

Original Research Article

Diversity and distribution of *Termitomyces* spp. in the ecological niches of Central and Northern Kerala, India

ABSTRACT

Kerala, situated in southern India, provides an ideal environment for *Termitomyces* mushrooms due to its warm temperatures, high humidity, and monsoon-driven rainfall. These termite-associated mushrooms thrive well in nutrient-rich soils like laterite, forest loam, and coastal sandy, supporting their symbiotic relationship with termites of the Macrotermitinae subfamily. The genus, documented by Heim (1942), showcases a unique ecological niche, with over 30 known species globally and 22 reported in India and 15 species in Kerala. A mushroom survey was carried out from October 2023 to October 2024 across Thrissur, Wayanad, and Kasaragod districts and identified 28 *Termitomyces* samples from different agro-ecological units. Morphological and molecular characterization revealed the prevalence of species such as *T. microcarpus*, *T. srilankensis*, *T. fuliginosus*, *T. heimii*, and *T. eurrhizus* in the surveyed locations. The study highlights significant variation in pileus size, stipe morphology, and microscopic features, such as basidia, basidiospores and cystidial structures. The most frequently occurred species was *T. microcarpus* in diverse habitats, while rare ones like *T. eurrhizus* occurred in isolated moist habitat. These findings contribute to understanding the biodiversity and ecological role of *Termitomyces* in Kerala, emphasizing their importance for further research and conservation.

Keywords: Biodiversity, Environment, Habitat, Kerala, Monsoon, Morphological and molecular characterization, Mushroom, *Termitomyces*

1. INTRODUCTION

Kerala, located at the southern part of the Indian Peninsula, is positioned between 8°18'–12°48' N latitudes and 74°52'–77°22' E longitudes. This small state is bordered by the Western Ghats to the east and the Arabian Sea to the west. The climatic conditions of Kerala provide an ideal environment for the growth of *termite* mushroom, owing to its warm temperatures, high humidity, and abundant rainfall. The state experiences significant precipitation during the South-west Monsoon (May–August) and the North-east Monsoon (October–December), maintaining the moist conditions essential for mushroom development. The annual temperature ranges from 25 °C to 27.5 °C in coastal regions and 20 °C to 22.5 °C in the eastern highlands, creating a favorable thermal regime for the mushroom growth. Moreover, soil types of

Kerala, which are rich in organic matter and retain moisture effectively, coupled with the diverse vegetation and undisturbed habitats provide an optimal substrate for the proliferation of this mushroom.

Termite mushroom classified under the genus *Termitomyces* of the family Lyophyllaceae and distinguished by their distinctive characteristics (Heim, 1942). These mushrooms show a symbiotic relationship with termites, of the subfamily Macrotermitinae. The mutualistic relationship between *Termitomyces* fungi and termites originated at least 31 million years ago (Nobre *et al.*, 2011), where, termites create a stable environment for fungal growth and aid in spore dispersal, while *Termitomyces* serves as a nutritional resource for the termites (Wood *et al.*, 1989). This unique group encompasses Agarics that necessitate an obligate association with termites, demonstrating a specialized ecological niche. The observation of *Termitomyces* occurrence within termite mounds was documented by the German researcher König (1779) at Tanjore, located in southern India and discovered brain-shaped formations within termite nests, named "Mushroom gardens". The predominant research on this paleotrophic mushroom and their symbiotic relationship with termites has been primarily explained by Heim (1977), mainly from Central Africa, Batra and Batra (1979) in India. To date, more than 30 species of this genus are known and were accepted in the 10th edition of the Dictionary of the Fungi (Frøslev *et al.* 2003, Kirk *et al.*, 2008).

The regional variations reflect the diverse ecological niches and habitats that support the growth of *Termitomyces* species across Kerala, India. In light of this, current study was carried out to document the diversity and distribution of different species of *Termitomyces* from Thrissur, Wayanad and Karasagod district of Kerala.

2. MATERIAL AND METHODS

2.1. Survey for the collection of *Termitomyces* spp.

Purposive sampling survey was conducted in Central and Northern parts of Kerala during the South-West and North-East monsoon seasons from October 2023 to October 2024. The survey covered different agro-ecological units of Thrissur (02, 06, 10), Wayanad (20, 21) and Kasaragod (07, 11) districts of Kerala. Out of seven AEUs, 10 locations were surveyed for the collection of fresh fruiting bodies of *Termitomyces* and location information of all the collected samples were recorded using Global Positioning Systems (GPS). During the survey, observations were made on its location, time and periodicity of occurrence, habitat and type of soil along with the sporocarp distribution.

The mushroom samples showing morphological characters of *Termitomyces* were photographed and carefully collected from the ground by digging into the soil to obtain the complete fruiting body along with pseudorhiza without disturbing the underground mycelial system, as the stipe base is often a crucial identification feature. The samples were then wrapped in paper bags to retain adequate moisture, colour, and stiffness of the fungus, and to prevent the samples from being crushed. The samples were then brought to the laboratory as quickly as possible and subjected to various morphological observations and later on dried in a hot air oven to bring the moisture content to below 10 per cent for long term storage.

2.2. Identification of different species of *Termitomyces* species.

2.2.1. Morphological characterization

The collected samples were morphologically characterized based upon the size, shape and colour of pileus, nature and colour of perforatorium, arrangement of lamellae, size of the stipe, and presence or absence of annulus and pseudorhiza as per the data sheet provided by Nair and Devi (1986). Micro-morphological characters were recorded using

fresh and mature sporocarps of *Termitomyces* spp. and thin sections of the gills were prepared and observed for the shape and size of basidia, cheilocystidia, pleurocystidia and basidiospores using compound microscope (Leica- ICC50 HD, USA) at 400 X and 1000 X magnification.

2.2.2. Molecular characterization

The collected samples were identified upto genus level based on the observable morphological characters. The species level identification was carried out using molecular techniques which included fungal DNA isolation by 2 per cent CTAB method (Doyle and Doyle, 1987), amplification of the DNA template followed gel electrophoresis, sequencing of the nuclear ribosomal internal transcribed spacer (nrITS) and nuclear large subunit of ribosomal DNA (nrLSU) regions and *in silico* analysis.

3. RESULTS AND DISCUSSION

3.1 Survey for the collection of *Termitomyces* species.

A purposive sampling survey was carried out for the collection of termite mushroom from three districts of Kerala, including Thrissur, Wayanad and Kasaragod. The survey details and recorded observations are presented in Table 1. Ten locations were surveyed in seven Agro-Ecological Units (AEUs) in the state, resulting in the collection of 28 mushroom samples (Fig 1). To document the most prevalent species in the Central and Northern parts of the state, their frequency of occurrence and abundance were assessed by repeated sampling of the same species from 10 locations, with particular emphasis on various locations within the Thrissur district.

Table 1. Details of survey and observations particulars

District	AEU	Location	Time of collection	Designated Code	Periodicity	Soil type
Thrissur	10	Vellanikkara	June- November	T1 to T4, T6, T8-T16	Once in a year	Laterite
		Chirakkakode	January	T5	Once in a year	Laterite
		Kadukutty	June	T7	Once in a year	Sandy
		Alathur, Mala block	October	T18	Continuously appeared for 3 weeks	Laterite
	06	Pazhuvil	October	T19	Continuously appeared for 3 weeks	Laterite
	02	Engadiyoor	October	T20	Once in a year	Sandy
Wayanad	20	Ambalavayal	August	W1-W5	Once in a year	Forest loamy
	21	Mathamangalam	September	W6	Three times in a year	Forest loamy
Kasaragod	07	Nileshwar	October	K1	Once in a year	Coastal sandy
	11	Periya, Kanhangad	October	K2	Once in a year	Coastal sandy





Figure 1. *Termitomyces* species collected during the survey period from Thrissur, Wayanad and Kasaragod districts of Kerala. *T. microcarpus* (1, 2, 4, 6, 9, 12, 18, 23, 28), *T. striatus* (3, 7, 19, 25), *T. schimperi* (5), *T. clypeatus* (8), *T. fuliginosus* (13, 20), *T. srilankensis* (11, 15, 16, 17, 27), *T. eurhizus* (10, 14, 26), *T. heimii* (21, 22), *Termitomyces* spp. (24)

3.2. Identification of the collected samples based on morphological and molecular characterization

1. *Termitomyces microcarpus*

Among the 28 collected samples, nine samples were identified as *Termitomyces microcarpus* based on the morphological and molecular characterizations techniques from all the three districts. Small fruiting bodies with conical to plano-convex, grayish white coloured pileus measured 1.7 to 3.8 cm in diameter. Perforatorium appeared as pointed to

conical, brown coloured. Lamellae were smooth, white, loosely arranged towards the centre, 1.2-1.2 cm long and 0.1-0.3 cm wide. Stipe was slender, cylindrical, solid, 3.5-7.8 x 0.4-0.9 cm in size with creamy white colour. Pseudorhiza and annulus were absent. Pileipellis layer, ixocutis form. Basidia were clavate, 18.45-24 x 7.4-10.57 μm in size bearing double walled, hyaline, ellipsoid basidiospores measuring 6.5-7.59 x 5.85-6.29 μm . Pleurocystidia were elongated, hyaline, thin walled, 45.69 x 23.49 μm in size. Cheilocystidia, clavate, hyaline, thin walled, 20.89-26.33 x 10.58-13.65 μm in size. Our observations align closely with the descriptions provided by Heim (1977), Nair (1989), and Anukrishna (2023).

2. *Termitomyces srilankensis*

A total of four basidiocarps of *T. srilankensis* were collected from Vellanikkara and Ambalayaval region of Thrissur and Wayanad district respectively. They had Medium to large sized solitary fruiting body plano-convex, white to brownish coloured pileus measuring 10.2-10.4 cm diameter with fragile margin were observed in the collected samples. Perforatorium appeared tiny, pointed, light brown coloured. Lamellae were whitish, thick (3.8-4.0 x 0.3-0.5 cm). Stipe was whitish, long, slender, cylindrical, solid, 12.8-13 x 2.4-2.6 cm in size. Annulus was absent. Pseudorhiza slender, cylindrical, solid, brown, 7.2-7.4 x 2.3-2.5 cm. Pileipellis layer, cutis form. Basidia were clavate, 17.03-22.71 x 5.82-6.32 μm in size, with double walled, ellipsoid basidiospores (4.31-5.67 x 4.13-5.11 μm). Pleurocystidia pyriform, hyaline, thin walled, 24.34-27.31 x 13.35-18.87 μm . Cheilocystidia ovoid to clavate, hyaline, thin walled, 35.52 x 18.93 μm in size. The observed macro- and micromorphological characteristics exhibit similarities to those species reported by Ediriweera *et al.* (2023) for a newly described species from Kegalle, Sri Lanka.

3. *Termitomyces fuliginosus*

Two samples collected from Vellanikkara region of Thrissur district had medium to large sized solitary fruiting body with plano-convex, grayish white coloured pileus measuring 9.8-10 cm diameter, without fragile margin. Perforatorium appeared as pointed, spiny form, light brown coloured. Lamellae were thick, whitish, compactly arranged towards the centre, 4.2-4.3 cm long and 0.3-0.5 cm width. Stipe was long, cylindrical, straight, solid, (7.4-7.5 cm x 3-3.2 cm). Annulus absent. Pseudorhiza dark brown, pointed towards the distal end, 4.8-5.0 x 1.2-1.4 cm. Pileipellis layer, cutis form. Basidia were oblong, 16-20.22 x 5.3-7.58 μm in size, bearing double walled, ellipsoid, hyaline basidiospores (4.6-7.29 x 3.27-4.68 μm). Pleurocystidia pyriform, hyaline, thin walled, 23-26.04 x 18.05-22.23 μm . Cheilocystidia reniform, thin walled, hyaline, 26.99-37.2 x 27.887-30.31 μm in size. These morphological and microscopic features is in congruence with the descriptions provided by Jannual *et al.* (2020) in Thailand, suggesting a strong similarity between our findings and the previously documented species.

4. *Termitomyces heimii*

Medium to large sized fruiting bodies with plano-convex shaped cap in matured basidiocarp. When immature, it was conical to convex, white to whitish brown, 6.8-7.3 cm in diameter, with fissile margin. Perforatorium appeared umbonate, dark brown coloured. Lamellae were thick, whitish, 2.9-3.1 cm long and 0.2-0.5 cm wide. Stipe was long, cylindrical, straight, solid, 2.6-3 x 5.2-5.4 cm in size and annulus present. Pseudorhiza was very long, slender, cylindrical, creamy white, 7.5-12.9 x 1.6-2.3 cm in size. Pileipellis layer, ixotrichodermium form. Basidia elongated, 29.89-32 x 10.63-11 μm in size bearing double walled ellipsoid, hyaline, basidiospores (7.18 x 6.95 μm). Pleurocystidia and cheilocystidia were elongated, hyaline, thin walled, 34.67-45 x 15.53-18.3 μm and 35.03-42 x 12.23-16.67 μm in size, respectively.

Similar macro-morphological observations were described by Natarajan (1979). For *T. heimii* comparable characteristics were subsequently documented by Nair (1989) from various forested regions of Kerala and by Mohanan (2011).

5. *Termitomyces eurrhizus*

Two samples out of 28 collected samples were identified as *T. eurrhizus*. It has medium sized solitary fruiting body with plano-convex, grayish white coloured pileus measuring 4.5-4.8 cm in diameter with fissile margin. Perforatorium pointed, spiny formed and dark brown coloured. Lamellae were whitish, thick (1.8-2.0 x 0.3-0.4 cm). Stipe was straight, cylindrical, solid, 5.2-5.3 x 1.7-1.8 cm in size. Annulus absent. Pseudorhiza dark brown, cylindrical, solid, 3.2-3.5 x 1.3-1.6 cm. Pileipellis layer cutis form. Basidia were clavate, 19.41-123.88 x 5.62-6.68 µm in size, bearing double walled, ellipsoid, basidiospores (6.13-6.40 x 4.81-6.2 µm). Pleurocystidia obovoid, hyaline, thin walled, 6.13-6.40 x 4.81-6.2 µm. Cheilocystidia were clavate to pyriform, thin walled, hyaline, 13.78-32.46 x 15.90-23.28 µm in size. Pahlevanlo and Janardhana (2012) also characterized *T. eurrhizus* in similar way, from Kodagu district of Karnataka and N'Golo *et al.* (2013) from Germany. Ye *et al.* (2019) categorized sixteen *Termitomyces* isolates based on macro morphological characteristics among them, *T. eurrhizus* showed similar morphological characteristics with our species.

6. *Termitomyces schimperi*

This species was observed in a cluster of five with medium to large fruiting bodies, collected from Chirakkekode, Thrissur district. Pileus was convex, grayish white, 8.3 to 8.5 cm in diameter, with fissile margin. Perforatorium appeared conical to convex, dark brown in colour. Lamellae were thick, white (3.2- 3.4 x 0.5-0.7 cm). Stipe long, slender, cylindrical, hollow, 8.9-9.2 x 2.1-2.3 cm in size, with creamy white colour. Annulus present. Pseudorhiza creamy white, hollow, 15.0-22.4 long and 1.6-1.8 cm thick. Pileipellis layer ixocutis. From around the perforatorium area, the cuticle appeared to be easily getting peeled off as and when the sporocarp matured. Basidia were clavate, 17.24-18.33 x 5.47-5.59 µm in size bearing double walled, ellipsoid, hyaline, basidiospores (5.73-6.16 x 4.28-5.3 µm). Pleurocystidia fusiform, 19.91 x 5.61 µm in size. Cheilocystidia ellipsoid, thin walled, hyaline, 20.03 x 17.57 µm in size. This species was first reported by Heim (1942), whose morphological observations were analogous with the findings of this study, as well as those recorded by Mohanan (2011).

7. *Termitomyces striatus*

Out of 28 samples three samples were morphologically identified as *T. striatus*, which were collected from Thrissur and Wayanad districts. Pileus appeared plano-convex, grayish white, 10.0 to 15 cm in diameter with split margin. Perforatorium appeared as small conical, brown in colour. Lamellae thick, white (3.9-4.2 x 0.6-0.8 cm). Stipe long, straight, cylindrical, solid, 9.6-9.8 x 2.4-2.6 cm, with creamy white colour. Annulus was absent. Pseudorhiza was cylindrical, solid, creamy white, 3.0-3.2 x 2.1-2.3 cm in size. Pileipellis layer was ixocutis. Basidia oblong, tetra-sterigmated, 15.9-17.03 x 5.43-5.92 µm in size, bearing double walled ellipsoid, hyaline, basidiospores (5.3-6.17 x 4.87-5.46 µm). Pleurocystidia fusiform, hyaline, thin walled, 25.34-27.98 x 8.42-10.25 µm. Cheilocystidia elongated, thin walled, hyaline, 26.88-44.50 x 13.05-17.93 µm in size. These observed morphological characters of *T. striatus* are exhibited

equivalent with the *T. striatus* species illustrated by Roy and Pati (1981) with the name as *Schulzeria striata* and, Pegler and Vanhaecke (1994).

8. *Termitomyces clypeatus*

This species was identified based on the morphological observations. It is medium to large sized solitary fruiting body from Kadukutty, Thrissur district. Pileus was plano-convex to near flattened, grayish white, 6.0 to 6.3 cm diameter, with fissile margin. Perforatorium appeared spiny form, dark brown. Lamellae were thick, whitish (2.8-3 x 0.4-0.7 cm). Stipe, long, straight, cylindrical, solid, 7.2-7.4 x 2.4-2.5 cm in size, and slightly bulged towards the pseudorhiza. Annulus absent. Pseudorhiza dark brown, pointed towards the distal end, 5.9-6.0 x 2.4-2.5 cm. Pileipellis layer was cutis form. Oblong basidia, 6.82-7.15 x 5.90-6.24 μm in size, with double walled, ellipsoid, basidiospores (4.96-5.15 x 4.63-4.8 μm). Pleurocystidia were clavate, hyaline, thin walled, 22.36-28.9 x 11.58-19.98 μm in size. Cheilocystidia were oblong, hyaline, thin walled, 18.0-19.35 x 12.06-13.68 μm in size. In the study conducted by Srivastava *et al.* (2012) a similar feature of strong spiniform perforatorium, with a pileus diameter of 6 cm was recorded. Our illustration about this species showed more equivalence to the observations of Karun and Sridhar (2013) from Western Ghats and the west coast of India, Majumder *et al.* (2016) from Indian forests.

3.3

3.2. Habitat, diversity, distribution and soil type of collected *Termitomyces* spp.

Termitomyces microcarpus was the most predominant species observed across the three surveyed districts of Kerala, which consistently occurred during both the South-west and North-east monsoon seasons. It was typically found in clusters of 15–70 fruiting bodies. The widespread distribution of this species throughout Kerala may be attributed to its preference for diverse habitats, including areas with mixed vegetation, termite mounds, and moist regions along the homesteads. This species was present across all soil types in the surveyed districts, underscoring its adaptability. The growth and distribution of *T. microcarpus* are strongly influenced by the tropical climatic conditions prevalent in these districts, characterized by warm temperatures, high humidity, and consistent rainfall. With an annual temperature range of 20°C to 30°C, the climate provides an optimal environment for fungal development. Nair (1989) also reported that this species occurred in widely scattered groups of up to 100 fruiting bodies above termite combs in Kerala. Similar observations regarding its occurrence and distribution were made by Bhavani Devi (1982), Heim (1977), and Anukrishna (2023) from the Thiruvananthapuram and Kollam districts of southern Kerala.

The findings of *Termitomyces srilankensis* in the Thrissur and Wayanad districts of Kerala align with the initial report by Ediriweera *et al.* (2023) from the Sabaragamuwa Province, Sri Lanka. The similar climatic conditions between these regions, particularly the influence of the South-west monsoon season, are crucial to the species distribution. Both locations experience continuous rainfall, leading to consistently high relative humidity (85–90%), which creates a moist environment for fungal growth. Additionally, the annual temperature range of 20-35°C in both regions provides optimal thermal conditions for *T. srilankensis*. The organic content of homestead soil of Thrissur and forest loamy soil of Wayanad mirrors the nutrient-dense substrates found in Sri Lanka, which support the growth and reproduction of this species.

Two samples from Thrissur district were identified as *Termitomyces fuliginosus*, occurred either in cluster of two or as solitary fruiting bodies on the surface of termite mounds in mixed vegetation areas with lateritic soil. The termite

mounds provide a nutrient-rich substrate, as termites incorporate organic matter such as decomposed plant material, creating ideal conditions for fungal growth. The microclimate within the mounds, characterized by stable temperature, high moisture levels, and controlled ventilation, further supports the development of *T. fuliginosus*. The environmental conditions of Thrissur, including continuous rainfall during the South-west monsoon and high humidity, enhance the moist microclimate and nutrient availability, promoting the seasonal occurrence of this species. This findings was in concurrence with Anukrishna (2023) reported the presence of *T. fuliginosus* under comparable climatic conditions in the southern region of Kerala, specifically from a Venganoor in Thiruvananthapuram during the South-west monsoon of 2022, highlighting its consistent ecological preferences across the state.

Two samples collected from Alathur, Mala block and Pazhuvil, Thrissur district were identified as *Termitomyces heimii*, a gregariously occurring species found in scattered groups of 60–70 fruiting bodies under mixed vegetation and in homesteads. This species thrives in soils rich in organic matter and requires moist climatic conditions, both of which are prevalent in Thrissur due to its soil type, with dense tree cover and consistent rainfall during the South-west monsoon. The gregarious growth pattern can be attributed to the nutrient-enriched substrate provided by termite activity. Wide spread gregarious occurrence of *T. heimii* across Kerala in forest soils, indicating its adaptability to organic-rich substrates was documented by Nair (1989) and Mohanan (2011).

Two samples of *Termitomyces eurhizus* collected from the Thrissur and Wayanad districts, exhibited distinct morphological variations due to differences in the climatic conditions and soil types of the two regions. The specimens from Wayanad appeared more brownish compared to those from Thrissur, likely due to the higher nutrient and humus content in Wayanad's forest loamy soils and the region's consistently moist conditions, influenced by heavier rainfall. In contrast, the specimens from Thrissur, growing in lateritic soils with relatively lower humus content and slightly drier conditions, exhibited paler characteristics. Interestingly, the *T. eurhizus* collected from Thrissur closely resembled the specimens identified by Anukrishna (2023) from various parts of Thiruvananthapuram during the South-west monsoon of 2022. This similarity suggests that the species maintains consistent morphological traits under comparable climatic conditions and soil substrates, while deviations occur in response to localized environmental factors.

Only one sample of *Termitomyces schimperi* was collected from the Thrissur district, which was observed in a cluster of five fruiting bodies on the surface of a termite mound in January 2024. The fruiting bodies emerged during bright sunny days with warm and humid conditions and soil enriched with organic matter through the activity of fungal-farming termites. Mohanan (2011) also recorded this species from Nilambur and Thrissur in Kerala during 2009, where it was also found on termite mounds. These findings emphasize the ecological importance of termites in regulating microclimatic conditions within mounds.

Three samples were morphologically identified as *Termitomyces striatus*, collected from Vellanikkara region of Thrissur and Ambalavayal region of Wayanad districts. The two samples from Thrissur were found growing under mixed vegetation in lateritic soil, while the sample from Wayanad was observed in forest loamy soil under similar vegetation. Morphologically, the Wayanad sample was larger and more brownish compared to the Thrissur samples, suggesting regional variations influenced by differences in soil type and environmental conditions. These observations align with the descriptions of Roy and Pati (1981), regarding similar climatic requirements for *T. striatus* in West Bengal.

The sample of *Termitomyces clypeatus* collected from the Kadukutty region of Thrissur district, was observed in undisturbed areas of banana field with sandy soil. This species was observed during the South-west monsoon season under warm and humid climatic conditions. Similarly, Account of *T. clypeatus* from the Vellanad region of Thiruvananthapuram district, during the South-west monsoon was given by Nair (1989). These findings underscore the importance of the monsoon season, and the consistent rainfall and high humidity provide the moist environment essential for the fruiting of *T. clypeatus*.

4. CONCLUSION

The current study revealed the rich species diversity and distribution patterns of *Termitomyces* mushrooms in the districts of Thrissur, Wayanad, and Kasaragod, Kerala. The findings highlighted the significant role of Kerala's climatic conditions, soil types, and diverse habitats in supporting the growth and proliferation of these fungi. Based on the collections, it was noted that, *T. microcarpus* was the most prominent species, thriving across varied habitats during both monsoon seasons. *T. srilankensis* and *T. heimii*, also showed substantial distribution during the South-west monsoon. The study reiterates the prevalence of various *Termitomyces* spp. in varied agroecological niches in Kerala and the need for their conservation in these biodiversity-rich regions.

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