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Morphology Based Taxonomic Characterization of Three Termitomyces Species from the Marathwada Region, Maharashtra, India

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Authors' contributions

This work was carried out in collaboration between both authors. Author GPM contributed to the methodology design, experiment execution, data collection, and data analysis. Author RBK contributed to draft writing, validation, review and editing, and supervision of the work. Both authors read and approved the final manuscript.

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Abstract

Background: *Termitomyces* species are ecologically important termite-associated macrofungi that contribute to lignocellulose degradation and nutrient cycling. However, their diversity and taxonomic characteristics remain inadequately documented in the Marathwada region of Maharashtra, India.
Aims: This study aimed to characterise and identify specimens of *Termitomyces heimii*, *Termitomyces clypeatus*, and *Termitomyces microcarpus* collected from the Marathwada region through detailed macro- and micromorphological examination.
Methods: The three species were collected during the 2024 monsoon season from Parbhani, Chhatrapati Sambhaji Nagar, and Nanded. Field observations documented habitat, substrate, locality, pileus form,

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perforatorium, lamellar arrangement, stipe features, annulus, pseudorrhiza, and spore-print colour. Laboratory examination included the measurement and assessment of basidiospores, basidia, cheilocystidia, pleurocystidia, pileus cuticle, stipe tissues, and hyphal characteristics. The observed features were compared with published taxonomic descriptions to confirm species identity and evaluate morphological variation.

Results: *Termitomyces heimii* was characterised by its large basidiomata, broad perforatorium, and persistent double funnel-shaped annulus. *Termitomyces clypeatus* was distinguished by its white pileus, striate margin, densely packed lamellae, and well-developed pseudorrhiza. *Termitomyces microcarpus* was differentiated by its small basidiomata, applanate pileus, obtuse umbo, hollow stipe, and absence of an annulus. The recorded macroscopic and microscopic characters were generally consistent with established descriptions of the respective taxa.

Conclusion: The findings document three *Termitomyces* species in Marathwada and contribute to the regional inventory of termite-associated macrofungi. Molecular confirmation and broader ecological sampling are required to strengthen future regional taxonomic assessments.

Keywords: Basidiomata; fungal diversity; Lyophyllaceae; macrofungi; macromorphology; Marathwada; micromorphology; morphological taxonomy; termite-associated fungi; Termitomyces.

1. Introduction

Fungi constitute one of the most diverse and ecologically important groups of organisms in the biological world, and India is recognised as a major centre of fungal diversity because of its varied climatic and ecological conditions. Although global fungal diversity is estimated to comprise nearly 1.5 million species, only about 50% have been characterised to date (Manoharachary et al., 2005). A substantial proportion of this diversity is believed to occur in India; nevertheless, much of the country's fungal wealth remains unexplored, emphasising the need for continued taxonomic and ecological investigations (Dwivedi et al., 2012). The genus *Termitomyces* R. Heim (Lyophyllaceae, Agaricales) comprises ecologically significant mushrooms characterised by an obligate symbiotic association with termites and plays essential roles in lignocellulose degradation, nutrient recycling, and the maintenance of ecosystem stability (Aanen et al., 2002; Heim, 1977; Hsieh et al., 2017; Kirk et al., 2008). The genus *Termitomyces* is distinguished by features such as siderophilous granulation in mature basidiocarps, a light-pink spore print, smooth, inamyloid and cyanophilous basidiospores, and the absence of clamp connections in the hyphae. The hymenophoral trama may be regular or bilateral. Members of this family are commonly termitophilous and form a unique symbiotic association with termites, which is particularly characteristic of *Termitomyces*. Some species also grow on decaying plant material or moist soil. According to Kirk et al. (2008), the family Lyophyllaceae comprises approximately eight genera and 157 species worldwide. In the present study, only one genus of this family, *Termitomyces*, was investigated. More than 30 species of *Termitomyces* have been reported globally (Kirk et al., 2008), whereas 29 species are known from India (Aryal & Budhathoki, 2014; Atri et al., 2005; Bilgrami et al., 1991; Karun & Sridhar, 2013). Verma et al. (1995) and Vesala et al. (2017) contributed valuable information on the ethnomycology and diversity of *Termitomyces*, respectively. Similarly, Tang et al. (2020) reported two new species, namely *T. floccosus* and *T. upsilocystidiatus*, from China and Thailand. Shaleh et al. (2021) further studied fungus-growing termites and their mutualistic association with *Termitomyces* species in Thailand. Members of the genus *Termitomyces* are well known for their obligate symbiotic association with termites and are recognised as ecologically and economically important mushrooms. These fungi serve as valuable dietary resources and have considerable potential for nutraceutical and ethnomedicinal applications, thereby contributing to the socio-economic well-being of local communities (Aryal & Budathoki, 2016; Koné et al., 2013; Njue et al., 2018; Pradhan et al., 2010). Nutritional studies have shown that *T. microcarpus* is rich in protein (30.69% dry weight), dietary fibre (11.60% dry weight), and essential minerals (Teke et al., 2018). Ethnomedicinally, *T. clypeatus* has traditionally been used to treat yellow fever in Nepal, pox in India, and gastrointestinal disorders, such as constipation and gastritis, in Ethiopia (Aryal & Budhathoki, 2016; Manna et al., 2014; Sitotaw et al., 2020). Recent regional studies have continued to use detailed macro- and micromorphological characters to document macrofungal diversity and support taxonomic identification (John et al., 2024). For *Termitomyces*, recent morpho-molecular investigations have demonstrated the value of combining morphological comparisons with DNA-based phylogenetic evidence when assessing species identity and variation (Tang et al., 2023; Das et al., 2024; Rehman et al., 2026). A comparable integrative approach has also been applied to the taxonomic characterisation of another wild edible mushroom (Rai et al., 2025). The systematic exploration, documentation, and identification of *Termitomyces* undertaken in this study are important for refining regional biodiversity inventories and assessing the potential of these macrofungi as sources of nutrition, income, and bioactive compounds for local communities. Despite their ecological and medicinal importance, the diversity and distribution of *Termitomyces* species in the Marathwada region of Maharashtra remain poorly investigated. Accordingly, the present study aims to characterise and accurately identify the collected *Termitomyces* specimens based on detailed morphological observations.

2. Materials and Methods

Termitomyces fruiting bodies were collected from various locations, including Parbhani, Chhatrapati Sambhaji Nagar, and Nanded, within the Marathwada region of Maharashtra during the monsoon season of 2024. Specimens were observed in natural habitats, including organic-rich soil, mixed leaf litter, garden areas, and sites around decaying woody substrates. Field notes were recorded at the time of collection and included the locality, habitat, substrate, date, and collection number (Lodge et al., 2004). Entire basidiomata were carefully excavated, with the basal portion intact, using a knife or small trowel (Largent, 1977). Fresh specimens were photographed in the field and placed in paper bags for transport to the laboratory (Mueller et al., 2004). For microscopic study, a small portion of fresh material was preserved in a formalin–alcohol solution (Hawksworth, 1991), while the remaining material was dried in a hot-air dryer at approximately 45°C. The dried specimens were stored for herbarium deposition at J.E.S. College, Jalna, Maharashtra, India, and for future reference.

Macromorphological characters were recorded from fresh specimens using standard methods for describing mushrooms. Features such as pileus size, shape, surface texture, colour, margin, lamellae, stipe, annulus, pseudorrhiza, and spore-print colour were noted. Colour terms were recorded during field observations and used as reference points for comparisons among the species (Kornerup & Wanscher, 1978). Microscopic examination was performed using thin, hand-cut sections from the fertile portions of the basidiomata. The sections were mounted in 10% KOH and, when required, stained with Congo red, lactophenol, iodine solution, or Melzer's reagent. Basidiospores, basidia, cystidia, hyphal elements, pileus cuticle, and stipe tissues were examined under a compound microscope. Measurements were obtained using a calibrated ocular micrometer, and microphotographs were taken using a digital camera attached to the microscope.

Species identification was performed by comparing the observed characters with published monographs, keys, and taxonomic descriptions of *Termitomyces* and related agarics (Kaur et al., 2023; Ranadive et al., 2022; Siegel & Schwarz, 2016). The diagnostic features were critically compared with earlier reports to confirm species identity and assess intraspecific variation.

3. Results and Discussion

The field photographs and macroscopic and microscopic details of the described mushrooms are provided in this paper. The investigated species are described in the sequence presented in the key below.

3.1 Key to the Investigated Species

1 Annulus present on stipe, funnel-shaped and persistent; pileus very large (up to 27 cm), with broad perforatorium; lamellae crowded, free; basidiospores 6.64–8.3 × 4.98–5.81µm ....***Termitomyces heimii***
1’. Annulus absent... 2

2 Pseudorrhiza present, dark brown to blackish, well developed; stipe solid; pileus up to 6.5 cm, surface white; basidiospores 7.2–8 × 3–6.1 µm............................ ***Termitomyces clypeatus***

2’. Pseudorrhiza absent; stipe hollow; pileus small (≈ 2.1 cm), applanate with obtuse umbo, white to creamish; lamellae free and unequal; basidiospores 5.81–8.3 × 4.15–6.64 µm...***Termitomyces microcarpus***

3.2 *Termitomyces heimii* Natarajan (Fig. 1)

Family: Lyophyllaceae

Morphological Details: Fructification: 9.0-18.5 cm. Pileus: 27 cm wide, convex to plano-convex and flattened, with a central depression and a broad perforatorium. The surface is white (1A1), silky, and covered with appressed fibrillose scales. The margin is irregular and splits at maturity, with a broad umbo. Gills: free, unequal, arranged in three lengths, and crowded (up to 21 lamellulae per cm); up to 0.5 cm wide; white (1A1), becoming orange-white at maturity (5A2); with smooth edges. Stipe: central, 6.6-18 cm long, 0.8-1.4 cm broad in the middle, and up to 0.7 cm wide at the broadest end; off-white, hollow, and longitudinally twisted; with a persistent, double, funnel-shaped annulus attached to the upper part. The surface is smooth.

Microscopic Details: Basidiospores measure 6.64-8.3 × 4.98-5.81 µm and are broadly ovoid to ellipsoid, thin-walled, inamyloid, hyaline, and provided with a 0.83 µm apiculus. Basidia measure 23.24-33.2 × 7.47-9.96 µm and are tetrasterigmate, clavate, granular, and thin-walled. Pleurocystidia measure 26.56-44.82 × 7.47-14.94 µm and are clavate and granular. Cheilocystidia measure 18.26-41.5 × 9.96-14.94 µm and are clavate to pyriform, with refractive walls; they are hyaline and sterile at the gill edges. The pileus cuticle is composed of projecting hyphae 3.32-6.64 µm wide. The stipe cuticle consists of parallel, gelatinised hyphae 4.98-8.3 µm broad. The stipe-context hyphae are broader, measuring 4.98-19.92 µm. Clamp connections are absent throughout.

Specimens Examined: Pathri (Dist. Parbhani), on mixed leaf litter, scattered; Latitude 19.255912°, Longitude 76.427204°, 12/08/2024, Musale, G.P; Collection no: PA 01 - PA 05.

The morphological and anatomical features of the collected specimen align closely with the description of *Termitomyces heimii* provided by Natarajan (1979). Key diagnostic traits include a broad perforatorium, an incurved, non-striated pileus margin, and a well-developed annulus. This species was previously reported from Punjab by Atri et al. (2005). In India, it has been reported under different names, including *Termitomyces albuminosus* (Patil et al., 1979) and *Termitomyces longiradicatus* (Borkar et al., 2015). Records from Maharashtra were documented by Hedawoo (2010) and Borkar et al. (2015). Considering the morphological, anatomical, and distributional data, the present collection was identified as *Termitomyces heimii*. In the present study, the stipe measured 6.6-18 cm in length and 0.8-1.4 cm in width; it was off-white, hollow, longitudinally twisted, and bore a persistent, double, funnel-shaped annulus. In contrast, Srivastava et al. (2011) reported a stipe measuring 5 cm in length and up to 1.5 cm in width, which was cylindrical, creamy, stuffed, and bore a thick annulus. The persistent double annulus remains a stable diagnostic trait across geographical populations, supporting its taxonomic significance.

This difference may reflect developmental plasticity associated with Marathwada's edaphic and climatic conditions. Specifically, deeper soil penetration by pseudorrhizae in response to the region's semi-arid soils may necessitate longer stipes for reproductive efficiency. *Termitomyces heimii* has been reported to produce fruiting bodies with an incurved margin and a smooth pileus; however, in the present study, the margin was irregular and split at maturity, and a broad umbo was present. This variation suggests that maturation stage and moisture availability during fruiting may influence margin morphology. The irregular splitting pattern may represent an adaptation to Marathwada's drier climate that facilitates spore dispersal through mechanical tension rather than through intact margins. Srivastava et al. (2011) reported the presence of a pseudorrhiza, whereas no pseudorrhiza was observed in the present study. They also reported crowded, free, whitish gills that became pinkish with age, which is consistent with the present observations. A similar macro- and micromorphological study of *Termitomyces heimii* was conducted by Kaur et al. (2022).

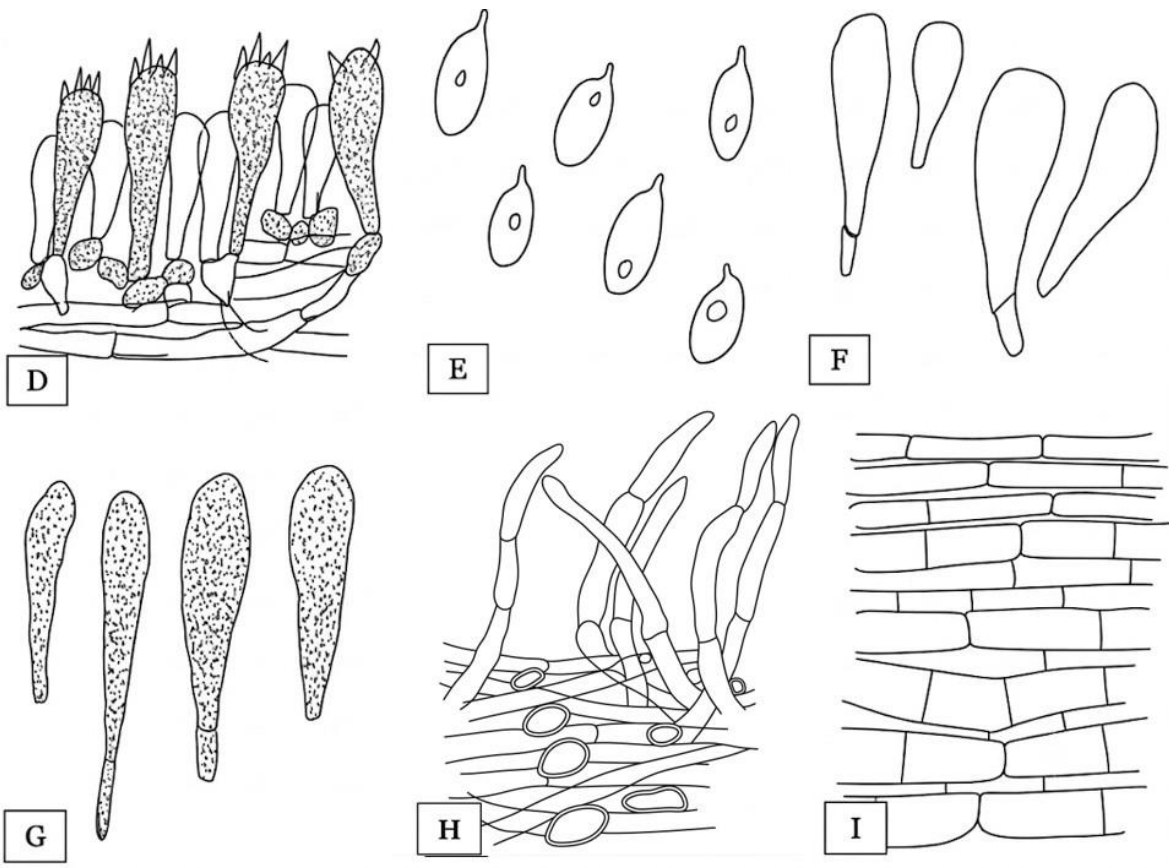


Fig. 1. *Termitomyces heimii*: A, B, C: natural habitat of carpophore; D: basidia; E: basidiospore; F: cheilocystidia; G: pleurocystidia; H: pileus cuticle; I: stipe cuticle. Scale bar 50 mm for macroscopic structures and 20 µm for microscopic structures

3.3 *Termitomyces clypeatus* R. Heim (Fig. 2)

Family: Lyophyllaceae

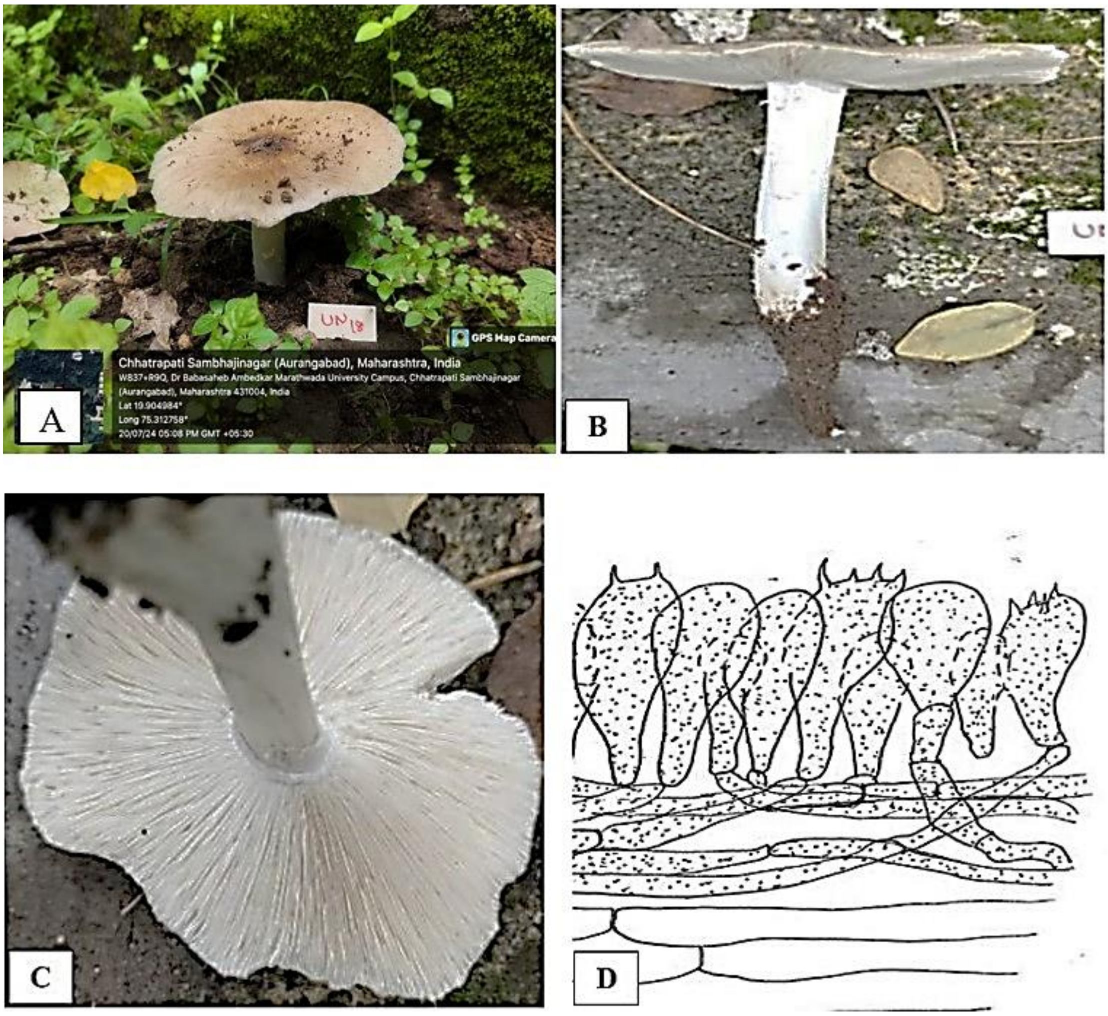
Morphological Characteristics: The carpophore is up to 14 cm high. The pileus is up to 6.5 cm in diameter and has a dry, white surface. The margin is regular and striate along the edge and splits at maturity. When young, the cuticle is gravid red (7B); at maturity, it is greyish brown (7D) at the centre. The lamellae are free, unequal, densely packed, white (2A), and number up to 14 per cm. The gill edge is smooth. The spore print is white. The stipe is central, up to 12 cm long and 0.7 cm wide, and has a fibrillose surface.

Microscopic Characteristics: Basidiospores measure 7.2-8 × 3-6.1 µm and are elongate to cylindrical, smooth, double-walled, inamyloid, and provided with an apiculus. Basidia measure 17-19 × 4-5 µm, are clavate, and bear four sterigmata. Cheilocystidia measure 18-25 × 8.1-13.4 µm and are clavate to pyriform. The gill edges are fertile. Pleurocystidia measure 18-31 × 15-19 µm and are broadly clavate to pyriform. The pileus cuticle consists of hyphal projections composed of septate hyphae 2.7-4.2 µm wide, while the context contains septate hyphae 13.9-21.9 µm wide. The stipe cuticle is composed of regular, septate hyphae 4.8-13.70 µm wide. Clamp connections are absent.

Specimen Examined: Dr. Babasaheb Ambedkar Marathwada University Campus, (Dist. Chhatrapati Sambhaji Nagar); habitat: growing solitary in a garden; Latitude 19.904984° Longitude 75.312758°, 05/07/2024; Musale, G. P; Collection no: UN 01 - UN 8.

The morphological and anatomical features of the collected specimen closely match the description of *Termitomyces clypeatus* R. Heim, originally described by Heim (1942) and later discussed by Pegler (1977) and Natarajan and Raman (1983). Key diagnostic traits include a smooth to spiniform perforatorium on the pileus, a striate margin that splits at maturity, free and densely packed lamellae, a long, tapering stipe with a well-developed pseudorrhiza, white spores, and the absence of clamp connections. Microscopically, the species exhibits elongate to cylindrical, smooth basidiospores; clavate, tetrasterigmate basidia; fertile gill edges; and clavate to pyriform cheilo- and pleurocystidia. In India, *Termitomyces* species have been widely reported from termite-associated environments (Natarajan, 1979; Natarajan & Raman, 1983). In Maharashtra, the genus has been recorded by Hedawoo (2010) and Borkar et al. (2015), indicating its presence in the region. Considering the combined morphological, anatomical, ecological, and distributional data, the present collection was identified as *Termitomyces clypeatus* R. Heim. Srivastava et al. (2011) reported a stipe 3-7 cm long, whereas the present specimen had a 12 cm stipe. This difference may suggest either that local termite colonies construct deeper mound structures, requiring longer pseudorrhizae for mycelial anchorage, or that Marathwada's soil conditions necessitate deeper penetration for moisture acquisition and structural stability. They reported an irregularly lobed margin, whereas the present specimen had a regular margin that was striate along the edge and split at maturity. They did not report cheilocystidia or pleurocystidia; however, both were described in detail in the present study. These observations provide additional data on gill-edge histology that may refine the species' diagnostic profile. The presence of both cystidial types is rare among *Termitomyces* and may represent an evolutionarily derived state. This observation adds diagnostic weight to the species' phylogenetic position within the genus. The gill characteristics observed in the present study were consistent with their report. A similar macro- and micromorphological study of *Termitomyces clypeatus* was conducted by Kaur et al. (2022).

Microscopically, the basidiospore morphology (5.81–8.3 × 4.15–6.64 µm; ellipsoid) remains consistent with published standards, reinforcing the stability of spore characters across geographically distant populations. The rarity or absence of pleurocystidia contrasts sharply with *T. heimii* and *T. clypeatus*, suggesting that gill-edge differentiation may be associated with fruiting-body size and ecological strategy.



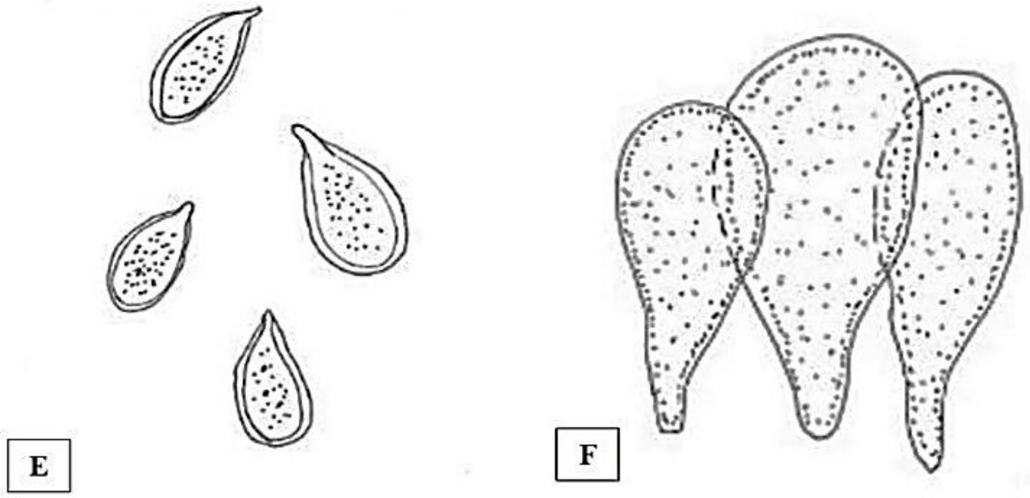


Fig. 2. *Termitomyces clypeatus*: A, B, C: Natural habitat of carpophore; D: basidia, E: basidiospore; F: cheilocystidia. Scale bar 50 mm for macroscopic structures and 20 μm for microscopic structures

3.4 *Termitomyces microcarpus* (Berk. and Broome) R. Heim (Fig. 3)

Family: Lyophyllaceae

Macroscopic Features: The fruiting body measures 7.8 cm in height and is white. The pileus is 2.1 cm wide and flat (applanate), with an obtusely pointed, cream-white surface and a greyish-brown umbo. The surface bears appressed scales, is slightly radially striate, and is 0.1 cm thick. The gills are free, white (1A1), and unequal. The stipe is 6.9 cm long and 0.4 cm wide, centrally positioned, white, smooth, and cylindrical.

Microscopic Features: Basidiospores measure 5.81-8.3 × 4.15-6.64 µm and are ellipsoid, inamyloid, hyaline, smooth-walled, and provided with an apiculus. Basidia measure 13.28-26.56 × 4.98-8.3 µm and are clavate, bi- to tetrasterigmate, hyaline or slightly granular, with a regular gill trama. Cheilocystidia measure 18.26-46.48 × 9.96-18.26 µm and are broadly clavate to pyriform, hyaline, thin-walled, and located at the gill edge. Pleurocystidia measure 24.9-41.5 × 9.96-19.92 µm and are clavate to pyriform, hyaline, thin-walled, and rarely present. The pileus cuticle consists of hyphal elements 4.15-8.3 µm thick and is composed of septate, hyaline to granular, smooth-walled hyphae that produce septate, granular, projecting hyphae. The pileus-context hyphae measure 6.64-19.22 µm and are septate, hyaline, and smooth-walled, with intermixed hyaline, irregular cells. The stipe-cuticle hyphae measure 4.98-9.96 µm and are septate, smooth-walled, and hyaline. The stipe-context hyphae are broader, measuring 6.64-26.56 µm. Clamp connections are absent throughout.

Specimens examined: Nanded (Dist. Nanded), on soil, scattered to solitary; Latitude 19.101026° Longitude 77.284647° 27/07/2024; Musale, G. P; Collection no: NA 01- NA 04.

Based on detailed macro- and micromorphoanatomical characters, the present collection closely conforms to *Termitomyces microcarpus* as described by Pegler (1986). Although it resembles *T. badius* in having small basidiomata, a fistulose stipe, and no annulus, the obtuse pileus apex serves as a critical taxonomic discriminator that separates *Termitomyces microcarpus* from the morphologically similar *T. badius*. In the present study, it was recorded from Parbhani, Chhatrapati Sambhaji Nagar, and Nanded, representing a new addition to the documented local fungal diversity. The observed characters also agree with those reported by Srivastava et al. (2011) and Manjula (2024), particularly in pileus form, lamellar arrangement, stipe morphology, and spore features.

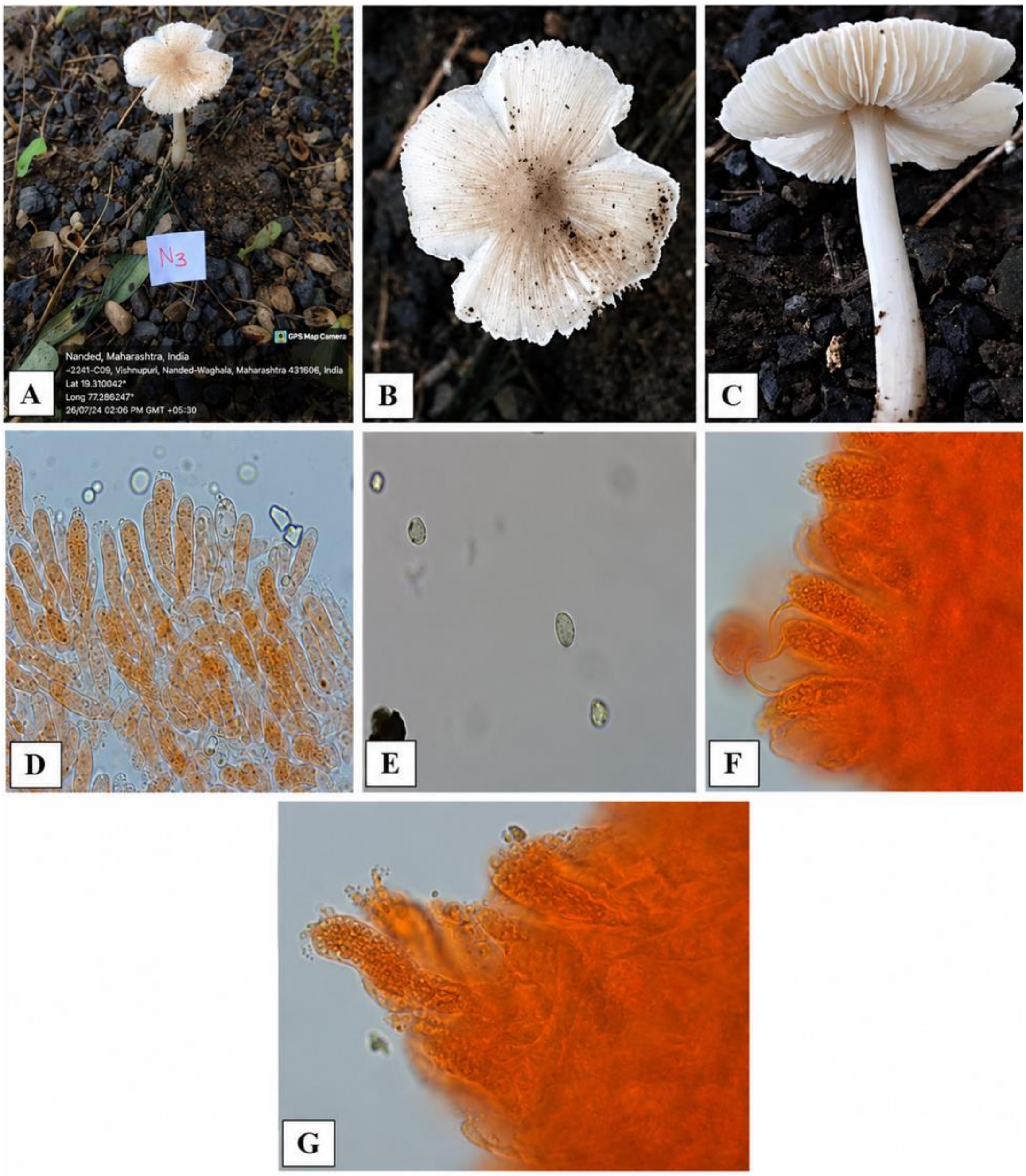


Fig. 3. *Termitomyces microcarpus*: A, B, C: Natural habitat of carpophore; D: basidia; E: basidiospore; F: cheilocystidia; G: gill Edge(100x). Scale bar 50 mm for macroscopic structures and 20 µm for microscopic structures

4. Conclusions

The study documented three termite-associated mushrooms, *Termitomyces heimii*, *Termitomyces clypeatus*, and *Termitomyces microcarpus*, from selected localities in the Marathwada region of Maharashtra. Identification was based on a combined assessment of macroscopic and microscopic characters, including pileus morphology, perforatorium, lamellar arrangement, stipe structure, annulus or pseudorrhiza, basidiospore dimensions, basidia, cystidia, and hyphal features. *Termitomyces heimii* was distinguished by its large basidiomata and persistent double funnel-shaped annulus, whereas *T. clypeatus* showed a striate pileus margin and a well-developed pseudorrhiza. *Termitomyces microcarpus* was characterised by small basidiomata, an applanate pileus, and the absence of an annulus. The observed characters were broadly consistent with published taxonomic descriptions and support the morphological identification of the specimens. These records expand the available information on *Termitomyces* diversity in Marathwada. Future studies should include molecular identification, larger specimen collections, detailed ecological observations, and clearly deposited voucher specimens to improve taxonomic confidence and regional biodiversity documentation.

5. Limitations

The study was primarily based on macroscopic and microscopic observations and did not include molecular confirmation. Herbarium accession numbers and detailed voucher-deposition information were not clearly provided. Some collection localities require more precise site descriptions. The limited number of specimens examined for each species may restrict the assessment of intraspecific variation. Ecological observations were also limited and did not include detailed information on associated termite species, soil conditions, or seasonal abundance. Therefore, larger collections, molecular analyses, and more detailed ecological documentation are required.

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Declaration of AI Use

This manuscript was prepared through the combined contributions of all author(s), including contributions to the study design, data, content development, results, interpretation, and related scholarly work. The author(s) acknowledge the use of Grammarly and ChatGPT to assist with grammar checking, language refinement, reference formatting. These AI-assisted tools were not used as authors and did not replace the intellectual contributions or scholarly judgment of the author(s). All AI-assisted outputs, including content, references, and interpretations, were carefully reviewed, revised, verified, and approved by the author(s). The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that no competing interests exist

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