Mycorrhiza (1997) 7:63–81

ORIGINAL PAPER

Lee Su Lee · I. J. Alexander · R. Watling

Ectomycorrhizas and putative ectomycorrhizal fungi of *Shorea leprosula* Miq. (Dipterocarpaceae)

Accepted: 26 March 1997

Abstract The ectomycorrhizas of *Shorea leprosula* Miq. are described and their putative fungal associates discussed. Of the 24 ectomycorrhizal types reported from seedlings, wildlings and 20-year-old trees of *Shorea leprosula,* 20 were associated with the Basidiomycotina, two with the Ascomycotina and two with either members of the Ascomycotina or the Russulaceae. The dominant group of fungi associated with *Shorea leprosula* ectomycorrhizas were members of the Russulaceae. This was confirmed by collections of fungal fruiting bodies made under adult *Shorea leprosula* trees in various parts of Peninsular Malaysia over a period of 3 years. Of the 28 species of putative ectomycorrhizal fungi collected, 15 were members of the Russulaceae.

Key words Ectomycorrhizas · *Shorea leprosula* (Dipterocarpaceae) · Identification

Introduction

Shorea leprosula Miq., known locally as *meranti tembaga,* is a common tree of mixed Dipterocarp forests in southern Thailand, the Malay peninsula (excluding seasonal areas), the islands of Banka, Sumatra and Billiton in Indonesia, and the island of Borneo (Ashton 1982). It is often abundant on deep clay soils at elevations below 700 m asl. *Shorea leprosula* is commercially popular, yielding timber classified as light hardwood of the red meranti group. In Malaysia it has been used quite extensively for enrichment planting and is now one of the indigenous species recommended for forest plantations.

Shorea leprosula forms ectomycorrhizas (Singh 1966; Becker 1983; Lee 1988; Bimaatmadja in Hadi et al. 1991) and several different ectomycorrhizal types have been observed (Becker 1983; Lee 1988, 1992). *Russula* spp. and *Amanita princeps* Corner & Bas have been observed associated with *Shorea leprosula* trees in the FRIM arboretum at Kepong (Hong 1979) but recent observations show many different associated fungi. Watling and Lee (1995) recorded the occurrence of basidiomes of putative ectomycorrhizal fungi growing in the vicinity of numerous dipterocarp hosts, including *Shorea leprosula,* but no descriptions of the associated ectomycorrhizas were made.

In this paper, we highlight the main fungal associations of the ectomycorrhizas of *Shorea leprosula* based on descriptions of the ectomycorrhizas and observations of basidiomes growing under and around parent trees.

Materials and methods

Roots were obtained from *Shorea leprosula* seedlings and wildings collected from logged-over forest in Gombak, regenerated forest in Ulu Langat and Sungai Lalang Forest Reserves, a *Shorea leprosula* plantation in the grounds of the Forest Research Institute Malaysia (FRIM), Kepong, and a selectively logged forest in Air Hitam Forest Reserve, all located in the state of Selangor, Malaysia. Roots were also collected from a planted stand of 20-year-old trees in Ulu Langat Forest Reserve. Details of the various sites are given elsewhere (Lee 1988, 1992).

Washed and cleaned roots were examined under stereo- and compound microscopes. The presence of ectomycorrhizas was confirmed by examining free-hand sections for the presence of the mantle and Hartig net (Marks and Foster 1973; Nylund et al. 1982). Anatomical features of the ectomycorrhizas were studied using 1–3 μm transverse sections of root tips embedded in Agar Resin 100/Araldite (Agar Scientific Ltd., UK). The surface view of the mycorrhizas was studied using peeling and squashing techniques. Ectomycorrhizal types were differentiated using the methods of Chilvers (1968), Agerer (1986) and Ingleby et al. (1990).

Lee Su See (✉)
Forest Research Institute Malaysia, Kepong,
52109 Kuala Lumpur, Malaysia
Fax: +60-3-636-77-53; e-mail: leess@frim.gov.my

I. J. Alexander
Department of Plant and Soil Science, University of Aberdeen,
Aberdeen AB9 2UD, UK

R. Watling
Royal Botanic Garden, Edinburgh EH3 5LR, UK

The colour terminology follows that of Kornerup and Wanscher (1978).

Fungal basidiomes were collected in the FRIM grounds at Kepong and in the forests at Sungai Lalang in Selangor, close to Ulu Langat, and Pasoh Forest Reserve, Negri Sembilan, approximately 170 km south of FRIM. Collecting was carried out according to methods described in Henderson et al. (1969).

Results and Discussion

Like the roots of other species of dipterocarps, *Shorea leprosula* roots have no root hairs. Uninfected roots are thin, light to slightly dark brown with very young roots pale to hyaline. The ultimate branches are usually up to 6 mm in length and in transverse section possess an outer layer of epidermal cells followed by 3–4 layers of cortical cells (Fig. 1). There is no radial elongation of the epidermal cells in uninfected dipterocarp roots.

The ectomycorrhizal short roots of *Shorea leprosula* are variously branched and generally much shorter than non-mycorrhizal roots. They are characterised by the presence of a well-developed mantle of varying thickness and hyphal arrangement, and one layer of distinctly radially elongated epidermal cells between which are located the hyphae of the Hartig net. This is followed by 1–2 layers of cortical cells compared to the numerous layers found in non-mycorrhizal roots. The paraepidermal Hartig net (Godbout and Fortin 1985) does not penetrate beyond the epidermal cells. Becker (1983) erroneously reported non-mycorrhizal dipterocarp roots as having radially elongated cortical cells ensheathed by a periderm-like layer of 3–4 tiers of cells. These were in fact ectomycorrhizal roots. It should also be noted that the structures reported by Becker (1983) as actinomycete filaments were actually sectional views of the Hartig net.

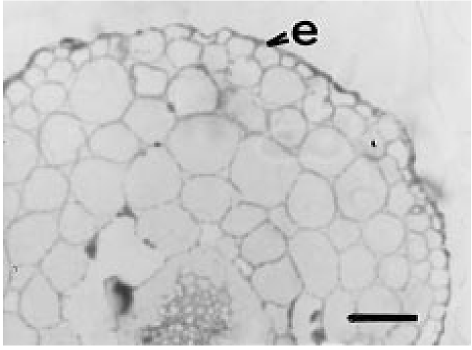


Fig. 1 Transverse section of a non-mycorrhizal root tip of *Shorea leprosula* (*e* epidermis); *bar* 20 μm

Twenty-four ectomycorrhizal types were associated with roots of *Shorea leprosula* seedlings, wildings and adult trees (Table 1). A key to these types and their descriptions are given below.

Key to ectomycorrhizas on S*horea leprosula* Miq.

1.	Ectomycorrhizas dark brown to black	2
1*.	Ectomycorrhizas paler: creamish, shades of yellow to dull brown	10
2.	Mantle a mixture of angular cells and plectenchyma	T13
2*.	Mantle pseudoparenchymatous	3
3.	Emanating hyphae simple septate	4
3*.	Emanating hyphae bearing clamp connections	8
4.	Hyphae smooth	5
4*.	Hyphae verrucose to warty	6

Table 1 Ectomycorrhizas of *Shorea leprosula* seedlings (*s*), wildings (*w*), and adult trees (*a*) and their possible fungal associates

Ectomycorrhizal type	Possible fungal associate	Source plant
T1	Russulaceae	s, w, a
T2	cf. *Fagirhiza fusca*	s, w, a
T3	*Russula* sp.	s, w
T4	Russulaceae	s, w
T5	cf. *Genea hispidula*/Russulaceae	s
T6	Russulaceae	s
T7	Russulaceae	s
T8	*Riessia radicicola*	s, w, a
T9	*Riessiella* sp.	s, w, a
T10	*Amanita* sp.	s, w
T11	*Scleroderma* sp.	s, w
T12	*Cenococcum geophilum*	s
T13	Russulaceae	s, w
T14	Paxillaceae	s
T15	Tuberaceae/Russulaceae	s
T16	Russulaceae	s
T17	Russulaceae	s
T18	*Russula* sp.	s, w
T19	*Scleroderma* sp.	s
T20	Boletaceae	s
T21	Tricholomataceae	s
T22	Boletaceae	s
T23	Russulaceae	s
T24	Tuberaceae	w, a

5.	Hyphae bearing terminal vesicles	T8
5*.	Hyphae not bearing terminal vesicles	T1
6.	Hyphae with yellow pigment	T19
6*.	Hyphae with dark pigment	7
7.	Hyphae long, straight, bristly, thick walled	T12
7*.	Hyphae short, setiform	T24
8.	Hyphae dark pigmented, bearing terminal vesicles	T9
8*.	Hyphae hyaline	9
9.	Mantle smooth, unornamented	T2
9*.	Mantle grainy, ornamented with club-shaped, inflated cells	T4
10.	Ectomycorrhizas white to dirty white or creamish	11
10*.	Ectomycorrhizas various shades of yellow, light olive to pale brown	14
11.	Hyphal tips not club shaped	12
11*.	Hyphal tips often branched, septate with terminal clavate to club-shaped tips	T20
12.	Emanating hyphae simple septate, hyaline	13
12*.	Emanating hyphae simple septate, pale to dark brown, curled, with rounded tips	T15
13.	White hyphal strands present	T6
13*.	White hyphal strands absent	T17
14.	Ectomycorrhizas various shades of light olive or brown	15
14*.	Ectomycorrhizas various shades of yellow	17
15.	Emanating hyphae frequent, with clamp connections, mantle surface not ornamented	T7
15*.	Emanating hyphae absent or rare, mantle surface ornamented with inflated cells or cystidia	16
16.	Inflated cells or cystidia flask shaped	T5
16*.	Inflated cells terminating in orange, round to ovate, conidia-like structures or vesicles	T23
17.	Emanating hyphae simple septate	18
17*.	Emanating hyphae with clamp connections	21
18.	White hyphal strands present	19
18*.	White hyphal strands absent	20
19.	Mycorrhizas densely ramified, twisted, pale to dark yellow, mantle surface shiny	T11
19*.	Mycorrhizas monopodially pinnate, short, twisted, dirty creamish-yellow, mantle surface dull	T21
20.	Mycorrhizas dark yellow-brown, unramified to monopodially pinnate	T18
20*.	Mycorrhizas pale creamish-yellow, irregularly pinnate, tortuous, clumped	T22
21.	Hyphal strands abundant	T10
21*.	Hyphal strands absent or few	22
22.	Cystidia on mantle surface	T3
22*.	No cystidia on mantle surface	23
23.	Emanating hyphae yellow, 6–8 μm diameter	T16
23*.	Emanating hyphae fluffy, hyaline, 4–6 μm diameter	T14

Descriptions of mycorrhizas

T1: Russulaceae

Morphological characteristics: The mycorrhizas were twisted, long and flexous, 2–6 mm in length. They were dark chocolate brown, with young roots usually having cream-coloured tips. No rhizomorphs were observed.

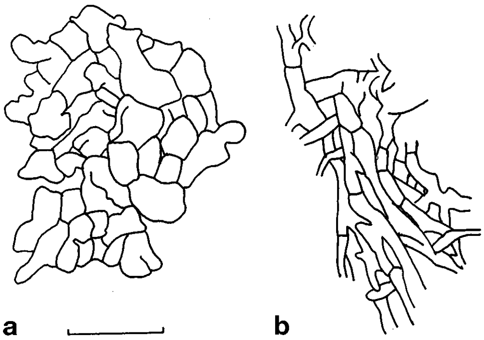


Fig. 2a, b Features of type T1, Russulaceae. **a** Pseudoparenchymatous mantle surface. **b** Inner mantle composed of net pseudoparenchyma with simple septate hyphae; *bar* 20 μm

Mantle edge: Smooth and compact when not obscured by emanating hyphae. The abundant emanating hyphae were hyaline, thin-walled, simple septate and 1.5–3.0 μm in diameter.

Mantle surface: Pseudoparenchymatous, composed of irregularly shaped hyphal elements which were angular and small (Fig. 2a). The inner mantle was composed of net-like pseudoparenchyma with distinguishable long, simple septate hyphal elements (Fig. 2b). The mantle was 15–23 μm thick in cross-section.

This mycorrhiza was most probably formed by a member of the Russulaceae as its features resembled those described for mycorrhizas formed by *Russula* spp. (Agerer 1987–1990), *Lactarius rufus* (Ingleby et al. 1990) and *Lactarius* spp. (Agerer 1987–1990).

T2 – similar to Fagirhiza fusca Brand

Morphological characteristics: The mycorrhizas were long, flexous, pyramidally branched when young, becoming irregularly pinnate with age. They were evenly dark chocolate brown. No rhizomorphs were observed.

Mantle edge: Smooth and compact. There were numerous long, hyaline emanating hyphae, 1.5 μm in diameter with clamp connections.

Mantle surface: Pseudoparenchymatous, composed of isodiametric cells (Fig. 3a) described as type L by Agerer (1995). The inner mantle was also composed of pseudoparenchymatous cells like that of the surface layer but the cells were of smaller diameter (Fig. 3b). The mantle was 15–30 μm thick in cross-section.

In plastic sections, the mantle stained pinkish-purple (metachromatically) with methylene blue. A morphologically very similar mycorrhiza with the same staining reaction was described by Berriman (1986) from roots of seedlings and adult trees of *Shorea lepidota* collected from Pasoh in Malaysia. T2 was also very similar to Becker's (1983) Type 4 from roots of *Shorea leprosula*

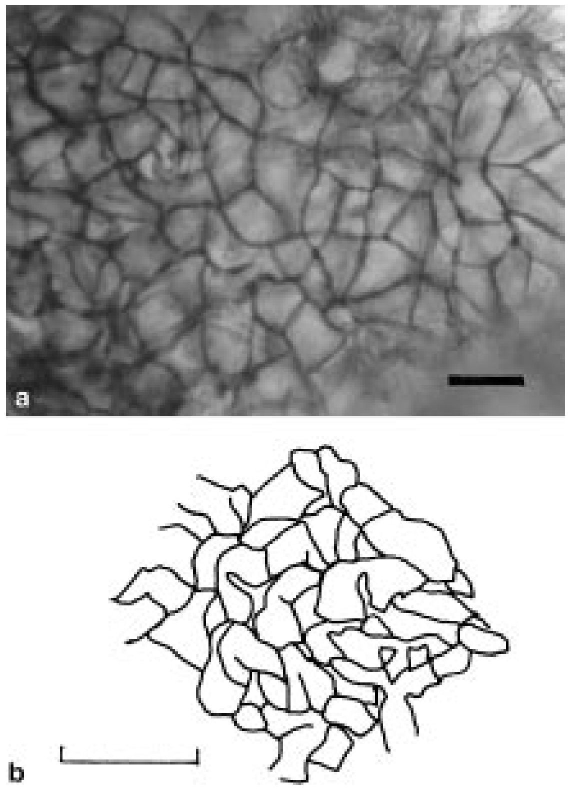


Fig. 3a, b Plan view of the pseudoparenchymatous mantle of type T2, resembling *Fagirhiza fusca* Brand. **a** Outer pseudoparenchymatous surface, composed of isodiametric cells. **b** Inner layer composed of smaller isodiametric cells; *bar* 20 μm

seedlings collected at Pasoh. T2 resembled *Fagirhiza fusca* (Brand 1991) in its mantle features and the possession of clamped hyphae. Mycorrhizas with similar mantles are also known to be formed by members of the Tuberaceae and Hygrophoraceae (Agerer 1995).

T3 – Russalu sp.

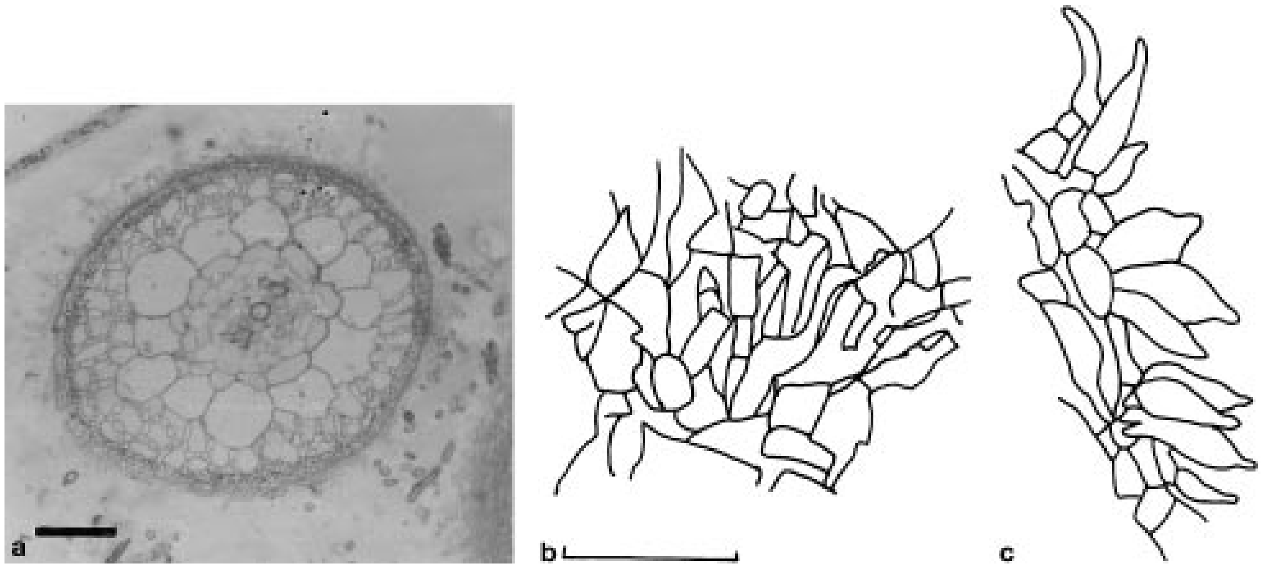
Morphological characteristics: The mycorrhizas were irregularly pinnate to dichotomously branched, thick, furry and pale yellowish-brown. No rhizomorphs were observed.

Mantle edge: Felty due to the presence of numerous cystidia (Fig. 4a). The abundant emanating hyphae were hyaline and bore clamp connections.

Mantle surface: Appeared to be a mixture of plectenchyma and pseudoparenchyma (Fig. 4b), resembling Agerer's type P (Agerer 1995), i.e. 'with angular cells bearing a delicate hyphal net'. The mantle was relatively thin, 8–20 μm in cross-section.

The mantle bore clavate- to flask-shaped cystidia on its surface (Fig. 4c). These cystidia resembled those described on mycorrhizas of Sitka spruce formed by *Russula aeruginea* (Taylor and Alexander 1989). However, T3 also had abundant clamped hyphae which according to Agerer et al. (1990) are not found in *Russula* ectomycorrhizas. Cystidia-bearing mantles have also been reported from mycorrhizas of *Pulveroboletus shoreae* on *Shorea robusta* (Bakshi 1974) and on mycorrhizas synthesised experimentally with *Descolea maculata* (Bougher and Malajczuk 1985). However, the former has simple septate hyphae while the cystidia of the latter are distinctly capitate. Since there are still many undescribed species of tropical Russulaceae of which little is known, it is possible that T3 may be formed by one of these species of *Russula.*

Fig. 4a–c Features of ectomycorrhiza type T3, *Russula* sp. **a** Transverse section showing clavate-shaped cystidia on the mantle surface; *bar* 40 μm. **b** Plan view of the mantle surface composed of a mixture of plectenchyma and pseudoparenchyma. **c** Clavate-shaped cystidia on the surface of the mantle; *bar* 20 μm



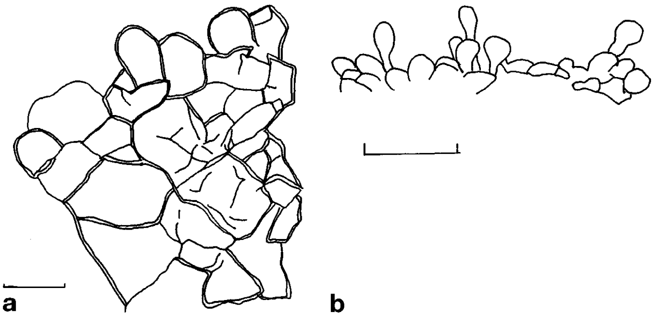


Fig. 5a,b Features of ectomycorrhiza type T4, Russulaceae. **a** Plan view of the mantle composed of thick-walled, angular pseudoparenchyma; *bar* 20 μm. **b** Club-shaped inflated cells or cystidia on the mantle surface; *bar* 40 μm

T4 – Russulaceae

Morphological characteristics: The mycorrhizas were short, stubby and blunt, often unramified with few branches but sometimes branching pinnately. They were very dark brown to black. No rhizomorphs were observed.

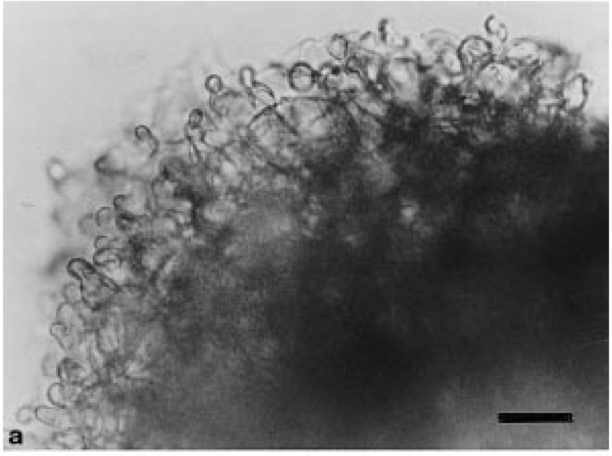
Mantle edge: Compact but grainy and emanating hyphae were rarely observed. Where present, the hyaline emanating hyphae had clamp connections.

Mantle surface: Composed of thick-walled, angular, regular pseudoparenchyma (Fig. 5a). The inner layers could not be clearly differentiated. In cross-section the mantle was 20–40 μm thick.

T4 was easily distinguished by its grainy surface due to the presence of club-shaped inflated cells or cystidia (Fig. 5b). T4 closely resembled mycorrhiza type ITE.5 formed by a basidiomycete on seedlings of *Picea* and *Betula* spp. (Ingleby et al. 1990) but the setae found on the mantle surface of ITE.5 were replaced in T4 by more club-shaped structures with obovate heads similar to those depicted by Chilvers (1968) for eucalypt mycorrhiza type 4. The associated fungus was not identified in both cases. The mantle characteristics of T4 closely resembled those of *Lactarius acris* described by Brand (1991), and of mantle type I described by Agerer (1995) formed by some species of *Lactarius* in the Russulaceae. As lacticifers were not observed, it could not be confirmed whether a species of *Lactarius* was involved. However, species of *Lactarius* are not uncommon in Malaysian forests (Watling and Lee 1995).

T5 – Geneaceae or Russulaceae

Morphological characteristics: The mycorrhizas were very similar to T4 except that they were a lighter muddy brown and with smaller dimensions. The mycorrhizas were unramified to monopodially or pinnately branched. No rhizomorphs were observed.



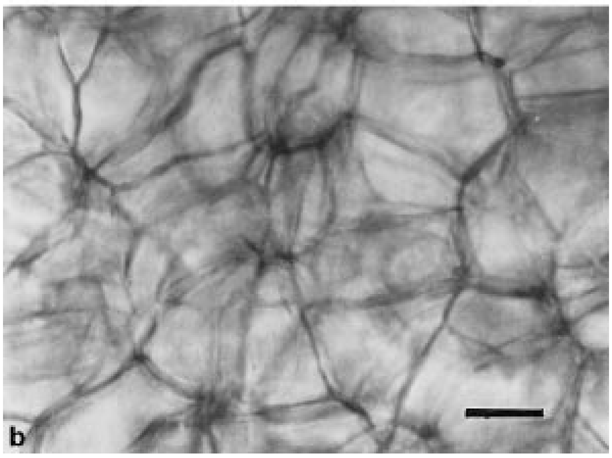


Fig. 6a,b Features of ectomycorrhiza type T5, Geneaceae or Russulaceae. **a** Flask-shaped inflated cells or cystidia on the mantle surface; *bar* 20 μm. **b** Plan view of the mantle surface showing large, angular pseudoparenchyma; *bar* 10 μm

Mantle edge: Compact but grainy due to the presence of clumps of flask-shaped inflated cells or cystidia on the mantle surface (Fig. 6a).

Mantle surface: Composed of angular, regular pseudoparenchyma (Fig. 6b). The inner layers could not be distinguished. The mantle was 15–20 μm thick in cross-section.

The external morphology and arrangement of the mantle tissue in T5 were very similar to that of *Genea hispidula,* a member of the Geneaceae described by Brand (1991). Agerer (1995) lists this type of mantle as type K described from members of the Geneaceae and Russulaceae. While members of the Geneaceae have yet to be discovered in Malaysia, members of the Russulaceae are among the most commonly occurring fungi in Malaysian forests.

T6 – Russulaceae

Morphological characteristics: The mycorrhizas were twisted, monopodial pinnate, and dirty dark cream.

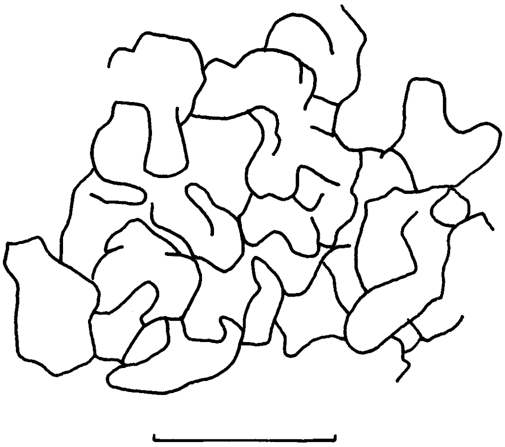


Fig. 7 Plan view of the pseudoparenchymatous mantle surface of type T6, Russulaceae, composed of thin-walled, interlocking irregular jigsaw puzzle-like cells; *bar* 20 μm

Loosely formed rhizomorphs composed of smooth, simple septate, undifferentiated hyphae were present.

Mantle edge: Indistinct due to the presence of abundant velvety, hyaline and simple septate emanating hyphae.

Mantle surface: Pseudoparenchymatous, composed of thin-walled, interlocking, irregular, jigsaw puzzle-like cells (Fig. 7). The inner layers were not easily discernible but appeared to be composed of more loosely arranged irregular pseudoparenchyma. The mantle was 6–18 μm thick in cross-section.

The thin mantle stained very lightly with methylene blue. The pseudoparenchymatous, jigsaw puzzle-like or epidermoid mantle and simple septate hyphae are characteristic of mycorrhizas of *Lactarius* spp. or *Russula* spp. (Agerer et al. 1991). This mantle type classified as type M by Agerer (1995) has been described from members of the Tuberaceae, Hygrophoraceae and Russulaceae. In Malaysia the most likely fungal associate would be a member of the widely occurring Russulaceae (Watling and Lee 1995).

T7 – Russulaceae

Morphological characteristics: The mycorrhizas were long, unramified and pale olive-brown. The emanating hyphae were hyaline and bore clamp connections. No rhizomorphs were observed.

Mantle edge: Smooth and compact.

Mantle surface: Composed of a layer of loosely arranged plectenchyma (Fig. 8a) overlying non-interlocking, irregular pseudoparenchyma (Fig. 8b) (=type P sensu Agerer 1995). The mantle was 10–15 μm thick in cross-section.

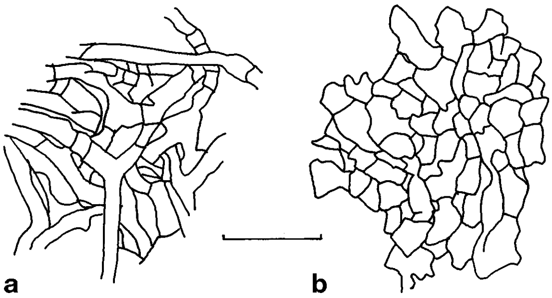


Fig. 8a,b Plan view of the mantle surface of type T7, Russulaceae. **a** Loosely arranged plectenchyma on the mantle surface. **b** Inner layer of irregular pseudoparenchyma; *bar* 20 μm

Such mantles have been described from mycorrhizas formed by members of the Russulaceae (Agerer 1995).

T8 – Riessia radicicola Julich

Morphological characteristics: The mycorrhizas were monopodially pinnate and bristly to hairy. The branches varied from short to long and were dark brown to black. Rhizomorphs were dark brown to black and composed of undifferentiated, simple septate hyphae.

Mantle edge: Smooth and compact and covered by a network of simple septate hyphae which bore conidiophore-like branches terminating in clubbed or lobed vesicles, giving the roots a bristle-like appearance (Fig. 9a). The emanating hyphae were smooth, dark pigmented and simple septate.

Mantle surface: The pseudoparenchymatous mantle surface (Fig. 9b) was composed of non-interlocking, irregular cells. The different layers in the mantle were not discernible. Cells of the root epidermis were scattered in a layer of orangish-yellow matrical material underneath the mantle. The mantle was 15–30 μm thick in cross-section.

Although T8 had the well-developed mantle characteristics of an ectomycorrhiza, there was no radial elongation of the epidermal cells and no evidence of the Hartig net (Fig. 9c). Morphologically, the presence of the bristly terminal vesicles distinguished T8 from other ectomycorrhiza types. Berriman (1986) described a similar mycorrhiza, Type J, on roots of *Shorea macroptera* from Pasoh, Malaysia. Roots resembling T8 have been described by Julich (1985) as being associated with *Riessa radicicola* spec. nov. He stated that *Riessia radicicola* was ectomycorrhizal with *Shorea laevis* and *Hopea sangal.* He called the terminal hyphal structures conidia but, as Hutchison (1989) pointed out, these structures were never observed to separate from the 'conidiogenous cell' and appeared rather to resemble the jack-shaped cells found in other fungi. The term

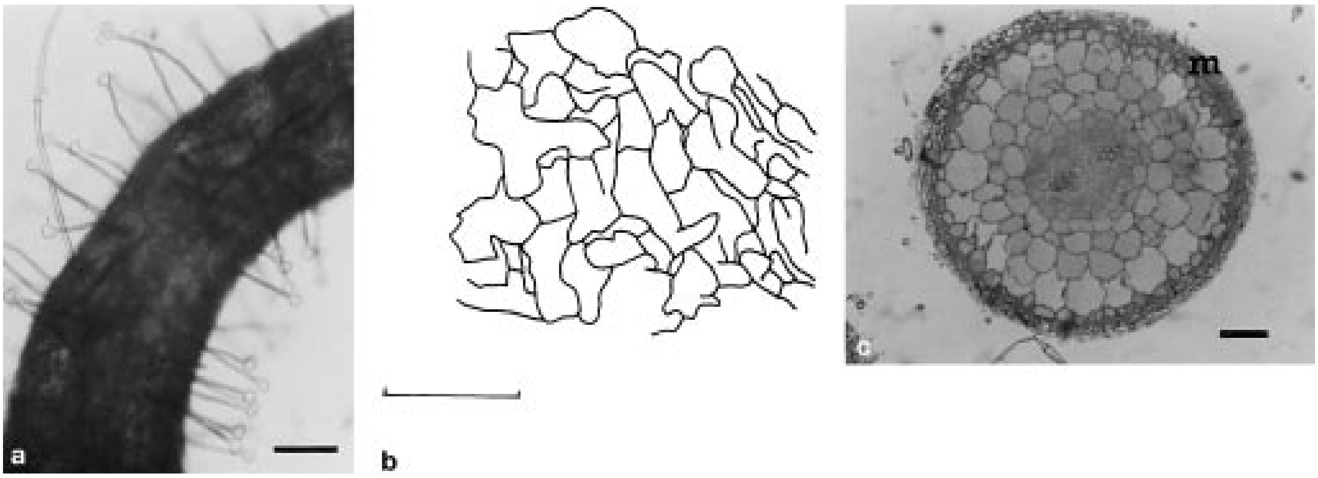


Fig. 9a–c Features of type T8, *Riessia radicicola* Julich. **a** Lobed vesicles borne on hyphae extending from the mantle surface resulting in bristly-looking roots; *bar* 50 μm. **b** The pseudoparenchymatous mantle. **c** Transverse section of a root tip showing the well-developed mantle (*m*) and absence of elongated epidermal cells and Hartig net; *bar* 20 μm

'vesicle' would be more appropriate for these structures. Agerer (1995) has classified these structures as cystidia.

The absence of the Hartig net and deposition of phenolic compounds were indicative of an interaction between an incompatible host and ectomycorrhizal symbiont (Molina 1981; Molina and Trappe 1982; Malajczuk et al. 1984; Duddridge 1987). On the other hand, mycorrhizas of *Pisonia grandis* have been found to develop transfer cells in place of the Hartig net (Ashford and Allaway 1982). *Riessia* associations were reported by Smits (1994) from roots of *Dryobalanops lanceolata* and *Shorea pauciflora* in Kalimantan, Indonesia. He stated that the plants responded positively to the infection as if it were a mycorrhizal association but this observation was not backed up by statistically analysed results. Until well-replicated and properly designed synthesis experiments have been conducted with *Riessia radicicola,* it is difficult to say whether the association functions like a mycorrhiza.

T9 – Riessiella Julich, gen. nov.

Morphological characteristics: The mycorrhizas were very similar in external morphology to those of *Riessiella radicicola.* The branches were long, monopodially pinnate, bristly, and dark brown to black. Rhizomorphs were undifferentiated, dark-pigmented and bore clamp connections.

Mantle edge: Smooth and compact, similar to that of *Riessiella radicicola* and emanating hyphae were smooth, dark pigmented and bore clamp connections. The mantle surface was covered by a loose network of frequently clamped hyphae, many bearing long, extending, conidiophore-like branches terminating in narrowly to broadly clavate vesicles (cystidia) (Fig. 10a). These vesicle-bearing hyphae were brownish to yellowish, thick walled, 2–4 celled and clamped. Such hyphae were reported by Fassi and Fontana (1961) from a mycorrhizal type associated with *Julbernardia seritii* in the Congo.

Mantle surface: The outer mantle was composed of interlocking, irregular pseudoparenchyma (Fig. 10b) described as type D by Agerer (1995), while the inner layer was composed of similar cells but with a larger diameter. The mantle was 15–30 μm thick in cross-section.

As in *Riessia radicicola* there was no radial elongation of the epidermal cells, and these cells were scattered in the layer of orange matrical material just below the mantle. The Hartig net was also absent (Fig. 10c). The morphological and anatomical features of T9 very closely resembled the mycorrhizal Type A described by Berriman (1986) on roots of seedlings of *Shorea lepidota, Shorea leprosula* and *Shorea macroptera.* Her specimens may have been obtained from older portions of the roots as no vesicle-bearing emanating hyphae were reported. T9 is also very similar to the mycorrhizal type described by Fassi and Fontana (1961) on *Julbernardia seritii* as *'micorrize con ife a vescicole terminali'* which also did not possess a Hartig net. Julich (1985) illustrated morphologically similar structures from several Malaysian *Shorea* species as mycorrhizal (without anatomical descriptions) and distinguished two species of fungi, *Riessiella clavata* and *Riessiella cylindrica,* associated with such roots. He differentiated the two fungi based on differences in the shape of the terminal vesicle; *Riessiella clavata* with a broader vesicle, *Riessiella cylindrica* with a cylindrical or narrowly clavate vesicle. T9 in this study includes roots possessing both sets of vesicle characteristics, as the two types were usually indistinguishable except upon microscopic examination of the vesicles. Moreover mantle characteristics considered most important for identification of ectomycorrhi-

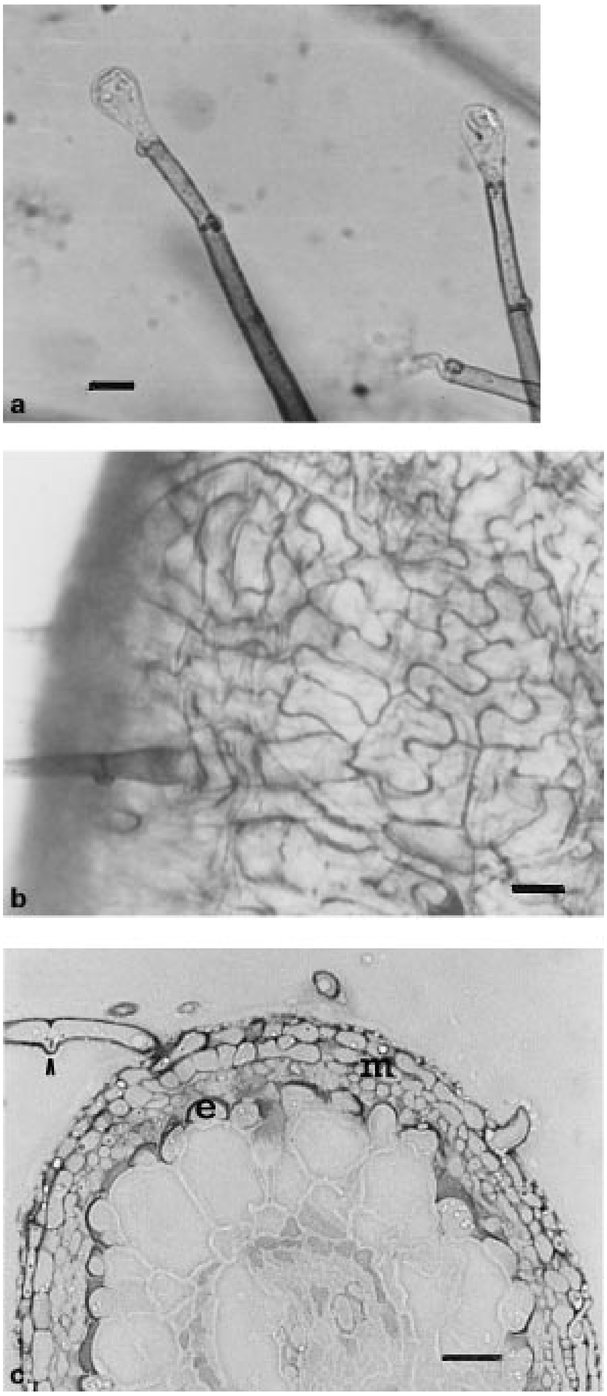


Fig. 10a–c Features of type T9, *Riessiella* Julich, gen. nov. **a** Rounded terminal vesicles borne on hyphae with clamp connections extending from the mantle surface. **b** Plan view of the mantle surface composed of interlocking irregular pseudoparenchyma. **c** Transverse section of a root tip showing a well-developed mantle (*m*), a clamped emanating hypha (*arrow*), epidermal cells (*e*) and absence of the Hartig net; *bar* 20 μm

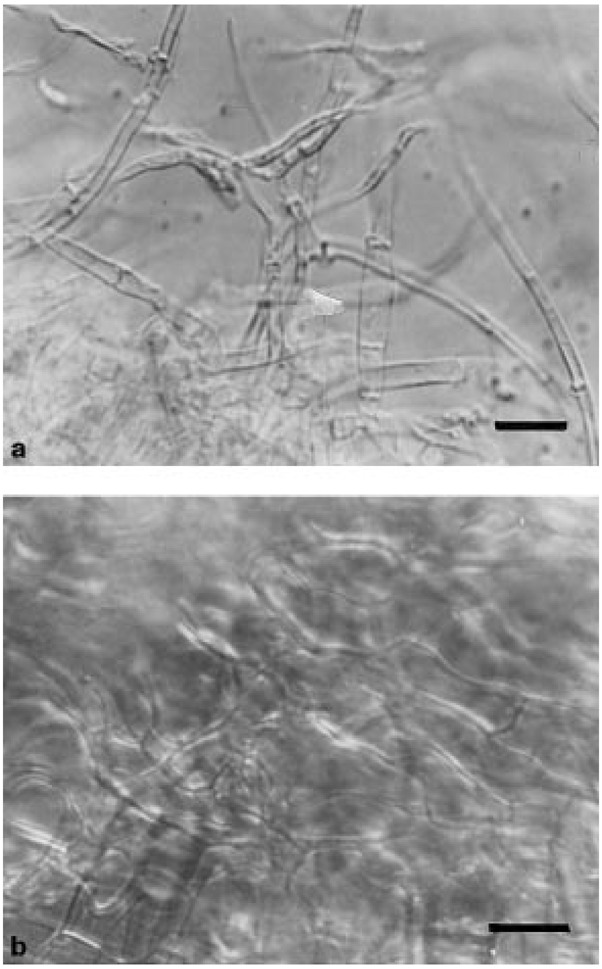


Fig. 11a,b Features of type T10, possibly *Amanita* sp. **a** Hyaline, clamped emanating hyphae; *bar* 20 μm. **b** Plan view of mantle surface composed of large open hyphae; *bar* 10 μm

zal types were the same in both cases. There is a possibility that the two species suggested by Julich (1985) could be variants of the same species. Smits (1994) has also observed similar associations in roots of several species of dipterocarps in Indonesia. As with *Riessia radicicola* the functional status of T9 awaits experimental investigation.

T10 – possibly Amanita sp.

Morphological characteristics: The mycorrhizas were monopodially pinnate, dull yellowish to light brownish, with a velvety to hoary surface. The rhizomorphs were thick, white strands with an untidy margin and composed of loosely woven, undifferentiated hyphae of equal diameter. The septation was not clear.

Mantle edge: Loosely formed with abundant hyaline emanating hyphae bearing clamp connections (Fig. 11a).

Mantle surface: Composed of large hyphae arranged in a net-like pattern (Fig. 11b) similar to Agerer's (1995) type E. The arrangement of the inner layer was not clear. The mantle was 10–20 μm thick in cross-section.

The whitish, frost-like surface, the type E mantle (Agerer 1995) and the presence of the thick, white strands were the distinctive features of T10. Berriman (1986) reported a mycorrhiza, Type D, of similar morphology, anatomy and hyphae from roots of seedlings and adults of *Shorea lepidota* from Pasoh, Malaysia.

Type E mantles are known from mycorrhizas formed by members of the Amanitaceae, Boletaceae, Cortinariaceae, and Rhizopogonaceae (Agerer 1995). According to Godbout and Fortin (1985), *Amanita* spp. form very distinctive ectomycorrhizas with a hoary surface due to abundant cystidium-like multiseptate hyphae and also have white, differentiated hyphal strands. Molina and Trappe (1982) and Ingleby et al. (1990) also reported thick, white hyphal strands as being characteristic of *Amanita muscaria* ectomycorrhizas. In transverse section, the large angular cells of the mantle in T10 also resembled those of synthesised *Amanita* spp. ectomycorrhizas (Godbout and Fortin 1985) and of ectomycorrhizas of *Amanita* aff. *rubescens* described on roots of *Uapaca guineensis* by Thoen and Ba (1989). However, hyphae of *Amanita* ectomycorrhizas may be simple septate or clamped depending on the species of the fungus involved. Godbout and Fortin (1985) and Ingleby et al. (1990) reported simple septate emanating hyphae from *Amanita muscaria* mycorrhizas while Largent et al. (1980) reported distinctively clamped emanating hyphae from ectomycorrhizas formed between *Amanita gemmata* and *Arctostaphylos manzanita* and *Pinus ponderosa.* Based on the high degree of similarity between T10 and some of the known *Amanita* ectomycorrhizas described above, there is a very high possibility that T10 is associated with a species of *Amanita.* Moreover, many species of *Amanita* are often found fruiting in the dipterocarp forest (Corner and Bas 1962; Watling and Lee 1995).

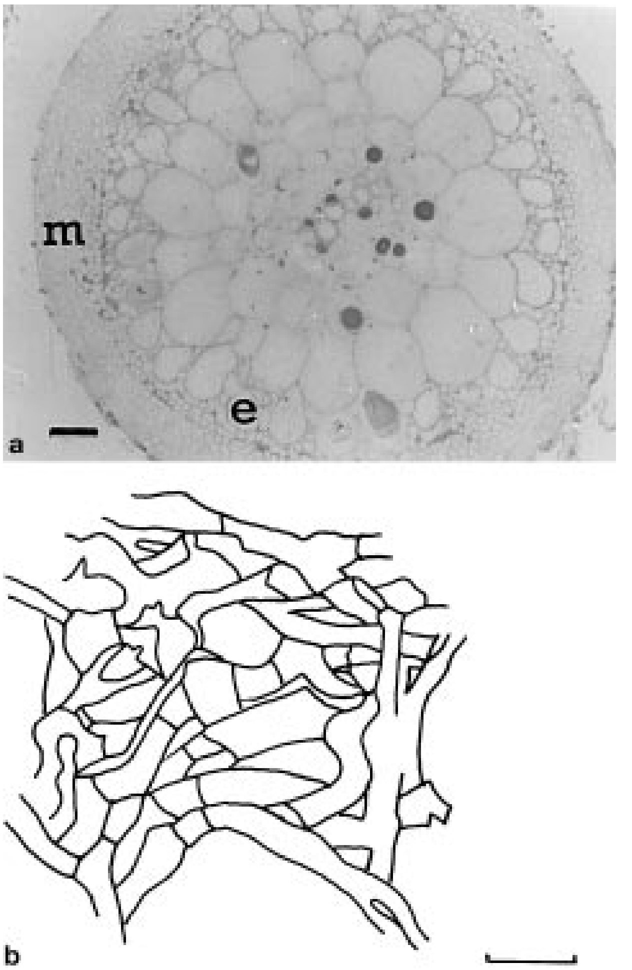


Fig. 12a,b Features of type T11, *Scleroderma* sp. **a** Transverse section of a root tip showing the compact mantle (*m*) and elongated epidermal cells (*e*) surrounded by hyphae of the Hartig net; *bar* 20 μm. **b** Plan view of the plectenchymatous mantle surface; *bar* 10 μm

T11 – Scleroderma sp.

Morphological characteristics: The mycorrhizas were densely but irregularly pinnate and twisted. They were pale to dark yellow or sometimes with yellow patches and the surface was shiny. Soil and sand were often attached. Occasionally white, string-like strands were present. The hyphae in the rhizomorphs were compactly arranged forming rhizomorphs with smooth margins like type B described by Agerer (1995).

Mantle edge: Smooth and compact (Fig. 12a), the roots appearing solid. There was abundant hyaline, simple septate emanating hyphae.

Mantle surface: Covered by a thin network of hyphae forming a net plectenchyma (Fig. 12b) similar to type A described by Agerer (1995). The inner layer was composed of closely woven, tortuous, irregular pseudoparenchyma. The mantle was 15–25 μm thick in cross-section.

While a range of fungi from several families form mycorrhizas with type A mantles (Agerer 1995), the string-like hyphal strands and the external morphology of T11 resembled ectomycorrhizas associated with *Scleroderma* spp. (Molina and Trappe 1982; Godbout and Fortin 1985) even though the mantle colour was different. The emanating hyphae of *Scleroderma* ectomycorrhizas may be simple septate or clamped depending on the fungus species (Godbout and Fortin 1985). A morphologically and anatomically similar mycorrhiza was described by Berriman (1986) on seedling roots of *Shorea lepidota* and roots of adult *Shorea lepidota* and *Shorea leprosula* from Pasoh, Malaysia. Several species of *Scleroderma* are known to occur in dipterocarp forests (Watling and Lee 1995).

T12 – possibly Cenococcum geophilum

Morphological characteristics: The mycorrhizas were unramified to simple, monopodially pinnate, short, club shaped, and resembled black, hairy sausages on the ends of non-mycorrhizal roots. They were black and usually entangled in a mass of long, dense, thick, black, hair-like hyphae (Fig. 13a). Rhizomorphs were not observed.

Mantle edge: Compact and uneven. Emanating hyphae were straight, dark brown, verrucose to warty, simple septate with thick cell walls and easily broken.

Mantle surface: Composed of a mosaic of thick-walled, isodiametric, regular pseudoparenchyma, arranged in radiating clusters (Fig. 13b). The inner mantle was composed of thin-walled net pseudoparenchyma embedded in a matrix of unidentified material. The mantle was 20–33 μm thick in cross-section.

Infiltration of roots for preparation of sections was always difficult. The thickened epidermal cells which contained deposits of phenolic materials were not radially elongated. Haustoria-like structures extended from the mantle into the cortex cells and a Hartig net was absent (Fig. 13c). The general appearance and mantle anatomy of T12 resembled mycorrhizas formed by *Cenococcum geophilum* (Chilvers 1968; Zak 1973; Ingleby et al. 1990). However, the inner mantle which appeared to be composed of thin-walled cells embedded in a matrix of dark material is uncharacteristic of *Cenococcum geophilum.* Roots possessing similar haustorial structures but with a Hartig net have been reported on *Julbernardia seretii* (Fassi and Fontana 1961) from the Congo and on *Shorea leprosula* (Becker 1983) from Malaysia. The *Julbernardia seretii* fungus was unidentified but an imperfect stage of a tropical *Elaphomyces* was suspected to be associated with Becker's specimen, although its similarity to *Cenococcum geophilum* was also noted. With the exception of the absence of the Hartig net, T12 appears to be the same mycorrhiza type described as Type 10 by Becker (1983). There is a possibility that more than one fungus was involved in the T12 association; one of which was *Cenococcum geophilum* producing the characteristic outer mantle and another which formed the inner mantle and haustoria. It was also possible that *Cenococcum geophilum* formed haustoria in association with *Shorea* roots. Another possibility was that the fungus involved was not *Cenococcum geophilum* but a closely related fungus. Haustoria-like structures formed by *Chroogomphus* spp. and *Gomphidius roseus* and *Gomphidius maculatus* have been found within the cortex cells of *Suillus* and *Rhizopogon* ectomycorrhizas (Agerer et al. 1991). Brand (1991) described intracellular infection of cortical cells by haustorial structures developing from the Hartig net in ectomycorrhizas of *Fagus sylvatica* associated with *Lactarius acris, Russula mairei* and *Tricholoma sciodes,* while Agerer et al. (1991) noted that such haustoria-like intrusions have been found in ectomycorrhizas of *Leccinum scabrum, Russula emetica, Russula mairei* and *Russula nana.* Their function is as yet unknown.

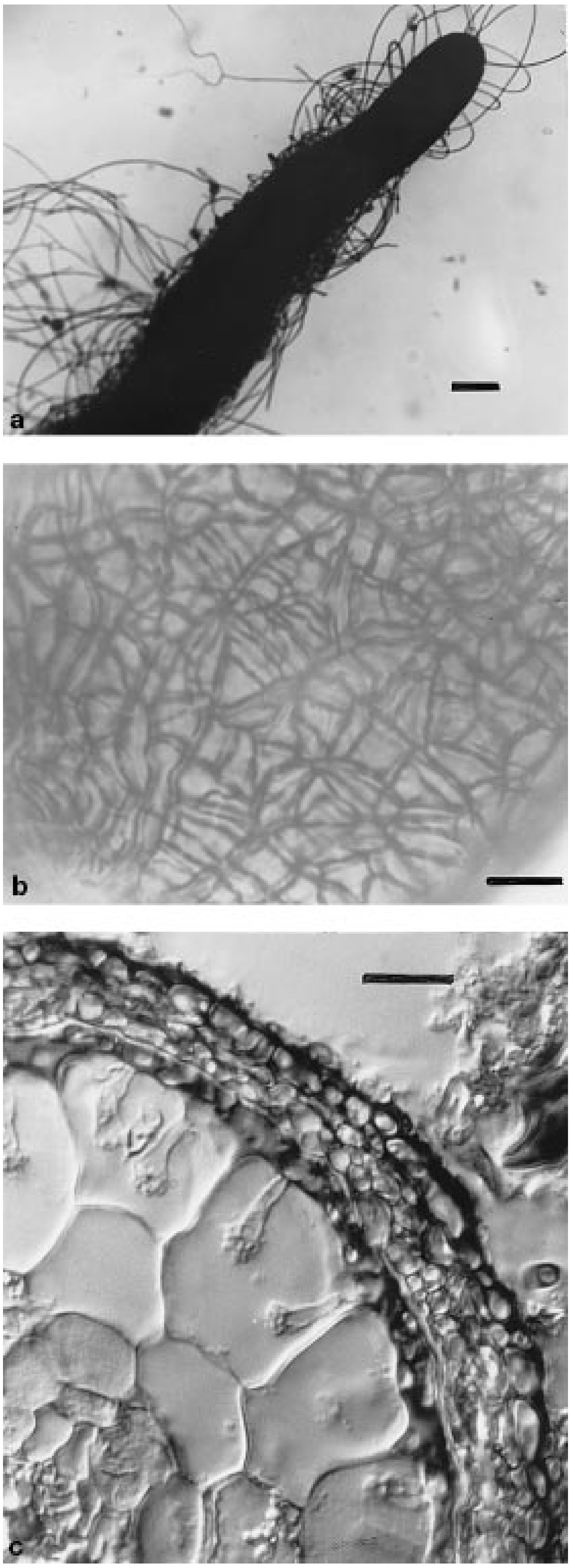


Fig. 13a–c Features of type T12, possibly *Cenococcum geophilum* Fr. **a** A root tip covered with thick-walled emanating hyphae. **b** Plan view of the mantle surface composed of thick-walled pseudoparenchyma arranged in radiating clusters. **c** Transverse section of a root tip showing the well-developed mantle and haustoria-like structures extending into the cortical cells; *bar* 20 μm

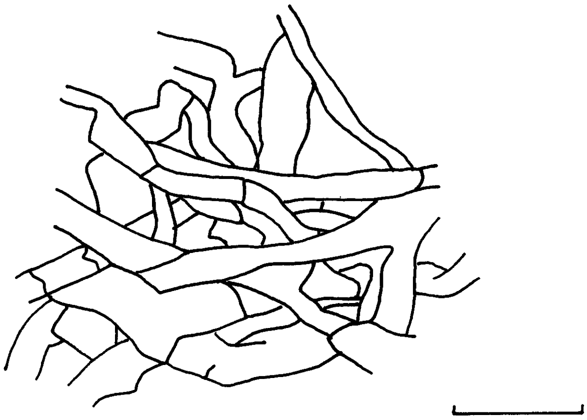


Fig. 14 Plan view of the mantle surface of type T13, Russulaceae, composed of angular cells intermingled with large-diameter, simple septate hyphae; *bar* 20 μm

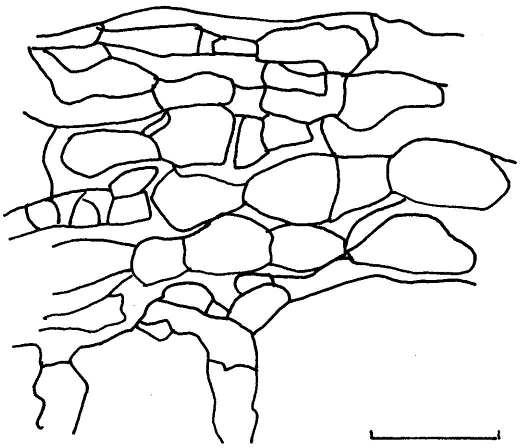


Fig. 15 Transverse section through the mantle of a root tip of type T14, possibly *Phylloporus* sp. showing the large-diameter pseudoparenchyma; *bar* 20 μm

T13 – Russulaceae

Morphological characteristics: The mycorrhizas were unramified, long, flexous and dark brown. No rhizomorphs were observed.

Mantle edge: Smooth and compact. Emanating hyphae were hyaline and simple septate.

Mantle surface: Composed of a mixture of angular cells and large-diameter, simple-septate and often dichotomously-branching hyphae (Fig. 14) similar to Agerer's (1995) type P. The mantle was 8–25 μm thick in cross-section.

The similarity of the large-diameter, simple septate and dichotomously branching hyphae to lactiferous hyphae and the features of the mantle indicated a possible association with a *Lactarius* sp. (Godbout and Fortin 1985; Ingleby et al. 1990; Agerer 1995).

T14 – possibly Phylloporus sp. (Paxillaceae)

Morphological characteristics: The mycorrhizas were twisted with short branches, creamish to light yellow. No rhizomorphs were observed.

Mantle edge: Loosely formed with abundant emanating hyphae forming a fluffy, cottony covering on the mycorrhizas. The hyphae were hyaline, 4–6 μm in diameter and bore clamp connections.

Mantle surface: Obscured by the presence of the abundant cottony hyphae. However, in transverse section the mantle was composed of large-diameter, irregular pseudoparenchyma (Fig. 15). The mantle was 25–42 μm thick in cross-section.

The external appearance and the microscopic features of the emanating hyphae and mantle showed some resemblance to *Paxillus* mycorrhizas (Ingleby et al. 1990). While species of *Paxillus* have not been recorded from Malaysian forests, species of the closely related *Phylloporus* are often found in dipterocarp forests (Watling and Lee 1995). Thus there is a possibility that this fungus could be involved.

T15 – Tuberaceae or Russulaceae

Morphological characteristics: The mycorrhizas were unramified to monopodially pinnate, the tips bulbous, varying from creamish when young to black with age. The mycorrhizas appeared woolly being covered with curling, pale to black, setae-like hyphae. There were no rhizomorphs.

Mantle edge: Uneven. The emanating setae-like hyphae were simple septate, with rounded tips (Fig. 16a), rising directly from large-diameter cells on the surface of the mantle (Fig. 16b).

Mantle surface: The mantle surface appeared to be composed of one distinct layer of large-celled, thin-walled, interlocking pseudoparenchyma (Fig. 16c) similar to Agerer's (1995) type D. The mantle was 10–25 μm thick in cross-section.

T15 was very distinctive and not easily mistaken for other types because of the setiform hyphae/cystidia on the mantle surface. Setiform hyphae have been reported by Dominik (1959) on two types of ectomycorrhizas, one on spruce and another on poplar. However, the structure of the setae in T15 was not similar. The setiform hyphae on the mantle surface resembled those found on mycorrhizas formed by members of the Thelephoraceae (A. Taylor personal communication) but *Thelephora* mycorrhizas have a plectenchymatous mantle and hyphae bearing clamps, which T15 did not possess. The hyphae also closely resembled the strongly curved, thick-walled cystidia of *Chroogomphus* spp. ectomycorrhizas. However, members of the Gomphidia-

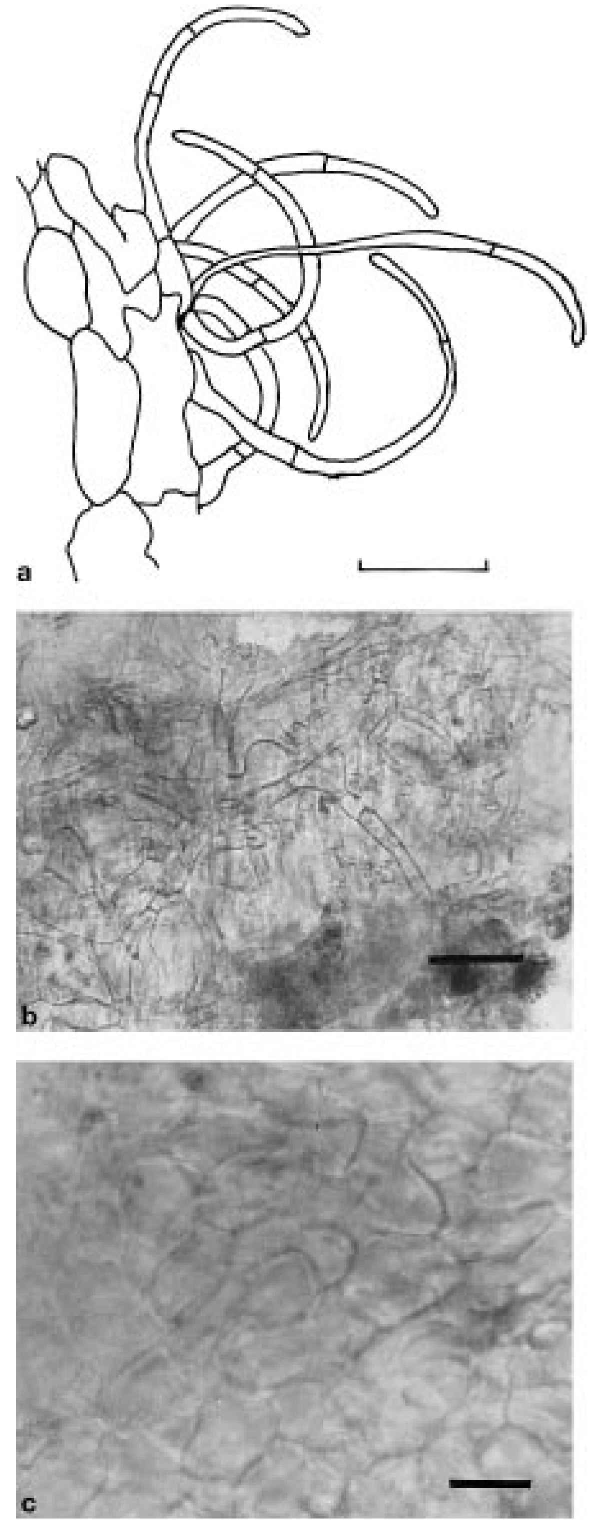


Fig. 16a–c Features of type T15, Tuberaceae or Russulaceae. **a** Curled, setae-like hyphae on the mantle surface; *bar* 20 μm. **b** Hyphae of large diameter at the bases of the curled setae-like hyphae on the mantle surface; *bar* 20 μm. **c** Plan view of the mantle composed of large-celled, thin-walled pseudoparenchyma; *bar* 10 μm

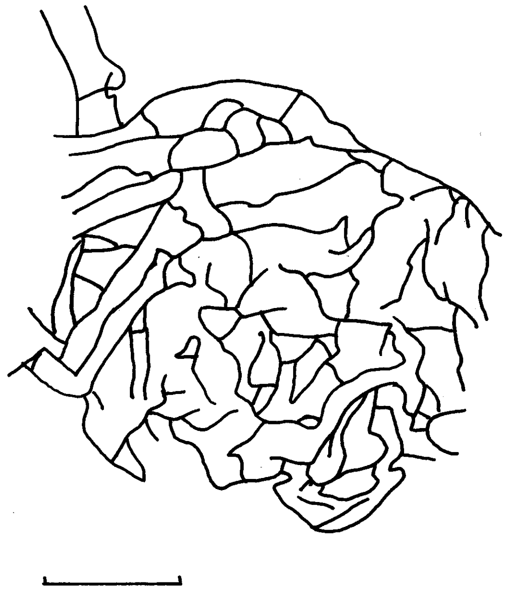


Fig. 17 Plan view of the mantle surface of type T16, Russulaceae, composed of large-celled tortuous pseudoparenchyma; *bar* 20 μm

ceae have so far been found only in the temperate northern hemisphere and in New Zealand, associated with pines (R. Watling unpublished data). Based on the mantle configuration, other possible associates are members of the Tuberaceae or the Russulaceae (Agerer 1995).

T16 – Russulaceae

Morphological characteristics: The dark yellow-brown to ochre mycorrhizas were pyramidally branched and sometimes almost coralloid with short, stubby and irregularly pinnate branches. The surface was velvety and there were no rhizomorphs.

Mantle edge: Smooth and compact. Emanating hyphae were up to 8 μm wide, hyaline yellow and bore clamp connections.

Mantle surface: Composed of a covering of net plectenchyma overlying a layer of large-celled (Fig. 17), tortuous pseudoparenchyma similar to Agerer's (1995) type Q. The inner mantle was composed of irregular pseudoparenchyma of smaller diameter than the cells of the surface. The mantle was 20–60 μm thick in cross-section.

The mantle features indicated that a member of the Russulaceae could be involved (Agerer 1995).

T17 – Russulaceae

Morphological characteristics: The mycorrhizas were monopodially pinnate with short, thick and felty

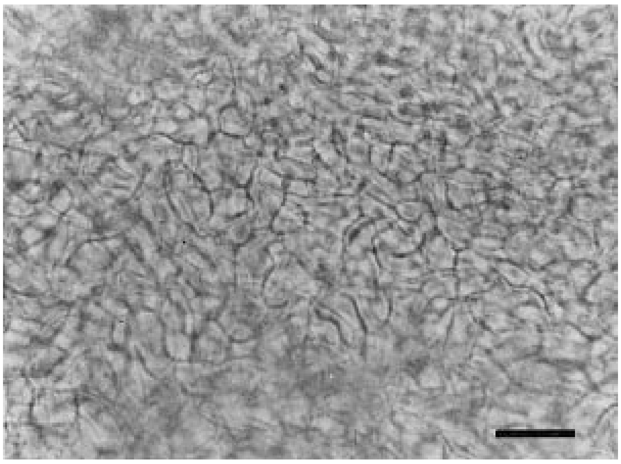


Fig. 18 Plan view of the mantle surface of type T17, Russulaceae, composed of non-interlocking pseudoparenchyma; *bar* 25 μm

branches. They were dirty white to cream and did not possess rhizomorphs.

Mantle edge: Compact but uneven. Emanating hyphae were hyaline and simple septate.

Mantle surface: Composed of non-interlocking pseudoparenchyma (Fig. 18) described as type H by Agerer (1995). The inner mantle was also composed of non-interlocking pseudoparenchyma but cells were of a smaller diameter than those of the surface layer. The mantle was 25–32 μm thick in cross-section.

The associated fungus was most probably a member of the Russulaceae (Agerer 1995).

T18 – Russula sp.

Morphological characteristics: The mycorrhizas were unramified to monopodial pinnate, furry and dark yellow-brown. There were no rhizomorphs.

Mantle edge: Compact but uneven. Emanating hyphae were hyaline and simple septate.

Mantle surface: Composed of irregularly shaped pseudoparenchyma (Fig. 19a) similar to Agerer's (1995) type H. There were numerous elongated cystidia (Fig. 19b) rising from bulbous bases on the mantle surface which gave the roots a furry appearance. The inner mantle was composed of non-interlocking, irregular pseudoparenchyma. The mantle was 10–35 μm thick in cross-section.

The surface cystidia were very distinctive and characteristic of that formed by *Russula* spp. as was the pseudoparenchymatous mantle with lobed cells (Agerer 1987–1990; Brand 1991; Agerer 1995). Agerer (1987–1990) described a similar mycorrhiza from *Larix decidua* associated with *Russula laricina,* while Taylor and Alexander (1989) described a similar mycorrhiza from *Picea sitchensis* associated with *Russula aeruginea.* A similar mycorrhiza was described by Becker (1983) on roots of *Shorea maxwelliana* and *Shorea leprosula* seedlings from Pasoh, Malaysia.

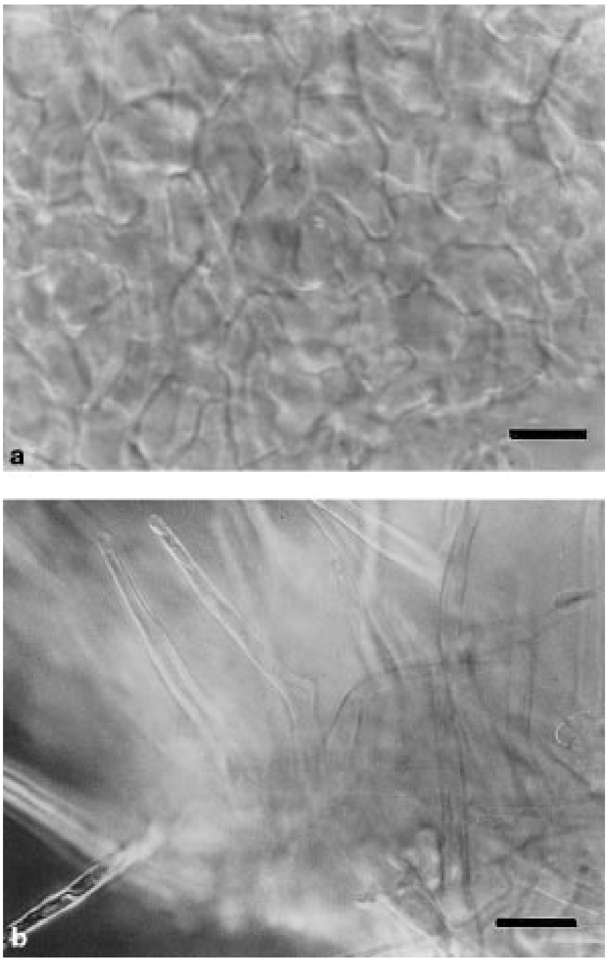


Fig. 19a, b Features of type T18, *Russula* sp. **a** Plan view of the mantle surface composed of irregularly shaped pseudoparenchyma; *bar* 10 μm. **b** Elongated cystidia on the mantle surface; *bar* 20 μm

T19 – Scleroderma sp.

Morphological characteristics: The mycorrhizas were twisted, monopodially branched and the branches short and hirsute. The mantle was dark brown but the whole root system was heavily covered by yellow emanating hyphae. There were numerous thick, yellow strands composed of undifferentiated hyphae.

Mantle edge: Loosely formed. Emanating hyphae were abundant, hyaline yellowish-green, simple septate and verrucose to rough.

Mantle surface: The surface of the mycorrhiza was covered by a loose network of simple septate, dichotomous-branching plectenchyma (Fig. 20a) overlying a

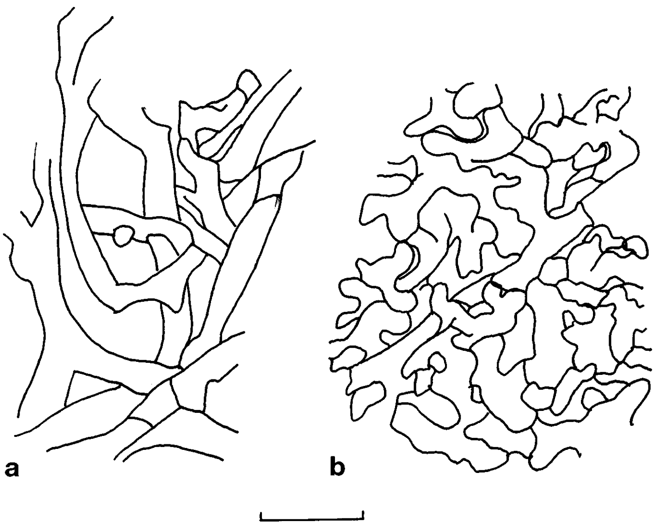


Fig. 20a, b Features of type T19, *Scleroderma* sp. **a** Loose network of simple septate plectenchyma on the mantle surface. **b** Mantle composed of elongated jigsaw puzzle-like pseudoparenchyma; *bar* 20 μm

mantle composed of elongated, jigsaw puzzle-like pseudoparenchyma of variable size (Fig. 20b). The mantle was 15–30 μm thick in cross-section.

T19 was easily recognised by its bright yellow emanating hyphae and strands. The bright yellow *Scleroderma sinnamariense* frequently observed under trees of *Dryobalanops aromatica* and *Neobalanocarpus heimii* in the FRIM compound (Watling and Lee 1995) is most probably the associated fungus. Fluffy, yellow emanating hyphae and strands from such fruit bodies have been found to be connected to dark brown mycorrhizas very similar to T19 (unpublished data). The hyphae from the fruit bodies also had the same characteristics as the emanating hyphae of T19. Moreover, undifferentiated strands are known to occur in ectomycorrhizas of *Scleroderma* spp. (Godbout and Fortin 1985).

T20 – Boletaceae

Morphological characteristics: The mycorrhizas were unramified to monopodially pinnate, the unramified branches usually bent and tortuous. The mycorrhizas were white to pale creamish with a transparent outer layer. There were no rhizomorphs.

Mantle edge: Loosely formed. Emanating hyphae were hyaline and simple septate. The surface of the mycorrhizas was covered by a transparent layer composed of hyaline, simple septate hyphae bearing clavate tips (Fig. 21a).

Mantle surface: Composed of plectenchyma (Fig. 21b), while the inner layer was composed of irregular pseudoparenchyma. The mantle was 4–18 μm thick in cross-section.

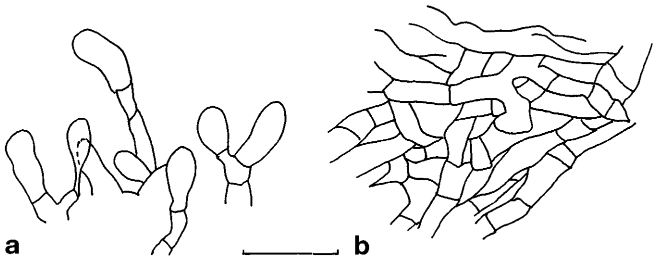


Fig. 21a, b Features of type T20, Boletaceae. **a** Clavate-tipped hyaline, simple septate hyphae borne on the surface of the mantle. **b** Plan view of the plectenchymatous mantle surface; *bar* 20 μm

T20 was readily distinguished by its small, white branches and the transparent, gel-like covering of clavate-tipped cells on the surface of the mycorrhizas. Godbout and Fortin (1985) described somewhat similar cystidium-like, multiseptate, sometimes branched hyphae on the surface of *Amanita* ectomycorrhizas which imparted a hoary appearance to the mantle surface. However, the surface of T20 mycorrhizas was not hoary. The hyphal arrangement of the mantle and the swollen hyphal tips indicate a member of the Boletaceae as the most probable associated fungus (Agerer 1987–1990, 1995). Members of the Boletaceae are common in Malaysian rainforests (Corner 1972, Watling and Lee 1995).

T21 – Tricholomataceae

Morphological characteristics: The mycorrhizas were monopodially pinnate, the branches short and twisted. They were coloured shades of dirty creamish to pale yellow. White, stringy undifferentiated hyphal stands were present.

Mantle edge: Smooth and compact with simple septate emanating hyphae.

Mantle surface: Composed of a reticulate network of long, loose, simple septate hyphae (Fig. 22a), with an inner layer of irregular pseudoparenchyma (Fig. 22b). The mantle was 15–30 μm thick in cross-section.

The mantle features of T21 closely resemble those described for mycorrhizas formed by *Tricholoma* spp. (Agerer 1987–1990, Brand 1991) and *Laccaria* spp. (Agerer 1987–1990; Ingleby et al. 1990). Species of *Laccaria* are commonly found in lowland dipterocarp forests (Watling and Lee 1995) and it is possible that a species of *Laccaria* was involved.

T22 – Boletaceae

Morphological characteristics: The mycorrhizas were irregularly pinnate, bent and tortuous, clumped, and pale creamish-yellow. There were no rhizomorphs.

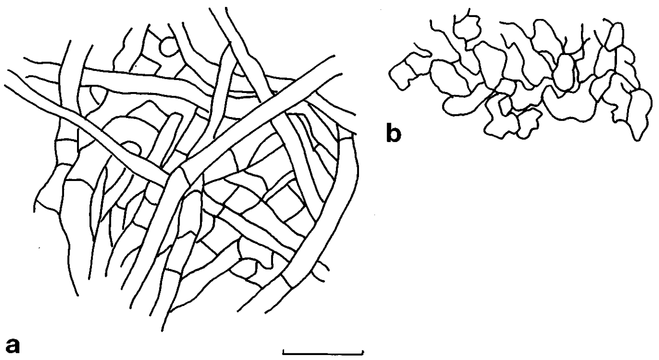


Fig. 22a, b Features of type T21, Tricholomataceae. **a** Reticulate network of long, loose simple septate hyphae on the mantle surface. **b** Underlying irregular pseudoparenchyma; *bar* 20 μm

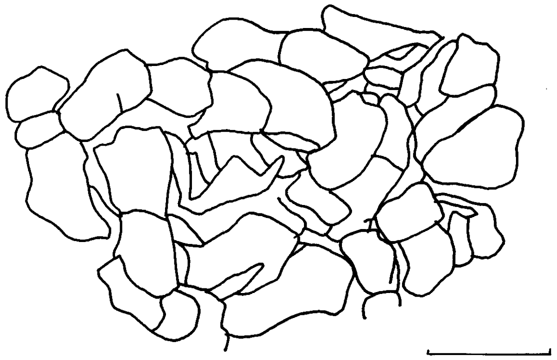


Fig. 23 Plan view of the mantle surface of type T22, Boletaceae, composed of large-celled irregular pseudoparenchyma; *bar* 20 μm

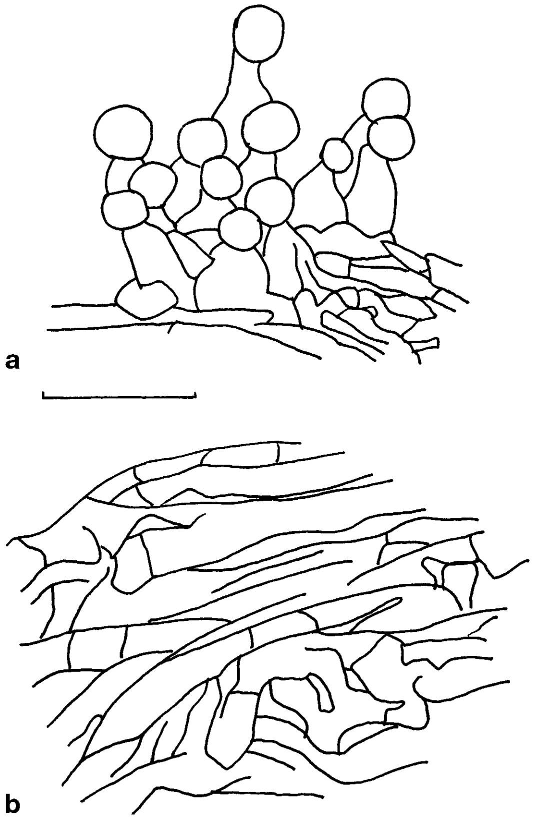


Fig. 24a, b Features of type T23, Russulaceae. **a** Rounded-tipped clavate cystidia(?) borne on the mantle surface. **b** Plan view of the mantle surface composed of elongated pseudoparenchyma; *bar* 20 μm

Mantle edge: Smooth and compact with abundant hyaline, simple septate emanating hyphae.

Mantle surface: Composed of large-celled, irregular, pseudoparenchyma (Fig. 23). The inner layer was composed of irregular pseudoparenchyma of smaller diameter than those of the surface. The mantle was 27–40 μm thick in cross-section.

The swollen hyphal cells in the mantle were similar to those described for *Leccinum scabrum* and *Boletus edulis* by Agerer (1987–1990). In view of the many species of the Boletaceae found in Malaysia (Corner 1972) and the similarities to mycorrhizas of the Boletaceae, it is highly likely that a member of the Boletaceae was associated.

T23 – Russulaceae

Morphological characteristics: The mycorrhizas were mostly monopodially pinnate with rough surfaces and ochre brown. There were no rhizomorphs.

Mantle edge: Compact but uneven and rough. Emanating hyphae were infrequent. The surface of the mantle was covered by clavate-protruding cells whose rounded tips were filled with an orange substance (Fig. 24a).

Mantle surface: Composed of elongated pseudoparenchyma (Fig. 24b). No inner layers were distinguishable. The mantle was 18–25 μm thick in cross-section.

The orange, round-tipped, clavate protrusions were very distinctive of T23. It was possible that these structures were protruding, stout and curved hyphal end cells which had been filled with oil droplets. A similar mantle type (=type I sensu Agerer 1995) is known to be associated with *Lactarius* ectomycorrhizas (Agerer et al. 1991; Brand 1991) and species of *Lactarius* are not uncommon in Malaysian rain forests (Watling and Lee 1995).

T24 – Tuberaceae

Morphological characteristics: The mycorrhizas were monopodially pinnate, with short branches borne at almost right angles to the main axis. They were dark brown to almost black, with a frosty surface. There were no rhizomorphs.

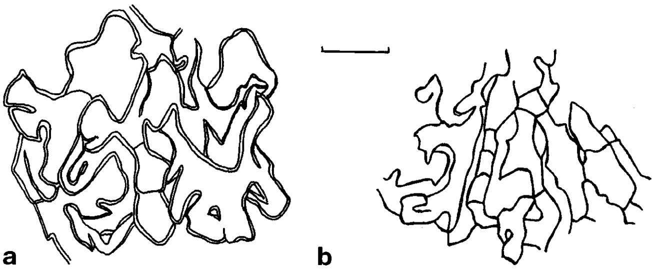


Fig. 25a, b Features of type T24, Tuberaceae. **a** Plan view of the mantle surface composed of thick-walled epidermoid pseudoparenchyma. **b** Inner mantle layer composed of thin-walled elongated net pseudoparenchyma; *bar* 20 μm

Mantle edge: Felty due to the presence of abundant emanating hyphae. The mantle bore setiform hyphae, with rounded tips, approximately 4.8 μm in diameter and up to 200 μm in length. The emanating hyphae were simple septate, thick-walled and rough to warty.

Mantle surface: Pseudoparenchymatous, composed of jigsaw puzzle-like, thick-walled epidermoid cells (Fig. 25a). The inner layer was composed of thin-walled, elongated net pseudoparenchyma (Fig. 25b). The mantle was 13–40 μm thick in cross-section.

Similar mycorrhizas were described by Berriman (1986) on seedling roots of *Shorea macroptera, Shorea lepidota* and *Shorea leprosula* and on roots of adult trees of *Shorea lepidota* from Pasoh, Malaysia. Becker (1983) also described a similar mycorrhiza on roots of *Shorea leprosula* seedlings from Pasoh, Malaysia. On the basis of the arrangement of its mantle hyphae and the coarse, thick-walled, simple septate emanating hyphae, the fungus associate of T24 was probably a member of the Ascomycotina. The mantle configuration of T24 closely resembled that of *Tuber* sp. mycorrhizas described by Agerer (1987–1990) and Ingleby et al. (1990).

Since these mycorrhizas were not synthesised, the identity of the fungal associates could only be based on morphological and anatomical features and comparisons with published descriptions. Twenty of the ectomycorrhizal types were found to be associated with members of the Basidiomycotina, two with members of the Ascomycotina, two with either members of the Ascomycotina (Geneaceae or Tuberaceae) or Russulaceae. Of the Basidiomycotina associates, 10 were placed in the Russulaceae, two in the Sclerodermataceae, two in the Boletaceae, one each in the Amanitaceae, Paxillaceae and the Tricholomataceae, while the remaining were *Riessia radicicola* Julich, *Riessiella* sp. and a type very closely resembling *Fagirhiza fusca* Brand (Table 1). Of those associated with the Ascomycotina, T12 closely resembled *Cenococcum geophilum,* at least externally, while T24 was very likely associated with a member of the Tuberaceae. The mantle features of T5 and T15 indicated associations with members of either the Ascomycotina or Russulaceae (Agerer 1995). If members of the Ascomycotina were involved, T5 could probably be associated with a fungus resembling *Genea hispidula* Berk. et Br. (Brand 1991) while T15 could probably be associated with a member of the Tuberaceae (Agerer 1995).

Putative ectomycorrhizal fungi

Data from our basidiome collections over 3 years show that species of *Russula* were the most common fungi fruiting under the canopy and in the vicinity of *Shorea leprosula* trees (Table 2). Of the 28 species of fungi recorded, 15 were members of the Russulaceae, 13 *Russula* spp. and two *Lactarius* spp. It was not surprising to find a predominance of Russulaceae associated with both the mycorrhizas and trees of *Shorea leprosula* as species of *Russula* are the most frequently encountered ectomycorrhizal fungi in the dipterocarp forests of Peninsular Malaysia (Watling and Lee 1995). Troups of *Russula virescens* are often found growing over a large area under the parent trees. Hong (1979) recorded unspecified numbers of species of *Russula* growing under *Shorea leprosula* trees in the FRIM arboretum. Identification of this group of fungi in the tropics, however, still remains problematical as many are new, undescribed species.

Members of other well-known ectomycorrhizal families such as the Amanitaceae, Boletaceae, Sclerodermataceae and species such as *Inocybe, Laccaria* and *Cantharellus* are also encountered in the dipterocarp forest although much less frequently and usually in much lower numbers than the Russulaceae.

In Malaysia many species of *Amanita* are found in mixed dipterocarp forests during the fungal fruiting season. *Amanita virginea* has been recorded from Singapore, Malaya, Sumatra and Java (Corner and Bas 1962) and it has now been located at both Kepong and Pasoh associated with *Shorea leprosula* and other dipterocarp hosts. *Amanita tjibodensis* was described from Java by Corner and Bas (1962) and has often been found in the FRIM grounds at Kepong associated with a variety of dipterocarp hosts including *Shorea leprosula.*

Boletus aureomycelinus is considered uncommon in Malaysia by Corner (1972) but is a feature of the grounds of the Forest Research Institute Malaysia at Kepong, where it is associated with a range of dipterocarp hosts (Watling and Lee 1995), including *Shorea leprosula. Boletus frians* which is common in Pasoh is known from the Malay peninsula, Singapore and Borneo (Corner 1972). *Pulveroboletus icterinus* which was found in Pasoh is known from several places in Peninsular Malaysia (Corner 1972; Watling and Lee 1995) and, more recently, the Philippines (Watling unpubl.). *Rubinoboletus ballouii* is a very common and variable bolete found in both Kepong and Pasoh (Watling 1994). It is known to be distributed in Malaysia, China,

Table 2 Basidiomes collected from under the canopy and in the vicinity of *Shorea leprosula* trees in FRIM, Kepong (*K*), Pasoh Forest Reserve, Negeri Sembilan (*P*) and in Sungai Lalang Forest Reserve, Selangor (*S*), and their herbarium numbers deposited: *Wat.* Royal Botanic Garden, Edinburgh, *S-S* FRIM, Kepong

Fungus species	Location collected	Herbarium number
Amanita virginea Mass.	K, P	S-S 973, Wat. 26729
Amanita tjibodensis Boedijn	K	S-S 55, 1155, Wat. 24878, 24884,24885
Boletus aureomycelinus Pat. & Baker	K	S-S 574, 583, 585, 1604
Cantharellus sp.	S	Wat. 25081
Craterellus cornucopioides (L.: Fr.) Pers.	P	Wat. 26355, 26356, 26742
Hydnum repandum L. ex Fr.	P	Wat. 26590-3
Laccaria vinaceoavellanea Hongo	K, P	S-S 1195, Wat. 24515
Lactarius hygrophoroides cf. sumstinei Peck	P	Wat. 24798
Pulveroboletus icterinus (Pat. & Baker) Watling	P	Wat. 24620
Russula cf. *castanopsidis* Hongo	K, P	S-S 1381, Wat. 25061
Russula alboreolata Hongo	K	S-S 857
Russula cf. *cyanoxantha* (Schaeff.) Fr.	K, P	S-S 1344, Wat. 25060
Russula cf. *crustosa* Peck	K	S-S 1345
Russula eburneoareolata Hongo	K	S-S 1152, 1154
Russula sect. Foetentinae	K, P	S-S 1562, Wat. 151A*
Russula sect. Heterophyllinae	P	Wat. 24536
Russula cf. *pectinata* (Bull.) Fr. agg.	S	Wat. 24501
Russula virescens (Schaeff.)	K, P	S-S 1481, 1629, Wat. 24453
Russula sect. Ingratae cf. *senecis* Imai	K	Wat. 24519
Russula sect. Nigricantinae	K	S-S 1628
Russula sect. Plorantinae	K, P	S-S 716, 1231, Wat. 141A*
Russula sp. (grey pileus)	P	Wat. 25064, 25067

[a] Field collection numbers, specimens yet to be processed

North America and Australia (Corner 1972) and was recently recorded from the Philippines (Watling 1994). *Strobilomyces velutipes* has been found in Kepong associated with the dipterocarp *Dryobalanops aromatica* and was associated with *Shorea leprosula* in Pasoh. This fungus was originally described from India and is known to occur from Australia to the Himalayas and Africa (Corner 1972; Watling 1994).

Corner (1960) states that while many species of *Cantharellus* await discovery in the tropics, *Craterellus cornucopioides* is undoubtedly widespread, with several varieties needing study. An unidentified species of *Hydnum* was collected from under *Shorea leprosula* in the northern state of Kedah and several collections of *Hydnum repandum* have since been made from Pasoh where *Shorea leprosula* is common. However, these collections were not specifically associated with *Shorea leprosula.* While we did not find *Laccaria laccata,* we found *Laccaria vinaceoavellanea* associated with *Shorea leprosula* and in lowland forests where *Shorea leprosula* is a common tree. *Inocybe sphaerospora* occurs with a range of dipterocarp hosts in Kepong (Watling and Lee 1995) and is known from Japan, Papua New Guinea and Singapore (Horak 1980). *Scleroderma* spp. are commonly encountered in lowland dipterocarp forests but none were specifically associated with *Shorea leprosula.*

Lactarius hygrophoroides cf. *sumstenei* which was found in Pasoh was described from North America, and ranges from Maine to Florida and Texas, and north to Canada. It is a complex of closely related taxa on which much more work is required. It is known from Japan and Europe and both deciduous and coniferous trees are considered putative hosts. While the identity of the *Lactarius* sect. Plinthogali found in association with *Shorea leprosula* in Pasoh has to be determined, another member of the same group, *Lactarius gerardii* has been found in both Kepong (Watling and Lee 1995) and Pasoh, although not in association with *Shorea leprosula. Russula alboareolata* which fruits frequently and abundantly in Kepong was described from Japan, where it occurred commonly in evergreen oak woods (Hongo 1979). The closely related *Russula eburneoareolata* which also occurs frequently at Kepong was first described from a *Castanopsis* forest in Papua New Guinea and recorded as a species new to Japan in deciduous oak forests and under *Abies homolepsis* (Hongo 1979). *Russula* cf. *castanopsidis,* which was found fruiting in abundance at Kepong under both *Shorea leprosula* and other dipterocarp hosts, was described from broadleaved forests in Japan chiefly associated with *Castanopsis* and *Quercus* (Hongo 1973). This and the fact that the pileipellis is slightly different, make us hesitant at the moment to consider the Pasoh collections as the species. *Russula cyanoxantha* and *Russula virescens* are widespread in Asia, North America and Europe. Macroscopically our collections are typical of European collections in all ways and this has been confirmed by microscopic analysis. There are many members of the Russula sect. Ingratae in Peninsular Malaysia; the two associated with *Shorea leprosula, Russula* cf. *pectinala* and *Russula* cf. *senecis* are closest to the Japanese fungi. In Asia *Russula senecis* is known from both deciduous and coniferous woodland while *Russula pectinata* is known from deciduous woods (Hongo 1960; Bi et al. 1993). The Malaysian collections of *Russula* *senecis* differ slightly in details of the pileipellis but the basidiospore morphology is in keeping with this disposition.

In Indonesia, ectomycorrhizas were successfully synthesised on *Shorea leprosula* seedlings and cuttings using chopped basidiomes of *Scleroderma columnare* Pat. (Hadi et al. 1991; Omon et al. 1994), *Laccaria laccata* (Scop.: fr.) Cooke and *Amanita* sp. (Omon et al., 1994) There is, however, no information regarding the natural occurrence of these fungi with *Shorea leprosula* trees in Indonesian forests.

We hope in future to be able to identify the ectomycorrhizal fungal partners more accurately as our knowledge of tropical macrofungal taxonomy and ecology improves.

Acknowledgements The results presented here are part of a larger study on the ecology of dipterocarp ectomycorrhizas jointly funded by the Forest Research Institute Malaysia (FRIM) and by a grant from the Commission for the European Community (CEC) under contract TS2*-0298 M(EDB).

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