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New record of *Podoscypha multizonata* (Berk. & Br.) Pat. from Garhwal Himalayas, Uttarakhand, India

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ABSTRACT

The Garhwal region under Uttarakhand state is very rich in natural biodiversity. This region is blessed by diverge range of climate and altitudinal variations coupled with varied ecological habitats. This fungus, *Podoscypha multizonata* was collected and described from this region first time. The morphological, microscopic and cultural studies on this fungal species were conducted to evaluate its characteristics. The fruiting bodies were collected from riparian forest of Tons river, Dehradun and Asan Barrage, Chakrata, Dehradun region of Uttarakhand state, India. The survey of macro-fungi was done during the month of June-August, 2018, *P. multizonata* was observed for the first time in the region. The fruiting bodies were in the form of wrinkled sheets, fused at edges forming a rosette structure. The upper surface was matt like in texture with short sharp longitudinal ridges whereas, the lower one was pale and longitudinally wrinkled. The fruiting body appeared translucent when observed in light and after drying, it changed to patchily black colour. The macro fungal composition of this region has been studied earlier by several workers, but *P. multizonata* was never reported before. The key objective of this study was to evaluate the morphological and cultural characteristics of *P. multizonata* isolated from Garhwal hills, Uttarakhand and its *in situ* and *ex situ* conservation.

Key words: Aphyllophorales**,** basiodiomata, biodiversity, macro-fungi, Garhwal hills.

INTRODUCTION

Fungi are diverse group of eukaryotic heterotrophy which grows in low moisture and low pH environment. Some of them cause diseases in plants, animals and human beings. Fungi produce secondary metabolites that can be used for different biological purposes. The hilly and valley areas of Garhwal region show an outstanding fungal richness of great importance for taxonomy and nomenclature. Many previous works and reports have stated the presence of various biologically important as well as rare fungi in this area (Prasher 2013; Vishwakarma *et al.* 2012). In this study, *Podoscypha multizonata* naturally occurring in Garhwal region under Uttarakhand state is reported first time and described in detail. It is a genus of poroid Aphyllophorales fungi in the family Meruliaceae. The fungal genus has a widespread distribution, especially in tropical regions and contains about 35 species. *Podoscypha* was first described by Berkeley and Broome in1865 under the name *Thelephora multizonata.* This fungal species was initially placed in the Polyporaceae, then Thelephoraceae. Later on *P. multizonata* has been

placed in its own family, Podoscyphaceae. It is, as the dialect name suggests, rosette like, with a number of thin, tough, erect, individual sporocarp, reddish to pinkish brown in colour. It usually measures between 50 and 250 mm across. The fungus is known to occur commonly on base of oak trees.

Taxonomically *Podoscypha multizonata* comes under the phylum basidiomycota, which is the second largest phylum of fungi, comprising of approximately 35000 species divided under three main subphylum namely; Agaricomycotina (Mushroom-forming fungi), Pucciniomycotina (rusts and allies), Ustilaginomycotina (smuts and allies). Agaricomycotina subphylum contains about 20000 described species in which 98% of the total species are in a clade, called Agaricomycetes which includes mushrooms, bracket fungi, puffballs and others. The Agaricomycetes class comprises of the order Polyporales which include the family- Meruliaceae and *P. multizonata* is one of important poroid fungal species.

The most recent record of *Podoscypha multizonata* was reported from Turkey-İstanbul, Belgrad Forest, Kömürcü Bent, on beech root (Akata and Sesli 2017). Environmental Protection Agency (EPA) and the European Council for Conservation of Fungi (ECCF) compiled a list of 33 threatened fungi in Europe which included *P. multizonata* as one of the threatened fungal species. The species is known to be distributed only in 8 countries, 5 European Union (EU15) countries and a total of 116 localities. Among these, only 29% of the localities are falling under protected areas. This fungal species is red listed in 3 countries (Hungary, Slovakia and Spain). The main threat to the species is habitat decline in area and quality due to change in land-use *e.g.* densification, forestry and exploitation of localities (Jahn and Müller 1976). The most frequent habitat for *P. multizonata* is reported to be Asperulo-fagetum beech forests (Dahlberg and Croneborg 2006). In republic of Serbia, the fungal species is enlisted to be strictly protected and protected fungal species under the Nature Conservation Law (2009) and Regulation on the proclamation and protection of strictly protected and protected wild species of plants, animals and fungi (2010) (Ivančević *et al.* 2012). Overall (2013) stated that *P. multizonata* is especially associated with old deer parks and generally sporulates around the roots of old or veteran oak or beech trees, generally in open areas. South East England is host to 80% of the world's population of this species precisely because of the type of habitat provided by areas such as Hampstead Heath (Overall 2013). Presence of this species is an indicative of the level of continuity of habitat that there is sufficient ecosystem structure and processes (Alexander and Butler 2004). *Podoscypha* species has also been isolated as one of the endophyte from host plant *Theobroma gileri* (Thomas *et al.* 2008). The mycelial culture of *P. multizonata* is known to show antibiotic activity against bacteria like *Sarcinalutea, Staphylococcus aureus, Bacillus subtilis* which are well known bacterial pathogens, thus, confirming the importance of this macro-fungus (Chung *et al*. 1988).

Therefore, the macro fungal composition is important from ecosystem point of view. The macro-fungi of Garhwal region under Uttarakhand state has been studied earlier by some workers, but *Podoscypha multizonata* was never reported before. So the key objective of this paper was to study the morphological and cultural characteristics of *P. multizonata* isolated from Garhwal hills, Uttarakhand and its *in situ* and *ex situ* conservation.

MATERIALS AND METHODS

Study area and sampling

The main sampling sites in this study were Tons river, (Reserve Forest area) Dehradun and Asan barrage region. Both of these sampling sites are located in Uttarakhand (Fig.1). The sampling site Tons river, Reserve Forest is located between 30°20′19″ N 78°00'03" E and Asan Barrage between 30°26'01" N 77°39'53" E.

Collection and study of material

The macro-fungal survey of the study area was conducted during the month of June-August, 2018 and fruiting bodies of *P. multizonata* were collected.

Fig.1. A. Map of study site, **B.** Tons river flowing through reserve forest, collection site of samples (Green balloon tag showing collection area).

Isolation of fungus

Isolation of fungus was performed by surface sterilization method (Kinkel and Andrews, 1988). Five bits (2×2 mm) of fruiting were submerged in 70% ethanol for 1 min, then transferred into 15% H_2O_2 for 1 min and again kept in 70% ethanol for 1 min. Thereafter, the bits were serially washed in 10 changes of sterile distilled water, blotted dry, then placed in each of five Petri plates (5 bits/plate) containing PDA (Potato Dextrose Agar) medium supplemented with Streptomycin (100 mg/l) and incubated at 25±1°C for 7 days. The fungal colonies that appeared after incubation period were isolated in fresh sterilized petri plates containing PDA medium. After that fungus grew on culture media, it was analysed for morphological and microscopic characteristics for final identification.

Microscopic examination

Concomitant morphological features of *Podoscypha multizonata* culture were studied in detail. The major and remarkable macroscopic features studied in fruiting body identification were the colony diameter, colour (conidia and reverse), exudates and colony texture. Microscopic characteristics for the identification were conidial heads, trama, colour and length, vesicles shape and seriation, conidia size, shape and roughness. After culturing on PDA medium, colony features were observed including diameter, colour of conidia, mycelia, exudates and reverse observation, colony texture and shape after 7 days. Finally, the morphological characteristics of isolated *P. multizonata* isolate/s were compared with those of the standard species/isolates with the help of literature (Sharma 2012) and Enet.

RESULTS AND DISCUSSION

The fruiting body (basidiocarp) was observed to be associated with the roots of *Bambusa* species. The samples of basidiocarp was analyzed and visualized under microscope. The basidiocarp was measured 4-4.5 cm high and 3.5-4 cm wide with white margin and stout stripes. Basidiocarp comprises of numerous sub-stipitate pilei arising from a common base and arranged in rosette pattern. Pileus was leathery, thin and pale brown with dark zones and white margin. The basidiocarp was observed to produce fleshy smell. Dimitic hyphal structures were observed which consists of generative and skeletal hypahe. The freely branched generative hyphae measured, 2-5 µm in diameter, thin walled, hyaline and consist of clamp-connections at the septal region. The skeletal hyphae were 2.5-5 µm in diameter, thick walled and unbranched. The basidiospores measured about 4.5 - 6.5 x 4 - 5 µm, subglobose to ovoid, hyaline, smooth, amyloidal double walled and pinched. Gloeocystidia were abundant, elongate, cylindrical, clavate with budding apex and finger like in shape. Cheilocystidia and Pileocystidia were also present but wereclavate and curved or setae like structures.

Cultural characteristics

The colony of the fungal species on PDA medium was reported with flat elevation, filiform margin and filamentous form. A felty and white coloured colony of *in vitro* fungal culture was observed in time duration of approximate two weeks which after one week started to form black droplets kind of structures in the culture (Fig.2).

Welden and Bennett (1973) studied the cultural characteristics and mating-type behaviour in *P. multizonata* and *P. ravenelii*. The results of this study were also found in accordance asdescribed by them with some variations such as Gloeocystidia were abundant, elongate, cylindrical, clavate with budding apex and Cheilocystidia and Pileocystidiaalso present but these were clavate and curved or setae like. Two similar species of *Podoscypha e.g. P. bolleana* (Mont.) Boidin and *P. moelleri* (Bres. &Henn.) D.A. Reid. have Caulocystidia. *Podoscypha bolleana* differs from *P. yunnanensis* by having larger Caulocystidia (40–125 × 7–15 µm)according to Drechsler-Santos *et al.*(2007). But *Podoscypha moelleri* differs in having smaller basidiospores (2.2–3.2 × 2–2.2 µm) (Reid 1965). The diversity of *Podoscypha* species is low in China and only two species have been reported previously *e.g. Podoscypha brasiliensis* D.A. Reid and *P. elegans* (G. Mey.) Pat. (Wu 2003, 2019; Dai 2010). However, morphologically, *P. brasiliensis* differs from *P. yunnanensis* by larger basidiospores (5–7 × 3.5–4.5 µm) (Reid 1965; Wu 2003; Drechsler-Santos *et al*, 2007). *Podoscypha elegans* can be distinguished by the presence of chlamydospores in the context and larger basidiospores (5–5.6 × 3.8–4.3 µm) (Patouillard 1900). The diversity of *Podoscypha* species in China is still not well known, especially in the subtropical and tropical regions and many taxa of wood-rotting fungi were recorded and described recently (Ren and Wu 2017; Yuan *et al*. 2017). *Podoscypha yunnanensis* is also reported from the subtropics only. Similarly, the natural occurrence, diversity and distribution of *Podoscypha* species in India particularly of Garhwal Himalayas, Uttarakhand is still not well known and reported earlier. Therefore, it is possible that new taxa of macro-fungi of importance will be found after further investigation in the region.

On the basis of above findings, it can be concluded that Dehradun valley under Garhwal Himalayas region of north India, being rich in flora and fauna; natural forest can be a good source of potential edible and medicinal mushrooms. Also, a preparation of checklist of threatened fungal species located in Garhwal Himalayas of Uttarakhand, India should also be undertaken in near future. Since, this macro-fungus was found in very less number in this region so the *in situ* conservation or *ex situ* conservation is must require for conserving and preserving this important and medicinal macro-fungal species so that it cannot be lost irrevocably.

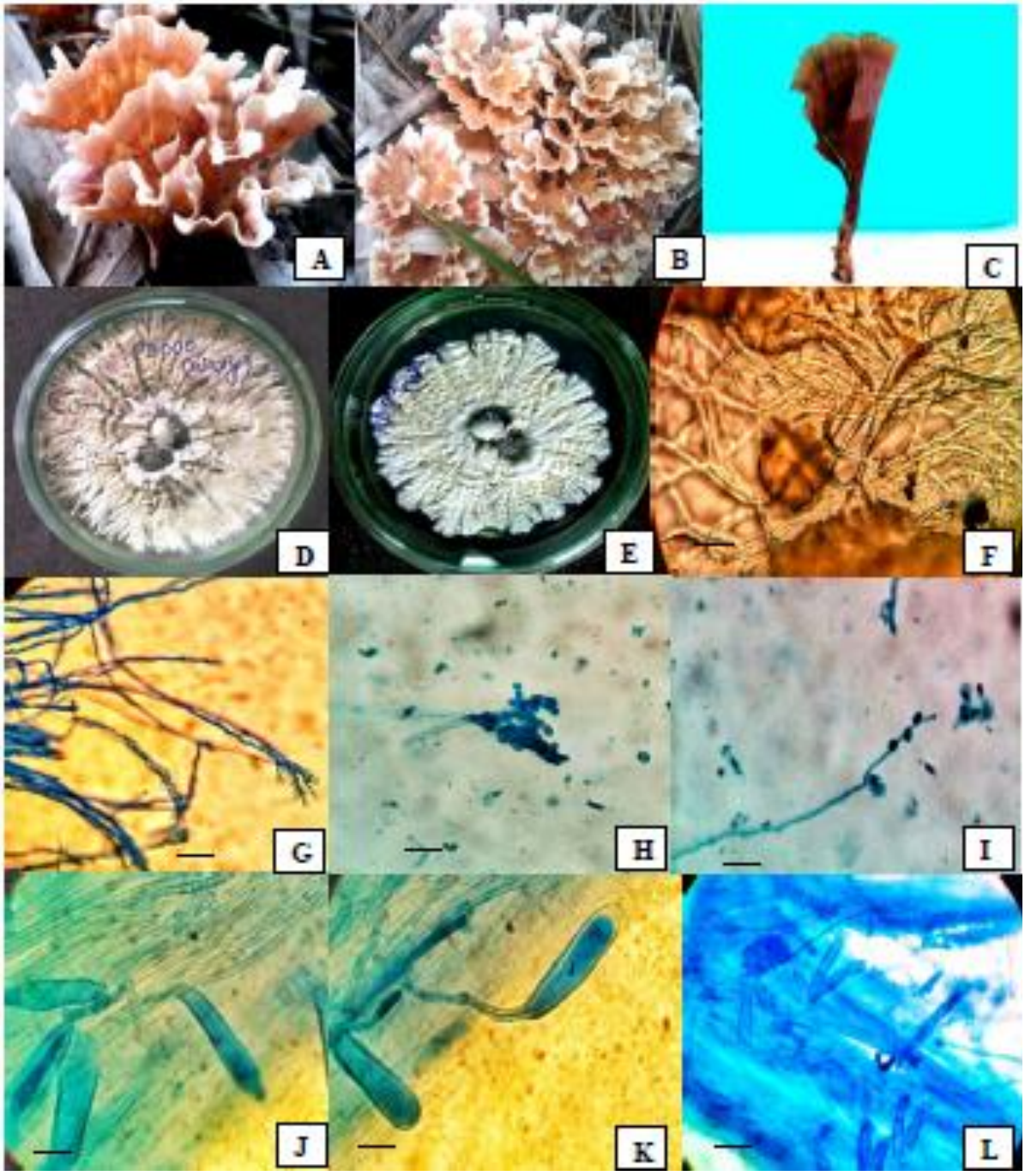

Fig. 2 A, B- Basidiomata, C. Single Basidiome, D,E. *In vitro* culture colony in medium, F.hyphal skeletal network in Basidiome, G. Bifurcated and forked hyphal tips, H, I. Basidiospores, J, K. Gloeocystidium, L. Cheilocystidia and Pileocystidia in Basidiomata. (All images from F-K at 400X, L-100X and cross bar - 20µm)

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