

New Report of Termitophilic Fungi from Nepal

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Abstract

Termitomyces fuliginosus R. Heim, *T. globulus* R. Heim and *T. heimii* Natarajan, collected within an altitudinal range of 500-1500 m a.s.l. in subtropical deciduous forest during 2010-2012 are described and illustrated. Their macro and microscopic features delimit these taxa as new species in Nepal.

Keywords: Agaricales; Symbiosis; Taxonomy

Abbreviations

VDC: Village Development Committee; WN: Ward Number.

Introduction

Termitophilous fungi are a monophyletic group [1] of tropical gilled mushrooms belonging to the genus *Termitomyces*. They belong to the basidiomycete family Tricholomataceae, latter named Lyophyllaceae (Agaricales, Agaricomycetidae) [2-4]. The family was first described by R. Heim for a group of termitophilous agarics. They are unique as they grow on or near Termitoria built by termites their excreta in various localities of Nepal. The mycelium of *Termitomyces* is a good source of food for termites and they are also used for culinary purposes by humans. They live in an obligate symbiosis with termites of the subfamily Macrotermitinae [5]. Their spore transfer is mainly by shedding from mushrooms, which protrude from the termite mounds [6]. These fungi grow on combs which are excreta from the termites, dominated by tough woody fragments. *Termitomyces* is a paleotropical genus of agarics interesting both to mycologists and entomologists [7].

The diagnostic characters of the *Termitomyces* species are the pinkish spores, the termite association and the subterranean elongation of the stipe called a pseudorrhiza, through which the fruit bodies of most species are connected to the comb in the termite nest. Furthermore, most of the species have a pronounced umbo or papilla called a perforatorium, which play a role during penetration of the soil.

Singer [8] placed this genus in the family Tricholomataceae, but Pegler [9] kept it in Pluteaceae. A molecular study [10] supported Singer's [8] classification. Heim was first to place this genus of epigeaic species (i.e. *T. microcarpus*) in the subgenus *Praetermitomyces* and all the other species in the subgenus *Eutermitomyces*. He further divided *Eutermitomyces* into six stirpes, more for convenience than to reflect relationships. This classification is followed by Pegler [9,11] and Pegler and Vanhaecke, [12], whereas Singer [8] places *T. microcarpus* in the genus *Podabrella* together with five small, neotropical, non-termitophilous species. Gomez [13] transferred *Podabrella* to *Termitomyces*. *Sinotermitomyces* of five species was described by Mu [14] from Yunnan, China. The genus *Sinotermitomyces* is distinguished from *Termitomyces* by comparing the expanding pileus, solid stipe and, non-ornamented pleurocystidia in the

presence of small conical pileus, ornamented pleurocystidia and hollow stipe.

Investigation and study of these fungi in Nepal was started in 1986 [15]. Since then several mycological investigations have been done [16-21] and 19 taxa that are taxonomically described till the present [22-27]. All the species are edible and highly priced due to their good nutritive value, in Nepal and elsewhere in the world [25,28].

The species are characterized by typical agaricoid carpophores pluteoid, entire and incurved margin, usually fleshy, large often sharply, differentiated umbo, stipe central, spore print brownish to pinkish, lamellae free to adnexed but with decurrent teeth, crowded, white to pink in colour. Their stipe is cylindrical, solid, fibrous and smooth with pseudorrhiza. It is cylindrical and widening at a depth of 5-7 cm, then narrowing down to the point of attachment to the termite nest, its length determined by the depth of the termite comb and with a simple veil, hyphae, inamyloids, with a hilum of the open pore type, ellipsoid, smooth, basidia normal, cystidia present, tramal hyphae inamyloids, with or without clamp connection, trame system monomitic, presence of thin walled pyriform cheilocystidia and hymenophoral trama hyaline. In India, the Government has restricted the collection of *Termitomyces* for the purpose of conservation of genetic diversity in Western Ghats that lies in the wildlife sanctuaries in Goa (http://www.mamud.com/Docs/Biodiversity_in_the_Western_Ghats.htm).

Key to Identification of Species

Basidiocarp small to large. Pileus 2-30 cm broad, with or without scales, smooth, dry to viscid, umbonate, with or without perforations. With or without annulus. Stipe with long pseudorrhiza. Hyphae without clamps. Spores pinkish in deposits - *Termitomyces*

- 1a. Fruit-body differentiated into prominent head and tail on mound
- 1b. Fruit bodies in groups, growing on the sandy soil or on the shaded ground in association with termites' nests
- 2a. Pileus medium, without papilla and annulus, ochraceous brown dark unicolor, concentric "scrobiculate" stipe not annulate - *T. badius*
- 2b. Pileus continuation of the Perforatorium, obtuse, not spiniform, no rhizomorphs, brownish orange, concentric scrobiculate, umbo obtuse - *T. fuliginosus*
- 3a. Pileus globose, smaller, cystidia dimorphic, pseudorrhiza brownish perforatorium low, small and poorly developed, stipe usually without annulus, grayish brown - *T. globules*
- 3b. Pileus medium, up to 10 cm diam, whitish, smooth, with grayish brown, broad umbo, without papilla, stipe with a thick, ochraceous brown, persistent annulus - *T. heimii*

Materials and Methods

Collections were made in the pre- and post- monsoon seasons, possibly as a result of its subtropical affinities, when most agarics grow in the study area which lies in central Nepal (Makawanpur district of Churiyamai VDC, WN-9) (Figure 1). Several samples of *Termitomyces* species were collected growing on termitaria among vegetation, dominated by members of the Dipterocarpaceae, Combretaceae and Leguminosae. The collection was entirely based on their sexual reproductive structures [29]. The samples were photographed in their natural habitat. They were well dried and packed in wax paper bags with proper tag numbers. The habit and habitat including ecological parameters were recorded by the accessory equipment.

The collections were examined from fresh material both macro- and microscopically with a compound microscope (Olympus CX 22). Xanthochoric reaction was determined by placing a small piece of materials in an aqueous solution containing 5% KOH. The cynophily was observed in 0.01% cotton blue mounted in lactophenol [3]. Amyloidity of spores was observed by using Melzer's reagent [30]. The measurements were taken with the help of an ocular micrometer (1div = 11.66 μ m under 100X, 3.75 μ m under 400X and 1.66 μ m under 1000X). At least 20 spores were measured from lamellae. Selected macrographs were taken using a Sony DSC-S 980 camera mounted on the microscope.

The voucher specimens were identified using publications

[8,31–34] and on line data base [35–38]. Herbarium specimens were rehydrated in an aqueous solution of 3 % KOH and have been deposited at the Natural History Museum (NHM), Tribhuvan University, Nepal.

Results and Discussion

Termitomyces fuliginosus R. Heim, *Arch. Mus. Hist. Nat. Paris, ser. 6, 18: 147 (1942)*

Description: This species has a prominent, cone-like pointed, dark coloured projection at the center of the cap i.e., spiniform papilla. Caps are ochraceous-orange, perforatorium bullate-umbonate rather than spiniform papilla, and grey, medium size. Sporophores, growing on the sandy soil in groups, or on the shaded ground in association with termite's nests, characterized by its obligate symbiont with termites; usually centrally stipitate white but olivaceous near the umbonal region (Figure 2).

Pileus: 4.75–5.50 cm in diameter, convexoapplanate, smooth, surface grayish brown to yellow brown, perforatorium spiniform, radially fibrillose, sometimes radially striate. Lamellae: free, crowded, white to pink, adnexed. Stipe: Buffy-brown, medium cylindrical, 5.0 \times 0.9 cm, solid, cylindrical or slightly tapering downward; surface white above and pale brown on the bulb, fibrous, pellicle thick, and below it grey in colour, annulus absent. Pseudorrhiza: Present, solid, fibrous, grey to brown in colour and size up to 3.5 \times 0.7 cm. Flesh: soft, buffy-brown, hyaline, thin walled, clamp connection absent.

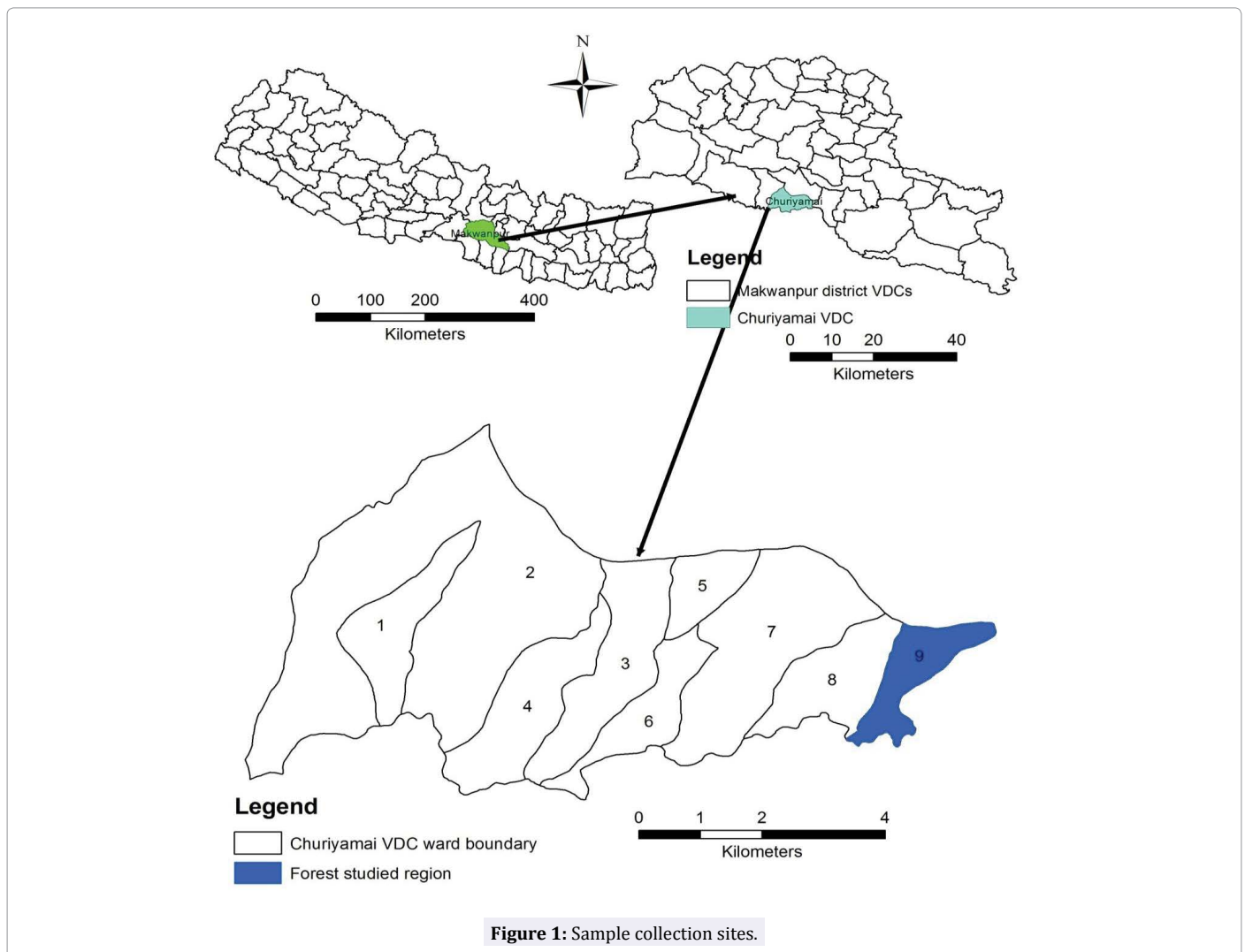
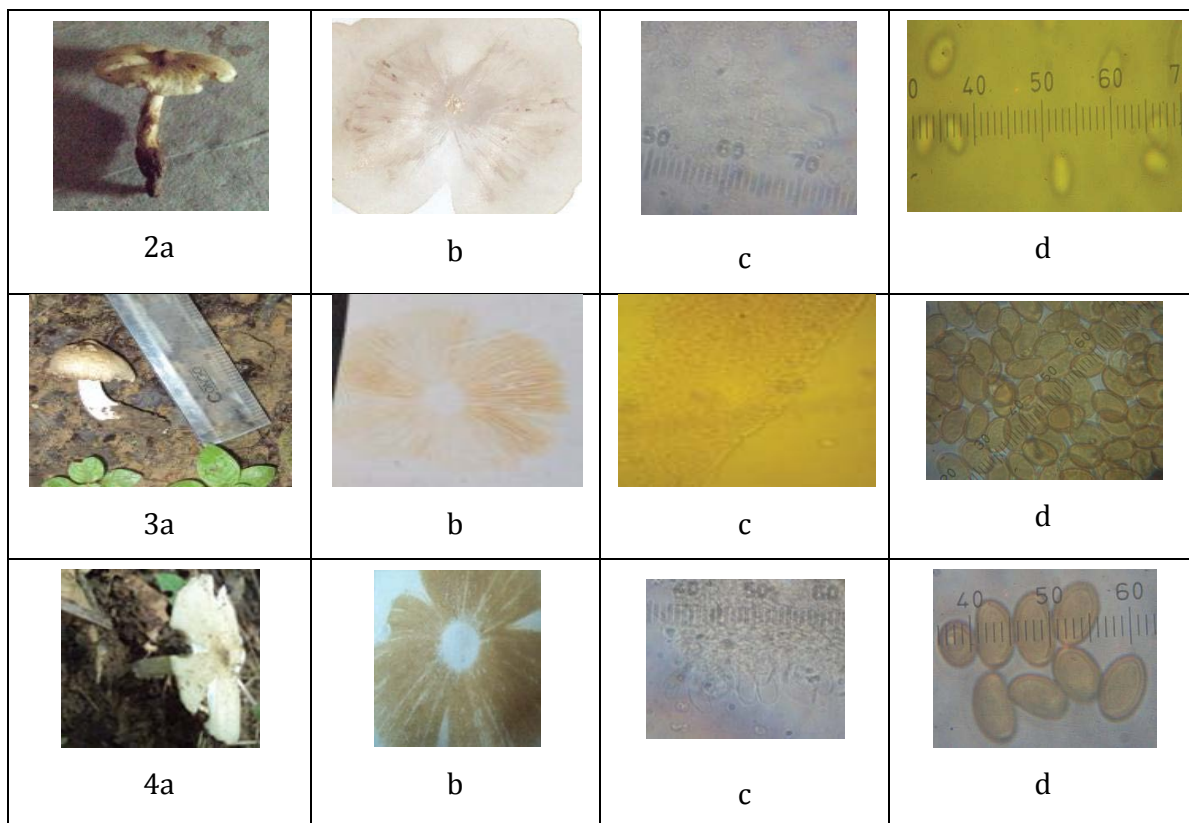


Figure 1: Sample collection sites.



Figures 2: 2 a-d is *T. fuliginosus*. 2 a = Basidiocarp, b = spore print, c = spores with basidium (1div = 3.75 μ m), d = spores under immersion oil (1div = 1.66 μ m). 3 a-d is *T. globulosus*. 3 a = Basidiocarp, b = spore print, c = spore with basidium (1 div = 11.66 μ m), d = spores under immersion oil (1div = 1.66 μ m). 4 a-d is *T. heimii*, 4 a = Basidiocarp, b = spore print, c = spores with basidium (1 div = 3.75 μ m), spores under immersion oil (1div = 1.66 μ m).

Spore print: purplish. Basidia: 29–31 \times 4.0–6.0 μ m, tetra sterigmata bearing 4 basidiospores. Basidiospores: 7.5–9.5 \times 4.0–6.0 μ m ovoid to sub cylindrical or ellipsoid, thick walled, hyaline, smooth, non-amyloid. Cystidia: 24–26 \times 18–20 μ m. Cheilocystidia: abundant, thin walled, pyriform. Pleurocystidia: similar to cheilocystidia. Hymenophoral trama: regular.

Habitat: On termite nests in tropical forests. Fruiting season: July–September. Edibility: Edible.

Species Examined: It grows on termites nest soil, in Sal (*Shorea robusta*) forest; Altitude: 560 m; longitude: 80.10818° E; latitude: 27.62274° N; Aspect: 198° S/W; Slope: 10° S/W; Temperature: 21.9–29.1°C; Humidity: 79–87 %; Soil pH: 6.5; Time: 9:10 am; Collected by Aryal, H.P. Date: 06.09.2011. Accession No. NHM TU 2-2-1676.

***Termitomyces globulus* R. Heim & Gooss., Bull. Jard. Bot. État 21: 216 (1951)**

Description: This species has prominent, cap gibbous with umbo, dark brown perforatorium at the center of the cap. Sporophores, grow in groups on the sandy soil or on the shaded ground in association with termites nests (Figure 3).

Pileus: 6.0–9.0 cm diam. subglobose, finally expanding but margin usually remaining incurved, surface pale ochraceous brown to tawny brown, paler at margin, glabrous and smooth, radially striate; margin lobed, often splitting. Lamellae: free, narrow, up to 8 mm broad, white to pale pinkish, densely crowded, with lamellulae of three lengths. Stipe: 6.0 \times 1.5 cm, cylindrical, solid; surface cream coloured, fibrous and smooth. Pseudorrhiza: elongate, up to 6 \times 1 cm or more, slender, tawny to rusty brown. Flesh: whitish in colour,

firm to spongy, consisting of thin-walled, inflated hyphae, clamp connection absent. Spore print: Pinkish. Basidia: 20.0–25.0 \times 9.0–11.0 μ m, clavate, bears four sterigmata. Basidiospores: 10.5–12.5 \times 9.0–11.0 μ m, obovoid to ellipsoidal, thick walled, non-amyloid. Cystidia: 28 \times 15 μ m. Cheilocystidia: crowded, polymorphic ranges from globose or pyriform to cylindrical and subcapitate, often septate wall. Pleurocystidia: similar to cheilocystidia. Hymenophoral trama: subregular, of thin walled, inflated hyphae.

Habitat: On termite nests in tropical forests. Fruiting season: July–September. Edibility: Edible.

Species Examined: It grows on termites nest soil, in mixed forest; Altitude: 1015 m; longitude: 80.18024° E; latitude: 27.68348° N, Temperature: 20.3–37.9° C, Humidity: 70–87 %; Soil pH: 5.5; Time: 16:54 pm; Date: 31-08-2011. Collected by Aryal, H.P, Accession No. NHM TU 2-2-1677.

***Termitomyces heimii* Natarajan, Mycologia, 71(4): 853–855 (1979)**

Description: The pellicle of the pileus peels off. Partial veil membranous, at first covering whole surface of the pileus and the stipe, then broken into tough squamules and forming a persistent double annulus on the upper part of the stipe; of narrow hyphae, parallel and perpendicular to the surface of the pileus and the stipe. Sporophores are growing on sandy soil in groups or on the shaded ground in association with termite's nests (Figure 4).

Pileus: Silky white, turns into the grey to brown-white-creamy in colour, Smooth, 7.5–9.0 cm in diameter, convex or campanulate at first, then expanding to plano-umbonate with a patch of veil at

the apex, perforatorium obtuse (umbo); surface pale brown to dark brown at center. Whitish grey at the umbonal, margin incurved and cracking (the cap splits at a few places from margin); covered at first by a firm partial veil, which disrupts to form persistent velar squamules at maturity; margin radially striate, often splitting; pileal surface an epicutis consisting of thin walled, repent hyphae. Lamellae: Free, surface white to pinkish cream with age, margin serrulate. Stipe: 5.0 × 1.5 cm, central, cylindrical, bulges near the pileus and possess a thick ring, surface creamish white stuffed, and smooth above thick and white persistent annulus, with pale brownish velar squamules below; solid, fibrous. Pseudorrhiza: Significantly long, 60 × 1.7 cm, up to 15 cm below ground level, fleshy, pale-grey, hollow, terminated by a disk connected to the termite comb; leathery, surface cream in colour. Flesh: white, fleshy, soft; of inflated, hyaline, thin-walled, septed with clamp connection hyphae. Spore print: Brown to pinkish grey. Basidia: 16.5–18.5 × 7.5–9.5 µm, sub hyaline and thick-walled, clavate, with four sterigmata Basidiospores: 10.5–12.5 × 7.5–9.5 µm, ovoid to ellipsoid, thick walled, hyphae non-amyloid, smooth, sub hyaline. Cystidia: 34–36 × 19–21 µm. Cheilocystidia: Rarely, clavate to pyriform. Pleurocystidia: clavate, similar to cheilocystidia, rare. Hymenophoral trama: regular, hyaline, thin walled, parallel hyphae.

Habitat: On termite nests in tropical forests. Fruiting Season: July to August. Edibility: Edible.

Specimens Examined: It grows on termites nest soil, in Sal (*Shorea robusta*) forest; Altitude: 1505 m; Longitude: 80.65415° E; latitude: 27.97291° N; Aspect: 22° N/E; Slope: 20° S/W; Temperature: 21.9–33.5° C; Humidity: 76–87 %; pH: 5.5; Time: 9:17 am; Date: 18.07.2010. Collected by Aryal, H.P, Accession No. NHM TU 2-2-1669.

There are 32 species in the genus [3,27] and 22 species have been documented in India [39–43], 11 in China [44] and 20 in Thailand [45]. They are distributed in Equatorial, Southern Africa, Madagascar, South Asia, and South East Asia including Nepal [5,22–28,31,46]. The taxonomic description and a survey of the literature [20,22–24,26,47–52], showed that *T. fuliginosus*, *T. globulus* and *T. heimii* are new additions to the macro fungal flora of Nepal.

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