

New ectomycorrhizas from Sakesar Hills

N. Afshan, A.N. Khalid and A.R. Niazi

*Department of Botany, University of the Punjab, Quaid-e-Azam Campus,
Lahore. 54590, Pakistan*

Abstract

The Sakesar hills fall in Dry subtropical submountaneous forest of salt range. The highest peak of this range is 1, 522m above sea level. Conifers especially *Pinus roxburghii* and *Pinus wallichiana* occupy the upper altitudinal zone of these hills. During the investigation of pines of Sakesar hills for the exploration of diversity of ectomycorrhizas, twenty-two root samples from the rhizosphere of these trees were taken from different sites of PAF Base, Sakesar. This investigation revealed fifteen different kinds of ectomycorrhizas. The isolated mycorrhizas are morphologically and anatomically described and are given tentative binomials considering each mycorrhiza as a distinct entity. These ectomycorrhizas fall in the category of "unidentified" mycorrhizas, as their possible mycobionts are not known.

Key Words: Sakesar hills, ectomycorrhizas, pines, PAF Base, *Pinus roxburghii*, *Pinus wallichiana*, Salt Range.

Introduction

Sakesar hills fall in Dry subtropical submountaneous forests of salt range in Pakistan. In these forests of Salt range (Sakesar hills) where Pines grow, temperature, rainfall, and number of rainy days vary greatly and these factors significantly affect the forest types and especially underground mycoflora. These areas receive appreciable amount of rainfall during wet months. This precipitation supports herbaceous vegetation on forest floor and rainfall along with high temperature make the environment conducive for the growth of mushrooms and other macrofungi. Most of these mushrooms /macrofungi form mutualistically symbiotic association with the root systems of pines called ectomycorrhiza. Ectomycorrhiza is a very diverse group of fungi. More than 5,000 fungal species form ectotrophic mycorrhizae throughout the world (Marx, 1991).

Ectomycorrhizas (ECM), the symbiotic organs of fungi and roots of needle and broad-leaved trees create distinct anatomical features (Agerer, 2003). All forest trees especially pines are completely dependent on ectomycorrhizal association (Wiemken et al., 2001).

The taxonomy of ECM fungi is based almost exclusively on characteristics of their sexual fruiting structures, which are produced intermittently in response to seasonal changes in environmental stimuli such as rainfall and temperature. (Brundrett *et al.*, 1996). The ectomycorrhizas whose possible mycobionts are not definitely known fall in the category of "unknown and unidentified". The unidentified

mycorrhizas are given binomials (Gronbach and Agerer, 1986).

The Sakesar hills are investigated very first time for the occurrence of these ectomycorrhizas. During this investigation, twenty-two samples were taken from the rhizosphere of *Pinus roxburghii* Sargent and *Pinus wallichiana* A.B. Jackson, from which fifteen different types of ectomycorrhizas were isolated, characterized and given tentative binomials until their mycobionts are unknown. Occurrence of these mycorrhizas with two Pines indicates the diversity and richness of fungal species in these forests of Pakistan.

Materials and Methods

Description of the sampling sites

Different sites of PAF Base, Sakesar, were visited to collect root samples of *Pinus wallichiana* and *Pinus roxburghii* for investigation of ectomycorrhizas.

Conifers especially *P. roxburghii* and *P. wallichiana* occupy the upper altitudinal zone of Sakesar hill. In area of Salt Range the dry season is long, rainfall takes place mostly in monsoon months of June to September and slightly in winter season. In these localities annual rainfall ranges from 10-36 inches. Humidity is generally low falling to about 15% in the summer afternoon. Frost may occur especially at the upper altitudinal limits. Mean annual temperature in these forests lies between 15°C and 22°C. Maximum temperature may go up to 37.7°C. The vegetation of Salt Range comprises of two dominant species i.e., *Olea cuspidata* Wall. ex DC. and *Acacia modesta* Wall., other species found in these forests are

Dodonaea viscosa (L.) Jacq., *Monotheca buxifolia* (Falc.) Dcne. ex Engler, *Gymnosporia royleana* (Wall.) Lawson, *Adhatoda vasica* Nees non L., *Pistacia integerrima* J.L. Stewart ex. Brandis, and at certain places groves of *Nannorrhops ritchieana* H. Wendl. are present. However, vegetation varies with the variations of height. The upper part of the Sakesar hill, from where the sampling was done is occupied by pines (*P. roxburghii*, *P. wallichiana*) intermixed with other tree species like *Quercus* spp., *Prunus domestica* L., *Pyrus malus* L., *Punica granatum* L., *Prunus armeniaca* L., *Prunus persica* (L.) Batsch, *Piper nigrum* L., etc., at lower height. *Pinus roxburghii* is dominant tree species, while *P. wallichiana* grows intermixed with *P. roxburghii* at the highest elevations (Hussain, 1995).

Sampling

Soil samples containing the mycorrhizal roots to be examined were taken either with intention of identifying the specimens by tracing rhizomorph or hyphal connections to fruit bodies (identification process) or with the intention of characterizing as many ectomycorrhiza as possible in order to obtain a picture of the "species" composition of ectomycorrhiza within the soil (blind probes).

A) Collection of Soil Samples

A sharp, robust knife with a long blade was used to take soil blocks cores to a depth of 10 cm. The blocks were wrapped in polythene bags to avoid evaporation and crumbling.

B) Designation

All samples of ectomycorrhiza were given special designations (collection No.), which allow referral to all observations from one ectomycorrhizal collection.

C) Washing roots

The soil blocks were soaked in tap water to loosen the adhering soil particles. The attached soil particles were removed with the help of camel hairbrush under a stereomicroscope. These washed roots were kept in small bottles with a few drops of washing liquid (Max-Colgate Palmolive

Pakistan Ltd.) to wash the roots more clearly. The roots were rinsed with distilled and sterilized water.

D) Selection

The ectomycorrhizal roots having specialized branching patterns were selected. The selected roots were photographed using macrolens and stereomicroscope.

Preparation of ectomycorrhiza

Morphological characterization

Morphological characterization was carried out on freshly isolated material, using lamps of day light quality and a black background. The purpose of morphological study was to characterize definitely recognizable mycorrhizal types and to relate these to different species of fungal symbionts for taxonomic purposes. Morphological characters were observed following Agerer (1990).

Anatomical studies

i) Fixation

The roots selected for anatomical studies were fixed in F.A.A. (formaline, acetic acid, alcohol; 5:5:90) in sample bottles. These fixed roots were used for section cutting.

ii) Section cutting

Free hand sections of fine root pieces were cut with the help of sharp razor. The fine sections were selected and mounted in 0.05 trypan blue in lactophenol. The sections were transferred to glass slide and labeled. A drop of trypan blue was added to the cross section and covered with a cover slip. These sections were examined and photographed microscopically.

Preparation of plane views

Mantle and rhizomorph preparations were made from freshly isolated material. Mantle preparations were prepared while observing the structure through a dissecting microscope at magnification of 25 or 50X and were traced using camera lucida and compound microscope. All features were checked in the fresh conditions (Agerer, 1990).

Results

Name of ECM	Morphology	Outer Mantle	Inner Mantle	Rhizomorph	Emanating Hyphae	Color reactions	Host Plant
<i>Pinirrhiza argentiana</i>	Dark brown to black; branching dichotomous irregularly pinnate; tips beaded; silvery and woolly. (Plate.1A)	Plectenchymatous; hyphae irregularly arranged (Plate.3A)	Plectenchymatous; hyphae irregularly arranged (Plate.3B)	Brown; branched; undifferentiated; 6.5-6.7 um wide. (Plate.3C)	Branched; 8.2 um wide. (Plate.3D)	Phenol-black lactic acid-n.r trypan blue-blue 10% KOH-yellow	<i>Pinus roxburghii</i>
<i>Pinirrhiza pinnata</i>	Blackish brown; irregularly pinnate to monopodial pinnate; bent; woolly. (Plate.1B)	Plectenchymatous; hyphae loosely arranged. (Plate.3E)	Plectenchymatous; hyphae loosely arranged. (Plate.3F)	Branched; differentiated; 5.6um wide. (Plate.3G)	Yellowish brown; branched; 6.5um. (Plate.3H)	Phenol-black lactic acid-yellow trypan blue-blue 10% KOH-black	<i>Pinus roxburghii.</i>
<i>Pinirrhiza nigra</i>	Black With dark brown swollen tips; simple to dichotomous; bent; grainy & glistening. (Plate.1C)	Plectenchymatous; clustered in a net like arrangement. (Plate.3I)	Plectenchymatous; arranged in a haphazard manner (Plate.3J)	Present in low frequency; branched; differentiated; 5-7 um. (Plate.3K)	Rarely branched; 6.8um. (Plate.3L)	Phenol-brown lactic acid-yellow trypan blue-blue 10% KOH-yellow	<i>Pinus roxburghii.</i>
<i>Pinirrhiza elongata</i>	Blackish brown; dichotomous; tips mostly straight, few bent; short spiny. (Plate.1D)	Plectenchymatous; hyphae loosely arranged. (Plate.3M)	Plectenchymatous.; hyphae irregularly arranged. (Plate.3N)	Unbranched; differentiated; 6-7um. (Plate.3O)	White; rarely branched; 11.7um. (Plate.3P)	Phenol-purple lactic acid-brown trypan blue-blue 10% KOH-brown	<i>Pinus roxburghii</i>
<i>Pinirrhiza dichotoma</i>	Dark brown to black with brown swollen tips; dichotomous; tips bent; short spiny. (Plate.1E)	Plectenchymatous; irregularly arranged forming a net (Plate.3Q)	Plectenchymatous; haphazardly arranged forming a net. (Plate.4A)	Absent.	Brown; rarely branched; 11.9um. (Plate.4B)	Phenol-purple lactic acid-n.r Typan blue-blue 10% KOH-n.r	<i>Pinus roxburghii.</i>
<i>Pinirrhiza monopodata.</i>	Blackish brown; monopodial pinnate; tips mostly straight, a few slightly bent; short spiny. (Plate.1F)	Plectenchymatous; hyphae irregularly arranged. (Plate.4C)	Plectenchymatous; hyphae irregularly arranged. (Plate.4D)	Rarely branched; differentiated 6.7um. (Plate.4E)	Brown; branched; 8.9um. (Plate.4F)	Phenol-purple lactic acid-n.r Trypan blue-blue 10% KOH-yellow	<i>Pinus roxburghii</i>
<i>Pinirrhiza granulata</i>	Black but young brown; Dichotomous; tips mostly straight, few bent; woolly & grainy; some times smooth & lustrous. (Plate.2A)	Pseudoparenchymatous; hyphal cells angular in shape. (Plate.4G)	Pseudoparenchymatous; hyphal cells angular in shape. (Plate.4H)	Brown; branched; differentiated; 5.9-6.2um. (Plate.4I)	Brown; branched; 9.2-9.5um. (Plate.4J)	Phenol-black lactic acid-n.r Trypan blue-blue 10% KOH-yellowish brown	<i>Pinus wallichiana</i>
<i>Pinirrhiza spinulata</i>	Dark brown with brown swollen tips; simple to dichotomous; tips straight; smooth to short spiny. (Plate.2B)	Plectenchymatous; irregularly arranged forming a net. (Plate.4K)	Plectenchymatous; arranged in longitudinal directions. (Plate.4L)	Absent	Whitish yellow; unbranched; 5.5-5.8um. (Plate.4M)	Phenol-yellow lactic acid-n.r Trypan blue-blue 10% KOH-n.r	<i>Pinus roxburghii</i>

<i>Pinirrhiza citrina</i>	Citrus to dark brown; dichotomous; tips mostly straight, few beaded; smooth with sugary crystals. (Plate.2C)	Pseudoparenchymatous; hyphal cells angular or roundish, in part elongated, with no definite pattern. (Plate.4N)	Densely Pseudoparenchymatous to parenchymatous; hyphal cells irregular, angular, elliptical, cubicular. (Plate.4O)	Absent	Brown; unbranched; 5.25-6um. (Plate.4P)	Phenol-black lactic acid-greenish Trypan blue-blue 10% KOH-n.r	<i>Pinus wallichiana</i>
<i>Pinirrhiza variegata</i>	Dark brown with light brown swollen tips; straight; mostly grainy few woolly. (Plate.2D)	Densely plectenchymatous; haphazardly arranged. (Plate.5A)	Densely Plectenchymatous (Plate.5B)	Absent	Brown; unbranched; forming hyphal fans; 6.0-6.5um. (Plate.5C)	Phenol-n.r. lactic acid-n.r Trypan blue-blue 10% KOH-light brown	<i>Pinus roxburghii</i>
<i>Pinirrhiza aggregata</i>	Brick yellow with light brown swollen tips; densely packed dichotomous coralloid type; tips straight; cottony. (Plate.2E)	Plectenchymatous; clustered in a net like arrangement. (Plate.5D)	Plectenchymatous; haphazardly arranged. (Plate.5E)	Brown; branched; differentiated; 5.8-6.0um. (Plate.5F)	Light brown; branched; 6.5-7.4um. (Plate.5G)	Phenol-n.r lactic acid-n.r Trypan blue-blue 10% KOH-yellow	<i>Pinus wallichiana</i>
<i>P.smoothiana</i>	Mustard color with light brown swollen tips; dichotomous; tips straight to beaded; smooth. (Plate.2F)	Plectenchymatous; cylindrical in shape. (Plate.5H)	Plectenchymatous; with no definite pattern. (Plate.5I)	Absent	Brown; rarely branched; 5.6um. (Plate.5J)	Phenol-reddish lactic acid-yellowish brown 10% KOH-n.r Trypan blue-blue	<i>Pinus roxburghii</i>
<i>Pinirrhiza irregulata</i>	Flavous to cigar brown color irregularly pinnate dichotomous like; tips mostly bent, few straight; reticulate with yellowish warts. (Plate.2G) Black;	Plectenchymatous; net like arrangement. (Plate.5K)	Plectenchymatous; ring like arrangement. (Plate.5L)	Absent	White; branched; 5.3-5.8um. (Plate.5M)	Phenol-n.r lactic acid-n.r Trypan blue-blue 10% KOH-brown	<i>Pinus wallichiana</i>
<i>Pinirrhiza Sakesariana</i>	Bay to purplish chestnut color Dichotomous; tips bent; grainy. (Plate.2H)	Pseudoparenchymatous; hyphal cells irregularly angular or elongated. (Plate.5N)	Pseudoparenchymatous; slightly angular or elongated. (Plate.5O)	Absent	Yellowish brown; unbranched; 5.3-5.7um. (Plate.5P)	Phenol-black lactic acid-n.r Trypan blue-blue 10% KOH-red	<i>Pinus roxburghii</i>
<i>Pinirrhiza reticulata</i>	Sienna to Cinnamon with few dark colored growing tips; irregularly pinnate with few dichotomies; tips mostly straight, few beaded; reticulate. (Plate.2I)	Densely plectenchymatous; haphazardly arranged. (Plate.5Q)	Pseudoparenchymatous; hyphal cells angular, cubicular and elongated. (Plate.5R)	Absent	Dark brown; branched; 5.6um. (Plate.5S)	Phenol-reddish lactic acid-n.r 10% KOH-n.r Trypan blue-blue.	<i>Pinus roxburghii</i>

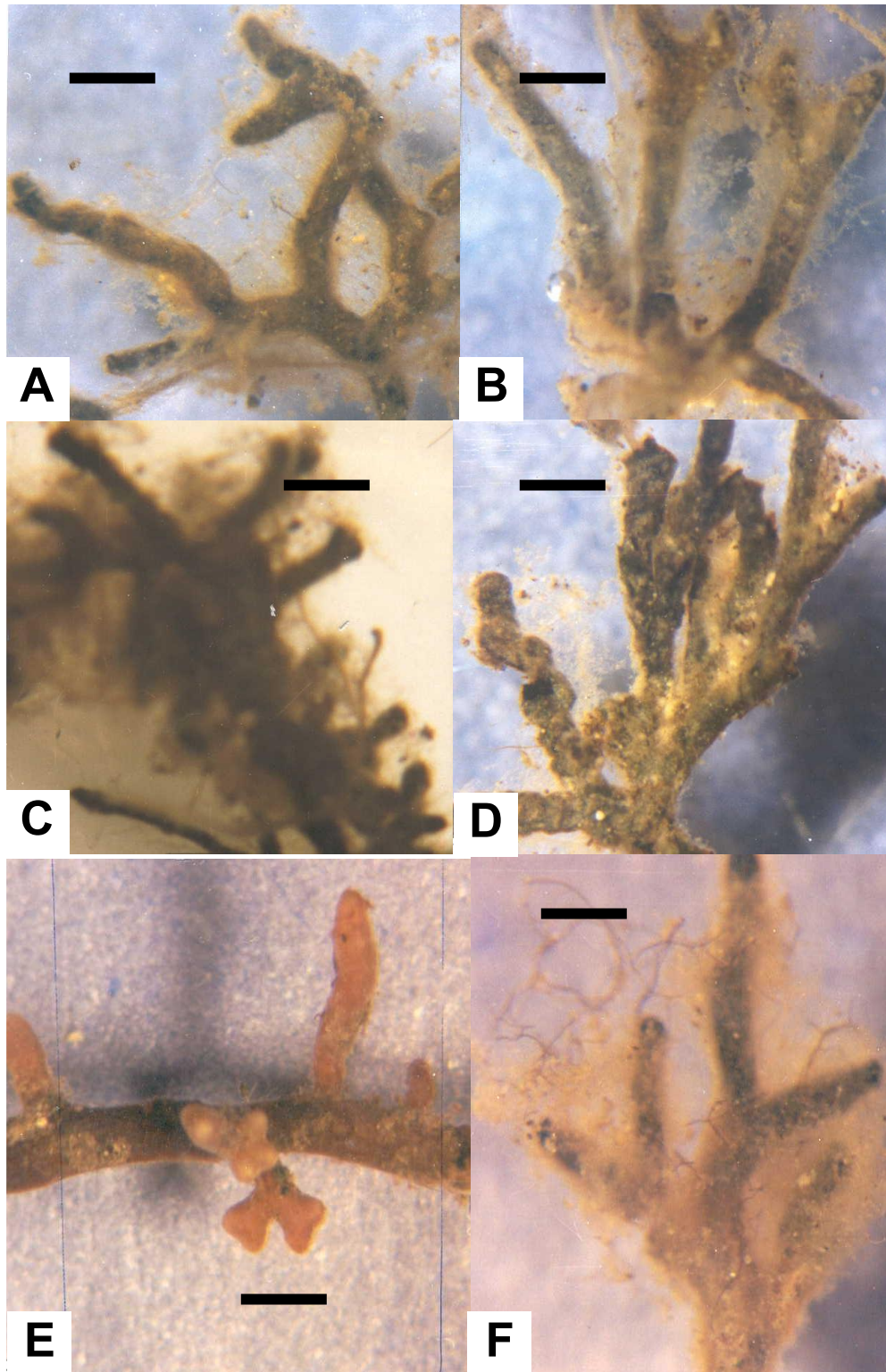


Plate 1: A. *Pinirrhiza argentiana*, B. *Pinirrhiza pinnata*, C. *Pinirrhiza nigra*, D. *Pinirrhiza elongata*, E. *Pinirrhiza dichotoma*, F. *Pinirrhiza monopodata*. Scale bar = 12 mm.

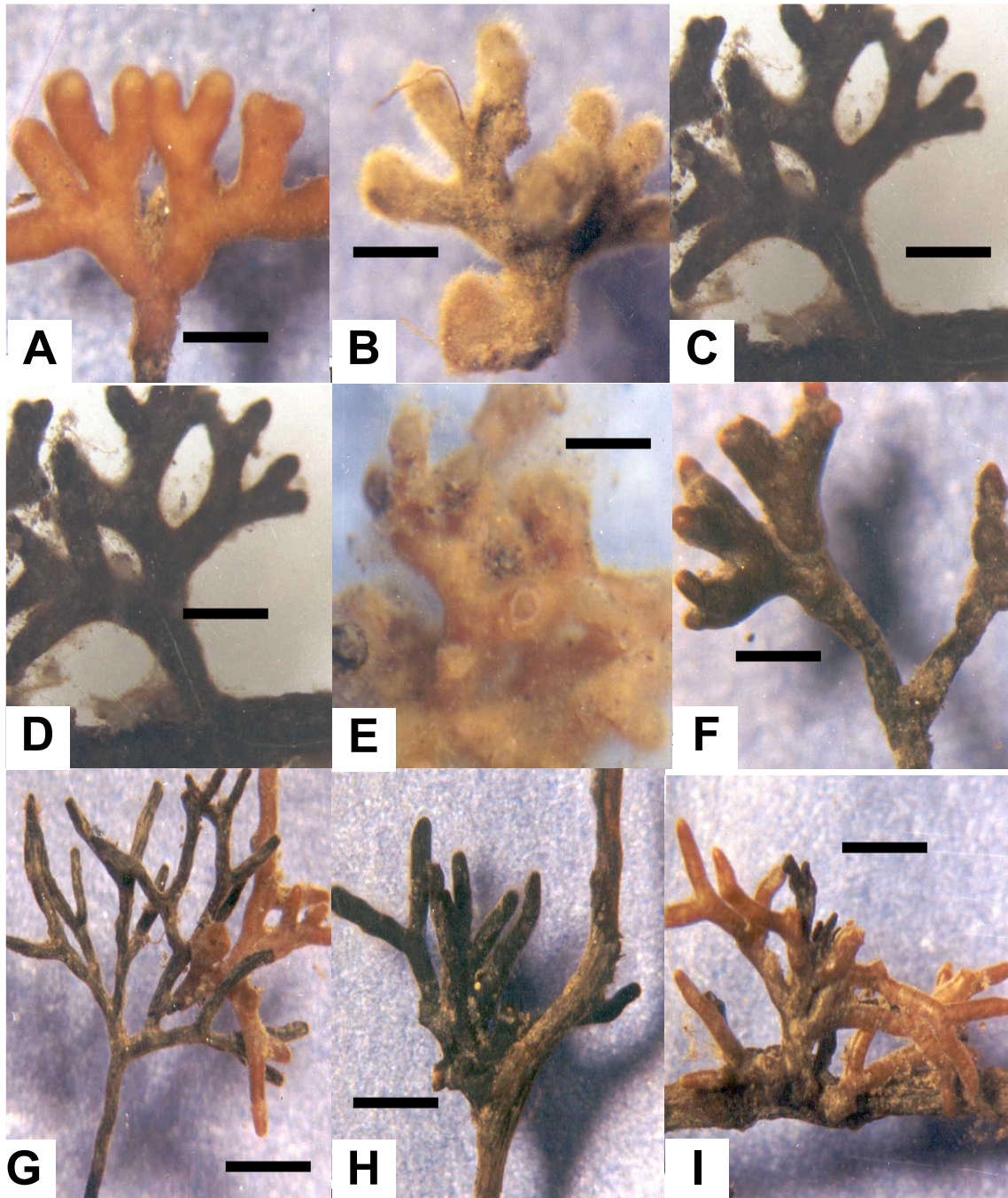


Plate 2: A. *Pinirrhiza granulata*, B. *Pinirrhiza spinulata*, C. *Pinirrhiza citrina*, D. *Pinirrhiza variegata*, E. *Pinirrhiza aggregata*, F. *Pinirrhiza smoothiana*, G. *Pinirrhiza irregularata*, H. *Pinirrhiza Sakesariana*, I. *Pinirrhiza reticulata*. Scale bar = 12 mm.

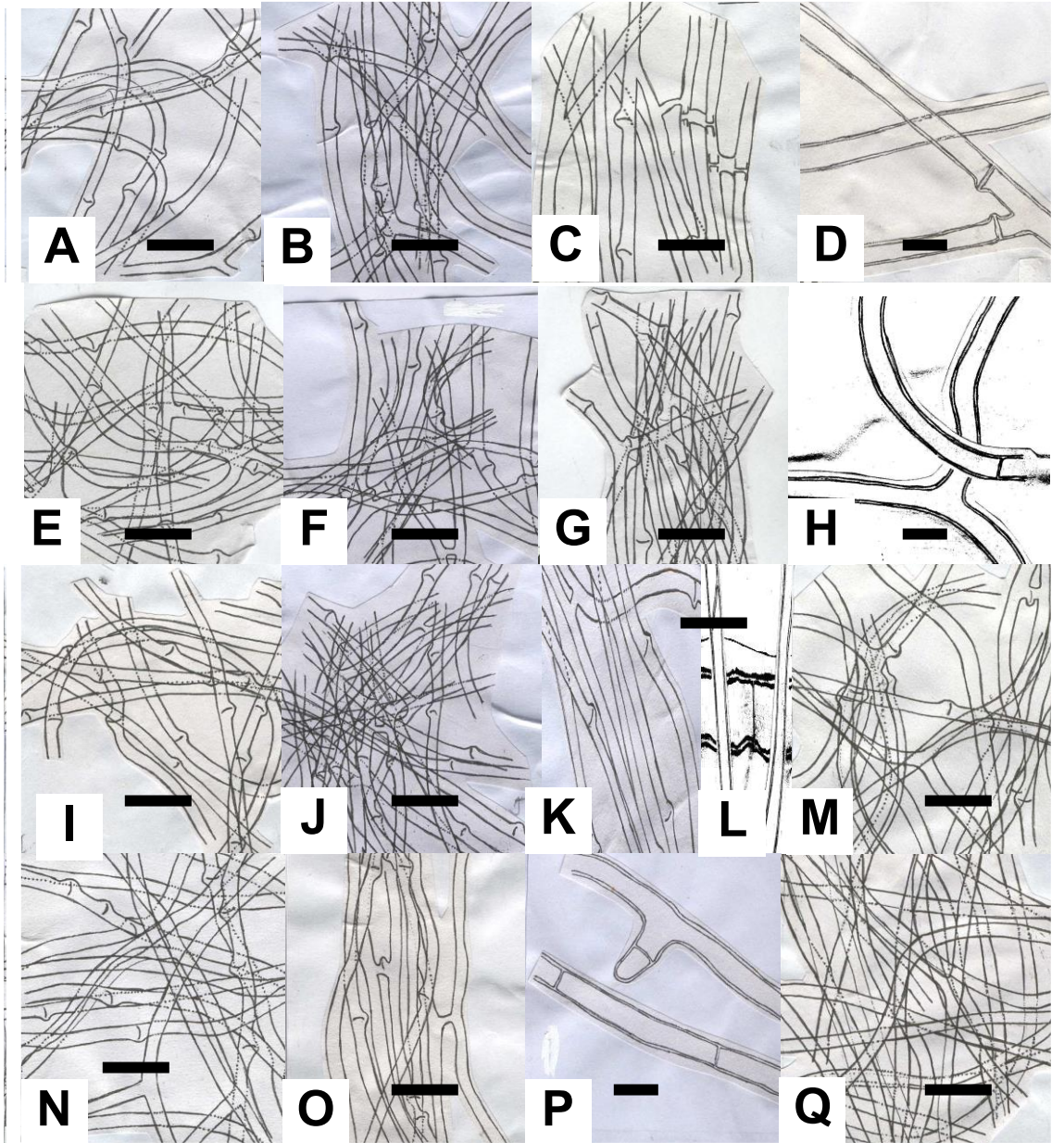


Plate 3: *Pinirrhiza argentina*: A (OMV), B (IMV), C (Rh), D (EH). *Pinirrhiza pinnata*: E (OMV), F (IMV), G (Rh), H (EH). *Pinirrhiza nigra*: I (OMV), J (IMV), K (Rh), L (EH). *Pinirrhiza elongata*: M (OMV), N (IMV), O (Rh), P (EH). *Pinirrhiza dichotoma*: Q (OMV). Scale bar for A, B, C, E, F, G, I, J, K, M, N, O, Q = 12 μ m; Scale bar for D, H, L, P = 10 μ m.

Abbreviations: OMV = Outer mantle view; IMV = Inner mantle view; Rh = Rhizomorph; EH = Emanating hyphae.

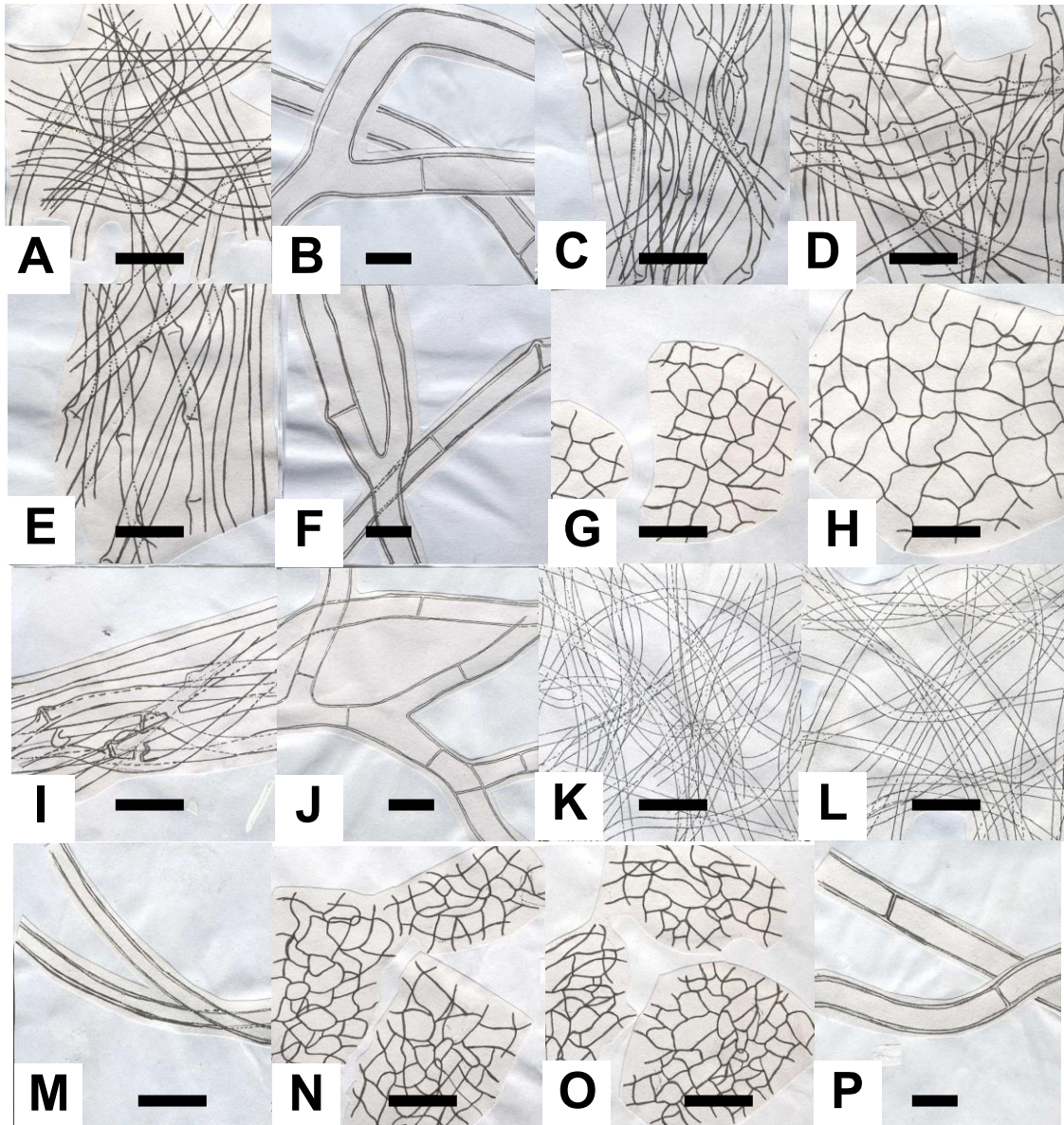


Plate 4: *Pinirrhiza dichotoma*: A (IMV), B (EH). *Pinirrhiza monopodata*: C (OMV), D (IMV), E (Rh), F (EH). *Pinirrhiza granulata*: G (OMV), H (IMV), I (Rh), J (EH). *Pinirrhiza spinulata*: K (OMV), L (IMV), M (EH). *Pinirrhiza citrina*: N (OMV), O (IMV), P (EH). Scale bar for A, C, D, E, G, H, I, K, L, N, O = 12 μ m; Scale bar for B, F, J, M, P = 10 μ m.

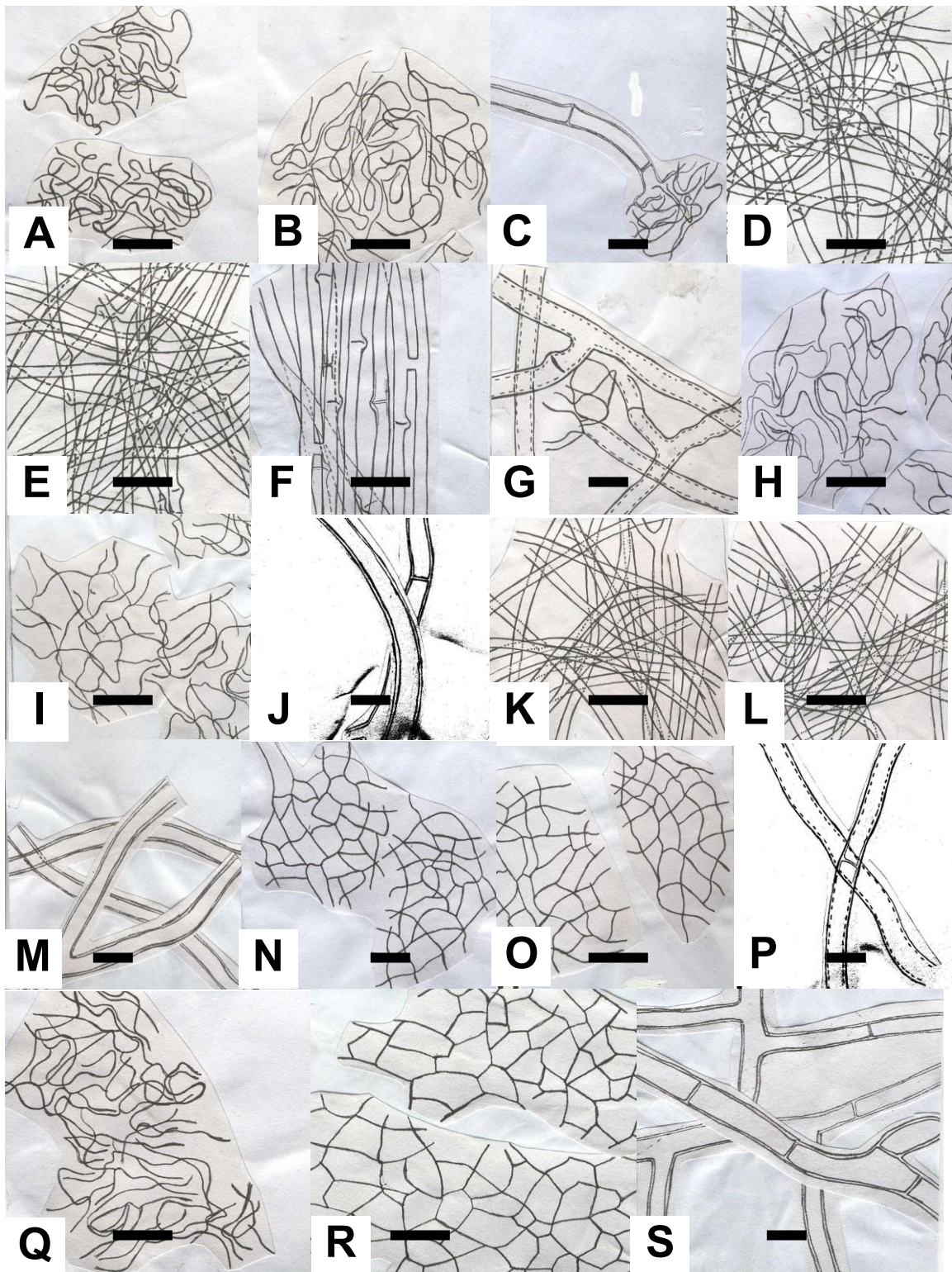


Plate 5: *Pinirrhiza variegata*: A (OMV: Outer mantle view), B (IMV: Inner mantle view), C (EH: Emanating hyphae). *Pinirrhiza aggregata*: D (OMV), E (IMV), F (Rh: Rhizomorph), G (EH). *Pinirrhiza smoothiana*: H (OMV), I (IMV), J (EH). *Pinirrhiza irregulata*: K (OMV), L (IMV), M (EH). *Pinirrhiza Sakesariana*: N (OMV), O (IMV), P (EH). *Pinirrhiza reticulate*: Q (OMV), R (IMV), S (EH). Scale Bar for A, B, D, E, F, H, I, K, L, N, O, Q, R (12 μ m); Scale bar for C, G, J, M, P, S (10 μ m).

Discussion

Ectomycorrhizas differ from one another by their characteristic shape, size, color, root geometry and mantle surface etc. They have been categorized as “identified” and “unidentified” depending upon the known and unknown identity of the mycobionts (Agerer, 1990). Known mycorrhizas have been given the same name as the fungus, whereas the ‘unknown’ mycorrhizas are given binomials in the same way as plants and fungi (Gronbach and Agerer, 1986). The first part of binomial represents the genus whereon the ectomycorrhizas are formed while the second part usually indicates a special feature.

Mycorrhiza isolated from the root system of *Pinus roxburghii* is characterized by dichotomous type of ramification. Color of the younger ectomycorrhiza is light brown while the older one is dark brown with woolly and silvery mantle surface. Emanating hyphae and rhizomorphs are abundantly present. Mantle surface is plectenchymatous having septa and clamps. This mycorrhiza is described for the first time with this host. None of the known ectomycorrhizae possesses these characteristics and it does not exist in literature (Agerer, 1997; Khurshid, 1997; Niazi, 2001). So this mycorrhiza lies in the category of “Unknown and unidentified mycorrhizae”, hence is given the binomial “*Pinirrhiza argentiana*” due to its silvery appearance (Plate 1A, 3A-D).

Pinirrhiza pinnata (Plate 1B, 2E-H)), another mycorrhiza, isolated from the same host but different site is characterized by irregularly pinnate to monopodial pinnate type of ramification, blackish brown color, woolly mantle surface and bent unramified ends. Emanating hyphae and rhizomorphs are abundantly present. Hartig’s net is present throughout the cortical region. Hyphae are plectenchymatously arranged on the mantle surface. Clamps and septa are present. This mycorrhiza is anatomically similar to *Pinirrhiza argentiana* but morphologically different. No mycorrhiza of this type has been reported earlier and it does not exist in literature (Agerer, 1997; Khurshid, 1997; Niazi, 2001).

An ectomycorrhiza isolated from the root system of *P. roxburghii* from two different sites of Sakesar hills is described morphologically as well as anatomically. This ectomycorrhiza is dichotomously branched having bent unramified ends, grainy mantle surface and dark black color. Rhizomorphs and emanating hyphae are present. The frequency of occurrence of sheath is 100% and has plectenchymatous type of mantle surface with septa and clamp connections. Hartig’s net is

also present. This mycorrhiza isolated from two different samples No .ARN 103 and ARN 106 is compared with other reported mycorrhizae. This ectomycorrhiza resembles morphologically with the ectomycorrhiza *Cedrirrhiza aggregata* isolated from the root system of *Cedrus deodara* from Murree hills (Niazi, 2001). But anatomy of *Cedrirrhiza aggregata* has not been described. The newly isolated mycorrhiza is described morphologically as well as anatomically, and is given the new name as *Pinirrhiza nigra* (Plate 1C, 3I-L) due to its dark black color.

Pinirrhiza nigra is also somewhat similar in anatomy with the *Nothofagihiza vinicolor* isolated from the *Nothofagus pumilio* (Agerer, 1997). Both have plectenchymatous type of arrangement of hyphae on the mantle surface. Septa and clamps are present in the hyphae of mantle surface, but morphologically these are different.. So, the *Pinirrhiza nigra* lies in the category of “Unknown and unidentified” ectomycorrhizae”.

Another mycorrhiza *Pinirrhiza elongata* (Plate 1D, 3M-P) isolated from the root system of *Pinus roxburghii* is characterized by dichotomous type of ramification and spiny mantle surface, straight unramified ends and blackish brown color. The frequency of occurrence of mycorrhizal sheath is 100%. Hypae are plectenchymatously arranged on the mantle surface. This mycorrhiza is similar morphologically with the ectomycorrhiza *Pyrirrhiza implicata* (Niazi, 2001) isolated from the root system of *Pyrus aucuparia* from Murree hills. But the *P. implicata* is not properly described anatomically with mantle preparation and also both have different hosts, so newly isolated mycorrhiza has given the new name as *Pinirrhiza elongata*.

A mycorrhiza *Pinirrhiza dichotoma* (Plate 1E, 3Q, 4A-B) isolated from the root system of *P. roxburghii* from Sakesar is characterized by dichotomous type of branching pattern, bent shaped unramified ends and spiny mantle surface. Color of the ectomycorrhizal root system is dark brown to black. Rhizomorphs are absent Hyphae are plectenchymatously arranged on the mantle surface. *Pinirrhiza dichotoma* is similar morphologically with the *Pinirrhiza himaliana* isolated from the root system of the same host *P. roxburghii* from Murree hills (Niazi, 2001), but the *P. himaliana* has not been described anatomically with mantle preparation, so the newly described mycorrhiza from Sakesar hills is given the new appropriate binomial *Pinirrhiza dichotoma*, *Pinirrhiza monopodata* (Plate 1F, 4C-F) isolated from the *Pinus roxburghii* from

Sakesar hills is characterized by monopodial pinnate type of branches pattern. The unramified ends are mostly straight but a few are bent and the mantle is short spiny. Color of the ectomycorrhizal root is blackish brown. Hyphae plectenchymatously arranged on the mantle surface with septa and clamps. Rhizomorphs rarely branched, hyaline, septate and differentiated having clamps. *Pinirrhiza monopodata* has morphology similar to the *fagirrhiza spinulosa* isolated from the *Fagus sylvatica* L. (Agerer, 1997) but the difference lies in anatomical characters, and the presence of cystidia in *Fagirrhiza spinulosa* which are absent in *Pinirrhiza monopodata*. *P. Monopodata* also resembles in few morphological characters with the *Tetraberlinia heterocysidia* isolated from the *Tetraberlinia bifoliolata* (Harms) Hauman (Agerer, 1997) but anatomical characters are totally different. So the *Pinirrhiza monopodata* is new to science and has not been described earlier.

A mycorrhiza isolated from the *Pinus wallichiana* is characterized by dichotomous type of branching pattern. The unramified ends are mostly straight but few are bent in shape. Younger ectomycorrhizas are light brown while the older are black in color. Younger ectomycorrhizas has woolly and lustrous mantle surface while the older one has grainy mantle surface. Emanating hyphae and rhizomorphs are present. Hyphae are pseudoparenchymatously arranged on the mantle surface. Hyphal cells are angular in shape. This ectomycorrhiza has not been described earlier and lies in the category of "Unknown and unidentified" mycorrhiza. So, it has given the new name "*Pinirrhiza grainulata*" (Plate 2A, 4G-J). This mycorrhiza is also new to science.

Pinirrhiza spinulata is isolated from *Pinus roxburghii* and is characterized by simple to dichotomous type of branching pattern. Mantle surface is smooth to short spiny and the unramified ends are straight in shape (Plate 2B, 4K-M). Color of the ectomycorrhizal root system is dark brown with the light brown swollen tips. Rhizomorphs and emanating hyphae are present. Hyphae are plectenchymatously arranged on the mantle surface. Clamps and septa are absent in the hyphae of mantle surface. Emanating hyphae are unbranched, hyaline and thick walled. This mycorrhiza is described for the first time with this host. None of the known mycorrhizae possess these characteristics and this mycorrhiza does not exist in literature. (Agerer, 1997; Khurshid, 1997; Niazi, 2001). So, this mycorrhiza lies in the category of "unidentified" mycorrhizae.

Another mycorrhiza isolated from root system of *P. wallichiana* is characterized by dichotomous type or ramification and smooth mantle surface. Unramified ends are mostly straight but a few are bended. Color of the younger ectomycorrhizal root is citrus brown while the older one is dark brown. Rhizomorphs are absent. Mantle surface has pseudoparenchymatous arrangement of hyphal cells. Hyphal cells are angular, elliptical, or elongated in shape. Emanating hyphae are unbranched, thin walled and septate having no clamps. This mycorrhiza has not been described earlier, so it has given a new name *Pinirrhiza citrina* (Plate 2C, 4N-P). *P. citrina* resembles morphologically with the *Pinirrhiza lutea* isolated from *Pinus sylvestris* L. (Agerer, 1997). Both have dichotomous type of branching pattern, smooth mantle surface, straight unramified ends and brown colored ectomycorrhizal root system. But anatomically both ectomycorrhizae are totally different from each other.

Pinirrhiza variegata (Plate 2D, 5A-C) is isolated from *Pinus roxburghii* and is characterized by dichotomous type of branching pattern, straight unramified ends and brown colored ectomycorrhizal system. Mantle surface is mostly grainy but a few unramified ends look woolly. Extensive emanating hyphae are present forming hyphal fans. Rhizomorphs are also present. The arrangement of hyphae is plectenchymatous on the mantle surface. This mycorrhiza is "unknown and unidentified" because it does not exist in literature (Agerer, 1997; Khurshid, 1997; Niazi, 2001). So it has given a new binomial *Pinirrhiza variegata*.

Pinirrhiza aggregata (Plate 2E, 5D-F) isolated from the *Pinus wallichiana* is characterized by densely packed dichotomous like coralloid type of ramification. The unramified ends are straight in shape and mantle surface is cottony. Color of the younger ectomycorrhiza is brick yellow, with light brown swollen tips. *Pinirrhiza aggregata* resembles in morphology with *Betulaerhiza hystrix* isolated from *Betula pendula* Roth. (Agerer, 1997). The difference lies in that the *Betulaerhiza hystrix* has cystidia that are absent in newly described mycorrhiza from the root system of *Pinus roxburghii*. *Pinirrhiza aggregata* resembles in morphological characters with the mycorrhiza *Inocybe terrigena* isolated from the *Pinus sylvestris* (Agerer, 1997), but is totally different anatomically. So *P. aggregata* has not been described earlier (Agerer, 1997; Khurshid, 1997; Niazi, 2001).

Pinirrhiza smoothiana is isolated from the *P. Roxburghii* and is characterized by dichotomous

type of branching pattern, straight to beaded shape unramified ends, smooth mantle surface and mustard colored ectomycorrhizal system. Rhizomorphs are absent. Hyphae are plectenchymatously arranged on the mantle surface (Plate 2F, 5H-J). This mycorrhiza has not been described earlier and does not exist in literature (Agerer, 1997; Khurshid, 1997; Niazi, 2001). So this mycorrhiza lies in the category of unknown and unidentified.

Pinirrhiza irregulata (Plate 2G, 5K-M) is isolated from the *Pinus wallichiana* and is very distinct anatomically as well as morphologically. This mycorrhiza has not been described earlier and is new to science.

Another mycorrhiza isolated from the *Pinus roxburghii* is characterized by dichotomous type of branching pattern, grainy mantle surface, bent shaped unramified ends and bay to chestnut colored ectomycorrhizal system. Rhizomorphs are absent. Hyphae are pseudoparenchymatously arranged on the mantle surface. This mycorrhiza is also new and unidentified and has been given a new name *Pinirrhiza Sakesariana* (Plate 2H, 5N-P).

Pinirrhiza reticulata is characterized by irregularly pinnate dichotomous like ramification and reticulate mantle surface. The unramified ends are mostly straight-- beaded in shape. Rhizomorphs are absent. Color of the ectomycorrhizal system is light to dark brown. Hyphae are pseudoparenchymatously arranged on the inner mantle surface while the arrangement is densely Plectenchymatous on the outer mantle layer. Emanating hyphae are branched, thick walled and septate (Plate 2I, 5Q-S).

Pinirrhiza reticulata is very similar morphologically to *Pinirrhiza irregulata* but anatomically these are different from each other. So, the *P. reticulata* does not exist in literature (Agerer, 1997; Khurshid, 1997; Niazi, 2001), so it lies in the category of unknown and unidentified mycorrhiza, and has been given a new name.

The Sakesar hills are investigated very first time for these ectomycorrhizas in association with pines. The sampling was carried out in the months of March and September, which is not the proper

season for the fruiting of these ectomycorrhizae. So, all these described ectomycorrhizas fall in the category of "unidentified mycorrhizas" and are given tentative binomials on the basis of plant as well as specific identity of the ectomycorrhizas.

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