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Diversity, properties and ecological significance of the genus *Termitomyces* associated with fungus farming termites in Africa

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Abstract

The fungus-cultivating termites constitute part of diverse termite fauna in Africa that include *Macrotermes, Odontotermes, Microtermes* among others. Mutualistic relationship between lineages of fungi (*Agaricomycetes, Lyophyllaceae, Termitomyces*) and the fungus cultivating termites remains obscure. The complex association between the *Termitomyces* symbionts and termites is due the distinct organization strategies of members in the genus *Termitomyces* and other micro-symbionts including *Xylaria/Pseudoxylaria* within the termite mounds. We have reviewed the diversity, properties, and ecological significance of the genus *Termitomyces* in Africa as a continent, which harbours diverse species of flora and fauna. The organization of these fungal species within the mounds is exceptional and leniently forms a distinct micro-environment that supports diverse species of micro-organisms. Enzymatic assays have also revealed that *Termitomyces* species play significant roles in balancing the ecosystem within the termite mounds. The review has established sixteen *Termitomyces* species that have been recently published in Africa. In comparison to the diverse fungus-farming termites, this is an indication of limited findings. The diversity of *Termitomyces* species is still underexplored despite its interesting symbiotic interactions; hence, newly emerging methods to improve its classification and mutualistic association should be explored.

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Introduction

Fungus-growing termites are known to occur throughout the old world tropics but they originated from the African rainforests (Nobre and Aanen, 2010). African tropics harbour more diversity of the fungus-growing termites of which one-third of the 2600 termite species recorded to date are from Africa (Aanen *et al.,* 2002). About 100 species of the one-third are fungus-farming termites (Aanen *et al.,* 2007). The fungus symbionts belong to the genus *Termitomyces* which are the higher fungus of the Class *Basidiomycetes* (Aanen *et al.,* 2007). The origin of these symbionts is associated to the ancient rain forests of central Africa (Aanen *et al.,* 2007). However, the existence of the termites and their *Termitomyces* symbionts is exceptional. They have a tight mutual relationship and existing of either partner independently has always remained a problem (Otieno, 1968). The ancient research on *Termitomyces* might have been possible from the rain forests due to the existence of the favourable climatic conditions allowing the growing of the fruiting bodies from the termite nests. Alasoadura (1967), describes *Termitomyces* as weather oriented organisms. The fruiting bodies are hardly seen during dry seasons. However, they are much available during rainy seasons. The usual appearance of the fruiting bodies is between April through October; rainy periods in Nigeria. Notably, this termite-fungus symbiotic relationship has always played a key role in the survival of both the termites and the *Termitomyces* symbionts. To the present knowledge, both partners (Termites and *Termitomyces*) cannot survive independently in the ecosystem. Termites provide a conducive environment, which is a standard microclimate, substrate and a free space for the fungal growth (Aanen *et al.,* 2002; Frøslev *et al.,* 2003). Alternatively, *Termitomyces* are primarily described to be providing certain enzymatic metabolites that enable them to predigest harder plant components, making such plants easier for the termites to assimilate (Khowala and Sengupta, 1992; Aanen *et al.,* 2007). In certain exceptional circumstances, nitrogen rich fungal nodules and old fungus combs serve as nutritious source of food to the termites. In addition, these substances (nitrogen rich fungal nodules and old fungus combs) have also been noted to stabilize the carbon/nitrogen ratios in termite diet (Wood and Thomas, 1989; Darlington, 1994; Hyodo *et al.,* 2003). However, the contribution of the symbiont fungi to the termites is not yet exhausted. In this review, we have studied the current status of *Termitomyces species* in Africa. Our review focuses on various issues which have been noted as limiting factors like the evolutionary history. There are also elaborated aspects of properties and organizational characteristics *Termitomyces species* in Africa. We have further highlighted the possible gaps that will facilitate full characterization and identification of *Termitomyces species* in Africa.

Organization of Termitomyces fungi within the termite nests

Most ancient studies on *Termitomyces* focused on the visible fruiting bodies which can be easily seen from the termite mounds. Through such studies, limited knowledge on the ecological systematics of these fungi was documented. Makonde *et al.* (2013), reveals the synergistic existence between the termites and the *Termitomyces* symbionts. The study outlines the coexistence of *Termitomyces* with other silent contaminant micro-symbionts like the *Xylaria* and *Pseudoxylaria.* However, these contaminant micro-symbionts always bloom when the termite-*Termitomyces* ecosystem is disturbed or when the termites are isolated from the combs. This is another aspect that remains complicated in the study of *Termitomyces* and their symbiont termites.

The current preliminary studies show that *Xylaria* is more active and grows within 24 hours when the fungus combs are removed from the termite nest. In the same process, fungus nodules also get oxidised losing their viability. These features show a complex relationship among the fungus farming termites, *Termitomyces, Xylaria/Pseudoxylari* and other micro-symbionts whose activities are little known. Another interesting finding from (Makonde *et al.,* 2013) is the coexistence of two different genera of fungus cultivating termites within a mound. These are the *Macrotermes* and *Microtermes species,* which colonize the bottom and top parts of the mound, respectively. The most important and difficult question is how these two termite genera manage to cultivate different *Termitomyces species* within a single mound (Makonde *et al.,* 2013) regardless of their foraging strategy in which the respective workers interact since *Macrotermes* workers have to pass through the *Microtermes* part of the mound. Moreover, the studied *Termitomyces* are not only ecto-symbionts but they are also the most abundant fungi group colonizing the termite guts. This has been shown by termite clone libraries of three genera (Makonde *et al.,* 2013). The case of host symbiont specificity within a mound needs to be resolved. However, the mutual relationship between the termites and their symbiont *Termitomyces* seems to be an eternal complex association. Some termite genera have been reported to cultivate more than a single species of *Termitomyces* in different mounds. Closely related fungus haplotypes cultivated by a single termite species have been reported from recent studies from Kenya, and South Africa (Aanen *et al.,* 2007). These findings have been closely related to earlier studies in Africa. However, findings by Osiemo *et al.* (2010) equally contradict the existing knowledge. The data suggest that the earlier information on termite-symbiont host low specificity is indirectly related to the diversity and distribution of *Termitomyces.* Osiemo *et al.* (2010), emphasizes that, the occurrence of *Termitomyces* is totally independent of the immediate surrounding environment. Their symbiont host termite provide fully favourable environment that support their life within the mound. This means that the longevity and existence of *Termitomyces* depend mainly on the termite host but not the environment. The *Termitomyces* fungal occurrences may depend on the necessary host activities and shared mutual benefits from the haplotypes and host termites.

Other recent study by Zeleke *et al.* (2013) suggests that the finding from (Osiemo *et al.,* 2010) is absolutely true. Findings by Zeleke *et al.* (2013) shows that, the ecological system of the fungus farming termite mound is inclusive of all the necessary nutrients needed by the *Termitomyces.* This further elaborates the hanging information on the existence of the pseudo-micro-symbionts within the host termite mound. The moisture content of the termite combs are in lower levels (52.7±2.55% w/w) and they are also acidic (pH, 4.5±0.05). Such conditions are not favourable for the growth of most bacteria and certain fungal genus. Most known bacteria prefer conditions of high moisture content and neutral pH conditions (Zeleke *et al.,* 2013). Such conditions are not found in any environmental conditions. However, termites that are also referred to as the ecosystem engineers are the only organisms that can create such conditions.

Studies elsewhere by (Nobre and Aanen, 2010), suggest that the occurrence of *Termitomyces* is rather dependent on the foraging strategies of their host termites. Colony of *Termitomyces species* can be established in a given mound through vertical or horizontal transmission (Nobre and Aanen, 2010). Horizontal transmission always occurs across different locations within single environmental or different environmental conditions. It always occurs when the host termites collect new *Termitomyces* colonies during their normal foraging strategies in the ecosystem (Nobre and Aanen, 2010). However vertical transmission is inclined to the species parental lineage within the micro-ecosystem (Nobre and Aanen, 2010).

The limited taxonomic classifications of *Termitomyces* have always created misunderstanding in the termite-*Termitomyces* association. The question posed by (Makonde *et al.,* 2013) on taxonomy of the *Termitomyces* still remains to be answered. Nevertheless, even if the taxonomic classification is poor among these fungus genera, we firmly believe that host termite symbionts provide the necessary requirements to support this fungus growth. Study by Zeleke *et al.* (2013) reveals that, the content of soluble proteins from the termite combs play important role in the development of the *Termitomyces.* The presence of the soluble proteins and nitrogen in the termite comb is associated with the number of the fungus nodules colonizing the comb (Zeleke *et al.,* 2013). High number of fungus nodules is associated with high nitrogen and soluble protein components in the comb.

Tibuhwa (2012) refers to the symbiont *Termitomyces* as cultivate of the farming termites. He used a Scanning Electron Microscope (SEM) that showed nodules (sporodochia) as white stuffs scattered over the surface of the termite comb. The microscope further revealed the sporodochia to be a massive component of big viable cell which under favorable conditions differentiate to form conidia. The cultivated *Termitomyces* colonizes the fungus comb or garden that is compost of dead plant materials broken down by the host termite symbionts within the caste (Tibuhwa, 2012).

Ecological and Physiological Impacts in the Ecosystem

There are several assays which have been carried out to establish the ecological and physiological significance of *Termitomyces* species within their inclined ecosystem with the termites (Rouland *et al.,* 1988; Nobre and Aanen, 2010; Zeleke *et al.,* 2013; Makonde *et al.,* 2013). However, there is continuous publication of contradicting results depending on the location and region of the study in Africa as a continent. Some *Termitomyces* species have been published to fully degrade a wide-range of substrate components from the plant materials that are found associated with the termite mounds (Zeleke *et al.,* 2013; Makonde *et al.,* 2013).

Generally, *Termitomyces* are known to contribute sufficiently in the degradation of cellulose substrates from plant materials (Martin and Martin, 1978; Rouland, 1988; Rouland-Lefevre, 2000). This suggests that these fungus species maintains the termite ecosystem by improving their assimilation and feeding strategies. Some studies from the African *Termitomyces* species indicate that they have xylanase and cellulase activities with limited degradation of wide-range carbon sources (Makonde *et al.,* 2013). This information is partially supported by results from elsewhere (Zeleke *et al.,* 2013). Zeleke *et al.* (2013) demonstrated that the comb extracts to have xylanase activity of 8.27±0.14 U g^{-1} with no cellulase activities detected. This has created a dilemma on the perceived cellulose utilization by these fungus species.

Some *Termitomyces species* are compared to fungus species due to capability of utilizing wide range of different substrate compounds (Kaura, 2010). *T. striatus* is one of the known species to degrade complex and toxic carbon and nitrogen compounds (Kaura, 2010). It resembles *Morchella hybrid* (Sharma, 2003) and *Ustilago esculenta* (Chung and Tzeng, 2004) by utilizing glucose, D (+) raffinose, D (-) fructose. By utilization of the toxic nitrite substrates, it is compared to the edible fungi, *Morchella esculenta* and other species within the same genus (Morton and MacMillan, 1954).

Termitomyces species have also been shown to maintain micro-biome cultures within the relative host mounds. The low moisture content and acidic conditions are apparently unfavorable conditions for the growth of competitive bacterial cultures (Zeleke *et al.,* 2013). This is equally important for the ecological balance between the fungus and the termites. Termites are able to carry out their foraging practices within their nests fully without interruption by other micro-cultures within their ecosystem.

Diversity of Termitomyces in Africa

Recent studies have diversified the existence of *Termitomyces* species in Africa. The diversity of this fungus genus is absolutely dependent on the possible symbiont relative termites. In Africa, the number of fungus-farming termites' species is approximately 165 that belong to 11 genera (Kambhampati and Eggleton, 2000), which are underestimated considering on the novel termite species that are still discovered (Makonde *et al*., 2013). However, there are more *Termitomyces* species since a single termite species can cultivate more than one *Termitomyces* symbiont (Makonde *et al.,* 2013). Studies by Osiemo *et al.* (2010) indicate that the diversity of *Termitomyces* species is defined by the genus of the host symbionts in an ecosystem.

The published *Termitomyces* species are about 30 (Kirk *et al.,* 2008). This shows limited information compared to the number of fungus-farming termite species. In addition, if one termite species can cultivate more than a single fungus species, then more is yet to be revealed in the continent. With the little research in Africa, results have been published to show state *Termitomyces* diversity. Data that have been drawn from current publications show that (Härkönen, 1995) sampled 5 *Termitomyces* species; (Katende *et al.,* 1999) sampled 4 *Termitomyces* species; (Makonde *et al.,* 2013) sampled 9 *Termitomyces* species; (Osiemo *et al.,* 2010) sampled 8 *Termitomyces* species; (Otieno, 1964) sampled 5 *Termitomyces* species; (Otieno, 1968) sampled 4 *Termitomyces* species; (Oyetayo, 2012) sampled 7 *Termitomyces* species; (Pegler, 1977) sampled 8 *Termitomyces* species; (Tibuhwa, 2012) sampled 10 *Termitomyces* species; (Wood and Thomas, 1989) sampled 2 *Termitomyces* species; (Botha and Eicker, 1991) sampled 5 *Termitomyces species*; and (Van der Westhuizen and Eicker, 1990) sampled 7 *Termitomyces* species as illustrated in Table 1.

Table 1. Species of *Termitomyces* species in Africa.

Species	Region of Collection	Source *
T. clypeatus	Nigeria, Kenya, Uganda, Tanzania, South Africa	1, 2, 7, 8, 9, 10
T. robustus	Nigeria, Kenya, Uganda	1, 10, 11
T. microcarpus	Nigeria, Kenya, Tanzania, Uganda, South Africa	1, 2, 5, 6, 7, 8, 9, 10, 11
T. aurantiacus	Tanzania, Uganda	5, 6
T. globulus	Kenya	10
T. letestui	Tanzania, Kenya, Uganda	5, 6, 12
T. mammiformis	Tanzania	10
T. rabuorii	Kenya	11
T. schimperi	Kenya, Ethiopia, Tanzania, South Africa	8, 9, 10, 11, 12
T. singidensis	Tanzania	5
T. tyleranus	Kenya	12
T. striatus	Kenya, Uganda, Congo, South Africa	1, 2, 8, 9
T. eurhizus	Kenya, Tanzania, Uganda, Congo	2, 5, 6, 11
T. umkowaani	Tanzania, South Africa	1, 3, 7, 8
T. titanicus	Tanzania	3
T. saggitiformis	Tanzania, South Africa	3, 7, 8
T. reticulatus	South Africa	7, 8
T. heimii	Kenya	2

Key: Source *

[1]Oyetaya, 2013; [2]Makonde *et al.,* 2013; [3]Tibuhwa, 2012; [4] Osiemo *et al.,* 2010; [5] Katende *et al.,* 1999; [6]Härkönen *et al.,* 1995; [7]Botha and Eicker, 1991; [8]Van der Westhuizen and Eicker, 1990; [9]Wood and Thomas, 1989; [10]Pegler, 1977; [11]Otieno, 1968; [12]Otieno, 1964.

Conclusion

Termitomyces symbionts and the fungus-farming termites have an exceptional relationship which benefits both the organisms. However, the existing problem of classifying fungal species of the genus *Termitomyces* remains unresolved. The recorded number of fungus-cultivating termites in Africa is relatively high compared to the established number of *Termitomyces species* that they cultivate. In this review we have recognized 17 *Termitomyces species* from Africa. This is fairly lower number compared to the approximated 100 fungus-farming termites in the entire continent. Considering both the vertical and horizontal transmission of the *Termitomyces species,* there could be a possibility of novel *Termitomyces* species to be discovered in the termite-symbiont ecosystem. The approaches that have been published on classification of *Termitomyces species* have not provided satisfying data so far. More promising research is now focusing on the macro-morphological and molecular methods. These however, have not provided substantial information to solve the taxonomic problem of the *Temitomyces* species and their complex mutualistic nature with their host termites. There is need to establish other methods that would address such problems in the future. Combined molecular and culture-dependent studies based on the extractable features from the culture material can be used. Teleomorphic characteristics could have answered the taxonomic question, nonetheless, some species in this fungus genus rarely or do not express the teleomorphic features. This would accelerate the biotechnological exploration of *Termitomyces* species with their extraordinary plant-biomass biodegradation potential.

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