APHYLLOPHORALES AND THEIR MORPHOTYPES IN PAKISTAN

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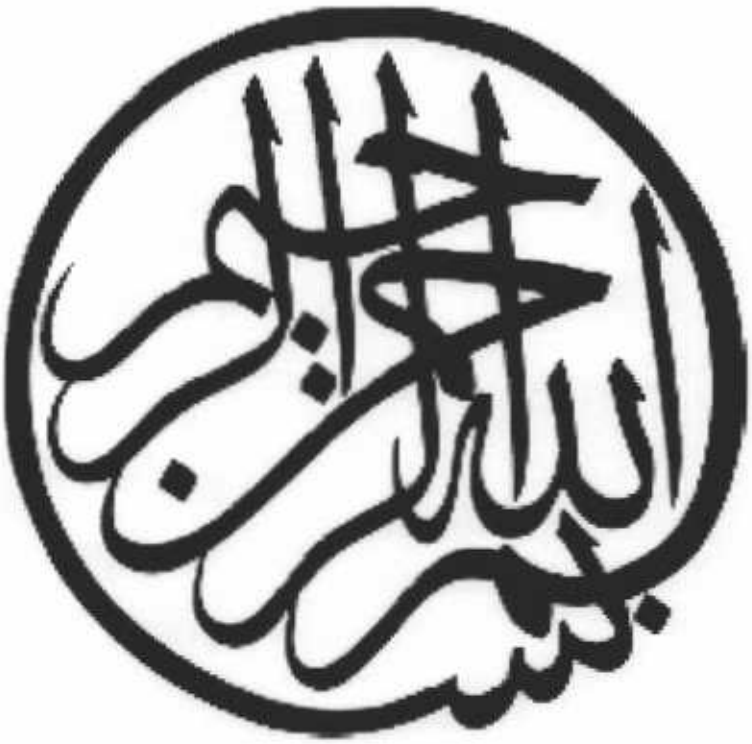
بسم الله الرحمن الرحيم

DEDICATED

To

My great & caring

FATHER 'MY IDEAL'

&

My sweet and endeared

LATE MOTHER

They are always the source of inspiration and motivation for me and

Whose love is

Precious boon

That God has conferred me with

And for which

I am destitute to defray.

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*alphabetical order

SUMMARY

SUMMARY

The main objective of this study is characterization and identification of Aphyllophorales and their morphotypes based on rDNA-ITS usage and morpho-anatomical approaches. The attempts have also been made to assess the diversity of Aphyllophorales associated with different host trees and below ground ectomycorrhizal structures, in Himalayan Moist Temperate forests (HMTF) of Pakistan distributed in seven stands. Other objectives were to find out the connections between above and below ground mycoflora, community structure of Aphyllophorales and to find out the phylogenetic relationship among the members of these fungi.

In the present investigation, sixty one (61) basidiomata of Aphyllophorales were analyzed. These were grouped into seventeen (17) species belonging to eight (08) genera viz; four of *Cantharellus* (*C. albiceratus* nom. prov., *C. cf. cibarius*, *C. lutescenioides* nom. prov., *C. pakistanicus* nom. prov.), one of *Clavariadelphus* (*C. subfastigiatus*), two of *Clavulina* (*C. cf. cinerea*, *C. minor* nom. prov.), two of *Gomphus* (*G. pukhtunkhawensis* nom. prov., *G. macrocarpus* nom. prov.), two of *Hydnum* (*H. repandum*, *H. pileoelevari* nom. prov.), three of *Ramaria* (*R. flavescenioides* nom. prov., *R. cymeioides* nom. prov., *R. alba* nom. prov.), one of *Sparassis* (*S. crispa*), two of *Thelephoara* (*T. horrida* nom. prov. and *T. palmata*). All species except five (*C. cf. cibarius*, *C. cf. cinerea*, *Hydnum repandum*, *Sparassis crispa* and *Thelephora palmata*) are new to science while *Clavariadelphus subfastigiatus* is first time reported from Pakistan. These Aphyllophorales were identified using morpho-anatomic features and partial Internal Transcribed Spacer regions (ITS1 and ITS2), complete 5.8s gene and sometimes with partial sequences of 18s and 28s genes. For this purpose, 97% cut-off similarity has been used as a proxy for a species level identification.

A total of 97 molecular operational taxonomic units (MOTUs)/species belonging to 35 genera from roots of different host trees were identified. Most of them were found associated with *conifers* while few were associated with deciduous trees.

The treated MOTUs/species fall in following 35 genera; *Amphinema* (01 species), *Bisporella* (01 species), *Cenococcum* (01 species), *Chalara* (01 species),

Clavulina (02 species), *Cortinarius* (01 species), *Davidiella* (01 species), *Entoloma* (01 species), *Helvella* (03 species), *Humaria* (01 species), *Hyaloscypha* (01 species), *Hydnobolites* (02 species), *Hymenoscyphus* (02 species), *Inocybe* (17 species), *Marasmius* (01 species), *Pachyphloeus* (01 species), *Peziza* (03 species), *Phialocephala* (01 species), *Phoma* (01 species), *Piloderma* (02 species), *Pochonia* (01 species), *Pseudotomentella* (01 species), *Rhizoscyphus* (02 species), *Russula* (09 species), *Sebacina* (05 species), *Sistotrema* (01 species), *Suillus* (01 species), *Tarzetta* (01 species), *Thelephora* (03 species), *Tomentella* (16 species), *Trichophaea* (03 species), *Tuber* (05 species), *Tylospora* (01 species) and *Wilcoxina* (01 species).

All these ninety-seven (97) MOTUs/species are first time reported as ectomycorrhizal from Himalayan Moist Temperate forests of Pakistan. On the whole, communities of all the stands are heterogeneous and composed of ectomycorrhizal species. Stand 01 was colonized by five (05) species which were not represented in rest of the sampling sites. These species include; *Helvella* sp. MHLAC-01, *Hymenoscyphus* sp. MHHYM-01, *Inocybe* spp., MHMUR-01, MHOBL-01, MHTAR-01 and MHUMB-01, *Lactarius* sp. MHSEM-01, *Peziza michelii*, *Peziza* sp. MHSUC-01, *Piloderma* sp. MHFAL-02, *Pseudotomentella* sp. MHTRI-01, *Rhizoscyphus* sp. MHERI-01 and *Russula livescens*.

Twenty six (26) species were found to be limited in occurrence in stand 02 viz; *Cenococcum* sp. MHGEO-01, *Clavulina* sp. MHCIN 01, *Cortinarius leucopus*, *Entoloma* sp. MHAME-01, *Helvella* sp. MHELA-02, *Inocybe* spp., MHGLA-01, MHNIT-01, MHSQU-01 and MHUMB-01, *Lactarius* sp. MHSEM-01, *Pachyphloeus* sp. MHMEL-01, *Peziza* sp. MHSUCS-01, *Piloderma* sp. MHFAL-02, *Russula cessans*, *Russula* spp., MHDIS-01, MHFOE-01, MHHET-01 and MHNAU-01, *Suillus sibiricus*, *Tarzetta* sp. MHCAT-01, *Thelephoraceae* sp. MHTBR-01, *Tomentella* sp. MHFER-01, *Tomentella* sp. MHVIR-01, *Trichophaea* sp. MHCFH-02, *Tuber aestivum* and *Wilcoxina rehmii*.

From Stand 03, *Hyaloscypha* sp. MHAUR-01, *Inocybe* sp. MHCRY-01, *Inocybe* sp. MHHIR-01 and *Tomentella* sp. MHSUB-01 were found to be unique as these did not occur in any other stand.

Ten (10) exclusive MOTUs/species were found in stand 04. These included; *Chalara* sp. MHSP-01, *Clavulina* sp. MHCRI 02, a member of *Corticiaceae* sp.

MHCOR-01, *Davidiella tassiana* , *Humaria* sp. MHHEM-01, *Hydnobolites* sp. MHCER-01, *Inocybe* sp. MHPRA-01, *Phialocephala* sp. MHFOR-01, *Piloderma* sp. MHOLI-01 and *Pochonia* sp. MHSUC-01.

From Stand 05, nine (09) ITS unique sequences were indentified. These included; *Bisporella* sp. MHCIT-01, *Inocybe* sp. MHMIC-01, *Sebacina aff. epigaea*, *Sebacina* spp., MHAFE-01 and MHSEB-02, *Sistotrema* sp. MHALB-01, *Tomentella* sp. MHSTU-02, *Tuber whetstonense* and *Tylospora* sp. MHTYL-01.

From Stand 06, six (06), unique ITS sequences were indentified and these included; *Inocybe* spp., MHPSE-01 and MHRUF-01, *Sebacina epigaea*, a member of *Sebacinaceae* sp. MHSEB-01, *Thelephora* sp. MHGAN-01 and *Tomentella* sp. MHFUS-01.

From Stand 07, seven (07) unique ITS sequences were indentified and these included; *Hydnobolites* sp. MHCER-02, *Lactarius sanguifluus*, *Marasmius* sp. MHHAE-01, *Russula lutea*, *Tomentella galzinii*, *Tomentella* spp., MHFUS-02 and MHGAL-01.

Most of the communities studied were found to be dominated by species belonging to Aphyllophorales (17-40.22%). Among them, members of Thelephoraceae were found to be more prominent and formed ectomycorrhizal association. The species richness of Stand 06 was observed maximum (3.53) and minimum for PU Suite and Stand 02, while the maximum diversity was observed for Stand 01 (0.037).

A total of twenty three (23) MOTUs/species are being reported in the present work belonging to Aphyllophorales. Three (03) species of Aphyllophorales were isolated and identified from stand 01, five (05) from stand 02, four (04) from stand 03, three (03) from stand 04, nine (09) from stand 05, eight (08) from stand 06 and five (05) of Aphyllophorales from stand 07, with different host trees. Stand 05 represented the maximum number of MOTUs/species of Aphyllophorales.

Among the hosts plants, *Abies pindrow* showed the greater affinity than other hosts towards ectomycorrhizal fungi (45 MOTUs/species) including 15 Aphyllophorales and this number reduced with other host trees.

Pakistan's part of Himalayan Moist Temperate Forests (HMTF) is rich in Aphyllophorales and many more new taxa are expected to be there if sampling effort are extended to less frequently surveyed areas.

For species richness and underground community of ectomycorrhizal MOTUS/species, there is much more need to obtain sample to reach maximum richness and complete community structure. This is the first serious attempt to assess the diversity and community structure of ectomycorrhizal fungi and Aphyllophorales in HMTF of Pakistan using above ground fruiting surveys as well ectomycorrhizal morphotypes. We recovered 17 species from fruiting bodies and 97 MOTUs/species from soil with no overlap between above and below ground communities. It is very essential to sample more from these sites to find the belowground Aphyllophorales matching the above ground community and vice versa. Documentation of Aphyllophorales is yet to complete which requires to explore less frequently surveyed areas.

CHAPTER 01
INTRODUCTION

INTRODUCTION

Pakistan is an oblong stretch of land between the Arabian Sea and Karakoram mountains lying between 24° N and 37°N latitude and 61°E and 75°E longitude, covering an area of 87.98 m hac (Pakistan Online Encyclopaedia, 2011).

The forests of Pakistan reflect great physiographic, climatic and edaphic contrasts in the country. There are twelve types of forests based on the climate, altitude and composition of forest species. These range from Tropical wet evergreen to sub-alpine and alpine forests (Champion *et al.,* 1968).

The Moist temperate forests are found along the moist Himalayan Mountains between the dry temperate and sub alpine zone (1,200-3,000 m). These are found in Kashmir, Murree-Hazara hills tracts, Swat, Dir, upper reaches of Kurram Agency, Gilgit and Baltistan districts. In moist Himalayan mountains, precipitation is derived from the South-Western monsoon, which falls during the months of July to September and due to westerly disturbances it also takes place during the winter and spring seasons. Rainfall ranges between 650-750mm or some times to about 1500mm. In these areas, large amount of precipitation takes place in the form of snow and is a factor of considerable importance in determining the types of forests, particularly where summer rainfall is low. Gradual melting of snow in early summer prolongs the season during which adequate moisture is available. The mean maximum temperature in the months of December-February is 9°C, -4°C and -1°C, respectively while mean minimum temperature in these months is -2°C, -4°C and -1°C. In the summer months of June, July and August mean maximum temperature is 30°C, 31°C and 30°C and 17°C as mean minimum temperature. Average humidity is 57% (Pakistan Meteorological Department, 2011).