

Full Length Research Paper

Additions to ectomycorrhizae associated with *Populus ciliata* Wall. Ex Royle from Pakistan

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Ectomycorrhizal roots sampled from the mycorrhizosphere of *Populus ciliata* from Himalayan moist temperate forests of Pakistan were characterized based on standard methodology for morphological and anatomical characters which include: morphology of ectomycorrhizal system, structure of mantle layers in plain view, structure of rhizomorphs, shape of cystidia and features of emanating elements. Two distinct types of ectomycorrhizae were found and briefly described. Characteristic features of these ectomycorrhizae were dichotomously branching pattern, pseudoparenchymatous mantle and branched emanating hyphae with septations. The two new types of ectomycorrhizal morphotypes are found undescribed previously and are given tentative names until their mycobiont is identified.

Key words: Deciduous, ectomycorrhiza, Himalayas, morphological, anatomical description.

INTRODUCTION

Ectomycorrhizal (ECM) symbiosis involves a number of plants and fungal taxa world wide. From the last 50 years, research on ectomycorrhizae has evolved greatly. Numerous descriptions of ectomycorrhizae have been published from all over the world. In 2005, de-Roman et al. reviewed 479 articles and concluded that the number of different morphotypes described was 814.

Among these, only 345 ECM were identified up to species level and 99 were unidentified ECM morphotypes published under a binomial name (Agerer, 1986, 1987-2008, 1994, 1999) or other name widely used in the literature (morphotypes AD and SB; Giraud, 1988), and 188 were unidentified ECM morphotypes lacking a name, 379 descriptions included drawings and 771 provided photographs.

The literature regarding ECM from Asia is scanty. Only few ECM have been reported from Pakistan in recent years. Up till now, from Pakistan there were 52 ECM morphotypes published, in either identified or unidentified

form from Pakistan (Afshan et al., 2003; Khalid and Niazi, 2003; Kazmi et al., 2004; Niazi et al., 2006, 2007, 2008, 2009, 2010; Hanif et al., 2012; Jabeen et al., 2012; Sarwar et al., 2011, 2012; Ashraf et al., 2012).

In the present investigation, 2 different types of ectomycorrhizae associated with Himalayan Poplar (*Populus ciliata* Wall. ex Royle) were isolated and characterized morpho-anatomically using Agerer's Methodology (Agerer, 1999) based on different hyphal features. The morpho-anatomical study is informative and widely used in structural identity and to recognize fungal relationships.

MATERIALS AND METHODS

Sampling site description

The sampling sites lies in Himalayan moist temperate forests of Pakistan. These forests contain dominant stands of *Abies pindrow*

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Royle which dominated, with varying stands share of *Pinus wallichiana* A.B.Jacks., *Cedrus deodara* (Roxb. ex Lambert) G. Don, *Picea smithiana* Boiss, *Taxus wallichiana* Zucc. along with intermixture of deciduous or broad leaf trees including different species of *Salix* L., *Quercus* L. and *Populus* L.

Sampling, cleaning and photography of ECM morphotypes

Selected sampling sites were visited periodically during rainy season (July-August), 2008-2009. Soil cores of size 10 cm³ were excavated with the help of digger at 10 to 90 cm away from tree trunk. Four samples per tree were collected and brought to the laboratory in polythene bags with proper labeling. Each soil block was dipped in water for overnight to loosen the soil particles and then transferred to the sieve of 3 mm pore size and further cleaned under shower. All fine roots were isolated from the soil after washing and then sorted into morphotypes under incandescent bulb. Adhering soil particles were removed under stereo-microscope with the help of squirrel hair brush and then photographed with camera associated with stereo-microscope. These morphotypes were stored in McCartney bottles for further process identification.

Morphological and anatomical characterization of ectomycorrhizae

Selected morphotypes systems belonging to each specimen were analyzed following methodology described in Agerer (1991). The following characteristics were observed under stereo microscope morphologically by examining branching pattern, branch shape, mantle color and texture, presence of emanating elements (rhizomorphs and emanating hyphae, cystidia) (Agerer, 1991).

During anatomical characterization, characteristics of emanating hyphae and rhizomorphs and structure of the mantle in terms of hyphal arrangement, hyphal dimensions, presence of specialized cells and mantle layering layers were analyzed (Agerer, 1991, 1987-2008) and drawn using Camera Lucida.

Identification of Ectomycorrhizae

All morpho-anatomical characters were then compared with the available literature. In general, all anatomical features that include hyphae can be applied to characterize ECM (Agerer, 1991, 1987-2008; Agerer and Rambold, 2004-2005), but structure of mantle layers, structure of rhizomorphs, presence and shape of cystidia and features of emanating hyphae are of utmost importance. The sequence of these characters indicates the decreasing taxonomic importance in identification of ECM. For unidentified ECM morphotypes, a binomial nomenclature system created by Agerer was followed to give tentative names to these mycorrhizae (Agerer, 1987-2008).

RESULTS

Populirrhiza epidermoida (Figure 1)

Morphological characters

Ectomycorrhizal system dichotomously branched, up to 6

mm long, with 0.6 mm thick main axis, unramified tips 2.4 mm long and about 0.5 mm thick, color of system is dark brown, younger tips are honey brown, rounded, surface of mycorrhizal system is smooth, host tissue is visible under mantle surface. Rhizomorphs is common, attached at restricted points, thick, dark brown to black, branched. *Emanating hyphae* are rare, brownish and straight.

Anatomical characteristics of mantle in plan views

Mantle is pseudoparenchymatous in all layers. Outer mantle layer pseudoparenchymatous, (type M, Agerer, 1987-2008); cells are irregular to epidermoid in shape, 10 µm in diameter and 11.5 µm in length, color of cells walls are light brown, no cell content is observed, no septa and clamp connections were presents. Inner mantle layer is pseudoparenchymatous (type M, Agerer, 1987-2008); cells are smaller than in the outer mantle, 11 µm in diameter and 11.3 µm in length, color of cells is honey brown, no matrix material, no septa and clamps.

Anatomical characteristics of emanating elements

Rhizomorphs is differentiated (type B, Agerer, 1987-2008); septa is present, cells are 4 µm in diameter, 23 µm in length, cells have thin wall, septa is common, clamps and clamp septa are absent. *Emanating hyphae* is branched, septate, septa are common, cells are long, wall is thick, septa is also thick, hyphae is smooth, cell are 6 µm in diameter, 43 µm in length.

Materials examined

Pakistan, Khyber Pakhtunkhwa, Khaira Gali, 2347 m a.s.l., ectomycorrhizal under *Populus cillata* Wall., 19 June 2008, Sarwar S. B. # 33ECM (Lah0608).

Populirrhiza himalayensis (Figure 2)

Morphological characters

Ectomycorrhizal system is dichotomous, 2.4 to 4 mm in length and 0.3 mm thick. Unramified ends straight to beaded, brown to black, 0.4-1.0 mm in length and 0.1-0.2 mm in diameter. Width of basal portion of tips is 0.2-0.4 mm and that of apical portion ranged from 0.4-0.5 mm. Tips of olive is yellow to brown, older mycorrhizae is grayish white to black. Mantle surface is woolly with many abundant emanating hyphae arising commonly from mantle.

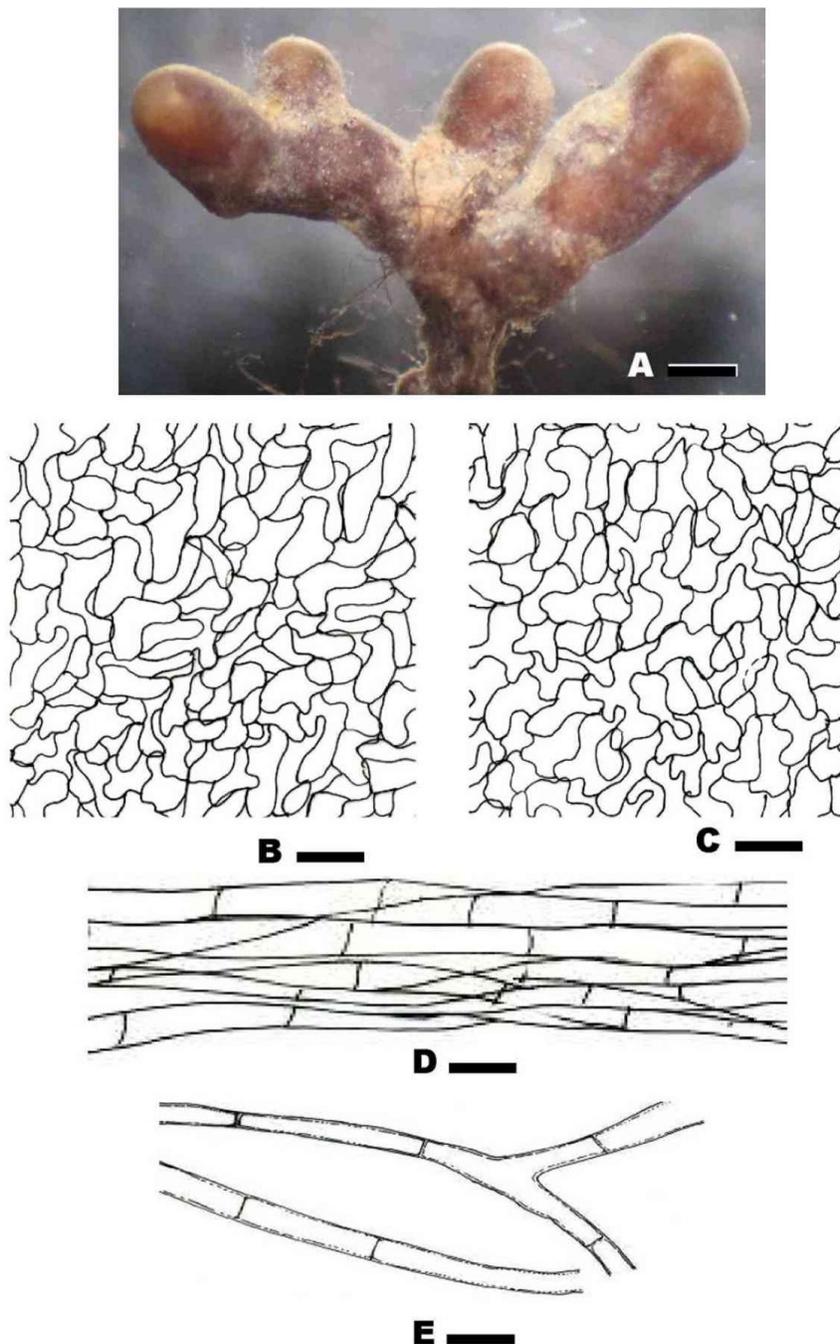


Figure 1. *Populirhiza epidermoida*. A, ECM (habit) showing important features; B, parenchymatous outer mantle; C, parenchymatous inner mantle; D, rhizomorph; E, emanating hyphae. Scale Bars: for A = 0.15 cm; B = 14 μ m; C = 11 μ m; D = 11.5 μ m; E = 17 μ m.

Anatomical characters of mantle in plan views

Outer mantle layer pseudoparenchymatous (type N, Agerer, 1987-2008), cells is angular to roundish with a

hyphal net. Cells of outer mantle is hyaline to olive yellow, 3.8-5.7 μ m in length and 2.4-3.3 μ m in diameter. Hyphae is densely arranged, rarely branched, olive yellow with smooth surface, hyphal width is upto 3.5 μ m.

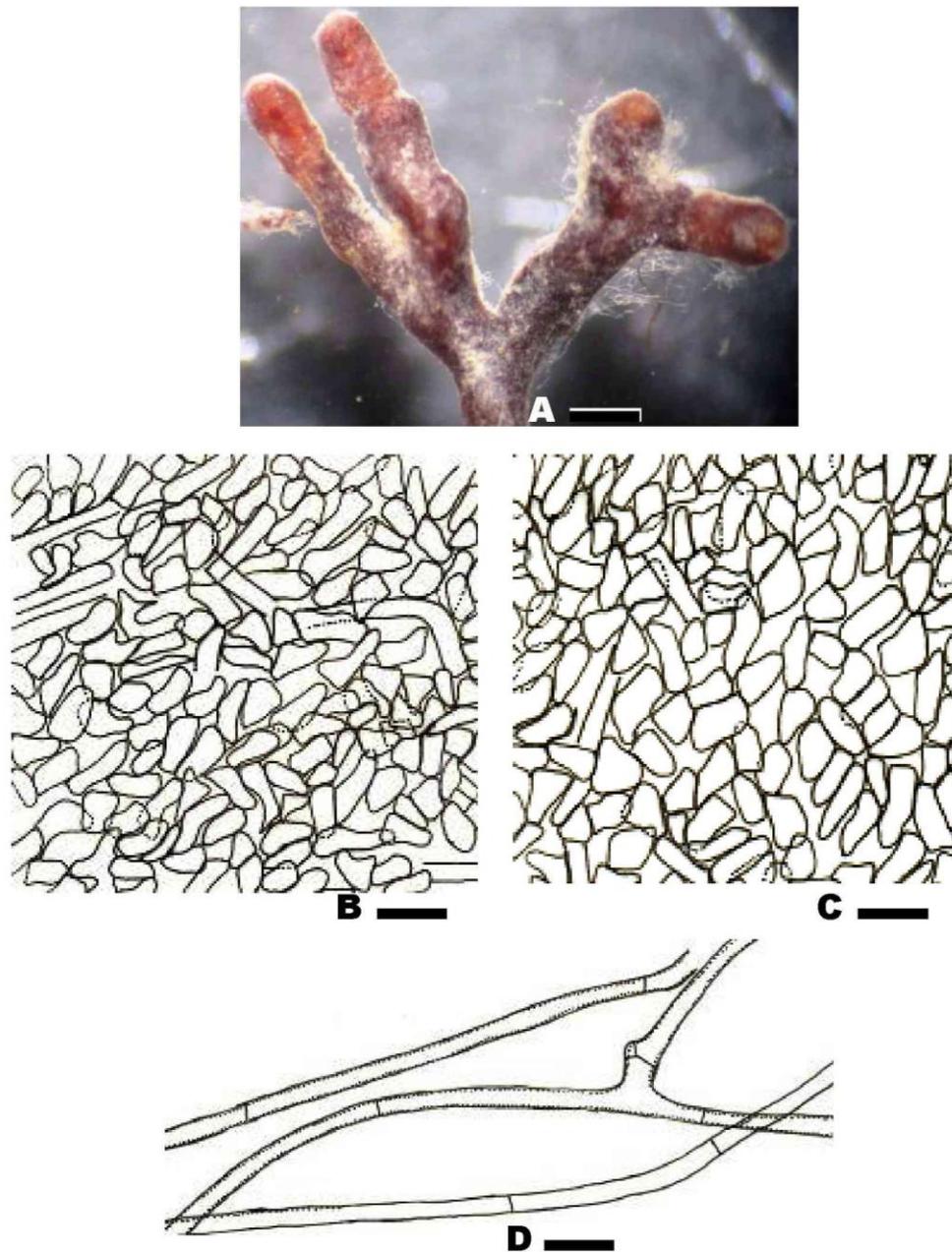


Figure 2. *Populirhiza himalayensis*. A, ECM (habit) showing important features; B, parenchymatous outer mantle; C, parenchymatous inner mantle; D, emanating hyphae. Scale Bars: for A = 0.05 cm; B = 9.5 μm ; C = 7 μm ; D = 12 μm .

Anastomosis and clamp connection was not observed among hyphae. Inner mantle layer pseudoparenchymatous (type N, Agerer, 1987-2008), cells are angular to roundish, with a hyphal net. Cells of inner mantle are hyaline to olive yellow, 3.8-4.5 μm in length and 1.8-2.9 μm in diameter. Hyphae are densely arranged, rarely branched, olive yellow with smooth surface, hyphal width is up to 2.8 μm . Anastomosis and clamp connection is

not observed.

Anatomical characteristics of emanating elements

Emanating hyphae hyaline has smooth surface, branched, septate, straight, hyphal width upto 3.1 μm , cell length is 53.2 μm , clamp connections are absent.

Material examined

Pakistan, Khyber Pakhtunkhwa, Helipad, 2350 m a.s.l., ectomycorrhizal under *Populus ciliata* Wall., 8th August 2009, Sarwar S.B. # 10ECM (Lah 0809).

DISCUSSION

Populirrhiza epidermoida and *Populirrhiza himalayensis* were found associated with roots of *Populus ciliata* where basidiomata of boletes were found in close proximity in Khaira Gali and Halipad Khanspur, respectively. These ECM morphotypes were characterized by pseudoparenchymatous mantle and branched septated emanating hyphae with septation. Both are different in having types of ectomycorrhize different in presence of smooth mantle surface, differentiated rhizomorphs in *P. epidermoida* while and wooly mantle surface of in *P. himalayensis* had wooly mantle surface and no rhizomorphs.

In previous studies, ECM fungal partner *Lactarius bicolor* Masee of *Populus* sp. has been reported (Pandey et al., 2007; Kemppainen et al., 2008). *Tomentella subtestacea* Bourdot and Galzin, *Tomentella pilosa* (Burt) Bourdot and Galzin, *Russula amoenolens* Romagn., *Lactarius controversus* (Pers.) Pers., and *Scleroderma bovista* Fr. were found associated with *Pityriasis alba* (Agerer, 1987-2008) but their ECM have either cystidia or laticifers. ECM of *Russula medullata* Romagn., was found to be associated with *Populus tremula* L. but had cystidia (Beenken, 2001). Jabeen et al. (2012) reported 18 different types of ECM associated with *Populus* spp. from Pakistan but these morphotypes are different from those in their morphology as well as anatomy.

Morphotypes of *Populirrhiza epidermoida* are distinguished from all the ECM described earlier in the presence of a particular mantle type M with epidermoid cells (Agerer, 1987-2008). These cells are characteristic feature of ECM of *Russula lepida*. But other morpho-anatomic characters distinguish these morphotypes from those of *R. lepida*. On the other hand, ECM morphotypes of *Populirrhiza himalayensis* consist of a particular type of morphology with dichotomous branching pattern and N type Mantle mantle (Agerer, 1987-2008). In most of the ECM of *Populus* spp. simple to irregular type of branching was observed (Jabeen et al., 2012). Presence of hyphal net in mantle layers also distinguish character of *Populirrhiza himalayensis* from other genera ectomycorrhizae in *Populus* spp.

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