

# New Ectomycorrhizas in association with Poplar from Himalayan moist forests of Pakistan

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## Abstract

*Populinirrhiza pinnata* and *populinirrhiza khanspurensis* are described and illustrated as new ectomycorrhizas from Himalayan moist temperate forests of Pakistan in association with the root system of *Populus ciliata*. *Populinirrhiza pinnata* has monopodial pinnate type of ramification. The young mycorrhizas are dark brown while the older ones are black. White sugary crystals are present on mycorrhizal system. Emanating hyphae surround mycorrhizas. Rhizomorphs are thick and branched. *Populinirrhiza khanspurensis* has a simple to monopodial type of ramification. The color of the young mycorrhiza is brown; some times with dark tips while the older ones are dark brown. Rhizomorph and emanating hyphae are absent. As so far no fungal partner of these mycorrhizas has been identified, these fall under the category of 'unknown' and 'unidentified' mycorrhizas.

**Key words:** *Populus ciliata*, *Populinirrhiza pinnata*, *Populinirrhiza khanspurensis*, Ramification, Salicaceae, Himalayan poplar.

## Introduction

*Populus* L. belongs to Salicaceae and is represented by 6 species in Pakistan. (Sheikh, 1993). Salicaceae has been reported as ectomycorrhizal. (Agerer, 1997). *Populus ciliata* Wall.ex Royle also known as Himalayan Poplar, a large deciduous tree 18-21m tall with diameter of 6-8m. The trunk is erect and the crown spreading.

The tree is native to the Subcontinent, Northern and the Central Asia and North America. In Pakistan, it is distributed at 2000-3000m above the sea level. It is common in mixed deciduous coniferous forests Dir, Swat, Chitral, Astore, Gilgit, Balti, Hazara, Murree hills and Kashmir (Stewart, 1972). During the survey of ectomycorrhizas of the trees of Himalayan temperate forests of Pakistan, two different kinds of mycorrhizas associated with the root system of *Populus ciliata* were found. These mycorrhizas seem to be undescribed previously.

Many types of identified and unidentified ectomycorrhizas occur with the root systems of plants of different families. Binomials are given to the mycorrhizas described, as the mycobionts are unknown. Tentative names (binomial) are given till such times the mycobionts are known.

## Materials and Methods

### 1. Collection and isolation of Ectomycorrhizas

The blocks of soil along with fine root system of *Populus ciliata* were dugout with the help of a digger. To check loss of water, the blocks were

wrapped with polythene bags and brought back to the laboratory. The soil samples were treated as follows:

**a) Washing:** The soil samples were soaked in water for 24 hours. This loosened the soil. The attached soil particles were removed with the help of camel hairbrush. Roots were magnified under stereomicroscope for better vision. To wash the roots more cleanly, Max washing liquid was used.

**b) Selection:** The ectomycorrhizal roots having special morphology, colour and ramification pattern were selected. Selected roots were studied morphologically under stereomicroscope and illustrations were made.

### 2. Morphological studies

Morphological studies were primarily concerned with the identification and classification of mycorrhizas. These were observed following Agerer (1991a,b).

### 3. Anatomical studies

**a) Section cutting:** Freehand sections of fine fresh roots were cut with the help of a sharp razor. The fine sections were selected and mounted in 0.05 % trypan blue in lactophenol. The sections were mounted on the glass slides and labeled. A drop of trypan blue was added and covered with a cover slip. These sections were examined microscopically. Following observations were recorded:

- i) % Frequency of occurrence of ectomycorrhizal sheath around cut section
- ii) Thickness of sheath was measured

iii) Penetration of hyphae of ectomycorrhizal fungus into cortical cells was noted. Fine root sections were photographed.

b) **Mantle preparation:** Outer and inner mantle surface views were prepared and illustrated with Camera Lucida

## Results

### *Populinirrhiza pinnata*

#### 1. Morphological characteristics (Plate 1A)

Ramification of ectomycorrhizal system was monopodial pinnate but dichotomies were also observed. Length of the mycorrhizal system was 10mm and the diameter of the main axis was 0.6mm. Unramified ends were 2mm long and their diameter was 0.4 to 0.6mm. The color of the mycorrhizal system was dark brown to black. The unramified ends were mostly straight but a few bent ends were also observed. White sugary crystals were found on the mantle surface. Mantle surface was rough and grainy.

A number of emanating hyphae were present surrounding the mycorrhizal system in some cases. In few places root tips appeared woolly due to extensive hyphal growth on these tips.

Rhizomorphs were also present. These were very thick and branched, originating from the restricted points as well as from the base of the system.

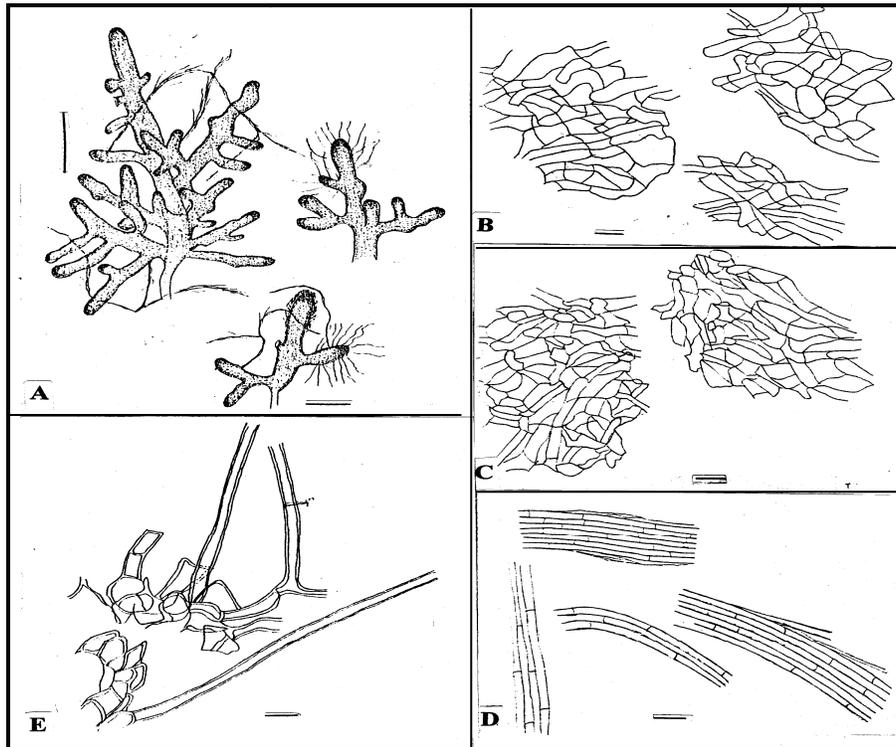
#### 2. Anatomical characteristics

Surface view of the mantle was grainy in appearance. Mycorrhizal sheath was 40µm thick. Frequency of occurrence of sheath around root cross section was 100%. Hyphae penetrated the cortex of the root. Hyphae formed a plectenchymatous mass in the mantle. Mantle surface was shiny due the presence of white sugary crystals.

#### Mantle Preportion

##### i) Outermost layer (Plate 1B)

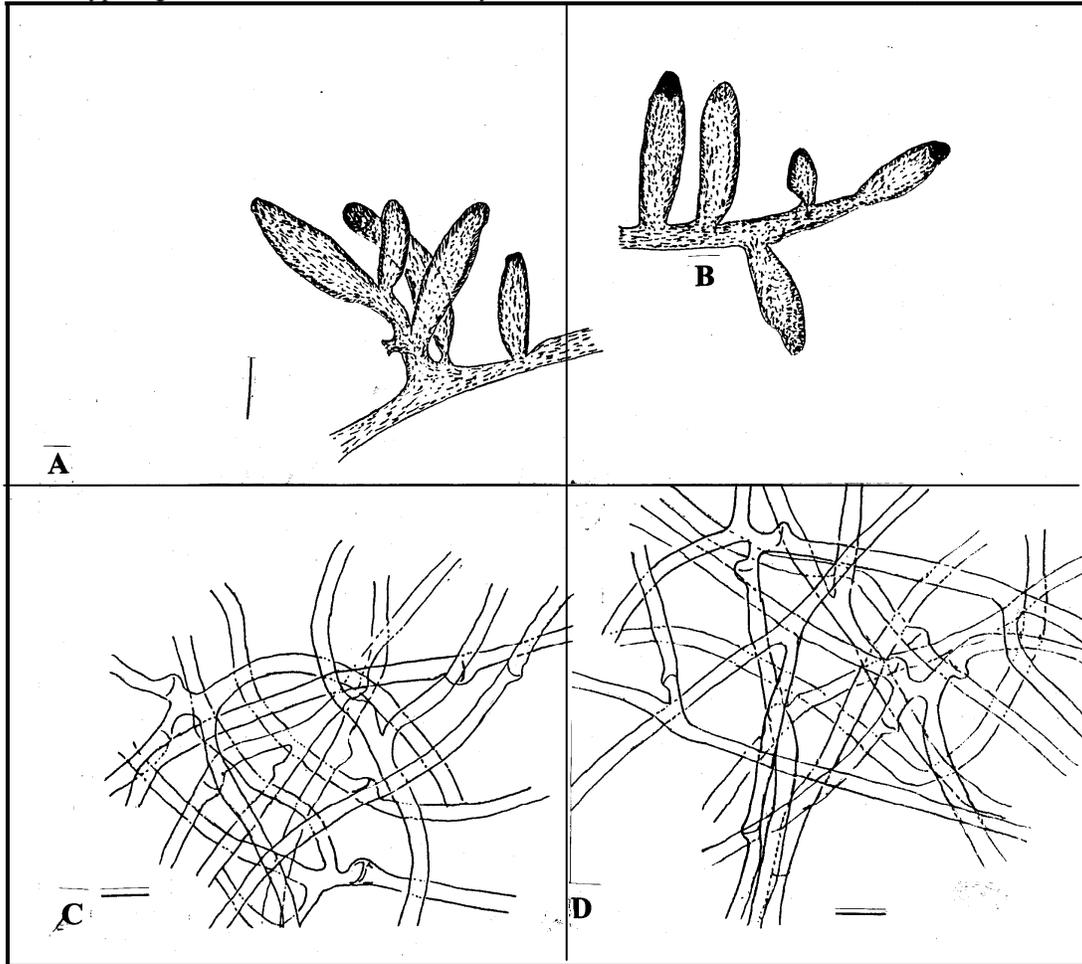
In plane view, the mantle surface was found to be a transitional type between the plectenchymatous and pseudoparenchymatous in which irregular shaped hyphae form a coarse net (Agerer, 1991) Hyphae were simple, thin walled and hyaline. Septation was present but near the point of origin. Septa were simple and there were no clamp connection in hyphae.



**Plate 1A-D:** A. *Populinirrhiza pinnata* (Habitat) showing rhizomorph originating from restricted points, and emanating hyphae at certain tips. B. Plane view of outer mantle surface. C. Plane view of inner mantle surface. D. Rhizomorph (different views). E. Emanating hyphae originating from mantle surface. Bar = 20 mm and 8 mm for A; 5 µm for B, C and D; 8 µm for E.

ii) Innermost layer (Plate 1C)  
 Inner layer of mantle was similar to outer layer but hyphal growth was more than outer layer.

Hyphae were almost of same size as that of outer layer. Clamps were absent. Hyphae were almost roundish.



**Plate 2A-D:** A-B. *Populinrhiza khanspurensis* (Habitat), C. Plane view of outer mantle surface. D. Plane view of inner mantle surface. Bar = 12 mm for A & B; 10  $\mu$ m for C & D.

#### Rhizomorph (Plate 1D)

Rhizomorphs were undifferentiated with rather smooth margins; hyphae were compactly arranged and were of uniform diameter. A few hyphae were protruding out of the rhizomorph, Rhizomorphs were mostly originating from the restricted points as well as from the base of the mycorrhizal system.

#### Color reactions

Aniline (Blue), Trypan blue. (Blue), KOH. (No reaction), Lactic acid. (No reaction), Phenol (orange).

#### *Populinrhiza khanspurensis*

1. Morphological characteristics (Plate A,B)  
*Populinrhiza khanspurensis* is characterized as simple but some times monopodial type of ramification. The color of the mycorrhizal system

was brown. Some times dark brown color was also observed in older system. A few tips of the mycorrhizal system were dark. Mantle surface was grainy in appearance but the older mycorrhizal systems had whitish surface due to the over growth of white coloring mantle hyphae. Length of the mycorrhizal system was 4mm and the diameter of the main axis was 0.3 mm. Unramified ends were 3mm in length and their diameter was 0.3 mm. Some times the tips of unramified ends were narrow and sharp. Surface of the mycorrhizal system was shiny. Rhizomorph and emanating hyphae were absent.

#### 2. Anatomical Characteristics

The frequency of occurrence of mycorrhizal sheath around the cross section was 100% and the

sheath was 23.6µm thick. The Hartig net was present through out the cortical region.

i) Mantle Preparation

Outer mantle view (Plate 2C)

Outer surface of the mantle was consisting of loosely woven hyphae forming a net like arrangement. The hyphae were branched and septate. Clamp connections were present and septation was also at point of clamp formation. Hyphae were of same diameter.

ii) Inner mantle view (Plate 2D)

The hyphae of the inner surface were same as that of outer but were in greater in number forming pseudoparenchymatous mass. These hyphae were also branched and often formed rings. They also had clamp connections and true septa at clamp formation.

Host plant: *Populus ciliata* Wall. ex Royle

Locality: Khanspur (NWFP)

Specimen no: ARN 300A,B.

## Discussion

Ectomycorrhiza *Populinirrhiza pinnata* is characterized by monopodial pinnate type of ramification. The color of the ectomycorrhiza is dark brown to black. Emanating hyphae are present. Rhizomorphs are branched and dark brown in color. Some times unramified ends are woolly due to the extensive growth of hyphae at the tip of mycorrhizal system.

So for ectomycorrhiza of *Populus nigra* L., formed by *Inocybe mfulcomarginata* has been reported (Agerer, 2003). The newly reported mycorrhiza has no resemblance with the mycorrhizas of *Populus nigra*. However *P.pinnata* differs from the already described mycorrhiza *Inocybe mfulcomarginata* in type of ramification i.e., *I. mfulcomarginata* has irregularly pinnate mycorrhizal system. Hyphae are without clamp connections and the color is dark brown to black. *Populinirrhiza pinnata* closely resembles morphologically with *Genea hispidula* Berk et Br., isolated from the host *Fagus sylvatica* (Agerer, 2003). Both mycorrhizas have monopodial type of ramification and are similar in color, and thick emanating hyphae, but the difference lies in the rhizomorph, present in *Populinirrhiza pinnata* which is thick and branched but absent in *Genea hispidula*.

*Populinirrhiza pinnata* closely resembles with another unidentified mycorrhiza of *Fagus sylvatica*, which is *Fagirrhiza spinulosa* (Agerer

.2003) due to ramification i.e. both have monopodial type of ramification, dark brown to black color and thick emanating hyphae. Both have plectenchymatous dense mantle. Presence of cystidia separates *Fagirrhiza spinulosa* from *Populinirrhiza pinnata*. In addition, both form association with different hosts.

*Populinirrhiza khanspurensis* has its own identity, due to simple ramification system but some times with monopodial type, light brown color, tips are dark brown, grainy surface, some times whitish due to overgrowth of mantle hyphae and clear clamp connections are also present. *Populinirrhiza khanspurensis* is different from already described mycorrhizas due to its ramification pattern and presence of emanating hyphae and thick rhizomorphs in *P.pinnata* which are absent in *P. khanspurensis*.

These mycorrhizas are reported for the first time with the root system of *Populus ciliata* from Pakistan. No mycorrhiza has been reported earlier with this host. So these mycorrhizas are new and "unknown". These also fall in the category of unidentified.

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