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All general correspondence should be addressed to **Prof. N.Raaman,** Secretary, MSI, C.A.S. in Botany, University of Madras, Guindy Campus, Chennai-600 025, India (*Email: raaman55@gmail.com*). Manuscripts for publication and books for review may be sent to **Editor, Prof. N.S. Atri**, Department of Botany, Punjabi University, Patiala-147002 Punjab, India (*Emails: narinderatri04@gmail.com; fungalbio2007@rediffmail.com*).

The name of the journal KAVAKA *is a Sanskrit word which means Fungus.*

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From the Editor's Desk

In today's world technology is one of the most important components of our life. The recent revolution in information technology has resulted in providing easy access and timely solutions to number of intricate time consuming issues which we come across at work place on daily basis. Keeping pace with time, the Mycological Society of India started the online publication of Kavaka in 2014. This important development has resulted in improved visibility of the Journal and excellent response of the contributors from India and abroad which can be judged from the standard of publications and the viewer ship of the journal from the website of Mycological Society of India (*http://www.fungiindia.co.in*). So as to add efficiency to the editorial process we are in the process of hiring online work space which will facilitate online manuscript submission, editorial processing and publishing. The adoption of open journal system will also improve both accessibility and citation of the research articles being published in Kavaka. Although we were already doing it since we started the online publication of the journal, but with lot of manual intervention. For online submission and further processing of the articles in Kavaka an independent portal *(http://*www.*kavaka.fungiindia.co.in*) is being created, which will be made functional in due course.

I would like to express my sincere gratitude to all the reviewers and the contributors of this issue.

June 30, 2016

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Relavance of Thermophilic Fungi in Omics Era!

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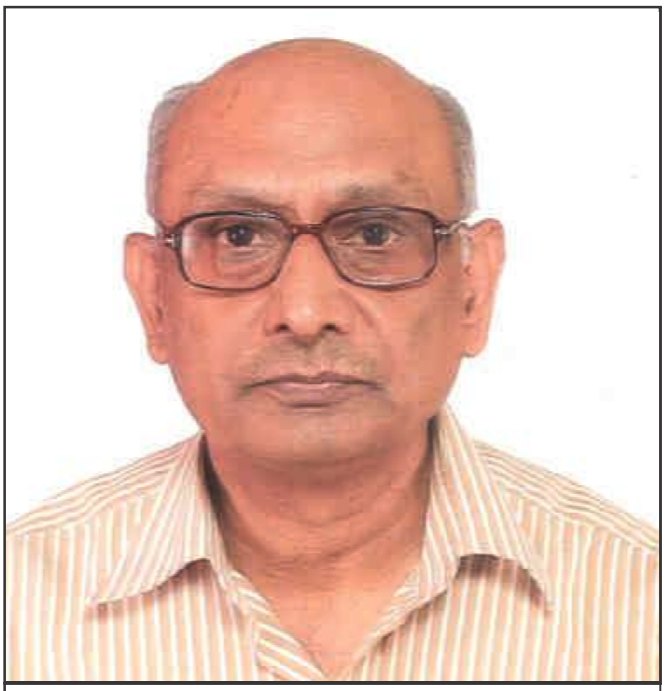

Prof. B.N. Johri
President MSI 2015-16

ABSTRACT

Microbiological thermogenesis has been discussed for well over a century since mankind required stored material for food and feed to tide over the environmental perturbations. That thermophiles were dominant in degraded plant matter was convincingly demonstrated by Hugo Miehe who described *Rhizomucor miehei* as a pioneer fungal species. Sporadic reports of recovery of thermophilic fungi kept appearing from other habitats including soil. However, it goes to the credit of well known aquatic mycologist, Ralph Emerson to discuss detailed distribution and activities of this group of fungi while working on retting guayule, the waste plant matter of rubber plant. In view of their association with self-heated habitats, thermophilic fungi have attracted a great deal of attention as producers of a variety of enzymes, chiefly carbohydrases. However, the first commercially exploited enzyme comprised of lipase of *Humicola lanuginosa* (*Thermomyces lanuginosus*) and *Rhizomucor miehei*. *Thermomyces lanuginosus* was found to secrete cellulase-free xylanase and has been a key fungus in genomics related investigations. In view of their efficient hemi-cellulose degrading ability, a consortium (The Genozyme Project) of Canadian industry and University has undertaken detailed genomic study of about twenty thermophilic fungi to exploit their enzymes in generation of bioethanol and bioenergy. Major thermophilic fungal species under detailed investigation for this purpose include *Humicola insolens*, *Melanocarpus albomyces*, *Myceliophthora thermophila*, *Sporotrichum thermophile*, *Thermoascus aurantiacus* and *Thielavia terrestris*. Relevance of these fungi in biotechnology are discussed.

Keywords: Thermophilic fungi, *Rhizomucor meihei*, biofuels, carbohydrases, phylogeny, genomics.

INTRODUCTION

Considerable data exists in the realm of microbial diversity estimates at global scale that include fungi although time and again revision of such estimates are made on account of yet unexplored habitats, primary being the endophytic populations (de Oliveira *et al*., 2015; Natvig *et al*., 2015). However, this does not in any manner reduces approximation of global fungal diversity estimates. Most existing fungi are mesophilic as they can happily grow within the temperature regimen of 25°C to 35°C; some are, however, cold loving or psychrophilic and there are few others that preferentially live in environments approaching a temperature profile of 30°C to 60°C. This latter group has been termed, 'thermophilic' and fungi falling within this group have been variously defined to differentiate them from their mesophilic counterparts (Cooney and Emerson, 1964). Truly thermophilic fungi can be counted on finger tips since their number hovers around three dozen species. It is natural therefore to question their existence in a repertoire of more than one million fungal species! Yet these handful species have attracted a great deal of attention of microbial biotechnologists on account of their industrial potential. So much so that nearly all of them are under genome sequencing platform; several genomes are well described and are being discerned to exploit especially their carbohydrases (Berka *et al*., 2011; van den Brink *et al*., 2013; 2015).

PAST IS THE TRUE REFLECTION OF FUTURE

The phenomena of thermogenesis was known to practicing scientists for long, however, first reports of truly thermophilic fungi emerged from the writings of Miehe (cited in Cooney and Emerson, 1964) wherein *Thermoascus* and *Malbranchea pulchella* var *sulfurea* were discussed. However, it must be emphasized that reports of *Mucor pusillus* and *Humicola lanuginosa* had earlier been described on bread and potato (Lindt, 1886; Tsiklinskaya, 1889 cited in Cooney and Emerson, 1964). Miehe's early work attracted considerable attention that lead to the search for the thermophilic fungi from a variety of habitats inclusive of soil, hay, compost, birds nest, waste of various kinds and other natural and man-made thermogenic habitats; these early researches were monographed in a volume written by Cooney and Emerson (1964). One must appreciate here the work of Ralph Emerson, a renowned aquatic mycologist, on the retting guayule during his stint during the war that laid the foundation of systematic and functional investigations of thermophilic fungi (Cooney and Emerson, 1964). Michael Tansey (1971) in particular described not only the distributional component but also discussed the basis of thermophily in these moulds (Brock, 1967). This was perhaps the first indication that eukaryotic thermophily is distinct from the prokaryotes since the upper limit of growth appeared to extend to only 62°C! This report opened up an area of science which until than had few active researchers in the field.

The recovery of thermophilic moulds from a variety of decomposing plant biomass opened up an arena of exocellular enzymes especially carbohydrases from these fungi are considered today an ideal system in bioenergy and biofuel sector of tomorrow (Straatsma *et al.*, 1994; Maheshwari *et al.,* 2000). Some of these developments of 1980's are compiled in a volume edited by Johri, *et al.* (1990).

PLANT BIOMASS DEGRADATION IS CENTRAL TO BIOPOTENTIALITY IN THERMOPHILIC FUNGI!

A great deal is known and written upon the cellulosic biomass available for effective utilization in industry therefore basics of such issues can be found elsewhere (Rajasekaran and Maheshwari, 1999; Rubin, 2008). However, the important questions that need attention from the view point of industrial exploitation through fungal system merits specific consideration of the following:

- Dispersed vs. point availability
- Seasonality of the system
- Ease of hydrolysis
- Effectivity and conversion efficiency
- Thermostability of enzymes
- Second step conversion of sugars to end product(s)

The above discussion also indicates the virtues by way of extracellular hemicellulolytic machinery of this fungal group. This work has been reviewed extensively therefore relevant points dealing with genomics, proteomics and transcriptomics issues shall only be highlighted (Rawat and Johri, 2014).

GENOMICS OF THERMOPHILIC FUNGI HAS PROGRESSED RAPIDLY!

Ever since the issue of thermal stability and conversion efficiency of cellulosics by various species belonging to the cartel of thermophilic fungi has been highlighted, genome sequencing work has begun very rapidly. So much so that a joint consortium of university and industry in Canada has decided to sequence nearly twenty thermophilic fungi whereas many of them are also covered within the purview of the Fungal Genome Initiative operating globally (Berka *et al.*, 2011; Morgenstern *et al.*, 2012; Zhou *et al.*, 2014).

I shall discuss in the following pages, characteristics of a few thermophilic fungal species and compare them with experimentally tested mesophilic fungi such as *Aspergillus niger, Neurospora crassa, Phanerochaete chrysosporium* and *Trichoderma reesei*. In a detailed study of genomics and transcriptomics of *Rhizomucor miehei* used commercially in production of aspartic protease (substitute of calf chymosin) and lipase (chiral hydrolysis), Zhou *et al.* (2014) bring out several interesting features. That this fungus broadly comprises of 110 glycoside hydrolases, 118 glycosyl transferases, 20 carbohydrate esterases, 155 proteases, 4 endoglucanases and 8 β-glucosidases; presence of β-glucanase, xylanase and fibrinolytic enzyme was earlier not associated with *R. miehei*. The molecular size of its genome (27.6Mb) is much smaller than that of thermophilic *Myceliophthora thermophila* (38.7 Mb) or *Trichoderma reesei* (33.9Mb). This is also reflected in its relatively lower G+C content (43.83%, cf 51.4% for *Myceliophthora thermophila*). But in terms of number of protein coding genes, *Rhizomucor miehei* (10,345 genes) stands out against *Myceliophthora themophila* (9,110 genes) and *Trichoderma reesei* (9,129 genes); *Neurospora crassa,* however, possesses 12,188 and *Aspergillus niger* with 33.9 Mb genome comprises of 14,165 coding genes. Thus, no specific conclusions can be drawn by simply looking at the size of fungal genome, mesophilic or thermophilic.

Berka *et al.* (2011) were the first to describe the genomes of *Myceliophthora thermophila* (MT) and *Thielavia terrestris* (TT), key thermophilic fungal species for bioenergy and biofuel research. There is a little difference in their genome size (TT, 369 Mb vs. 38.7 Mb in MT) and G+C content (54.7 TT; 51.4 MT) as also the number of genes (TT, 9.813; MT, 9.110). The predicted secretome contains approximately 180 carbohydrases, 40 peptidases, 65 oxidoreductases and 230 proteins of unknown function. Xylanases of MT and TT were cloned and expressed in mesophilic *Aspergillus niger* which resulted in expression of active proteins with T_{opt} of 40°C to 70°C. When crude enzyme preparations were used for hydrolyses of alfalfa plant biomass, appreciably higher amount of sugars were released compared to preparation from mesophilic *Chaetomium globosum* and *Trichoderma reesei*. In this context, it is also relevant to refer to a paper on deconstruction of plant biomass by an enzyme from *Thermoascus aurantiacus*, another fungus with high conversion efficiency for hemicelluloses under thermophilic condition (Mc Clendon *et al.,* 2012). The authors have reported greater saccharification of ionic liquid pretreated switchgrass (*Panicum virgatum*) by preparation derived from *Thermoascus aurantiacus* compared to even *Thielavia terrestris*; the proteins were more thermostable than those available commercially (Novozyme, Cellic CTec2).

In the context of plant biomass hydrolyses by enzymes derived from thermophilic fungi, two other paper also deserve attention. The first of these refer to the expression of a thermostable xylanase of *Malbranchea pulchella* in *Aspergillus nidulans* (Ribeiro *et al.,* 2014). These enzyme preparation exhibits activity up to 80°C and is stable at 65°C even after 24 h. The stability is attributed to the presence of 8 cysteine residues at the active site (Kaur *et al,.* 2015) On the other hand, these authors describe functional and structural diversity in α-L-arabinofuranosidases of *Scytalidium thermophilum* and report that carbohydrate degrading genes *abf62, abf62B* and *abf62C* of this thermophilic fungus are actively expressed on straws from barley, alfalfa, triticale and canola.

An interesting thermophilic fungus whose genomics and proteomics investigation have made it an important eukaryotic experimental system in structural biology domain is, *Chaetomium thermophilum* (Bock *et al.,* 2014). The genome of this fungus is much smaller than the thermophiles discussed earlier since it stands at only 28.3Mb. The protein coding genes number 7,277 of which 2,853 genes have been functionally characterized. Based on structural investigation

it appears that thermophily in *C. thermophilum* primarily occurs at the individual protein level. However, van Noort *et al.* (2013) have earlier reported on the nature of eukaryotic thermophily in *C. thermophilum*, *Thielavia terrestris*, and *T. heterothallica* based on their genomes and mutational path and arrive at the conclusion that "many protein required for eukaryotic cell function are absent from bacteria and archea". According to these workers a common change in proteins comprises of the substitution of lysine by arginine; this study has also thrown light on the approaches "to engineer protein" stability.

TAXONOMIC AND PHYLOGENETIC UPDATE!

There are umpteen reports that discuss the phylogenetic relationship of this small, rather artificial group since thermophilic fungi exist only in seventeen genera within the approx 0.5 million fungal species known currently (de Oliveira *et al.,* 2015). These include: *Canariomyces, Chaetomium, Coonemeria, Corynascus, Dactylomyces, Malbranchea, Paecilomyces, Rhizomucor, Scytalidium, Stilbella, Talaromyces, Thermoascus, Thermomyces* and *Thielavia*. With the recent induction of One Fungus = One Name dictum, several changes have occurred in the designation of thermophilic fungi since their earlier identities (Mouchacca, 2000). Some representative changes include; *Corynascus thermophilus* (old) = *Myceliophthora fergusii* (new); *Corynascus heterothallicus* (old) = *Myceliophthora heterothallica* (new); *Talaromyces thermophilus* (old) = *Thermomyces dupontii* (new); *Talaromyces byssochlamydoides* (old) = *Rasamsonia byssochlamydoides* (new).

On the phylogenetic front, heat loving fungi have been found to form a single paraphyletic group although thermophily is thought to have multiple independent origins (Morgenstern *et al.,* 2012). Detailed analysis of thermophilic fungi in *Chaetomiaceae* depicts origin from a common ancestor with multiple gene loss (van Noort *et al.,* 2013).

ADAPTATION AND *IN SITU* RESPONSES

Mechanism of adaptation in a handful of thermophilic fungi have been investigated that suggest involvement of melanization; formation of saturases and macromolecular stability as responsible for thermophily (Powell *et al.,* 2012; Van Noort *et al.*, 2013). However, detailed analysis of primary and secondary metabolism, excluding secretion of carbohydrases, is scanty (Mchunu *et al.*, 2013; Maheshwari *et al.,* 2000; Langarica Fuentes *et al.*, 2014). An interesting investigation in this context is that of Powell *et al.* (2012) that deal in a detailed manner the thermophilic fungi of Sevilleta National Wildlife Refuge, New Mexico, USA. This study spread over nearly ten years of field research has examined the distribution of thermophilic fungi on a seasonal basis and conclude that these fungi show distinct fluctuation during spring and summer; majority of the fungal isolates belonged to the order *Eurotiales* and *Sordariales* (*Chaetomiaceae*). However, there was no specific association with either the substrate or the habitat suggesting niche exclusion. There was clearly marked phylogenetic diversity as also genetic diversity within a phylo group. *In vitro* growth vs. temperature tests showed an optima for most strains at 45°C-50°C; furthermore while most isolates grew at 60°C, none showed any detectable growth at 65°C, thus reverting to the contention of an upper temperature eukaryotic growth at 62°C! (cf. Tansey and Brock, 1972). According to the author of this comprehensive study, their data "supports the hypothesis that the fungal thermophily is an adaptation to transient seasonal and diurnal high temperature, rather than simply an adaptation to specialized high temperature environment" (Powell *et al.,* 2012). In view of the use of metagenomic tools in microbial ecology and the emergence of "One Fungus = One Name" concept, biosystematics of thermophilic fungi requires a fresh look (Hibbett and Taylor, 2013; Taylor, 2011).

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Some New Records of Russulaceous Mushrooms from North West Himalayas

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ABSTRACT

The present paper deals with taxonomic diversity of russulaceous mushrooms in Himalayas. During the survey, seven taxa, namely *Russula cremeoavellanea* Singer, *R. peckii* Singer, *Lactarius salmoneus* var. *curtisii* Peck., *L. rubrilacteus* Hesler & Smith, *L. eburneus* var. *eburneus* Thiers, *L. badiopallenscens* Hesler & Smith and *Lactifluus luteolus* var. *luteolus* (Peck) Verbeken have been recorded and described for the first time from India.

Keywords: Russulaceous mushrooms, taxonomy, *Russula*, *Lactarius*, *Lactifluus*.

INTRODUCTION

The family *Russulaceae* is one of the 12 families under order *Russulales* (Kirk *et al*., 2008). The members of this family are most diverse, containing a remarkable variety of sporophore forms ranging from resupinate to pleurotoid, pileate and stipitate with lamellate or gasteroid hymenophore. The vast majority of the known species in family *Russulaceae* are agaricoid belonging to the traditional genera *Russula*, *Multifurca*, *Lactarius*, and *Lactifluus*. (Buyck *et al*., 2008; Verbeken *et al*., 2011)). There are 750 species of genus *Russula* and 450 species of genus *Lactarius* documented worldwide (Kirk *et al*., 2008). As compared from India 125 species of *Russula* and 80 species of *Lactarius* are known so far. (Atri *et al*., 1994; Das and Sharma, 2005; Bhatt *et al*., 2007; Dar *et al*., 2009; Das *et al*., 2010; Das and Verbeken, 2011; 2012; Das *et al*., 2013). While investigating the diversity of russulaceous mushrooms in North West Himalayas, seven taxa which are new records for India were documented. These include *Russula cremeoavellanea* Singer, *R. peckii* Singer, *Lactarius salmoneus* var. *curtisii* Peck., *L. rubrilacteus* Hesler & Smith, *L. eburneus var. eburneus* Thiers, *L. badiopallenscens* Hesler & Smith and *Lactifluus luteolus var. luteolus* (Peck) Verbeken.

MATERIAL AND METHODS

The macromorphology and micromorphology was studied in accordance to the methodology given by Atri *et al.* (2005). The terminology used for describing the colour tone of sporophore parts and spore print is after Kornerup and Wanscher (1978). Microscopic line drawings were drawn with the aid of camera lucida at 1000X. Basidiospore measurement excludes the height of ornamentation and length of apiculus. The spore shape quotient (Q=L/W) was calculated considering the mean value of length and width of 20 basidiospores. Spore ornamentation was studied both under compound microscope and SEM. Basidium length excludes the length of sterigmata. Specimens are deposited in the Herbarium of Botany Department, Punjabi University, Patiala under PUN.

Taxonomy

Russula cremeoavellanea Singer, *Revue Mycol.*, Paris **1**: 288, 1936.

Figs. 1 (A-H) & 2 (A-G)

Sporophore 2.5-8.8 cm in height. Pileus 1.5-8.5 cm broad, plano convex to applanate; umbo present in young specimens; margin regular when young, splitting at maturity;

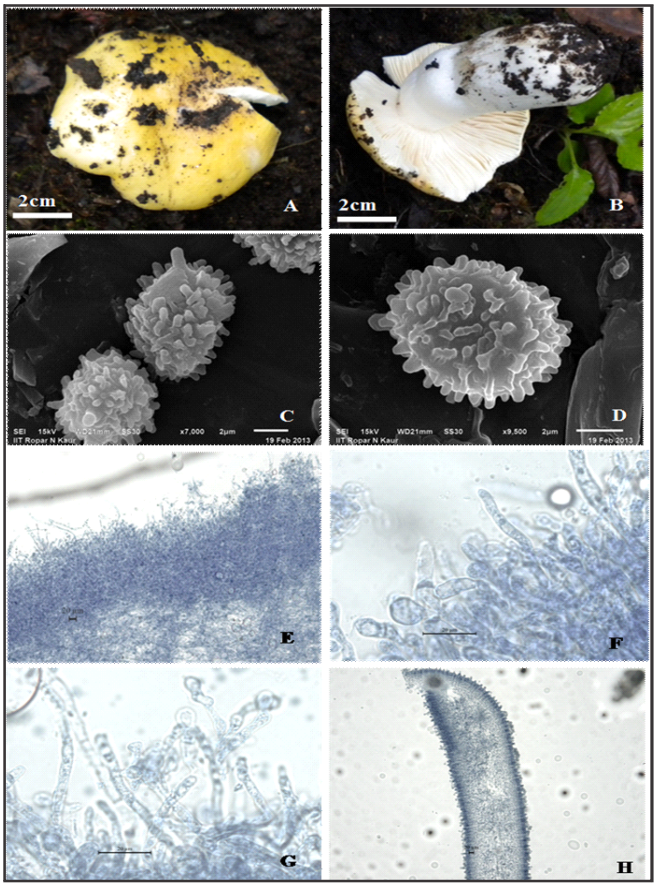

Fig. 1 (A-H) ***Russula cremeoavellanea***: A & B. Carpophores, C & D. SEM photographs of basidiospores, E. Trichodermal turf on pileus cuticle, F & G. Pileus cuticular elements, H. Cellular trama.

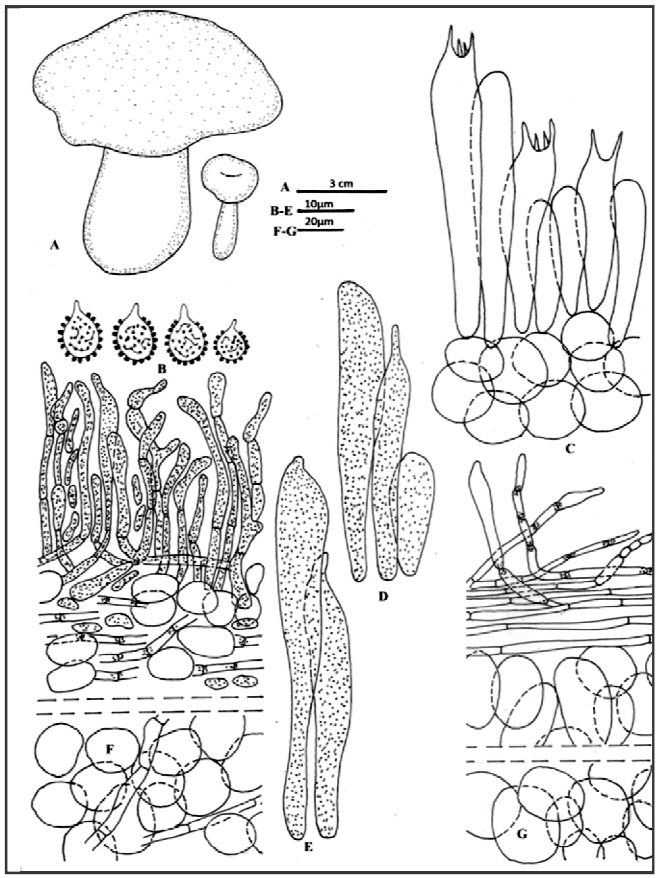

Fig. 2 (A-G) ***Russula cremeoavellanea***: A. Carpophores, B. Basidiospores, C. Hymenophore showing basidia, D. Cheilocystidia, E. Pleurocystidia, F. Cross section through pileus showing cuticular details and context, G. Cross section through stipe showing cuticular details and context

surface light yellow ($4A_4$), moist, sticky, shiny, unchanging; cuticle fully peeling; flesh 0.1-0.5 cm thick, white ($1A_1$), unchanging; taste and odour mild. Lamellae adnate, equal, distant, branched, moderately broad (0.5 cm), white ($1A_1$), gill edges smooth, fragile. Stipe central, 2.4-9.0 cm long, 0.5-2.5 cm broad above, 0.8 cm broad in the middle, 1.0-4.0 cm broad below, obclavate, white ($4A_1$), slightly grayish brown on bruising, hollow, smooth.

Spores 6.0-8.6 × 5.0-7.2 µm (excluding ornamentation), broadly ellipsoidal (Q=1.2), warty, ornamentation in the form of isolated warts, few warts joined, ornamentation type VI, VII, amyloid; plage amyloid, distinct; apiculate, apiculus 1.4-2.1 µm long. SEM study reveals the presence of isolated warts and wart to wart connections, warts 0.4-1.4 µm high with blunt apices; plage area with low diffused ornamentation. Basidia 28.6-54.34 × 7.2-10.0 µm, clavate, granular, 2-4 spored, abundant (4 basidia/100 µm); sterigmata 2.8-7.2 µm long; pleurocystidia: macrocystidia 54.3-71.5 × 7.2-13.0 µm, subcylindrical to clavate with flame shaped, tubular, acute, blunt and capitate tips, granular, 2 cystidia/100µm; cheilocystidia 22.8-57.2 × 5.7-10.0 µm broad, similar in details to pleurocystidia; cystidial elements sparcely present on the sides and edges of the lamellae. Pileus cuticle up to 166.5 µm broad, consist of an ixotrichodermal layer of inflated to tapered tipped closely septate 3.7-5.5 µm broad hyphae having incrustrations and adhering debris, arising from the subcellular layer having scattered hyphae; context made up of rosettes of sphaerocysts and tangled hyphae. Hymenophoral trama cellular; subhymenium indistinct. Stipe cuticle made up of longitudinally running 1.8-3.7 µm broad hyphae with occassional projecting elements and small sized fusoid to ventricose caulocystidia measuring 21.4-22.8 × 4.3-5.0 µm in size; context made up of rosettes of sphaerocysts. Clamp connections absent.

Collection examined: Uttarakhand: Kedarnath, Rambada (3583 m), scattered on humicolous soil in association with mosses, Samidha Sharma PUN 6994, August 17, 2010. Kedarnath, Chopta (2600 m), solitary on humicolous soil, Samidha Sharma PUN 6987, August 20, 2010.

Chemical colour reaction: stipe surface pinkish with $FeSO_4$

Discussion: The above collection belongs to *R. cremeoavellanea* Singer. The characteristic features of present collection are in agreement with the description of *R. cremeoavellanea* given by Romagnesi (1967) and Rayner (1970) except in the present collection wart to wart connections in basidiospores are common which are reported to be rare in *R. cremeoavellanea* (Rayner, 1970). This species looks like *R. ochroleuca* Fr., *R. fellea* (Fr.) Fr., *R. claroflava* Grove, *R. solaris* Ferd. & Winge and *R. smaragdina* Quél. morphologically, however, there are sharp differences in other diagnostic features. From *R. ochroleuca* and *R. fellea,* the present collection differs in possessing white gills, mild taste and unreticulated basidiospores where as *R. ochroleuca* and *R. fellea* possess cream coloured and ochraceous honey coloured gills, hot taste and partially reticulated basidiospores. The studied specimen differs from *R. claroflava* by its unchanging carpophores, as all parts become blackish on bruising in *R. claroflava.* From *R. solaris* it differs by its mild taste and odour as *R. solaris* possesses hot taste and fruity odour. *R. smaragdina* possess distant gills and basidiospores with mostly isolated warts while *R. smaragdina* have subcrowded gills and basidiospores with numerous meshes.

The identifying features of the present collection are its shiny sticky yellowish white cap, distantly spaced forked lamellae, obclavate stipe and pileus cuticle with erect cylindrical multiseptate thick walled inflated and incrusted primodial hyphae. Romagnesi (1967) found this fungus associated with birch trees from Europe and Rayner (1970) from Britain. The present collection has been has found associated with mosses. This fungus constitutes a new record for India.

Russula peckii Singer, *Mycologia* **35**(2): 147, 1943

Figs. 3 (A-E) & 4 (A-H)

Sporophore up to 9.5 cm in height. Pileus 7.0 cm broad, flattened depressed; umbo absent; margin regular; translucent striate; surface red ($9A_6$) in the centre, yellowish white ($4A_2$) along the periphery, moist, apex slightly depressed, brownish on bruising; flesh 0.4 cm thick, yellowish white ($2A_2$),

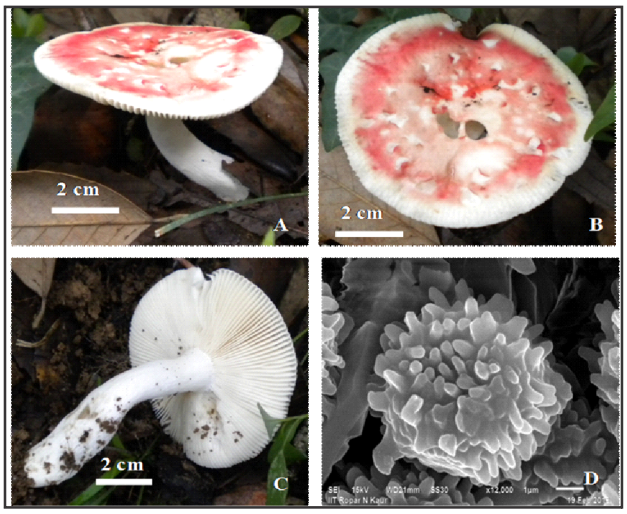

Fig. 3 ***Russula peckii***: A-C. Carpophores, D. SEM of Basidiospores

unchanging; taste and odour mild. Lamellae adnate, equal, distant, branched towards the margin, broad (0.7 cm); yellowish white ($4A_2$); gill edges smooth, normal. Stipe central, 7.0 cm long, 1.5 cm broad, equal in diameter throughout, yellowish white ($2A_2$), grayish brown on bruising, hollow, smooth. Spore deposit yellowish white ($1A_2$).

Spores 5.7-7.2 x 5.0-6.4 µm (excluding ornamentation), subglobose (Q=1.12-1.14), warty, warts 0.7-1.4 µm high, ornamentation in the form of isolated warts, ornamentation type VI, amyloid; plage amyloid, distinct; apiculate, apiculus 1.4 µm long. Basidia 17.16-28.6 x 5.7-10.0 µm, clavate, granular, 2-4 spored; sterigmata 4.3-7.2 µm long; pleurocystidia: macrocystidia 65.8-85.8 x 5.7-7.2 µm, cylindrical to vermiform, granular, thick walled with flame shaped, tubular and capitate tips, cystidia extends 24.3-35.8 µm from the hymenium; cheilocystidia 35.3-64.3 x 4.3-8.6 µm broad, clavate, granular, thick walled with blunt and capitate tips, abundant, rendering the edges sterile. Pileus cuticle 111-148 µm broad, cellular, partially gelatinized; from the cutis arises a discontinuous turf of multiseptate 3.0-4.3 µm broad hyphae; context made up of rosettes of sphaerocysts. Hymenophoral trama heteromerous; subhymenium distinct. Stipe cuticle consist of continuous turf of thick walled septate projecting 3.0-5.7 µm broad hyphae with broad rectangular basal region intermingled with clavate granular thick walled caulocystidia measuring 34.3-43.0 x 3.0-4.3 µm in size; context made up of rosettes of sphaerocysts. Clamp connection absent.

Collection examined: Uttarakhand: Dehradun, Korba (3025 m), scattered on humicolous soil under *Quercus leucotrichophora*, Munruchi Kaur and Samidha Sharma PUN 6988, July 21, 2010.

Discussion: The details of examined collection goes well with the description given by Shaffer (1970) for *R. peckii* Singer, however, in the present collection stipe is yellowish

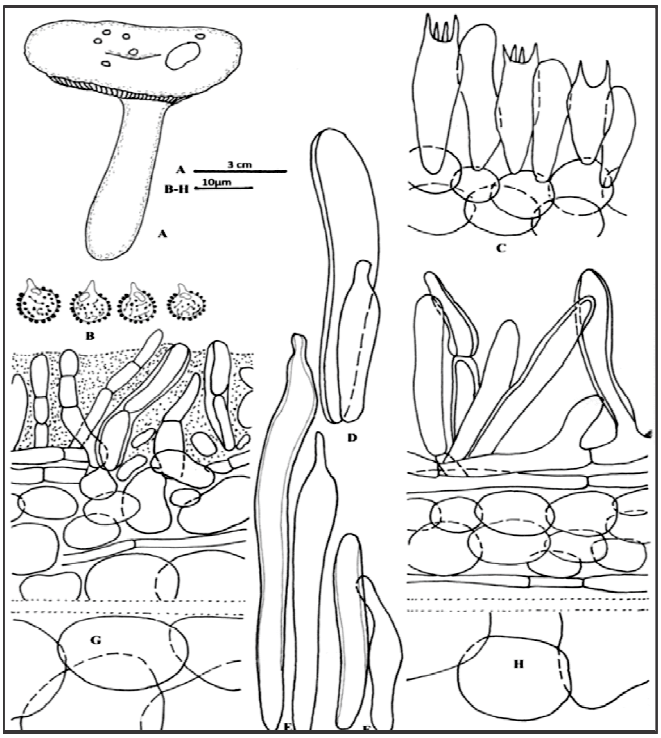

Fig. 4 (AH) ***Russula peckii***: A. Carpophores, B. Basidiospores, C. Hymenophore showing basidia, D. Cheilocystidia, E. Pleurocystidia, F. Caulocystidia, G. Cross section through pileus showing cuticular details and context, H. Cross section through stipe showing cuticular details and context

white instead of pinkish as documented for *R. peckii* (Shaffer 1970). The prominent features of the present collection are its red pileus surface with yellowish white periphery, distant and forked lamellae; basidiospores with mostly isolated warts, wart to wart connections on spore surface forming chain like rows, pileus cuticle with turf of projecting hyphae arising from inflated basal cells and stipe surface with caulocystidial elements. Shaffer (1970) reported this species growing solitary, scattered or gregariously on humus in coniferous and deciduous woods. The present collection was found in association of *Quercus leucotrichophora*. This fungus constitutes a new record for India.

Lactarius salmoneus **var.** ***curtissi*** Peck., *Bull. Torrey Bot. Club* **25**: 369, 1898

Figs. 5 (A-C) & 6 (A-G)

Sporophore 5.5-7 cm in height. Pileus 3.5-4.5 cm broad; convex with depressed to infundibuliform centre; exumbonate; margin regular , inrolled; surface reddish brown ($9E_5$) covered with felt like white to greyish white pruina, at some places greenish grey, eroded, feebly zonate; moist to dry; scaly, scales pruinose fibrillose; apex depressed. Latex reddish brown to blood red, unchanging; mild; cuticle not peeling; flesh 0.6 cm broad, creamish white, changing, turns greenish; taste and odour mild. Lamellae adnate to broadly adnate, unequal, distant (8-12 gills/cm), lamellulae in 3-4 rows, reddish brown ($9E_5$),

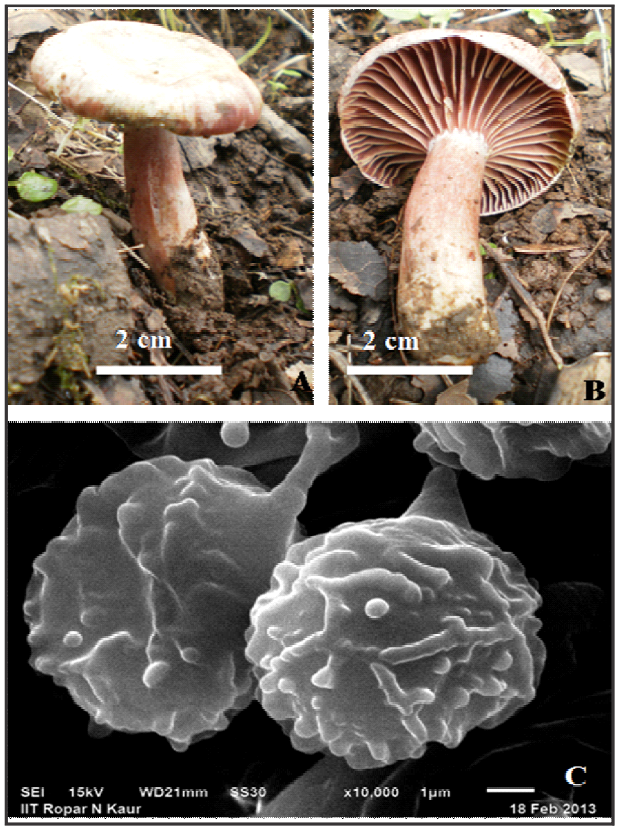

Fig. 5 (A-C) ***Lactarius salmoneus* var. *curtissi***: A & B Carpophores, C. SEM of basidiospores

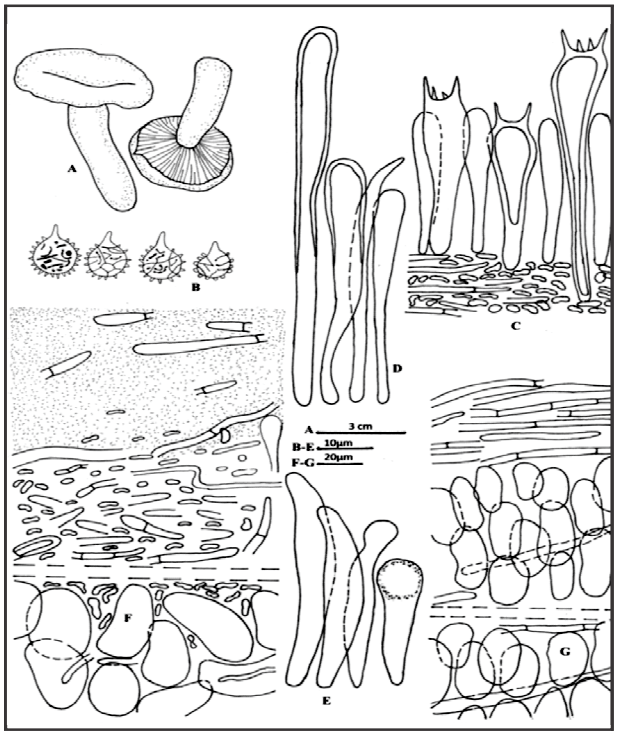

Fig. 6 (A-G) ***Lactarius salmoneus* var. *curtissi***: A. Carpophores, B. Basidiospores, C. Hymenophore bearing basidia, D. Pseudocystidia, E. Cheilocystidia, F. Cross section through pileus showing cuticular details and context, G. Cross section through stipe showing cuticular details and context.

greenish on bruising, 0.3 cm broad in the centre; gill edges smooth, fragile to normal. Stipe excentric, 45 cm long, 11.3 cm broad, tough, stout, cylindrical, cartilaginous, equal in diameter throughout with tapering base covered with white mycelium, orangish brown to concolorous with the pileus with grayish white felt like covering on the surface, greenish on bruising, solid with persistant pith to hollow, smooth.

Spores 5.7-8.6 × 5.7-7.2 µm (excluding ornamentation), globose to broadly ellipsoidal (Q=1.0-1.19), warty, some warts isolated, at places 2-3 warts connected by thick lines forming incomplete reticulum, ornamentation type IIIb, IV, VI, amyloid; plage distinct, weakly amyloid; apiculate, apiculus up to 1.4 µm long. SEM study reveals the presence of semicircle shaped low warts measuring 0.5-0.7 µm in height; isolated warts visible as rounded buttons on the surface, wart to wart connections forming partial reticulum; plage area with low ornamentation. Basidia 34.3-57.2 × 7.2-8.6 µm, 2-4 spored, clavate, granular, thick walled, 12 basidia/100 µm, sterigmata 2.8-7.2 µm long. Pleuromacrocystidia absent, pseudocystidia 50.05-88.7 × 4.3-7.2 µm, clavate to vermiform with capitate to inflated tips, thick walled; pseudocystidia occasional. Cheilocystidia 28.6-50.0 × 5.7-8.6 µm, clavate with acute, capitates to blunt tips, few densely granular with thick walls. Pileus cuticle 74-148 µm broad, partially gelatinized, made up of repent 3.7-7.4 µm broad hyphae; context made up of rosettes of sphaerocysts intermingled with 3.7-11.1 µm broad hyphae. Hymenophoral trama hyphoid; subhymenium indistinct. Stipe cuticle gelatinized, composed of 3.7-7.4 µm broad hyphae; context made up of rosettes of sphaerocysts and tangled hyphae. Laticifers present throughout the context of the carpophores. Clamp connections absent.

Collection examined: Uttarakhand, Deoban (2865 m), solitary on humicolous soil in association with *Quercus dilatata*. Samidha Sharma, PUN 7012, July 18, 2010. Himachal pradesh, Mandi, Futtakal (2500 m), solitary on humicolous soil in mixed forest, Samidha Sharma, PUN 7044, July 27, 2012.

Discussion: The fungus belongs to *Lactarius salmoneous* var. *curtisii* Peck of subgenus *Piperites* due to its reddish brown latex, presence of felt like white to greyish prunia on the surface, inrolled margins and cuticle an ixotrichoderm (Heilmannclausen *et al*., 1998). Earlier Hesler and Smith (1979) classified it under subgenus *Lactarius*. The presently examined collection is characterised by the presence of dry white cottony felt both on the pileus and stipe, blood red latex, surface staining greenish where bruised, excentric stipe and absence of pleurocystidia, which are typical of *L. salmoneous* var. *curtisii*. Except for the broadly adnate lamellae instead of subdecurrent and slightly larger spores measuring 6.5-7.5 × 5.0-6.0 µm as compared to 5.7-8.6 × 5.7-7.2 µm in the Indian collection, rest all features are in conformity. Green staining of the surface where bruised takes it away from the type

variety in which the bruised area of the basidiocarp do not stain green (Hesler and Smith, 1979). This fungus has been reported on moist low lying area under Pine or mixed-Pine hardwood forests from Tennessee and North Carolina (Hesler and Smith, 1979). Presently, it was collected from mixed coniferous forest in association with *Quercus dilatata*, in the month of July. It constitutes a new record from India.

Lactarius rubrilacteus Hesler & A.H. Smith, *North American Species of Lactarius*: 76, 1979.

Figs. 7 (A-D) & 8 (A-H)

Sporophore up to 7.5 cm in height. Pileus up to 5.5 cm broad, infundibuliform; exumbonate; azonate to feebly zoned along the periphery; margin regular, translucent striate; surface brownish orange ($5C_4$), at places flushed greenish, moist, apex depressed; cuticle not peeling; flesh 0.2 cm thick in the centre, brownish on bruising; taste and odour mild. Latex

Fig.7 (A-D) ***Lactarius rubrilacteus***: A & B. Carpophores, C & D. SEM of basidiospores

reddish brown, unchanging, mild. Lamellae adnate, close to crowded, up to 0.6 cm broad in the centre, grayish orange ($5B_3$), flushed greenish, unequal, lamellullae in 3-4 rows, trabeculae present, forking towards the margin; gill edges smooth, greenish on bruising, normal. Stipe central, 4 cm in length, 0.6 cm broad, concolorous with the pileus, equal in diameter throughout, flushed greenish, hollow, smooth.

Spores 6.4-8.6 × 5.7-7.2 µm (excluding ornamentation), subglobose to broadly ellipsoidal (Q =1.12-1.2), warty, warts 0.7-1.4 µm high, ornamentation in the form of isolated warts and catenulations, some warts joined to form ridges on the surface, some warts connected by thick lines forming incomplete reticulum, ornamentation type IIIb, VI,VIII amyloid; plage hyaline, indistinct, apiculate, apiculus up to 1.4 µm long. SEM studies reveal ridges forming wing like structures, isolated warts measuring 0.9-1.4 µm in height and catenulations forming incomplete reticulum on the surface of spores. Basidia 35.7-43.0 × 5.7-8.6 µm, clavate, 4 spored, 3

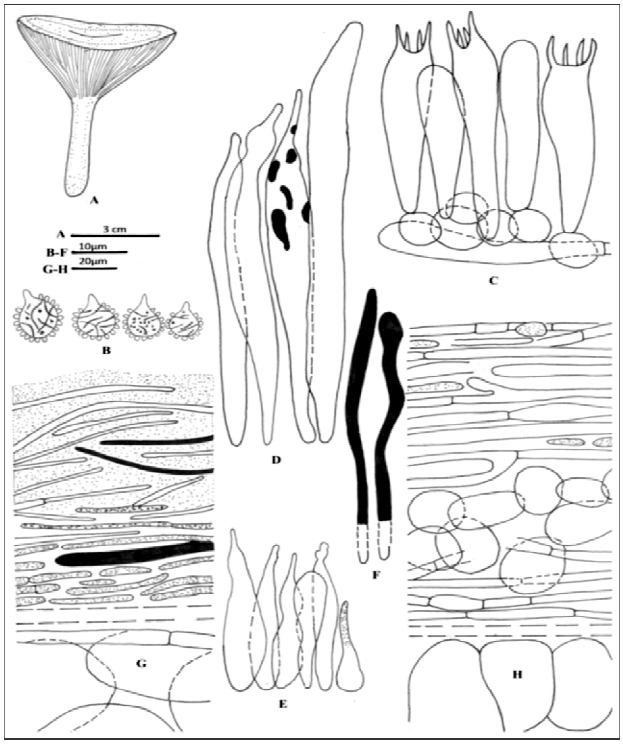

Fig. 8 (A-H) ***Lactarius rubrilacteus***: A. Carpophore, B. Basidiospores, C. Basidia, D. Pleurocystidia, E. Cheilocystidia, F. Pseudocystidia, G. Cross section through pileus showing cuticular details and context, H. Cross section through stipe showing cuticular details and context

basidia/ 100 µm; sterigmata 4.3-7.2 µm long; pleurocystidia: macrocystidia 64.4-93.0 x 4.3-7.2 µm, clavate with tubular, flame shaped, blunt to capitate granular tips; pseudocystidia 3.0-4.3 µm broad, vermiform, deeply seated with broadened to acute tips; cheilocystidia 20.0-35.8 × 4.3-8.6 µm; similar in details to pleurocystidia. Pileus cuticle 140-210 µm broad, gelatinized, made up of 1.4-3.0 µm broad hyphae, slimy layer present on the surface; context made up of rosettes of sphaerocysts intermingled with 4.3-7.2 µm broad hyphae. Hymnophoral trama formed of rounded, closely septate elements giving it a cellular appearance, rosettes not formed, laticifers present in the trama, subhymenium indistinct. Stipe cuticle partially gelatinized, made up of 1.4-4.3 µm broad longitudinally running branched hyphae; context made up of rosettes of sphaerocysts. Clamp connection absent.

Collection examined: Himachal Pradesh: Kangra, Palampur (1219 m), solitary on humicolous soil in *Pinus roxburghii* forest, Samidha Sharma, PUN 7025, July 27, 2009.

Discussion: This collection has been identified as *L. rubrilacteus* Hesler & Smith. It belongs to subgenus *Piperites* because of its feebly zonate pileus, reddish brown mild latex and presence of ixocutis on the pileus surface. The fungus is characterized by feebly zonate orange brown pileus with depressed disc and translucent striate margin, adnate close to crowded lamellae, reddish brown latex and carpophore parts

stained greenish with age. In most of these features including overall carpophore morphology and latex color, the presently examined collection is quite close to *L. subpurpureus* Peck and *L. paradoxus* Beardslee & Burlingham as described by Hesler and Smith (1979). From *L. subpurpureus*, it differs in having orange brown feebly zonate pileus and close to crowded gills as compared to vinaceous red zonate pileus and subdistant gills. Also the spores are much larger in *L. subpurpureus* in comparison. In *L. paradoxus*, the lamellae are close to crowded as is the case in *L. rubrilacteus*, but the pileus is azonate and dingy pale bluish, latex is dark vinaceous brown and flesh slowly becoming slightly acrid in *L. paradoxus*. Comparatively, in the presently examined collection the pileus is feebly zonate with or without orange zones, carpophore parts are flushed greenish with age, latex is reddish brown and flesh is mild in taste. Hesler and Smith (1979) reported this species growing gregarious to scattered under 23 needle pine throughout California and New Mexico. Presently, a solitary carpophore was recorded growing on humicolous soil. It is a new record for India.

Lactarius eburneus* var. *eburneus Thiers, *Mycologia* **49**:715, 1957

Figs. 9 (A-E) & 10 (A-J)

Sporophores 2.2-3.6 cm in height. Pileus 0.7-2.0 cm broad, convex; margin regular; surface grayish orange ($5B_4$), moist, apex umbonate; cuticle not peeling; flesh grayish orange

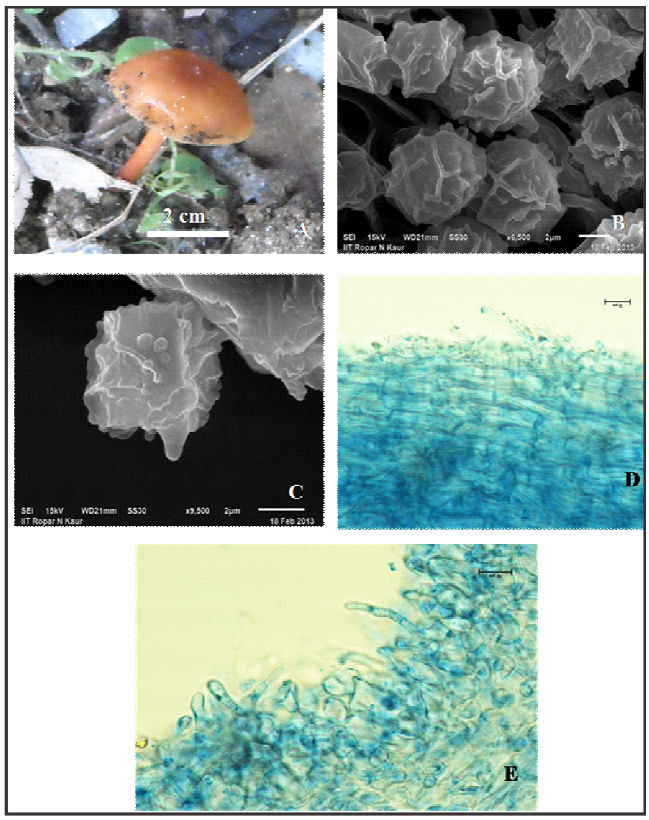

Fig. 9 (A-E) ***Lactarius eburneus* var. *eburneus***: A. Carpophore, B & C SEM of basidiospores, D. Stipe surface, E. Pileus surface.

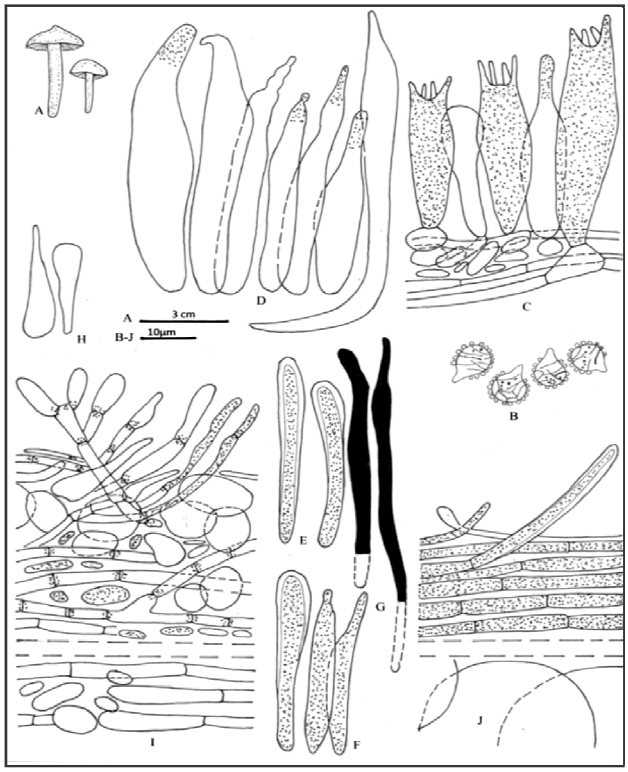

Fig.10 (A-J) ***Lactarius eburneus* var. *eburneus***: A. Carpophores, B. Basidiospores, C. Hymenophore with basidia and cystidium, D. Pleurocystidia, E.Caulocystidia, F. Cheilocystidia, G. Pseudocystidia, H. Pilocystidia, I. Cross section through pileus Surface, J. Cross section through stipe surface.

($5B_4$), unchanging, 0.2 cm broad; taste and odour mild. Latex milky, unchanging, mild. Lamellae decurrent, unequal, close, offwhite, later dark cream with brown spotting, brownish on bruising, gill edges smooth, normal, not differently colored than faces. Stipe central, 2.2-3.5 cm long, 0.2-0.4 cm broad, equal in diameter throughout, bulbous towards the base in some, concolorous with the pileus, hollow. Spore deposit orange white ($5A_2$).

Spores 6.4-7.2 × 5.7-7.2 µm (excluding ornamentation), subglobose (Q=1.1), warty, incomplete reticulum formed by catenulations and few scattered isolated warts, ornamentation type IIIb, IV,VIII, amyloid; plage hyaline and indistinct; apiculate, apiculus up to 1.43 µm long, nodulose type. SEM details reveal the presence of subcylindrical blunt warts measuring 0.5-1.4 µm in height, catenulations and isolated warts clearly visible, warts interconnected to form incomplete network on the surface. Basidia 28.6-45.8 × 7.2-11.4 µm, 2-4 spored, clavate, 3 basidia/ 100 µm; sterigmata 2.9-5.7 µm long. Pleurocystidia: 40.0-85.8 × 7.2-11.4 µm, clavate with tubular, capitate, flame shaped, acute and blunt tips. Pseudocystidia 2.9-5.7 µm broad, cylindrical to vermiform. Cheilocystidia 28.6-40.0 × 4.3-5.7 µm, similar in detail to pleurocystidia. Pileus cuticle up to 490 µm thick, a trichopalisade in the form of discontinuous turf of projecting hyphae and a thin layer of septate elements overlapping a cellular zone, projecting hyphae 2.9-4.3 µm in width

intermingled with versiform pilocystidia having inflated and tubular tips measuring 28.6-35.8 × 7.2-8.6 µm in size; context made up of rosettes of sphaerocysts. Hymenophoral trama hyphal, subhymenium indistinct. Stipe cuticle of longitudinally running septate hyphae measuring 2.86-7.15 µm in width, from the cutis arises a turf of projecting hyphae and caulocystidia measuring 35.8-40.0 × 5.7-7.2 µm in size, clavate, thick walled; context made up of rosettes of sphaerocysts. Laticifers present throughout the context of carpophores. Clamp connections absent.

Collection examined: Uttarakhand, Mukteshwar (2286 m), scattered on humicolous soil, in association with mosses under *Rhododendron* tree, Samidha Sharma and Munruchi Kaur, PUN 7034, August 20, 2011.

Discussion: This fungus is a thin fleshed variant of *L. eburneus* var. *eburneus* of subgenus *Plinthogalus* (Heilmanclausen *et al*., 1998; Hesler and Smith, 1979). It is characterized by grayish orange moist pileus surface, umbonate apex, milky white mild unchanging latex, close decurrent off white lamellae which later turns dark cream with brown spotting, pileus cuticle a trichopalisade formed of discontinuous turf of projecting hyphae intermingled with pilocystidial element and stipe with few caulocystidial elements. This variety differs from *L. eburneus* var. *ervinii* Hesler and Smith by its dark cream coloured lamellae at maturity instead of pinkish grey lamellae of *L. eburneus* var. *ervinii*. Earlier, documentation of fungus is from humicolous soil in hard woods by Hesler and Smith, (1979). Presently the collection has been found growing under broad leaved forest in association with *Rhododendron* and Mosses from Uttarakhand. This fungus constitutes new record for India.

Lactarius badiopallenscens Hesler & Smith, *North American species of Lactarius*: 526-527, 1979.

Figs. 11 (A-D) & 12 (A-H)

Sporophores up to 5.5 cm in height. Pileus up to 3.5 cm broad; convex; umbonate; margin regular; surface light brown ($6D_6$), dark brown towards the centre, moist, azonate; cuticle not peeling; flesh 0.1 cm thick, light brown ($6D_6$) unchanging; taste and odour mild. Latex milky, unchanging, mild. Lamellae subdecurrent, unequal, crowded, narrow (0.1 cm), pale orange ($5A_3$), changing, brownish on bruising; gill edges smooth, normal. Stipe central, 5 cm long, 0.4 cm broad, concolorous with the pileus, equal in diameter throughout, smooth, hollow.

Spores 7.2-8.6 × 5.7-7.2 µm (excluding ornamentation), broadly ellipsoidal (Q=1.20-1.25), warty, warts and ridges connected to form incomplete reticulum, some warts isolated, some joined to form catenulate rows , ornamentation type IIIa, IIIb, VI, VIII, amyloid, plage hyaline without amyloid incrustations, apiculate, apiculus up to 1.4 µm long, nodulose type. SEM study reveals the presence of subcylindrical 0.76-1.4 µm high warts with blunt apices, most of the warts connected to form catenulations and ridges which make incomplete network on the surface; plage area with low diffused ornamentation. Basidia 28.6-40.0 × 5.7-8.6

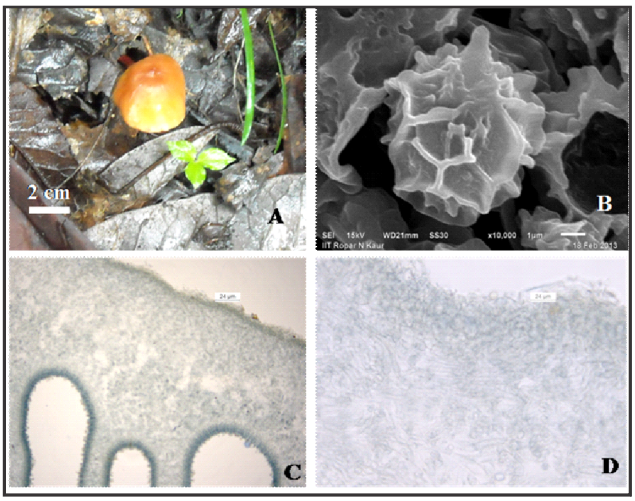

Fig. 11 (A-D) ***Lactarius badiopallenscens***: A. Carpophore, B. SEM of basidiospore, C. Pileus surface and context, D. Pileus epithelium.

µm, 2-4 spored, clavate, granular, 3 basidia /100 µm; sterigmata 2.86-4.3 µm long. Pleurocystidia 25.7-45.8 × 4.3-10.0 µm, clavate, granular with capitate, tubular, acute and blunt tips. Pseudocystidia 2.8-64.3 µm broad, hyphoid ,not projecting beyond the hymenium. Cheilocystidia 21.5-28.6 × 3.0-5.7 µm, clavate to fusiform, some with constricted apical portion. Pileus cuticle 55-74 µm broad; epicutis cellular, 3-6 layered, with few appressed hyphoid elements on the surface,

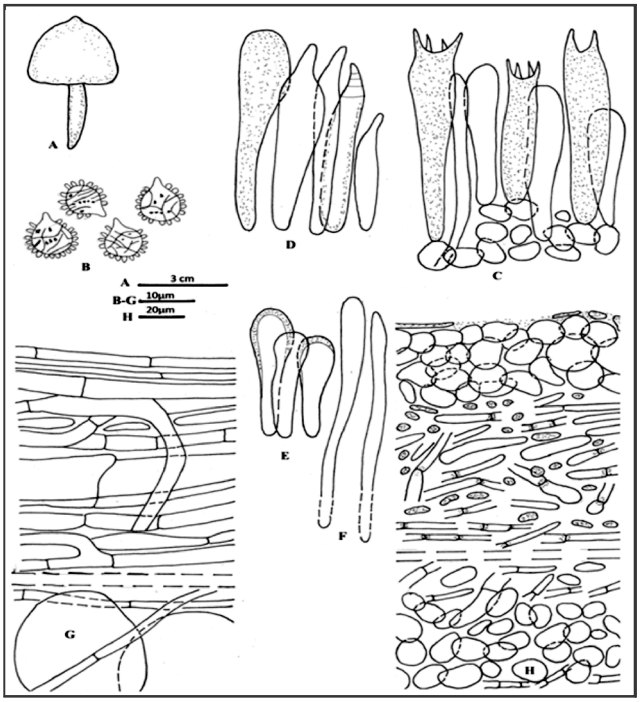

Fig. 12 (A-H) ***Lactarius badiopallenscens***: A. Carpophore, B. Basidiospore, C. Hymenophore showing basidia, D. Pleurocystidia, E. Cheilocystidia, f. Pseudocystidia, G. Cross section through stipe showing cuticular details and context, H. Cross section through pileus showing cuticular details and context.

subcutis made up of loosely placed 3.7-11.5 µm broad hyphae; context made up of rosettes of sphaerocysts intermingled with 3.7-11.5 µm broad hyphae. Hymenophoral trama hyphal, subhymenium indistinct. Stipe cuticle made up of longitudinally tangled septate 4.3-11.4 µm broad hyphae; context made up of rosettes of sphaerocysts and laticifers. Clamp connections absent.

Collection examined: Uttarakhand, Kedarnath, Rambada (3586 m), solitary on humicolous soil, Samidha Sharma, PUN 7018, August 18, 2010.

Discussion: The above collection has been identified as *Lactarius badiopallenscens* Hesler & Smith. It belongs to subgenus *Russularia* because of its azonate light brownish pileus surface and epithelial cuticle. The taxonomically important features of the present collection are its convex cap, umbonate moist azonate pileus, milky unchanging mild latex, crowded pale orange gills which become brownish on bruising and cellular pileus cuticle. Hesler and Smith (1979) documented it growing under beech. However, presently it has been found growing solitary on humicolous soil in the month of August. It constitutes a new record from India.

Lactifluus luteolus* var. *luteolus (Peck.)Verbeken, *Mycotaxon* **120**: 446

Figs. 13 (A-D) & 14 (A-D)

Sporophores 3.7-8.5 cm in height. Pileus 4.3-8.3 cm broad; convex when young, flattened depressed at maturity, margin regular; surface light yellow ($4A_5$), rugose to almost velvety, dark brownish on bruising, azonate, dry; cuticle not peeling; flesh 0.8 cm thick, yellowish white ($2A_2$), first changes to yellowish brown than dark brown; taste and odour mild. Latex abundant, grayish beige ($4C_2$), gum like in appearance, unchanging, mild. Lamellae adnate, unequal (in 6 series), subdistant, forking towards the margin, trabeculae present, narrow 0.3-0.4 cm broad, yellowish white ($2A_2$), first

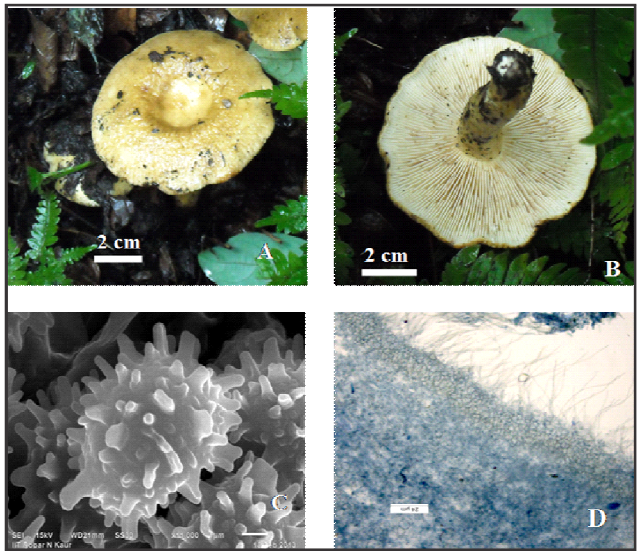

Fig. 13 (A-D) ***Lactifluus luteolus* var. *luteolus***: A & B. Carpophores, C. SEM photograph of Basidiospores, D. Pileus surface.

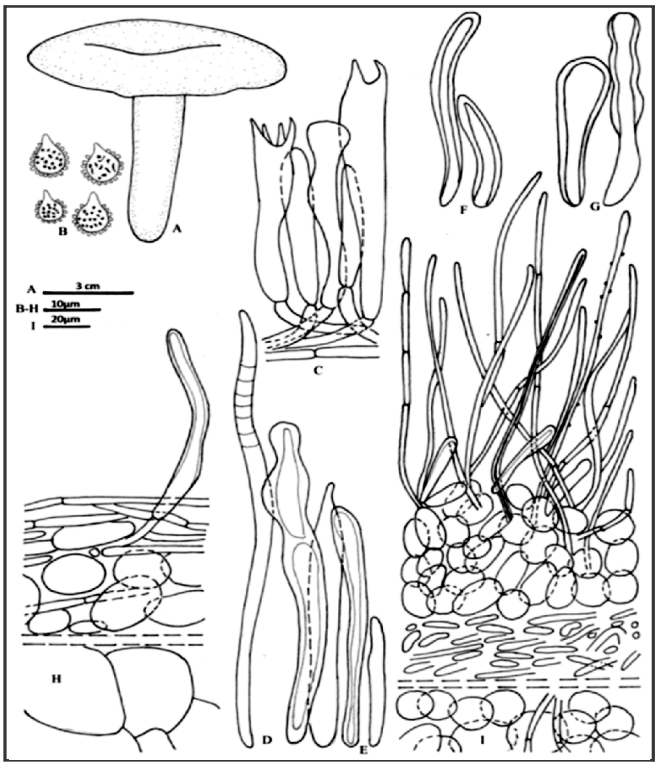

Fig. 14 (A-I) ***Lactifluus luteolus* var. *luteolus***: A. Carpophore, B. Basidiospores, C. Hymenophoral tama showing basidia, D. Pleurocystidia, E. Caulocystia, F. Pilocystidia, G. Cheilocystidia, H. Cross section through stipe showing cuticular details and context, I. Cross section through pileus showing cuticular details and context.

changes to light brown than dark brown on bruising; gill edges smooth, normal. Stipe central to excentric, smooth, 5.5-6.0 cm long, 1.4-2.0 cm broad above, 0.5-1.2 cm broad below, slightly tapering downwards, light yellow ($4A_5$), first yellowish than dark brown on bruising, hollow. Latex same as exuded from gills.

Spores 5.0-7.2 × 5.0-5.7 µm (excluding ornamentation), globose to broadly ellipsoidal (Q=1.0-1.25), warty, warts mostly isolated, some warts joined by fine connections to form catenulations, ornamentation type VI, VIII, amyloid; plage hyaline, indistinct; apiculate, apiculus up to 1.43 µm long, nodulose type. SEM study of the surface ornamentation reveals the presence of isolated cylindrical blunt warts measuring 1.3-1.4 µm in height. Plage area with very low ornamentation. Basidia 34.3-51.5 × 4.3-8.6 µm, 2-4 spored, clavate, 2 basidia/100 µm; sterigmata 4.3-5.7 µm long. Pleurocystidia pseudocystidioid, 45.8-85.8 × 2.9-5.7 µm, flexuous, clavatefusoid with inflated, tubular to blunt tips. Cheilocystidia 28.6-50.0 × 2.9-4.3 µm, fusoid to ventricose, filamentous, thin walled. Pileus cuticle up to 74 µm broad, cellular, subcutis made up of hyphal elements, from the cutis arises a continuous turf formed of pilocystidia and granular trichodermal projecting hyphae measuring up to 3.7 µm in width; pilocystidia 28.6-35.8 × 5.7-10.0 µm in size, clavate thin walled with broadly capitate to blunt tips, granulation present on the surface of some projecting hyphae; context

made up of rosettes of sphaerocysts intermingled with 3.7-7.4 µm broad hyphae. Hymenophoral trama hyphal, subhymenium indistinct. Stipe cuticle gelatinized, from the cuticle arises a turf of granular, closely septate, 2.8-4.3 µm broad hyphae intermingled with caulocystidial elements measuring 24.3-43 × 2.9-4.3 µm, clavate, caulocystidia thin walled with capitate, acute to blunt tips, some cystidia shows granulations on their surfaces, some of the laticiferous hyphae also projecting from the surface; context made up of rosettes of sphaerocysts. Clamp connections absent.

Collection examined: Uttarakhand, Pighla Pani (2600 m), in groups on humicolous soil, Samidha Sharma, PUN 7051, August 20, 2010.

Discussion: This fungus belongs to *Lactifluus luteolus* var. *luteolus* (Peck.) Verbeken due to its rugose to almost velvety, light yellow, azonate, dry pileus; grayish beige, gum like abundant unchanging mild latex; thin walled pleurocystidia; cellular pileus surface and all parts of the fungus changing to light brownish to dark brown on bruising (Verbeken *et al.*, 2012). Many of its characters are similar to *Lf. echinatus* from which it differs in its mild tasting latex. In its older dispensation before the carving out of genus *Lactifluus,* Hesler and Smith (1979) described it as species of genus *Lactarius* from the deciduous and mixed forest of North America and Texas in the month of June to November. Presently it has been found in association with *Quercus semecarpifolia* and *Rhododendron* in broad leaved forest in the month of August. It constitutes a new record from India.

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Two new records of Himalayan wild mushrooms for Indian mycobiota

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ABSTRACT

Two species, namely, *Bovista nigrescens* (*Agaricaceae*), a gasteroid mushroom collected from the subalpine grassland of Sikkim and *Pulveroboletus auriflammeus* (*Boletaceae*), a fleshy poroid mushroom collected from subtropical to temperate broadleaf forest of Uttarakhand are reported for the first time from India with their macro- and micromorphological details coupled with the illustrations.

Key words: *Agaricaceae, Boletaceae, Bovista,* macrofungi, *Pulveroboletus*, Sikkim, Uttarakhand

INTRODUCTION

Bovista is a gasteroid genus which is represented by about 56 species from the world (Kirk *et al.,* 2008), however, from India 11 species namely, *Bovista limosa* Rostr., *B. plumbea* Pers., *B. bovistoides* (Cooke & Massee) S. Ahmad, *B. fulva* Massee, *B. oblongispora* (Lloyd) Bottomley, *B. citrina* (Berk. & Broome) Bottomley, *B. dryina* (Morgan) Demoulin, *B. cunninghamii* Kreisel, *B. pusilla* (Batsch) Pers., *B. trachyspora* (Lloyd) Kreisel and *B. lycoperdioides* (Cooke) S. Ahmad are documented so far (Bilgrami *et al.,* 1991; Bisht, 2008).

The other genus *Pulveroboletus* is a fleshy poroid mushroom covered by flocculent to almost powdery veil of mostly bright yellow coloration and pulverulent consistency (Singer, 1947; Smith and Thiers, 1971); About 31 species of *Pulveroboletus* are recorded till date worldwide (http://www.indexfungorum.org/) and from India only a single species, namely *Pulveroboletus shoreae* Singer & B. Singh is reported (Bilgrami *et al.,* 1991).

Recently, during 2009-2015, while undertaking a couple of macrofungal explorations to different Himalayan areas of the state of Uttarakhand and the North district of the state of Sikkim (a small Hiamalayan state) in India two of us (KCS & KD) came across a large number of macrofungi. After thorough morphological examination of few of these collections revealed two species: *Bovista nigrescens* and *Pulveroboletus auriflammeus*. They are described and illustrated here for the first time from India.

MATERIAL METHODS

Macromorphological features were recorded from young to mature fresh basidiomata in the field and in basecamp. Specimens were dried with hot air using a portable field dryer. Field photographs were captured with the aid of Nikon D300s camera and Nikon Coolpix P510. Microphotographs were taken with the aid of a dedicated camera of Stereo Zoom Dissecting Microscope, Nikon SMZ 1500 and a compound Microscope, Olympus CX 41. Color codes and terms mostly follow the Colour identification chart of British Fungus Flora (Henderson *et al.* 1969, here prefixed by "a:") and Methuen Handbook of Color (Kornerup and Wanscher 1978, here prefixed by "b:"). Micromorphological characters were recorded with the aid of a compound microscope (Nikon Eclipse N*i*-U) from free-hand sections of dry samples mounted in 5% KOH, or stained in a mixture of 5% KOH and phloxin and mounted in 30 % glycerol or distilled water. Drawings were made with a drawing tube (attached to Nikon-DS-N*i*1) at 400× and 1000× magnifications. Basidium length excludes length of sterigmata. Spore-measurements were recorded from twenty basidiospores. Spore-size measurements and length/width ratios (Q) are given as minimum-mean-maximum. Herbarium acronym follows Holmgren *et al.*,1990. Scanning electron micrographs of basidiospores were obtained from dry spores (in gleba) directly mounted on double sided adhesive tape, pasted on a metallic specimen-stub, gold coated, and subsequently scanned in high vacuum mode at different magnifications to observe spore-ornamentations. The electron microscopy was carried out with a FEI's Quanta 200 model scanning electron microscope (SEM) at the S.N. Bose National Centre for Basic Sciences, Kolkata (India).

TAXONOMY

Bovista nigrescens Pers. *Neues Mag. Bot.* **1**:86(1794)

Figs. 1 & 2

Basidiomata 25-50 × 22-49 mm, globose to subglobose, with rhizoids, firmly attached to the soil, opening with an apical slit or through big irregular furrows. Peridium double. Exoperidium membranous, smooth, gradually with fine conical to blunt verrucae, white when young, slowly becoming buff (a: 52) to sienna (a: 11), umber (a: 18) to bay (a: 19) or purplish chestnut (a: 21) to black, becoming cracked or broken into small appressed squamule-like pieces with maturity. Endoperidium papery, smooth, white when young, becoming sienna (a: 11) to dark brown or rust (a: 13) or blackish with maturity. Gleba white when young, extended up to base, becoming buff initially then olive-brown to snuff brown (a: 17). Subgleba absent or never forming pseudostipe. Odor indistinct. Taste unknown. Spore print olive-brown.

Basidiospores 4.0-5.1-6.0 × 3.9-4.7-5.3 µm, globose to subglobose, rarely broadly ellipsoid (Q = 1.00-1.08-1.16), nearly smooth under light microscope, but, distinctly warty under SEM; warts up to 0.25 µm high, conic to blunt, mostly isolated, some connected by connectors; pedicel 6-14 µm, straight, truncate (never tapering or pointed). Capillitium to 11 µm broad, bovista-type, with discrete, regularly branched units tapering gradually in narrow tips, aseptate, non-pitted,

Fig. 1 (a-e) *Bovista nigrescens*: a. Mature and immature fresh basidiomata. b. Dehiscence through regular to irregular slits and furrows. c. Slit at the apex of basidiomata. D. Longitudinal section through basidiomata showing absence of subgleba. e. SEM images of a basidiospore with long pedicel. Bars: c & d = 5000 µm (5 mm); e = 5 µm.

sienna (a: 11) to rust (a: 13) in KOH, thick-walled (25 µm thick), walls dark, brownish. Paracapillitium absent. Exoperidium mainly with pyramidal to conic verrucae in regular to irregular interval; verrucae to 240 µm high, pseudoparenchymatous in nature, composed mainly of globose to ellipsoid thick-walled cells of 8-27 × 8-20 µm, cell-wall to 0.9 µm thick. Endoperidium narrow, to 42 µm thick, composed of hyaline septate branched hyphae.

Specimens examined: INDIA, Sikkim, North district, Shib-mandir, alt. 3964 m, N 27°51'11.1" E 88°41'33.8", on subalpine grass-land, 31 Aug., 2011, K. Das, KD-11-166 (CAL); ibid., below shib-mandir, alt. 3828 m, N 27°50'23.5" E 88°41'36.7", on subalpine grass-land, 31 Aug., 2011, K. Das, KD-11-178 (CAL).

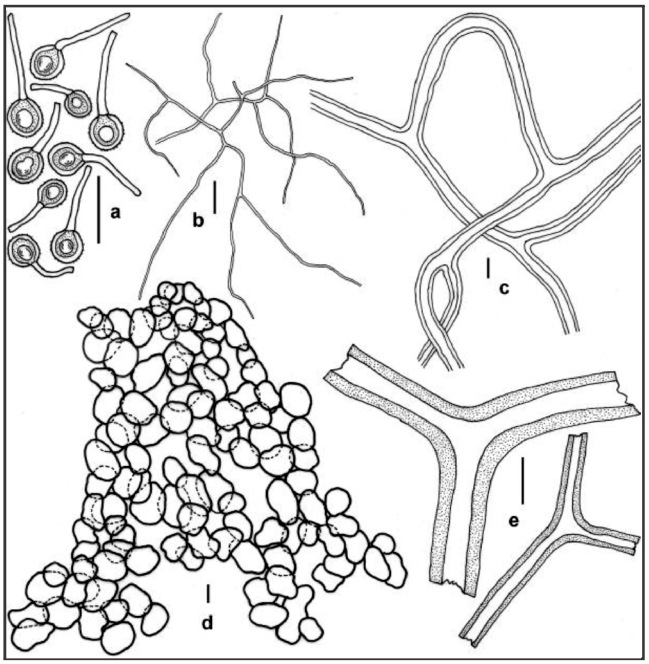

Fig. 2 (a-e) *Bovista nigrescens*. a. Basidiospores with long pedicel. b, c & e. Capillitia. d. Cross-section through exoperidium (verrucae) showing thick-walled cells. Bars: a, c, d & e = 10 µm, b = 100 µm.

REMARKS

Bovista nigrescens is also known as *Bovista montana* Morgan and *Lycoperdon nigrescens* (Pers.) Vittad. The macro- and micromorphological characters like globose to subglobose basidiomata with rhizoids, white to buff coloured exoperidium, absence of sterile base like subgleba and pseudostipe, presence of basidiospores with straight and truncate pedicels, bovista-type capillitium with regularly branched tapering units place the present species under the genus *Bovista* (Miller and Miller, 1988). Moreover, the combination of features namely, occurrence on grass-land, opening of basidiomata by a slit or irregular furrow, brown to blackish endoperidium, non-poroid bovista type capillitium, spores with straight truncate pedicel make this species distinct as *Bovista nigrescens*.

Morphologically, some species like, *Bovista paludosa* Lév., *B. plumbea* Pers. (also reported from India) and *B. graveolens* appear to be quite close to *B. nigrescens*. Though like the present species *B. plumbea* and *B. paludosa* both have same type (Bovista-type) of capillitium, a strong taxonomical character (Kreisel, 1964;1967; Krüger, *et al.* 2001) but, *B. paludosa* is separated from *B. nigrescens* by the presence of distinct subgeba (Pegler *et al.* 1995; Calonge 1998) whereas, *B. plumbea* has "lead-grey" endoperidium (Thind andThind 1982; Pegler *et al.* 1995) and ellipsoid spores with pointed (never truncate) pedicel (Calonge, 1998). *B. graveolens* Schwalb can be distinguished micromorphologically by its spores with distinctively curved ("C" shaped) pedicel (Calonge, 1998).

Pulveroboletus auriflammeus (Berk. & M.A. Curtis) Singer. *The American Midland Naturalist* **37**:10(1947)

Figs. 3 & 4

Pileus 45-70 mm. diam., convex when young, plano-convex, becoming flat to nearly uplifted with age, sometimes depressed in the middle in matured fruitbodies due to solid context turned into fluffy stuff with age, surface covered with yolk yellow to yellowish orange, dark yellow (b: 4B8-4A8, 4C8) cottony-fibrils which then convert into pointed powdery scales in matured specimens, often staining yellow color on finger and cloths during handling, sometimes cracked due to expansion of fruit body; margin plane, sometimes flaring with velar remnants. Pore surface yellow to light yellow, butter yellow (b: 3A5-4A5,-6) to pale orange yellow becoming pale yellowish to yellowish white (b: 4A3-2) at maturity, unchanging on bruising, pores often up to 2 mm broad at maturity, distinctly angular to elongated and decurrently stretched towards stalk, gradually compound towards cap margin. Tubes 2-4 mm long, decurrent, concolorous to pore surface. Stipe 57-80 × 6-15 mm, central, terete or more or less tapering downwards and slightly expended at the apex, concolorous with cap, surface distinctly pruinose or powdery, faintly reticulated at the apex, white mycelial elements at the base. Context light yellowish (b: 3A5-4), unchanging, solid at first, fluffy with age. Odor mushroomoid. Taste indistinct. Spore print light brown (b: 5D-6).

Fig. 3 (a-g) *Pulveroboletus auriflammeus*. a & b. Mature and fresh basidiomata showing dorsal and ventral view. c & d. Hymenial cystidia. e & f. Hyphae of pileipellis with yellow crystaloid incrustations. g. Cross section through pileipellis. h. Cross section through tube edge showing cheilocystidia. i. Basidiospores. Bars: c f & i = 10 µm; g & h = 50 µm.

Basidiospores 9.4-10.39-12 × 4.6-4.3-5.8 µm, (n= 20, Q= 1.81-2.09-2.19), elliptic to oblong, slightly thick-walled, smooth under light microscope. Basidia 27-31 × 11-12 µm, 2- and 4-spored, subclavate to clavate; sterigmata to 5-6.5 × 2-2.5 µm. Pleurocystidia 43-65 × 9-12 µm, subcylindrical to broadly ventricose or clavate, thin-walled with dense content, emergent up to 35 µm. Tube edge fertile with basidia and cystidia. Cheilocystidia 35-45 × 10-12 µm, fairly common, subclavate to clavate, content dense. Tube trama divergent,

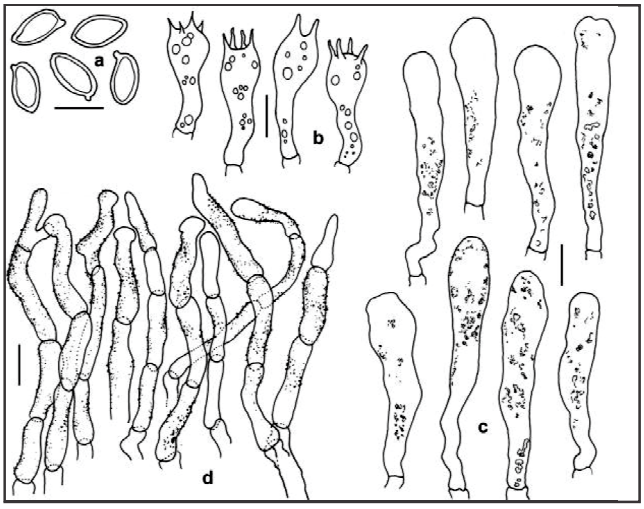

Fig. 4 (a-C) *Pulveroboletus auriflammeus*. a. Basidiospores. b. Basidia. c. Hymenial cystidia. d. Pileipellis showing encrusted hyphae. Bars: a, b & c = 10 µm, d = 25 µm.

gelatinous. Pileipellis 300-350 µm thick, cutis to trichoderm, composed of erect branched septate hyphae (8.5-20 µm with yellow crystal like incrustations; terminal cell cystidoid to cylindrical with rounded to capitate or fusoid apex.

Specimens examined: INDIA, Uttarakhand, Champawat, On the way to Hingli Mata Mandir, under the canopy of *Quercus leucotrichophora* and *Rhododendron arboreum* in subtropical to temperate broad leaved forest, 26 Aug. 2009, K.C. Semwal, KCS 1267; ibid., Uttarakhand, Pauri Garhwal, Dandapani Forest, 2000 m, N30°09'12" E78°46'11", under *Myrica esculenta* and *Quercus leucotrichophora,* 1 Aug. 2015, K.C. Semwal, KCS 2422; Mundneshwar 1820 m, N30°01'5" E78°44'32", under *Q. leucotrichophora* 12 Aug. 2015, K.C. Semwal, KCS 2454.

REMARKS

Pulverboletus auriflammeus is also known as *Boletus auriflammeus* Berk. & M.A. Curtis, *Ceriomyces auriflammeus* (Berk. & M.A. Curtis) Murrill, *Suillus auriflammeus* (Berk. & M.A. Curtis) Kuntze (http://www.speciesfungorum.org). It mostly grows in the middle Himalaya region of Uttarakhand Himalaya (India). Basidiomata were mostly collected from broad leaved forest in association with *Quercus leucotrichophora, Rhododendron arboreum* and *Myrica esculenta* and assumed to be an ectomycorrhizal partner for aforesaid mentioned tree species. It has never been observed in coniferous forest in Uttarakhand Himalaya. Growing in the wall type ridges and slopes which are sometimes colonized by moss is another feature which distinguishes it from other similar contender in this group. It grows solitary to scattered and appeared garish due to its bright yellowish to yellowish orange fruitbody which easily attract the hunter's eyes in the forest. Micromorphologically, strongly divergent trama and yellow pigmented hyphae in pileipellis is also very characteristic. Similar looking species: *Pulveroboletus curtisii* (Berk.) Singer (≡*Boletus curtisii* Berk.) has viscid to glutinous pileus and stipe surface and longer basidiospores (9.5-17 × 4-6 µm) as mentioned by Bessette *et al.* (2010). *Boletus aurantiosplendens* Baroni is also quite close to the present species but, the earlier has yellowish context which becomes darker when exposed. Micromorphologically, ventricose to ventricose rostrate hymenial cystidia is also very distinct in *B. aurantiosplendens* (Bessette *et al.* 2010).

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Studies on Genus *Peziza* from Ladakh (Jammu & Kashmir), India

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ABSTRACT

Five species of genus *Peziza* (*P. ammophila* Durieu & Lev., *P. ampliata* Pers., *P. badia* Pers., *P. succosa* Berk. and *P. vesiculosa* Pers.) are described and illustrated in the present communication. *Peziza ammophila* Durieu & Lev. and *P. ampliata* Pers. are new to India while *P. badia* Pers., *P. succosa* Berk. and *P. vesiculosa* Pers. are first authentic records from Trans-Himalayan Ladakh region of Jammu and Kashmir, India.

Key words: *Peziza*, new records, taxonomy, Leh, Ladakh

INTRODUCTION

The genus *Peziza* Dill. ex Fr. (*Pezizaceae, Pezizales, Pezizomycotina, Ascomycota*) that produces epigeous, sessile or stipitate, cupulate, turbinate, pulvinate or sparassoid apothecia is the largest genus within the order *Pezizales*. The genus occupies a wide range of ecological niches, ranging from different types of soil including humus soil, sand, clay and limestone to fire places, dung and woods (Dimitrova and Gyosheva, 2009; Barseghyan and Wasser, 2011). According to Maia *et al.* (1996), majority of *Peziza* spp. are considered to be saprotrophs where as a few species are claimed to be ectomycorrhizal in nature. Nutritionally, many species *viz.*, *Peziza badia* Pers., *P. succosa* Berk. and *P. vesiculosa* Pers. are edible (Rinaldi and Tyndalo, 1974; Kumar and Sharma, 2010) and constitute the most relished food commodities amongst the number of non-conventional foodstuffs. While Hawksworth *et al.* (1995) mentioned 80 species in this genus, the number was subsequently raised by Kirk *et al.* (2008) to 104 species. The genus has been described from North America, South America and European countries (Larsen and Denison, 1978; Hohmeyer, 1986; Dissing, 2000; Gamundi, 2010). In India, approximately 32 species of *Peziza* are known to exist (Sarbhoy *et al.*, 1975; Bilgrami *et al.*, 1979; 1981;1991; Jamaluddin *et al.*, 2004; Kumar and Sharma, 2008; Dar *et al.*, 2009; Sheikh *et al.*, 2014). The present study was conducted in unexplored areas of Ladakh from where five species of *Peziza* were located *viz.*, *P. ammophila* Durieu & Lev., *P. ampliata* Pers., *P. badia* Pers., *P. succosa* Berk. and *P. vesiculosa* Pers. Out of these, the first two being new addition to India's mycobiota while the remaining three were recorded first time from Ladakh province of Jammu and Kashmir, India.

MATERIALS AND METHODS

Ladakh (Jammu & Kashmir), India, is located between 32°15′ to 36° N Latitude and 75°15′ to 80°15′ E Longitude with an altitude ranging from 2900 to 5,900 meters above the mean sea level and covers an area of 45,110 sq. km. Ladakh experiences extremely cold arid climate and is among the climatically very inhospitable regions for human habitation. The region witnessed fluctuating temperature (-35°C to 35°C), scarce precipitation (80-300 mm) in the form of snowfall/rainfall and low relative humidity (45%-50%). The soil is predominantly sandy to sandy-loam and pure clay. Indus river and its tributaries constitute the principal water resources in the region. Besides, melting ice and glacial, natural springs and lakes (Pangong Tso, Tsomoriri and Tsokar) aid to the water resources. The vegetation of the region varies from temperate to alpine mesophytes and desert shrubs. Herbaceous and shrubby vegetation form the major component. Tree vegetation is little representing small deciduous forests of *Salix* and *Populus*, mixed forests of *Hippophae rhamnoides* and *Salix*, Juniper (shukpa) forests at higher altitude, trees of *Prunus armeniaca* (chulli), *Juglans regia* (starga) and *Pyrus malus* (kushu) represent the main arboreal vegetation.

Collections of the specimens were made during July and August 2011 to 2012 from different locations of Leh district of Ladakh. Field records were made for macroscopic features of fruiting bodies and habitat. Microscopic observations were made by mounting the dried materials in 5% ethanol and 3% aqueous KOH solution, and then staining with 1% aqueous solution of Congo red. Colour notations are as per Ridgway (1912). Measurements were made for each character for description of average dimension. For field study, identification and description, work of Soothill and Fairhurst 1978; Dennis 1978; Smith *et al.*, 1981; Purkayastha and Chandra, 1985; Arora, 1986; Atri *et al.*, 2003 was employed. The examined specimens have been deposited in the Herbarium of Botany Department, University of Jammu with accession numbers.

RESULTS AND DISCUSSION

1. ***Peziza ammophila*** Durieu & Lév., in Durieu, *Expl. Sci. Alg.* **1**: tab. 28, fig. 2 (1848)

Synonymy: *Geopyxis ammophila* Sacc., *Syll. fung.* (Abellini) **8**: 70 (1889) *Tarzetta ammophila* (Sacc.) Theodor., *Badania Przyrodnicze Pomorskie*, Tow. nauk. **2**: 11 (1936)

Figs.1(a,b); 2 (Ia-e)

Apothecia: cupulate, 0.6-1.2 cm in diameter, army brown (XL i. 13'''. OY-O.) inner surface to dark brown (honeycomb) from outer side, attached to ground with rhizoids, outer surface slightly furfuraceous but glabrous shiny inside; **Asci:** cylindrical, 248.0-264.0 µm long, 8.0-10.0 µm wide at the top, 10.0-12.0 µm at middle and 6.0-8.0 µm at the bottom, thick walled, hyaline, 8-spored, arranged obliquely in uniseriate manner; **Ascospores:** ellipsoidal, 13.6-16.0 × 6.4-8.0 µm (a_vL=21.6, a_vW=7.2, Q= 2.1-2.0), smooth, thick

Fig. 1 a) Apothecia of *Peziza ammophila* in natural habitat; b) Magnified view of an apothecium of *P. Ammophila;* c) Apothecia of *P. ampliata* in natural habitat; d) Magnified view of apothecia of *P. Ampliata*; e) Apothecia of *P. Badia* in natural habitat; f) Magnified view of apothecia of *P. Badia;* g) Apothecium of *P. succosa*; h) Apothecium of *P. Vesiculosa.*

walled, hyaline, mono- to multiguttulate; **Paraphyses:** filiform, 4.0-6.0 wide, thin walled, hyaline, septate, slightly swollen (4.0-8.0 µm wide) towards tip; **Pubescent hairs:** up to 20.0 µm wide, thick walled, branched, septate.

Edibility: Not known

Collection examined: Jammu and Kashmir, Ladakh, Leh, Khaltse, Skurbuchan, bryophilous, scattered, among mosses under *Populus*, Konchok Dorjey and Y.P. Sharma, BHJU-257 (Holotype), August 2011.

Distribution: Earlier recorded on sandy soil or sand dunes in Israel, China, New Zealand, Europe and Africa (Binyamini, 1986; Barseghyan and Wasser, 2011).

Remarks: *Peziza ammophila* is characterised by cup-shaped apothecia buried in sandy soil when young, emerging and typically splitting margins at maturity, pale to dark brown on upper and under surfaces. The species is recorded first time from India.

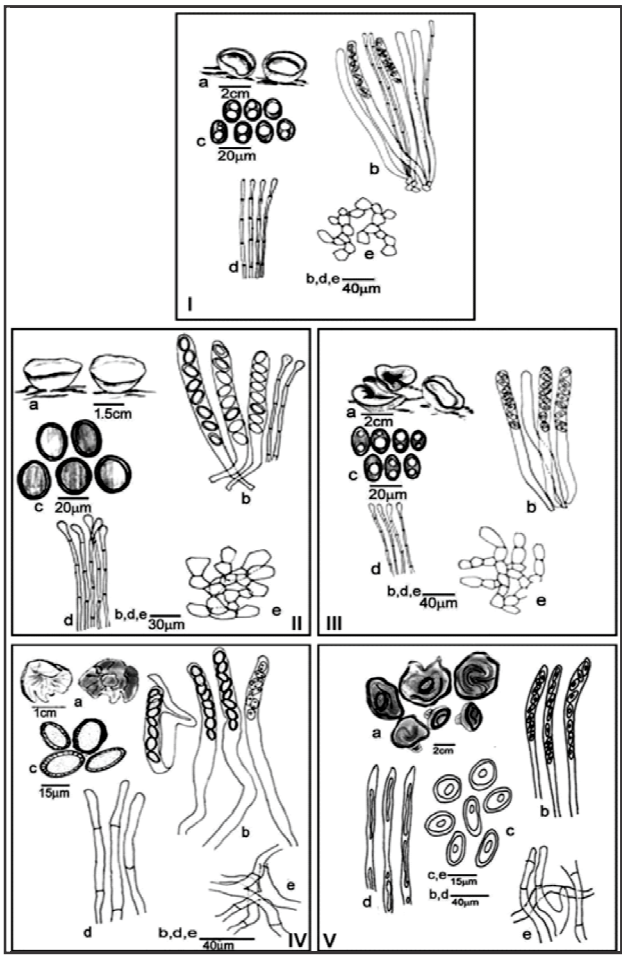

Fig.2 I) *Peziza ampliata*: a) habit; b) asci; c) ascospores; d) paraphyses; e) pubescent hairs
II) *P. ampliata*: a) habit; b) asci; c) ascospores; d) paraphyses; e) pubescent hairs
III) *P. badia*: a) habit; b) asci; c) ascospores; d) paraphyses; e) pubescent hairs
IV) *P. succosa*: a) habit; b) asci; c) ascospores; d) paraphyses; e) pubescent hairs
V) *P. vesiculosa*: a) habit; b) asci; c) ascospores; d) paraphyses; e) pubescent hairs

2. ***Peziza ampliata*** Pers., in Pant, *Mycol. eur.* (Erlanga) **1**: 227 (1822)

Synonymy: *Aleuria ampliata* (Pers.) Gillet, *Champignons de France*, Discom. **2:** 47 (1879) *Aleuria palustris* (Boud.) Le Gal, *Revue Mycol.*, Paris 6 (Suppl. Colon. no. **2:** 71 (1941) *Galactinia ampliata* var. *costifera* (Boud.) Le Gal, *Bull. Jard. bot. État Brux.* **29:** 81 (1959)

Figs. 1(c,d); 2(IIa-e)

Apothecia: cupulate or bowl shaped, 0.8-1.8 cm in diameter, sayal brown (XL k. 17". O-Y.) to brown (Nut-brown) inner surface, shiny, wet, glabrous, margins entire, smooth, sessile, directly growing in soil through small hair like rhizoids; **Asci:** elongated to cylindrical, 220.0-240.0 µm in length, 12.0-16.0 µm wide at the distal end, 14.0-18.0 µm at the middle and 8.0-12.0 µm at the bottom, thick walled, hyaline, eight spored,

obliquely arranged; **Ascospores:** ellipsoidal to broadly ellipsoidal, 16.0-20.0 × 10.4-12.0 µm (a_vL=18.0, a_vW= 11.2, Q=1.5-1.7), thick walled, hyaline; **Paraphyses:** filiform, 4.0-6.0 µm wide, thin walled, hyaline, septate, darker and swollen (8.0-10.0 µm wide) at the tip; **Pubescent hairs:** up to 10.4 µm wide, hyaline, branched, septate.

Edibility: Not known

Collection examined: Jammu and Kashmir, Ladakh, Leh, Wanla, bryophilous, scattered to gregarious, among mosses in deciduous forests of *Salix* species, Konchok Dorjey and Y.P. Sharma, BHJU-258 (Holotype), July 2011.

Distribution: The species was earlier reported from Europe (Dennis, 1978; Soothill and Fairhurst, 1978).

Remarks: *Peziza ampliata* is recognised in the field by large, cupulate or bowl-shaped, reduced stipitate, glabrous apothecia with brownish outer to light brownish under surface. The species constitute new record for India.

3. ***Peziza badia*** Pers., *Observ. mycol.* (Lipsiae) **2**: 78 (1800) [1799]

Synonymy: *Galactinia badia* (Pers.) Arnould, *Bull. Soc. mycol. Fr.* **9:** 111 (1893) *Helvella cochleata* Bolton, *Hist. fung. Halifax* (Huddersfield) **3:** 99, tab. 99 (1790) [1789] *Plicaria badia* (Pers.) Fuckel, *Jb. nassau. Ver. Naturk.* **23-24**: 327 (1870) [1869-70]

Figs. 1 (e,f); 2(IIIa-e)

Apothecia: cupulate when young turning turbinate or inverted hemispherical at maturity, 1.5-2.5 µm in diameter, sessile, natal brown (XL k. 13'''. OY-O.) from inside surface to light brown (honey comb) from outside, surface glabrous, wet, dull; **Asci:** cylindrical with rounded ends, 260.0-288.0 µm in length, 8.0-12.0 µm wide at the top, 12.0-14.0 µm at the middle and 8.0-10.0 µm at the bottom, thin walled, hyaline, eight spored, obliquely arranged in uniseriate fashion; **Ascospores:** ellipsoidal, 14.4-18.4 × 7.2-8.8 µm (a_vL=16.4, a_vW=8.0, Q=2.0-2.10), thick walled, hyaline, biguttulate; **Paraphyses:** filiform, 4.0-6.0 µm wide, thin walled, hyaline, unfurcate, septate, swollen at tips; **Pubescent hairs:** up to 34.0 µm wide, thick walled, hyaline, branched, septate.

Edibility: *Peziza badia* is reported to be edible in some parts of Jammu Province (Kumar and Sharma, 2010) but not consumed in study area.

Collection examined: Jammu and Kashmir, Ladakh, Leh, Skurbuchan, bryophilous, scattered, among mosses, Konchok Dorjey and Y.P. Sharma, BHJU-259 (Holotype), August 2012.

Distribution: *Peziza badia* has wide distribution in India (Subramaninam, 1952; Thind and Batra, 1957).

Remarks: The species is characterised by apothecia which is cupulate when young to irregularly wavy with age, sessile, inner surface olive brown to outer reddish brown and slightly scurfy. The species constitute first authentic record from Ladakh region of Jammu and Kashmir.

4. ***Peziza succosa*** Berk., *British Fungi* : No. 156 (1841)

Synonymy: *Galactinia succosa* (Berk.) Sacc., *Syll. Fung.* (Abellini) **8**: 106 (1889)

Figs.1(g); 2(IVa-e)

Apothecia: cupulate, often wrinkled, up to 3.0 cm wide, shortly stipitate, circular, slightly incurved margins, light brown, dark brown inside, flesh juicy and the juice turn yellow in colour when exposed to air; **Asci:** cylindrical, 312.0-368.0 µm long, 14.0 -16.0 µm wide at the top, 16.0-24.0 µm wide in the middle, 8.0-10.0 µm wide at the base; 8-spored, uniseriate, oblique to irregularly placed, few overlapping; **Ascospores:** ellipsoidal, 16.0-20.8 × 9.6-12.0 µm (a_vL=18.4, a_vW=16.4, Q= 1.6-1.7), rough walled, pale yellowish, biguttulated; **Paraphyses:** 4.8-7.2 µm wide, 1-2 septate, unbranched; **Pubescent hairs:** 8.0-16.0 µm wide, septate and branched.

Edibility: The species is reported to be edible (Rinaldi and Tyndalo, 1974) but not consumed in the study area.

Collection examined: Jammu and Kashmir, Ladakh, Leh, Tya-Tingmosgang, humicolous, scattered, deciduous forests, Konchok Dorjey and Y.P. Sharma, BHJU-260 (Holotype), July-August 2011.

Distribution: *Peziza succossa* is commonly found growing on soil under mixed forests and have been recorded from Mussoorie, U.P. (Thind and Batra, 1957).

Remarks: Apothecia cupulate, often wrinkled with inrolled margins, inner surface pale yellowish-buff to brownish, outer light brownish and furfuraceous, often in clusters, sessile.

The species is new record from Jammu and Kashmir State.

5. ***Peziza vesiculosa*** Bull., *Herbier de la France* **10**: tab. 457, fig. 1 (1790)

Synonymy: *Scodellina vesiculosa* (Bull.) Gray, *Nat. Arr. Brit. Pl.* (London) (1821) *Pustularia vesiculosa* (Bull.) Fuckel, *Jb. Nassau. Ver. Naturk.* **23-24** (1870) [1869-70] *Galactinia vesiculosa* (Bull.) Le Gal, *Discom. De Madag-ascar*: 33 (1953)

Figs.1(h); 2(Va-f)

Apothecia: cupulate or turbinate, 1.5-3.5 cm, initially subglobose, barely open at the top, urn-shaped, later becoming hemispherical with incurved margin, clearly crenulate and furfuraceous, then distended and cracked, light yellowish ochre inside (XV k. 17. O-Y) cinnamom brown (XV k 15. Y-O) outside, sessile or with short stipe; **Flesh:** light ochre brown, thick, juicy, fragile; **Asci:** cylindrical, 240.0-288.0 µm long, 8.0-19.2 µm wide at the top, 8.0-16.0 µm in the middle and 6.0-8.0 µm at the base, apex obtuse, 8-spored; **Ascospores:** broadly ellipsoid or oblong with rounded ends, 11.2-22.4 × 6.4-9.6 µm (a_vL=176.8, a_vW=8.0, Q= 1.7-2.3, uniseriate, parallel to oblique, often overlapping, monoguttulate; **Paraphyses:** filiform, 184.4-273.6 µm long, 8.0-12.8 µm wide at the top, 7.2-14.4 µm in the middle and 6.4-8.0 µm at the base, septate, unbranched; **Pubescent**

hairs: 3.2-9.6 µm wide, branched.

Edibility: The species is not edible in the study area. However, its edibility has previously been reported by Rinaldi and Tyndalo (1974).

Collection examined: Jammu and Kashmir, Ladakh, Leh, Khaltse, Tya-Tingmosgang, humicolous, scattered to gregarious, growing among temperate forest, Konchok Dorjey and Y.P. Sharma, BHJU-261(Holotype), July-August 2012.

Distribution: *Peziza vesiculosa* was reported on humus and heavily manured soil from Punjab province of Indian subcontinent (Thind and Waraitch, 1964).

Remarks: Apothecia sessile, globose when young turning cupulate or turbinate, often contorted when clustered; margin incurved often eroded at maturity; pale brownish, frequently convoluted or wrinkled. The collection is first record from Ladakh province of Jammu and Kashmir.

DISCUSSION

Survey of literature revealed that two species (*Peziza ammophila* and *P. ampliata*) constituted new records for India. Likewise, *Peziza succosa* represents first time record from Jammu and Kashmir; earlier this species was recorded from mixed forests of Mussoorie, Uttarakhand (Thind and Batra, 1957). *Peziza badia* and *Peziza vesiculosa* have wide distribution in Jammu and Kashmir, Punjab plains and other states of Indian subcontinent (Subramanian, 1952; Thind and Waraitch, 1964; Thind and Batra, 1957; Kumar and Sharma, 2010). However, they constitute first authentic record from Ladakh region of Jammu and Kashmir.

In Ladakh, all the five species of *Peziza* are referred to as 'Koreh' locally. However, *Peziza ammophila* is particularly known as 'Balti Koreh' (Balti is tribe in Ladakh and Koreh means cup) since the apothecia of *P. ammophila* resembles the cup used by the people of Balti tribes in Ladakh. Surprisingly, none of these species were found to be edible in Ladakh inspite of the fact that *Peziza badia*, *P. succosa* and *P. vesiculosa* are commonly consumed in other areas of world (Rinaldi and Tyndalo, 1974; Kumar and Sharma, 2010).

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Some Interesting Hyphomycetous fungi from Himachal Pradesh

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ABSTRACT

In this paper four species of hyphomycetes, namely *Vanakripa menglensis* D. M. Hu, L. Cai and K. D. Hyde, *Repetophragma inflatum* (Berk.; Ravenel) W.P. Wu, *Alysidium resinae (Fr.) M.B. Ellis* and *Fusariella concinna* (Syd.) S. Hughes have been described from Himachal Pradesh (North-Western Himalayas).*Vanakripa menglensis* and *Repetophragma inflatum* constitutes new records for India, while *Alysidium resinae* and *Fusariella concinna* are being reported for the first time from North-Western Himalayas.

Keywords: Anamorphic fungi, taxonomy, new records, Himalayas, India.

INTRODUCTION

This communication is in continuation with our earlier reports on new records of anamorphic fungi from North India/ North-Western Himalayas (Adamčik *et al.*, 2015; Prasher and Verma, 2012a; b; 2014; 2015a; b; c; Prasher and Singh, 2012; 2013; 2014a; b; 2015; Prasher and Sushma, 2014). During the survey of conidial fungi from forests of Himachal Pradesh, four interesting hyphomycetous fungi *viz. Vanakripa menglensis*, *Repetophragma inflatum, Alysidium resinae* and *Fusariella concinna* have been collected and described, out of which *Vanakripa menglensis* and *Repetophragma inflatum* constitutes new record for India where as *Alysidium resinae* and *Fusariella concinna* are being reported for the first time from North-Western Himalayas (Bilgrami *et al.*, 1991; Jamaluddin *et al.*, 2004).

MATERIALS AND METHODS

Bark of different tree species were collected in ziplock plastic bags and taken to the laboratory. The specimens were mounted in 4%KOH, Lactophenol and Cotton blue 0.01% in lactophenol (Kirk *et al.*, 2008). The specimens were studied microscopically under Matrix stereo trinocular microscope (VL-Z60) and transmission microscope (VRS-2*f*) for macroscopic and microscopic characters. All the measurements were taken with the help of Pro MED software. The specimens were deposited in the herbarium of Botany Department, Panjab University, Chandigarh, India (PAN).

TAXONOMY

Vanakripa menglensis D. M. Hu, L. Cai & K. D. Hyde, 2010, *Sydowia* **62** (2): 191-203.

Fig. 1(a-g)

Colonies on natural substratum forming compact sporodochia, black, scattered. Setae and hyphopodia absent. Mycelium mostly immersed in substratum. Conidiophores micronematous, short. Separating cells hyaline, clavate to vermiform, 21.8-35 × 3-7 µm. Conidia solitary, clavate to obpyriform, smooth, dark brown in colour, aseptate, 16.6-20.8 × 11.2-14.7 µm.

Collection Examined: India, Himachal Pradesh, Kangra, Baijnath, on fallen twigs. November 07; 2013, Sushma, PAN 31512.

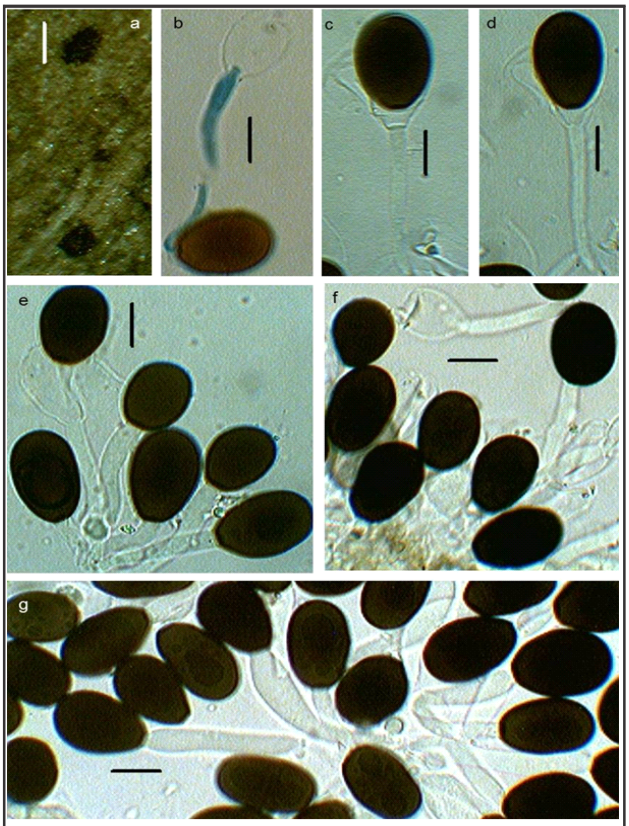

Fig.1 (a-g) *Vanakripa menglensis:* a- Sporodochia on substrate, b to d- Conidia with vesicles, e to g- Conidia attached with separating cells. Bars b-g = 10 µm.

Fig.1 (a-g) *Vanakripa menglensis:* a- Sporodochia on substrate, b to d- Conidia with vesicles, e to g- Conidia attached with separating cells. Bars b-g = 10 µm.

Remarks: The genus *Vanakripa* was established by Bhat, W.B. Kendrick and Nag Raj (Bhat and Kendrick, 1993) with type species *Vanakripa gigaspora*. The genus is characterized by the presence of sporodochial conidiomata arising from pseudoparenchymatous stromata and a separating cell attached at the base of conidia. The genus comprises of 8 species known till date *viz.V. fasciata* R.F. Castañeda-Ruiz, M. Stadler & Decock (Castañeda-Ruiz *et al.*, 2005), *V.*

gigaspora Bhat, W.B. Kendrick & Nag Raj (Bhat and Kendrick, 1993), *V. inexpectata* S.M. Leão & Gusmão (Leão *et al.*, 2013), *V. inflata* (Hol.-Jech.) Mel'nik (Mel'nik, 2011), *V. menglensis* D.M. Hu, L. Cai & K.D. Hyde (Hu *et al.*, 2010), *V. minutiellipsoidea* Pinnoi (Pinnoi, 2003), *V. parva* Bhat, W.B. Kendrick & Nag Raj (Bhat *et al.*, 1993), *V. rhizophorae* R.M. Arias-Mota, Heredia & R.F. Castañeda-Ruiz (Arias *et al.*, 2008). The name *Vanakripa ellipsoidea* K.M. Tsui, Goh & K.D. Hyde (Tsui *et al.*, 2003), is considered nom. inval., because the authors' did not indicate the type material. The above described species matches well with the description of *V. menglensis* (Hu *et al.*, 2010) except for slight variation in size of conidia and separating cells, which was first time reported on submerged wood in China from Yunnan Province. This species is first time reported from India as a terrestrial species.

Repetophragma inflatum (Berk.; Ravenel) W.P. Wu, in Wu & Zhuang, *Fungal Diversity Res. Ser.* **15**: 82 (2005).

Fig. 2(a-g)

= *Helminthosporium inflatum* Berk.; Ravenel [as 'Helmisporium'], in Berkeley, *Grevillea* **3**(no. 27): 104 (1875)

= *Sporidesmium inflatum* (Berk.; Ravenel) M.B. Ellis, *Mycol. Pap.* **70**: 70 (1958)

Colonies olivaceous brown. Conidiophores pale to mid brown, up to 200 µm in length and 5.35-8.23 µm in width. Conidia usually sigmoid when mature, 3-5 septate, often constricted at the septa, smooth, subhyaline or pale brown except for the second and occasionally the third cell which is brown to dark brown, 46.71-80.04 µm long, 13.06-18.96 µm thick in the broadest part, 5.33-8.18 µm wide at the base.

Collection Examined: India, Himachal Pradesh, Shimla, Narkanda, on fallen twigs, October 02; 2013, Sushma, PAN 31506.

Remarks: The genus *Repetophragma* was established by Subramanian in 1992 with type species *Repetophragma biseptata* to accommodate *Sporidesmium* species with euseptate conidia produced on monoblastic, integrated, indeterminate conidiogenous cells with several percurrent extensions. There are a total of 36 species known till date (Subramanian, 1992; Castañeda-Ruiz *et al.*, 2011; 2013; Ma *et al.*, 2014). The above described species matches well with the description of *Repetophragma inflatum* which was first time reported on rotten bark of an un-identified dicotyledonous tree; near Rabaul, New Britain, Papua, New Guinea in 1970. This species is reported for the first time from India.

***Alysidium resinae* (Fr.)** M.B. Ellis, *Dematiaceous Hyphomycetes:* **90** (1971)

Fig. 3(a-g)

Colonies effuse, black. Mycelium partly superficial, partly immersed. Conidiophores variable in length, brown to dark blackish brown in colour, branched, 4.97-6.31 µm in width. Conidia formed in simple and branched chains terminally and laterally on the conidiophores, spherical and 6.76-8.72 µm in

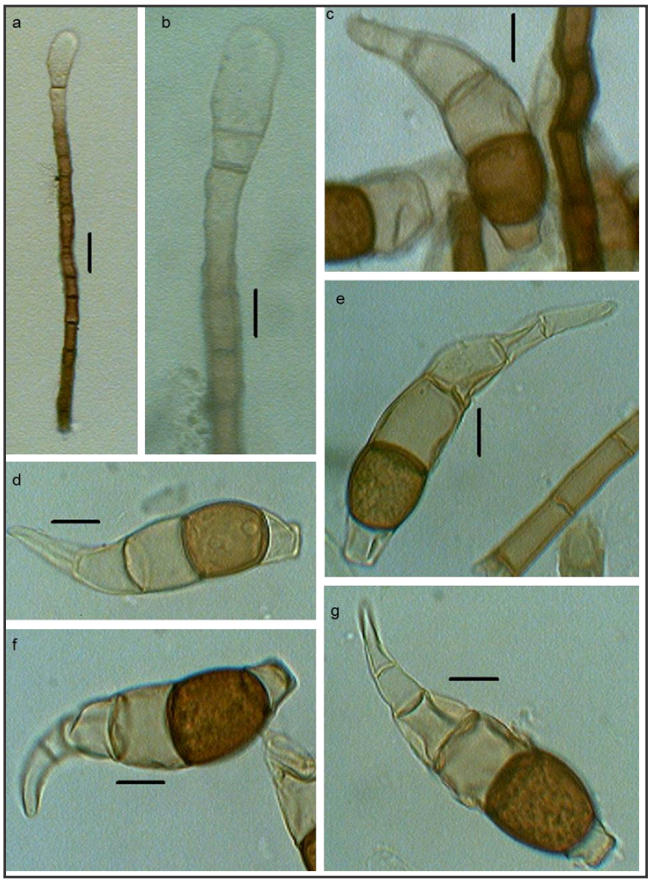

Fig.2 (a-g) *Repetophragma inflatum:* a & b- Developing conidia on conidiophores, c to g- Conidia in different planes. Bars a = 20 µm; b-g = 10 µm.

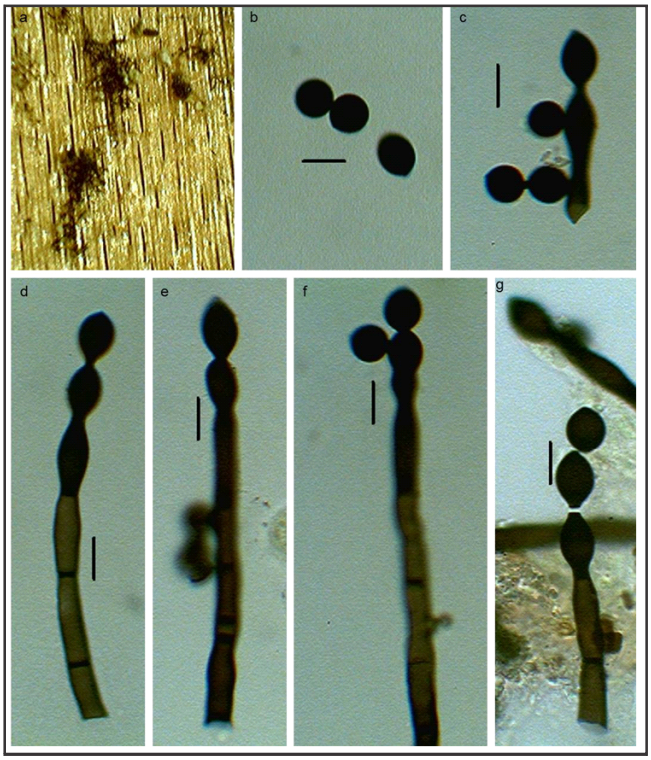

Fig.3 (a-g) *Alysidium resinae* a: Colony on natural substrate, b- Conidia, c-g: Conidia attached to conidiophores. Bars b-g=10µm.

diameter and limoniform, ellipsoidal or oblong 9.16-13.47 × 6.77-8.72 µm, brown to dark blackish brown, smooth, non-septate.

Collection Examined: India, Himachal Pradesh, Chamba, Khajjiar, on fallen wood log, June 20; 2014, Sushma, PAN 31530.

Remarks: The above described species matches well with the description of ***Alysidium resinae.*** This species is first time reported from North-Western Himalayas. It was earlier reported from Mt. Abu, Rajasthan (Jamaluddin *et al.*, 2004).

Fusariella concinna (Syd.) S. Hughes, *Mycol. Pap.* **28**: 8 (1949) = *Clasterosporium concinnum* Syd. *Annls mycol.* **31** (1/2): 94 (1933)

Fig. 4(a-h)

Colonies effuse, dark blackish brown, hairy. Mycelium partly superficial, partly immersed. Stroma none. Conidiophores irregularly or sometimes dichotomously branched, flexuous, smooth 2.11-3.46 µm wide. Conidiogenous cells integrated and terminal. Conidia smooth, pale to light brown, 1-3 septate, slightly curved, often fusiform pointed at the apex blunt at the base but sometimes cylindrical, 18.42-23.10 × 4.05-5.30 µm.

Collection examined: India, Himachal Pradesh, Mandi, Sarkaghat, on dead and decaying twigs, July 17; 2015, Sushma, PAN 31533.

Remarks: The species in its morphological range agrees well within *Fusariella concinna*. Earlier it has been reported from Uttar Pradesh and Karnataka, so this constitutes a new record for North-Western Himalayas (Jamaluddin *et al.*, 2004).

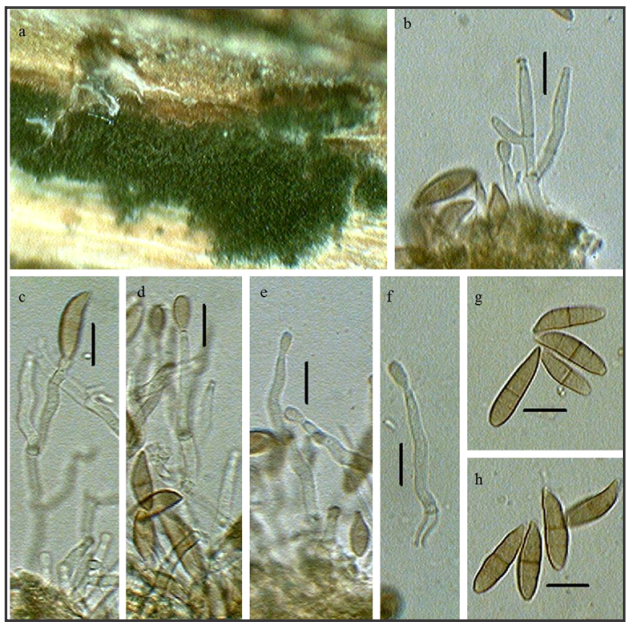

Fig.4 (a-h) *Fusariella concinna:* a- Colony on natural substrate, b- Conidiophore, c & d- Conidia attached to conidiophores, e & f- Developing conidia on conidiophores, g & h- Conidia. Bars b-h = 10 µm.

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KAVAKA 46: 27-29(2016)

A new species of *Didymocyrtis* (*Phaeosphaeriaceae*, *Ascomycota*) growing on *Thamnolia vermicularis* from India

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ABSTRACT

A new species, *Didymocyrtis thamnoliicola*, colonizing thallus of *Thamnolia vermicularis* is described from India and is compared with closely related species.

Keywords: Ascomycota, anamorphic fungi, lichenicolous fungi, taxonomy

INTRODUCTION

Didymocyrtis Vain. is a genus of ascomycetous fungi and is currently included in the family *Phaeosphaeriaceae* M.E. Barr (Ertz *et al.,* 2015). The genus is distinguished by perithecioid ascomata with a hamathecium of septate paraphysoids, transversally 1-3 septate pale brown to brown ascospores and *Phoma*-like conidiomata lacking conidiophores and having hyaline, simple, smooth walled conidia with guttules (Ertz *et al.*, 2015). As like most of the lichenicolous genera, the species concept in the genus *Didymocyrtis* is to some extent host based and appears to be narrowly host specific to particular species, genera or families, with some exceptions (**Table 1**). Currently the genus comprises twelve species and in this paper we describe a new species of *Didymocyrtis* collected from the thallus of *Thamnolia vermicularis* (SW) Schaer., belonging to family *Icmadophilaceae* Triebel and compared with other closely related species.

This work is a further contribution to the knowledge of the lichenicolous fungi of India, which is the result of our current study focusing on the taxonomy and diversity of secondary fungi associated with lichens of India (Joshi *et al.,* 2015a;b; 2016).

MATERIALS AND METHODS

Macroscopical examination was carried out using a dissecting microscope (OLYMPUS SZ2-ILST) and microscopical studies of handmade sections were made using a CX21iLeDFS1 microscope. Measurements of conidia refer to material examined in tap water. Conidial size of a large number of conidia were measured for specimen, and are indicated as (minimum) low mean-high mean (maximum), followed by the number of measurements (*n*). The type specimen has been deposited in CSIR-National Botanical Research Institute (LWG).

Taxonomy

Didymocyrtis thamnoliicola Y. Joshi, R. Bajpai & Upreti sp. nov.

(Fig. 1 A-E)

Mycobank MB 816871

Diagnosis: The species is characterized by its occurrence in anamorphic stage, biguttulate conidia having conidial width <3 µm, and differs from other known species in having different host preference, i.e. *Thamnolia vermicularis*.

***Etymology*:** Named after the host lichen *Thamnolia* on which it was found growing.

Ascomata unknown. Conidiomata several per thallus (in stroma), scattered, completely immersed at first, later protruding over the host thallus, dark brown, subspherical to

Table 1. Host specificity shown by various *Didymocyrtis* species against lichen family, genera and species

Didymocyrtis species	Lichen		
	Family	Genus	Species
D. bryonthae (Arnold) Hafellner	-	-	*Lecanora epibryon*
D. cladoniicola (Diederich, Kocourk. & Etayo) Ertz & Diederich	*Parmeliaceae*	*Cladonia, Flavoparmelia, Parmelina, Ramalina, Squamarina*	-
D. consimilis Vain.	-	*Caloplaca, Cladonia, Heterodermia, Melanohalea*	-
D. epiphyscia Ertz & Diederich s.s.	*Physciaceae*	-	-
D. epiphyscia Ertz & Diederich s. l.	-	*Physcia, Xanthoria*	-
D. foliaceiphila (Diederich, Kocourk. & Etayo) Ertz & Diederich	-	*Cladonia, Parmelia*	-
D. infestans (Speg.) Hafellner	-	*Teloschistes*	-
D. kaernefeltii (S.Y. Kondr.) Hafellner	-	-	*Teloschistes chrysophtalmus*
D. melanelixiae (Brackel) Diederich, Harris & Etayo	*Parmeliaceae*	-	-
D. pseudeverniae (Etayo & Diederich) Ertz & Diederich	-	*Pseudevernia*	-
D. ramalinae (Roberge ex Desm.) Ertz, Diederich & Hafellner	-	*Ramalina*	-
D. slaptoniensis (D. Hawksw.) Hafellner & Ertz	-	-	*Xanthoria parietina*
D. thamnoliicola Y. Joshi, R. Bajpai & Upreti	-	-	*Thamnolia vermicularis*
D. xanthomendozae (Diederich & Freebury) Diederich & Freebury	-	*Xanthomendoza*	-

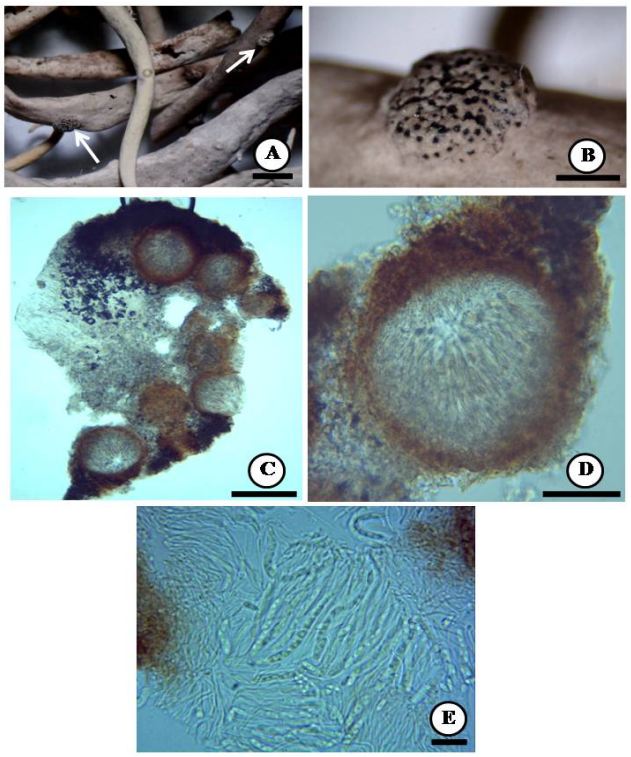

Fig. 1 *Didymocyrtis thamnoliicola* (A-E). A) Arrows indicating conidiomata on *Thamnolia vermicularis*; B) Enlarged view of conidiomata (in stroma), C) Section through pycnidia in stroma, D) Enlarged view of a single pycnidium, and E) Hamathecium (in KOH) with several biguttulate conidia. *Scale bars*: A = 1 mm, B = 3 mm, C = 80 µm, D = 100 µm, E = 8 µm.

pyriform to subglobose, 70-100(140) µm in diam.; wall of conidiomata dark brown in upper part, pale brown to subhyaline towards the base, c. 7.5-10 µm in diam., composed of several layers of cells, outer cells dark brown, inner cells hyaline. Conidiogenous cells short-ampulliform, 3-7 µm high, 2-3 µm wide. Conidia biguttulate, with a small guttule near each apex, ellipsoid, (6)7-7.5×2-2.5(3) µm (n=25).

Host and Distribution: The species is so far confined to the thallus of *Thamnolia vermicularis* that was collected growing over mosses from Arunachal Pradesh at an altitude of ca. 4100 m.

Specimen examined: India, Arunachal Pradesh, Tawang district, Sela Pass, alt. 4135 m, 27°30'47.1" N, 92°05'45.0" E, colonizing thallus of *Thamnolia vermicularis* growing over mosses, 17 June 2015, R. Bajpai, 15-026585 (LWG Holotype).

Discussion: Presently no species of *Didymocyrtis* is recognized as occurring on *Thamnolia* across the world and, hence, it is the first lichenicolous fungus reported from species of *Thamnolia*. Besides, *Didymocyrtis thamnoliicola*, one of the Indian specimens of *Thamnolia vermicularis* from Sikkim is hosting another lichenicolous fungus - *Geltingia associata* (Th. Fr.) Alstrup & D. Hawksw., which differs from the new taxon in having aseptate, subglobose to shortly ellipsoid ascospores.

Didymocyrtis bryonthae, D. cladoniicola, D. epiphyscia s.l., *D. ramalinae, D. slaptoniensis* and *D. xanthomendozae* are some of the species with which the new taxon needs not to be confused (**Table 2**). *D. bryonthae* differs from the new taxon in having multiguttulate, broader conidia (>3 µm) and different host (*Lecanora epibryon*). *D. Epiphyscia* s.l., generally having biguttulate conidia (rarely multiguttulate) makes it confusing with the new taxon, but can easily be demarcated in having smaller conidia and different hosts (*Physcia adscendens, P. tenella, Xanthoria parietina*). *Didymocyrtis cladoniicola*, which used to colonize members of *Cladonia, Ramalina, Squamarina* and *Parmeliaceae* differs from the new taxon in its host preference and smaller conidial size (**Table 2**). Large pycnidial size, broader conidia and occurrence on *Xanthomendoza*, separates *Didymocyrtis xanthomendozae* from the new taxon (**Table 2**). *D. slaptoniensis* differs from the new taxon in having l/b ratio approximately 2 and a different host (*Xanthoria parietina*) **(Table 2).** *Didymocyrtis ramalinae* differs from the new taxon in having conidial width >3 µm and used to colonize *Ramalina*

Table 2. Comparison between some closely related species

Characters	*D. bryonthae*	*D. cladoniicola*	*D. epiphyscias.l.*	*D. ramalinae*	*D. slaptoniensis*	*D. thamnoliicola*	*D. xanthomendozae*
Pycnidia size (µm)	80–100 × 100–130	(40–)50–100(–140)	(50–)100–150	105–135	80–120(–150)	70–100(–140)	140–160
Conidia breadth (µm)	>3	<3	<3	>3	<3	<3	>3
Number of guttules	Multiguttulate	Biguttulate	Bi- to multi-guttulate (rarely)	Biguttulate	Biguttulate	Biguttulate	Uni- to bi-guttulate
Conidia size (µm)	(6)7-8 × 3-4	(3.8)4.7-5.9(7.3) × (2.0)2.4-3.0(3.5)	(3.7)4.6-6.4(8.0) × (2.0)2.5-3.1(3.5)	5-7 × 3-4	6-8 × 2.5-3.5	(6)7-7.5 × 2-2.5(3.0)	(4.5)5.6-7.1(8.6) × (2.9)3.3-4.3(4.6)
l/b ratio	2-2.3	(1.4)1.7-2.2(2.8)	(1.2)1.6-2.3(3.5)	1.5-2	ca. 2	2.5-3	(1.2)1.4-2.0(2.5)
Host(s)	*L. epibryon*	*Cladonia, Ramalina, Squamarina, Parmeliaceae*	*P.adscendens, P. tenella, X.parietina*	*Ramalina*	*X. parietina*	*T. vermicularis*	*Xanthomendoza*

species (**Table 2**). In view of the presence of major differences in the presently examined collection from the closely allied taxa, a new species, *D. thamnoliicola,* has been named after its lichen host.

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KAVAKA 46: 30-34(2016)

Isolation and Characterization of Pigment Producing Fungi from Natural Habitat

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ABSTRACT

Seven pigment producing fungi were isolated from different regions of Jalandhar, Punjab (India). The fungi were identified as *Aspergillus niger, Aspergillus flavus, Aspergillus nidulans, Pencillium expansum, Pencillium digitatum, Fusarium udum* and *Acrothecium* sp. Effect of different carbon, nitrogen and amino acid sources were assessed under submerged fermentation conditions. It was observed that a pronounced increase in pigment production was there in case of all the isolates on adding up to 3-5 % (w/v) peptone and yeast extract. Also, the addition of up to 7% of glucose and maltose resulted in enhanced pigment production, although the impact was less as compared to that of nitrogen sources (peptone and yeast extract). Addition of amino acids like Methionine, Cysteine and Aspartic acid in the basal medium also showed significant enhancement in pigment production.

Key words: Secondary metabolites, fungal pigment, carbon source, nitrogen source

INTRODUCTION

Fungi are eukaryotic organisms and possess the capability of producing various natural pigments, with utility as food colorants. Recently, the concern related to the possible harmful effects of commonly used chemical food colorants has fuelled researchers to explore the extraordinary chemical diversity and biodiversity of these pigmented fungi which are regarded as an alternative source for the biotechnological production of safe colorants (Duran *et al.*, 2002; Mapari *et al.*, 2005). It has also been observed that one fungal species can synthesize mixture of pigment molecules viz. carotenoids, melanins, sporopollenins, flavins, phenazines, etc. with variety of applications in food and medicine biotechnology (Dufossé *et al.*, 2014). The objective of the present research effort was to isolate various pigment producing fungi from their natural habitats and optimise conditions for enhancing pigment products by using various amino acids, nitrogen sources and carbon sources as supplements in the basal medium.

MATERIAL AND METHODS

Collection of sample: A total of nine samples were collected from different areas of Punjab, viz. Lovely Professional University Phagwara (LPU), Santokhpura (Jalandhar), Chaheru (Jalandhar), Kadhar (Hoshiarpur), Hardaspur (Kapurthala) and Dakhoha Phatak (Kapurthala). The soil samples were taken from a depth of 5-10 cm in sterile plastic bags and kept under refrigerated conditions till further use (Pradeep *et al.*, 2013). Similarly the plant samples were also asceptically collected and stored at 4^0C in refrigerator till further use. The details of samples are provided in **table-1**.

Table-1 Samples for Isolation of Pigment Producing Fungi

S. No.	Sample type	Site of Sample Collection
1	Soil sample	Plant pot soil, Lovely Professional university(LPU), Phagwara.
2	Plant sample (Flower)	Flower from Garden, LPU, Phagwara.
3	Soil sample	Agricultural land of Baba Bhudha Enclave,Dhakoha, Jalandhar.
4	Plant sample (Leaf)	Garden of Lovely Professional University, Phagwara.
5	Soil sample	Paddy Field near School of Biosciences, LPU,Phagwara.
6	Leaf sample (Leaf)	Plant Nursery Baba Bhudha Enclave Dhakoha Jalandhar.
7	Leaf sample (Leaf)	Plant Nursery Chaheru, Phagwara, Punjab
8	Soil sample	Plant Nursery Baba Bhudha Enclave Dhakoha Jalandhar
9	Soil sample	Agricultural field Sham Churasi, Hoshiarpur Punjab.

Cultivation and isolation of fungi: Soil sample were serially diluted and inoculated through spread plate method onto Czapek Dox Agar (CDA) media plates supplemented with streptomycin. Plant samples (Leaf, sepals and petals) were inoculated by directly touching the leaf and flower bases on the surface of the same medium (CDA). Inoculation was carried in laminar air flow and the inoculated plates were incubated in BOD incubator at 28±2 °C for 6 days (Pradeep *et al.*, 2013; Pradeep and Pradeep, 2013).

Screening of pigment producing fungi

Physical screening: Isolated fungal strains were cultured on Czapek Dox Agar. These inoculated fungal plates were then incubated in a BOD incubator in which the temperature was maintained at 28±2 ^{0}C for 6 days. The colored fungal colonies were then pure cultured individually on the same medium and were again incubated in a BOD incubator at 28±2°C for 6 days. These pure cultures were then preserved at 4°C for further analysis.

Qualitative and quantitative screening of pigment: The isolated colored fungal colonies were inoculated in Czapek Dox Broth (CDB) in which a 0.8 cm^2 plug from outer zone of colony was punched with a sterile well cutter (Fusaro, 1972). This mycelium plug was then transferred into a 100 ml CDB in 250 ml Erlenmeyer flask and grown at 28±2°C for 6 days in a shaking BOD incubator at 200 rpm. The culture broth was then filtered through Whatman No. 1 filter paper. Mycelia were dried in hot air oven at 50^O C for 48 h (Olsson and Nielsen, 1997). Pigment extraction was performed by using methanol (5/20:w/v). Optical density was measured by first calculating the λ_{max} for each isolate depicted in **Table 2.** Changes in pigment production were recorded by calculating the OD Units (Dhale and Raj, 2009).

Morphological characterization of isolated fungi: The isolated fungi, grown on CDA, were identified by observing their macroscopic (color, texture, appearance, and diameter of colonies) and microscopic (microstructures) characteristics as per Gilman's Manual of Soil Fungi (Gilman, 1994). The microscopic examination was made by observing the slide after staining with lactophenol cotton blue (Leck, 1999).

Pigment extraction

All the fungal isolates were then inoculated into the liquid medium with 0.8 cm^2 plug from outer zone of colony growing on CDA individually. Pigments were extracted through methanol extraction process. All the submerged cultures were filtered through Whatman No.1 filter paper. The mycelia collected were then dried in a hot air oven till the whole moisture content got vaporized and then the weights of mycelia were measured (Olsson and Nielsen, 1997). Pigment extraction process was performed by adding methanol to the dried mycelia in the ratio of 5/20 (w/v). Process was carried out for 60-70 minutes with periodic shaking. The mixture was then filtered through Whatman No.1 filter paper. Pigment production was evaluated by measuring the absorbance of all the filtrates at the wavelength showing maximum absorption. The pigments extracted from submerged fermentation were expressed as OD Units/ml. The extraction procedure was performed as per the method of Dhale and Raj (2009) with slight modification.

$$\text{OD Units/ml} = \frac{\text{OD X Total volume of Solvent X Dilution}}{\text{Volume of substrate}}$$

Optimization and quantification of pigments : Two different carbon sources (maltose and glucose), two nitrogen sources (peptone and yeast extract) and four amino acids (methionine, cysteine, aspartic acid and proline) were used as additives to the basal medium so as to evaluate their impact on pigment production by fungal isolates.

Effect of carbon and nitrogen source on pigment production : Two different carbon sources, Glucose and Maltose were used as additives for the pigment production. Different concentrations of these two carbon sources *viz*., 1%, 3%, 5% and 7% were added separately to the basal CDB medium. The unsupplemented CDB was used as control. The flasks were inoculated with 0.8 cm^2 plug from outer zone of colony growing on CDA. These inoculated samples were placed in a shaking incubator at 200 rpm for 6 days at 28±2 °C (Pradeep and Pradeep, 2013). Extraction was done as per the method of Dhale and Raj (2009) with slight modification. Similarly experiment was performed with Peptone and Yeast extract as additives to the basal medium separately at different concentrations (1%, 3%, 5% and 7%) with unsupplemented CDB serving as control (Gunasekaran and Poorniammal, 2008).

Effect of Amino acids : Different amino acids like methionine, cysteine, proline and aspartic acid were added in same concentration (0.5%) to the liquid medium (CDB) individually and the effect on pigment production was observed.

RESULTS

Out of the total eleven isolates, seven pigmented fungi were screened out based upon their physical apperance and their ability to produce pigments under submerged culture conditions (Pradeep and Pradeep, 2013; Dhale and Raj, 2009) . These pigmented fungi were incubated at 28±2 ^{0}C for 6 days at 200 rpm in a shaking BOD incubator (Pradeep and Pradeep, 2013) . Optical densities were measured at their respective maxima (λ_{max} wavelengths) as depicted in **Table 2**. The calculated OD units of these seven isolates are given in **Fig. 1**. Also, it was observed that in case of all the isolates no color change was recorded in the inoculated liquid medium.

Table-2 Identification and λ_{max} *of the seven screened fungal isolates*

Isolate	Fungi	Lambda max (λ_{max})
F1	*Aspergillus niger*	440nm
F2	*Aspergillus flavus*	580 nm
F4	*Aspergillus nidulans*	200 nm
F6	*Penicillium expansum*	360 nm
F7	*Penicillium digitatum*	400 nm
F10	*Fusarium udum*	372 nm
F11	*Acrothecium* sp.	426 nm

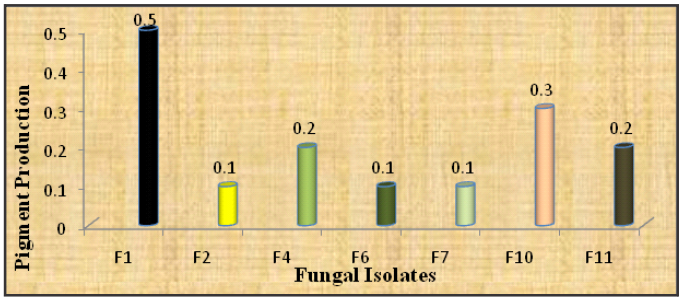

Fig 1. Screening of Pigment production in Fungal isolates

Characterization of isolated fungi : The isolated fungi (growth on CDA) were identified by observing their macroscopic (color, texture, appearance, and diameter of colonies) and microscopic (hyphae, conidia etc.) characteristics by using Gilman's manual of soil fungi (Gilman, 1994). Fungal slides were prepared in Lactophenol cotton blue dye and examined under microscope at 40X and 100X magnification (Leck, 1999). Isolates F1, F2 and F3 were found to be members of *Aspergillus* genus and F6 and F7 were found to belong to the genus *Penicillium* with F1 identified as *A. niger*, F2 as *A. flavus*, F4 as *A. nidulans*, F6 as *Penicillium expansum* and F7 as *P. digitatum*, respectively. The isolates F10 and F11 were identified as *Fusarium udum* and *Acrothecium* sp., respectively.

Effect of Carbon sources on pigment production : Different concentrations of glucose were amended into the basal medium(1%, 3%, 5% and 7%). It was observed that by increasing the concentration of glucose in the basal medium Czepak Dox Broth (CDB) pigment production increased in most of the fungal isolates. However, in case of isolate F7, increase in pigment production was observed only with glucose concentration of 3% and higher (**Fig. 2**).Similary, supplementation of maltose also resulted in enhanced pigment production in all the seven isolates with maximum pigment production in case F1 (**Fig. 3**). In fact, this isolate showed pigment production (2.3 OD units) with 7% maltose as comapred to 7% glucose (2.0 OD units). Also, supplentation of up to 7% of both the carbon sources resulted in enhanced pigment production.

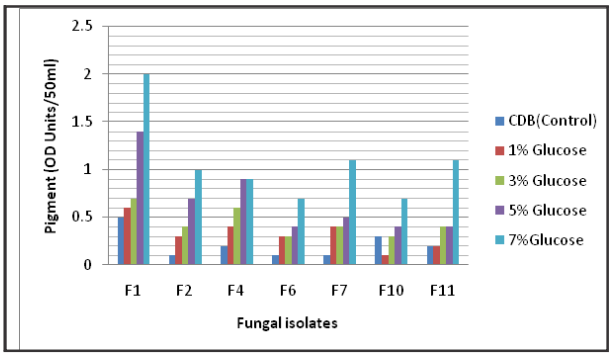

Fig 2. Effect of different concentrations of Glucose on pigment production

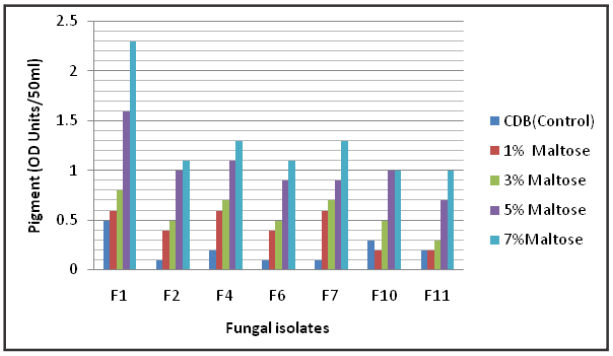

Fig 3. Effect of different concentrations of Maltose on pigment production.

Effect of Nitrogen sources on pigment production : Different concentrations of Peptone were amended into the basal medium (CDB). Variation in pigment production was observed at different concentrations of peptone, no linearity in pigment production was observed. All the isolates showed variations in pigment production at different concentrations of peptone (**Fig. 4**). At 1% peptone, F7 isolate showed maximum pigment (3.4 OD units/50ml) production and at 3% peptone F4 (9.7 OD units/50ml) had the maximum pigment production. Maximum pigment production was observed at 5% peptone in all isolates except for F10 and F11 in which the pigment production decreased by half as compared to the pigment production at 3% peptone. Pigment production

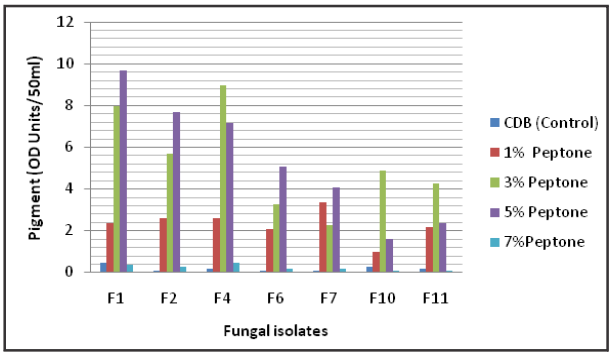

Fig 4. Effect of different concentrations of Peptone on pigment production

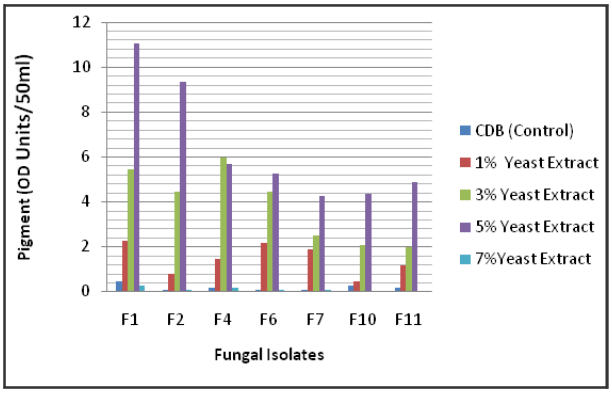

Fig 5. Effect of different concentrations of Yeast extract on pigment production

increased in all the isolates by adding up to 5% of yeast extract (**Fig. 5**). Maximum pigment production was observed in F1 isolate at 5% yeast extract (11.1 OD units/50ml). However, when the concentration was further increased the pigment production decreased.

Impact of different amino acids on pigment production : Methionine and cysteine have shown maximum effect on pigment production in F1, F2 and F4 isolates, whereas aspartic acid had shown remarkable effect on pigment production in 4 fungal isolates such as, F2, F4, F10 and F11. Proline showed relatively lesser impact in all the fungal isolates (**Fig. 6**).

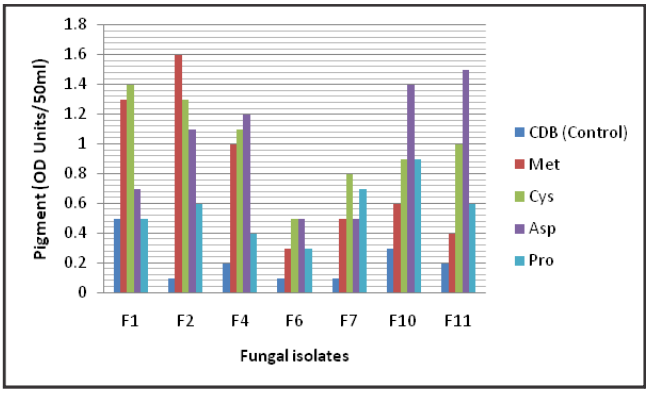

Fig 6. Effect of different amino acids on pigment production

DISCUSSION

Eleven pigment producing fungi were isolated from different regions of Punjab (India) in which after screening only seven were found to be potent pigment producers. These seven isolates were identified as members of *Aspergillus* sp., *Pencillium* sp., *Fusarium* sp. and *Acrothecium* sp. The isolated fungal strains were grown in the CDB (basal medium) in which an increasing concentrations of carbon and nitrogen sources were amended. Further, the effect of a few amino acids were also assessed. In different carbon sources (glucose and maltose) pigment and biomass production increased continuously in all fungal isolates and the maximum pigment production was observed in case of F1

isolate. Similar results were also observed in different fungal species by Demain (1986).

When peptone and yeast extract were added separately to the basal medium at concentrations like 1%, 3%, 5% and 7%, pigment production increased by adding up to 5% of both peptone and yeast extract in most of the fungal isolates except F10 and F11. Chen and Johns (1993) have also found peptone to be a good nitrogen source for pigment production by *Monoascus pupureous*. Cho *et al.* (2002) have also reported peptone to be very effective in enhancing pigment production by *Paecilomyces sinclairii.* Carels and Shepherd (1977) have reported addition of yeast extract to result in pigment production in *Monoascus* sp.

However, on increasing the concentration of peptone and yeast extract beyond 5%, a drastic drop in both pigment and biomass production was observed in all isolates. Fungal biomass increased with increasing concentrations of peptone. But after adding 7% peptone, biomass production also decreased in all the fungal isolates. This may be due to some toxic effects of extra nitrogen sources that might be blocking some important biochemical pathways. Also, on comparing the impact of carbon sources with nitrogen sources, it was evident that the nitrogen sources were much more effective in enhancing the pigment production. This observation is in agreement with the studies conducted by Subhashree *et al.* (2011) in *Monoascus purpureus*.

Four different amino acids *viz.* methionine, cysteine, aspartic acid and proline were added separately to the CDB at same concentration (0.5%). The amino acids supplemented may have important role because of sharing their carbon ring or nitrogen skeleton in primary and secondary metabolic processes (Noaman *et al.*, 2004; Pradeep and Pradeep, 2013). However, the individual amino acids produced relatively lesser enhancement in pigment production as compared to the supplementation with peptone and yeast extract (**Fig. 4-6**) which may be attributed to the fact that both peptone and yeast extract are rich source of many growth factors including various amino acids, vitamins, etc which might be playing a crucial role in pigment production.

Pigments produced by fungus vary from species to species and also depends upon the substrate used (Noaman *et al.*, 2004). Pigments produced by fungi have very important applications in food industry, textile industry and pharmaceutical industry (Jung *et al.*, 2003). In recent years, pigments isolated from biological sources are becoming an extensive area of research. There were a lot of side effects by using synthetic pigments. To overcome those side-effects, natural food colorants are extracted from various fungi like *Fusarium* sp., *Pencillium* sp. and *Monascus* sp. (Delgado-Vargas, 2000). However, there may be more industrially important fungal species that are not still reported. So, it is necessary to carry more research programs on the isolation and characterization of such fungal species.

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Gelatinoamylaria gen. nov. (*Dermateaceae*, *Helotiales*) from Bhutan

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ABSTRACT

***Gelatinoamylaria* gen. nov. (*Dermateaceae*, *Helotiales*) is proposed for the material collected from Bhutan. The single species *G. thimphuensis* is described and illustrated. The genus is characterized by non-ionomidotic gelatinous apothecia and amyloid ascospores.**

Keywords: Eastern Himalayas, fungi, inoperculate discomycetes, amyloid

INTRODUCTION

Extensive fungal explorations organized by one of us (R.S.) in the Himalayas led to a rich collection of higher fungi. A collection made in Bhutan, marked by unique features, could not be accommodated in any of the already known genera of *Discomycetes*. Therefore a new genus *Gelatinoamylaria* is proposed with the single species *G. thimphuensis*. The Holotype has been deposited in the Mycology Herbarium, Botany Department, Panjab University, Chandigarh (PAN) and a part of the collection in the Plant Pathology Herbarium, Cornell University, Ithaca (CUP). The description is based on the fresh as well as dry material. The color standards are according to Kornerup and Wanscher (1967).

MATERIALS AND METHODS

The material examined in this study was collected from Bhutan, on decaying leaf stalk of some broad leaved angiosperm. The specimen for study was revived in 4% KOH and stained and mounted in Cotton blue -Lactophenol and Melzer's reagent (Kirk *et al*. 2008). Amyloid reaction of spores was performed with Melzers' reagent. The specimen was studied microscopically under Matrix stereo trinocular microscope (VL-Z60) and transmission microscope (VRS-2*f*) for macroscopic and microscopic characters. All the measurements were taken with the help of Pro MED software. The examined material has been deposited in the herbarium of Botany Department, Panjab University, Chandigarh, India (PAN) and in the Plant Pathology Herbarium, Cornell University, Ithaca (CUP).

RESULTS

Gelatinoamylaria I. B. Prasher & R. Sharma, gen. nov.

MycoBank no.: MB817325

Type species: *Gelatinoamylaria thimphuensis* I.B. Prasher & R. Sharma

Etymology: After the gelatinous nature of the apothecium and amyloid ascospores.

Diagnosis :Apothecia black, cupulate to plane, gelatinous, up to 1 mm in diameter. Asci 8-spored, J+, clavate-cylindric. Ascospores ellipsoid, hyaline and non-septate when young, becoming 1-2 septate and brown at maturity, turning light blue to deep blue in Melzer's reagent. Paraphyses hyaline, filiform, branched, septate. Ectal excipulum textura angularis, brown, gelatinized, the outermost cells running out into short, unicellular processes ("hairs"); medullary excipulum textura intricata, light brown, not gelatinized; hypothecium indistinct.

Gelatinoamylaria thimphuensis I. B. Prasher & R. Sharma, gen et sp. nov.

Figs 1 and 2

MycoBank no.: Mb817326

Apothecia gregarious, subsessile, gelatinous, cupulate to plane, margin incurved, external surface black, hymenium light grey, becoming coal black on drying, up to 1 mm in diameter. Asci 8-spored, J+, 80-102 × 12-14 µm, clavate-

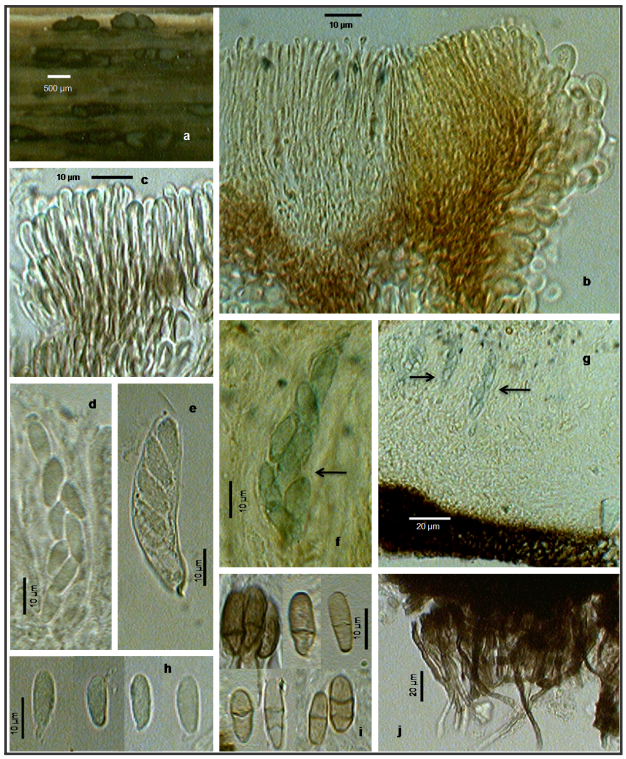

Fig. 1 *Gelatinoamylaria thimphuensis* **a** Apothecia; **b** Vertical section of apothecium; **c** Gelatinized ectal excipular cells; **d,e** Asci and ascospores; **f,g** Ascospores turning blue in melzer's reagent; **h** Hyaline ascospores; **i** Mature brown septate ascospores; **j** Basal hyphae.

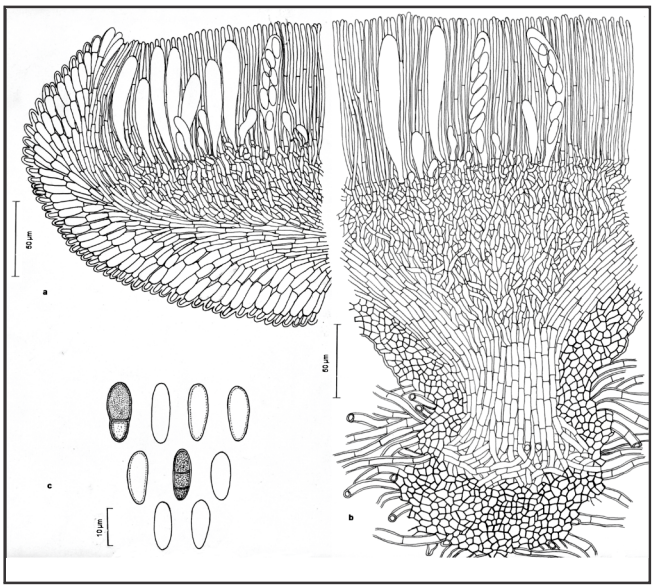

Fig. 2 Microscopic structures of *Gelatinoamylaria thimphuensis* (drawn from the holotype). a Vertical section of apothecium margin; b. Vertical section of apothecium central; c Ascospores part.

cylindric, apex round, base stem-like, arising from croziers. Ascospores 11-17 × 5-7 µm, ellipsoid, irregularly biseriate, remaining hyaline and non septate for a long time, becoming brown and 1-2 septate much later at maturity, the larger upper cell being darker than the lower cell; guttulate, guttule large, filling whole of the ascospore, disappearing at maturity, amyloid, turning light blue to deep blue in Melzer's reagent. Paraphyses hyaline, filiform, branched, septate, up to 3 µm wide at the top, projecting up to 10 µm beyond the tips of asci. Ectal excipulum gelatinized, textura angularis, light brown to dark brown, up to 60 µm thick, cells up to 25 × 9 µm, elongated and radially arranged, more or less appearing textura prismatica, with the terminal cells conspicuously smaller, covered up to the base of the apothecium by a gelatinous sheath up to 11 µm thick. Tissue of the ectal excipulum textura angularis, up to 45 µm thick with isodiametric cells in the middle or base of the apothecium, cells up to 8 × 6 µm; medullary excipulum non-gelatinized, light-brown, textura prismatica at the base, with parallel, vertical rows of rectangular cells up to 11× 5 µm, extending above as a thin layer of parallel textura porrecta along the ectal excipulum towards the margin. Medullary excipulum hyaline, conspicuously densely textura intricata in the middle, with hyphae somewhat oriented upward, with hyphal cells up to 18 × 4 µm. Attaching hyphae at the base of the apothecium thick-walled, blackish brown, septate, cells up to 20 × 6 µm.

***Etymology*:** The species name is after the place from where collection was made.

Specimen examined: Bhutan, Thimphu, Begana, 27°28′00″N 89°38′30″E, on decaying leaf stalk of some broad leaved angiosperm, 7.8.1981, R. Sharma, Holotype 24080 (PAN).

DISCUSSION

The Bhutan collection belongs to the family *Dermateaceae* of the order *Helotiales* as recognized by Korf (1973), Dennis (1978) and other leading workers of *Discomycetes*. It is characterized by the gelatinous apothecia and amyloid ascospores. The family *Dermateaceae*, according to Lumbsch and Huhndorf (2007) comprises of 76 genera. The medullary excipulum in *Gelatinoamylaria* is non-gelatinized unlike *Coleosperma* Ingold (Nauta and Spooner, 1999), *Dermea* Fr. (Nauta and Spooner, 2000a), *Hysteronaevia* Nannf., *Pyrenopeziza* Fuckel and *Micropeziza* Fuckel (Nauta and Spooner, 2000b), where it is gelatinized. However, it resembles *Micropeziza* in having gelatinized ectal excipulum. However, the present collection differs from *Micropeziza* and other known genera of *Dermateaceae* in having amyloid ascospores as well as gelatinous apothecium. The genus name *Gelatinoamylaria* proposed is quite suggestive of its unique features. It differs from the other genera with gelatinous tissue by its non-ionomidotic apothecia and amyloid ascospores.

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Diversity of mangrove fungi on *Rhizophora* wood in Puducherry, Southeast coast of India

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ABSTRACT

Decomposing prop roots of *Rhizophora apiculata* collected from Ariankuppam mangroves of Puducherry, Southeast coast of India were examined for marine fungi. Among 26 fungi, 10 ascomycetes, 1 was basidiomycetes and 15 were anamorphic fungi. *Hydea pygmea* and *Periconia prolifica* were very frequent (16.9% and 18.2%, respectively) while *Halenospora varia, Phoma* sp. and *Verruruculina enalia,* were frequent (7.9%) followed by *Monodictys pelagica, Trichocladium alopallonellum* and *Verticillium* sp. (5.4%). Others were infrequent with less than 5% occurrence. At the study site the man-made disturbances could be attributed for recording a higher percentage of anamorphic fungi than ascomycetes. Fourteen fungi constitute new records for this region and these include *Aniptodera chesapeakensis* Shearer & Miller, *Dactylospora haliotrepha* (Kohlm. & E. Kohlm) Hafellner, *Halomassarina thalassiae* (Kohlm. & Volkm. Kohlm.) Suetrong, Sakayaroj, E.B.G. Jones, Kohlm., Volkm. Kohlm. & C.L. Schoch, *Lignincola laevis* Hohnk, *L. tropica* Kohlm., *Marinosphaera mangrovei* K.D. Hyde, *Saccardoella rhizophorae* K.D. Hyde, *Cytospora rhizophorae* Kohlm. & E. Kohlm., *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., *Phomopsis mangrovei* K.D. Hyde, *Monodictys pelagica* (T.W. Johnson) E.B.G. Jones, *Periconia prolifica* Anastasiou, *Phaeosaria clematidis* Fuckel (S. Hughes), *Trichocladium. alopallonellum* (Meyerss & R.T. Moore) Kohlm. & Volkm. Kohlm.

Key words: Mangroves, marine fungi, diversity, *Rhizophora apiculata*

INTRODUCTION

Recently marine mycology has evolved as a specialized branch of science. Jones (2011) predicted that there could be over 10,000 marine fungi worldwide. They play an important role in the processing of organic matter facilitating energy flow to the marine animal community (Kohlmeyer and Kohlmeyer, 1979; Jones and Alias, 1997). Besides fungi, wood borers and bacteria also contribute as decomposers of woody and herbaceous substrata entering marine ecosystems (Fell and Master, 1980). Marine fungi grow on diverse substrata other than woody material (e.g. sediments, algae, dead corals, calcareous tubes of mollusks, decaying leaves, seedlings, prop roots and pneumatophores, swards and living animals (Hyde *et al.*, 2000; Kohlmeyer and Kohlmeyer, 1979). In addition to wood naturally occurring in marine habitat, man-made structures in the sea and mangroves were also studied for colonization/decomposition by fungi (Hyde *et al.*, 2000; Kohlmeyer and Kohlmeyer, 1979;Fell and Master, 1980).

Mangroves are tropical and subtropical swampy forests comprising a variety of trees species (Kohlmeyer and Kohlmeyer, 1979). Terrestrial fungi and lichens occupy the aerial parts of mangrove plants while marine fungi occur at lower parts where their trunks and roots are permanently or intermittently submerged in water (Kohlmeyer and Kohlmeyer, 1979). At the high tide mark there will be an interface and overlapping of marine and terrestrial fungi. Higher marine fungi occurring on mangroves of West coast of India (e.g., Borse, 1988; Patil and Borse, 1985; 2001; Chinnaraj and Untawale, 1992; Chinnaraj, 1993; Prasannarai and Sridhar, 2001;Maria and Sridhar, 2002; Ananda and Sridhar, 2004; Nambiar and Raveendran, 2008) and east coast ((Ravikumar and Vittal, 1996; Sarma and Vittal, 2001;Sarma *et al.*, 2001) have been studied by several workers. There is a gap in our knowledge on the mangrove fungi of Puducherry mangroves, hence the present study gives a comparison of fungi in Puducherry mangroves with earlier studies.

MATERIAL AND METHODS

Samples of decaying wood of *Rhizophora apiculata* were randomly collected from Ariankuppam mangrove stand in Puducherry (11°47N; 79°49E) along the Bay of Bengal region, India. The particular site is influenced by leakage of petroleum, diesel and engine oil waste as there is a garage nearby in addition to various human settlements and their domestic waste also being leaked into the back waters where the mangrove formation is located. Three collection trips (January and October 2012; July 2013) were undertaken and samples were placed in plastic bags and were examined in the laboratory directly and after incubation up to several months. Fungi were examined and identified based on the latest keys (Kohlmeyer and Volkmann-Kohlmeyer 1991; Hyde and Sarma 2000; Sarma and Hyde, 2000; Jones *et al.*, 2009). The percentage occurrence of each fungus was calculated based on the total occurrences of sporulating fungi divided by number of occurrences of a particular fungus x 100. Subsequently the percentage occurrence data was used to convert into different frequency groupings viz., (i) very frequent (above 10%), frequent (5-10%) and infrequent (below 5%).

RESULTS AND DISCUSSION

The present study revealed that the diversity of mangroves is relatively poor in Ariankuppam region of Puducherry. **Table 1** gives details of fungi recorded on woody litter of Puducherry (10 ascomycetes, 1 basidiomycete and 15 anamorphic fungi). *Periconia prolifica* and *Hydea pygmea* were very frequent with higher percentage occurrence (18.2% and 16.95%, respectively). The frequent fungi were *Halenospora varia, Phoma* sp. and *Verruruculina enalia,*(7.9% each) followed by *Monodictys pelagica., Trichocladium alopallonellum* and *Verticillium* sp. (5.4% each). Rest of the fungi were quite infrequent.

In the present study the fungi constitute 38.5% ascomycetes, and the remaining anamorphic fungi. In most of the earlier

Table 1. Frequency of occurrence of fungi recorded associated with decaying prop roots of *Rhizophora apiculata*, Ariankuppam mangroves, Puducherry.

Name of the species	No. of sporulating fungal occurrences	% occurrence	VF, F or IF
Periconia prolifica Anastasiou*	14	18.2	VF
Hydea pygmea (Kohlm) K.L. Pang & E.B.G. Jones	13	16.9	VF
Halenospora varia (Anastasiou) E.B.G. Jones	6	7.9	F
Phoma sp.	6	7.9	F
Verruculina enalia (Kohlm.) Kohlm. & Volkm.–Kohlm.	6	7.9	F
Monodictys pelagica (T.W. Johnson) E.B.G. Jones*	4	5.4	F
Verticillium sp.*	4	5.4	F
Trichocladium. alopallonellum (Meyers & R.T. Moore) Kohlm. & Volkm.–Kohlm.*	4	5.4	F
Lasiodiplodia theobromae (Pat.) Griffon & Maubl*	3	3.9	IF
Lignincola laevis Hohnk*	2	2.6	IF
Halocyphina villosa	2	2.6	IF
Aniptodera chesapeakensis Shearer and Miller*	1	1.3	IF
Aniptodera sp.*	1	1.3	IF
Dactylospora haliotrepha (Kohlm. & E. Kohlm) Hafellner*	1	1.3	IF
Fusarium sp.*	1	1.3	IF
Halomassarina thalassiae (Kohlm. & Volkm.–Kohlm.) Suetrong, Sakayaroj, E.B.G. Jones, Kohlm., Volkm.–Kohlm. & C.L. Schoch,*	1	1.3	IF
Lignincola tropica Kohlm.*	1	1.3	IF
Marinosphaera mangrovei K.D. Hyde*	1	1.3	IF
Saccardoella rhizophorae K.D. Hyde*	1	1.3	IF
Unidentified ascomycete 1	1	1.3	IF
Trimmatostroma sp.*	1	1.3	IF
Phaeosaria clematidis Fuckel (S. Hughes)*	1	1.3	IF
Cytospora rhizophorae Kohlm. & E. Kohlm.*	1	1.3	IF
Phomopsis mangrovei K.D. Hyde*	1	1.3	IF
Phomopsis sp. *	1	1.3	IF
Xylomyces sp.-like*	1	1.3	IF
Total	78		
*= Reported for the first time from Pondicherry mangroves; VF= Very frequent; F = Frequent; IF = Infrequent			

reports it has been revealed that ascomycetes occur more than 80% in mangroves, whereas the anamorphic fungi are recorded with 20-30% (Sarma and Hyde, 2001). Though a particular reason could not be assigned for this phenomenon in this mangrove the fact that the mangrove stand has man-made disturbances including human settlements and a garage nearby leaking hydrocarbons could be one of the reasons that could be attributed to. Five of the 15 anamorphic fungi form fruit bodies i.e. they belong to the coelomycetes group. The remaining 10 are hyphomycetes.

The following fourteen fungi are recorded for the first time from this region viz., *Aniptodera chesapeakensis* Shearer & Miller, *Dactylospora haliotrepha* (Kohlm. & E. Kohlm) Hafellner, *Halomassarina thalassiae* (Kohlm. & Volkm.Kohlm.) Suetrong, Sakayaroj, E.B.G. Jones, Kohlm., Volkm.Kohlm. & C.L. Schoch, *Lignincola laevis* Hohnk, *L. tropica* Kohlm., *Marinosphaera mangrovei* K.D. Hyde, *Saccardoella rhizophorae* K.D. Hyde, *Cytospora rhizophorae* Kohlm. & E. Kohlm., *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., *Phomopsis mangrovei* K.D. Hyde, *Monodictys pelagica* (T.W. Johnson) E.B.G. Jones, *Periconia prolifica* Anastasiou, *Phaeosaria clematidis* Fuckel (S. Hughes), *Trichocladium. alopallonellum* (Meyerss & R.T. Moore) Kohlm. & Volkm.Kohlm. With the addition of these 14 fungi at species level and others at genus level, the overall list of fungi reported from Pondicherry, east coast of India now stands at 47 after adding to those included in the list by Borse *et al.* (2013) from Puducherry.

So far from the east coast of India *Verruculina enalia, Eutypa bathurstensis, Lophiostoma mangrovei, Rhizophila marina, Dactylospora haliotrepha, Halorosellinia oceanica* and *Halocyphina villosa* were frequently recorded fungi as per the earlier reports (Sarma and Vittal, 2000; 2001; Vittal and Sarma, 2006; Chinnaraj, 1993). It is interesting to note that though some of these fungi were recorded in the present study also they were not very frequent excepting *Verruculina enalia*. When compared to other mangroves on the east coast of India where fungal diversity has been investigated viz., Pichavaram in Tamil Nadu, Godavari and Krishna deltaic mangroves on the east coast of India, it could be mentioned that while the ascomycetes are predominant in pristine mangrove forests (Pichavaram, Godavari and Krishna deltaic mangroves) those of anamorphic fungi seem to be dominant in the disturbed mangrove forests like the present one. Further studies are required to focus on isolation of the fungi and investigate their reaction to different hydrocarbons and other pollutants.

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Diversity of Genus *Phanerochaete* in Punjab and adjoining areas

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ABSTRACT

An account of 7 species of genus *Phanerochaete* P. Karst. (*P. chrysosporium* Burds., *P. jose-ferreirae* (D.A. Reid) D.A. Reid, *P. leptoderma* Sheng H. Wu, *P. sordida* (P. Karst.) J. Erikss. & Ryvarden, *P. tropica* (Sheng H. Wu) Hjortstam, *P. tuberculata* (P. Karst.) Parmasto and *P. xerophila Burds.*) has been given. All these are being described for the first time from Punjab and adjoining areas. Of these, *P. chrysosporium* and *P. xerophila* are new records for India.

Key words: *Basidiomycota, Agaricomycetes, Phanerochaetaceae.*

INTRODUCTION

Genus *Phanerochaete* P. Karst. (*Phanerochaetaceae, Polyporales, Agaricomycetes, Basidiomycota*), is characterized by ceraceous basidiocarps, monomitic hyphal system, simple-septate generative hyphae, clavate basidia, and smooth, thin-walled, inamyloid basidiospores. It was proposed by Karsten (1889) with *P. alnea* as the type species. It is represented by 65 species worldwide (Kirk et al. 2008). From India, earlier workers (Bagchee and Bakshi, 1954; Thind and Rattan, 1973; Rattan, 1977; Bhosle *et al.*, 2005; Dhingra, 2005; Ranadive *et al.*, 2011; Sharma, 2012; Prasher and Ashok, 2013; Ranadive, 2013; Dhingra *et al.*, 2014 and Sanyal, 2014) have reported 22 taxa of the genus from different parts of India. Present account of seven species (*P. chrysosporium* Burds., *P. jose-ferreirae* (D.A. Reid) D.A. Reid, *P. leptoderma* Sheng H. Wu, *P. sordida* (P. Karst.) J. Erikss. & Ryvarden, *P. tropica* (Sheng H. Wu) Hjortstam, *P. tuberculata* (P. Karst.) Parmasto and *P. xerophila Burds.*) of this genus is based on the collections made from different parts of Punjab and adjoining areas in the state of Haryana and Union territory of Chandigarh during the years 2012-2015. It is pertinent to mention here that all the seven species are being described for the first time from the study area with two highlighted in bold as new records for India. The material pertaining to all the taxa has been deposited at the Herbarium, Department of Botany, Punjabi University, Patiala (PUN). The color standards used are as per Kornerup and Wanscher (1978). A key to all the seven species has also been given.

KEY TO THE SPECIES

1. Cystidia absent.....2
1'. Cystidia present.....5
2. Hymenial surface smooth; subicular hyphae with encrustation; basidiospores ellipsoid to subcylindrical.. .....*Phanerochaete jose-ferreirae*
2'. Hymenial surface smooth to tuberculate; subicular hyphae without encrustation; basidiospores ellipsoid to broadly ellipsoid.....3
3. Basidiospores ellipsoid, 3.5-4.5 µm wide.....*P. xerophila*
3'. Basidiospores broadly ellipsoid.....4
4. Basidia 35-50 × 6.5-7.1 µm.....*P. tropica*
4'. Basidia 23-32 × 5.6-6.8 µm .....*P. tuberculata*
5. Cystidia cylindrical, without encrustation....*P. chrysosporium*
5'. Cystidia subfusiform to fusiform, encrusted.....6
6. Cystidia subfusiform to fusiform, thick-walled; basidiospores subcylindrical to suballantoid.....*P. leptoderma*
6'. Cystidia subcylindrical to subfusiform, thin- to thick-walled; basidiospores ellipsoid .....*P. sordida*

TAXONOMIC DESCRIPTIONS

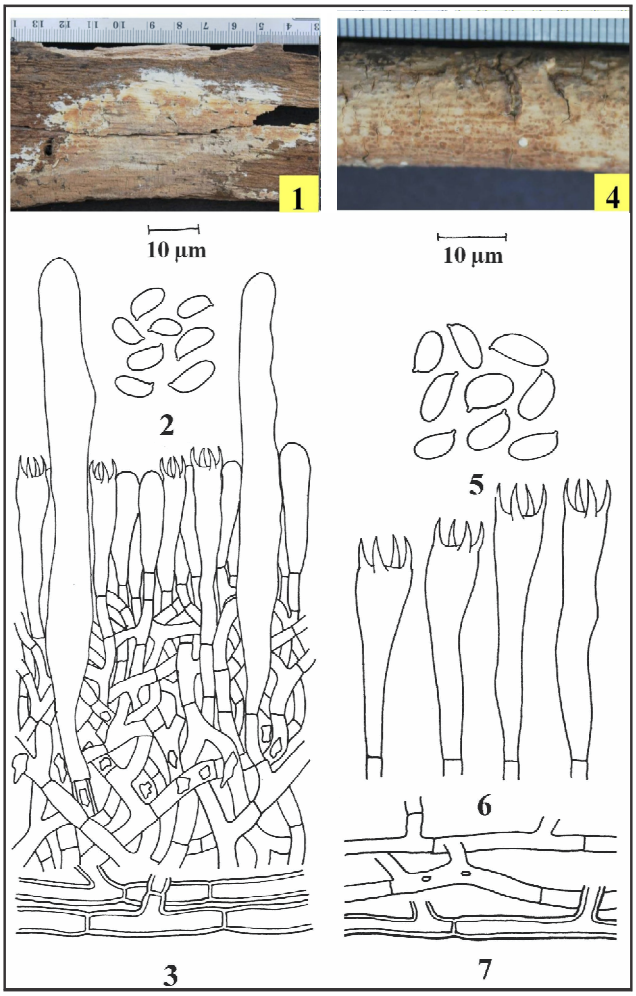
1. *Phanerochaete chrysosporium* Burds., *Mycotaxon* **1** (2): 124, 1974.

Figs. 1-3

Basidiocarp resupinate, effused, adnate, ≤325 µm thick in section; hymenial surface smooth, orange white to pale orange to greyish orange to brownish orange when fresh, not changing much on drying; margins thinning, fibrillose, paler than the colour of the hymenial surface or indeterminate. **Generative hyphae** branched, simple septate; basal hyphae ≤5.6 µm wide, generally thick-walled, branched at wide angles in all directions into an open context; subhymenial hyphae ≤5 µm wide, thin- to somewhat thick-walled, vertical, intricately branched. **Cystidia** 53-99 × 6.8-9.2 µm, subcylindrical to cylindrical, with obtuse apex, sinuous, thin-walled, without basal clamp; projecting ≤65 µm out of the hymenium. **Basidia** 18-24.2 × 4.7-5.7 µm, clavate, 4-sterigmate, without basal clamp; sterigmata ≤3.1 µm long. **Basidiospores** 5.3-9.5 × 2.8-3.4 µm, ellipsoid, smooth, thin-walled, inamyloid, acyanophilous.

Collection examined India: Punjab, Anandpur Sahib, Dabri, on stump of *Mangifera indica*, Gurpreet and Avneet, 7667 (PUN), September 13, 2015.

Remarks: This species is marked by smooth, orange white to pale orange to greyish orange to brownish orange basidiocarps, subicular hyphae branched at wide angles and big, subcylindrical to cylindrical, thin-walled cystidia. Earlier, it has been reported from Southern Arizona, North America, Iran and Europe (www.mycobank.org, 2016). However, presently it is being described for the first time from India.



Figs 1-3. ***Phanerochaete chrysosporium*****:** 1. Basidiocarp showing hymenial surface; 2. Basidiospores; 3. Vertical section through basidiocarp

Figs 4-7. ***Phanerochaete jose-fererriae*** **:** 4. Basidiocarp showing hymenial surface; 5. Basidiospores; 6. Basidia; 7. Generative hyphae

2. ***Phanerochaete jose-ferreirae*** (D.A. Reid) D.A. Reid, *Acta bot. croat.* **34**: 135, 1975.– *Corticium jose-ferreirae* D.A. Reid, *Revta Biol., Lisb.* **5**(1-2): 140, 1965.

Figs. 4-7

Basidiocarps resupinate, adnate, effused, ≤400 µm thick in section; hymenial surface smooth, pale red to greyish red to light brown when fresh, not changing much on drying; margins thinning, paler than the colour of the hymenial surface or indeterminate. **Generative hyphae** ≤4 µm wide, branched, simple septate; subicular hyphae parallel to the substrate, less branched, thin- to somewhat thick-walled, encrusted; subhymenial hyphae, vertical, richly branched, thin-walled. **Cystidia** absent. **Basidia** 16-28 × 6.2-6.8 µm, clavate, 4-sterigmate, without basal clamp; sterigmata ≤6.2 µm long. **Basidiospores** 5.2-6.8 × 3.1-3.7 µm, subcylindrical, smooth, thin-walled, inamyloid, acyanophilous.

Collections examined India: Punjab, Patiala, Punjabi University, GoI market, on branch of *Thevetia peruviana,* Gurpreet 7665 (PUN), October 2, 2013; Back of Kala Bhawan, on an angiospermous branch, Gurpreet and Navpreet 7664 (PUN), October 2, 2013.

Remarks: This species is characteristic in having simple-septate hyphae, absence of cystidia and ellipsoid to subcylindrical basidiospores. Earlier from India, it has been listed by Ranadive *et al.* (2011) from the Western Ghats, whereas described/listed by Priyanka (2012) and Dhingra *et al.* (2014) from Himachal Pradesh. Presntly it is being recorded for the first time from Punjab.

3. ***Phanerochaete leptoderma*** Sheng H. Wu, *Acta Botanica Fennica* **142**: 45, 1990.

Figs. 8-12

Basidiocarp resupinate, effused, adnate, ≤160 m thick in section; hymenial surface smooth to grandinioid, orange white to pale orange when fresh, not changing much on drying; margins thinning, somewhat fibrillose, paler than the colour of the hymenial surface or indeterminate. **Generative hyphae** branched, septate, thin- to thick-walled; basal hyphae ≤4.7 µm wide, branched, rarely clamped, smooth to encrusted; subhymenial hyphae ≤2.8 µm wide, highly branched, without clamps. **Cystidia** 40-73 × 7.8-10 µm, subfusiform to fusiform, thick-walled, without basal clamp, with crystalline encrustation which dissolves in 3% KOH solution. **Basidia** 23-38 × 4.3-5.7 µm, subclavate to clavate, 4-sterigmate, without basal clamp; sterigmata ≤4.1 µm long. **Basidiospores** 5.9-9.4 × 2.5-3.7 µm, subcylindrical, smooth, thin-walled, acyanophilous, inamyloid.

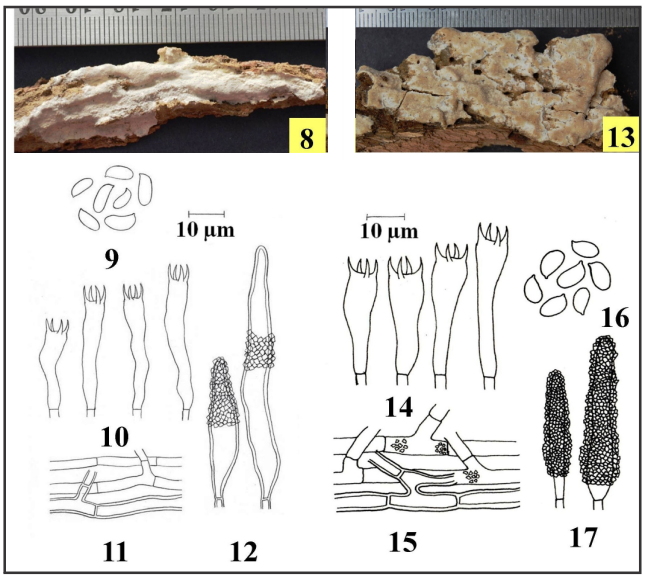
Collection examined India, Punjab: Patiala, Punjabi University, Nursery, on angiospermous log, Gurpreet and Jyoti 7669 (PUN), August 24, 2014.

Remarks This species was first described by Wu (1990) from Taiwan and is characterized by subfusiform to fusiform, encrusted cystidia and subcylindrical basidiospores. From India, it was earlier reported by Sanyal (2014) from Uttarakhand. However, from Punjab plains it is being reported for the first time.

4. ***Phanerochaete sordida*** (P. Karst.) J. Erikss. & Ryvarden, *The Corticiaceae of North Europe (Oslo)* **5**: 1023, 1978. *Corticium sordidum* P. Karst., *Meddelanden af Societas pro Fauna et Flora Fennica* **9**: 65, 1882.

Figs. 13-17

Basidiocarps resupinate, effused, adnate, ≤300 µm thick in section; hymenial surface smooth to tuberculate, orange white to pale orange to greyish orange to brownish orange when fresh, not changing much on drying; margins thinning, fibrillose, paler than the colour of the hymenial surface or indeterminate. **Generative hyphae** branched, simple septate; basal hyphae ≤5.0 µm wide, thin- to somewhat thick-walled, branched at wide angles in all directions into an open context; subhymenial hyphae ≤2.5 µm wide, thin-walled, vertical. **Cystidia** 40-72 × 6.8-11.2 µm, subcylindrical to subfusiform, thin- to thick-walled, without basal clamp, usually encrusted with crystalline matter; projecting ≤25 µm out of the hymenium. **Basidia** 18-22 × 4.3-5.6 µm, clavate, 4-



Figs 8-12. ***Phanerochaete leptoderma*:** 8. Basidiocarp showing hymenial surface; 9. Basidiospores; 10. Basidia; 11. Generative hyphae; 12. Cystidia

Figs 13-17. ***Phanerochaete sordida*:** 13. Basidiocarp showing hymenial surface; 14. Basidia; 15. Generative hyphae; 16. Basidiospores; 17. Cystidia

sterigmate, without basal clamp; sterigmata ≤3.1 µm long. **Basidiospores** 4.2-7.8 × 3.1-3.5 µm, ellipsoid, smooth, thin-walled, with oily contents, inamyloid, acyanophilous.

Collections examined India: Chandigarh (UT), Sector 14, Panjab University, opposite gate no. 3, on angiospermous wood, Gurpreet and Dhingra 7675 (PUN), October 6, 2013; Sector-1, Backside of Rock garden forest, on angiospermous host, Gurpreet and Dhingra 7663 (PUN), August 16, 2013; Pinjore, Bitna road, on bark of *Morus alba*, Gurpreet 7673 (PUN), July 14, 2013; Patiala, Punjabi University, Botanical Gardens, on bark of *Thevetia peruviana*, Gurpreet and Avneet, 7674 (PUN), September 8, 2014.

Remarks: This species is being reported for the first time from the study area. Earlier from India, it has been described/listed by Singh (2007) and Priyanka (2012) from Himachal Pradesh, Sharma (2012) from Himachal Pradesh and Uttarakhand, Sanyal (2014) from Uttarakahand and Dhingra *et al.* (2014) from Himachal Pradesh.

5. *Phanerochaete tropica* (Sheng H. Wu) Hjortstam, *Mycotaxon* **54**: 189, 1995. *Efibula tropica* Sheng H.Wu, *Acta Botanica Fennica* **142**: 25, 1990.

Figs. 18-21

Basidiocarp resupinate, adnate, effused, ≤335 µm thick in section; hymenial surface smooth, orange white to pale orange to greyish orange to brownish orange when fresh, not changing much on drying; margins thinning, somewhat fibrillose, paler than the colour of the hymenial surface or indeterminate. **Generative hyphae** ≤3.6 µm wide, branched, simple septate; basal hyphae parallel to the substrate, less branched, thin- to thick-walled; subhymenial hyphae vertical, more branched, thin-walled. **Cystidia** absent. **Basidia** 35-50 × 6.5-7.1 µm, clavate, 4-sterigmate, without basal clamp; sterigmata ≤5.6 µm long. **Basidiospores** 6.5-8.1 × 4.2-4.6 µm, broadly ellipsoid, smooth, thin-walled, inamyloid, acyanophilous, with oily contents.

Collection examined India: Haryana, Panchkula, Pinjore, Bitna road, on bark of *Morus alba,* Gurpreet 7668 (PUN), July 14, 2013.

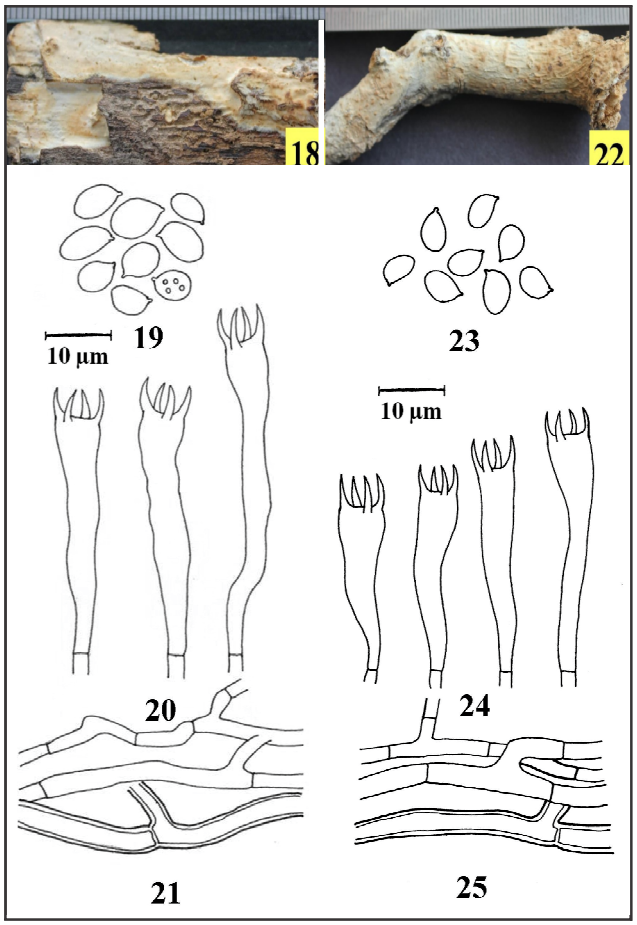
Remarks: This species is characterized by the presence of broadly ellipsoid basidiospores and absence of cystidia. It was earlier described/listed from India by Priyanka (2012) and Dhingra *et al.* (2014) from Himachal Pradesh. However, presently it is being reported for the first time from Punjab.

6. *Phanerochaete tuberculata* (P. Karst.) Parmasto, *Conspectus Systematis Corticiacearum (Tartu)*: 83, 1968. *Corticium tuberculatum* P. Karst., *Hedwigia* **35** (1): 45, 1896.

Figs. 22-25

Basidiocarps resupinate, adnate, effused, ≤225 µm thick in section; hymenial surface smooth to tuberculate, orange white to pale orange to greyish orange to brownish orange when fresh, not changing much on drying; margins thinning, fibrillose, paler than the colour of the hymenial surface or indeterminate. **Generative hyphae** ≤3.4 µm wide, branched, simple septate, thin- to somewhat thick-walled; basal hyphae parallel to the substrate; subhymenial hyphae vertical. **Cystidia** none. **Basidia** 23-32 × 5.6-6.8 µm, clavate, somewhat sinuous, 4-sterigmate, without basal clamp; sterigmata ≤3.8 µm long. **Basidiospores** 6.2-8.2 × 3.6-5 µm, broadly ellipsoid, smooth, thin-walled, inamyloid, acyanophilous, with oily contents.

Collections examined – India: Punjab, Hoshiarpur, Hariana, Hoshiarpur bye pass road, on angiospermous branch, Gurpreet and Avneet 7671 (PUN), August 15, 2014; Bathinda, Bir Talab forest, on stick of *Morus alba*, Gurpreet and Avneet 7660 (PUN), September 13, 2014, 2014; Bir Talab forest, on stick of *M. alba*, Gurpreet and Avneet 7653 (PUN), September 13, 2014; From G.T. road towards Joga Nand village, on stick of *M. alba*, Gurpreet and Avneet 7658 (PUN), September 13, 2014, 2014; Patiala, Gol market, on angiospermous stick, Gurpreet 7659 (PUN), August 28, 2014; Hostel number 2, on angiospermous stick, Gurpreet and Navpreet 7657 (PUN), September 16, 2014; Ludhiana, Tiger Safari, on stick and bark of *M. alba*, Gurpreet and Navpreet, 7656 (PUN), September 8, 2013; Tiger Safari, on angiospermous log, Gurpreet and Navpreet 7655 (PUN), September 8, 2013; Tiger Safari, on bark of *M. alba*, Gurpreet and Navpreet 7672 (PUN), September 8, 2013; Roopnagar, Naugavan, near forest nursery, on bark of *M. alba*, Gurpreet and Avneet 7661 (PUN), September 5, 2012; Forest Rest House, on bark of *M. alba*, Gurpreet and Avneet 7670 (PUN), September 5, 2012; Patiala, Horticulture department, Punjabi University, on branch of *Bauhinia tomentosa* Gurpreet and Harpreet 7662 (PUN), September 10, 2012; Nursery, on branch of *Morus alba*, Gurpreet and Navpreet 7652, (PUN), September 10, 2012.



Figs 18-21. ***Phanerochaete tropica*****:** 18. Basidiocarp showing hymenial surface; 19. Basidiospores; 20. Basidia; 21. Generative hyphae

Figs 22-25. ***Phanerochaete tuberculata*****:** 22. Basidiocarp showing hymenial surface; 23. Basidiospores; 24. Basidia; 25. Generative hyphae

Remarks: It is the first report of this species from Punjab. Earlier from India, it was documented by Dhingra (1983) from the Eastern Himalaya; Rattan (1977), Singh (2007), Priyanka (2012) from Himachal Pradesh, Sharma (2012) from Himachal Pradesh and Uttarakhand and Sanyal (2014) from Uttarakhand. It was also listed by Dhingra *et al*. (2011) from the Eastern Himalaya, and Dhingra *et al*. (2014) from Himachal Pradesh.

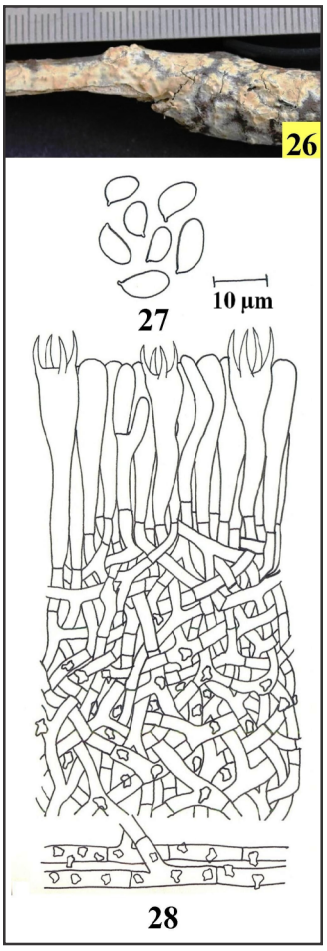
7. Phanerochaete xerophila Burds., *Mycologia Memoirs* **10**: 141, 1985.

Figs. 26-28

Basidiocarp resupinate, effused, adnate, ≤290 m thick in section; hymenial surface smooth to somewhat cracked, pale orange to greyish orange to brownish orange when fresh, not changing much on drying; margins thinning, fibrillose, paler than the colour of the hymenial surface or indeterminate. **Generative hyphae** ≤3.1 µm wide, thin-walled, simple septate; basal hyphae branched at wide angles in all directions; subhymenial hyphae vertical, branched, with crystalline encrustation. **Cystidia** absent, but thin-walled hyphidia present. **Basidia** 17-26×5.9-7.5 µm, clavate, 4-sterigmate, without basal clamp; sterigmata ≤6.8 m long. **Basidiospores** 5.6-9.4×3.5-4.5 m, ellipsoid to broadly ellipsoid smooth, thin-walled, acyanophilous, inamyloid.

Collection examined India, Punjab: Bathinda, Bir Talab mini zoo cum Deer safari, on sticks of *Morus alba*, Gurpreet and Avneet 7666 (PUN), September 13, 2014.

Remarks This species is characteristic in its occurrence in the xerophytic localities, lack of cystidia and ellipsoid to broadly ellipsoid basidiospores. Earlier, it was described from Arizona, U.S.A., Argentina, Uruguay, Russia and the Caucasus (www.mycobank.org, 2016). It is the first report of this species from India.



Figs 26-28. ***Phanerochaete xerophila*****:** 26. Basidiocarp showing hymenial surface; 27. Basidiospores; 28. Vertical section through

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KAVAKA 46: 45-47(2016)

Two species of Genus *Lentinus* Fr. from India

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ABSTRACT

The present paper deals with the taxonomy of two species of the genus *Lentinus* Fr., namely *L. kashmirinus* sp. nov and *L. nigroosseus* Pilát from India.

Key words Basidiospores, cheilocystidia, hymenophoral trama.

INTRODUCTION

Genus *Lentinus* Fr. belongs to the family *Polyporaceae* Fr. ex Corda. It is known by 597 species the world over (Mycobank, 2015), while only 40 species of *Lentinus* are known from India (Sharma and Atri, 2015). *Lentinus* usually grows in caespitose clusters in groups usually xeromorphic, lignicolous to rarely graminicolous in habitat. Carpophores are centrally stipitate, lamellae decurrent, sometimes intervened to rarely furcated, gill edges entire, denticulate or coarsely serrate. Spore print white to cream. Microscopically the sporophore context with dimitic hyphal construction, basidiospores cylindrical, aporous, inamyloid, non-dextrinoid, lamellae edges always sterile, either with emergent skeletal elements in the form of cystidia which may be metuloids, gloeocystidia, skeletocystidia or cheilocystidia, pleurocystidia and hyphal pegs rarely present. Pileus cuticle usually undifferentiated forming a repent epicutis of radially parallel hyphae, sometimes trichodermial.

MATERIALS AND METHODS

The collections were worked out as per the standard methodology given by Atri *et al.* (2005). The classification, terminology and generic concept used are as given by Kirk *et al.* (2008) and Mycobank (2015). The colour terminology used is after Kornerup and Wanscher (1978).

Taxonomic observations

Lentinus kashmirinus Munruchi Kaur and Nazir Ahmad Malik sp. **nov.**

Fig. 1(A-D); Fig. 2 (A-G)

Mycobank no.: MB 808717

Etymology- The name of the species is based on the area from where this new species was collected.

Diagnosis-Sporophore tough with convex to depressed thick fleshed pileus. Lamellae decurrent, denticulate. Hymenophoral trama of descending hyphae. Gill edges sterile, crowded with Cheilocystidia some of which have metulloidal apices. Hyphal system dimitic, clamp connections present. Carpophore lacks any resinous amber coloured secretion.

Sporophore 12 cm in height. Pileus 8.2 cm broad, convex to applanate; without umbo; surface white ($30A_1$), moist, scaly, scales appressed, covering the entire pileus, squamules in

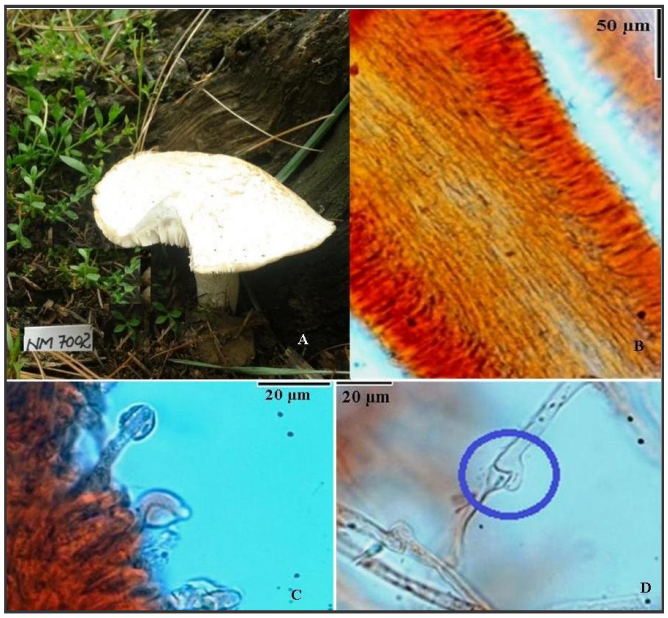

Fig. 1 (A-D) ***L. kashmirinus:*** A) Growing solitary on the dead stumps of *Cedrus* lying on the forest floor; B) Hymenophoral trama regularly descending hyphae (40x); C) Metulloid cheilocystidia (100x); D) Clamp connections (100x).

patches present in the centre, light orange ($5A_5$); latex absent; margin irregular, splitting at maturity, non striated cuticle half peeling; flesh white, unchanging, tough, up to 1.8 cm broad; odour mild. Lamellae decurrent, descending down up to 2cm forming a fine ridge down the stipe, close, broad (up to 0.9 cm), grayish orange ($5B_6$), unchanging; lamellulae unequal, not in series; normal; gill edges serrate. Spore print grayish yellow ($2B_6$). Stipe central, 10 cm long, up to 2 cm broad, almost equal in diameter, concolorous with the pileus, tapering downwards; scaly, scales applanate to patchy; solid, fleshy; exannulate.

Basidiospores 8-13 x 3.32- 4.15 µm (excluding apiculus), oblong to cylindrical; inamyloid; wall granular, thin, double; apiculate, apiculus up to 0.8 µm long, excentric. Basidia 19.92-25 x 4.15-6.64 µm, clavate, granular, bi-tetra sterigmate; sterigmata 3.32-4.98 µm long, pointed at the apices, granular. Pleurocystidia 41.5-55 x 2.49-4.15 µm, deeply seated, cylindrical with inflated tip, granular, thin walled. Cheilocystidia rarely metulloidal, 46-75 x 2.49-3.32 µm, fusoid, capitate to blunt tipped, granular, thin to thick walled, crowded. Gill edges sterile. Pileus cuticle hyphal;

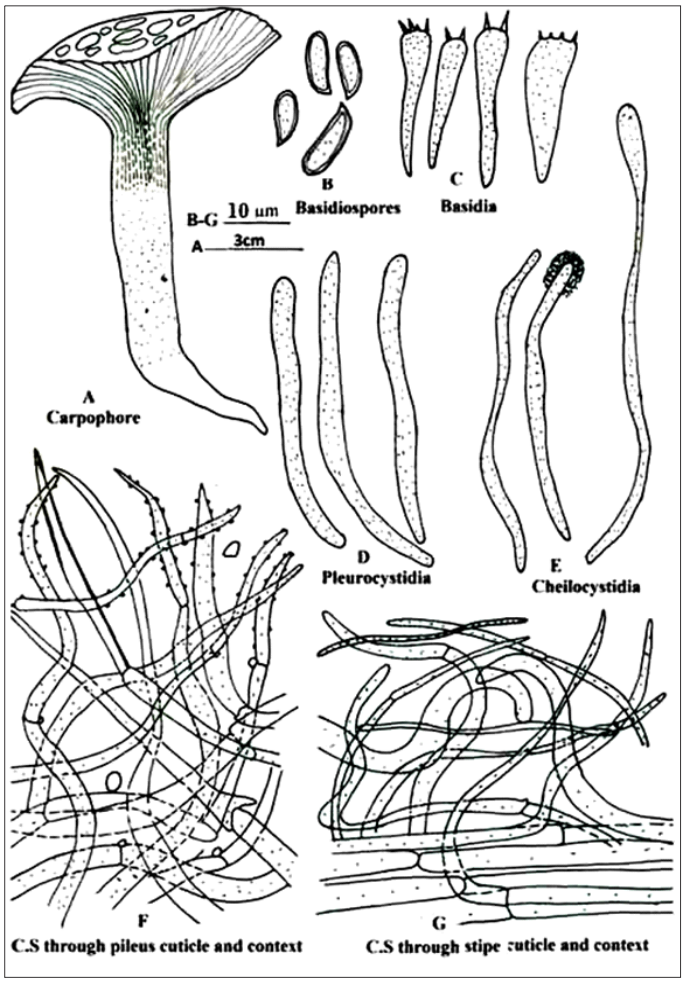

Fig. 2 **(A-G) *L. kashmirinus* sp. nov :** A) Carpophores. B) Basidiospores. C) Basidia D) Pleurocystidia. E) Cheilocystidia. F) C.S through pileus cuticle and context. G) C.S through stipe cuticle and context.

hyphae thin to thick walled, unbranched, septate, granular, clamped, intermixed with few granulated small sized cells; generative hyphae 1.6-5.8 µm broad; on the surface cuticular hyphae densely arranged with encrustations forming a regular turf, hyphal apices rounded to pointed; pileus context hyphal, some hyphae thick walled, unbranched, septate, granular, skeletal hyphae 2.49-9.96 µm broad, densely irregular, rarely clamped. Hymenophoral trama formed of regularly descending hyphae. Stipe cuticle made up of 2.4-7.5 µm broad, septate, granular, unbranched hyphae forming a dense irregular turf, few granulated small sized cells intermixed; stipe context made up of longitudinally tangled, thin walled, septate, granular, 5-10 µm broad hyphae. Clamp connections rarely present.

Chemical colour reaction: The surface of the stipe changes from white ($30A_1$) to reddish brown ($9E_7$) with Phenol.

Collection examined: Jammu and Kashmir, Damhal Hanji Pora, village Khull (2390m), growing solitary on the dead stumps of *Cedrus* lying on the forest floor towards the end of spring season, 3rd May 2013, Nazir Ahmad Malik, PUN 6595 (**Holotype**).

Remarks: The presently examined collection based upon some of its diagnostic features including metuloidal Cheilocystidia; thick walled unbranched skeletal hyphae, denticulate lamellae having hymenophoral trama formed of descending almost regularly arranged hyphae falls under the taxonomic limits of subgenus *Panus*, section *Pulverulenti* (Pegler 1983). In this section, the presently examined collection is quite close to *L. adharens* (Alb. & Schewein.) Fr. in possessing clamps, deccurent gills running 1-2 cm down the stipe and presence of some cheilocystidia with metulloidal apices. However, the Indian collection lacks resinous amber coloured secretion from the carpophores and also differ in spore size. The basidiospores of the presently examined species are much larger i.e. 8-13 x 3.32-4.15 µm while 6-10 x 2.5- 3.5 µm in *L. adharens*. In view of the above the present collection is described as new species.

Lentinus nigroosseus Pilát, *Ann. Mycol.* **34**: 122, 1936.

Fig. 3(A-C); Fig. 4 (A-H)

Sporophores up to 7.0 cm in height. Pileus 4.4-6.7 cm broad, funnel shaped, finally flattened depressed; umbo absent; margin irregular, inrolled; surface dry; cuticle fully peeling; flesh white, unchanging, up to 0.5 cm thick; taste and odour mild. Lamellae deeply decurrent, unequal, subdistant, moderately broad (0.3- 0.4 cm broad), whitish ($3A_1$) when young, light brown ($5D_5$) at maturity; unchanging on bruising; lamellulae present; gill edges denticulate; normal. Spore print creamish white ($1A_2$). Stipe lateral, up to 2.2 cm long, cylindrical, 0.8 cm broad near the apex, 0.6 cm in the middle and up to 0.9 cm towards the base; whitish ($5A_1$), scaly, scales similar as on cap; solid; fleshy; exannulate.

Basidiospores 5.9-7.16 × 3.58- 4.47 µm (excluding apiculus), elliptical, thin walled, smooth, inamyloid; apiculate, apiculus excentric, inconspicuous 0.7-0.9 µm long. Basidia 25-28 × 8-10 µm, clavate, granular, bi-tetra stergimate; sterigmata 3.5-4.5 µm long. Cheilocystidia 21-42 × 5-10 µm, claviform to

Fig. 3 **(A-C) *L. nigroosseus:*** A) Carpophore growing in caespitose clusters on the cut trunk of willow (*Salix* sp.); B) Lamellae deeply decurrent; C) Microphotograph showing inamyloid basidiospores (100x).

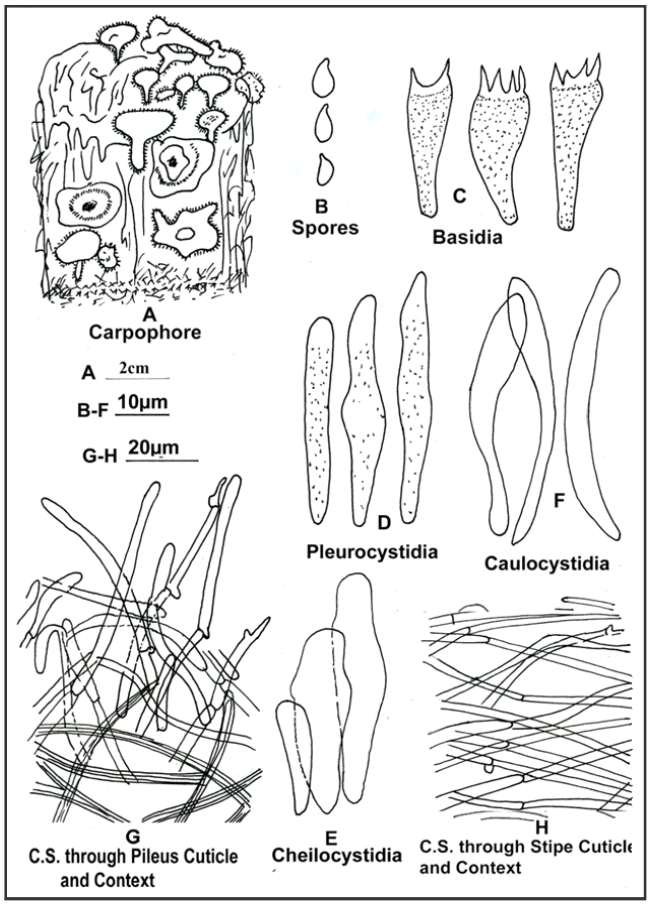

Fig. 4 **(A-H) *L. nigroosseus*:** A) Carpophores. B) Basidiospores. C) Basidia D) Pleurocystidia. E) Cheilocystidia. F) Caulocystidia. G) C.S through pileus cuticle and context. H) C.S through stipe cuticle and context.

fusoid, with blunt rounded tips, hyaline, abundant. Pleurocystidia 37-45 × 4- 7 µm, fusoid, ventricose, granular, less abundant. Gill edges sterile. Pileus cuticle hyphal, made up of loosely arranged, thin walled, highly branched 7-9 µm broad, clamped generative hyphae with capitate, rounded apices giving rise to a regular turf; pileus context hyphae thick walled, unbranched, 4-7µm broad. Hymenophoral trama irregular. Stipe cuticle hyphae made up of longitudinally tangled, 2-7 µm broad, septate, clamped hyphae bearing caulocystidia. Caulocystidia 44-49 × 5-7 µm, fusoid-ventricose with tubular blunt to capitate tips, abundant. Clamp connections present throughout.

Collection examined: Jammu and Kashmir, Baramulla, village Logriwalpora, Tragpora (1593m) growing in caespitose clusters on the cut trunk of willow on September 3th2012, Hilal Ahmad Rather, PUN 5189.

Remarks: The macroscopic and microscopic details match well with the description given for *L. nigroosseus* Pilát by Pegler (1983, 1986). It was also compared with another allied species *L. tigrinus* (Bull.) Fr. from which the presently examined collection is quite different. The clamps are more prominant and the spores are slightly narrower in the present collection which are typical of *L. nigroosseus* Pilát. Presently, this species has been described for the first time from India.

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KAVAKA 46: 48-54(2016)

Diversity and status of Arbuscular Mycorrhizal Fungi in two semi-arid grasslands of Gujarat, India

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ABSTRACT

Two grasslands of Gujarat (Rampura and Hingolgadh) belonging to semi-arid region were surveyed for the status of arbuscular mycorrhizal fungi (AMF) among selected grasses. A total 38 species of AMF belonging to 3 families and five genera were recorded. *Glomus* (25 species) is the most dominant genus followed by *Acaulospora* (8 species), *Scutellospora* (3 species) and *Gigaspora* (2 species) in the surveyed grasslands. A significant positive correlation was found between frequency of occurrence and relative abundance of AMF in both the grasslands. Although, lower value of Sorenson's coefficient revealed less similarity in AMF species composition in selected grasslands but Simposon's and Shannon Weiner diversity index studies carried out in both the grasslands exhibit less variation in both the sites. The spore count and per cent root colonization was significantly higher in Hingolgadh than in Rampura. The spore density (10-271 spores per 10 g soil) and degree of colonization (30.94 to 76.12%) was highly variable in the selected plants. Moreover, no significant correlation was found between root colonization and spore density.

Key words: Semi-arid grasslands, arbuscular mycorrhiza, diversity, grasses, spore density, root colonization

INTRODUCTION

A mycorrhiza is a symbiotic association between plant and fungus characterized by energy flow from plant to fungus and inorganic nutrients from fungus to plant. AM fungi are the mycorrhizal fungi from the fungal phylum *Glomeromycota* (Schüßler *et al.,* 2001) known to be associated with about 80% of the plant families in the world (Giovanneti and Sbrana, 1998). *Glomus*, the largest genus of the arbuscular mycorrhizal fungi (*Glomales*), is non-monophyletic (Schwarzott *et al.*, 2001). The diverse forms of AMF associated with majority of plants need conservation and efficient utilization for sustainable production of plant system (Giovanneti and Gianinnazi-Pearson, 1994). Moreover, diversity of plants and plant community structure in ecosystems are also influenced by AMF diversity (Bever *et al.*, 2001). AM fungi play an important role in mobilizing phosphorous (Baqual *et al.*, 2005), improving water relations (Sheng *et al.*, 2008) and drought tolerance (Devachandra *et al.*, 2008). Moreover, AM fungi also improve overall growth of plant, accumulation of secondary metabolites in roots (Chandarana and Jasrai, 2011), rooting and overall growth of micro-propagated plants (Thaker and Jasrai, 2002) including salt and heavy metal tolerance (Khare *et al.*, 2008).

Gujarat lies between 20^0 07' N - 24^0 43' N latitude and 68^0 10' E - 74^0 29' E longitude in the western coast of India. In Gujarat 90,520 (46.18 %) and 62, 180 (31.72 %) km^2 area belongs to semi-arid and arid regions, respectively where the scarcity of water is a serious problem. Grasslands play an important role in performing ecological functions including the cattle management and maintenance of biodiversity. They help in maintaining humus in the soils which paves the way for establishment of other forms (Tyagi *et al.*, 2010). The grassland in India represents 10% of the total grassland area of the world. In India, the grasslands cover 72 million ha area which is equivalent to about 4% of the total geographical area of India. In Gujarat, grasslands cover an area of about 8, 49,000 ha (Dabadghao and Shankamarayanan, 1973). Moreover, grasses are most important group of flowering plants as they provide food, shelter, medicine, and fodder for animals.

Various studies have been undertaken to assess the diversity of AM fungi in semi-arid and arid regions of India and all over the world (Zhao and Zhao, 2007; Panwar and Tarafdar, 2006 a;b; Tao and Zhiwei, 2005). Many attempts have also been made by various researchers to evaluate the diversity and status of AMF from different grasslands (Su and Guo, 2007; Lugo and Cabello, 2002; Bever *et al.*, 1996). In India, diversity and status of AMF have been examined for plants growing in the Western Ghats (Radhika and Rodrigues, 2010; Muthukumar and Udaiyan, 2000), coastal sand dunes of west - coast of India (Beena *et al.*, 2000), forest soils of Arunachal Pradesh, north east India (Singh *et al.,* 2003). AMF association among common grasses of Delhi has also been evaluated (Gupta and Mukerji, 1996).Yet, no reports have been found on the diversity of AMF from semi-arid grasslands of Gujarat State, India. It is in this context, that the present study was aimed to explore the diversity of AMF in two major grasslands of Gujarat (Rampura and Hingolgadh) and association of AMF with selected grasses from the semi-arid regions of the Gujarat.

MATERIALS AND METHODS

Study Site : The study was carried out in two major grasslands of Gujarat (Rampura and Hingolgadh). Rampura sanctuary is a protected grassland of savannah type located between 22^0 49' N and 074^0 10' E. The annual rainfall ranges between 411-1188 mm. The selected sites Rampura and Hingolgadh were savannah type of grassland and quite distinct from each other in species composition. In Rampura grassland, the dominant tree forms are *Butea monosperma* (Lam.) Taub., *Azadirachta indica* A. Juss., *Zizyphus nummularia* (Brum, F.) W. & A., *Soymida febrifuga* (Roxb.) A. Juss. The major grass species are *Dichanthium annulatum* (Forsk.) Stapf., *Themeda triandra* Forsk., *Themeda quadrivalvis* (L.) O. Ktze., *Heteropogon contortus* (L.) P. Beauv. ex R. & S., *Cymbopogon martini* (Roxb.) Wats., *Chrysopogon fulvus* (Spr.) Chiov., *Apluda mutica* L., *Sehima sulcatum* (Hack.) A. Camus. In Hingolgadh grassland, the dominant tree species are *Acacia leucophloea* (Roxb.) Willd., *Acacia nilotica* (L.) Del., *Balanites aegyptiaca* (L.) Del.,

Bauhinia purpurea L., *Prosopis cinerea* (L.) Druce. The major grass species are *Heteropogon contortus* (L.) P. Beauv. ex R. & S., *Aristida adscensionis* L., *Apluda mutica* L., *Melanocenchris jacquemontii* J. & S., *Sehima sulcatum* (Hack.) A. Camus., *Chrysopogon fulvus* (Spr.) Chiov., *Themeda triandra* Forsk., *Cymbopogon martini* (Roxb.) Wats., *Dichanthium annulatum* (Forsk.) Stapf. The grass is harvested after monsoon and stored for future use. Hingolgadh, a nature education sanctuary, is savannah type of grassland, located between 22° 10' N and 91° 21' E. The annual rainfall ranges between 375-425 mm. The grass is allowed for cattle grazing after sufficient growth in the growing season.

Sampling : The root and soil samples were randomly collected to a depth of 10-15 cm from rhizosphere of selected grasses in October, 2010. For each species, 3-5 samples were collected as per the availability of plants in selected grasslands. Minimum 30 m distance was kept between two samples. Soil samples were placed in polythene bags for transport. Soil samples were air-dried and stored at room temperature before use. Roots were immediately fixed in FAA solution (5 ml formalin, 5 ml acetic acid, and 90 ml of 70% alcohol) and diluted twice when used (1/2 FAA).

Soil Analysis : The samples were sieved through 2 mm sieve to remove larger soil particles and analyzed. The soil was suspended in water (1:2 soil and water) to measure the pH with pH meter (Chemiline, CL-110). Electrical conductivity (EC) was calculated using conductivity meter (Equiptronics, EQ-660A) at room temperature in 1:5 soil suspensions (Aery, 2010). Rapid titration method (Walkley and Black, 1934) was employed for determination of soil organic carbon (SOC). Bray and Kurtz (1945) and Toth and Prince (1949) methods were employed for determination of available phosphorus (AP) and available potassium (AK), respectively.

Isolation and Identification of Arbuscular mycorrhizal fungal spores : AMF spores were isolated from 10 g dried soil sample by wet sieving and decanting technique (Gerdemann and Nicholson, 1963) followed by centrifugation in 60% sucrose. Isolated spores were mounted in poly vinyl alcohol lacto glycerol (Koske and Tessier, 1983) and stained in mixture (1:1 v/v) of PVLG and Melzer's reagent (Brundrett *et al*., 1994) and observed under microscope (Lawrence and Mayo, LM-52-1804). AM fungi were identified based on spore morphology and wall characteristics (Schenck and Perez, 1990; Morton and Benny, 1990). Taxonomic positions of identified AM were confirmed by matching the descriptions based on the international culture collection (http://invam.caf.wvu.edu.), AMF phylogeny website (http://www.amfphylogeny.com) and related literature (http://www.agro.ar.szczecin.pl/~jblaszowski/).

Diversity Studies : The isolation frequency (IF) and relative abundance (RA) of AMF was determined following Zhao and Zhao (2007). Using the data obtained diversity indices of species structure was also assessed. Index of general diversity, Shannon diversity index and evenness (Shannon and Weaver, 1949) and Index of dominance Simpson's diversity index (Simpson, 1951) were calculated in two selected semi-arid grasslands.

Root Colonization : The root colonization (Sylvia, 1994) and total percentage colonization by the AMF symbiont were also determined (McGonigle *et al*., 1990).

Data Analysis : Differences in the five rhizospheric edaphic factors, mean spore count and mean per cent root colonization between two selected grasslands were analysed by employing independent sample t-test. Differences were considered significant when $P \leq 0.05$. Pearson correlation coefficient was carried out to assess the relationship between root colonization and spore density; frequency of occurrence and relative abundance. All statistical analysis was carried out by SPSS software (IBM SPSS Statastics 20).

RESULTS

Soil physiochemical properties : Physiochemical properties of soil from selected grassland have been depicted in **Table 1**. The soil pH of both the grasslands was slightly neutral and EC ranged from 0.26-0.27. AP ranged from 53.54-58.50 kg/ha and AK ranged from 289.46-296.40 kg/ha in selected grasslands. SOC varied from 1.15-1.28 and soil of both the grasslands has loamy sand textural class loamy sand. Among the soil parameters analysed by t-test, significant differences were noted in soil pH (P=0.031) of the selected grasslands. Although, no significant difference was found in EC, SOC, AP and AK between selected grasslands.

Table1. Soil analysis of selected grasslands Rampura and Hingolgadh (mean±SE)

Site	Parameters					
	pH	EC mhos/cm	OC (%)	AP kg/Ha	AK kg/Ha	Textural class
Rampura	7.05±0.09	0.26±0.01	1.28±0.08	53.54±2.78	289.46±13.09	Loamy sand
Hingolgadh	7.3±0.06	0.27±0.01	1.15±0.07	58.50±2.75	296.40±23.59	Loamy sand

Diversity of AMF : Two grasslands of Gujarat (Rampura and Hingoldagh) were found to be highly diverse in AMF diversity. A total of 38 morpho - species of AMF belonging to 3 families and 4 genera were recorded (**Table 2**). Members of the genus *Glomus* (25 species) were most dominant followed by *Acaulospora* (8 species), *Scutellospora* (3 species) and *Gigaspora* (2 species). Both sites were quite different in AMF species composition. In Rampura grassland 26 species of AMF belonging to 4 genera were recorded viz. *Glomus* (16 species), *Acaulospora* (6 species), *Gigaspora* (2 species) and *Scutellospora* (2 species). In comparison, Hingolgadh grassland exhibited 23 AMF species including *Glomus* (16 species), *Acaulospora* (5 species), *Gigaspora* (1 species) and *Scutellospora* (1 species).

Occurrence of AMF species in selected grasslands and their IF and RA are given in **Table 2**. The IF in Rampura grassland ranged from 4.17-37.5 per cent. AMF species with highest IF in Rampura grassland was *Glomus etunicatum* (37.5%) followed by *Acaulospora scrobiculata* (25%), *Glomus versiforme* (20.83%) and *Scutellospora biornata* (20.83%) and fifteen species showed 4.17 percentage of frequency of occurrence. In Hingolgadh grassland, IF ranged from 4.76 to 66.7 per cent. *Glomus constrictum* was the most dominant species with 66.7 % IF followed by *G. rubiforme* (42.86 %), *G. aggregatum* (38.1 %), *G. intraradices* (38.1 %) and four species showed 4.76 percentage of frequency of occurrence. However, 11 AMF species were common in both the selected grasslands.

Table 2. Isolation frequency (IF) and relative abundance (RA) of arbuscular mycorrhizal fungi

Sr. No.	Arbuscular mycorrhizal fungal species	Rampura		Hingolgadh	
		IF %	RA %	IF %	RA %
1	*Acaulospora bireticulata* F.M. Rothwell & Trappe	12.5	2.14	-	-
2	*Acaulospora delicata* C. Walker, C.M. Pfeiff. & Bloss	8.33	1.41	9.52	0.65
3	*Acaulospora foveata* Trappe & Janos,	-	-	9.52	0.88
4	*Acaulospora lacunosa* J.B. Morton	-	-	9.52	2.25
5	*Acaulospora mellea* Spain & N.C. Schenck	4.17	1.75	-	-
6	*Acaulospora rehmii* Sieverd. & S. Toro,	4.17	0.45	14.29	1.33
7	*Acaulospora scrobiculata* Trappe	25	9.58	9.52	1.16
8	*Acaulospora undulata* Sieverd.	4.17	0.39	-	-
9	*Gigaspora decipiens* I.R. Hall & L.K. Abbott	4.17	0.73	4.76	0.44
10	*Gigaspora margarita* W.N. Becker & I.R. Hall	4.17	0.23	-	-
11	*Glomus aggregatum* N.C. Schenck & G.S. Sm.	4.17	0.34	38.10	17.32
12	*Glomus ambisporum* G.S. Sm. & N.C. Schenck	16.67	7.49	4.76	3.81
13	*Glomus arenarium* Blaszk., Tadych & Madej	4.17	1.63	-	-
14	*Glomus australe* (Berk.) S.M. Berch, in Berch & Fortin	12.5	2.14	-	-
15	*Glomus clarum* T.H. Nicolson & N.C. Schenck	-	-	9.52	0.82
16	*Glomus clavisporum* (Trappe) R.T. Almeida & N.C. Schenck	-	-	9.52	0.85
17	*Glomus constrictum* Trappe	8.33	2.37	66.7	7.66
18	*Glomus coronatum* Giovann.	4.17	0.34	-	-
19	*Glomus epigaeum* B.A. Daniels & Trappe	4.17	0.06	-	-
20	*Glomus etunicatum* W.N. Becker & Gerd.	37.5	23.49	9.52	10.11
21	*Glomus fasciculatum* (Thaxt.) Gerd. & Trappe	4.17	6.25	28.57	16.68
22	*Glomus geosporum* (T.H. Nicolson & Gerd.) C. Walker	-	-	4.76	0.41
23	*Glomus globiferum* Koske & C. Walker	-	-	9.52	1.97
24	*Glomus heterosporum* G.S. Sm. & N.C. Schenck	16.67	13.80	14.29	5.34
25	*Glomus intraradices* N.C. Schenck & G.S. Sm.	-	-	38.10	19.78
26	*Glomus leptotichum* N.C. Schenck & G.S. Sm.,	8.33	0.56	-	-
27	*Glomus macrocarpum* Tul. & C. Tul.	4.17	3.04	-	-
28	*Glomus maculosum* D.D. Mill & C. Walker	4.17	2.59	-	-
29	*Glomus manihotis* R.H. Howeler, Sieverd. & N.C. Schenck	4.17	0.68	-	-
30	*Glomus multicaule* Gerd. & B.K. Bakshi	-	-	4.76	0.20
31	*Glomus pansihalos* S.M. Berch & Koske	4.17	0.45	23.81	2.93
32	*Glomus reticulatum* Bhattacharjee & Mukerji	-	-	9.52	0.71
33	*Glomus rubiforme* (Gerd. & Trappe) R.T. Almeida & N.C. Schenck	-	-	42.86	2.55
34	*Glomus sinuosum*(Gerd. & B.K. Balshi) R.T. Almeida & N.C. Schenck	-	-	9.52	0.85
35	*Glomus versiforme* (P. Karst.) S.M. Berch, in Berch & Fortin	20.83	14.14	-	-
36	*Scutellospora biornata* Spain, Sieverd. & S. Toro	20.83	3.66	-	-
37	*Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders	4.17	0.28	-	-
38	*Scutellospora Fulgida* Koske & C. Walker	-	-	9.52	1.29

Further, in Rampura grassland the RA of AMF ranged between 0.06-23.49 per cent. Species with highest percentage of RA was *Glomus etunicatum* (23.49 %) followed by *Glomus versiforme* (14.14 %), *Glomus heterosporum* (13.8 %) and *Glomus epigaeum* which was least abundant species with 0.06 RA. The RA of isolated AMF ranged between 0.2 to 27.63 % in Hingolgadh grassland. In Hingolgadh grassland, most abundant species was *Glomus intraradices* with 19.78 % RA followed by *Glomus aggregatum* (17.32 %), *Glomus fasciculatum* (16.68 %) and least abundant species was *Glomus multicaule* (0.2%). The present study revealed that there is a significant positive correlation between IF and RA in Rampura ($r = 0.838$; $P< 0.01$) and Hingolgadh ($r = 0.722$; $P< 0.01$) grasslands. The Shannon - Weiner Index, Simpson's Index and species evenness is slightly higher in Rampura grassland as compared to Hingolgadh grassland (**Fig. 1**). Moreover, no host specificity was observed in AMF.

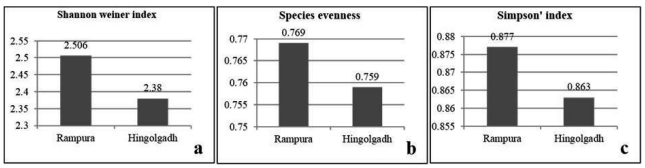

Fig. 1 Diversity indices of AM fungi among the study sites

Spore density and per cent root colonization : Details of spore count and root colonization from selected grasses are given in **Table 3**. By employing independent sample t-test the mean spore count ($P= 0.000$) and per cent root colonization ($P= 0.017$) in Hingolgadh and Rampura grasslands differ significantly. The mean spore count in Hingolgadh grassland was 149 spores which was significantly higher than Rampura having mean spore count of 70 spores per 10 g soil. Mean per cent colonization in Hingolgadh (69.28±3.01) was also

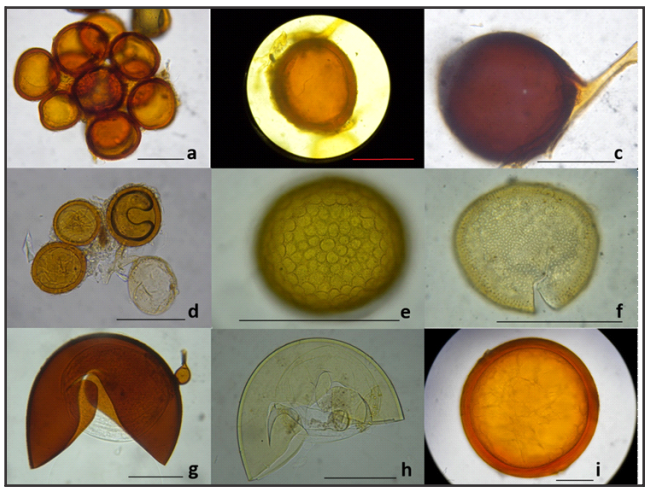

Fig. 2 AM fungal species a. Sporocarp of *Glomus heterosporum*, b. Spore of *Glomus multicaule* showing hyphal attachment at opposite side, c. *Spore of Glomus constrictum*, d. Sporocarp of *Glomus fasciculatum*, e. Spore of *Acaulospora undulata* showing large pits f. *Spore of Acaulospora scrobiculata* showing pits, g. Spore of *Scutellospora biornata* showing bulbous suspensor and inner flexible wall, h. Spore of *Scutellospora calospora* showing bulbous suspensor, germination shield and inner flexible wall, i. spore of *Gigaspora decipiens*. Bar = 100 µm

significantly higher than Rampura grassland (57.28±3.82). Ten grass species were selected from the study sites. Result of mean spore density and per cent root colonization **(Table 3)** of selected grasses revealed that plants belonging to family *Poaceae* were potentially colonized by AMF.

Table 3. Spore density and root colonization in selected 10 grasses (mean±SE)

Species	Spore density/10 g soil		Root colonization (%)	
	Rampura	Hingolgadh	Rampura	Hingolgadh
Bothriochloa glabra (Roxb.) A. Camus	-	*132*±25	-	60.04±5.45
Bothrichloa pertusa (L.) A. Camus	26±7	58±26	75.75±7.62	76.49±3.85
Chrysopogon fulvus (Spr.) Chiov.	54±12	258±35	67.49±15.7	77.93±4.14
Dichanthium annulatum (Forsk.) Stapf	72±33	160±49	56.36±6.11	57.39±7.09
Heteropogon contortus L.	100±30	141±21	41.59±2.82	50.13±2.52
Ischaemum rugosum salisb.	10±3	-	30.94±3.21	-
Pennisetum purpureum Schum. & Thonn.	*111*±8	-	66.52±7.83	-
Sehima nervosum (Rottl.) stapf	-	171±26	-	85.97±4.45
Themeda cymbaria (Roxb.) Hack.	122±41	118±33	58.05±25.81	72.01±5.71
Themeda triandra Forsk.	79±12	-	66.87±7.96	-

The spore density varied between 10-258 spores per 10 gm of soil in the rhizosphere of selected grasses. In Rampura grassland, AMF spore density varied between 10-118 spores per 10 g soil. The maximum number of spores were noted in *Themeda cymbaria* (122) followed by *Pennisetum purpureum* (111), *Heteropogon contortus* (100) and least number of spores were found in *Ischaemum rugosum* (10). In Hingolgadh grassland, AMF spore density varied from 58 to 258 per 10 g soil. The highest number of spores were found in *Chrysopogon fulvus* (258) followed by *Sehima nervosum* (171), *Dichanthium annulatumi* (160) and least number of spores were found in *Bothriochloa pertusa* (58).

The per cent root colonization of AMF varied from 30.94 to 85.97 in selected grasses. High degree of variation was noted in both the selected semi-arid grasslands. In Rampura, the per cent root colonization varied between 30.94-75.75 per cent. Maximum colonization was found in *Bothrichloa pertusa*

(75.75%) followed by *Chrysopogon fulvus* (67.49%), *Themeda triandra* (66.87%), *Pennisetum purpureum* (66.52%) and lowest colonization was found in *Ischaemum rugosum* (30.94%). In Hingolgadh, the per cent root colonization was varying from 50.13 to 85.97 per cent. Maximum colonization was found in *Sehima nervosum* (85.97%) followed by *Chrysopogon fulvus* (77.93%), *Bothriochloa pertusa* (76.49%) and lowest was found in *Heteropogon contortus* (50.13%). No significant correlation was noted between per cent root colonization and spore density.

DISCUSSION

In Rampura, the grass was cut after monsoon and stored for the future use, where as in Hingolgadh the grass was not harvested. After the sufficient growth period sanctuary is left open for the villagers to cut grass or graze their cattle. In this context, we can say that level of disturbance in Rampura grassland is more than Hingolgadh grassland because, in Rampura grasses are harvested before their sufficient growth, whereas in Hingolgadh grasses are allowed to attain maturity and then are grazed or cut for the cattle.

In present study, dominance of genus *Glomus* was observed in both the selected sites. Pre-dominance of genus *Glomus* was also recorded in non-grazed, restored and over-grazed grassland in Inner Mongolia steppe (Su and Guo, 2007). The pre dominance of genus *Glomus* was also recorded from various semi-arid and arid regions of India (Panwar and Tarafdar, 2006a;b) and from all over the world (Zhao and Zhao, 2007; Tao and Zhiwei, 2005). Moreover, dominance of genus *Glomus* was also recorded in India by various workers in different ecosystems (Beena *et al.*, 2000; Singh *et al.*, 2003; Muthukumar and Udaiyan, 2000). The possible reason for this dominance of genus *Glomus* is the ability of its spores to germinate in varied temperature and pH (Wang *et al.*, 1997) and to attain early maturity because spores of *Glomus* species possess small size as compared to members belonging to *Gigasporaceae* family during the short period of moisture in semi-arid areas (Tao *et al.*, 2004). Significant positive correlation between IF and RA observed in present study suggests that the AMF possesses strong capacity of sporulation and had wide distribution. Similarly, Zhao and Zhao (2007) as well as Yang *et al.* (2011) also noted significant positive correlation between IF and RA.

Simpson's and Shannon Weiner diversity index studies carried out in both the grasslands exhibited less variation in both the sites. The higher index of general diversity in Rampura grassland (2.506) than Hingolgadh grassland (2.38) suggests high diversity of AM fungi in Rampura grassland. Index of dominance in Rampura grassland (0.887) is also high which revealed dominance of few species in Rampura grassland where slightly lower value (0.863) in Hingolgadh grassland indicates shared dominance of AM fungi in Hingolgadh grassland. The evenness was also higher in Rampura grassland (0.769) than in Hingolgadh grassland (0.759). However, level of disturbance is quite high in Rampura grassland than in Hingolgadh grassland but no considerable differences were found in diversity index. This could be due to the fact that the impact of disturbance seems to be higher on spore production and then on root colonization and species richness (Beena *et al.*, 2000) but disturbance did not affect parameters of diversity index like Shannon-weaver, evenness and Simpson's index (Lugo and Cabello, 2002). Although, no significant difference was found in diversity index but the lower value of Sorenson's coefficient of similarity (0.41) between selected grasslands revealed less similarity between AMF species composition. This could be due to the reason that plant community existing in an ecosystem, environmental factors (Brundrett, 1991) and soil physico-chemical properties (Panwar and Tarafdar, 2006b) are responsible for AM fungal species composition.

In the present study, AMF species showed no host specificity. This is in agreement with the observations of Radhika and Rodrigues (2010) and Muthukumar and Udaiyan (2000) who found that AMF shows little or no host specificity. This could be due the fact that AMF community is highly dependent on the environmental factors and vegetation (Brundrett, 1991). During the survey of AMF from Rampura and Hingolgadh grasslands in all 26 and 23 species were recorded, respectively. This observation is also in conformity with Radhika and Rodrigues (2010) who isolated 27 and 26 different species of AMF from South and North Goa. Muthukumar and Udaiyan (2000) also reported 6-22 different species of AMF per site in the Western Ghats Region, however, in comparison Singh *et al.* (2003) reported 42 and 34 different AMF species in natural forest and jhum fallow soils from Arunachal Pradesh, North eastern India, which is not in consonance with the presently obtained results.

The possible reason for the high spore count and per cent root colonization in Hingolgadh grassland over Rampura grassland was the level of disturbance which is quite higher in Rampura grassland in comparison to Hingolgadh grassland. Beena *et al.* (2000) also reported the drastic reduction in spore number and per cent root colonization in severely damaged dunes in comparison to moderately disturbed dunes. Su and Guo (2007) also found less spore density in over-grazed grassland than in non-grazed grassland in the Inner Mongolia steppe. All the selected grass species associated with AMF suggest that they are dependent on AM fungal association. Sathiyadesh *et al.* (2010) also found that the AM fungal association is quite common in South Indian grasses and 88 % species of selected grasses were colonized by AM fungi. However, the level of association varied in different species. Earlier, Gupta and Mukerji (1996) also demonstrated that common grasses of Delhi were also potentially colonized by AM fungi.

It was noted that the degree of spore count per 10 gm soil is highly variable in the rhizosphere of selected grasses. Similarly, Radhika and Rodrigues (2010) also found large variation in AMF spore count in rhizosphere of medicinal plants of Western Ghats in Goa region. This can be attributed to several reasons like different sporulation ability of different AMF species (Bever *et al.*, 1996), interspecific competition between AMF (Brundrett and Kendrick, 1990), competition between AMF and environmental factors which also influence spore production in natural communities (Koske and Gemma, 1988). Spore populations are also reported to be

influenced by soil physicochemical properties in the natural ecosystems (Panwar and Tarafdar, 2006b). Lugo and Cabello (2002) found that spore production is strongly influenced by season. Another possible reason for the high spore number in Hingolgadh grassland in comparison to Rampura grassland was the pH of the soil which was significantly higher in Hingolgadh than in Rampura. Khanam *et al.* (2006) found a positive correlation between pH and spore density. The probability of mycorrhizal association have also been reported to increase with increase of soil pH by Brady (1990).

Earlier, Radhika and Rodrigues (2010) and Muthukumar and Udaiyan (2000) also reported that different species have varied level of AMF colonization. Moreover, during the present survey we found that same species when collected from different site had different level of colonization. Miller (2000) also found that single species may also have different level of colonization when collected from different sites in the same season which shows that soil chemical composition can also strongly affect the AMF colonization. Soil fertility and pH are also reported as important factors which affect the efficiency of interaction between AM fungi and host plants for the acquisition of nutrients by number of investigators.

In the present study, non-significant correlation was found between per cent root colonization and mean spore density. Similar results were observed by various workers (Radhika and Rodrigues (2010) and Panwar and Tarafdar (2006b). The possible reason for no correlation between spore density and root colonization is spore number which may be reflecting the poor colonization potential of AMF in soil (Hayman and Stovold, 1979) and they are not always correlated with the degree of mycorrhizal colonization. Non-significant correlation could be because the AM fungal sporulation and root colonization is highly dependent on host range and environmental factors (Tommerup, 1983; Koske and Gemma, 1988).

CONCLUSIONS

This study is the first report on identification of AMF community in semi-arid grasslands of Gujarat, India. *Glomus* was found to be the most dominant genus followed by *Acaulospora* in selected grasslands. Moreover, no host specificity was found in AMF which indicated that the community and association of AMF was highly dependent on environmental factor and soil physico-chemical properties of soil. Disturbance was also a major factor which affects the spore number and per cent root colonization in selected grasslands. The grasses selected for the study were potentially colonized by AMF which suggests dependency of selected grasses on AMF associations in semi-arid region.

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OBITUARY

Professor C.V. Subramanian
(11-08-1924 to 05-02-2016)

Professor Chirayathumadom Venkatachalaier Subramanian (affectionately referred to as CVS), former Director, CAS in Botany, University of Madras; internationally renowned and most distinguished mycologist; and past President of Mycological Society of India passed away on 5th February 2016, at the age of 92. He was born on 11th August, 1924, at Ernakulum in Kerala state. After his early education at Ernakulum, he joined Presidency College, Madras, for B.Sc. (Hons.) degree in Botany which he received with a first class and first rank, and was awarded Pulney Andi gold medal by the University of Madras. He obtained his M.A. degree by research from the same college (1941-1944). After post-graduation, he joined the Madras University Botany Laboratory to work for his Ph. D. degree under the supervision of Prof. T. S. Sadasivan. He was awarded the Ph.D. degree in 1948 for his thesis on "Soil Conditions and Wilt Diseases in Plants with Special Reference to *Fusarium vasinfectum* Atk. on Cotton." Subsequently, he received the D. Sc. degree from Madras University (1957) for his published work on "Floristic and Taxonomic Studies on Fungi imperfecti." He started his academic career in 1951 as a Senior Lecturer in University Botany Laboratory and promoted as Reader in 1953. Later, he moved to IARI (New Delhi) to take up the newly created position of Professor of Plant Pathology (1958-1960). Later he served as Professor and Head of the Department of Botany, established by him, in the University of Rajasthan (1960-1964). Subsequently, he moved to the University of Madras as Professor of Botany in 1964 and later became the Director of Centre for Advanced Studies in Botany in 1973, the post which he held until retirement in 1985. Prof. Subramanian's early researches were in soil mycology. During 1948-1950, he undertook an extensive study of *Fusaria* in south Indian soils under ICI research fellowship, and published a series of papers. His first visit to Commonwealth Mycological Institute, Kew, UK (1950-1951), gave him an opportunity to examine hundreds of type materials of conidial fungi located in the herbarium and developed interest in fungal floristics and taxonomy of Hyphomycetes. Prof. CVS made extensive collections of fungi from forests of Eastern and Western Ghats, India, and described numerous new and interesting species of fungi published in a series of papers eventually culminating in writing a monograph entitled "*Hyphomycetes*" published by ICAR (1971). For the first time he introduced Sanskrit names for some of the new taxa which was considered as a novel and bold step. Prof. CVS developed an active school of mycology at CAS in Botany and 24 students received Ph.D. degrees under his supervision working on different aspects of mycology. These include studies on floristics and ecology of fungi occurring in diverse habitats such as rhizosphere, decomposing plant litter, marine and mangroves, coprophilous fungi; effluents discharged from paper mills, fertilizer factories, oil refineries and cooling towers; developmental morphology and anamorph and teleomorphs of a range of taxa in the *Euroteales* and Hypocrealean fungi; taxonomic studies of *Coronophorales* (a less-known group of Ascomycota). Prof. CVS proposed a hierarchical system of classification of Hyphomycetes in 1962 on the basis of "conidium ontogeny" and "spore types" which he revised in 1983. Post-retirement, Prof. CVS travelled extensively in south-east Asia and collected specimens of fungi from Singapore, Malaysia, Thailand and Western Australia and described many new genera and species. Prof. Subramanian's research contributions have been well recognized both nationally and internationally and he received many honours and awards including Shanthi Swarup Bhatnagar Award for Biological Sciences in 1965, Birbal Sahni Medal of the Indian Botanical Society in 1972, Rafi Ahmed Kidwai Prize of the ICAR for 1972-1973, E. K. Janaki Ammal National Award for Taxonomy by Government of India in 2000. He served as a member of University Grants Commission. He has been associated with several international bodies. He served as both Vice President (1971-1977) and President of International Mycological Association (1977-1983), a rare distinction conferred on an Indian mycologist. He was also a member of the Executive Committee of the International Union of Biological Sciences (1979-1982). He was a corresponding member of the Belgian Royal Academy of Foreign Sciences (1978) and an honorary member of the Mycological Society of America and the British Mycological Society. He was an elected fellow of Indian Academy of Sciences, Bangalore, Indian National Science Academy, Delhi, and National Academy of Agricultural Sciences, New Delhi. He was a recipient of the prestigious Jawaharlal Nehru Fellowship during the tenure of which he wrote a book titled "*HYPHOMYCETES: Taxonomy and Biology*" published by the Academic Press, London, in 1983. He was instrumental in the formation of Mycological Society of India (MSI) in 1973, in the august presence of a galaxy of mycologists from India and abroad, during the International Symposium on "Taxonomy of Fungi" organized by him at the CAS in Botany, University of Madras. Prof. CVS founded the journal KAVAKA, the transactions of the MSI and also edited the journal since its inception till 1998. He was conferred the "Lifetime Achievement Award" by the MSI. Prof. Subramanian is survived by his wife and two sons. Prof. CVS is admired for his soft-spoken and helping nature. He will always be remembered for his significant contributions to taxonomic mycology. The discipline of mycology in India has lost a very distinguished teacher and researcher in the death of Prof. C. V. Subramanian. May God grant eternal peace to the departed soul and enough strength to his family members, close associates and friends to bear this irreparable loss.

Professor B. P. R. Vittal (Retd.)
CAS in Botany, University of Madras,
Guindy Campus, Chennai, Tamil Nadu

INSTRUCTIONS TO THE AUTHORS

The word Kavaka represents the Sanskrit word for fungus. Kavaka is the official journal of the Mycological Society of India. Kavaka is an international journal and publishes peer-reviewed, original articles and reviews on all aspects of mycology.

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