Diversity of fungus-growing termites (Macrotermes) and their fungal symbionts (Termitomyces) in the semiarid Tsavo Ecosystem, Kenya

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ABSTRACT

Fungus-growing termites of the subfamily Macrotermitinae together with their highly specialized fungal symbionts (*Termitomyces*) are primary decomposers of dead plant matter in many African savanna ecosystems. The termites provide crucial ecosystem services also by modifying soil properties, translocating nutrients, and as important drivers of plant succession. Despite their obvious ecological importance, many basic features in the biology of fungus-growing termites and especially their fungal symbionts remain poorly known, and no studies have so far focused on possible habitat-level differences in symbiont diversity across heterogeneous landscapes. We studied the species identities of *Macrotermes* termites and their *Termitomyces* symbionts by excavating 143 termite mounds at eight study sites in the semiarid Tsavo Ecosystem of southern Kenya. Reference specimens were identified by sequencing the COI region from termites and the ITS region from symbiotic fungi. The results demonstrate that the regional *Macrotermes* community in Tsavo includes two sympatric species (*M. subhyalinus* and *M. michaelseni*) which cultivate and largely share three species of *Termitomyces* symbionts. A single species of fungus is always found in each termite mound, but even closely adjacent colonies of the same termite species often house evolutionarily divergent fungi. The species identities of both partners vary markedly between sites, suggesting hitherto unknown differences in their ecological requirements. It is apparent that both habitat heterogeneity and disturbance history can influence the regional distribution patterns of both partners in symbiosis.

Key words: Basidiomycota; habitat ecology; Lyophyllaceae; Macrotermitinae; savanna; specificity; symbiosis.

ASSOCIATIONS BETWEEN THE FUNGUS-GROWING TERMITES (MACROTERMITINAE, TERMITIDAE, BLATTODEA) AND THEIR MUTUALISTIC FUNGI (TERMITOMYCES, LYOPHYLLACEAE, BASIDIOMYCOTA) are among the most sophisticated symbiotic interactions in the insect world. Termitomyces fungi are actively cultivated by the termites, and they sustain the insect colony by decomposing plant matter collected by the termite foragers. Foraged and partly digested plant material is stored in sponge-like structures (fungal combs) located in several specialized underground galleries within the nest, and fungal decomposition of the plant material takes place in the combs (Wood & Thomas 1989). Termites regulate the nest temperature, humidity, and gas exchange by building elegant ventilation structures, so that the growth conditions for fungi remain favorable throughout the year (Korb & Linsenmair 1998, Korb 2003). All termites in the colony are nourished by small spherical structures (nodules), containing asexual spores of the fungal symbiont and/or by plant material decomposed by the fungus (Wood & Thomas 1989). By feeding on Termitomyces mycelium termites also ingest fungal enzymes that enable effective decomposition of lignocelluloses and other biopolymers to start already in guts of termites together with insect and gut bacteria-derived enzymes (Martin & Martin 1978, Nobre & Aanen 2012, Poulsen et al. 2014). The symbiotic relationship between the fungus-growing termites and their Termitomyces symbionts is obligatory since none of the partners can survive without each other.

Termites are primary litter decomposers and soil ecosystem engineers in arid and semi-arid African savannas. In many regions, the majority of all dead wood and plant litter produced is transported into the nests of fungus-growing termites where it is effectively decomposed by the symbiotic fungus (Jones 1990, Dangerfield et al. 1998, Jouquet et al. 2011). Thus, the presence or absence of fungus-growing termites has profound effects on litter decomposition rates, soil horizonation, and soil nutrient content (e.g., Jones 1990, Jouquet et al. 2011, Erens et al. 2015). Termites also facilitate the succession of savanna vegetation (Sileshi et al. 2010, Joseph et al. 2013, Traoré et al. 2015) and can increase the robustness of the ecosystems toward desertification (Bonachela et al. 2015).

Phylogenetic reconstructions indicate that the initial establishment of the fungal symbiosis among termites occurred only once, most likely in African rain forests approximately 30 million years ago (Aanen et al. 2002, Aanen & Eggleton 2005, Brandl et al. 2007, Nobre et al. 2011a, Roberts et al. 2016). Since then, the fungus-growing Macrotermitinae have radiated into 11 genera with ca 330 described species (Kambhampati & Eggleton 2000, Aanen et al. 2002). Several

lineages have expanded into savannas and semiarid shrublands, enabled by their climate controlled nests, where both the termites and their fungi are protected against environmental extremes (Aanen et al. 2002, Aanen & Eggleton 2005).

Fungus-growing termites of the genus Macrotermes build large termite mounds that characterize many savanna landscapes in sub-Saharan Africa. In southern Kenya, *Macrotermes subhyalinus* and *Macrotermes michaelseni* are thought to be the two dominant species. They are closely related and morphologically nearly identical, but can usually be easily distinguished on the basis of mound architecture: the mounds of *M. subhyalinus* have open ventilation shafts, while those of *M. michaelseni* are closed, with gas exchange taking place through the rough and porous outer surface of the mound (Arshad 1981, Darlington 1984, 1985, Bagine et al. 1994). The species are believed to be largely sympatric, but with *M. michaelseni* being more common at higher and *M. subhyalinus* at lower elevations, presumably reflecting species-specific differences in their climatic optima (Bagine et al. 1994, Pomeroy 2005).

Macrotermes species are known to associate with several different taxa of *Termitomyces*, many of which appear to represent distinct species (Aanen et al. 2002, Rouland-Lefevre et al. 2002, Froslev et al. 2003, Osiemo et al. 2010, Makonde et al. 2013). Each termite nest has been found to always contain only one *Termitomyces* species, maintained as a monoculture of a single heterokaryotic clone in all galleries (Aanen et al. 2002, 2009, Katoh et al. 2002, De Fine Licht et al. 2005, Moriya et al. 2005, Long et al. 2010, Makonde et al. 2013). *Macrotermes* species seem to have their own set of symbiotic *Termitomyces* species that are not farmed by other genera of fungus-growing termites (Aanen et al. 2002). Several different *Macrotermes* species have been found to associate with the same fungal symbiont, some *Macrotermes* species can culture more than one Termitomyces species in different nests even within seemingly homogenous habitats (Aanen et al. 2002, Osiemo et al. 2010). However, so far, no studies have tried to determine patterns of symbiont specificity at the landscape-level, nor tried to elucidate possible correlations between specificity patterns and habitat variability. Such studies are clearly needed to deepen our understanding of the ecology and ecosystem impacts of fungus-culturing termites.

Here, we study the regional diversity of Macrotermes species and their Termitomyces symbionts with DNA methods. The biological material was collected from eight different savanna and shrubland habitats within the semiarid Tsavo Ecosystem in Southern Kenya. We focused on the species of the genus *Macrotermes* because of their high abundance in the study area. Mature *Macrotermes* nests are also easy to locate because of their visible above-ground mounds that

enabled us to determine reliably the total host-symbiont diversity in each studied site. Grasslands and environments with abundant woody vegetation represent contrasting habitats for wood-, litter- and grass-consuming termites and their symbiotic fungi. Knowing how differences in vegetation and other habitat variation possibly affects the diversity patterns of termites, and their fungi is essential for evaluating the dependence and ecological impacts of fungusgrowing termites on variable savanna landscapes.

MATERIAL AND METHODS STUDY AREA AND SAMPLING.—A total of 143 *Macrotermes* mounds were excavated from eight locations in the Tsavo Ecosystem in 2014–2015. All study sites were in the semi-arid plains around the Taita Hills and Mt Kasigau in Taita-Taveta County, southern Kenya (Fig. S1). The elevation of the plains is between 500 and 1100 m a.s.l., with two mountain regions, the Taita Hills and Mt Kasigau, rising up to 2208 and 1641 m, respectively. The mean annual temperature is ca 25°C and the mean annual precipitation is ca 600 mm, with two rainy seasons in November–December and March–May, respectively. The study sites were selected to represent a wide range of savanna, shrubland, and woodland vegetation types (Table 1). At each site, we first searched all *Macrotermes* mounds and the location of each mound was georeferenced with GPS (Garmin, Oregon 550). Mounds that were obviously actively maintained by the termites and/or had elevated CO_2 levels in their ventilation shafts were classified as active nests, while the others were classified as inactive. Carbon dioxide levels of the ventilation shafts were measured with a portable CO_2 meter (Testo 535, accuracy +/– 75 ppm within the scale 0 to 5000 ppm). Active nests typically showed values more than 1000 ppm in one or several outflow shaft, whereas CO_2 levels of the inactive mounds were always <500 ppm. In cases of closed mounds, we made small holes into the turret walls and then inserted the sensor into the exposed ventilation shaft.

We collected biological specimens for DNA analysis from most active nests at each study site. At least one fungal gallery of each termite colony was accessed by digging from the base of the mound, generally without major damage to the mound structures. The termite colonies seemed to tolerate the disturbance well as in most cases the chambers were sealed within a few days. Several termites (soldiers and workers) and a large quantity (dozens) of fungal nodules were collected from the first opened fungal gallery of each nest and preserved in absolute ethanol. In several cases (16 of the studied nests), two sample sets were collected from different galleries at opposite sides of the same mound in order to detect possible symbiont diversity within single colonies.

We believe that our sampling covered the vast majority of well-established and active *Macrotermes* nests at all study sites. However, we were not able to find any small, recently established mounds, presuming that such nests existed in the studied sites.

MOLECULAR METHODS.—Amplification of ribosomal ITS1-5.8SITS2 DNA region from the fungal specimens and the mitochondrial cytochrome c oxidase subunit 1 coding gene (COI) from the termite specimens was performed by using a direct PCR method (Thermo Scientific, Phire Animal Tissue Direct PCR Kit for the termite and Phire Plant Direct PCR Kit for the fungal samples). One fungal nodule or termite leg was placed in Dilution buffer, dilution was performed by following the manufacture's protocol and 1 ll of termite or 0.5 ll of fungal dilution was used as a template in direct PCR reaction. Universal primer pair ITS1 and ITS4 (White et al. 1990) was used for the fungal samples and termitespecific COI primers TL1862 and TH2877 (Aanen et al. 2002) for the termite specimens. PCR program for both organisms was as follows: initial denaturing in 98°C for 5 min followed by 40 amplifying cycles (98° for 5 s, 55°C for 5 s, 72° for 20 s) and the final elongation (72°C for 1 min). Amplified PCR products were purified prior sequencing by using the Exo I/FastAPTM protocol (Thermo Scientific; Werle et al. 1994). Purified PCR products were sequenced in two directions in FIMM Sequencing Unit.

Sequencing chromatograms were aligned using CodonCode Aligner 6.0.2 for Windows. Alignment of termite COI sequences was straightforward and unambiguous. COI sequences from 141 different Macrotermes colonies have been deposited in GenBank (accession numbers KY197485–KY197625). The alignment of fungal ITS sequences from the heterokaryotic Termitomyces specimens often required manual checking because the chromatograms contained double peaks caused by slight differences in the ITS copies of the two nuclei. Short indels in one copy complicated reading because the otherwise identical strands were not aligned downstream from the mutation site (cf. De Fine Licht et al. 2005, 2006). In most of such cases, the two ITS haplotypes could be separated manually by first identifying the mutation site and then deducing the two strands from the chromatogram base by base. A total number of 83 ITS sequences each originating from different nests have been deposited in GenBank with accession numbers KY197626–KY197708.

As our interest was to identify the *Termitomyces* species of each termite colony, and not to obtain the complete ITS sequence from every fungal specimen, we used two short lineage specific marker sites in the ITS1 region to identify some difficult sequences (Fig. 1). The marker sites

showed no variation within each fungal lineage but were clearly distinct in the different Termitomyces species, as confirmed by PlutoF workbench (Abarenkov et al. 2010; http://plutof.ut.ee) and a phylogenetic analysis of all *Termitomyces* ITS sequences published in GenBank (see below). The marker sites were always easy to read even from chromatograms with double peaks. A total of 41 *Termitomyces* specimens were identified to species based on these two marker sites.

SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSES.—To compare our new sequences with previously published Termitomyces sequences, all Termitomyces ITS sequences available in UNITE and NCBI GenBank were downloaded using the search function of PlutoF workbench. In order to detect and remove multiple identical sequences from single geographical locations and hosts, all the downloaded sequences were first sorted by country and termite species. All sequences originating from one country were first aligned with MUSCLE in SeaView 4.5.4. The maximum likelihoods of alignments for each country were calculated in RAxML 8 (Stamatakis 2014) by using model GTR + GAMMA with 10 bootstraps. On the basis of the obtained trees, all but one copy of 100 percent identical Termitomyces sequences from each country and associated with the same termite species were removed from the data set.

The remaining sequences and three representatives of our own new sequences were combined and aligned by using MUSCLE in SeaView 4.5.4. Three Lyophyllum spp. ITS sequences from GenBank were used as outgroup. Alignment was improved manually and by selecting short site sets and using the site-specific alignment function of the program. All short or unreliable sequences were left out from the final alignment to achieve maximal support for the branches. Maximum-likelihood analysis was run in RAxML v. 8 by using model CTR + CAT with 1000 bootstraps.

The monophyletic group of *Macrotermes*-associated *Termitomyces* was analyzed separately. Twelve of our new ITS sequences (Table 2) and all previously published GenBank ITS sequences that clustered into the *Macrotermes*-associated *Termitomyces* group in the previous analysis (Table S1) were included. Three *Odontotermes*-associated Termitomyces ITS sequences were used as outgroup. Alignment and the maximum-likelihood analysis were performed in SeaView and RAxML similarly as earlier except the model was GTR + GAMMA.

The resulting phylogenetic tree was compared with Species Hypothesis groups (SHs) separated by the cutoff levels 97 and 98.5 percent in PlutoF workbench (Abarenkov et al. 2010,

Köljalg et al. 2013; http://plutof.ut.ee) in order to evaluate the minimum number of different fungal species among the group of *Macrotermes*-associated *Termitomyces*. ITS similarity of 98.5 percent is currently regarded as a default threshold for species delimitation for Agaricales in UNITE.

Termite COI sequences from 141 host colonies were aligned with MUSCLE in SeaView 4.5.4., and maximum-likelihood analysis was performed in RAxML (model GTR + GAMMA) with 1000 bootstraps. Sequences were sorted in two groups based on the analysis, and genetic differences within and between the groups were calculated by dividing the number of substitutions found in the COI region with the length of the region.

ASSESSMENT OF HABITAT CHARACTERISTICS.—The eight study sites were delimited based on clear geographical boundaries (roads, railroads, fences, etc.) or, when this was not possible, by drawing a line that enclosed the outermost mapped nests buffered with half of the overall mean nearest-neighbor distance calculated separately for each study site (cf. Korb & Linsenmair 2001). Cover of woody vegetation was evaluated roughly in QGIS 2.8.1–Wien by using Google satellite images in OpenLayers Plugin (QGIS Development Team 2016). Tree and shrub canopies were digitized manually, and cover of woody vegetation was calculated for each study site by dividing the area of digitized canopy by the total area of the delimited study site. Abundance and distribution of woody vegetation vs. grassland was used to assess habitat heterogeneity: canopy woodlands and shrublands with closed canopies, and open grasslands were considered less heterogeneous habitats than patchy mosaics of woody vegetation and grassland.

RESULTS

Analysis of the termite COI sequences revealed that our material included two closely related but clearly distinguishable *Macrotermes* species with COI sequence similarity >99.5 percent within and 98–99 percent between the two groups of sequences. The first species (*Macrotermes* sp. 1) was dominant at all study sites, while the second species (*Macrotermes* sp. 2) was less common and found from only three study sites (Fig. 2). As nearly all (119/ 122) sequences of *Macrotermes* sp. 1 originated from the termite mounds with open ventilation shafts the first taxon was tentatively identified as *Macrotermes subhyalinus* Rambur 1842, and as all but one (18/19) sequence of

Macrotermes sp. 2 originated from closed termite mounds the second taxon was tentatively identified as *Macrotermes michaelseni* Sjöstedt 1914 (Bignell & Jones 2014). Reliable reference COI sequences of these two *Macrotermes* species are not presently available. The taxonomy of these and related African taxa is in need of thorough revision, as several cryptic *Macrotermes* species are known to exist (Brandl et al. 2007).

We obtained unambiguous *Termitomyces* ITS sequences from 83 *Macrotermes* nests. Phylogenetic analysis revealed that each of the sequences belonged to one of three lineages, each representing a distinct *Termitomyces* species (here named *Termitomyces* sp. A– C). All ITS sequences of each *Termitomyces* species were more or less identical except for a limited number of polymorphic sites which corresponded to those in two haplotypes of heterokaryotic fungal genotypes as previously described. In all cases where several parallel termite and/or fungal sequences were obtained from single termite colonies, all the parallel samples were always identical in sequence.

The maximum-likelihood analysis of all available *Termitomyces* ITS sequences grouped all *Macrotermes* symbionts into one wellsupported monophyletic group (data not shown). A further analysis focusing solely on this group placed *Termitomyces* sp. A and C in two different main lineages of the group (Fig. 3). These two lineages also included most previously published sequences of *Macrotermes*-associated *Termitomyces*. In contrast, *Termitomyces* sp. B formed a distinct lineage that did not cluster with any previously published ITS sequence. Depending on the threshold value used for species delimitation, the available sequences of *Macrotermes*-associated *Termitomyces* were found to represent 9–14 different species. The threshold of 97 percent ITS similarity suggests six African and three Asian species, while the cutoff level of 98.5 percent, which is currently the default threshold for Species Hypothesis groups in UNITE, suggests nine African and five Asian species (Fig. 3). Regardless of which threshold was used, our new sequences unambiguously belonged to three different *Termitomyces* species.

Figure 2 shows the spatial distribution of the two different *Macrotermes* hosts and the three Termitomyces symbionts within the eight sample sites. *Termitomyces* species A and C were cultured by both *Macrotermes* species. *Termitomyces* sp. A was by far the most common fungal symbiont as it occurred in many termite nests at all sites. *Termitomyces* sp. C was less common, but also it was found from most sites. *Termitomyces* sp. B was relatively rare, only locally frequent at the Maktau site and it was only found associated with *Macrotermes subhyalinus* (Fig. 2A). The

overall diversity of *Termitomyces* was highest in Maktau (Fig. 2A) and lowest in Bungule (Fig. 2H) where only *M. subhyalinus* was found and it cultured *Termitomyces* sp. A in all of its nests.

There were clear differences in vegetation and level of habitat heterogeneity between the different study sites (Fig. 2; Table 1). The proportion of woody vegetation in relation to grass correlated with the overall density of termite mounds and especially that of active mounds, with woodlands and shrublands supporting much higher mound densities than open grasslands. While there was no clear-cut distinction between the termite hosts and/or fungal symbionts of grassland and shrubland sites, respectively, complex vegetation mosaics with relatively high levels of human and/or animal disturbance (Figs. 2A, B, C and G) appeared to support more diverse biotas than monotonous grasslands (Fig. 2D and F) or shrublands (Fig. 2E and H).

DISCUSSION

HABITAT ECOLOGY OF MACROTERMES.—Our results revealed that there are two sympatric, closely related but genetically distinct *Macrotermes* species in the study area. The termite species were named to species on the basis of mound architecture, the differences in which almost always corresponded to what has previously been described for *Macrotermes subhyalinus* and *M. michaelseni*. The mounds of the former species typically have several open ventilation shafts, while the mounds of the later species are closed (Darlington 1984, 1985, Bagine et al. 1994). Still, in three separate cases, we did collect *Macrotermes subhyalinus* from closed mounds, and in one case, *M. michaelseni* was collected from an open mound. We suspect that all these discrepancies were caused by de novo re-colonization of recently abandoned termite mounds by the 'wrong' termite species. In any case, the results show that ventilation type can in some cases be misleading and should not be trusted blindly when identifying resident *Macrotermes* species.

Although the geographical ranges of the two *Macrotermes* species largely overlap, *Macrotermes michaelseni* is believed to prefer somewhat higher elevations and slightly more mesic habitats than *M. subhyalinus* (Bagine et al. 1994, Pomeroy 2005). This indicated that the open mounds of *M. subhyalinus* may offer better protection against high maximum temperatures than the closed mounds of *M. michaelseni*. Within the hot and semiarid Tsavo Ecosystem, *M. subhyalinus* was clearly more common and the dominant species at all sites (Fig. 2).

We did not find any clear explanation for the unequal distribution of the two *Macrotermes* species at different sites. For example, both species co-existed at Kasigau Road (Fig. 2G) and Salt Lick (Fig. 2D), which represent very different habitat types: the former being dense *Commiphora* and *Acacia* woodland with little grass and the latter representing almost pure grassland. Thus, even major differences in available food sources (i.e., woody material vs. grass) were not reflected as the presence or absence of either termite species. It has been suggested that in the hot conditions of low elevations, *M. michaelseni* would only live in habitats where the mounds are either shaded by vegetation or exposed to the wind (Bagine et al. 1994). The woodland sites (especially Bungule and Kasigau Road) supported much higher mound densities than the grasslands (Salt Lick and Mwashoti B) (Fig. 2; Table 1). These differences are most probably related to much higher amounts of carbon available in woodland environments. The conspicuous prevalence of inactive *Macrotermes* mounds in Taita Hills Wildlife Sanctuary (Fig. 2D and F), with most nests abandoned in the relatively recent past, probably coincides with a still ongoing decrease in woody vegetation at this site. Thus, we relate the exceedingly high number of abandoned termite nests to recent disturbance history, especially the effects of severe elephant overgrazing over the past decade and particularly during the drought of 2008–2009.

ECOLOGY OF TERMITOMYCES.—In congruence with the results obtained in all earlier studies (e.g., Katoh et al. 2002, Moriya et al. 2005, Aanen et al. 2009), only one *Termitomyces* species was always found when multiple galleries were analyzed from one nest, indicating that each *Macrotermes* colony always cultivates only one *Termitomyces* symbiont at any given time. Our results also confirm the findings of Osiemo et al. (2010) who reported that more than one species of *Termitomyces* was farmed in different nests of the same termite species in a seemingly uniform environment. The mechanism leading to a monoculture of only one *Termitomyces* genotype per colony is thought to result from positively frequency- dependent propagation and continuous reinoculation by the nursing termites within the fungal combs (Aanen 2006, Aanen et al. 2009).

Most *Macrotermes* species, including *M. subhyalinus* and *M. michaelseni*, are believed to rely on horizontal symbiont transmission, meaning that a newly established termite colony acquires a compatible symbiotic partner from the environment, presumably as *Termitomyces* basidiospores (Johnson et al. 1981, Sieber 1983, Korb & Aanen 2003, De Fine Licht et al. 2006, Nobre et al. 2011b). Only one *Macrotermes* species (*M. bellicosus*) is known to rely on vertical symbiont transmission, i.e., the swarming males transmit the symbiotic inoculum from the

paternal colony (Johnson et al. 1981, Korb & Aanen 2003, Nobre et al. 2011b). In that case, the initial selection of a particular *Termitomyces* genotype has already taken place within the lineage of paternal colonies, possibly many generations ago. Even in the case of vertical transmission, the favored fungal symbiont may occasionally be replaced by a new fungus acquired from the environment (Nobre et al. 2011b).

In horizontal transmission, and presuming that several *Termitomyces* genotypes are acquired from the local environment, the selection of particular symbiont genotypes probably takes place in the primordial fungus comb of a newly formed colony. Either one fungal genotype is so dominant in the environment that the frequency-depended dominance is determined from the start, or the selection takes place within the first primordial comb and can be directed by environmental clues, such as temperature, moisture availability, and/or substrate type. In the latter case, the primordial fungus comb might be a test ground for several *Termitomyces* genotypes, most of which are finally outcompeted by the symbiont that is best adapted to the prevailing conditions.

In our study area, three different *Termitomyces* species were cultured in the nests of the two *Macrotermes* species. *Termitomyces* species A and C were both common and seemed to be farmed equally by both termite species. The maximum-likelihood analysis placed the species in two different clusters that also include most other *Macrotermes*-associated *Termitomyces* ITS sequences in Gen- Bank (Fig. 3, groups 1.2 and 7.1). Hence, based on the available sequence data, the two locally most frequently found *Termitomyces* species must be common and widespread also in other parts of Africa. Indeed, the clade including *Termitomyces* sp. A (group 1.2 in Fig. 3) includes sequences from almost all of Africa. Also the clade including *Termitomyces* sp. C (group 7.1 in Fig. 3) has been found from several African countries but all of them from equatorial regions, suggesting that this fungus might be, for example, more sensitive to low temperatures. Both *Termitomyces* species have been found to associate with *Macrotermes subhyalinus*, *M. michaelseni*, *M. jeanneli*, and *M. bellicosus*. In addition, *Termitomyces* sp. A has also been reported from nests of *M. natalensis* and *M. herus*.

Termitomyces species B, in contrast, is a novel symbiont that has not been detected from other regions or associated with other termite species. In our study area, it was only found in *M. subhyalinus* nests and predominantly from one site. On the basis of the shared occurrence of the two other *Termitomyces* species in the nests of both *Macrotermes* species, one could presume that also species B could sometimes occur in *M. michaelseni* nests. However, this can only be resolved with more sampling. The presence of a previously unknown, regionally rare but locally

common symbiont in our study area indicates that the full diversity of *Macrotermes*-associated *Termitomyces* species can be higher than is presently known and that many 'local endemics' may exist especially in environments that are somehow marginal but still characterized by relatively long habitat continuity (cf. groups 3 and 4 in Fig. 3). Systematic samplings from a wide range of environments are clearly needed.

RELATION TO HABITAT VARIABILITY.—The unequal distribution of the three *Termitomyces* species in our sample sites may indicate that there are some hitherto unknown ecological differences between different fungal symbionts. Landscape-level diversity was lowest in Bungule, with only one *Termitomyces* species (sp. A), and highest in Maktau where all three *Termitomyces* species coexisted in different nests. Predictors of the diversity of soil fungi were recently analyzed by Tedersoo et al. (2014), who emphasized the importance of climate (especially mean annual temperature and precipitation), soil pH, and calcium and phosphorus concentrations. Large-scale climatic differences could not have played a major role in generating the observed differences between our sample sites, especially as possible differences in annual temperatures and precipitation are effectively buffered by the architecture of termite nests. However, edaphic factors and differences in soil moisture may well have played some role in generating the observed local differences in *Termitomyces* diversity. The most obvious difference between the eight study sites was in the cover of woody vegetation. Trees and tall shrubs provide shade and thus influence the local microclimate and especially the prevailing soil surface temperatures during the day. Thermal differences in turn have direct and indirect effects on soil moisture, which must be a crucial factor for termites and their symbiotic fungi in any tropical semiarid region. Together these factors may also influence the success of different fungal species. Naturally the type of the vegetation also influences food availability and quality.

Stable carbon isotope studies have shown that *Macrotermes michaelseni* feeds on both woody and herbaceous plant tissues and that the composition of its diet can vary depending on the habitat (Boutton et al. 1983). Obviously, the readily available food sources for termites in woodlands and shrublands are quite different from those in open grasslands. This has a great effect on the substrate provided to the fungal symbionts, which may in turn have implications for symbiont selection, especially if the different fungal species differ in their ability to break down complex biopolymers. As a whole, plant decomposition by *Macrotermes* and their symbionts is a

complex process managed complementarily by enzymes produced by the insect hosts, their gut microbiota, and the cultured *Termitomyces* symbiont (Poulsen et al. 2014, Poulsen 2015).

The highest *Termitomyces* diversity was found at the Maktau site where all three fungal species coexisted. The most obvious feature that distinguishes this habitat from most of the others is overall heterogeneity and patchiness; the local vegetation is a fine-scaled mosaic of woody vegetation and open grassland (Fig. 2A). This type of habitat heterogeneity may well promote high Termitomyces diversity as the small-scale variation in vegetation structure is also reflected in soil temperatures and other physical factors. In any case, the many different types of plant matter available for termites open several different substrate niches, and their optimal exploitation may potentially require a different *Termitomyces* species.

CONCLUSIONS

Our study confirms that different *Macrotermes* species can associate with several different Termitomyces species and that the association patterns between the symbionts can vary across tropical landscapes. As the termites and their fungal symbionts have evolved and specialized during millions of years, it seems likely that different *Termitomyces* species now have different biological abilities. Also different symbiont combinations may have adapted to exploit different resources within the variable savanna landscape. Clearly, we are only beginning to unravel the complex network of interactions and evolutionary processes that can influence the ecology and evolution of fungus-growing termites and their symbiotic partners.

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DATA AVAILABILITY

DNA sequences produced in this study have been deposited in GenBank (accession numbers KY197484–KY197709).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article: FIGURE S1. Location of the study sites, TABLE S1. Additional sequences and their GenBank accession numbers, TABLE S2. Host and symbiont identities and GenBank accession numbers.

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TABLES

TABLE 1. Physical features and vegetation of the study sites.

Study site	Coordinates	Vegetation	Size (ha)	Elevation (m.a.s.l.)	Woody vegetation cover (%)	Density of active termite mounds (colonies/ha)
Bungule	3°50'6"S, 38°41'50"E	Dense woodland with large *Acacia* and lower shrubs.	5.9	540	80	4.6
Kasigau Road	3°38'14"S, 38°28'34"E	Semi-open woodland with large *Commiphora* and *Acacia* trees.	5.6	815	66	5.7
Maktau	3°22'14"S, 38°8'41"E	Fine-scale mosaic of grassy areas and woody vegetation (incl. *Acacia* and *Commiphora* spp.).	18.7	1215–1270	43	1.6
Mgeno	3°28'21"S, 38°27'31"E	Fallow agricultural land with grass and isolated trees and shrubs. Heavily grazed by cattle.	32.9	720	25	0.8
Mbula	3°23'56"S, 38°11'1"E	Dense shrubland with scattered small trees (incl. *Acacia* and *Grewia* spp.).	17.9	1050	63	1.5
Salt Lick	3°32'37"S, 38°12'43"E	Open grassland with scattered and predominately damaged trees.	37.0	890	1	0.2
Mwashoti A	3°28'32"S, 38°13'58"E	Dense shrubland heavily grazed by cattle. Protected from elephants by sanctuary fence.	14.1	1040	70	0.8
Mwashoti B	3°28'43"S, 38°14'6"E	Grassland with decreasing amount of dead wood. Trees and shrubs killed recently by elephants.	20.3	1040	0.5	0.1

TABLE 2. Origin and GenBank accession numbers of the new *Macrotermes* and *Termitomyces* sequences used in phylogenetic analysis. See Table S2 in supporting information to find information and accession numbers of the produced sequences from all the studied 143 *Macrotermes* mounds.

Colony	**Study site**	***Macrotermes* species**	***Termitomyces* species**	**GenBank accession number**	
				***Macrotermes* (COI)**	***Termitomyces* (ITS)**
TK08	Bungule	*M. subhyalinus*	A	KY197491	KY197628
TK17	Bungule	*M. subhyalinus*	A	KY197500	KY197634
TR164	Kasigau Road	*M. michaelseni*	A	KY197611	KY197644
TR175	Kasigau Road	*M. michaelseni*	C	KY197614	KY197697
TR09	Kasigau Road	*M. subhyalinus*	C	KY197507	KY197693
TM14	Maktau	*M. subhyalinus*	A	KY197523	KY197647
TM35	Maktau	*M. subhyalinus*	B	KY197535	KY197689
TM39	Maktau	*M. subhyalinus*	B	KY197539	KY197690
TM41	Maktau	*M. subhyalinus*	B	KY197540	KY197691
TM37	Maktau	*M. subhyalinus*	C	KY197537	KY197700
TS14	Salt Lick	*M. subhyalinus*	A	KY197604	KY197684
TS16	Salt Lick	*M. michaelseni*	C	KY197622	KY197708

FIGURES

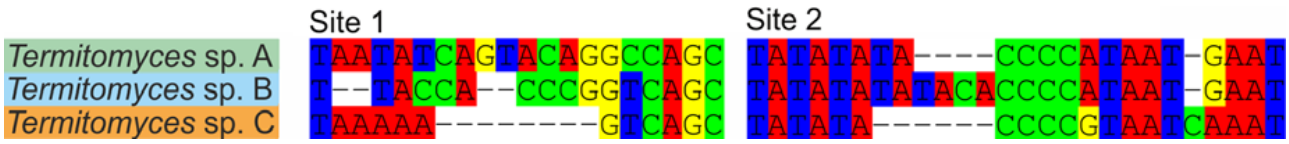

FIGURE 1. Two polymorphic sites in ITS1 that were used for determining the species identity of some *Termitomyces* symbionts (*Termitomyces* sp. A, B and C). They were used in cases where unambiguous sequences could not be obtained due to complicated indel polymorphisms between two ITS copies of the heterokaryotic genotypes.

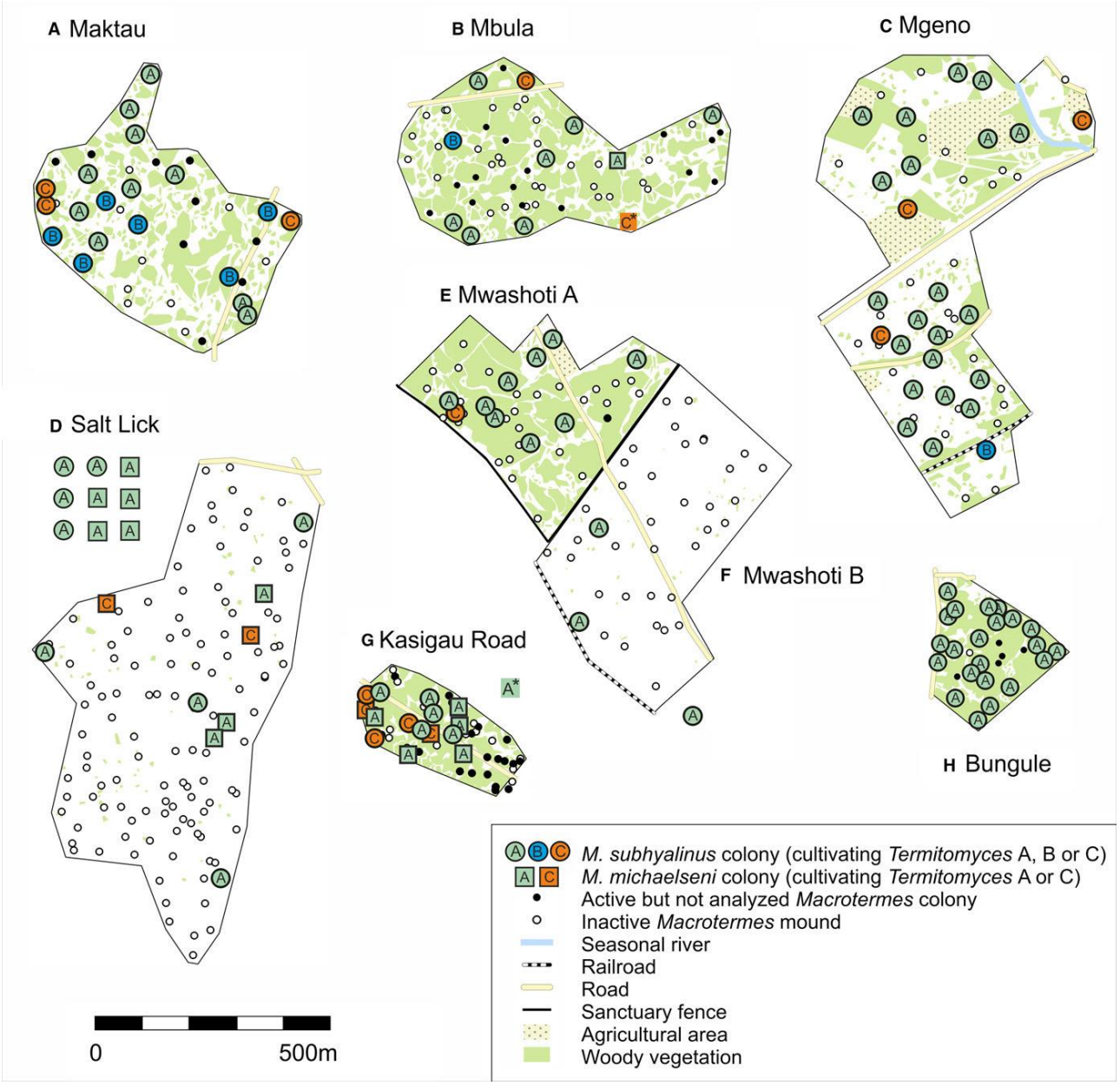

FIGURE 2. Distribution of the two species of *Macrotermes* and the three species of *Termitomyces* in the eight study sites: (A) Maktau, (B) Mbula, (C) Mgeno, (D) Salt Lick, (E) and (F) Mwashoti (two different study sites separated by the sanctuary fence), (G) Kasigau Road, and (H) Bungule. Circular symbols indicate *M. subhyalinus* and squares *M. michaelseni* colonies; colonies where the *Macrotermes* species could not be identified due to lack of host insects in sampled galleries are marked with asterisk (*). Letters and colors of the symbols help to identify the three fungal species: Green = *Termitomyces* sp. A; Blue = *Termitomyces* sp. B; Orange = *Termitomyces* sp. C. Symbols outsides the borders of sample sites D, G and F represent sampled termite mounds slightly outside the mapped area. Vegetation characteristics were mapped from satellite images (legend in figure). The white background represents grassland and/or bare soil.

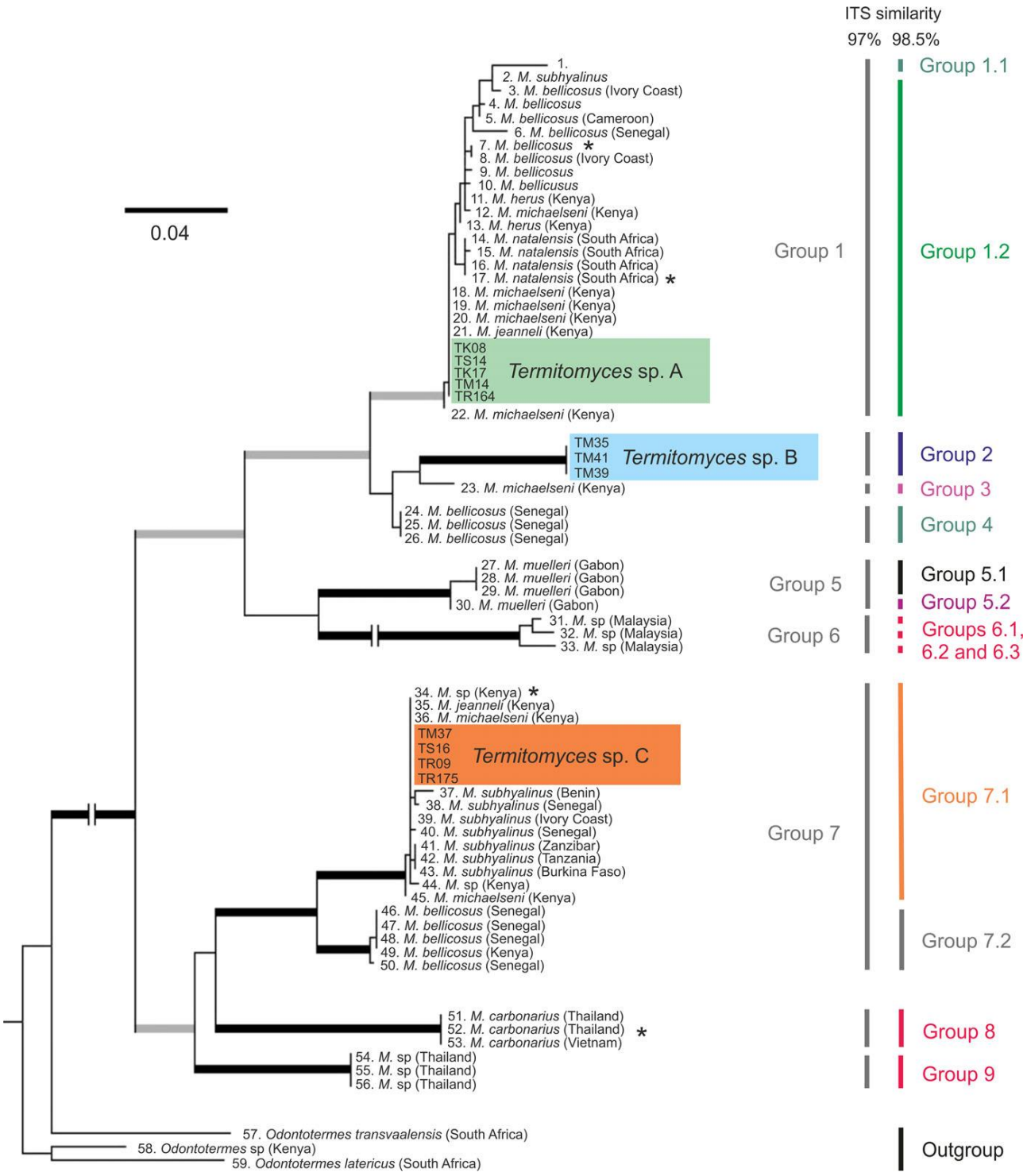

FIGURE 3. Maximum likelihood tree of *Macrotermes*-associated *Termitomyces* based on the new ITS sequences and all previous sequences in GenBank. Bootstrap values: thin line <85, grey thick line 85–98, black thick line 98–100. Accession numbers are given in Supplementary Table S1. Asterisks (*) indicate that additional 100 percent identical sequences from the same country and the same termite species exist. The new sequences produced in this study are highlighted in color: Green = *Termitomyces* sp. A; Blue = *Termitomyces* sp. B; Orange = *Termitomyces* sp. C. Vertical bars on the right indicate distinct fungal species based on two different levels of ITS similarity: 97% and 98.5%. Latter is currently regarded as the default threshold for species delimitation for Agaricales in UNITE whereas the earlier represents undoubtedly the minimum number of species among the group of *Macrotermes*-associated *Termitomyces*.

SUPPORTING MATERIAL

Table S1. Additional sequences and their GenBank accession numbers used in the final alignment and maximum likelihood analysis of the *Macrotermes*-associated *Termitomyces* (see Fig 3).

	Host termite species	Sampling country	GenBank accession number
1.	unknown	unknown	AF357024
2.	*Macrotermes subhyalinus*	unknown	HQ902227
3.	*Macrotermes bellicosus*	Ivory Coast	JF302815
4.	*Macrotermes bellicosus*	unknown	HQ902230
5.	*Macrotermes bellicosus*	Cameroon	HQ902218
6.	*Macrotermes bellicosus*	Senegal	GQ922682
7.	*Macrotermes bellicosus*	unknown	HQ902232
8.	*Macrotermes bellicosus*	Ivory Coast	HQ902219
9.	*Macrotermes bellicosus*	unknown	HQ902229
10.	*Macrotermes bellicosus*	unknown	HQ902224
11.	*Macrotermes herus*	Kenya	GQ383684
12.	*Macrotermes michaelseni*	Kenya	GQ383687
13.	*Macrotermes herus*	Kenya	GQ383683
14.	*Macrotermes natalensis*	South Africa	DQ436957
15.	*Macrotermes natalensis*	South Africa	AY764149
16.	*Macrotermes natalensis*	South Africa	DQ436940
17.	*Macrotermes natalensis*	South Africa	DQ436950
18.	*Macrotermes michaelseni*	Kenya	GQ383680
19.	*Macrotermes michaelseni*	Kenya	GQ383681
20.	*Macrotermes michaelseni*	Kenya	GQ383682
21.	*Macrotermes jeanneli*	Kenya	GQ383679
22.	*Macrotermes michaelseni*	Kenya	GQ383685
23.	*Macrotermes michaelseni*	Kenya	GQ383686
24.	*Macrotermes bellicosus*	Senegal	GQ922679
25.	*Macrotermes bellicosus*	Senegal	GQ922680
26.	*Macrotermes bellicosus*	Senegal	GQ922681
27.	*Macrotermes muelleri*	Gabon	GQ922688
28.	*Macrotermes nobilis*	Gabon	GQ922687
29.	*Macrotermes nobilis*	Gabon	AF321373
30.	*Macrotermes muelleri*	Gabon	AF321368
31.	*Macrotermes* sp.	Malaysia	GU001666
32.	*Macrotermes carbonarius*	Malaysia	AB051889

33.	*Macrotermes* sp.	Malaysia	GU001670
34.	*Macrotermes* sp.	Kenya	JQ088160
35.	*Macrotermes jeanneli*	Kenya	GQ383677
36.	*Macrotermes michaelseni*	Kenya	GQ383676
37.	*Macrotermes sybhyalinus*	Benin	HQ902240
38.	*Macrotermes sybhyalinus*	Senegal	AF321362
39.	*Macrotermes sybhyalinus*	Ivory Coast	JF302816
40.	*Macrotermes subhyalinus*	Senegal	GQ922686
41.	*Macrotermes subhyalinus*	Zanzibar	AF321370
42.	*Macrotermes subhyalinus*	Tanzania	AF321369
43.	*Macrotermes subhyalinus*	Burkina Faso	EU816416
44.	*Macrotermes* sp.	Kenya	JQ088143
45.	*Macrotermes michaelseni*	Kenya	GQ383678
46.	*Macrotermes bellicosus*	Senegal	GQ922683
47.	*Macrotermes bellicosus*	Senegal	GQ922684
48.	*Macrotermes bellicosus*	Senegal	GQ922685
49.	*Macrotermes bellicosus*	Kenya	GQ383675
50.	*Macrotermes bellicosus*	Senegal	AF321371
51.	*Macrotermes carbonarius*	Thailand	AB073515
52.	*Macrotermes carbonarius*	Thailand	AB073516
53.	*Macrotermes carbonarius*	Vietnam	EU816418
54.	*Macrotermes* sp.	Thailand	HM230659
55.	*Macrotermes* sp.	Thailand	AY244623
56.	*Macrotermes* sp.	Thailand	EF091678
57.	*Odontotermes transvaalensis*	South Africa	EF636905
58.	*Odontotermes* sp.	Kenya	KY197709
59.	*Odontotermes latericius*	South Africa	EF636912

Table S2. Host and symbiont identities and GenBank accession numbers (COI and ITS) for 143 *Macrotermes* colonies analyzed in this study and one *Odontotermes* colony that was used as an outgroup member in phylogenetic analysis. The *Termitomyces* species in parentheses were identified based on two polymorphic regions in ITS1 (Fig. 1) and their full ITS sequences were not deposited in GenBank.

				GenBank accession number	
Colony	**Study site**	***Macrotermes* species**	***Termitomyces* species**	***Macrotermes* (COI)**	***Termitomyces* (ITS)**
TK01	Bungule	*M. subhyalinus*	A	KY197485	KY197626
TK02	Bungule	*M. subhyalinus*	(A)	KY197486	
TK04	Bungule	*M. subhyalinus*	(A)	KY197487	
TK05	Bungule	*M. subhyalinus*	(A)	KY197488	
TK06	Bungule	*M. subhyalinus*	A	KY197489	KY197627
TK07	Bungule	*M. subhyalinus*	(A)	KY197490	
TK08	Bungule	*M. subhyalinus*	A	KY197491	KY197628
TK09	Bungule	*M. subhyalinus*	A	KY197492	KY197629
TK10	Bungule	*M. subhyalinus*	A	KY197493	KY197630
TK11	Bungule	*M. subhyalinus*	(A)	KY197494	
TK12	Bungule	*M. subhyalinus*	A	KY197495	KY197631
TK13	Bungule	*M. subhyalinus*	A	KY197496	KY197632
TK14	Bungule	*M. subhyalinus*	A	KY197497	KY197633
TK15	Bungule	*M. subhyalinus*	(A)	KY197498	
TK16	Bungule	*M. subhyalinus*	(A)	KY197499	
TK17	Bungule	*M. subhyalinus*	A	KY197500	KY197634
TK18	Bungule	*M. subhyalinus*	A	KY197501	KY197635
TK19	Bungule	*M. subhyalinus*	A	KY197502	KY197636
TK20	Bungule	*M. subhyalinus*	(A)	KY197503	
TK21	Bungule	*M. subhyalinus*	A	KY197504	KY197637
TK22	Bungule	*M. subhyalinus*	(A)	KY197505	
TK23	Bungule	*M. subhyalinus*	(A)	KY197506	
TR09	Kasigau Road	*M. subhyalinus*	C	KY197507	KY197693
TR10	Kasigau Road	*M. subhyalinus*	A	KY197508	KY197638
TR83	Kasigau Road		A		KY197639
TR149	Kasigau Road	*M. michaelseni*	A	KY197607	KY197640
TR151	Kasigau Road	*M. michaelseni*		KY197608	
TR154	Kasigau Road	*M. subhyalinus*	A	KY197509	KY197641
TR156	Kasigau Road	*M. subhyalinus*	(A)	KY197510	
TR159	Kasigau Road	*M. michaelseni*	A	KY197609	KY197642
TR160	Kasigau Road	*M. michaelseni*	(A)	KY197610	
TR161	Kasigau Road	*M. subhyalinus*	A	KY197511	KY197643
TR164	Kasigau Road	*M. michaelseni*	A	KY197611	KY197644
TR166	Kasigau Road	*M. michaelseni*	C	KY197612	KY197694
TR167	Kasigau Road	*M. subhyalinus*	(A)	KY197512	
TR168	Kasigau Road	*M. subhyalinus*	C	KY197513	KY197695

TR172	Kasigau Road	*M. subhyalinus*	C	KY197514	KY197696
TR173	Kasigau Road	*M. michaelseni*	(A)	KY197613	
TR175	Kasigau Road	*M. michaelseni*	C	KY197614	KY197697
TM01	Maktau	*M. subhyalinus*		KY197515	
TM02	Maktau	*M. subhyalinus*	A	KY197516	KY197645
TM04	Maktau	*M. subhyalinus*	B	KY197517	KY197687
TM05	Maktau	*M. subhyalinus*		KY197518	
TM06	Maktau	*M. subhyalinus*		KY197519	
TM07	Maktau	*M. subhyalinus*	(B)	KY197520	
TM08	Maktau	*M. subhyalinus*	C	KY197521	KY197698
TM10	Maktau	*M. subhyalinus*	A	KY197522	KY197646
TM14	Maktau	*M. subhyalinus*	A	KY197523	KY197647
TM15	Maktau	*M. subhyalinus*	A	KY197524	KY197648
TM16	Maktau	*M. subhyalinus*	A	KY197525	KY197649
TM17	Maktau	*M. subhyalinus*		KY197526	
TM19	Maktau	*M. subhyalinus*	B	KY197527	KY197688
TM22	Maktau	*M. subhyalinus*		KY197528	
TM23	Maktau	*M. subhyalinus*	A	KY197529	KY197650
TM25	Maktau	*M. subhyalinus*	A	KY197530	KY197651
TM26	Maktau	*M. subhyalinus*	A	KY197531	KY197652
TM28	Maktau	*M. subhyalinus*		KY197532	
TM33	Maktau	*M. subhyalinus*	A	KY197533	KY197653
TM34	Maktau	*M. subhyalinus*		KY197534	
TM35	Maktau	*M. subhyalinus*	B	KY197535	KY197689
TM36	Maktau	*M. subhyalinus*	C	KY197536	KY197699
TM37	Maktau	*M. subhyalinus*	C	KY197537	KY197700
TM38	Maktau	*M. subhyalinus*		KY197538	
TM39	Maktau	*M. subhyalinus*	B	KY197539	KY197690
TM41	Maktau	*M. subhyalinus*	B	KY197540	KY197691
TM42	Maktau	*M. subhyalinus*	A	KY197541	KY197654
TB02	Mbula	*M. subhyalinus*	(B)	KY197542	
TB03	Mbula	*M. subhyalinus*	(A)	KY197543	
TB04	Mbula	*M. subhyalinus*	C	KY197544	KY197701
TB05	Mbula	*M. subhyalinus*		KY197545	
TB06	Mbula	*M. subhyalinus*	(A)	KY197546	
TB07	Mbula	*M. subhyalinus*	(A)	KY197547	
TB08	Mbula	*M. michaelseni*	(A)	KY197615	
TB10	Mbula		C		KY197705
TB17	Mbula	*M. subhyalinus*		KY197548	
TB18	Mbula	*M. subhyalinus*		KY197549	
TB19	Mbula	*M. subhyalinus*		KY197550	
TB22	Mbula	*M. subhyalinus*		KY197551	
TB23	Mbula	*M. subhyalinus*		KY197552	
TB24	Mbula	*M. subhyalinus*		KY197553	
TB25	Mbula	*M. subhyalinus*		KY197554	
TB26	Mbula	*M. subhyalinus*	A	KY197555	KY197655
TB33	Mbula	*M. subhyalinus*	A	KY197556	KY197656

TB35	Mbula	*M. subhyalinus*	(A)	KY197557	
TB36	Mbula	*M. subhyalinus*	(A)	KY197558	
TB37	Mbula	*M. subhyalinus*		KY197559	
TA01	Mgeno	*M. subhyalinus*	A	KY197574	KY197657
TA02	Mgeno	*M. subhyalinus*	B	KY197575	KY197692
TA05	Mgeno	*M. subhyalinus*	A	KY197576	KY197658
TA06	Mgeno	*M. subhyalinus*	A	KY197577	KY197659
TA11	Mgeno	*M. subhyalinus*	A	KY197578	KY197660
TA12	Mgeno	*M. subhyalinus*	A	KY197579	KY197661
TA19	Mgeno	*M. subhyalinus*	A	KY197580	KY197662
TA21	Mgeno	*M. subhyalinus*	A	KY197581	KY197663
TA22	Mgeno	*M. subhyalinus*	A	KY197582	KY197686
TA26	Mgeno	*M. subhyalinus*	A	KY197583	KY197664
TA29	Mgeno	*M. subhyalinus*	C	KY197584	KY197702
TA30	Mgeno	*M. subhyalinus*	(A)	KY197585	
TA31	Mgeno	*M. subhyalinus*	A	KY197586	KY197665
TA32	Mgeno	*M. subhyalinus*	A	KY197587	KY197666
TA36	Mgeno	*M. subhyalinus*	(A)	KY197588	
TY01	Mgeno	*M. subhyalinus*	A	KY197589	KY197667
TY02	Mgeno	*M. subhyalinus*	A	KY197590	KY197668
TY04	Mgeno	*M. subhyalinus*	C	KY197591	KY197703
TY06	Mgeno	*M. subhyalinus*	A	KY197592	KY197669
TY09	Mgeno	*M. subhyalinus*	C	KY197593	KY197704
TY11	Mgeno	*M. subhyalinus*	(A)	KY197594	
TY12	Mgeno	*M. subhyalinus*	A	KY197595	KY197670
TY13	Mgeno	*M. subhyalinus*	(A)	KY197596	
TY14	Mgeno	*M. subhyalinus*	A	KY197597	KY197671
TY17	Mgeno	*M. subhyalinus*	A	KY197598	KY197672
TFA11	Mwashoti A	*M. subhyalinus*	(A)	KY197560	
TFA12	Mwashoti A	*M. subhyalinus*	A	KY197561	KY197673
TFA13	Mwashoti A	*M. subhyalinus*	(A)	KY197562	
TFA14	Mwashoti A	*M. subhyalinus*	A	KY197563	KY197674
TFA18	Mwashoti A	*M. subhyalinus*	(A)	KY197564	
TFA21	Mwashoti A	*M. subhyalinus*	(A)	KY197565	
TFA28	Mwashoti A	*M. subhyalinus*	(A)	KY197566	
TFA35	Mwashoti A	*M. subhyalinus*	C	KY197567	KY197706
TFA43	Mwashoti A	*M. subhyalinus*		KY197568	
TFA48	Mwashoti A	*M. subhyalinus*	A	KY197569	KY197675
TFA49	Mwashoti A	*M. subhyalinus*	(A)	KY197570	
TFB20	Mwashoti B	*M. subhyalinus*	(A)	KY197571	
TFB34	Mwashoti B	*M. subhyalinus*	(A)	KY197572	
TFB50	Mwashoti B	*M. subhyalinus*	(A)	KY197573	
TS01	Salt Lick	*M. michaelseni*	A	KY197616	KY197676
TS02	Salt Lick	*M. michaelseni*	A	KY197617	KY197677
TS03	Salt Lick	*M. michaelseni*	A	KY197618	KY197678
TS04	Salt Lick	*M. michaelseni*	(A)	KY197619	
TS05	Salt Lick	*M. subhyalinus*	(A)	KY197599	

TS08	Salt Lick	*M. subhyalinus*	A	KY197600	KY197679
TS09	Salt Lick	*M. subhyalinus*	A	KY197601	KY197680
TS10	Salt Lick	*M. subhyalinus*	A	KY197602	KY197681
TS12	Salt Lick	*M. michaelseni*	A	KY197620	KY197682
TS13	Salt Lick	*M. subhyalinus*	A	KY197603	KY197683
TS14	Salt Lick	*M. subhyalinus*	A	KY197604	KY197684
TS15	Salt Lick	*M. michaelseni*	A	KY197621	KY197685
TS16	Salt Lick	*M. michaelseni*	C	KY197622	KY197708
TS18	Salt Lick	*M. michaelseni*	(A)	KY197623	
TS19	Salt Lick	*M. michaelseni*	(A)	KY197624	
TS20	Salt Lick	*M. subhyalinus*	(A)	KY197605	
TS22	Salt Lick	*M. michaelseni*	C	KY197625	KY197707
TS56	Salt Lick	*M. subhyalinus*	(A)	KY197606	
TM03	Maktau	*Odontotermes* sp.	*Termitomyces* symbiont of *Odontotermes* sp.	KY197484	KY197709

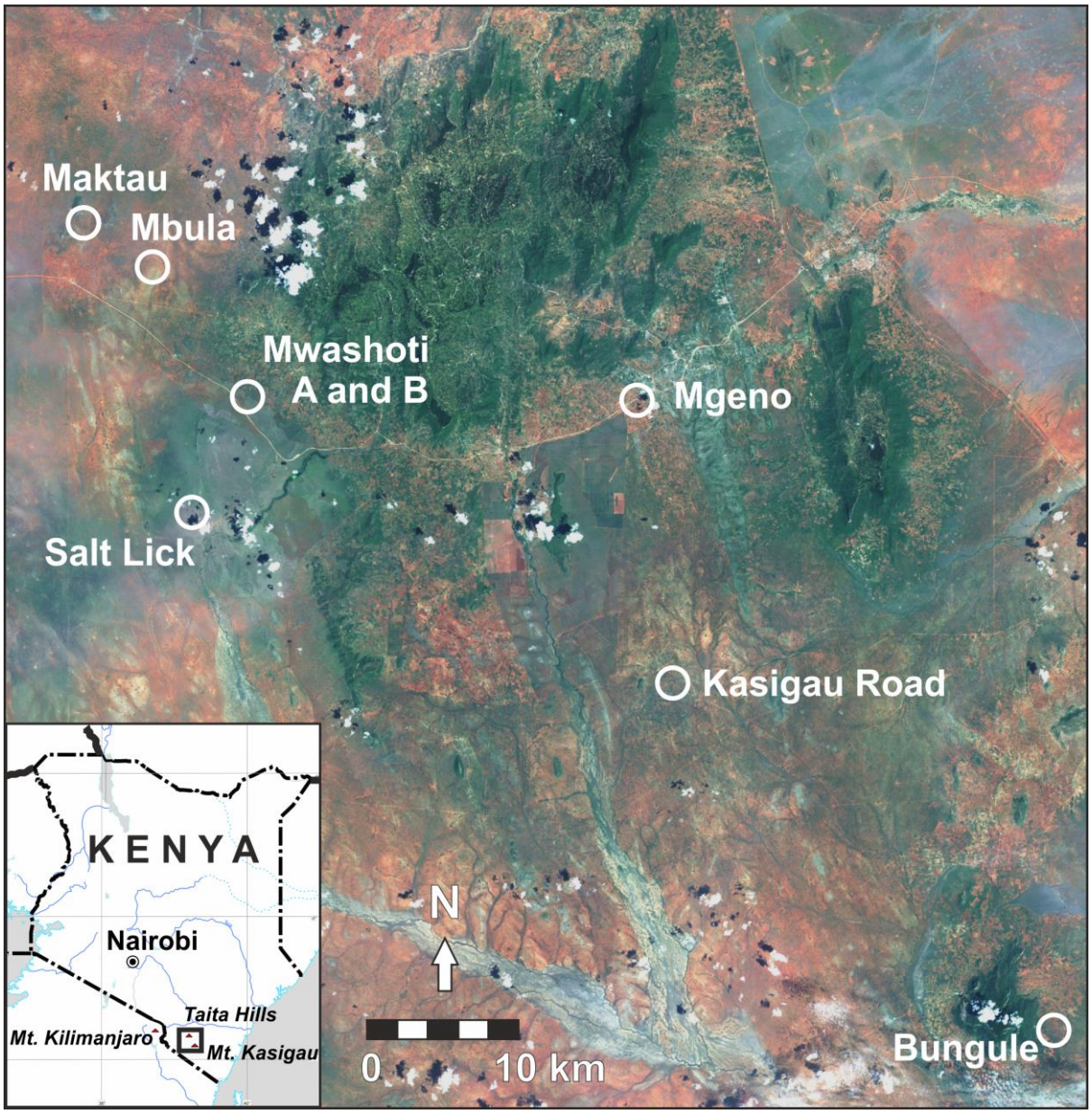

FIGURE S1. Location of the study sites around Taita Hills and Mt. Kasigau (satellite image: Landsat 8/2014).