Гриби і грибоподібні організми
Fungi and Fungi-like Organisms

https://doi.org/10.15407/ukrbotj78.02.112

RESEARCH ARTICLE

Characterisation and identification of ectomycorrhizae formed by the species of *Asproinocybe* (*Tricholomataceae*) and *Inocybe* (*Inocybaceae*) with the roots of the tropical sal tree *Shorea robusta* (*Dipterocarpaceae*)

Jitender KUMAR[1]*, Narender Singh ATRI[2]

[1]Rajiv Gandhi Government Degree College, Chaura Maidan, Shimla 171004, India

[2]School of Biological and Environmental Sciences, Shoolini University of Biotechnology and Management Sciences, Solan 173229, India

Abstract. In the course of the present study, surveys on occurrence and distribution of ectomycorrhizal (EcM) fungi in tropical sal forests of foothills of the Himalayas, India, were undertaken. The species of two genera of agarics, namely *Asproinocybe* and *Inocybe*, were found organically associated with the roots of *Shorea robusta* (sal tree). However, prior to our study the genus *Asproinocybe* has not been reported from India. In this article, the morpho-anatomical details of mycorrhizal roots of *Shorea robusta* associated with *Asproinocybe lactifera* and *Inocybe purpureoflavida* are provided for the first time. The EcM colonized roots of the two species are distinguished by differences in the shape and colour of the roots, surface texture, size and shape of cystidia, type of mantle, as well as different chemical reactions. *Asproinocybe lactifera* EcM is mainly characterised by a monopodial pinnate mycorrhizal system with the dark brown to reddish brown and loose cottony surface, while in *Inocybe purpureoflavida* it is irregularly pinnate to coralloid, silvery grey to reddish brown, with densely woolly surface. The outer mantle layer is heterogeneous with obclavate to awl-shaped cystidia in *Asproinocybe lactifera*, whereas *Inocybe purpureoflavida* EcM have a plectenchymatous outer mantle with subcylindrical to obclavate metuloidal and non-metuloidal cystidia. The presence of lactifers in the mantle is a unique feature in *Asproinocybe lactifera* as compared to *Inocybe purpureoflavida*.

Keywords: ectomycorrhiza, Hartig net, mantle, mushrooms, rhizomorphs, sporophores, sal, Shiwalik

Article history. Submitted 28 January 2021. Revised 09 April 2021. Published 30 April 2021

Citation. Kumar J., Atri N.S. 2021. Characterisation and identification of ectomycorrhizae formed by the species of *Asproinocybe* (*Tricholomataceae*) and *Inocybe* (*Inocybaceae*) with the roots of the tropical sal tree *Shorea robusta* (*Dipterocarpaceae*). *Ukrainian Botanical Journal*, 78(2): 112–122. https://doi.org/10.15407/ukrbotj78.02.112

*Corresponding author (e-mail: *jitenderthakur2010@gmail.com*)

Introduction

The ectomycorrhiza (EcM), also called ectotrophic or sheathing mycorrhiza, represents a mutualistic association between the roots of vascular plants and some of ascomycetous or basidiomycetous fungi interconnected by soil-borne mycelia or rhizomorphs (Agerer, 2006; Kumar, Atri, 2017). EcM associations are formed predominantly on the fine root tips of the host plant and are recognised by the presence of a mantle, which is a network of interwoven hyphae on the root surface, and a Hartig net, consisting of a labyrinth of highly branched hyphae between the cells of the root epidermis or cortex. The Hartig net is the place of bidirectional exchange of nutrients between the host plant and the fungus. These roots and their associated fungal hyphae form a complex network in the topsoil layers providing a larger area for absorption of nutrients (Smith, Read, 2008). The fungal

mycelium in these associations forms an underground web, or wood wide web. This complex network is reported to enhance the redistribution of nutrients among the organically connected plants through the mycelial connections amongst them and even from mother plant to the young seedlings in the forested areas. This may even result in the alteration of interspecific and intraspecific competition amongst such interconnected plants (Shi et al., 2017).

It is a well-established fact since the times of Frank (1885) that various EcMs differ greatly in their morphology and anatomy. The emanating hyphae radiating into the soil and the rhizomorphs organisation can also vary considerably. Besides, the exploration of soil by different fungi for a plant host is also known to vary from species to species (Agerer, 1987–2002). A great diversity is reported to exist in ectomycorrhizal structures in different species which plays an important role in the identification of associated species (Agerer, 1987–2002; Agerer, Rambold, 2004–2020). Before the works of Agerer and his co-investigators (Agerer, 1986, 2006; Agerer, Rambold, 2004–2020), the methods for describing the EcM associations varied greatly. Agerer and his associates through their series of publications have established a standard norm for the identification and characterisation of EcM which is now being widely followed throughout the world.

There are ca. 20,000 EcM fungal species, mainly belonging to *Basidiomycota*, followed by *Ascomycota* which are reported to form symbiotic associations with the plants of some families, including *Betulaceae*, *Caesalpiniaceae* (a paraphyletic group belonging to *Fabaceae* s. l.), *Dipterocarpaceae*, *Ericaceae*, *Fagaceae*, *Myrtaceae*, *Nothofagaceae*, *Pinaceae*, and *Salicaceae* (Smith, Read, 2008; Tedersoo et al., 2010). Ectomycorrhizal fungi are reported to be common in the temperate and boreal ecosystems and in large forested areas of tropical and subtropical regions. These are considered as key ecological factors in governing and maintaining the terrestrial ecosystems (Wang et al., 2017). Amongst the Gymnosperms, most of the investigators have so far focused their research on the study of EcM association of *Picea* Mill. and *Pinus* L. (*Pinaceae*). Among the angiosperms, *Quercus* L. and *Fagus* L. belonging to the family *Fagaceae* are the commonest genera investigated in this regard. In comparison, hardly any work on EcM studies is available on the other genera of angiosperms (Roman et al., 2005; Agerer, Rambold, 2004–2020). In India EcM studies are also exclusively focused on the temperate and boreal ecosystems (Mohan et al., 1993a, b, c), whereas there is little information on EcM communities of tropical and subtropical ecosystems. The tropical moist deciduous forests of India are largely dominated by the economically important sal tree *Shorea robusta* Gaertn. (*Dipterocarpaceae*), which is a major source of commercial timber (Singh, Singh, 1992). Plants of *S. robusta* are reported to form obligatory ectomycorrhizal association with a number of fungal species. Some of the agaricoid and boletoid genera, with which the sal tree is reported to form putative EcM associations, include *Russula* Pers., *Boletus* L., *Agaricus* L. *Amanita* Pers., *Lactarius* Pers., *Laccaria* Berk. & Broome, *Pisolithus* Alb. & Schwein., *Suillus* Gray, and *Cantharellus* Adans. ex Fr. (Natarajan et al., 2005; Tapwal et al., 2013; Kumar, Atri, 2016, 2019, 2020a). As far as study of EcM association of sal with these fungi is concerned, not much work is available in this regard (Alexander, Selosse, 2009; Kumar, Atri, 2018, 2019, 2020a).

During the survey of a sal forest in the Shiwaliks, two new ectomycorrhizal associates, viz. *Asproinocybe lactifera* R.Heim. and *Inocybe purpureoflavida* K.B.Vrinda & C.K.Pradeep, were noted as forming organic connections with the roots and young seedlings of sal. For the study purpose, the associated mushroom sporocarps and the EcM colonized roots of sal were collected from pure sal forests by tracing the hyphal or rhizomorphs connections with *Shorea robusta* roots in the soil underneath. Tracing the mycelial or rhizomorph connections in association with a fruit body and EcM colonized roots is reported to be the most reliable way of assessing the EcM status in the field (Agerer, 1986, 2006). Besides taxonomically investigating and identifying the associated mushroom sporocarps, the morpho-anatomical details of natural mycorrhizal roots of sal associated with these agarics were also studied. This ultimately resulted in the establishment of a putative ectomycorrhizal association of these mushrooms in nature with *S. robusta.*

Materials and methods

Study area. The area selected for undertaking surveys for the present study is the Shiwalik (also known as Sivalik or Shivalik) mountain range of India falling in the jurisdiction of Sirmour District of Himachal Pradesh State and adjoining parts of Uttrakhand State, which represent the geologically lowest and youngest mountain range of Himalaya dominated by pure formations of *Shorea*

robusta. The study area is located between 29°58'–31°20′ N, 77°34'–78°18′ E. 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).

Sampling, identification and characterization of sporophores. EcM root tips and associated epigeous sporophores of putative EcM genera were collected from different sites in pure sal forests, during the rainy season (July–October) in 2013–2015. The *Asproinocybe lactifera* and *Inocybe purpureoflavida* sporophores and their EcM colonized roots were collected by tracing the hyphal connections between *Shorea robusta* roots and sporophores. Morphological characters of each specimen were noted on the field key (Atri et al., 2005). Sporophores were air dried at 40–45 °C in a drier specially designed for drying mushroom specimens (Atri et al., 2005) and finally packed in cellophane packets for permanent preservation in the Punjabi University Herbarium (PUN). The macroscopic and microscopic details of the investigated taxa were examined using standard methods (Singer, 1986; Atri et al., 2017); species were identified using standard literature (Heim, 1970; Vrinda et al., 1997).

Sampling, identification and characterization of EcM roots. Mycorrhizal roots underneath sporophores 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. Morphological characterization of EcM roots was performed under a stereomicroscope (Magnus MSZ-TR), photographed and described by careful examination following Agerer (1987–2002) and Agerer & Rambold (2004–2020). The mycorrhizal roots were fixed in FAA [5 mL formalin (37%) + 5 mL acetic acid (100%) + 90 mL alcohol (50%)] for anatomical characterization. EcM colonization was confirmed by preparation of cotton blue stained hand cut thin sections of EcM roots, and microscopic examination by checking for the presence of mantle and Hartig net. The cross section and longitudinal section of EcM roots were examined and drawn under a compound microscope and photomicrographed under digital microscope (Leica DM4000 B LED) for the presence of the mantle, Hartig net, hyphal and rhizomorphs characteristics. The colour terminology used is that of Kornerup and Wanscher (1978). Microchemical reactions on EcM roots were performed using $FeSO_4$, sulphovanillin, ethanol, KOH, Melzer, and cotton blue.

Results

Description of ectomycorrhizae: *Asproinocybe lactifera* Heim. + *Shorea robusta* Gaertn.

Morphological characters. Mycorrhizal system monopodial pinnate to simple with one order of ramification, 2–6 mm long; main axes 0.15–0.3 mm in diameter. Unramified ends straight to slightly bent to sinuous, not inflated, cylindric, 2.0–6.8 mm in length and 0.4–0.6 mm in diameter, apex approximately half-circular or ellipsoid (Fig. 1). Surface of unramified ends shiny, mycorrhizae dark brown to reddish brown, older mycorrhizae dark reddish brown, loosely woolly to loosely cottony at some places, also covered with soil particles here and there, unchanging, not secreting latex or any other fluid where injured; mantle not transparent; mantle hydrophobicity absent, lactifers present, tip shows the same colour as rest of the mycorrhiza. Rhizomorphs present, frequent, up to 150 µm thick. Emanating hyphae frequent, not specifically distributed. Cystidia present. Sclerotia not observed.

Anatomical characters of mantle in plan view. Mantle 81.5–102.6 µm thick, distinct, differentiated into outer mantle layer and inner mantle layer. Outer mantle layer 32–50 µm, gelatinised, heterogeneous, compactly arranged with broad streaks of almost granulated to hyaline interwoven hyphae mixed with irregularly shaped, 5.0–10.0 × 2.5–5.7 µm cells representing type C arrangement (Agerer, 1987–2002; Agerer, Rambold, 2004–2020); hyphal cells 1.6–3.0 µm in diameter, cylindrical, compactly arranged, smooth, light brown, septate, thin walled (0.5 µm), not constricted at the septa, clamped, septa as thick as hyphal wall (Figs 1, 2). Inner mantle layer 50.0–55.5 µm, plectenchymatous, compactly arranged with broad streaks of almost interwoven granulated hyphae as observed in carpophores, representing type B pattern (Agerer, 1987–2002; Agerer, Rambold, 2004–2020); hyphal cells 1.6–2.5 µm in diameter, compactly arranged, smooth, light brown, septate, thin walled (0.5 µm), constricted at the septa, clampless, septa as thick as hyphal wall (Figs 1, 2). Pigmentation membranaceous**,** the pigment is located within the cell wall, therefore the walls appear dark, some hyphae with oily droplets which do not stain in sulphovanillin.

Anatomical characters of emanating elements. Rhizomorphs present, up to 150 µm thick, light brown, frequent, branched, rounded, oblique, margin smooth with few emanating hyphae, extraradical hyphae emanating from the surface, at some places covered

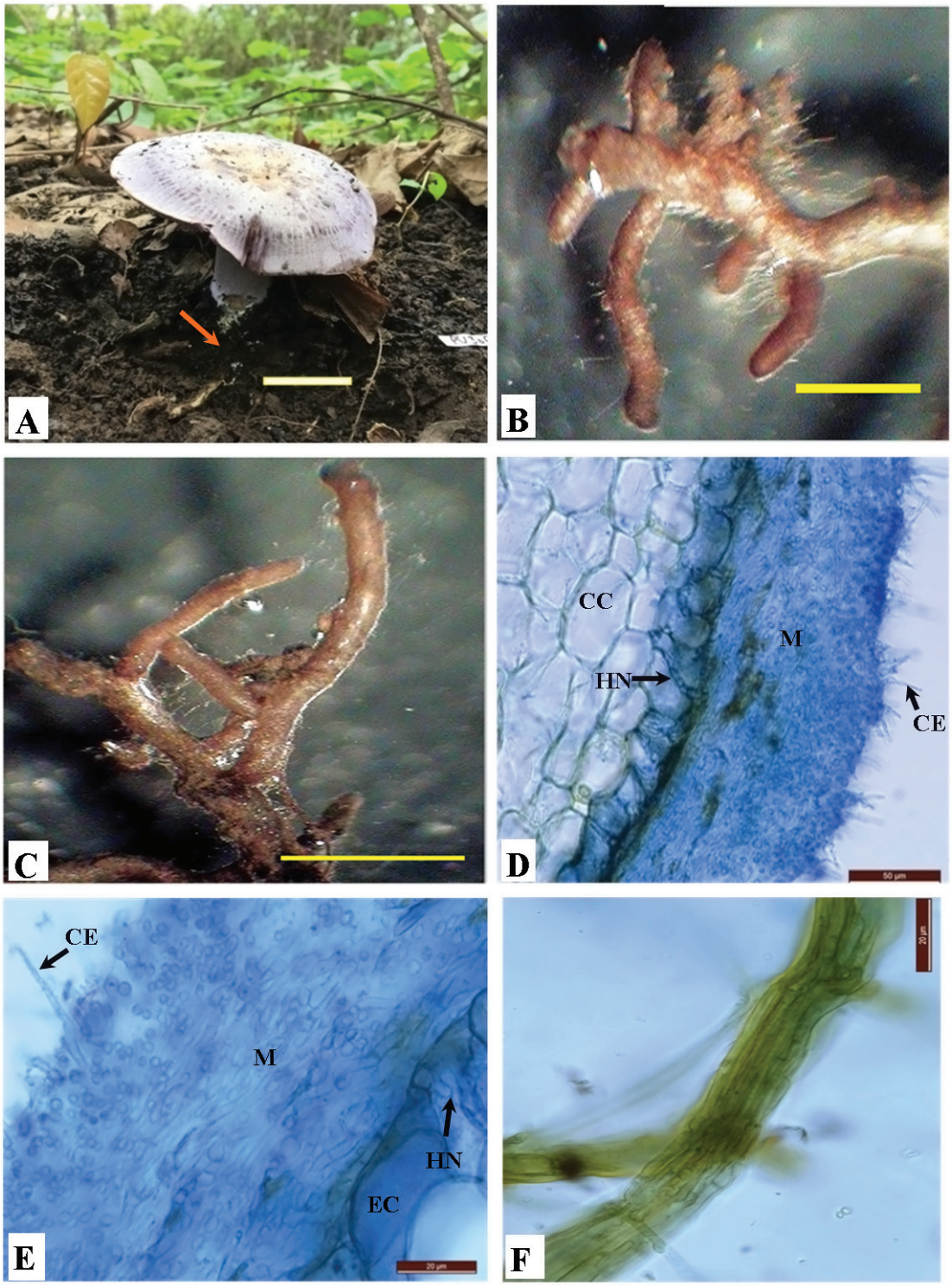

Fig. 1. *Asproinocybe lactifera* + *Shorea robusta*. A: sporophore in association with *Shorea robusta* root and seedlings; B, C: mycorrhizal system; D: cross section of ectomycorrhizal root showing mantle (M), cystidial element (CE) and Hartig net (HN); E: longitudinal section of ectomycorrhizae showing mantle and radially elongated epidermal cell (EC) with Hartig net; F: rhizomorphs. Scale bar: 3 cm (A), 1 mm (B, C)

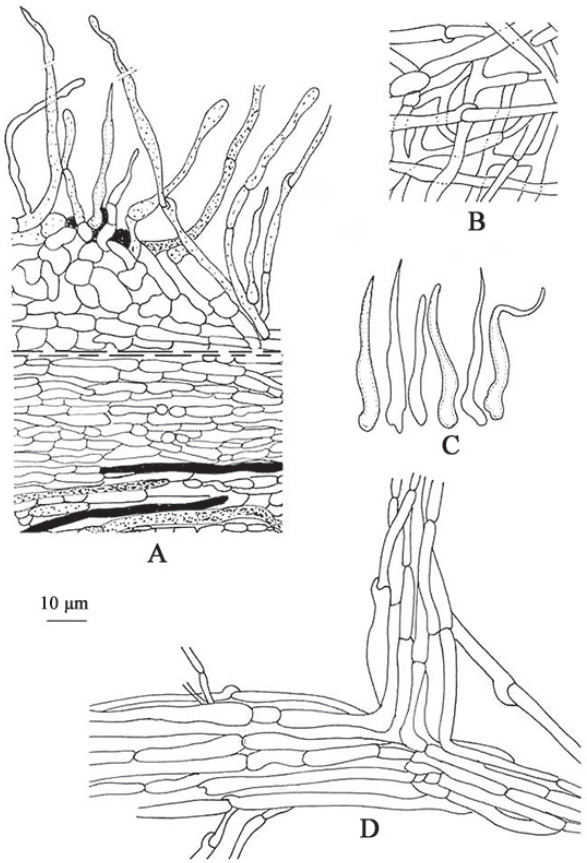

Fig. 2. *Asproinocybe lactifera* + *Shorea robusta*. A: mantle; B: root tip mantle; C: cystidial elements; D: rhizomorphs

with soil particles; nodia and internodia present; hyphae compactly arranged or sometimes loosely woven, variable in diameter (1.6–3.0 µm), septate, clamped, septa as thick as hyphal wall. The pigment is located within the cell wall, therefore the walls appear dark, and in hyphal surface view the margins of the hyphae appear darker. Emanating hyphae originating from mantle 1.6–3.0 µm, simple, smooth, septate, clamped, hyaline, not constricted at the septa (Figs 1, 2).

Anatomical characters in longitudinal section. Mantle 65.0–81.5 µm thick, compact, differentiated into outer mantle layer and inner mantle layer. Outer mantle layer 24–32 µm, gelatinised, plectenchymatous, compactly arranged with broad streaks of almost granulated to hyaline interwoven hyphae mixed with irregularly shaped, 5–10 × 2.5–5.7 µm cells representing type C (Agerer, 1987–2002; Agerer, Rambold, 2004–2020); hyphal cells 1.6–3.0 µm in diameter, compactly arranged, smooth, light brown, septate, thin walled (0.5 µm), constricted at the septa, clamped, septa as thick as hyphal wall. Inner mantle layer 46.0–50.5 µm, plectenchymatous, more compactly arranged with broad streaks of almost interwoven granulated hyphae representing type A pattern (Agerer, 1987–2002; Agerer, Rambold, 2004–2020); hyphal cells 1.6–2.5 µm in diameter, compactly arranged, smooth, light brown, septate, thin walled (0.5 µm), constricted at the septa, clampless. Hartig net one cell deep, made up of elongated cylindrical septate, 5–13 × 3–5 µm sized hyphal cells and is restricted to the anticlinal walls of the cortex cells (paraepidermal). Root tip mantle up to 179 µm, quite different and thicker than the rest of the mantle, of the same hyphal organisation, hyphae comparatively less compact, individual hyphae clearly observable. Root tip outer mantle plectenchymatous, 1.6–5.0 µm thick, interwoven, septate, clamped, hyphae rather irregularly arranged, no special pattern discernible representing type B pattern (Agerer, 1987–2002; Agerer, Rambold, 2004–2020). Inner mantle almost plectenchymatous made up of 1.6–5.0 µm interwoven, septate, clamped, hyphal cells, hyphae rather irregularly arranged with no special pattern clearly discernible, but almost representing type B pattern (Agerer, 1987–2002; Agerer, Rambold, 2004–2020). Hartig net also paraepidermal at the very root tip. Epidermal cells 8–15 µm tangentially and 8–10 µm radially, oval to elliptic or cylindrical, and oriented obliquely. Tannin cells not observed (Figs 1, 2).

Colour reactions with different reagents. $FeSO_4$: brown; sulphovanillin: no reaction (n. r.); ethanol (70%): n. r.; KOH (10%): dark brown; lactic acid: n. r.; Melzer: light yellow; acetic acid (50%): n. r.; cotton blue: hyphae dark blue.

Collection examined. Uttrakhand: Lachhiwala (525 m alt.), 24 September 2015, Jitender Kumar, PUN 9167.

Description of ectomycorrhizae: *Inocybe purpureoflavida* Vrinda & Pradeep + *Shorea robusta* Gaertn.

Morphological characters. Mycorrhizal system irregularly pinnate to coralloid with zero to one order of ramification, 2.5–7.0 mm long; main axes 0.2–0.5 mm in diameter. Unramified ends sinuous to slightly bent, occasionally tortuous, 1–3 mm in length and 0.1–0.4 mm in diameter, tips rounded not swollen (Fig. 3). Surface of unramified ends rough, densely woolly, occasionally with soil particles, younger mycorrhizae silvery grey to greyish brown and older dark brown to reddish brown (5D8), unchanging, not secreting latex or any other fluid when injured; mantle not transparent; mantle hydrophobicity absent; tip dark brown, rounded, slightly inflated in the centre. Rhizomorphs present, frequent,

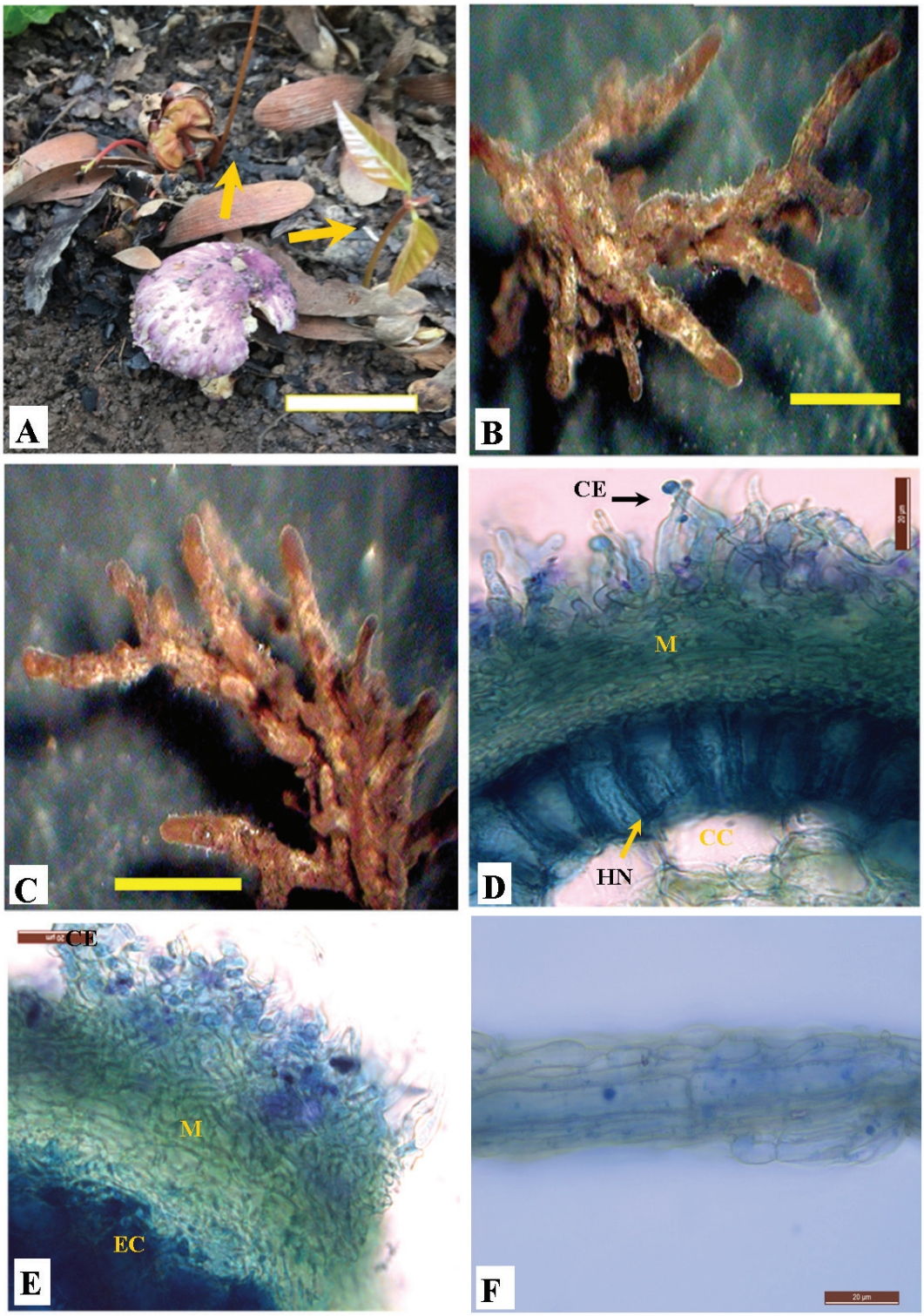

Fig. 3. *Inocybe purpureoflavida* + *Shorea robusta*. A: sporophore in association with *Shorea robusta* root and seedlings; B, C: mycorrhizal system; D: cross section of ectomycorrhizal root showing mantle (M), cystidial element (CE) and Hartig net; E: longitudinal section of ectomycorrhizae showing mantle and radially elongated epidermal cell (EC) with Hartig net; F: rhizomorphs showing clamp connection (Arrow). Scale bar: 3 cm (A), 1 mm (B, C)

compact, with distinct connection to the mantle, frequently and repeatedly branched, smooth, roundish to slightly flat, 16–32 µm thick. Emanating hyphae rarely observed. Cystidia present. Sclerotia not observed.

Anatomical characters of mantle in plan view. Mantle 81–105 µm thick, differentiated into outer mantle layer and inner mantle layer. Outer mantle layer 52–65 µm, plectenchymatous, compactly arranged, slightly gelatinized, representing type C pattern (Agerer, 1987–2002; Agerer, Rambold, 2004–2020); hyphal cells 3–5 µm, compactly arranged, smooth, inflated, hyaline, septate, thin walled (0.5 µm), cell wall light yellow to yellow, constricted at the septa, clampless; septa as thick as hyphal wall. Inner mantle layer 24–32 µm, compact, pseudoparenchymatous representing type K (Agerer, 1987–2002; Agerer, Rambold, 2004–2020), hyphal cells, epidermoid angular to irregularly lobed, bearing mounds of roundish cells representing type K, colourless, homogenously granulated, thin walled, 3.0–4.5 µm broad (Figs 3, 4).

Anatomical characters of emanating elements. Rhizomorphs present, rounded, frequent, flat, oblique, with smooth surface, without emanating hyphae, 24–65 µm, milky-white, compact, thicker rhizomorphs undifferentiated to slightly differentiated, representing type C pattern (Agerer, 1987–2002; Agerer, Rambold, 2004–2020), thicker hyphae differentiated in the centre, central hyphae almost parallel, 3.0–8.0 µm in diameter, cylindrical to inflated and ampullate, broader than peripheral hyphae, thick-walled (up to 1 µm), septate, septa complete, constricted at the septa, without clamps, septa as thick as hyphal wall; peripheral hyphae 1.6–5.0 µm, curled to twisted, intermingled, mostly clamped, clamps thin walled. Anastomoses between hyphae not observed, nodia and internodia present, ramification with one or two branches at nodia. Thinner rhizomorphs undifferentiated, all hypae almost parallel and equal in diameter, cylindrical not inflated and ampullate as observed in thicker rhizomorphs. Emanating hyphae 3.0–5.7 µm, thin-walled (0.8 µm), septate, slightly constricted at the septa, without clamps, septa as thick as hyphal wall (up to 1 µm). Cystidia 16–36 × 3.0–6.5 µm, present on the outer mantle layer, most distinct and often infrequent, type 1 pattern (Agerer, 1987–2002; Agerer, Rambold, 2004–2020), subcylindrical to obclavate with acute to rounded apex and swollen or rounded base, some are metuloidal as observed in sporophore, hyaline to homogenously granulated, smooth, thick-walled (up to 1.5 µm), aseptate without clamps (Figs 3, 4).

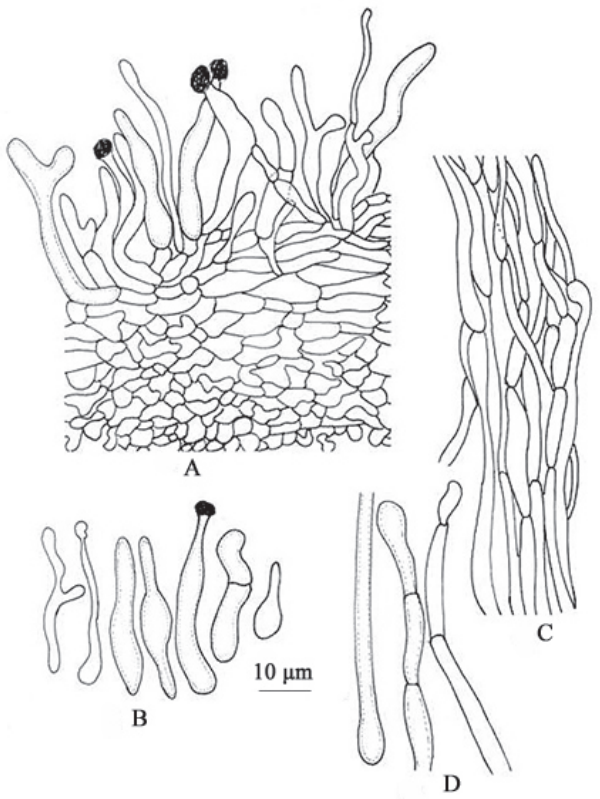

Fig. 4. *Inocybe purpureoflavida* + *Shorea robusta*. A: mantle; B: cystidial elements; C: rhizomorphs; D: emanating hyphae

Anatomical characters in longitudinal section. Mantle 81–105 µm, differentiated into outer and inner mantle layer. Outer mantle layer 52–65 µm, loosely plectenchymatous, slightly gelatinized at very surface, becoming denser towards middle layer, representing type C (Agerer, 1987–2002; Agerer, Rambold, 2004–2020); hyphal cells 3–4 µm broad, cylindrical to inflated, without any content and clamp connections, cell wall light yellow to light brown. Inner mantle layer 24–32 µm, pseudoparenchymatous; hyphal cells 3–4 µm broad, epidermoid angular to irregularly lobed cells, bearing mounds of roundish cells, representing type K pattern (Agerer, 1987–2002; Agerer, Rambold, 2004–2020), light brown, homogenously granulated, thin walled, 3.0–4.5 µm broad. Hartig net one cell deep, palmetti type with one row of 1.6–3.5 µm broad cylindrical hyphal cells, restricted to the anticlinal walls of the cortex cells (paraepidermal). Root tip mantle up to 195.6 µm, different from rest of the mantle (on the contrary, no layers discernible), plectenchymatous, 3.0–5.7 µm broad, interwoven, septate, hyphal cells hyaline, broader than rest of the mantle; hyphae rather irregularly arranged, no special pattern discernible, representing type C pattern

(Agerer, 1987–2002; Agerer, Rambold, 2004–2020). Hartig net also paraepidermal at very root tip with one row of cylindrical hyphal cells. Epidermal cells radially elongated to increase the area available for the Hartig net, 40–48 × 13–20 µm, tangentially oval to elliptical or cylindrical, and oriented obliquely. Tannin cells not observed (Figs 3, 4).

Colour reactions with different reagents. $FeSO_4$: n. r. (no reaction); sulphovanillin: n. r.; KOH (10%): n. r.; ethanol (70%): n. r.; acetic acid: n. r.; Melzer: n. r.; cotton blue: pale blue to dark blue.

Collections examined. Uttrakhand: Dehradun, Lachhiwalla (525 m alt.), 2 September, 2013, Jitender Kumar, PUN 9153; Dehradun, Asharodi (686 m), 23 July 2015, Jitender Kumar, PUN 9154.

Discussion

Several species of basidiomycetous fungi have been reported from sal forests as EcM associates of *Shorea robusta* roots based on sporophore surveys (Pyasi et al., 2011, 2013; Tapwal et al., 2013, 2015). Out of these, only *Russula michiganensis* Shaffer., *R. amoena* Quél. and *Lycoperdon compactum* G. Cunn. were clearly confirmed as EcM associates of *Shorea robusta* by synthesizing EcM in field experiments, while the rest of data is based on unsubstantiated observations. Some other mushrooms, including *Russula azurea* Bres., *R. chlorinosma* Burl., *R. cremeoavellanea* Singer, *R. cyanoxantha* (Schaeff.) Fr., *R. feugiana* Singer, *R. nigricans* (Bull.) Fr., *R. romagnesiana* Shaffer, and *Lactifluus volemus* (Fr.: Fr.) Kuntze var. *volemus,* were also confirmed as EcM associates of sal roots by observing the direct hyphal connection between sal roots and mushrooms besides examining the morpho-anatomical details of these roots (Kumar, Atri, 2016, 2019, 2020a). In fact, EcM fungi are poorly studied in tropical sal forests as compared to other forests in India (Riviére et al., 2007; Tapwal et al., 2013). In recent years, we have been carrying out a study on the EcM diversity, ecology, and biology of mushroom species occurring in direct association with *Shorea robusta* roots from Northwestern India. In the present study, it is reported for the first time that *Asproinocybe lactifera* and *Inocybe purpureoflavida* were found to form mycorrhizal association with sal roots. Earlier none of these mushrooms were known to form EcM association with any of the host plant. We have recorded the genus *Asproinocybe* for the first time from India (Kumar, Atri, 2020b). This genus was previously recorded from subtropical and tropical Africa (Heim, 1970), South America (Heinemann, 1977), Malaysia (Guzmán et al., 2004), and Australasia (Lebel, 2020). We have reported *Asproinocybe lactifera* growing in association with *Shorea robusta* from India (Kumar, Atri, 2020b), which was earlier recorded from subtropical and tropical Africa and South America without any specific host (Heim, 1970; Heinemann, 1977).

The examined mycorrhizal roots of *Shorea robusta* showed both well-developed fungal sheaths and the Hartig nets. The intimacy and the type of association were confirmed by observing direct hyphal or rhizomorph connection between *Shorea robusta* roots and mushrooms in addition to other morpho-anatomical details of the roots. Zak (1973) pointed out that the mantle surface can range from thin to profuse and its texture may vary from smooth, cottony, woolly, velvety, spiny and warty to granular. Apart from texture and thickness, the mantle can differ in organisation, colour, and presence or absence of cystidia on the mantle surface, and the Hartig net, depending on the host and EcM fungus identity (Agerer, 1986; Smith, Read, 2008; Tedersoo et al., 2010). During the present study, it was observed that with the change in mycorrhizal associate there is a variation in the morphology of the mycorrhizal system. Ectomycorrhizal association formed by *Asproinocybe lactifera* and *Inocybe purpureoflavida* species with sal roots is well characterised by the presence of numerous cystidia on the outer mantle surface which resemble in their morphology the cystidial elements present in the respective sporophore. As is the case presently, cystidia bearing mantle is reported to be quite common in the EcM association of various other species with *Shorea leprosula* (Lee et al., 1997) and *S. robusta* (Bakshi, 1974). *Asproinocybe lactifera* EcM roots are mainly characterised in having a monopodial pinnate to simple mycorrhizal system with a dark brown to reddish brown, loose woolly to cottony surface, heterogeneous to plectenchymatous thick mantle, obclavate to awl-shaped cystidia with almost acute apex having a swollen or rounded base, and the hyphal characteristics which were quite similar to those present in the sporophore of this mushroom. The presence of lactifers in the mantle is a unique feature, as similar types of lactifers were also present in the sporophore of *A. lactifera*. The simple septate agaricoid hyphae and lactifers in the inner mantle layer of EcM roots infected by *A. lactifera* are similar to those present in the sporophore which is an indication of this mushroom forming putative EcM association with

the roots of *Shorea robusta*. The EcM of *A. lactifera* in association with any host plant is described here for first time.

Another mushroom, *Inocybe purpureoflavida*, has an irregularly pinnate to coralloid mycorrhizal system, silvery grey to greyish brown to reddish brown surface, subcylindrical to obclavate cystidia with acute to rounded apex, and some of cystidia are metuloid as well. Similar metuloid cystidia were also observed in the sporophore of this mushroom. This species was recorded growing under *Hopea parviflora* Bedd. (*Dipterocarpaceae*) from Kerala, India, and subsequently described as a new species by Vrinda et al. (1997). Later on, a putative EcM status of this mushroom with *Hopea parviflora* was also described but without much details (Pradeep, Vrinda, 2010).

Thus both the presently examined species were described for the first time as EcM associates of *Shorea robusta*. Of the total EcM descriptions published so far, only 16 descriptions are available for different species of *Shorea* in family *Dipterocarpaceae* (Agerer, Rambold, 2004–2020; Roman et al., 2005; Rinaldi et al., 2008). Out of these, as many as 8 descriptions are of *S. robusta* (Kumar, Atri, 2016, 2019, 2020a). Hence, with the present study the number of EcM descriptions for *Dipterocarpaceae* has gone up to 18 and for *S. robusta* up to 10. There are a number of mushrooms which grow in close association with the members of the family *Dipterocarpaceae* in general and *S. robusta* in particular. They need to be investigated for their EcM details so as to understand their role in the growth and survival of this multipurpose tree.

Conclusions

The present study enhances our knowledge of *Shorea* mycorrhizal biology. Both reported species of fungi were found in direct organic connection with *Shorea robusta* roots and there is similarity in hyphal features of their sporophores and the mantle. Hence the presently investigated species, viz. *Asproinocybe lactifera* and *Inocybe purpureoflavida*, are confirmed EcM associates of *Shorea robusta*. 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 *Shorea robusta* seedlings which hardly survive without their EcM associates.

Acknowledgments

We are grateful to the Head of the Department of Botany, Punjabi University, Patiala, for providing laboratory facilities and to the Council of Scientific and Industrial Research (CSIR), New Delhi, India, for financial assistance under the CSIR-JRF Fellowship Scheme.

References

Agerer R. 1986. Studies on ectomycorrhizae III. Mycorrhizae formed by four fungi in the genera *Lactarius* and *Russula* on spruce. *Mycotaxon*, 27: 1–59.

Agerer R. 1987–2002. *Colour Atlas of Ectomycorrhizae*. 1st–15th editions. Schwäbisch Gmünd, Germany: Einhorn Verlag.

Agerer R. 2006. Fungal relationships and structural identity of their ectomycorrhizae. *Mycological Progress*, 5(2): 67–107. https://doi.org/10.1007/s11557-006-0505-x

Agerer R., Rambold G. 2004–2020. DEEMY – An Information System for Characterization and Determination of Ectomycorrhizae. München, Germany. Available at: www.deemy.de (Assessed 4 March 2020)

Alexander I., Selosse MA. 2009. Mycorrhizas in tropical forests: a neglected research imperative. *New Phytologist*, 182(1): 14–16. https://doi.org/10.1111/j.1469-8137.2009.02798.x

Atri N.S., Kaur A., Kour H. 2005. Wild Mushrooms – Collection and Identification. In: *Frontiers in Mushroom Biotechnology.* Eds R.D. Rai, R.C. Upadhyay, S.R. Sharma. Chambaghat, Solan, India: NRCM, pp. 9–26.

Atri N.S., Kaur M., Sharma S. 2017. Characterization of Lamellate Mushrooms – An Appraisal. In: *Developments in Fungal Biology and Applied Mycology*. Eds T. Satyanarayana, S. Deshmukh, B.N. Johri. Springer, Singapore, pp. 471–500.

Bakshi B.K. 1974. *Mycorrhiza and its role in forestry.* [Project report – P.L. 480]. Dehradun: Forest Research Institute, 89 pp.

Champion H.G., Seth S.K. 1968. *A revised survey of the forest types of India*. New Delhi Manager of Publications, Govt. of India, 404 pp.

Frank B. 1885. Ueber die auf Wurzelsymbiose beruhende Ernährung gewiser Bäume durch unterirdishe Pilze. *Berichte der Deutschen Botanischen Gesellschaft*, 3: 128–145.

Guzmán G., Ramirez-Guillan F., Contu M., Rodríguez O., Guzmán-Davalos L. 2004. New records of *Asproinocybe* and *Tricholosporum* (*Agaricales*, *Tricholomataceae*). *Documents Mycologiques*, 33: 23–28.

Heim R. 1970. Breves diagnoses latinae novitatum genericarum specificarum que nuper descriptarum. *Revue de Mycologie*, 34(4): 343–347.

Heinemann P. 1977. Un nouvel *Asproinocybe* (*Tricholomataceae*) du Zaire. *Bulletin de Jardin Botanique National de Belgique*, 47: 265.

Kornerup A., Wanscher J.A. 1978. *Methuen Handbook of Colour*. 3rd ed. London: Eyre Methuen, 252 pp.

Kumar J., Atri N.S. 2016. Characterisation of ectomycorrhiza of *Russula* and *Lactifluus* (*Russulaceae*) associated with *Shorea robusta* from Indian Shiwaliks. *Nova Hedwigia*, 103(3–4): 501–513. https://doi.org/10.1127/nova_hedwigia/2016/0368

Kumar J., Atri N.S. 2018. Studies on ectomycorrhiza: an appraisal. *The Botanical Review*, 84: 108–155. https://doi.org/10.1007/s12229-017-9196-z

Kumar J., Atri N.S. 2019. Characterisation and identification of ectomycorrhizae of *Russula* (*Russulaceae*: *Basidiomycota*) associated with *Shorea robusta*. *Journal of Tropical Forest Science*, 31: 114–124. https://doi.org/10.26525/jtfs2019.31.1.114124

Kumar J., Atri N.S. 2020a. Ectomycorrhizal biology of *Shorea robusta* roots in association with *Russula* Pers. (*Russulaceae*, *Basidiomycota*) from foothills of Himalayas, India. *Annals of Plant Sciences*, 9(4): 3798–3811. https://doi.org/10.21746/aps.2020.9.4.3

Kumar J., Atri N.S. 2020b. New records of lamellate mushrooms associated with Sal from Shiwaliks, India. *Kavaka*, 54: 30–37. https://doi.org/10.36460/Kavaka/54/2020/30-37

Lebel T., Syme K., Barrett M. Cooper J.A. 2020. Two new species of *Asproinocybe* (*Tricholomataceae*) from Australasia. *Muelleria*, 38: 77–85.

Lee L.S., Alexander I.J., Watling R. 1997. Ectomycorrhiza and putative ectomycorrhizal fungi of *Shorea leprosula* Miq. (*Dipterocarpaceae*). *Mycorrhiza*, 7(2): 63–81. https://doi.org/10.1007/s005720050165

Mohan V., Natarjan K., Ingleby K. 1993a. Anatomical studies on ectomycorrhizas. I. The ectomycorrhizas produced by *Thelephora terrestris* on *Pinus patula*. *Mycorrhiza*, 3(1): 39–42. https://doi.org/10.1007/BF00213466

Mohan V., Natarajan K., Ingleby K. 1993b. Anatomical studies on ectomycorrhizas. II. The ectomycorrhizas produced by *Amanita muscaria*, *Laccaria laccata* and *Suillus brevipes* on *Pinus patula*. *Mycorrhiza*, 3(1): 43–49. https://doi.org/10.1007/BF00213467

Mohan V., Natarajan K., Ingleby K. 1993c. Anatomical studies on ectomycorrhizas. III. The ectomycorrhizas produced by *Rhizopogan luteolus* and *Scleroderma citrinum* on *Pinus patula*. *Mycorrhiza*, 3(2): 51–56. https://doi.org/10.1007/BF00210692

Natarajan K., Senthilrasu G., Kumaresan V., Riviera T. 2005. Diversity in ectomycorrhizal fungi of a dipterocarp forest in Western Ghats. *Current Science*, 88(12): 1893–1895.

Pradeep C.K., Vrinda K.B. 2010. Ectomycorrhizal fungal diversity in three different forest types and their association with endemic, indigenous and exotic species in the Western Ghats forests of Thiruvananthapurum district, Kerala. *Journal of Mycopathological Research*, 48(2): 279–289.

Pyasi A., Soni K.K., Verma R.K. 2011. Dominant occurrence of ectomycorrhizal colonizer *Astraeus hygrometricus* of Sal (*Shorea robusta*) in forest of Jharsuguda Orissa. *Journal of Mycology and Plant Pathology*, 41(2): 222–225.

Pyasi A., Soni K.K., Verma R.K. 2013. Effect of ectomycorrhizae on growth and establishment of Sal (*Shorea robusta*) seedlings in central India. *Nusantara Bioscience*, 5(1): 44–49. https://doi.org/10.13057/nusbiosci/n050107

Riviére T., Diedhiou A.G., Diabate M., Senthilarasu G., Natarajan K., Verbeken A., Buyck B., Dreyfus B., Bena G., Ba A.M. 2007. Genetic diversity of ectomycorrhizal basidiomycetes from African and Indian tropical forests. *Mycorrhiza*, 17: 415–428. https://doi.org/10.1007/s00572-007-0117-6

Roman M.D., Claveria V., Miguel A.M.D. 2005. A revision of the descriptions of ectomycorrhizas published since 1961. *Mycological Research*, 109(10): 1063–1104. https://doi.org/10.1017/S0953756205003564

Rinaldi A.C., Comandini O., Kuyper T.W. 2008. Ectomycorrhizal fungal diversity: separating the wheat from chaff. *Fungal Diversity*, 33: 1–45.

Shi N.N., Gao C., Zheng Y., Guo L.D. 2017. Effects of ectomycorrhizal fungal identity and diversity on subtropical tree competition. *Journal of Plant Ecology*, 10: 47–55. https://doi.org/10.1093/jpe/rtw060

Singer R. 1986. *The Agaricales in Modern Taxonomy.* 4th ed. Koenigstein, Federal Republic of Germany: Koeltz Scientific Books, 981 pp.

Singh J.S., Singh S.P. 1992. *Forests of Himalaya*: *Structure, Functioning and Impact of Man.* Naini Tal, India: Gyanodaya Prakashan, 294 pp.

Smith S.E., Read D.J. 2008. *Mycorrhizal Symbiosis.* 3rd ed. Academic Press, New York, 815 pp.

Tapwal A., Kumar R., Borah D. 2015. Effect of mycorrhizal inoculations on the growth of *Shorea robusta* seedlings. *Nusantara Bioscience*, 7: 1–5. https://doi.org/10.13057/nusbiosci/n070101

Tapwal A., Kumar R., Pandey S. 2013. Diversity and frequency of macrofungi associated with wet evergreen tropical forest in Assam, India. *Biodiversitas*, 14(2): 73–78. https://doi.org/10.13057/biodiv/d140204

Tedersoo L., May T.W., Smith M.E. 2010. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza*, 20(4): 217–263. https://doi.org/10.1007/s00572-009-0274-x

Vrinda K.B., Pradeep C.K., Mathew S., Abraham T.K. 1997. *Inocybe purpureoflavida* sp. nov. (*Cortinariaceae*) from Western Ghats of Kerala State, India. *Mycotaxon*, 64: 1–6.

Wang X., Liu J., Long D., Han Q., Huang J. 2017. The ectomycorrhizal fungal communities associated with *Quercus liaotungensis* in different habitats across northern China. *Mycorrhiza*, 27(5): 441–449. https://doi.org/10.1007/s00572-017-0762-3

Zak B. 1973. Classification of ectomycorrhizae. In: *Ectomycorrhizae*. Eds. G.C. Marks, T.T. Kozlowski. New York: Academic Press, pp. 43–78. https://doi.org/10.1016/B978-0-12-472850-9.50008-6

Recommended for publication by V.P. Hayova

Кумар Дж., Атрі Н.С. 2021. **Характеристика та ідентифікація ектомікоризи, утвореної видами родів *Asproinocybe* (*Tricholomataceae*) та *Inocybe* (*Inocybaceae*) з корінням тропічного дерева *Shorea robusta* (*Dipterocarpaceae*).** *Український ботанічний журнал*, 78(2): 112–122 [In English].

Державний коледж імені Раджива Ганді, Чаура Майдан, Шимла 171004, Індія: Дж. Кумар. Факультет біологічних наук і наук про довкілля, Університет Шуліні з біотехнології та управління, Солан 173229, Індія: Н.С. Атрі

Реферат. Під час досліджень ектомікоризних грибів, проведених у тропічних лісах передгір'я Гімалаїв (Індія), були знайдені види двох родів агарикоїдних грибів – *Asproinocybe* та *Inocybe*, органічно пов'язані з корінням салового дерева (*Shorea robusta*). У результаті цих досліджень види роду *Asproinocybe* були вперше виявлені в Індії. Крім того, було вперше проведено вивчення морфолого-анатомічних особливостей мікоризних коренів *Shorea robusta*, асоційованих із *Asproinocybe lactifera* та *Inocybe purpureoflavida*. Встановлено, що ектомікоризи, утворені цими двома видами грибів, відрізняються за формою та кольором, текстурою поверхні, розміром і формою цистид, типом мантії, а також різними хімічними реакціями. *Asproinocybe lactifera* утворює моноподіальну пірчасту мікоризу з пухкою ватоподібною поверхнею, темно-коричневого до червонувато-коричневого кольору, тоді як у *Inocybe purpureoflavida* вона неправильно пірчаста до коралоїдної, має щільно-повстисту поверхню і сріблясто-сіре до червонувато-коричневого забарвлення. Зовнішній шар мантії у *Asproinocybe lactifera* неоднорідний, з обернено-булавоподібними до шилоподібних цистидами, тоді як для *Inocybe purpureoflavida* характерна зовнішня мантія плектенхімної структури з субциліндричними до обернено-булавоподібних, метулоїдними чи неметулоїдними цистидами. Наявність у мантії судиноподібних гіф із молочним соком є унікальною особливістю *Asproinocybe lactifera*, невластивою для *Inocybe purpureoflavida*.

Ключові слова: гриби, ектомікориза, мантія, плодові тіла, ризоморфи, салове дерево, сітка Гартіга, Шивалік (Сивалік)