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Original Research

Morphological and Molecular Studies on *Termitomyces* Species of Menge District, Asossa Zone, Northwest Ethiopia

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Abstract

Despite the extensive study on plant and animal biodiversity in Ethiopia, our knowledge of microbial diversity in general and macrofungal diversity in particular is very limited. Thus, as part of the ongoing study on macrofungal diversity of the country, this is the first report on morphological and molecular taxonomy of the genus *Termitomyces* in the country. In this particular report, we identified seven *Termitomyces* species collected from Menge Districts, Asossa Zone, Benshangul Gumuz region. Identification was based on morphological characteristics and partial LSU rDNA sequences. The results of morphological identification of each of our collection were supported with molecular investigation. Phylogenetic analysis of partial LSU rDNA sequences of 7 Ethiopian *Termitomyces* species using Distance, Parsimony measurements and Maximum Likelihood presented similar inferred trees that only had minor differences. Based on these phylogenetic analyses of the partial LSU rDNA sequences, 7 species of *Termitomyces* were identified as *T. aurantiacus, T. clypeatus, T. eurrhizus, T. letestui, T. microcarpus, T. robustus* and *T. schimperi.* The inferred ML cladogram revealed both Asian and African *Termitomyces* samples in our analysis demonstrated a well-supported monophyletic group with bootstrap value of 99%. Moreover, the monophyletic tree from pure Ethiopian *Termitomyces* collection and a combination of African and Asian *Termitomyces* samples suggested their common origin. However incorporating more samples, more DNA markers and extensive analyses may reveal the true link among the sequences from different regions of the world. All the species reported are used for culinary purposes by the native community and few of them (*T. microcarpus* and *T. clypeatus*) are used for treatment of indigestion and malnutrition.

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INTRODUCTION

Termitomyces R. Heim 1942 comprises a group of gilled mushrooms termed termitophilic which live in association with a particular family of termites *Macrotermitinae (Isoptera*) commonly found in Africa and Asia with a warm and humid climate (Kirk *et al*., 2008). Once this group of macrofungi were reported as an independent genus by Heim (1942) with 10 species, a number of species of this genus have been described predominantly from tropical areas (Heim, 1952, 1977) mainly Africa (Heim 1942; Pegler 1977; Otieno 1964; Van der Westhuizen & Eicker 1990; Mossebo *et al*., 2002; Pegler and Rayner, 1969; Moriss, 1986), South America (Otieno 1964, Alasoadura 1966, Gómez, 1995) and South East Asia (Zhang *et al*., 1986; Wei *et al*., 2003, 2004; Tang *et al*., 2006). Altogether, 68 taxa have been published in this genus, with 81 names containing combinations and autonyms (Kirk *et al*., 2008), however the taxonomic statuses of some of them are still doubtful.

Termitomyces species are typically characterized by the termite association, pinkish spore print, smooth basidiospores, conspicuous perforatorium on pileus and underground pseudorhiza connected to the comb in the termite nest (Heim 1977, Frøslev *et al*., 2003). Recent molecular phylogenetic analysis on species in this genus showed that, they form a monophyletic clade in Agaricales (Moncalvo *et al*., 2000, Aanen *et al*., 2002), although this genus genetically show geographic variation between Asia and Africa species (Frøslev *et al*., 2003).

Although Ethiopia is known to have highly diverse flora and fauna (Friis and Sebsebe 2001) the diversity, ecology, and distribution of macrofungi in general and genus *Termitomyces* in particular has been poorly explored. However the genus comprises species which are the choice edible mushrooms, it has not been a focus of taxonomic investigation among mycologists in the country. The reports on this genus so far were mainly

based on the local use of these fungi for food and medicine among most communities in rural areas of South and South-West part of the country (Teferi *et al*., 2013). Any of these reports did not provide a detailed morphological description or any molecular data of the species in the genus, thus the taxonomic status of these species is not clear. However, there has been a recent molecular taxonomic work by Sitotaw *et al*., (2015) on the genus *Agaricus*.

From the ongoing study on the diversity of macrofungi of the country, this is the first report on taxonomic study based on morphological and molecular characteristics of *Termitomyces* collections from Menge districts, North-west Ethiopia. This paper provides a description of seven *Termitomyces* collections based on morphological diagnosis and molecular data analyzed using LSU rDNA. The results reported here will provide points of reference to facilitate taxonomic, ethnomycological, ecological and economic studies on Ethiopian *Termitomyces* species.

MATERIALS AND METHODS

Collection Site

Specimens were collected form Menge District, Asossa Zone, Benishangul Gumuz region, located in the Northwest part of Ethiopia. It is found 720 km North West of Addis Ababa and 40 km to the north of Asossa town (the capital of the region). The District is geographically located between 34° 30` to 35° 10`E and 10° 00 to 10° 30 N. Topography of the region is composed of mainly low land and plains and a few mountainous and gorges, altitude ranges from about 600 -1700 m a.s.l. About 55% of the total land area of the district is covered by natural vegetation, especially bamboo thickets, broad-leaved deciduous woodlands and acacia woodlands, grazing and cultivated land (BRFSS, 2004).

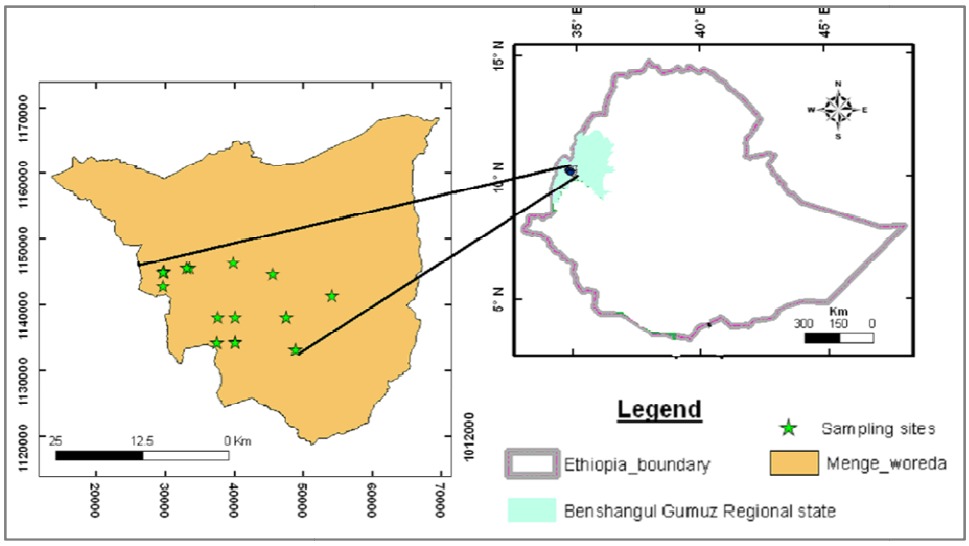

Figure 1: Map of Ethiopia and the study area

Meteorological data obtained from Ethiopian Meteorological Agency (Asossa Branch Office), the surrounding area has mean minimum and maximum temperatures of 12°C and 32°C, respectively. The temperature reaches a daily maximum of 20°C to 25°C in the rainy season and rises to 35°C to 40°C in the dry season (February to April). The rainfall in the area is unimodal and obtains high rainfall from May to October. Annual rainfall varies from 800 to 2000 mm. Generally, the rainfall is erratic from year to year (BRFSS, 2004).

Morphological Observation

Field collections were made during June–September, 2012–2014 in side forest, grazing land, around farm fields and backyards. Basidiomata were photographed (Canon A470) and their morphological and ecological characteristics were noted, labelled and brought to Mycology Laboratory, Addis Ababa University for detail microscopic and macroscopic characterization. Small pieces from the inner tissues of each specimen were collected using sterile forceps and stored at - 20°C for DNA extraction.

Microscopical observations were made from specimens mounted in 5% KOH and stained with 1% aqueous Melzer`s reagent and Congo red. Sections of pileus, lamellae and context were prepared with a razor blade and then observed under light microscope. At least twenty basidiospores were measured from each specimen. All the collections were dried and deposited in the department of Microbial, Cellular and Molecular Biology, Addis Ababa University, Ethiopia and duplicates are preserved in the Fungarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS). Morphological identification was assisted with basic literatures including Pegler (1977), Pegler and Piearce (1980) and Singer (1986).

DNA Extraction, PCR Amplification, and Sequencing

Total genomic DNA was extracted using the modified CTAB method described by Yao *et al.* (1999). The fungal universal primer pair LR0R/LR5 were used to amplify the nuclear larger subunit RNA (LSU) region (White *et al*., 1990). The PCR reaction mixture was comprised 25 µl Taq PCR MasterMix, 0.5 µl of 10 µM each primer, 1 µl diluted DNA template, and RNase-Free water to bring the total volume to 50 µl. The PCR conditions were set as follows: 94 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 53 °C for 1 min, 72 °C for 1 min and final extension step of 72 °C for 10 min on a GenAmp PCR System 9700 thermocycler (Vers. 3.03).

PCR products were sequenced from both directions using the same primers on an Applied Biosystems 3730 Analyzertm by the Beijing Genomics Institute (Beijing, China). Sequence ends were manually edited and assembled using DNAStar Lasergene SeqManTM II vers. 6.1. Sequences were then aligned using Clustal W algorithm (Thompson *et al*., 1994) and manually edited with BioEdit vers. 7.1.9 (Hall 1999).

Phylogenetic Analysis

Sequences of LSU were obtained from 10 *Termitomyces* collections in this study and aligned together with 35 closely related LSU rDNA sequences of *Termitomyces* species and 2 out-group taxa (*Lyophyllum semitale* and *Lyophyllum atratum)* which were downloaded from GenBank (Table 1). The LSU sequences obtained in this study were submitted to GenBank as KU933604 – 933612 and 933614. Evolutionary analyses were conducted in MEGA6 (Tamura *et al*., 2013). The evolutionary history was inferred by using the Maximum Likelihood (ML) method based on the Kimura 2-parameter model (Kimura, 1980). The tree with the highest log likelihood (-1318.5755) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying the Maximum Parsimony method. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 47 nucleotide sequences. There were a total of 595 positions in the final dataset.

Table 1: LSU sequences of *Termitomyces* and out-group species used in the phylogenetic analysis

Species	Voucher/isolate	Origin	GenBank* LSU	References
Termitomyces aurantiacus	tgf82	Tanzania	AY127804	Aanen *et al*. (2003)
	tgf89	Cameroon	AY232690	Froslev *et al*. (2003)
	tgf85	Thailand	AY232691	Froslev *et al*. (2003)
	HMAS273464	**Ethiopia**	**KU933605**	**This study**
	HMAS273466	**Ethiopia**	**KU933608**	**This study**
T. clypeatus	JMleg.MUID	Asia	AF261398	Moncalvo *et al*. (2002)
	tgf93	Burundi	AY127803	Aanen *et al*. (2002)
	PG	Malaysia	HM036342	Tan *et al*. (2010)
	HMAS273462	**Ethiopia**	**KU933604**	**This Study**
T. cylindricus	JM/leg.R.S	USA	AF042585	Moncalvo *et al*. (2000)
T. aff. entolomoides	tgf10	Malaysia	AY232692	Froslev *et al*. (2003)
T. entolomoides	tgf103	Africa	AY232693	Froslev *et al*. (2003)
T. eurhizus	tgf101	Burundi	AY232694	Froslev *et al*. (2003)
	HMAS273459	**Ethiopia**	**KU933607**	**This study**
	HMAS273458	**Ethiopia**	**KU933609**	**This study**
T. globulus	tgf11	Cameroon	AY232695	Froslev *et al*. (2003)
T. heimii	JM/leg.S	Asia	AF042586	Moncalvo *et al*. (2000)
	tgf9	Malaysia	AY232696	Froslev *et al*. (2003)
	TB	Malaysia	HM036345	Tan *et al*. (2010)
T. letestui	tgf16	Zimbabwe	AY127800	Aanen *et al*. (2002)
	tgf5	Cameroon	AY232699	Froslev *et al*. (2003)
	tgf83	Denmark	AY232698	Froslev *et al*. (2003)
	HMAS273763	**Ethiopia**	**KU933606**	**This study**
T. mammiformis	tgf100	Burundi	AY232701	Froslev *et al*. (2003)
	tgf102	Burundi	AY232700	Froslev *et al*. (2003)
	tgf92	Burundi	AY232703	Froslev *et al*. (2003)
T. medius	dka138	Cameroon	AY127796	Aanen *et al*. (2003)
	tgf70	Cameroon	AY232704	Froslev *et al*. (2003)
	tgf7	Cameroon	AY232705	Froslev *et al*. (2003)
T. microcarpus	tgf	Tanzania	AY127799	Froslev *et al*. (2003)
	tgf88	Tanzania	AY232708	Froslev *et al*. (2003)
	tgf80	Tanzania	AY232707	Froslev *et al*. (2003)
	tgf86	Zimbabwe	AY232706	Froslev *et al*. (2003)
	HMAS273461	**Ethiopia**	**KU933610**	**This study**
T. robustus	tgf95	Burundi	AY127797	Aanen *et al*. (2003)
	tgf72	Tanzania	AY232710	Froslev *et al*. (2003)
	tgf81	Tanzania	AY232709	Froslev *et al*. (2003)
	HMAS273465	Ethiopia	**KU933611**	This study
	HMAS273456	Ethiopia	**KU933612**	This study
T. schimperi	tgf18	Zimbabwe	AY232712	Froslev *et al*. (2003)
	HMAS273460	**Ethiopia**	**KU933614**	**This study**
T. singidensis	tgf74	Tanzania	AY232713	Froslev *et al*. (2003)
T. striatus	tgf99	Burundi	AY232714	Froslev *et al*. (2003)
T. subhyalinus	–	Switzerland	AF223174	Moncalvo *et al*. (2002)
T. titanicus	tgf96	Burundi	AY232715	Froslev *et al*. (2003)
	tgf94	Burundi	AY127801	Aanen *et al*. (2003)
Lyophyllum semitale	HC85/13	USA	AF042581	Moncalvo *et al*. (2000)
Lyophyllum atratum	CBS 709.87	Switzerland	AF223210	Moncalvo *et al*. (2002)

*** Sequences produced in this study in bold.**

RESULTS AND DISCUSSION

Phylogenetic Analyses

Phylogenetic analysis of partial LSU rDNA sequences of 10 Ethiopian *Termitomyces* collections using Distance, Parsimony measurements (trees not shown) and Maximum Likelihood presented similar inferred trees that only had minor differences. Based on these phylogenetic analyses of the partial LSU rDNA sequences, 7 species of *Termitomyces* were identified as *T. aurantiacus, T. clypeatus, T. eurrhizus, T. letestui, T. microcarpus, T. robustus* and *T. schimperi.* The inferred ML cladogram shown (Figure 2) both Asia and African *Termitomyces* samples in our analysis demonstrated a well-supported monophyletic group with bootstrap value of 99%. This result was corresponding with the previous phylogenetic studies on this genus (Aanen *et al*., 2002; Rouland-Lefevre *et al*., 2002; Froslev *et al*., 2003). Moreover, the monophyletic tree from pure Ethiopian *Termitomyces* collection and a combination of African and Asian *Termitomyces* samples may suggest their common origin.

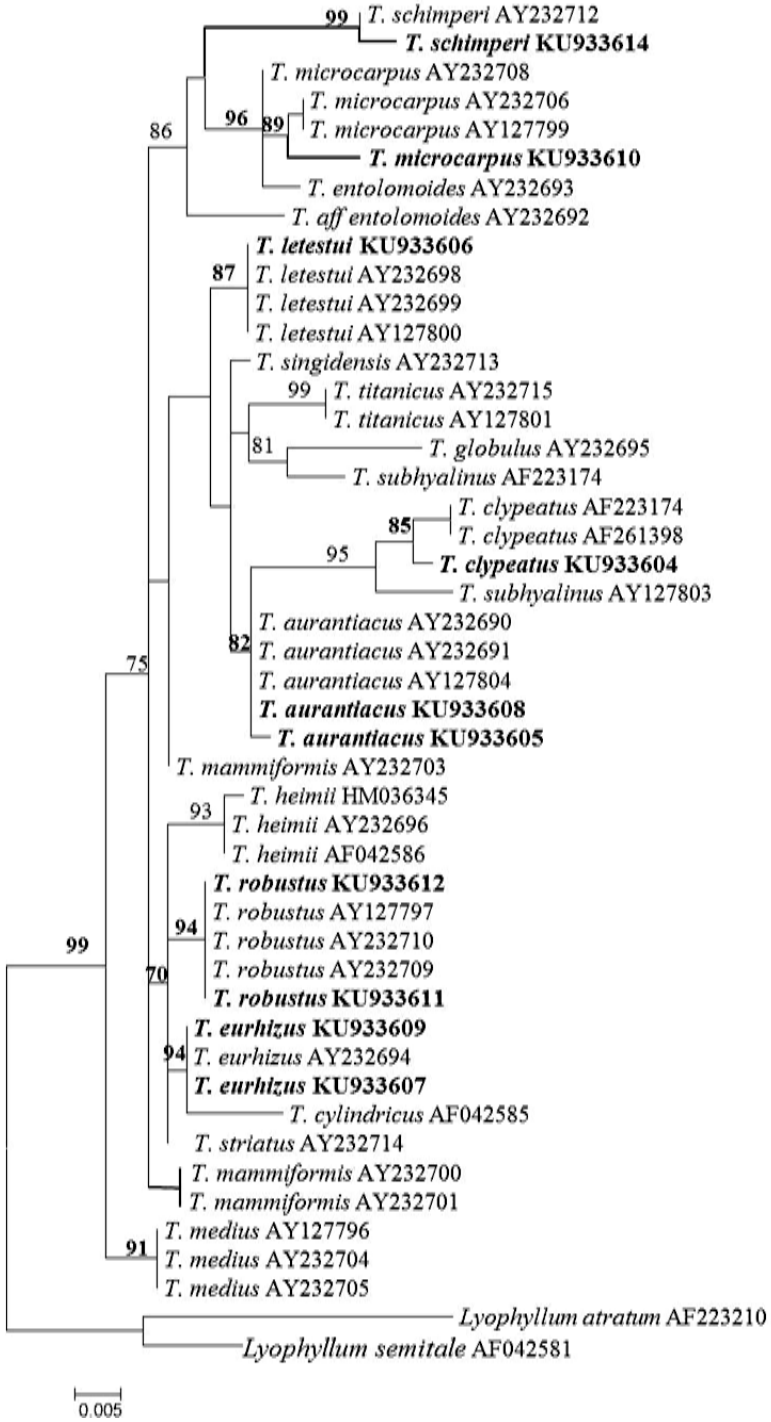

Figure 2: ML tree based on nrDNA LSU region sequences. The species in bold were sequenced by the authors. The percentage of trees in which the associated taxa clustered together is shown above the branches. Evolutionary analyses were conducted in MEGA6

Results of LSU sequence alignment showed that the two sequences of *T. eurhizus* (KU933607, KU933609) which were collected from different environments (Table 2) have identical sequences and similar results were observed with *T. robustus* (KU933611, KU933612). *Termitomyces microcarpus* (KU933610) and *T. clypeatus* (KU933604) collected from Menge have low LSU sequence similarity with both African and/or Asian collections, however they form a sister group with bootstrap support value of 89 and 85 respectively for the clade.

Except *T. robustus* and *T. schimperi* the rest 5 species of *Termitomyces* were also commonly found in the other region of Ethiopia as reported by Teferi *et al*., (2013). During the present study it was documented that all *Termitomyces* species are in use for culinary purposes during the rainy season by the native community but only few of them (*T. microcarpus* and *T. clypeatus*) are known for medicinal utility for treatment of indigestion and malnutrition.

Table 2: *Termitomyces* specimen examined from Menge District

Voucher	Taxa	Habitat	Geographical coordinates	Altitude (m.a.s.l)	Use	Vernacular Name
HMAS273464	*T. aurantiacus*	FA	10° 19' 51"E 34° 42' 33"N	1238m	E	Abenega
HMAS273466	*T. aurantiacus*	GR	10° 19' 48"E 34° 42' 32"N	1174m	E	Abenega
HMAS273462	*T. clypeatus*	FA	10° 18' 50"E 34° 42' 33"N	1238m	E & M	Akukufi
HMAS273458	*T. eurrhizus*	GR	10° 19' 48"E 34° 42' 31"N	1174m	E	Tsergunu
HMAS273459	*T. eurrhizus*	FA	10° 20' 41"E 34° 48',04"N	1174m	E	Tsergunu
HMAS273763	*T. letestui*	FA	10° 20' 41"E 34° 48' 04"N	1174m	E	Afifi
HMAS273461	*T. microcarpus*	FA	10° 17' 50"E 34° 44' 00"N	1174m	E & M	Aburalu
HMAS273465	*T. robustus*	FA	10° 19' 47"E 34° 51' 10"N	1135m	E	Gultse
HMAS273456	*T. robustus*	GR	10° 20' 10"E 34° 44' 35"N	1278m	E	Gultse
HMAS273460	*T. schimperi*	FO	10° 14' 05'E 34° 48' 18"N	1332m	E	Zoma

FO= Forest, FA= Farm area, GR= grazing land; In Vernacular name, quoted names are in Arutana (widely spoken language in the study area). E= Edible, M= Medicinal

Taxonomic Description of *Termitomyces* Collected from Menge District

Based on morphological characteristics, the habitat, geographic location and vernacular name all our collection provided in table 2. All identified species are described in detail and illustrated.

***Termitomyces aurantiacus* (R. Heim)** R. Heim in Termites et Champignons (Paris): 56 (1977). (Figure 3 (A1- 4))

Pileus 6–10 cm in diam., conical– applanate, with a small and pointed perforatorium; surface bright reddish/ orange to brown and darker at the centre, glabrous. Margin radially striate and splitting, slightly viscid when moist. Lamellae free, 3–6 mm wide, white and crowded, with few lamellulae. Stipe 6–7 × 1.5–2.0 cm, central; surface greyish white to brownish, smooth, solid; pseudorrhiza white, up to 15 cm, cylindrical but tapering downward the base, sometimes slightly swollen before tapering. Context white, thick and firm. Basidiospores deposit pinkish cream. Taste mild. Odor mushroomy.

Basidiospores 5–7.5 × 3.5–4.5 µm, average length (avl) × average width (avw) = 6.25 × 4 µm, Q (avl/avw)= 1.4 –1.6, avQ =1.5 ovoid to ellipsoid, thin-walled and subhyaline. Basidia 16–20 × 5–7.5 µm, clavate, bearing four sterigmata, hyaline and thin-walled. Lamella margin heterogeneous. Cheilocystidia 22–40 × 10–25 µm, clavate, thin-walled and hyaline. Hymenophoral trama subregular, thin-walled and hyaline hyphae, 4–15 µm diam. Clamp connection absent.

Remark: According to He (1985), *T. aurantiacus* can easily be distinguished from other species of the genus by its cylindrical pseudorrhiza and bright reddish to orange pileus and its firm texture (Pegler and Vanhaecke 1994) which is in line with our observation. However in this study authors didn't observe small squamules cited by He (1985) on the surface of the stipe. This may be due to the nature of the ephemeral remains of partial veil that can not be observed in mature material of *T. aurantiacus*. It is a well- known edible species sometimes appear in the local market during mid of the rainy season.

Termitomyces clypeatus R. Heim, Bull. Jard. bot. État Brux. 21: 207 (1951) (Figure 3. (B1- 4))

Pileus 4–6 cm in diam., conical becoming applanate with strongly spiniform to acutely pointed umbo and irregularly lobed margin; umbo at first brownish fading to ash-brown, lighter towards the margin, smooth, fibrillose, silky and viscid when wet, otherwise dry; context of pileus white, thin. Lamellae white to pinkish, free, crowded. Stipe 5–11 × 0.5–1 cm, whitish, solid, central, cylindrical and with a slightly bulbous base, with tapering pseudorrhiza. Annulus absent. Basidiospores deposit pinkish cream. Taste mild, pleasant. Odour mild, weak.

Basidiospores 5.5–7.0 × 3.5–4.5 µm, avl x avw = 6.25 x 4 µm, Q= 1.57 – 1.55, avQ= 1.56, broadly ellipsoidal, hyaline and smooth. Basidia 20–25 × 7–9 µm, clavate, bearing four sterigmata. Lamella margin heteromorphous with crowded cheilocystidia and basidia. Cheilocystidia 18–25 × 8–18 µm, pyriform, hyaline with a faintly thickened wall. Hymenophoral trama regular, hyaline. Clamp connection absent.

Remark: *Termitomyces clypeatus* is one of the smallest species of the genus. It partially resembles *T. tylerianus* in fruit body dimensions, but it differs in exhibiting a spiniform to acute umbo with a silky pileal surface and with greyish brown cap, whitish gills and long stipe with black pseudorrhiza (Pegler 1977). It is edible and used as medicine among the local people to treat

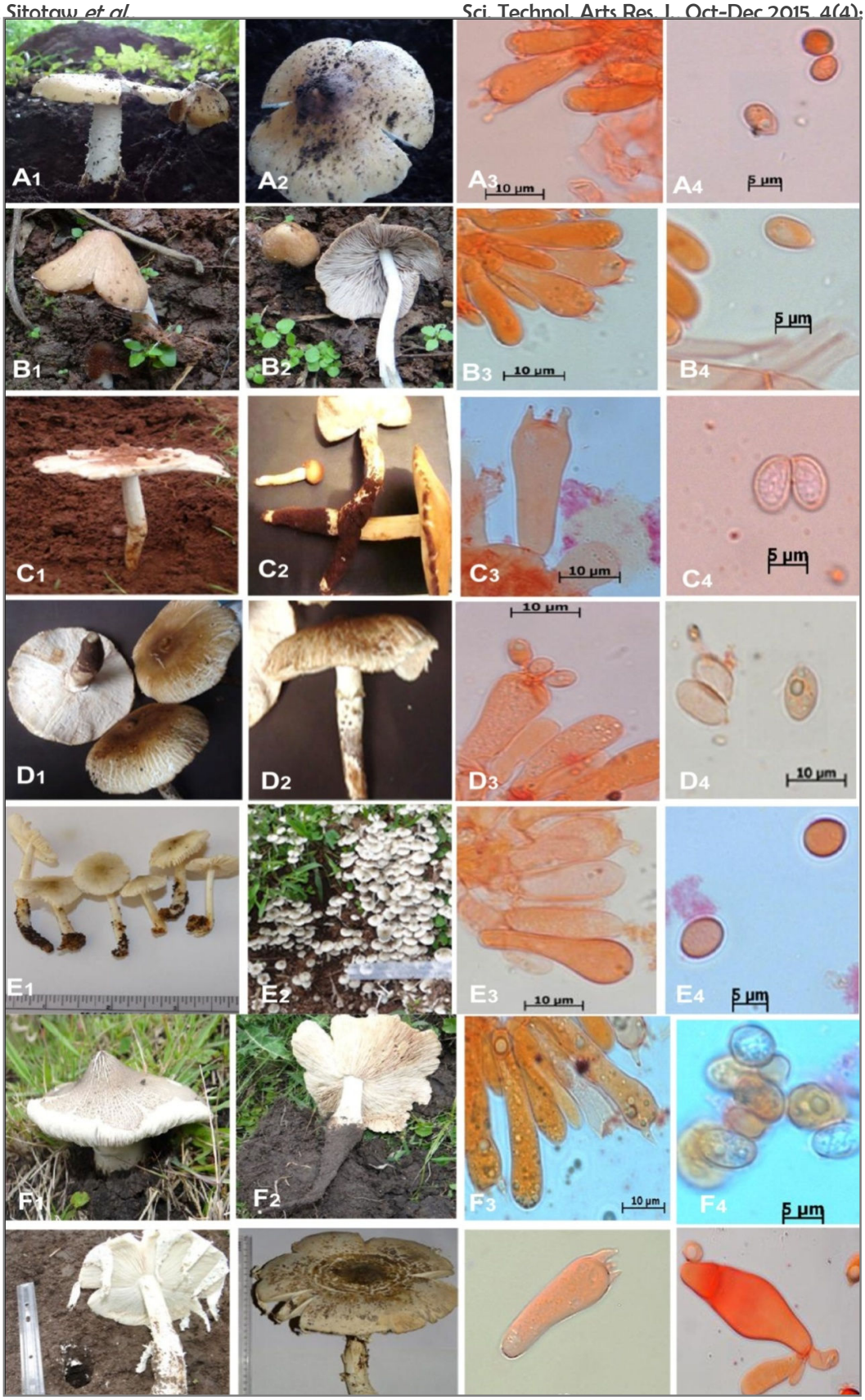

Figure 3: A1-A4: *Termitomyces aurantiacus,* B1-B4: *T. clypeatus,* C1-C4: *T. eurrhizus*, D1-D4: *T. letestui,* E1-E4: *T. microcarpus,* F1-F4: *T. robustus*, G1-G4: *T. schimperi*. 1& 2 represent- Basidiomata, 3- Basidia and Cheilocystida, 4- Basidospores

problems related to gastric and constipation in adults and to treat underweight children. The health benefit of this species also indicated on reports from West Africa and Asia (Anderson *et al*., 2013; Adhikari *et al*., 2005; Boa, 2004; Okhuoya and Akpaja, 2005).

Termitomyces eurrhizus (Berk.) R. Heim [as 'eurhizus'], Arch. Mus. Hist. Nat. Paris, ser. 6 18: 140 (1942) (Figure 3. (C1-4)).

Pileus 5–22 cm in diam., conico-campanulate then to nearly plane with a broadly ambonate prforatorium; surface greyish brown over the perforatorium becoming usually paler near the margin, silky striat towards the margin. Margin slightly incurved, splitting at maturity. Lamellae sub-free to adnexed, whitish cream to pinkish, 8-10 mm wide, moderately crowded with few lamellulae. Stipe 5–20 × 1–4 cm above ground, central, solid, cylindrical, surface whitish, attenuating as an elongate pseudorrhiza, up to 0.25m. Context fleshy, white, becoming thin towards the margin, thin-walled-hyphae, 3–5 µm diam. Basidiospores deposit brownish pink. Taste and odor mild, pleasant.

Basidiospores 6.0–8.5 × 4.0–5.5 µm, avl × avw = 7.5 × 5.0 µm, Q = 1.3 –1.4, avQ = 1.35 ellipsoid, hyaline, inamyloid, smooth, thin-walled. Basidia 20–25 × 6–10 µm, clavate, bearing four sterigmata. Lamella margin with crowded cheilocystidia. Cheilocystidia pyriform or rarely obovoid, 20–40 × 9–25 µm, hyaline with a slightly thickened wall. Hymenophoral trama regular of hyphae of 3-5 µm in diam., hyaline. Clamp connection absent.

Remarks: The special character of *T. eurrhizus* is the thick pseudorrhiza, which is black below the ground level. The structure of this species has been dealt with considerable detail by Petch (1913) who accepted two distinct forms; with persistent annulus and with out annulus commenting that the two forms were identical in size, shape, structure of pileus and gills, size and colour of spores. No persistent annulus was clearly observed in all of our *T. eurrhizus* collections in this study. However it is quite clear that the two forms are simply represent the same species. As described in Pegler (1977) the presence or absence of a ring is a more or less an accidental phenomenon brought about by a difference in the point of dehiscence of the universal veil. *T. eurrhizus* is a common species at grassland, edges of forests and cultivated fields in the study area. It is esteemed as delicacy by the indigenous people but not common at the local market.

Termitomyces letestui (Pat.) R. Heim, Arch. Mus. Hist. Nat. Paris, ser. 6 18: 109 (1942). (Figure 3. (D1-4))

Pileus 12–20 cm in diam., convex, with mammillate perforatorium, surface cream to light brown becoming dark brown or rust brown towards the center, finely squamulose at the disk. Margin incurved, splitting radially. Lamellae free, up to 6 mm wide, cream to pinkish, thin, crowded with numerous lamellulae up to 12mm broad. Stipe 8–15 × 1–3.5 cm, central, cylindrical, surface whitish, prolonged below in to tapering pseudorrhiza. Context white, firm, up to 25mm thick. Annulus white to whitish, membranous, superior and pendant. Basidiospores deposit pinkish cream. Taste mild and Odor strong and pleasant.

Basidiospores 5.5–8.5 × 4.0–5.5 µm, avl × avw = 7 × 4.7 µm, Q = 1.37 –1.5, avQ =1.4 obovoid to ellipsoid, hyaline, thin-walled. Basidia 20–25 × 5 –7 µm, clavate, bearing four sterigmata. Lamella margin heteromorphous with crowded cheilocystidia. Cheilocystidia 20–45 × 9–22 µm, mostly broadly clavate but sometimes cylinderic, with a fairly thick wall, hyaline. Subhymenial layer fairly broad, 7– 10 µm wide. Hymenophoral trama sub regular to vaguely bilateral, 2–6 µm diam., hyaline, with thin-walled hyphae. Clamp connection absent.

Remarks: It is recognized mainly by its large and fleshy pileus with a characteristic mammillate perforatorium and by the sheathing annulus. *Termitomyces letestui* is wide spread throughout Menge district and represent one of the largest species. It is one of the first mushrooms to appear at the beginning of the rainy season (mid of June). It is edible and highly prized due to its good test. It is offered for sale in local markets and on roadsides at quite an expensive price.

Termitomyces microcarpus (Berk. & Broome) R. Heim, Arch. Mus. Hist. Nat. Paris, ser. 6 18: 128 (1942). (Figure 3. (E1-4))

Pileus 1–1.5(2.5) cm in diam., campanulate to convex then expanding, broad convex to almost applanate, often umbonate, upper surface whitish to cream, darkening at the center, dry. Margin incurved or straight. Lamellae free, thin, white, 1-2 mm wide, moderately crowded with lamellulae. Stipe 2–4(–5) × 0.1–0.3 cm, central, solid, cylindric, sometimes with small bulbose base, surface whitish, smooth, lacking pseudorrhiza. Context white, thin. Annulus absent. Basidiospores deposit light pinkish. Taste mild. Odor mild, sometimes odourless.

Basidiospores 6.0–8.5 × 3.5–4.5 µm, avl × avw = 7.5 × 4.15 µm, Q = 1.5 –1.8, avQ = 1.65, ovoid to ellipsoid, hyaline, with usually one refractive guttule, inamyloid, thin-walled. Basidia 20–25 × 7–9 µm, clavate, bearing four sterigmata. Lamella margin fertile, sometimes with few cheilocystidia. Cheilocystidia and pleurocystidia similar, inconstant and often rare on the lamella edge, 15–40 × 9–16 µm, pyriform to cylindric, slightly thickened wall. Hymenophoral trama regular, hyaline hyphae. Hyphae of pileipellis 3–4 µm in diam., radially paralled hyphae. Clamp connection absent.

Remarks: Distinctive characteristics of this *Termitomyces* species is its small size, occurrence in dense troops and the absence of pseudorrhiza, however its association with termite, the presence of pinkish spore and other micromorphological characters that it shares with other species in the genus lead mycologists to put it under this genus. In contrast to previous researches (Pegler, 1977; Pegler and Vanhaecke 1994), *T. microcarpus* from Menge has cream colour spore deposit instead of the pink spore deposit. It is common and abundance around farming field and semi-opening bamboo forest in Menge District from end of June to August. It is edible and well-liked for its test and flavour and medicinal benefit, however due to its small size elder people do not prefer it since it takes much time to collect even for one meal.

Termitomyces robustus (Beeli) R. Heim, Bull. Jard. bot. État Brux. 21: 210 (1951). (Figure 3. (F1-4))

Pileus 7-11 cm diameter, at first conical then expanding to convex, pointed to perforatorium, surface grey with scrobiculate. Lamellae free, crowded 5-6 mm wide, ivory. Stipe 6-8 × 3-7 cm thick, white, solid, central, cylindrical slightly tapering downward. Context white, firm, up to 12mm thick. Annulus absent in mature specimens. Basidiospores deposit pinkish-cream. Taste and Odor mild.

Basidiospore 4-8×3-4.5 µm, avl × avw = 6 × 3.7 µm, Q = 1.3 –1.7, avQ =1.5 ovoid to ellipsoid, thin-walled, inamyloid, hyaline. Basidia 18–25 × 6 –8 µm, clavate, bearing four sterigmata. Lamella margin heteromorphous thin-walled, subhyaline. Cheilocystidia 25–40 × 10–20 µm, clavate, with a fairly thick wall, hyaline. Hymenophoral trama vaguely bilateral, 3–6 µm diam., hyaline, with thin-walled hyphae. Clamp connection absent

Remarks: *Termitomyces robustus* is readily recognized by its large, tough basidiomata with brown cap surface conical umbo and a swollen stipe and blackish psudorrhiza. The fruiting body appear after good rains from mid-July until end of September. It is edible and tasty.

Termitomyces schimperi (Pat.) R. Heim, Arch. Mus. Hist. Nat. Paris, ser. 6 18: 114 (1942) (Figure 3. (G1-4))

Pileus 18-40 cm in diam., convex to flat, lacking umbo, covered with thick large persistent scales concentric and forming plate-like covering at disc, surface white but stained light brown to reddish-brown by the soil of the mound. Margin entire, splitting radially, with persistent, long and membranous partial veil. Lamellae free to adnexed, up to 10 mm wide, whitish to cream, moderately crowded. Stipe 30–40 × 3–4 cm, cylindric, swollen towards the base then tapering to a long pseudorrhiza. context thick, white. Annulus absent. Basidiospores deposit cream with pinkish touch. Taste mild. Odor pleasant.

Basidiospore 5-9 × 4-5.5 µm, avl × avw = 7 × 4.7 µm, Q = 1.25 –1.6, avQ =1.4 ovoid to ellipsoid, thick-walled, inamyloid, hyaline. Basidia 18–30 × 5–8.5 µm, clavate, bearing four sterigmata. Lamella margin heteromorphous. Cheilocystidia 35–55 × 15–20 µm, clavate, with thick wall, hyaline. Hymenophoral trama bilateral, 4–6 µm diam., hyaline, with thick-walled hyphae. Clamp connection absent.

Remark: *Termitomyces schimperi* is well known for its large robust fruiting body, usually the cap may reach about 40 cm. in diam (Pegler, 1977; Pegler and Piearce, 1980; Singer, 1986). It is easily recognized by its rough scaly cap and stem, the brown scales of the cup usually in the form of radiating concentric rings unlike many other *Termitomyces* species it lacks an umbo. Usually found on top of termite hills. It usually appear after heavy rains from July to mid September which is the main rainy season in the study area.

CONCLUSION

Termitomyces is a paleotropical genus and many more new taxa of *Termitomyces* are likely to be reported from this part of the world. Being in tropics, there is a high possibility of finding new *Termitomyces* species to the world and new records to the country this underscores the need for further study of the genus. As this is the first report on morphological and molecular taxonomy of this genus, we hope that many more reports on *Termitomyces* species will follow. This study also confirmed that phylogenetic analysis of LSU rDNA sequences useful approach which helps to identify *Termitomyces* species in a combination with morphological classification.

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Conflict of Interest

None Declared.

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