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BIODIVERSITY OF MUSHROOMS AND ECTOMYCORRHIZAS. 1. *RUSSULA BREVIPES* PECK., AND ITS ECTOMYCORRHIZA- A NEW RECORD FROM HIMALAYAN MOIST TEMPERATE FORESTS OF PAKISTAN

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Abstract

During the investigation on Biodiversity of Mushrooms and ectomycorrhizas from Himalayan Moist Temperate Forests of Pakistan, *Russula brevipes* was found associated with *Pinus wallichiana*. *Russula brevipes* and its morphotypes/ectomycorrhiza have been described and illustrated. The fungus and its mycorrhiza are new records for Pakistan.

Introduction

Himalayan Moist Temperate Forests (HMTF) of Pakistan are characterized by the luxurious growth of conifers and are located at an elevation of 1373 to 3050 m. In these forests maximum temperature during summer varies from 10.7- 18C^{o}. Mean annual rainfall is 59.3cm, while average humidity in these areas is 57%. The most important feature of these forests is coniferous trees i.e., *Abies pindrow* Roxb. (Royle), *Cedrus deodara* (Roxb.) Loud., *Pinus wallichiana* A.B. Jackson, *P.roxbughii* Sargent, *Picea smithiana*(Wall.) Boiss., *Taxus wallichiana* Zucc.. Among the deciduous trees, *Acer acuminatum* Wall., *Aesculus indica* (Wall. Ex camb) Hooker, *Alnus rubra* Bongn, *Salix denticulata* N.J. Anderss, *Ulmus campestris* L., *Pyrus communis* L., are found as dominant vegetation. Among the shrubs, different species of *Rosa* L. *Rubus* L. *Indigofera hebepetala* Bth. Ex Baker. *Vibernum cotonifolium* D. Don., are typical. A large number of medicinal plants occur in this zone. These include *Zizyphus vulgaris* Lam., *Berberis lycium* Royle., *Skimmia laureola* (DC.) Sieb. & Zucc. ex Walp., *Viola serpens* Wall. Ex Roxb.and *Mentha pipertia* L. (Hussain, 1995).

These forests receive an appreciable amount of rainfall during monsoon (July-August). This rainfall and high temperature make environment conducive for the production of colorful mushrooms. Most of these mushrooms form mutualistic symbiotic association with forest trees in the form of ectomycorrhiza which is the most important for their growth, nutrient absorption and protection of roots from Pathogens (Marx, 1997).

Ectomycorrhizas create distinct features in roots of forest trees. These characters are preferentially dependent, influenced and fashioned by the fungal hyphae of these essentially important structures of root system (Agerer, 2002). Many species of fungi are normally involved in ectomycorrhizal association with a single tree or a single species may involve in this association with more than one tree (Marx, 1997).

The genus *Russula* (Pers. Ex. Fr.) S.F. Gray accounts in a large measure for our monsoon mushroom flora in these forests. It is cosmopolitan and largely ectomycorrhizal genus with a wide range of Gymnosperms and Angiosperms (Richardson, 1970; Agerer, 2002). Twenty-three speceies of the genus *Russula* have been reported which are

associated with different forest trees of Pakistan (Ahmad, *et al*., 1997) but their morphotypes/ectomycorrhizas have not been distinctly described. During the study of ectomycorrhizal communities in HMTF of Pakistan, we have found the members of Russulaceae to be common and conspicuous conifer associates. These mushrooms come on the forest floor during the month of July to September. Ectomycorrhizas formed by species of *Russula* are relatively distinct and several species of Russulaceous fungi have been shown to form mycorrhizae with the species of the genus *Abies*, *Fagus* and *Picea* (Agerer, 2002; Alexander, 1981; Molina & Trappe, 1982; Kraiger *et al*, 1995; Pillukat & Agerer, 1992).

Russula brevipes Peck., is widely distributed throughout North America, and mainly associated with species of *Abies, Picea, Tsuga and Pseudosuga* (Stanis, 1979; Kraiger *et al*, 1995). It was found very common on the ground in our Himalayan Moist Temperate forests under conifers in late fall, and can easily be identified by its large size, and white coloration which does not stain when handled. It is also known as short-stem Russula. *Russula brevipes* and its Ectomycorrhizas with *Pinus wallichiana* are for the first time reported from Pakistan.

Materials and Methods

Sampling of sporocarp and morphotypes: The sporocarps and blocks of soil (5x10cm) exactly beneath the fruiting bodies were excavated from the rhizosphere of *Pinus wallichiana.* The sporocarps were air dried while the soil blocks containing morphotypes/ectomycorrhizas were wrapped in polythene bags and brought to the lab for further analysis.

Characterization and photography: Sporocarps were characterized macro and microscopically. Morphotypes/ectomycorrhizas were isolated from the soil by wet sieving soil sample method (Core *et al*., 1979). Morphological and anatomical characterization was carried out under Stereo and Compound microscopes (Agerer, 1991), photographed and illustrations were made with the help of Camera Lucida. Identification of the mycobiont and ectomycorrhiza was carried out by following Kernaghan *et al*., (1997), Arora, (1986), Hoark, (1970).

Results and Discussion

Russula brevipes Peck.
New York St. Mus. Ann. Rept. 43: 20. 1890.

Pileus: Cap (4) 6-12 (20) cm broad, broadly convex with depressed center to nearly infundibuliform at maturity; surface smooth, dry, subviscid to viscid when wet, white, sometimes yellowish to brownish in age; margin plane to decurved; flesh thick, solid, crisp, brittle, white, becoming yellowish in age; odor mild, taste slightly acrid. **Lamellae** adnate to adnexed, decurrent with age, close; white, often pale yellow or pale tan in age; lamellulae present. **Stipe.** (2) 4 - 6 (8) cm long, (1.5)2 - 3(4.5) cm thick, equal or tapered towards the base, solid; surface dry white, with yellowish to brownish discolorations in age. **Spores.** (6) 8 – (10) 10.5 x (5.5) 6 -9 (9.5) µm, subglobose to subovoid to subellipsoid, with amyloid ornamentation of warts and ridges. Spore print; white to pale yellow. Smell not distinctive or faintly foul.

Ecology: Solitary to scattered to gregarious in soil of our Moist Temperate forests, often in great abundance; most common in late fall.
Edibility; Edible, but mediocre in taste

Distribution: Khanspur-Ayubia, Kuzagali, Nathiagali, NWFP. August 21, 2005, August 13,2006.

Specimen examined: # ARN 82105S, Sultan Ahmad Mycological Herbarium, Pakistan.

Description of Ectomycorrhizas

Morphological characters (Fig. 2A)

Ectomycorrhizal system dichotomously branched, branches 11-18 mm long, profusely branched in the form of clusters, 9-14 mm in diameter over main root, off-white- cream to light brownish tint. Main axis minute 2-4 mm long, 1-2 mm thick. Apices straight, 1-3(8) x 0.5-1(3) mm, shiny, whitish- silvery in appearance due to air trapped between mantle hyphae, brownish after displacement of the air. Ectomycorrhizas enclosing soil and dead woody material as well as humus particles. Mantle surface, fuzzy, hyaline to whitish to cream- copper color, densely short spiny, easily peeled off, host tissue visible under mantle surface.Rhizomorphs and emanating hyphae not observed.

Anatomical characters

Outer surface (Fig. 2B) of the mantle loosely plectenchymatous, weakly net-like, hyphae rarely septate, (1) 1.25-1.85 µm in diameter, clamps like structures rare, slightly thick-walled (up to 0.2 µm), hyphae whitish to off-white, surface of hyphae smooth; Somewhat rounded cells observed among hyphae, dense mat of long cystidia protruding from outer mantle surface. Mantle surface becoming dense inwards. Outer mantle surface of the tip transitional type i.e. between plectenchymatous to pseudoparenchymatous.

Inner mantle surface (Fig. 2D) densely plectenchymatous, hyphal cells irregular in shape, 1 -1.8µm in diameter, colorless, variable in length, walls up 0.5µm, **Very tip** with densely plectenchymatous arrangement of hyphae, no pattern discernable, hyphal cell straight, some time bent, 2-3µm thick. A few pseudoparenchymatous nests of cells visible among hyphal network.

Mantle appears thick and plectenchymatous throughout in cross section, forming a distinct sheath, 4.0 – 4.6 µm thick. Hartig's net dense, visible up to 3 layers of cortex, Profuse in 1st and 2nd layers. Extensive cystidia, (3.5) 4 – 5.0 (6.5) x (13.8)15 – 30.2 (34) µm looking to protrude out of the sheath,. *Cortical cells*: rounded to angular to cubical, 4-6 x 3-4(6) µm.

In longitudinal section the mantle hyphae running parallel to the root length. Sheath thicker at apex (4.2- 4.8 µm), slightly thinner on sides (4-4.4 µm). Hartig's net visible up to stellar region, very thick and profuse in first two layers. *Cortical cells*: mostly rectangular or cubical in shape, (2) 3-5 (5.5) µm long and (1) 2-3 (4) µm in diameter.

Cystidia (Fig. 2C), extensive at outer surface, (3.5) 4 – 5.0 (6.5) x (13.8)15 – 30.2 (34) µm, narrow towards distal end, slightly bulbous towards base.

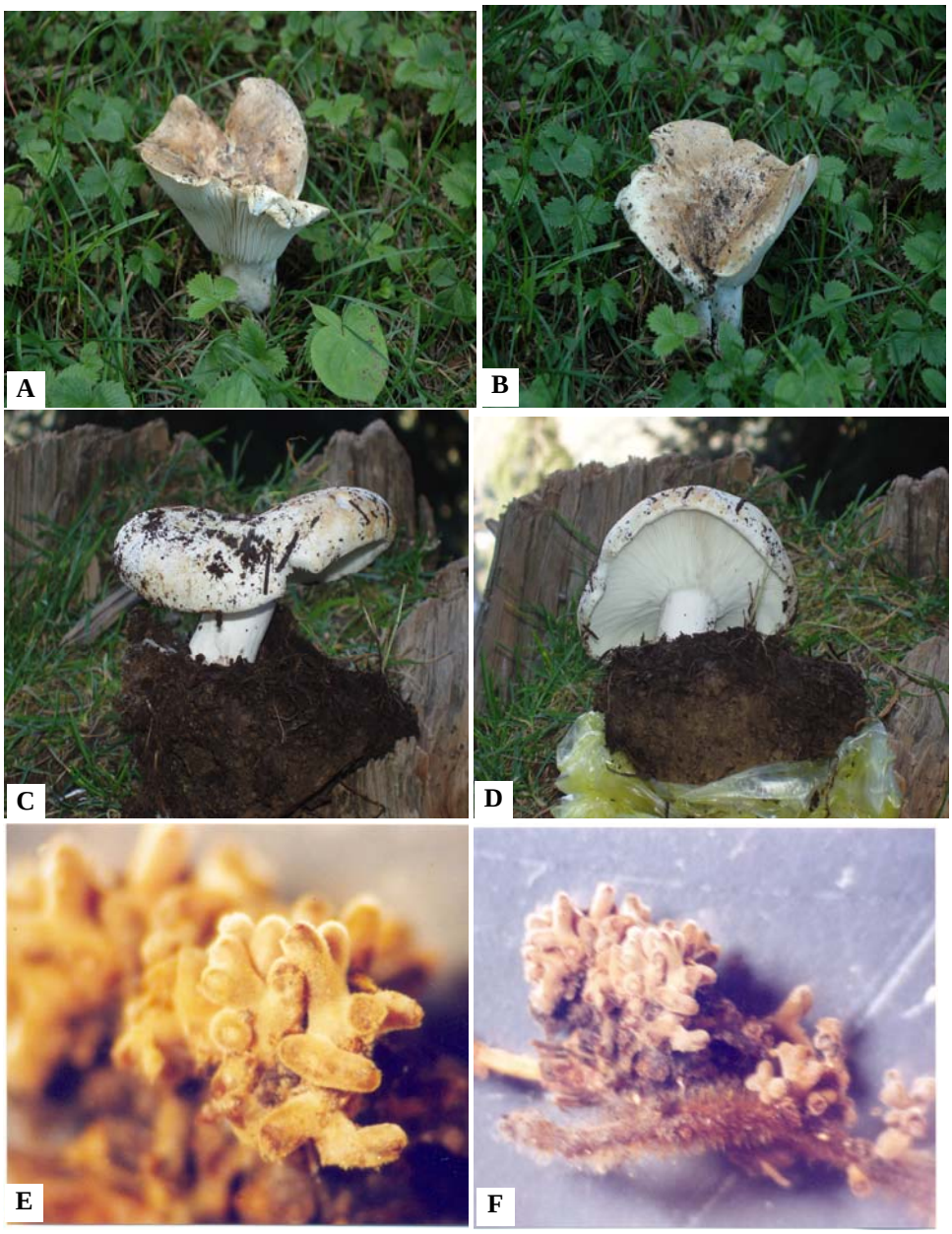

Fig. 1. A–D. Different Morphological views of Sporocarp (*Russula brevipes*). E – F. Ectomycorrhizae of *R. brevipes*. Scale bar: 2cm for A,B,C,D. 3mm for E, F.

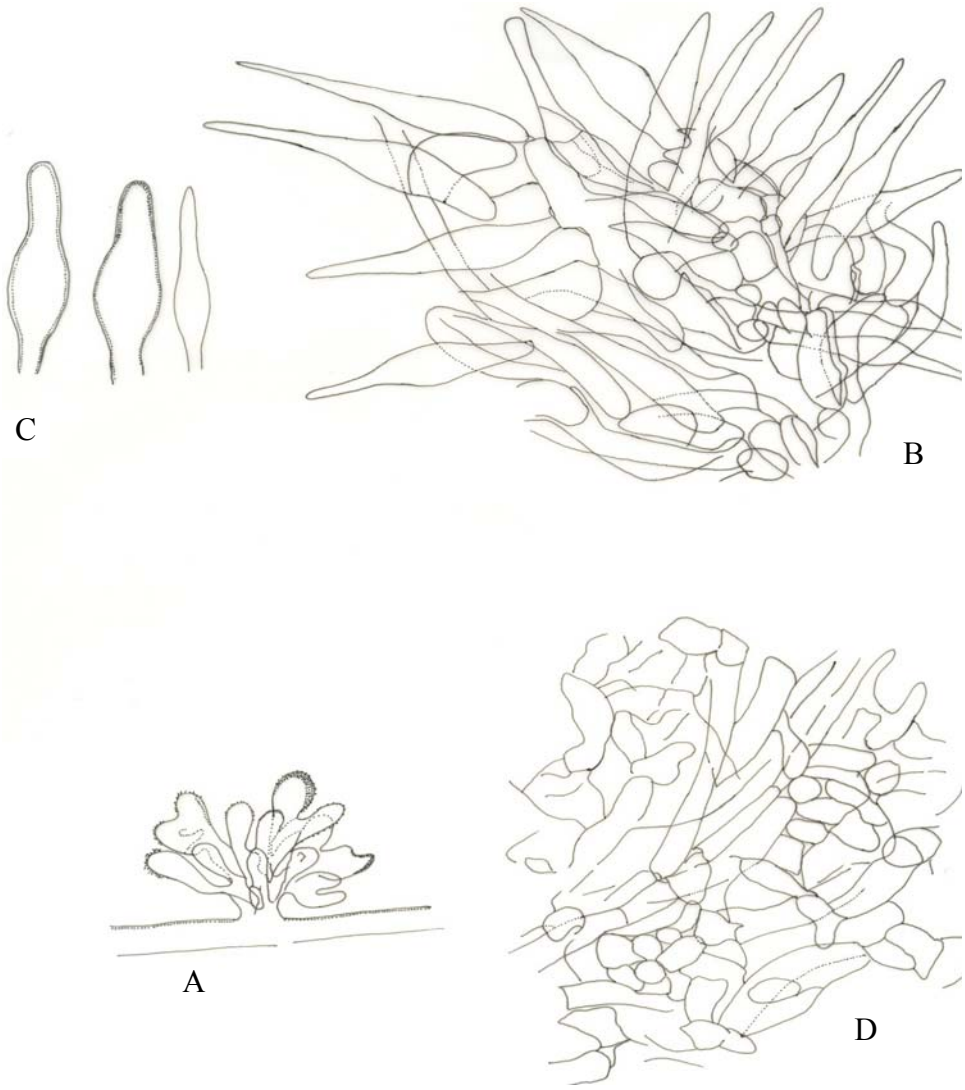

Fig. 2. A. Illustration of Morphotypes (*R. brevipes*) B.Outer Mantle View showing Cystidia. C. Cystidia D. Inner Mantle view, Scale Bar(A) 2mm, (B, C & D)= 15µm

Chlamydospores not observed.

Color reactions

Melzer's reagent: - orangish red
10% KOH: - reddish brown
Specimen # ARN 21805E, Sultan Ahmad Mycological Herbarium, Pakistan.

Discussion

A large number of fungi can be seen growing in the vicinity of trees in the forest during monsoon. Most of these form Ectomycorrhizas with a number of trees. Some fungi are host specific while others enter in this association with more than one tree (Valentine *et al*., 2002). These Mycorrhizas differ from one another by their characteristic shape, size, color, root geometry and mantle surface (Agerer, 1990).

A number of *Russula* species form ectomycorrhizas with different tree species. Ectomycorrhizae of *Russula brevipes* with *Pinus wallichiana* is characterized by its dichotomous – coralloid type of ramification, short spiny fuzzy mantle surface, which is easy to peel off. The color of the mycorrhizal system is off-white to cream to copper color, with loosely plectenchymatous inner mantle layer. Profuse bulbous base cystidia are observed all over the mycorrhizal system. (Fig. 2B).

Russula brevipes also forms ectomycorrhizal association with *Abies lacicarpa* (Kernaghan *et al*., 1997). Both mycorrhizas are much similar even with different host species and from different regions. Both have fuzzy appearance of their mantles due to many erect cystidia which look like short spines, hyaline to copper colored mantle and plectenchymatous arrangement of mantle hyphae. Both mycorrhizas lack emanating hyphae and rhizomorphs.

The only difference between them is the type of ramification. *Russula brevipes* forms dichotomous to coralloid type of ramification when it is associated with *Pinus wallichiana* while in association with *Abies lacicarpa* monopodial pinnate ramification is observed (Kernaghan *et al*., 1997). Pillukat & Agerer (1992) have regarded plant genus as the most important component in this partnership to determine the shape of ectomycorrhizas, the minor component may be the fungus.

Pinus wallichiana is reported a new ectomycorrhizal host for *Russula brevipes*. Mycobiont is a new record and an addition to the macromycetous flora of Pakistan.

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