *et al.* 2009), it was not

Table 1 The similarity of FP160 compared with the sequences of 42 other isolates of thelephoroid fungi in the EMBL, UNITE and NCBI GenBank databases

Species of blast match	Database	Accession No.	Similarity (%)	Length of sequence match (bp)
Tomentella atramentaria	UNITE	UDB000235	84	489
Tomentella atramentaria	UNITE	UDB000236	84	489
Tomentella badia	UNITE	UDB001656	89	531
Tomentella lateritia	UNITE	UDB000954	83	483
Tomentella lateritia	UNITE	UDB000268	83	488
Tomentella stuposa	UNITE	UDB001660	86	518
Tomentella bryophila	UNITE	UDB000035	87	525
Tomentella sp.	UNITE	UDB001658	88	539
Tomentella galzinii	UNITE	UDB000260	83	485
Tomentella fuscocinerea	UNITE	UDB000960	83	488
Tomentella ellisii	NCBI	AF272913	79	458
Thelephora penicillata	NCBI	U83484	82	494
Thelephora regularis	NCBI	U83485	78	464
Thelephoraceae sp.	NCBI	AY751562	83	500
Uncultured Thai ECM	NCBI	DQ146381	82	491
Uncultured Thai ECM	NCBI	DQ146384	83	504
Uncultured Thai ECM	NCBI	DQ146368	81	488
Tomentella sp.	NCBI	AJ534912	86	516
Tomentella sp.	NCBI	AJ534914	87	526
Tomentella sp.	NCBI	AF430289	86	519
Tomentella sp.	EMBL	AM412294	86	547
Tomentella sp.	EMBL	AM412295	83	503
Tomentella sp.	EMBL	AM412296	84	505
Tomentella sp.	EMBL	AM412297	81	484
Tomentella sp.	EMBL	AM412298	81	486
Tomentella sp.	EMBL	AM412299	82	494
Tomentella sp.	EMBL	AM412300	83	499
Tomentella sp.	EMBL	AM412303	82	495
Thelephoroid sp9*	EMBL	AM412291	80	485
Thelephoroid sp18*	EMBL	AM412287	81	484
Thelephora sp.	NCBI	AB453032	82	495
Pseudotomentella tristis	NCBI	GQ267480	62	410
Thelephora palmata	NCBI	EU819443	78	470
Thelephora ganbajun	NCBI	EU696946	86	521
Thelephora pseudoterrestris	NCBI	AB453027	84	508
Thelephora terrestris	NCBI	GQ267490	86	516
Pseudotomentella sp.	NCBI	GQ267479	63	414
Pseudotomentella mucidula	NCBI	AF274769	57	375
Pseudotomentella humicola	NCBI	AM490946	64	389
Pseudotomentella vepallidospora	NCBI	AF274773	68	353
Pseudotomentella nigra	NCBI	AF274770	58	381
Pseudotomentella larsenii	NCBI	AF326981	59	379

*Thelephoroid sp9 was closely matched to *Tomentella bryophila* and Thelephoroid sp18 to *Thelephora caryophyllea* (Tedersoo *et al.* 2007)

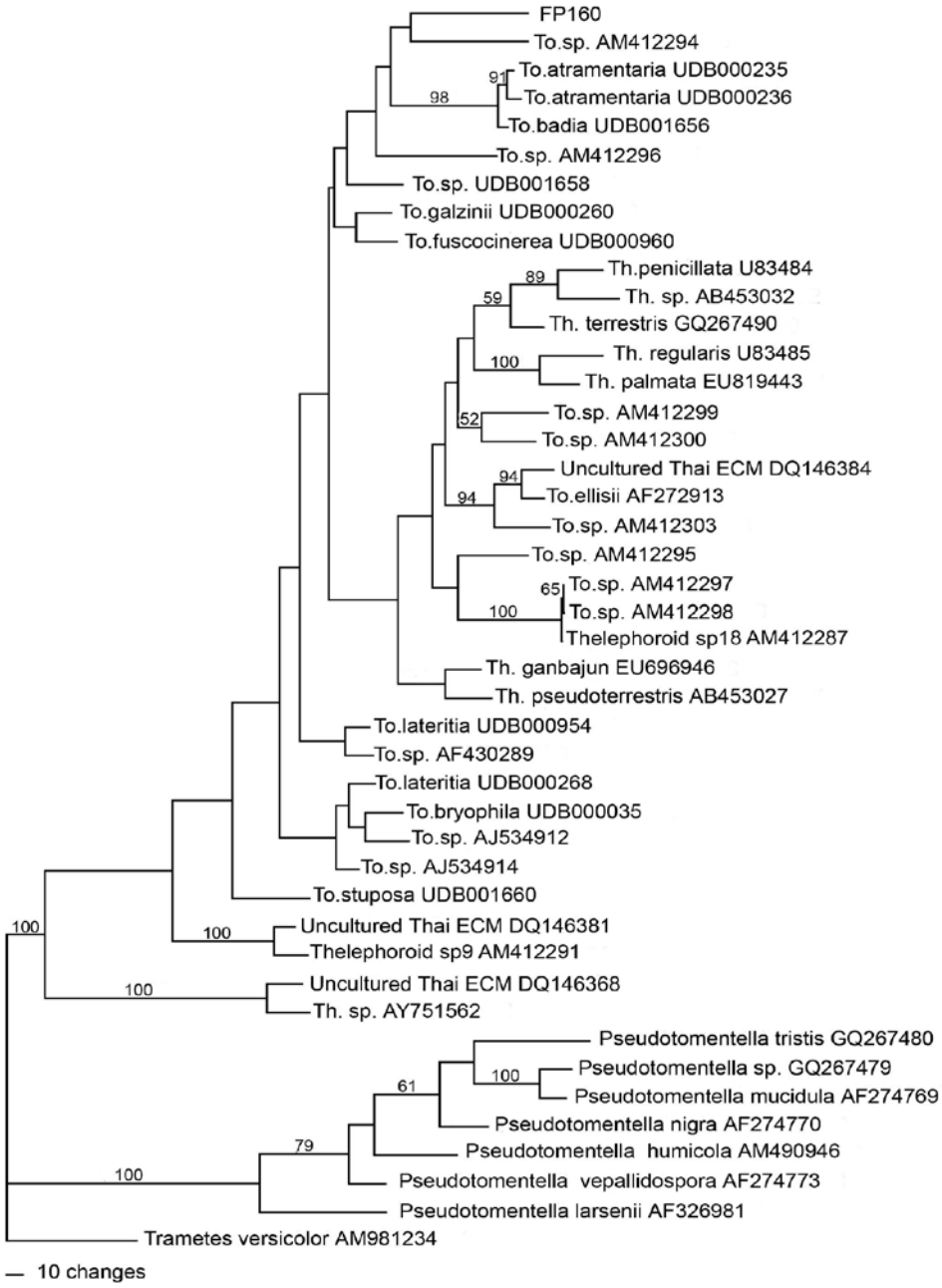

Figure 2 The most parsimonious tree for the ITS region of FP160 in comparison with 42 other isolates of thelephoroid fungi with *Trametes versicolor* as outgroup. The numbers above the branches indicate the bootstrap value of 1000 replicates (values lower than 50% are not shown). Th = *Thelephora*, To = *Tomentella*.

possible to obtain a better match for FP160 or to identify it more precisely. Mycorrhizal genera such as *Tomentella* are also represented by the highest number of insufficiently identified ITS sequences in GenBank (Ryberg *et al.* 2009). Much more research needs to be conducted on tropical Thelephoraceae.

Species of *Tomentella* are common in temperate (Taylor & Bruns 1999) and boreal forests (Kõljalg *et al.* 2000) but have not been well studied in the tropics. The tropics are known to be rich in members of the closely related genus *Thelephora* (Corner 1968) which are regularly encountered in tropical mushroom fruit body surveys (SS Lee, unpublished data). However, species of *Tomentella* have not been encountered although they have been reported to be present on ectomycorrhizal dipterocarp roots (Sirikantaramas *et al.* 2003, Peay *et al.* 2009). Members of *Tomentella* are easily overlooked as their fruit bodies are resupinate

and often located on the underside of dead logs and other woody debris. In the forest of the Guayana Region of Venezuela, a species of *Tomentella* was reported to be associated with the ectomycorrhizal tree *Aldina* (Moyersoen 2006). Results of molecular studies in Thailand (Yuwa-Amornpitak *et al.* 2006) and in Malaysia (Sirikantaramas *et al.* 2003, Peay *et al.* 2009) show that members of the Thelephoraceae including species of *Tomentella* are some of the main ectomycorrhizal fungi associated with the Dipterocarpaceae. In the Seychelles, the thelephoroid clade was found to be the most species rich on the dipterocarp *Vateriopsis seychellarum*; six thelephoroid species were associated with this host (Tedersoo *et al.* 2007). In addition, the ectomycorrhizal anamorphic genus *Riessiella* which is frequently associated with dipterocarps in Malaysia and Indonesia (Julich 1985, Smits 1994, Lee *et al.* 1997) and also found in the Seychelles, has now been recognised as a member of the genus *Tomentella* (Tedersoo *et al.* 2007). This further illustrates the widespread and common occurrence of Thelephoraceae in the tropics and its ectomycorrhizal association with the dipterocarps.

CONCLUSIONS

From both morphological and molecular methods the ectomycorrhizal fungus FP160 isolated from *S. parvifolia* which could form ectomycorrhizas with the dipterocarps *H. odorata* and *S. leprosula* and the exotic legume *A. mangium* can be identified as a member of the family Thelephoraceae, most likely a species of *Tomentella*. Our results show that it has low similarity with *Pseudotomentella*. These results further confirm the occurrence of thelephoroid fungi forming ectomycorrhizas on roots of the Dipterocarpaceae. This fungus is most likely an indigenous fungus that could have originated from the dipterocarp forests surrounding the Lentang nursery, which is located in a valley surrounded by selectively logged and unlogged dipterocarp forests. It could also have been introduced through the composted mesocarp fibre that was used as the potting medium at the Lentang nursery. Further collections of tropical thelephoroid fungi are needed for taxonomic and molecular studies for a better understanding of their identity, diversity, distribution, biology, ecology and potential utilisation.

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