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Three species of *Termitomyces* (*Lyophyllaceae*, *Agaricales*) from Punjab, India

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ABSTRACT

Three *Termitomyces* samples were collected in fungal surveys during 2008–2012 at different localities and sub-localities of district Ludhiana, Punjab. Detailed morphological examination showed the one of these collections represent a new species and remaining two species are new to district Ludhiana, Punjab. The macroscopic and microscopic characters with camera lucida established the new species which is described and illustrated in this paper. The new species, *Termitomyces punjabensis* sp. nov. has been taxonomically described and illustrated for the first time from India and reported as new to science.

Key words: India, *Termitomyces, Lyophyllaceae*, taxonomy

The Genus *Termitomyces* belongs to family Lyophyllaceae Jülich, characterized by presence of siderophilous granulation in mature carpophores; spore print light pink; spores smooth, inamyloid, cyanophilous; hyphae without clamp connections; hymenophoral trama either regular or bilateral; often termitophilous (a symbiotic relationship with the termites only in *Termitomyces*); otherwise on dead plant material, or on humid soil. This family includes 8 genera spread over 157 species (Kirk *et al*., 2008). During the present work only one genus belonging to this family has been studied. The world over 30 species of genus *Termitomyces* Heim are recorded so far (Kirk *et al.,* 2008) of which 29 species are known from India (Bilgrami *et al.,* 1991, Atri *et al.,* 2005a, Karun and Sridhar, 2013; Aryal and Budhathoki, 2014). Aryal *et al*. (2016) collected *Termitomyces palpensis* Aryal and *T. arghakhanchensis* Aryal, within an altitudinal range of 800-1500 m in subtropical deciduous forest in Nepal. Vesala *et al*. (2017) and Verma *et al*. (2019) studied about ethnomycology and diversity of *Termitomyces*, respectively. Usman and Khalid (2020) detailed another type of *Termitomyces* for example *T. acriumbonatus* from Pakistan. Two new species of *Termitomyces* for example *T. floccosus* and *T. upsilocystidiatus* reported from China and Thailand by Tang *et al*. (2020). Shaleh *et al*. (2021) Identified fungus-growing termites and mutualistic *Termitomyces* from two provinces in Thailand. In the present work three collections were found to be belonging to this genus, out of these *T. clypeatus* and *T. badius* are earlier reported from Punjab (Atri *et al*., 2005a). *T. punjabensis* sp. nov. has been reported as a new species to science.

MATERIALS AND METHODS

Fungal forays were made during monsoon season to various localities and sub- localities of Punjab. The

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taxonomic details of the above examined collections with respect to their gross morphology and internal structures were studied in accordance with the methodology given by Atri *et al*. (2005b). The terminology used for describing the color tone of carpophores parts and spore print is after Kornerup and Wanscher (1978). The identified specimens have been deposited in the Herbarium, Department of Botany, Punjabi University, Patiala.

Area of Investigation – Punjab state is located between 29º32' to 32º32'N latitude and 73º55' to 76º70'E longitude, in the north-western part of India and it occupies an area of 50,362 sq. km., which constitutes 1.57% of the total area of country. Punjab has four main seasons: summer, monsoon, winter and autumn season. Rainfall in Punjab ranges between 250 mm to 1000 mm. Maximum annual rainfall is experienced during the advent of southwest monsoon in the region. About 80% of the total rainfall is fixed during July, August and September and the rest occurs during the winter months. The study area has 7-8 months of mean monthly temperature of >20ÚC. It is primarily an agrarian state having diverse flora and fauna.

Preservation of Dried mushroom specimens: All descriptions are based on the specimens collected from different localities in Punjab explored during the years 2008 to 2012. The macroscopic features of the collected materials including pileus and stipe dimensions, surface features, colour, lamellar spacing, and attachment, habit and dung substrate, etc. were observed and noted from the fresh material and documented on the field key to mushroom collector (Atri and Saini 2000, Atri *et al*. 2005). The terminology of Kornerup and Wanscher (1978) was used for recording the color of various parts of the basidiocarp, and spore print, etc. Then the collected mushrooms were hot air dried in a wooden drier especially designed for this purpose (Atri *et al*. 2005). The mushrooms were finally packed in the moisture proof cellophane paper packets along with the collection number. To avoid insect infestations small packs of crystals of 1-4 Para-dichlorobenzene were placed in these packets (Smith 1949, Atri and Saini 2000). The hand cut sections of various parts of carpophore such as pileus, gills, stipe and basidiospores details were studied under the microscope after reviving a part of the dried specimens in 10% KOH solution and staining the sections in 1% Cotton blue or 2% Congo red. The Line drawings of microscopic details were drawn with the aid of Camera Lucida under oil lens. The magnification of each drawing has been mentioned at appropriate place on the plate of individual taxon. All the examined collections have been deposited in the Herbarium of Botany Department, Punjabi University, Patiala, Punjab, India under PUN. The correct names, basionym and synonyms of the investigated taxa are as per the latest version of Dictionary of Fungi by Kirk *et al*. (2008) and the information available on MycoBank (www.mycobank.org).

RESULTS AND DISCUSSIONS

Taxonomic observations

Termitomyces punjabensis Baljit Kaur, Munruchi Kaur and Harwinder Kaur sp. nov.

MycoBank # 840276

Fig. 1 (A–F), 2 (A–C).

Etymology: The species name is based on the name of collection site.

Fructification 38-45 cm in height including pseudorrhiza. Pileus 5.4-9.5 cm broad, convex when young, applanate at maturity; surface white ($6A_1$); scaly, scales present in the form of squamules covering the center, scattered towards the margin; margin regular, splitting at maturity; cuticle fully peeling; flesh white, unchanging, up to 0.6 cm thick; pileal veil present, patchy; taste fruity; odour fragrant. Lamellae free, unequal, crowded, up to 0.8 cm broad, orangish white ($6A_2$), unchanging, edges serrate, normal. Spore deposit greyish orange ($5B_4$). Stipe central, 3.9-7.5 cm long, 0.6-1.3 cm broad, tapering downward so as

to form pseudorrhiza, concolorous with pileus, unchanging, solid, smooth above the annulus, scaly below; annulate, annulus double, ring type, white, persistent; Pseudorrhiza 0.3-0.5 cm broad, tapering downwards, with reddish brown, fibrillose scales, throughout the length.

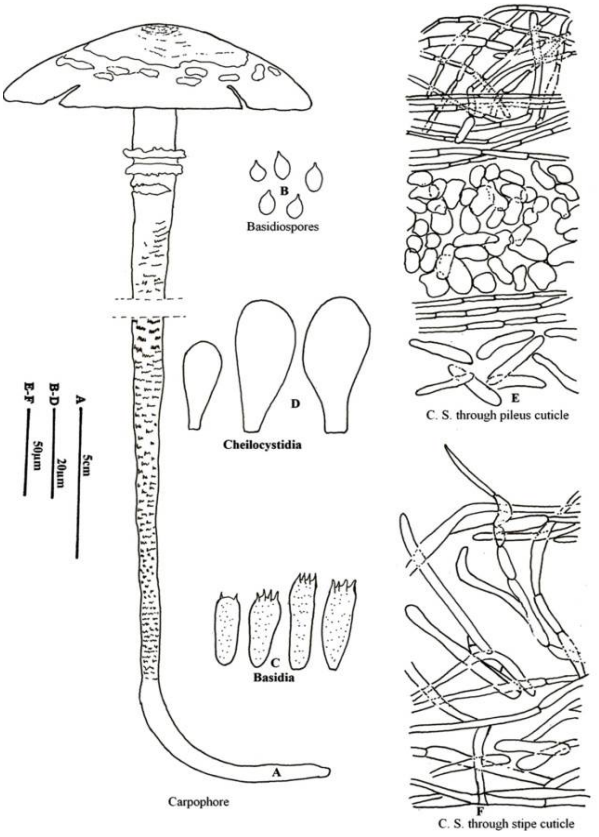

Fig. 1–A–F. *Termitomyces punjabensis* sp. nov. A Carpophore. B Basidiospores. C Basidia. D Cheilocystidia. E C.S. through pileus cuticle & context F C.S. through stipe cuticle

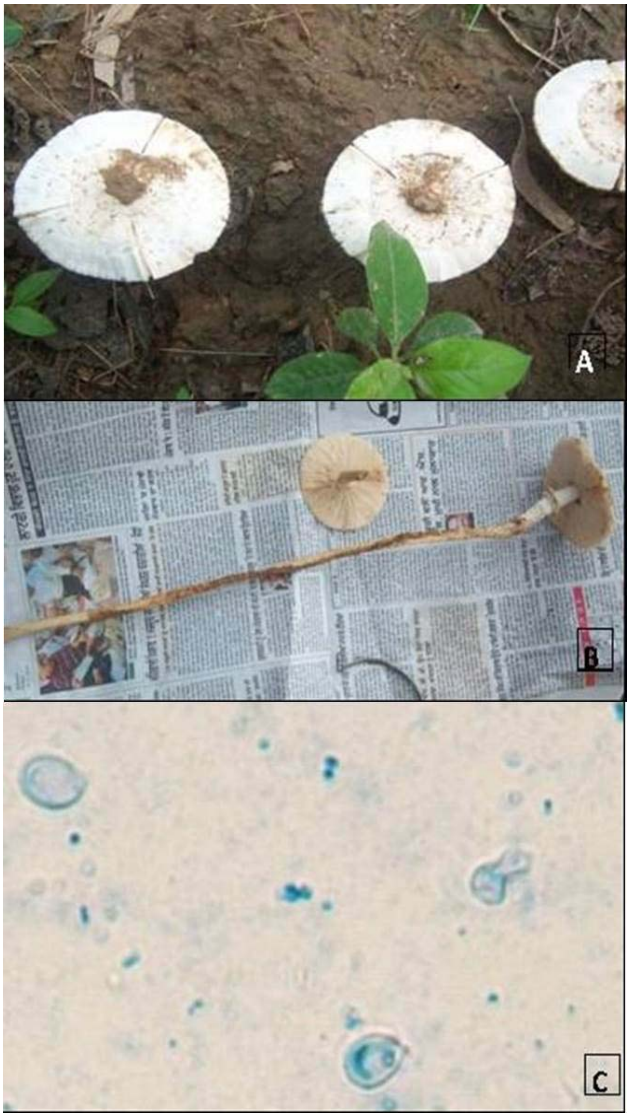

Fig. 2–A–C. *Termitomyces punjabensis* sp. nov. A Carpophores in their natural habitat. B Carpophore with long pseudorrhiza. C Basidiospores

Table 1. Comparison of Characteristic features of present collection *Termitomyces punjabensis* sp. nov. and *Termitomyces heimii*

Characteristic feature	***Termitomyces punjabensis* sp. nov.**	***Termitomyces heimii***
Color of Carpophore	White	Creamy (Srivastava *et al*., 2011)
Prominent large patch in thecentre of Cap	Yes	No
Pileus margin	Regular, splitting at maturity	Incurved smooth (Srivastava *et al*., 2011)
Stipe length	3.9-7.5 cm	5 cm (Srivastava *et al*., 2011)
Stipe and pseudorrhiza	Scaly	Smooth (Natarajan, 1975)
Color of gills	Skin	White (Natarajan, 1975)
Spore print	Greyish orange ($5B_4$)	Purplish (Srivastava *et al*., 2011)
Spore size range	3.4-5(6) x 3.4-4.2 µm	6-8.2 x 4.5-5.3 µm (Natarajan, 1975)
Pleurocystidia	Absent	Present (Natarajan, 1975)

Basidiospores 3.4-5(6) x 3.4-4.3 µm (excluding apiculus), (Q=1.1) broadly ellipsoid, thin walled, inamyloid; apiculate, apiculus up to 0.8 µm long. Basidia 15.3-20.4 x 5.8-6.8 (8.5) µm clavate, granular throughout, 2-4, spored; sterigmata 1.7-3.4 µm long. Pleurocystidia absent. Cheilocystidia 20.4-30.6 x 8.5-13.6 µm, clavate to pyriform. Gill edges sterile.

Carpophore context homoiomerous. Pileus cuticle made up of irregularly arranged, 2.4-4.7 µm broad, septate hyphae; context made up of 2.4-4.7 µm broad, septate hyphae, intermingled with cells. Hymenophoral trama regular, parallel. Stipe cuticle hyphal, made up of longitudinally tangled, 2.4-7 µm broad, septate hyphae, giving rise to a loose turf of uplifted, 4.7-15 µm broad, septate hyphae. Clamp connections absent throughout.

Collection Examined

Punjab: Ludhiana (254 m), Village Kumkallan, growing scattered on humicolous soil, along road sides under *Cassia fistula,* Baljit Kaur and Harwinder Kaur, PUN 3923, September 1st, 2009.

Remarks

The details of the present collection were matched with those given for *Termitomyces heimii* Natarajan by Natarajan (1975) and Srivastava *et al*. (2011). and striking differences were found in some of the key characters, such as in the presently worked out specimen, the umbo is lacking where as a broad umbo is well known in *T. heimii*, also the stipe and pseudorrhiza are scaly in this collection, instead of smooth known for *T. heimii,* color of gills is strikingly different in being skin color rather than white known for *T. heimii*. The spore range is quite different, in the present collection the range is 3.4-5(6) x 3.4-4.2 µm where as it is 6-8.2 x 4.5-5.3 µm given for *T. heimii*. Pleurocystidia are absent in the present collection where as they are known to be present in *T. heimii*. (Table-1) Although in the taste being fruity, odour being fragrant, double ring annulus, details of the pileus and stipe cuticle the two species are similar. To accommodate this collection a new species *Termitomyces punjabensis* sp. nov. is being proposed.

Termitomyces badius Otieno. *Sydowia* **22**:162, 1969.

Fig. 3 (A–G), 4 (A).

Fructification 2.9-4.6 cm in height. Pileus 1-2 cm in diameter, convex to applanate with a prominent perforatorium; surface yellowish white ($4A_2$) with greyish brown, pointed perforatorium; scales absent; margin irregular, splitting at maturity; surface moist; cuticle fully peeling; flesh 0.1 cm thick, white, unchanging; taste and odour mild. Lamellae free, unequal, crowded, 0.2 cm broad, yellowish white ($4A_2$), unchanging, edges serrate, normal. Spore deposit pale yellow ($4A_3$). Stipe central, 4.1 cm long, 0.2 cm broad, concolorous with pileus, unchanging, equal in diameter throughout, hollow, smooth surface; annulus absent.

Basidiospores 5-8.5 x 3.4-5 µm (excluding apiculus) (Q=1.6) ellipsoid, thin walled, inamyloid; apiculate, apiculus 0.8 µm long. Basidia 18.7-27.2 x 5-8.5 µm clavate, -2, -4, spored, granular throughout. Pleurocystidia 18.7-30.6 x 5-13.6 µm clavate, granular throughout. Cheilocystidia 20.4-30.6 x 10.2-13.6 µm clavate, granular throughout. Gill edges heteromorphous.

Carpophore context homoiomerous. Pileus cuticle gelatinized, made up of subhorizontal, granular, 2.4-4.7 µm broad, radially arranged, septate hyphae; context cellular made up of loosely arranged cellular elements. Hymenophoral trama regular. Stipe cuticle hyphal, made up of longitudinally tangled 2.4-7 µm broad, granular, septate hyphae. Clamp connections absent throughout.

Collection Examined

Punjab: Ludhiana (254 m), Nutt village, growing scattered in groups on wet termitophilous soil, Baljit Kaur, PUN 3929, August 30th 2009.

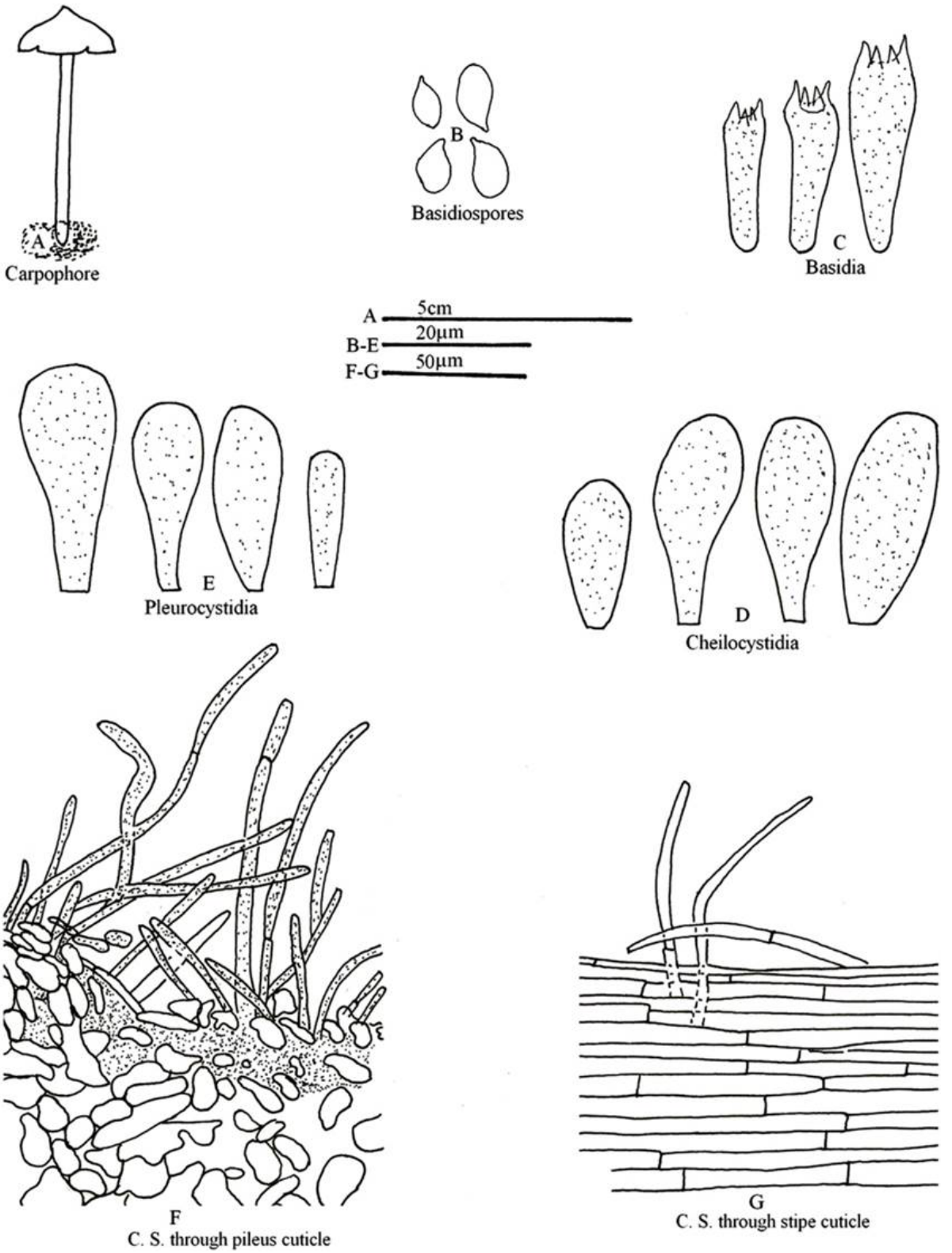

Fig. 3–A–G. *Termitomyces badius* Otieno A Carpophore. B Basidiospores. C Basidia. D Cheilocystidia. E. Pleurocystidia. F C.S. through pileus cuticle & context G C.S. through stipe cuticle

Fig. 4–A. *Termitomyces badius* Otieno: Scattered groups of carpophores growing on wet termitophilous soil

Remarks

The gross morphology and microscopic details of the presently examined collection match with those given for *Termitomyces badius* Otieno by Pegler (1977). This species was reported for the first time from India by Natrajan (1975) and from Punjab it was reported for the first time by Atri *et al.* (2005a). Presently, it is being reported from Ludhiana.

Termitomyces clypeatus R. Heim, *Bull. Jard. Bot. Etat*. **21**: 207, 1951.

Fig. 5 (A–F), 6 (A–B).

Fructification 10.5-15.5 cm in height. Pileus 9.5-11.8 cm broad; convex than expanding with a prominent perforatorium; yellowish white ($4A_2$); margin regular, splitting at maturity; cuticle fully peeling; flesh up to 0.6 cm thick, creamish white, unchanging; taste and odour mild. Lamellae free, crowded, unequal, 0.3 cm broad, creamish white, unchanging, edges serrate, normal. Spore deposit white. Stipe central, 9.5-14 cm long (excluding pseudorrhiza), 0.6-1.5 cm broad, tapering downward so as to form pseudorrhiza, concolorous with pileus, unchanging, solid, surface smooth to fibrillose; annulus absent.

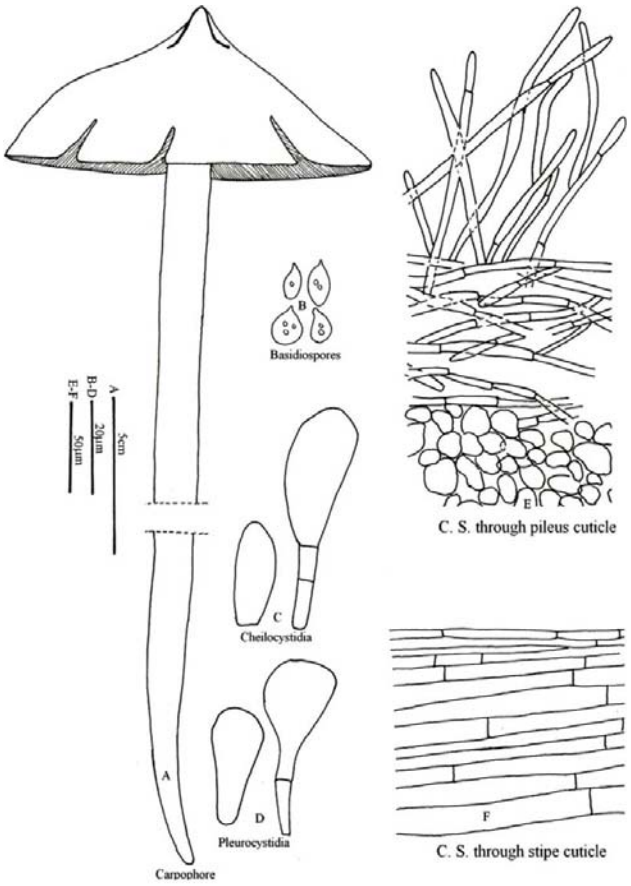

Fig. 5–A–F. *Termitomyces clypeatus* Heim. A Carpophore. B Basidiospores. C Cheilocystidia. D Pleurocystidia. E C.S. through pileus cuticle & context F C.S. through stipe cuticle

Basidiospores 6.8-8.5(10.2) x 3.4-6.8 µm (excluding apiculus), (Q=1.5) ellipsoid, thin walled, guttulate, inamyloid; apiculate, apiculus 0.85 µm long. Pleurocystidia 25.5-37.4 x 11-15.3 µm broadly clavate, abundant, basal cell present in one tiers. Cheilocystidia 22.1-47.6 x 10.2-17 µm, clavate, abundant, basal cell present 1 to 2 tiers. Gill edges sterile.

Carpophore context homoiomerous. Pileus cuticle made up of interwoven septate 2.4- 4.7 µm broad, hyphae, context made up of interwoven, septate hyphae, Subhymenium cellular. Hymenophoral trama regular, parallel. Stipe cuticle consists of longitudinally tangled septate 4.7-14.2 µm broad, hyphae. Clamp connections absent throughout.

Fig. 6–A–B. *Termitomyces clypeatus* Heim. A Carpophore growing solitary in its natural habitat, showing perforatorium. B Under view of the carpophore showing splitting margin and crowded gills

Collection Examined

Punjab: Ludhiana (254 m), Salempur village, growing scattered on humicolous soil, Baljit Kaur and Harwinder Kaur, PUN 3914, August 12th 2009.

Remarks

The gross morphological details and microscopic details of present collection match with those given for *Termitomyces clypeatus* R. Heim by Pegler (1977). *T. clypeatus* has been reported from Malaysia by Pegler and Venhaecke (1994); Bhavani Devi (1995) collected this species from Trivandrum during the month of May-June; Verma *et al*. (1995) reported this species from North East Hills of India. Leelavathy *et al.* (1983) recorded this species from the state of Kerala. Atri *et al*. (2005a) reported it from North India. Presently it has been collected from District Ludhiana and new to the collection area.

Key to the Investigated species of *Temitomyces*

1 Carpophore more than 35 cm long; spore deposit greyish orange; pseudorrhiza with red scales present throughout; perforatorium not prominent; annulus present.....***T. punjabensis***

1' Carpophore less than 16 cm long; spore deposit white to light yellowish; prominent perforatorium present; annulus lacking.....**2**

2 Pileus up to 2.0 cm broad; spore deposit pale yellow; pseudorrhiza lacking.....***T. badius***

2' Pileus up to 11.8 cm broad; spore deposit white; pseudorrhiza present with no scales on it .....***T. clypeatus***

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