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CHARACTERISATION AND IDENTIFICATION OF ECTOMYCORRHIZAE OF *RUSSULA* (RUSSULACEAE: BASIDIOMYCOTA) ASSOCIATED WITH *SHOREA ROBUSTA*

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The tropical moist deciduous forests of India are largely dominated by the dipterocarp tree *Shorea robusta* (also known as sal). Ectomycorrhizal (ECM) fungi form symbiotic association with sal trees and play an important role in the stability of the sal forests. A study was carried out to determine the occurrence and distribution of ECM fungi in tropical sal forests at the Shiwalik mountain range, north-west India. The genus *Russula* was the dominant fungi. In this paper the morphoanatomical details of mycorrhizal roots of *S. robusta* associated with three *Russula* species, namely, *R. cremeoavallanea, R. romagnesiana* and *R. nigricans* were investigated for the first time. The ECM colonised roots were distinguished by differences in the shape and colour of mycorrhizal system, surface texture, shape and size of cystidia, cell shape of mantle, as well as the differing chemical reactions. ECM roots of sal associated with *R. cremeoavallanea* were mostly greyish brown and have almost plectenchymatous outer mantle layers having subcylindrical to awl-shaped cystidia. Ectomycorrhizal roots of *R. romagnesiana* were reddish brown with silvery patches, and have purely plectenchymatous outer mantle layers having obpyriform to obclavate cystidia. *Russula nigricans*-associated ectomycorrhizal roots have greyish brown to black mycorrhizal system and have almost pseudoparenchymatous dark brown mantle with capitate cystidial elements.

Keywords: Dipterocarpaceae, Hartig net, sporocarps, rahizomorphs, sal, Shiwalik

INTRODUCTION

Shorea robusta which is commonly known as sal, is an important source of hardwood timber tree in India. It is a major constituent of moist deciduous broad-leaved tropical forests in India. The species is dominantly distributed on the plains, lower foothills and valleys of the Himalayas. Mycorrhizal fungi are ubiquitous and essential components of most ecosystems and are considered key ecological factors in governing and maintaining the terrestrial ecosystem (Wang et al. 2017). Ectomycorrhizal (ECM) associations are also considered key factors for the survival and growth of sal seedlings and trees by supplying nutrients to host plants, particularly immobile nitrogen and phosphorus (Tapwal et al. 2015).

Based on surveys of sporocarps, sal trees have been reported to be putatively associated with species of various fungal genera such as *Russula, Boletus, Agaricus, Amanita, Lactarius, Laccaria, Pisolithus, Suillus* and *Cantharellus* (Natarajan et al. 2005, Tapwal et al. 2013). Many ECM genera have been proposed, but in most of the studies, evidence for hypothesised ECM species is lacking.

The genus *Russula* is one of the highly diverse ECM groups in Agaricomycetes and plays a critical role in maintaining forest ecosystems and biodiversity (Henkel et al. 2011, Corrales et al. 2016). The dominance of *Russula* in low nutrient soil in terrestrial ecosystem has been linked to its unique role in nutrient uptake from the soil (Malysheva et al. 2016, Uesugi et al. 2016). To date, approximately 1100 *Russula* species have been reported worldwide (Kirk 2014) and distributed across a wide range of habitats from the tropics to arctic zones. In an extensive study of ECM fungi of *S. leprosula* occurring in Malaysia, Lee et al. (1997) reported 28 ECM fungi of which, 15 were members of the genus *Russula*. *Russula* was the dominant component of ECM communities associated with *Castanopsis fargessii* in subtropical evergreen broad leaved forest (Wang et al. 2011). Corrales et al. (2016) documented *Russula* in association with *Oreomunnea mexicana* (Juglandaceae) in a neotropical montane forest in western Panama. Thus, *Russula* species are important members of many well studied ECM fungal communities worldwide, indicating that this genus plays significant role in sustaining the forest ecosystem.

Russula is represented by about 158 taxa from India (Sharma et al. 2018). In India, however, information on native ECM fungi has been based on the occurrence of fungi fruiting under putative ECM trees without confirming the actual ECM association below ground. Hence, most native ECM hosts and their ECM fungal assemblages have not been studied and described. However, we also lack information on the morphoanatomic features of ectomycorrhizae, with only few available descriptions (Mohan et al. 1993a, b, c, Kumar & Atri 2016). ECM fungi are poorly studied in tropical sal forest compared with other forests in India (Tapwal et al. 2013). Of the total ECM descriptions published so far only 13 descriptions are available for different *Shorea* spp. (Roman et al. 2005, Agerer & Rambold 2004–2016, Rinaldi et al. 2008) with only two descriptions available for *S. robusta* (Kumar & Atri 2016). Hence, the present study was taken up to characterise and identify the ECM diversity of *S. robusta.*

In the present study, sporocarps and their ECM colonised roots were collected by tracing the hyphal or rhizomorphs connections in association with *S. robusta* from pure sal forests. Tracing the mycelial or rhizomorphs connections in association with fruit body and ECM colonised roots is the most reliable way of assessing ECM status in the field (Agerer 2006). The macroscopic and microscopic details of each investigated taxa was worked out and identified up to species level. The aim of this paper is to characterise the ectomycorrhizae of *Russula cremeoavallanea, R. romagnesiana* and *R. nigricans* collected in their natural habitat associated with the roots of *S. robusta.* In this study, the evidence of symbiotic association between the host and the fungal species were provided.

MATERIALS AND METHODS

Study area

Area selected for present investigation is sal forests of Shiwalik mountain range in north-west India, which is the geologically lowest and youngest mountain range of Himalaya. Dipterocarp tree *S. robusta* purely dominates the sal forests. The average elevation of the area is 400–1500 m and vegetation of the area is typical of tropical moist deciduous forests (Champion & Seth 1968). The monsoon arrives at the beginning of July and extends up to October, with average annual rainfall 1386 mm.

Sampling, identification and characterisation

ECM root tips and all epigeous sporocarps of putative ECM were collected from different sites in pure sal forests at about 700 m above sea level during the rainy season (July till October) in 2013 and 2015. The *R. cremeoavallanea, R. romagnesiana* and *R. nigricans* and their ECM colonised roots were collected by tracing the hyphal connections between *S. robusta* roots and sporocarps from Malsi (Uttrakhand), Tokiyon (Himachal Pradesh) and Asarodi range (Uttrakhand). Spore print of each specimen was taken. After noting down the morphological characters on the field key (Atri et al. 2005), small bits of the sporocarps were preserved in liquid preservative (25 mL rectified alcohol (95%) + 5 mL formalin (37%) + 70 mL distilled water) for studying the microscopic characters by adopting the standard procedures (Singer 1986, Atri & Saini 2000). The rest of the sporocarps were air dried at 40–45 °C in a drier specially designed for drying mushroom specimens (Atri et al. 2005) and packed in cellophane packets for permanent preservation in the herbarium. Macroscopic and microscopic details of each investigated taxa was worked out as per standard methodology (Singer 1986, Atri & Saini 2000) and identified up to species level using standard literature (Romagnesi 1967).

Mycorrhizal roots underneath sporocarps were collected and wrapped in polythene bags and brought to the laboratory for further analysis. The collected roots were first gently washed with flowing tap water on a 250 μm mesh to remove soil and attached debris. Before morphological characterisation ECM samples were submerged in distilled water in a Petri dish. Morphological characterisation of ECM was performed under a stereomicroscope, photographed and described by careful examination following Agerer (1987–2012) and Agerer and Rambold (2004–2016) and compared with other available descriptions of

Russula ectomycorrhizae. The mycorrhizal roots were fixed in FAA (5 mL formalin (37%) + 5 mL acetic acid (100%) + 90 mL alcohol (50%)) for anatomical characterisation. Confirmation of ECM colonisation was done by preparation of cotton blue stained semi-thin sections of ECM roots. Cross-section and longitudinal section of ECM were examined and drawn under a compound microscope and photographed under digital microscope for the presence of mantle, Hartig net, hyphal and rhizomorphs characteristics. The colour terminology used was that of Kornerup and Wanscher (1978). Microchemical reactions were performed using $FeSO_4$, sulphovanillin, ethanol, potassium hydroxide (KOH), Melzer's reagent and cotton blue.

RESULTS

Description of ectomycorrhizae: *Russula cremeoavallanea* + *Shorea robusta*

Morphological characters: Mycorrhizal system monopodial pinnate to irregularly pinnate, dichotomous-like with one order of ramification, up to 6 mm long; main axes 0.2–0.3 mm in diameter (Figures 1 and 2). Unramified ends slightly bent to straight, cylindrical, 0.2–2.0 mm in length and 0.1–0.3 mm in diameter, tips rounded. Surface of unramified ends smooth, occasionally with soil particles, younger mycorrhizae greyish brown (5D5 according to Kornerup and Wanscher (1978)) and older, reddish brown (5D8), unchanging, not secreting latex or any other fluid when injured; mantle not transparent; tip shows the same colour as rest of the mycorrhiza. Rhizomorphs present, 15–30 µm thick. Emanating hyphae rarely observed; cystidia present, sclerotia not observed.

Anatomical characters of mantle in plan view: Mantle thickness 22–30 (exceptional upper reading was 34) µm, differentiated into outer and inner mantle layers. Outer mantle layer 16–20 (24) µm thick, more or less plectenchymatous, compactly arranged, representing type D (Agerer 1987–2012, Agerer & Rambold 2004–2016). Hyphal cells 3–5 µm in diameter, compactly arranged, smooth, hyaline, septate, thin walled (0.5 µm), constricted at septa, clampless; septa as thick as hyphal wall. Inner mantle layer 6.5–8.2 (9.8) µm, pseudoparenchymatous representing type K (Agerer 1987–2012, Agerer & Rambold 2004–2016); hyphal cells colourless, hyaline, thin walled, variable in shape measuring 1.6–5.0 µm tangentially and 1.6–3.5 µm radially.

Anatomical characters of emanating elements: Rhizomorphs present, infrequent, rounded, oblique and with rough surface having extraradical hyphae emanating from surface. Hyphae almost equal in diameter (2.5 µm), thick walled, septate, constricted at septa, without clamp, septa as thick as hyphal wall (up to 1 µm). Cystidia 24–57 (65) × 3–5 µm, present on the outer mantle layer, the most distinct and often infrequent and type 1 (Agerer 1987–2012, Agerer & Rambold 2004–2016), subcylindrical to awl shaped with almost acute apex and swollen or rounded base (Agerer 1987–2012, Agerer & Rambold 2004–2016), hyaline, smooth, thick walled (up to 0.8 µm), aseptate to septate without clamp.

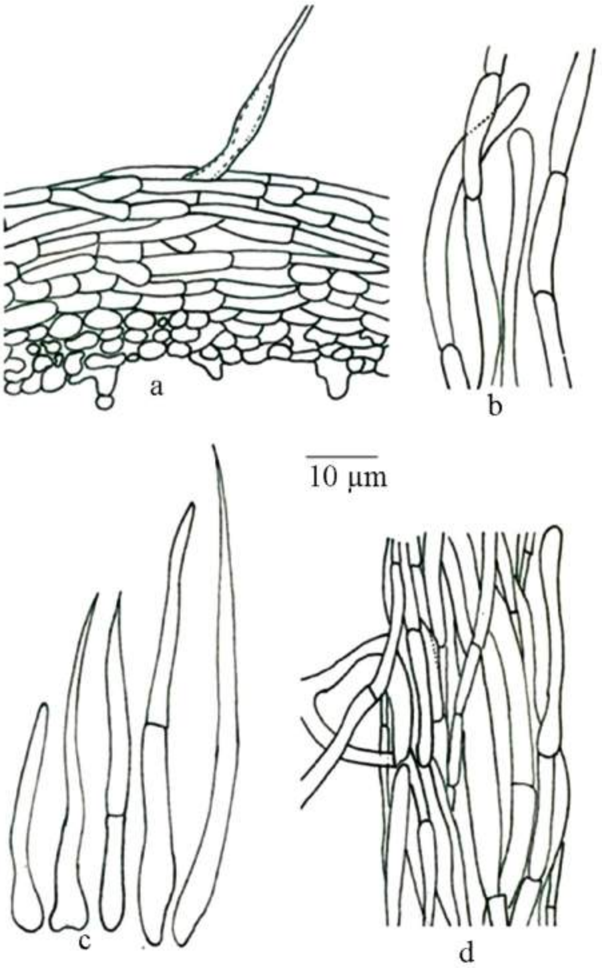

Figure 1 *Russula cremeoavallanea* + *Shorea robusta*; (a) mantle, (b) emanating hyphae, (c) cystidial elements and (d) rhizomorphs

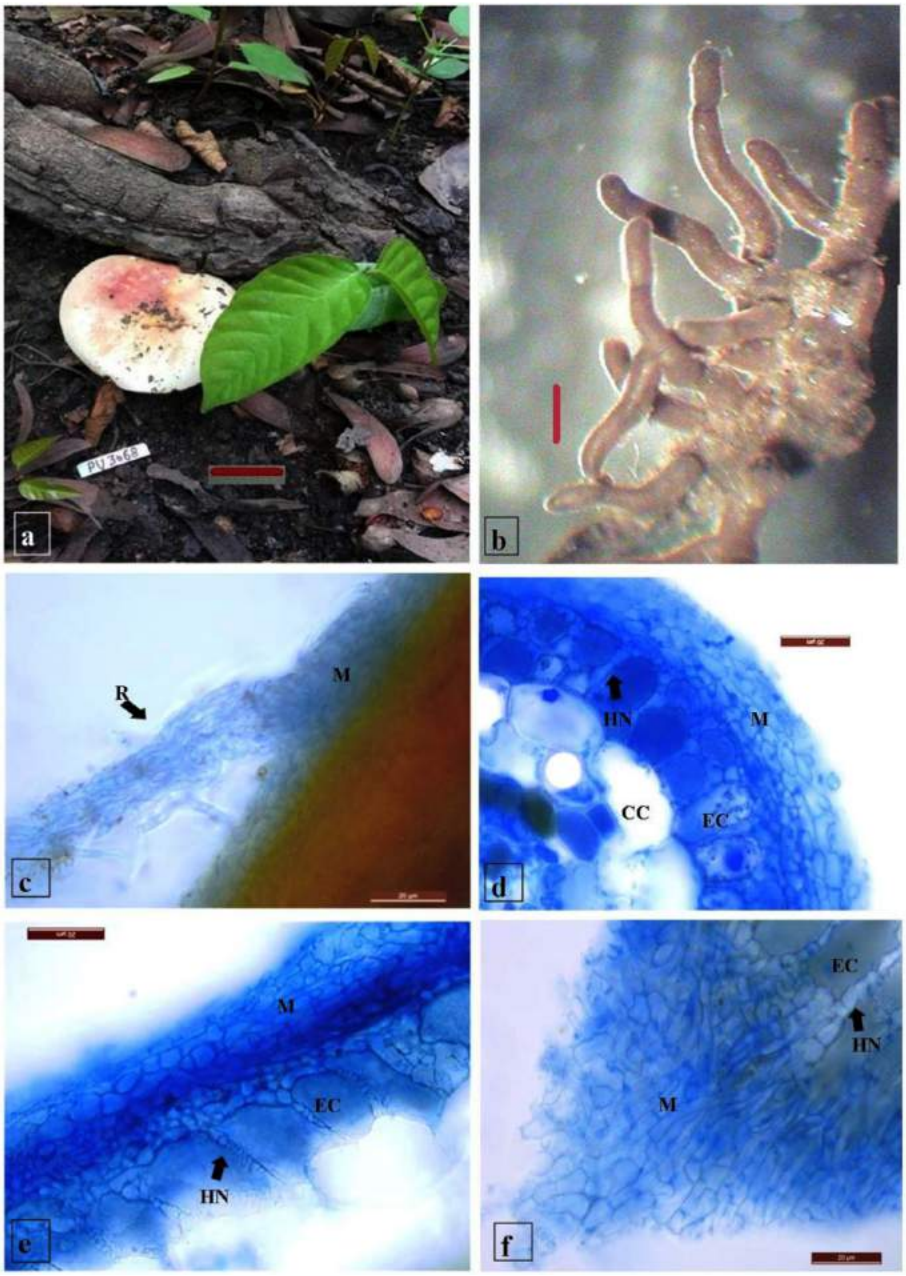

Figure 2 *Russula cremeoavallanea* + *Shorea robusta*; (a) carpophore in association with *Shorea robusta* root and seedlings, (b) mycorrhizal system, (c) surface view of unramified end showing rhizomorphs in connection with mantle, (d) cross-section of ectomycorrhizal root showing mantle (M) and Hartig net (HN), (e) longitudinal section of ectomycorrhizae showing mantle and radially elongated epidermal cell (EC) with Hartig net (HN) and (f) longitudinal section of root tip showing plectenchymatous mantle (M); scale bar a = 3 cm, b = 1 mm

Anatomical characters in longitudinal section: Mantle 25–30 (35) µm, differentiated into outer and inner mantle layer. Outer mantle layer 16.3–24.5 µm, more or less plectenchymatous, compact, representing type D (Agerer 1987–2012, Agerer & Rambold 2004–2016), hyphae 3–5 µm in diameter. without any content and clamp connection. Inner mantle layer 6.5–8.2 (9.8) µm, pseudoparenchymatous. Hyphal cells 1.6–5.0 µm tangentially and 1.6–3.5 µm radially. Hartig net one cell deep, the Hartig net palmetti type with one row of 3–8 µm radially and 3.5 tangentially roundish to cylindrical hyphal cells and is restricted to the anticlinal walls of the cortex cells (paraepidermal). Root tip mantle much thicker, up to 81.5 µm, different from rest of the mantle, plectenchymatous, having 3–4 µm interwoven septate hyaline hyphal cells, hyphae rather irregularly arranged and no special pattern discernible representing type B (Agerer 1987–2012, Agerer & Rambold 2004–2016). Hartig net also paraepidermal at very root tip with one row of roundish cells measuring 3.0–9.8 µm tangentially 3–5 µm radially. Hartig net cells have larger diameter at root tip as compared with the rest of the Hartig net. Epidermal cells become radially elongated to increase the area available for the Hartig net, 19.6–26.0 (29) × 9.8–13.0 µm, tangentially oval to elliptic or cylindrical, and oriented obliquely. Tannin cells not observed.

Colour reactions with different reagents: $FeSO_4$: n. r. (no reaction); sulphovanillin: brown, ethanol (70%): n. r., KOH (10%): n. r., Melzer: light yellow, cotton blue: cell wall blue.

Description of ectomycorrhizae: *Russula romagnesiana* + *Shorea robusta*

Morphological characters: Mycorrhizal system monopodial pinnate with one order of ramification, 2.5–4.8 (5.6) mm long; main axes 0.3–0.4 mm in diameter (Figures 3 and 4). Unramified ends slightly bent, cylindrical, 0.4–1.6 (2) mm in length and 0.1–0.2 (0.3) mm in diameter, tips rounded. Surface of unramified ends not smooth, densely cottony occasionally with soil particles, younger mycorrhizae white or silvery (1A1) and older greyish brown (5D5), unchanging, not secreting latex or any other fluid when injured. Mantle not transparent; hydrophobicity absent, tip shows the same colour as rest of the mycorrhiza. Rhizomorphs present, 16–32 µm thick. Emanating hyphae rarely observed. Cystidia present. Sclerotia not observed.

Anatomical characters of mantle in plan view: Mantle thickness 32.6–48.9 (53.8) µm, differentiated into outer mantle layer and inner mantle layer. Outer mantle layer 24.5–44.0 (47) µm, plectenchymatous, compactly arranged, representing type D (Agerer 1987–2012, Agerer & Rambold 2004–2016). Hyphal cells 1.6–3.5 (4.8) µm in diameter, compactly arranged, smooth, hyaline, septate, thick walled (0.8 µm), constricted at septa, clampless; septa as thick as hyphal wall. Inner mantle layer 6.5–8.2 (9.8) µm, pseudoparenchymatous representing type K (Agerer 1987–2012, Agerer & Rambold 2004–2016), hyphal cells granulated with light yellow content, thin walled, variable in shape measuring 3.3–5.0 (6.5) µm tangentially and 1.6–3.2 µm radially.

Anatomical characters of emanating elements: Rhizomorphs present, frequent, rounded, oblique and with rough surface having extraradical hyphae emanating from surface; hyphae almost equal in diameter (2.5–4.0 µm), thick walled (up to 1 µm), septate, constricted at septa, without clamp, septa as thick as hyphal wall. Emanating hyphae 3.5–4.5 µm, frequent, thick walled (1.2 µm) hyaline, septate without clamp slightly constricted at septa. Cystidia 24.5–57.0 (65.2) × 3.3–5.0 µm, present on the outer mantle layer, the most distinct and often frequent with type 1 (Agerer 1987–2012, Agerer & Rambold 2004–2016), bottle shaped with almost straight neck and swollen or rounded base to obpyriform, obclavate, representing type B (Agerer 1987–2012, Agerer & Rambold 2004–2016) to even ampulliform, representing type F (Agerer 1987–2012, Agerer & Rambold

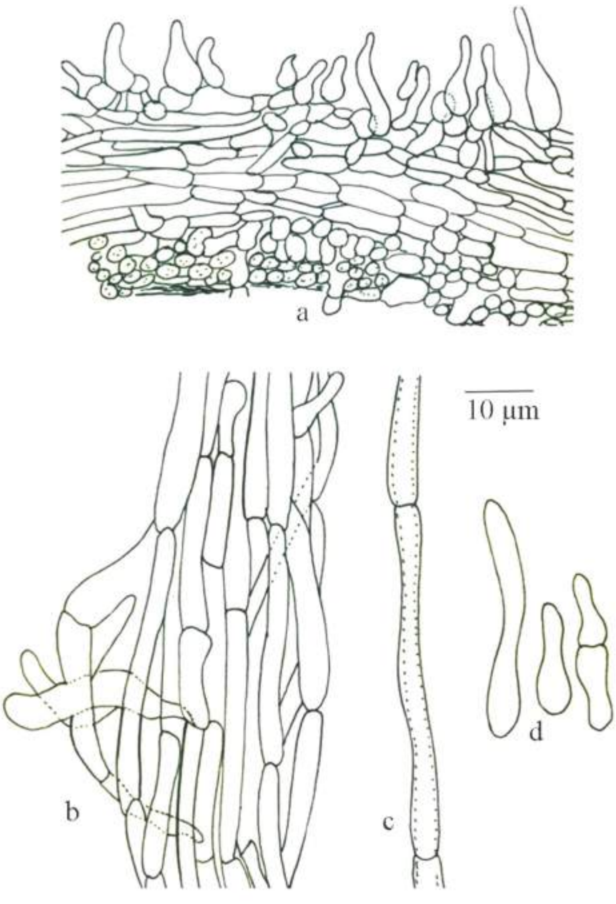

Figure 3 *Russula romagnesiana* + *Shorea robusta*; (a) mantle, (b) rhizomorphs, (c) emanating hyphae and (d) cystidial elements

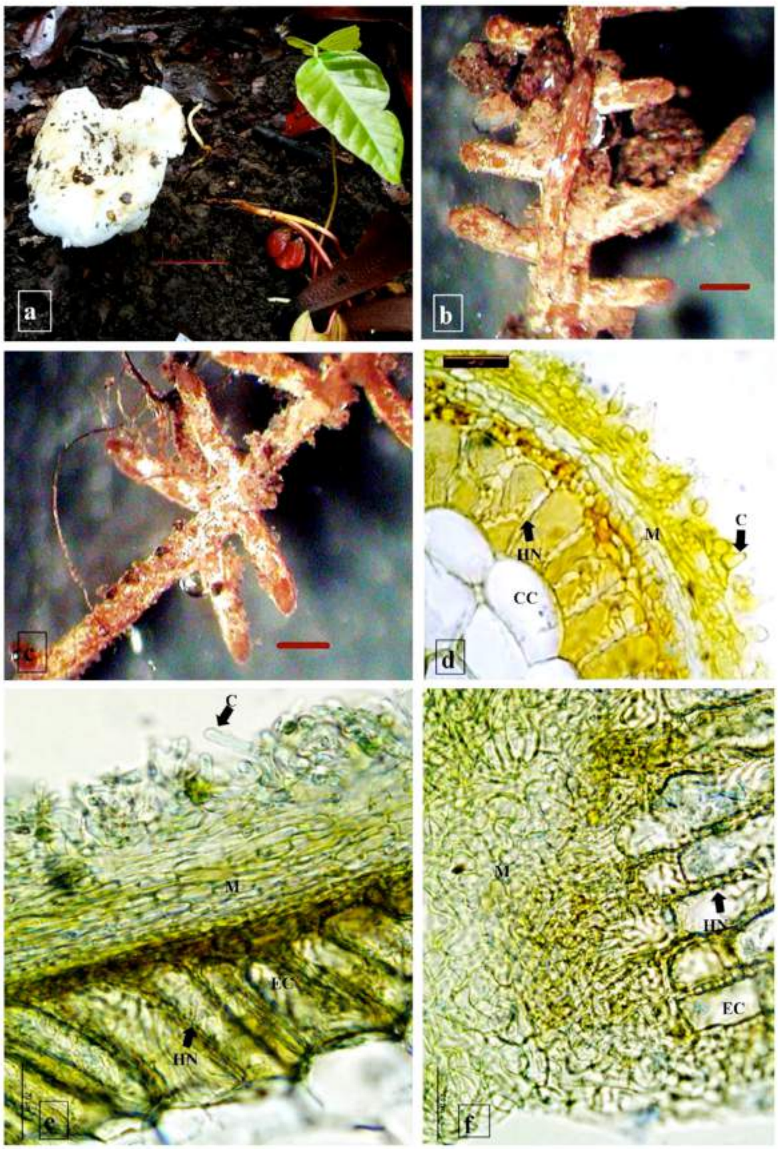

Figure 4 *Russula romagnesiana* + *Shorea robusta*; (a) carpophore in association with *Shorea robusta* root and seedlings, (b and c) mycorrhizal system, (d) cross-section (TS) of ectomycorrhizal root showing mantle (M) and Hartig net (HN), (e) longitudinal section of ectomycorrhiza showing mantle and radially elongated epidermal cell (EC) with Hartig net (HN) and (f) longitudinal section of root tip showing pseudoparenchymatous mantle (M); scale bar a = 3 cm, b–c = 1 mm

2004–2016), with oily droplets which do not stain in sulphovanillin, smooth, thick walled (up to 0.1 µm), aseptate to septate without clamp.

Anatomical characters in longitudinal section: Mantle 39–48 (52) µm, differentiated into outer and inner mantle layer. Outer mantle layer 29.5–34.5 (40.8) µm thick, plectenchymatous with broad streaks of more or less parallel hyphae, compact, slightly gelatinised representing type D (Agerer 1987–2012, Agerer & Rambold 2004–2016), hyphae 3–5 µm in diameter without any content and clamp connection. Inner mantle layer 6.5–9.8 (11) µm, pseudoparenchymatous. Hyphal cells 1.6–5.0 µm tangentially and 1.6–3.5 µm radially, cells ovoid, cylindric to round in shape. Hartig net one cell deep, palmetti type with one row of 1.6–8.0 (9.8) µm radially and 1.6–4.8 tangentially roundish to cylindrical hyphal cells and is restricted to the anticlinal walls of the cortex cells (paraepidermal). Root tip mantle up to 100 µm thick, different from rest of the mantle, plectenchymatous, having 4–5 µm interwoven, septate, hyaline hyphal cells, hyphae rather irregularly arranged and no special pattern discernible, representing type B (Agerer 1987–2012, Agerer & Rambold 2004–

2016). Hartig net paraepidermal at very root tip. Epidermal cells radially elongated 29–35 × 5–8 µm, tangentially oval to elliptic or cylindrical, and oriented obliquely. Tannin cells not observed.

Colour reactions with different reagents: $FeSO_4$: white and silvery mycorrhizae changes to pink; sulphovanillin: n. r., ethanol (70%): n. r., KOH (10%): n. r., Melzer: pale yellow; cotton blue: cell walls blue.

Description of ectomycorrhizae: *Russula nigricans* + *Shorea robusta*

Morphological characters: Mycorrhizal system irregularly pinnate with 0–1 order of ramification, up to 0.5–4.0 mm long (Figures 5 and 6). Main axes 0.3–0.4 mm in diameter. Unramified ends straight, cylindrical, 0.5–1.6 mm in length and 0.2–0.5 mm in diameter, tips rounded to pointed. Surface of unramified ends not smooth, loosely cottony, younger mycorrhizae reddish brown to greyish brown, and older dark brown to black, mycorrhizae changes to black on bruising, not secreting latex or any other fluid when injured. Mantle not transparent, mantle hydrophobicity absent, root tip rounded, straight, not swollen, mostly black, mantle carbonisation absent. Rhizomorphs not observed. Emanating hyphae present, not specifically distributed, present throughout the surface except at root tip. Cystidia present. Sclerotia not observed.

Anatomical characters of mantle in plan view: Mantle thickness 21–29 (32) µm, differentiated into outer mantle layer and inner mantle layer. Outer mantle layer 10–16 µm thick, almost pseudoparenchymatous, giving rise to abundant emanating hyphae and cystidia, compactly arranged, representing type D (Agerer 1987–2012, Agerer & Rambold 2004–2016). Hyphal cells 6.5–13.5 µm tangentially and 5–8 µm radially, smooth, granulated with light brown content as observed in sporophore hyphal cells, septate, thick walled (0.8 µm), not constricted at septa, clampless; septa as thick as hyphal wall. Inner mantle layer 9–14 µm, pseudoparenchymatous representing type K (Agerer 1987–2012, Agerer & Rambold 2004–2016), hyphal cells granulated, thin walled, variable in shape measuring 4–11 µm tangentially and 2–4 µm radially.

Anatomical characters of emanating elements: Rhizomorphs absent, Emanating hyphae 3.5–5.0 µm, frequent, thick walled (1 µm), hyaline, septate without clamp, not constricted at septa. Cystidia 11.5–57.0 (65.2) × 3.5–8.0 µm, present on the outer mantle layer, the most distinct and often frequent with type 1 (Agerer 1987–2012, Agerer & Rambold 2004–2016), unramified, clavate, subcylindrical to capitate representing type N (Agerer 1987–2012, Agerer & Rambold 2004–2016), granulated to agranulated, which do not stain in sulphovanillin, smooth, thick walled (up to 0.1 µm), aseptate to septate without clamp.

Anatomical characters in longitudinal section: Mantle thickness 21–29 (32) µm, differentiated into outer mantle layer and inner mantle layer. Outer mantle layer 10–16 µm thick, almost pseudoparenchymatous, variously shaped cells staining in sulphovanillin representing type N (Agerer 1987–2012, Agerer & Rambold 2004–2016), compactly arranged, hyphal cells 6.5–13.5 tangentially and 5–8 µm radially, smooth, granulated with light brown content plasmatically as observed in case of sporophore

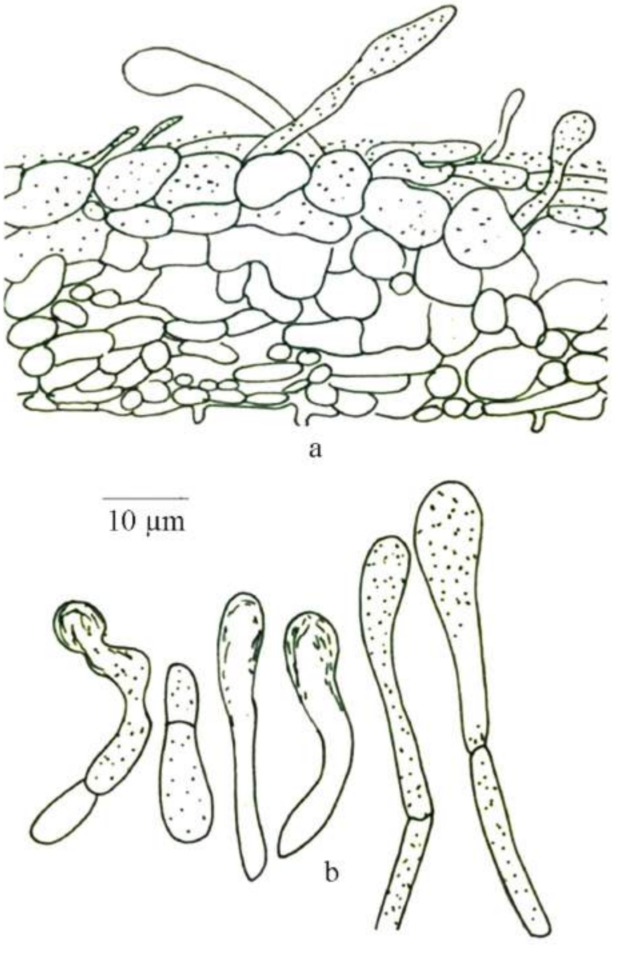

Figure 5 *Russula nigricans* + *Shorea robusta*; (a) mantle and (b) cystidial elements

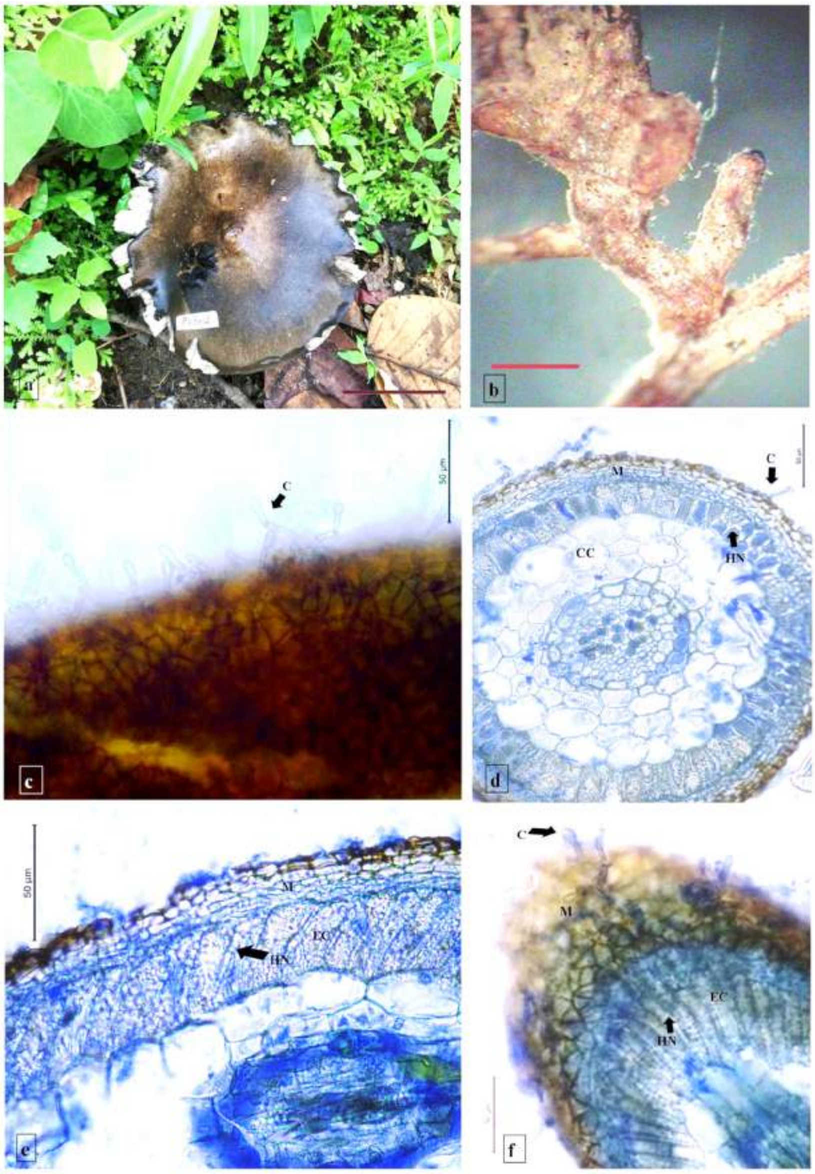

Figure 6 *Russula nigricans* + *Shorea robusta*; (a) carpophore in association with *Shorea robusta* root and seedlings, (b) mycorrhizal system, (c) surface view of unramified end showing cystidia (C) on outer mantle, (d) cross-section of ectomycorrhizal root showing mantle (M) and Hartig net (HN), (e) longitudinal section of ectomycorrhiza showing mantle and radially elongated epidermal cell (EC) with Hartig net (HN) and (f) longitudinal section of root tip showing pseudoparenchymatous mantle (M) with cystidial (C) elements; scale bar a = 6 cm, b = 1 mm

hyphal cells, giving rise to abundant emanating hyphae and cystidia. Inner mantle layer 9–14 µm, pseudoparenchymatous bearing epidermoid to roundish cells representing type K (Agerer 1987–2012, Agerer & Rambold 2004–2016), hyphal cells granulated, thin walled, variable in shape measuring 4–11 µm tangentially and 2–4 µm radially, cells ovoid, cylindric to rounded in shape. Labyrinthine Hartig net hyphae one cell deep around epidermal cells, with one row of 1.6–8.0 (9.8) µm radially and 1.6–4.8 tangentially, roundish to cylindrical hyphal cells and is restricted to the anticlinal walls of the cortex cells (paraepidermal), hyphal cells branched in Hartig net to increase more absorptive area. Root tip mantle up to 81.5 µm thick, different from rest of the mantle, pseudoparenchymatous, with angular cells representing type L (Agerer 1987–2012, Agerer & Rambold 2004–2016). Hartig net also paraepidermal at very root tip. Epidermal cells radially elongated 29–35 (29.3) × 5–8 µm, tangentially oval to elliptic or cylindrical, and oriented obliquely. Tannin cells not observed.

Colour reactions with different reagents: $FeSO_4$: n. r., sulphovanillin: dark brown, ethanol (70%): n. r., KOH (10%): n. r., Melzer: n. r., cotton blue: hyphal cell wall dark brown and cystidial granulation becomes dark brown.

DISCUSSION

The current knowledge of *S. robusta* symbionts is predominantly based on sporophore surveys. From dipterocarp forest Basidiomycete fungi including *Russula adusta, R. cinerella, R. congoana, R. delicula, R. michiganensis, R. amoena, R. delica, Amanita hemibapha, A. verna, Astraeus hygrometricus, Boletus edulis, B. fallax, Geastrum fimbriatum, G. triplex, Lycoperdon compactum, Scleroderma bovista, S. geaster, S. verrucosum* and *Agaricus trisulphoratus* are reported as ECM associates of *S. robusta* (Pyasi et al. 2011, 2013, Tapwal et al. 2013, 2015). Of these only *L. compactum, R. michiganensis* and *R. amoena* were confirmed as ECM associates of *S. robusta* using in-vitro synthesis between sal and these fungi. The rest of the fungi were identified based on unsubstantiated observations during sporocarps survey. *Russula feugiana* and *Lactifluus volemus* var. *volemus* were confirmed as ECM associates of sal roots by observing the direct hyphal connection between the roots and fungi besides examining the morphoanatomical details of these roots (Kumar & Atri 2016).

In the study of diversity, ecology and ECM biology of mushroom species occurring in direct association with *S. robusta* from north-west India, this is the first time that *R. cremeoavallanea, R. romagnesiana* and *R. nigricans* were recorded to form mycorrhizal association with sal roots. All the mycorrhizas examined were ECM, because they showed both well-developed fungal sheaths and Hartig nets but no intracellular penetration of hyphae. The intimacy and the type of association were confirmed by observing direct hyphal or rhizomorphs connection between *S. robusta* roots and mushrooms besides examining the morphoanatomical details of these roots. ECM types are fully characterised from a morpho-anatomical point of view and also comparing ECM anatomical characters with those known from related taxa so that the combination of morphological characters of basidiomes and mycorrhizas contribute to a reliable taxonomy in genus *Russula*. Such a combined approach could be of general help when dealing with the characterisation and identification of ECM associations without any fungal partner fruiting.

Russula cremeoavallanea had been reported in association with mosses (Sharma et al. 2016) while *R. romagnesiana*, with *Vateria indica* and *Hopea parviflora* (Vrinda & Pradeep 2010) from India but without any mycorrhizal description or morpho-anatomical detail. Morpho-anatomical detail of ECM of *R. cremeoavallanea* and *R. romagnesiana* are presented for the first time in association with a host. However, morpho-anatomical detail of *R. nigricans* had been presented in association with *Pinus densiflora* and *Betula platyphylla* from Japan (Yamada & Katsuya 1996, Yamada 1998 respectively), *Pseudotsuga menziesii* from Canada (Hagerman et al. 2001), *Pinus sylvestris* from Poland (Mleczko 2004), *Quercus* spp. from Europe (Beenken 2004), *Dicymbe corymbosa* from Guyana rainforest (Smith et al. 2011), *Abies alba* and *P. sylvestris* from Poland (Wazny et al. 2014), indicating its multihost specificity.

Mycorrhizal system of *R. cremeoavallanea* is monopodial pinnate to irregularly pinnate and greyish brown to reddish brown, while it is monopodial pinnate and white silvery to brown for *R. romagnesiana*. Mycorrhizal system was irregularly pinnate, greyish brown, or dark brown to black for *R. nigricans*. However, Beenken (2004) observed monopodial pinnate and brownish or ochre, yellowish brown mycorrhizal system in *R. nigricans* associated with angiosperm tree *Quercus* spp. Outer mantle layer is plectenchymatous, compactly arranged and inner mantle layer pseudoparenchymatous in *R. cremeoavallanea* and *R. romagnesiana* while outer and inner mantle layer is almost pseudoparenchymatous in *R. nigricans*. In the association of *Quercus* spp., *R. nigricans* was reported to be densely plectenchymatous to almost pseudoparenchymatous (Beenken 2004). The pseudoparenchymatous or epidermoid mantle and simple septate hyphae are common features of ECM formed by plants with various species of *Russula* (Agerer 1986). ECM of *R. cremeoavallanea* and *R. romagnesiana* mostly differed in cystidial shape and size on the outer mantle surface which is reported to be quite common in the ECM association of various *Russula* species with *S. leprosula* (Lee et al. 1997) and *S. robusta* (Kumar & Atri 2016). In the current study, similar features were observed in the ECM formed by different species of *Russula* with *S. robusta.* Cystidia are subcylindrical to awl shaped with almost acute apex and swollen or with rounded base and aseptate to septate in case of *R. cremeoavallanea.* Cystidia are frequent, bottle shaped with almost straight neck and swollen or rounded base to obpyriform, obclavate, to even ampulliform in ECM of *R. feugiana* in association with *S. robusta* (Kumar & Atri 2016). Cystidia in *R. nigricans* were also frequent and unramified but clavate, subcylindric to capitates, dark brown, granulated to agranulated, and aseptate to septate without clamp. Similar cystidial details were observed by Beenken (2004) in *R. nigricans* in association with *Quercus* spp. from Europe. Rhizomorphs were observed in *R. cremeoavallanea* and *R. romagnesiana* but these were not in *R. nigricans.* Hyphal morphology in carpophores and their respective ECM in all the studied samples were exactly alike. Hartig net was paraepidermal in all the ECM observed. This is a typical character of angiosperm plants and epidermal cells become radially much elongated compared with non-mycorrhizal roots which help to increase the area available for exchange of nutrients in the Hartig net region.

CONCLUSIONS

Russula cremeoavallanea, R. romagnesiana and *R. nigricans* were found in direct organic connection with *S. robusta* roots and there was similarity in hyphal features of the sporophores and mantle. Hence, all three *Russula* species were ECM associates of *S. robusta.* Close examination of ECM features revealed differences amongst different *Russula* species ECM. In the future, it would be interesting to test the proposed ECM associates for synthesis of ECM in nursery for better survival, growth and establishment of *S. robusta* seedlings which hardly survive without its ECM associates.

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