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Morphological and molecular characterisation of termite species in Taita Taveta County, Kenya

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The diversity of termites remains obscure despite having seized the equatorial ecological community with numerous effects both environmental and solvent. In the current study, morphology and DNA based techniques were used to appraise the heterogeneity of termites in Taita Taveta County. Soldier termites were arbitrarily fragmented from the five main plant lives in the study region. A dissecting microscope was used to discern the external features of the termites and was noted. The features were compared to the identification keys and then transformed into quasi-characters to generate a dendrogram. DNA was isolated from the 31 specimens from which mitochondrial cytochrome oxidase II (COX II) gene was sequenced and probed. We tested whether or not molecular characterisation underpins morphological characterization and then obtained similar sequences from the Genebank Repository of National Center for Biotechnology Information (NCBI) and used them together with our sequences for the phylogenetic tree construction. The results were supported by genetic specifications such as nucleotide composition and dichotomized genetic range. Termite of three different genera; *Macrotermes, Amitermes* and *Odontotermes* were identified based on their mound structure and soldier morphology. Phylogenetic analysis also placed the termites into three clusters, which were affiliated with the genera above. Genera *Amitermes* and *Odontotermes* were confined to distinct vegetation whereas the genus *Macrotermes* were all-pervasive. The study confirmed that both approaches identified the termite genera but a combination of both is necessary for confirmation and higher taxonomic classification.

Key words: COII, diversity, Macrotermitinae, termites, phylogeny.

INTRODUCTION

Termites (Isoptera) are known both as ecological drivers and significant menace to the environment (Vidyashree et

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al., 2018). They contribute to various ecosystem processes such as decomposition of organic matter into nutrients, and also modify soil properties decomposition of organic matter and nutrient cycling (Arif et al., 2019). Moreover, their termitaria provide special microhabitat for a number of organisms (Enagbonma and Babalola, 2019). However, they are also pests of agricultural crops and household items made of cellulosic material, a trait that tends to conceal their role in offering ecosystem services (Jouquet et al., 2018). Both positive and negative effects of termites arise from their feeding activities abetted by symbiotic microorganisms such as fungi and bacteria which facilitate cellulose digestion (Adaobi and Abiye, 2019).

Identification of termites still poses challenges due to inadequate taxonomic knowledge as well as their eusocial behavior coupled with distinct caste system (Murthy, 2020). The latter is because termites present limited or no species-specific morphological traits which allows identification only up to the genus level (Korb et al., 2019). In addition, there is a shortage of experts in most of the equatorial and subtropical countries (Mugerwa et al., 2014). As a result, there is scanty information on prevalence, identity, diversity and distribution of termites in Kenya and the African continent as a whole. Research leading to character level designation is thus necessary for accurate biodiversity assessment (Murthy et al., 2017).

Currently there are about 3106 sentient and remnant identified termite species (Effowe et al., 2021) allied to 330 genera, 12 families, 21 subfamilies and eleven families (Krishna et al., 2013). The order Isoptera is formally divided into inferior (underdeveloped) and superior (more advanced) termites (Zhu et al., 2012). The latter belong to the family Termitidae which have varying and sometimes conflicting characteristics. Members in the family Termitidae are capable of building termitaria albeit some just make galleries and tunnels underground. As a matter of fact, the family Termitidae contains the greatest number of studied genera and described species that is more than 2/3 in each case (Kanwal et al., 2011). The African continent leads in the number of species and this is due to its favourable climatic conditions (van Huis, 2017). There are over 650 species in the family Termitidae. The subfamilies under this family have grown to eight over the years (Engel, 2011). Majority of the described species belong to the eight families. East Africa alone has over 177 termite species and more could yet be identified (Ahmed et al., 2011). The lower termites which are majorly dependent on protozoa and bacteria (Huda et al., 2019) are*; Rhinotermitidae, Kalotermitidae, Hodotermitidae, Termopsidae, Archotermopsidae, Stolotermitidae, Stylotermitidae, Serritermitidae and Mastotermitidae.* The number of species in these families reduces respectively with the last two families having only one species each (Namanda, 2015; Nkunika, 1998).

External morphology of termites has for a long time been given importance in systematics and classification studies (Mandal et al., 2014). Taxonomic characters found in soldier caste present conspicuously reliable features for diversity investigations (Himmi et al., 2020). However, without keen conduction, morphological identification could present some ambiguity when solely applied. It should therefore be complemented with other methods (Patel and Jadhav, 2019). Many studies have opted to couple morphological identification with molecular characterization to avoid opaque identification (Schyra et al., 2019). The use of mitochondrial DNA (mtDNA) sequences has been successfully complemented with morphological characterisation for species identification. Mitochondrial COII gene in particular has been established to be reliable in species identification of members of different invertebrate groups as well as in establishing phylogenetic relationships among these groups (Garrick et al., 2015). To analyze mtDNA, little DNA samples is required and gives accurate results (Kapli et al., 2017); thus it remains a marker of choice for species identification. In addition, the COII gene has adequate representation of posted sequences in the GenBank database (Ghesini et al., 2020). This study aimed to determine termite colonies of the equatorial savannah of Taita Taveta County, Kenya based on the two approaches.

MATERIALS AND METHODS

Study site and sample collection

Collection of termites was done in four main districts of Taita Taveta County. The county is situated northwesterly of Mombasa city in the coast and 360 km southeast of Nairobi. Its expanse stretches an area of about 17,000 km^2 and is within a tropical savannah with various plant life systems. We highlighted Herbaceous Grassland (HG) [Mwatate district Latitude: -3° 29′ 59.99″ S, Longitude: 38° 22′ 59.99″ E]; Rain Forest (FS) [Wundanyi district Latitude: -3° 24′ 6.95″ Longitude 38° 21′ 50.47″]; Shrub Savanna (SV) [Voi district Latitude: -3° 23′ 45.78″ S, Longitude: 38° 33′ 21.92″ E]; Rain Fed Trees (RFT) [Taveta district Latitude: -3° 39′ 63.20″ S, Longitude: 37° 67′ 36.20 E″]; and Disturbed Areas (DL) all over of the county.

Aggregately, 54 active termite assemblages from the highlighted plant life systems were haphazardly chosen for sampling. Within each vegetation type we sampled mounds, which were at most 100 m apart. To obtain the exact points of reference of the collection points, Gamin software was installed in the phone and used to take the points (Table 1). Live termitaria were able to be distinguished by the absence of plant establishment as they cause developing soils around to harden and become impregnable (Yamashina, 2013). In order to get to the termite territory, the mounds had to be dug to a vertical extent of 50 cm downwards or depending on how far the nest was. 20 soldier termites were picked using pincers and preserved in sterile falcon tubes containing absolute ethanol. The collected samples were put in a cool box after which they were taken to the institution's research laboratory. In the laboratory, they were kept in the refrigerator for subsequent morphological and molecular characterization.

Table 1. GPS co-ordinates of termite sampling locations in Taita Taveta County in July 2013 and February 2014.

Sample site	Vegetation type	Latitude	Longitude
Mwatate TTUC	Disturbed land	3° 25' 17.24″ S	38° 30' 13.15″ E
Mwatate TTUC	Disturbed land	3° 25' 10.21″ S	38° 30' 07.98″ E
Mwatate TTUC	Herbaceous grassland	3° 25' 10.21″ S	38° 30' 07.98″ E
Mwatate TTUC	Disturbed land	3° 25' 10.22″ S	38° 30' 07.75″ E
Mwatate TTUC	Disturbed land	3° 25' 09.10″ S	38° 30' 07.11″ E
Mwatate KHS	Disturbed land	3° 29' 29.92″ S	38° 30' 08.44″ E
Mwatate KHS	Disturbed land	3° 22' 28.81" S 38°27'38.39'' E	38° 22' 40.40" E
Tausa	Rain fed trees	3° 20' 07.08" S	38° 29′ 41.02″ E
Konenyi	Rain fed trees	3° 20' 07.08" S	38° 29' 42.02 " E
Tausa	Rain fed trees	3° 20′ 06.59″ S	38° 29′ 41.94″ E
Konenyi	Rain fed trees	3° 22′ 28.81″ S	38° 27′ 38.39″ E
Mwambirwa	Forest	3° 21′ 11.36″ S	38° 25′ 54.36″ E
Taveta town	Disturbed land	3° 23′ 47.54″ S	37° 40′ 42.60″ E
Malukiloriti	Shrub savanna	3° 21′ 59.60″ S	37° 42′ 24.42″ E
Malukiloriti primary school	Disturbed land	3° 21′ 47.51″ S	37° 42′ 22.99″ E
Timbila	Rain fed trees	3° 23′ 52.49″ S	37° 43′ 05.09″ E
Luduwhai	Rain fed trees	3° 25′ 13.28″ S	38° 10′ 09.94″ E
Salaita	Shrub savanna	3° 25′ 13.28″ S	37° 45′ 51.02″ E
Bura forest	Forest	3° 30′ 30.24″ S	38° 22′ 41.01″ E
Mwaktau (Tsavo west)	Shrub Savanna	3° 25′ 13.28″ S	38° 10′ 19.94″ E
Tsavo east	Shrub savanna	3° 20′ 07.08″ S	38° 29′ 42.02″ E
Tsavo east	Shrub savanna	3° 22′ 28.81″ S	38° 27′ 39.38″ E
Buguta	Disturbed land	3° 40′ 59.35″ S	38° 29′ 41.94″ E
Tsavo East	Herbaceous grassland	3° 22′ 05.48″ S	38° 35′ 01.11″ E

Assessment of mound characteristics, morphological characterisation and analysis

Termite mound structure and features including height; size, presence and absence of ventilations, as well as shapes of the mounds were observed and noted. These features were collated with the identification features posted by Roonwal (1977). Morphological characterisation of soldier termites was conducted under a dissecting microscope. We measured the breath and linear distance of the soldier heads, entire linear distance, breath and linear distance of prothorax as well as the shape, and similarly for the mesothorax and metathorax. Apart from these characteristics, we also used; sensory features on the head by counting them and the parts thereof, head features including size and shape and maxillary characteristics. A combination of these characteristic features was employed in the generic level classification and identification of the termite samples. From every active mound sampled, only four termites were used to take measurements and features, and an average of the four was worked out. A high accurate vernier caliper (Mitutoyo 530-312) was used for taking measurement. We used the criterion published by Sornuwat et al. (2004) and Wijerathna and Dias (2012) respectively to ultimately identify the termites to the genus level. Further, Euclidean distance measure was employed and a hierarchical cluster analysis with average linkage method was performed. The impact brought about by scale was avoided by standardization of crude phonological information observed to zero mean and unit variance. The resulting covariance was employed in working out the interrelation amidst termite communities with cluster examination through hierarchical cluster analysis (HCA) with UPGMA (unweighted pair-group method using arithmetic averages). Paired range for all the sample (covariant equal advantage) were calculated. This was in a bid to establish those specimens with the smallest genetic range, which was done repeatedly for successive couples of specimens having trivial pair-wise range. These ranges are thus grouped repeatedly in a continuous manner. Proceeds from this continuous clustering were concocted to generate a rooted dendrogram with branching values using Euclidean diversity estimate in DARwin v.6.1 software. The dendrogram was generated using GenStat 16th Edition statistical software. Quality pictures of the exemplary termite galleries and termite samples were taken with an electric camera (OPPO A 93). A comparison of our findings with the records available in the National Museum of Kenya was done after which our representative specimens bearing the name and identity number of the researchers were conserved at the Entomology Department of the Kenyan archives.

DNA based delineation

Extraction of DNA

Slightly moderated Phenol:Chloroform method (Sambrook et al., 1989) of DNA extrication was used to extract complete DNA from

the heads of soldier termites. The heads were sterilized, combined and crushed in 200 µl of TE (Tris EDTA pH 8) with mortar and pestle. For easy and faster disintegration of the tissues, we used 500 µl of lysis buffer (400 µl of TE and 100 µl of 5% SDS) and 10 µl of Proteinase K. The blend was inoculated at 65°C for 1 h. A mixture of 120 µl of phenol: chloroform: isoamyl alcohol (25: 24: 1) was put in the tubes followed by swirling for 30 s, and then centrifugation for 10 min at 10,000 rpm. The uppermost watery film was cautiously taken and put in a sanitized Eppendorf tube ensuring that the protein layer remained intact at the interphase. For precipitation of DNA, 500 µl of isopropanol was appended to the separated watery film and stored at -4°C for a whole night after which centrifugation at 12,000 rpm for 10 min was done. This led to the formation of a pellet which was cleaned severally by first getting rid of the supernatant followed by rinsing with 70% ethanol and sterile water. The resulting pellet was dried in air for half an hour or thereabout and eluted in 40 µl of TE buffer to stabilize it against disintegration. To confirm whether the gDNA was up to standard, we ran the extract in 0.8% agarose gel. The DNA was stored at -20°C for subsequent use (Sambrook et al*.,* 1989).

Amplification and sequencing of COII gene

Using these set of primers; A-tLeu (5'-CAG ATA AGT GCA TTG GAT TT-3') and B-tLys (5'-GTT TAA GAG ACC AGT ACT TG-3'), forward and reverse directions respectively (Miura et al., 1998), the extracted genomic DNA was used as a prototype to multiply the targeted COII gene portion. The mix therefore comprised of 2.5 µl of 10X PCR buffer, 2.0 µl $MgCl_2$ (2.5 mM), 2.0 µl dNTPs (200 µM), 0.25 µl of *Taq* Polymerase (5 U/µl), 1 µl of each forward and reverse (5 Pico moles) and 0.5 µl of DNA. This was topped up with 15.75 µl of PCR water making a final volume of 25 µl. A reaction check with all the components except the prototype was also set for internal validity. Temperature cycles consisted of; primary denaturation at 95°C for five minutes, denaturation at 95°C for 30 s, annealing at 52°C for 45 s, and extension at 72°C for 1 min using automatic PCR unit. This was repeated 30 times, followed by 5 min of extension at 72°C. The resulting PCR by-products were authenticated by gel electrophoresis using 1.5% agarose in 1x TAE buffer stained with ethidium bromide and visualized under ultraviolet light. The by-products were refined using the QIAquick PCR purification Kit protocol (Qiagen, Germany). The refined by-products were preserved at - 20°C. This was then sent for sequencing through international courier services to Macrogen Netherlands, which is a commercial service provider (http://www.macrogen.com). The amplified DNA fragments were sequenced employing Sanger sequencing, the most common dideoxy method using COII universal primers, whereby there was use of redesigned dideoxy bases (ddNTP's). The sequence data of the termites were recovered in the form of chromatograms which were extracted and saved for editing and further analyses.

Phylogenetic analyses

Out of the effectual 31 sequences amplified, only four were utilized in the eventual probe to maximize on the quality of the results. These sequences were manually edited in CLC main workbench version 7.2.6. It was done by first trimming the sequences to eliminate reads of poor quality, followed by assembling the forward and reverse sequences to align them where they overlap to get a contiguous sequence. Conflicts arising as a result of mismatch in overlapping regions of the forward and reverse sequences were assembled. Using Mallard software (Ashelford et al*.,* 2006), we looked out for any chimerical structures and artefacts present. The edited sequences were submitted to the Genebank to obtain the following accession numbers; KT845956, KT845957, KT845958, KT845959, KT845960, KT845961, KT845962 and KT845963. Further, we did an exploration of identical sequence arrangement using BLASTN. Closest relatives of the sequences with 99-100% identity were retrieved from the GenBank for subsequent phylogenetic analysis. The sequences were aligned with the help of CLUSTAL Omega program (http://www.clustal.org). The evolutionary history was hypothesized employing UPGMA formula (Sneath and Sokal, 1973) after which it was possible to highlight optimal tree with total branch length that was equal to 1.77017127. The rate percent of duplicate trees in which the related species were grouped simultaneously in the bootstrap test (1000 replicates), were presented above the nodes (Tamura and Kumar, 2002). The tree was drawn to scale, using similar units like the units of the branch length in evolutionary distances for phylogenetic tree inference. Using Maximum Composite Likelihood formula (Tamura et al., 2004), we determined the genetic range in the units of the number of base interchanges in each site. The differentiation in constitution partiality among sequences was given consideration in evolutionary differentiation (Tamura and Kumar, 2002). We incorporated 15 homolog sequences. Codon sites considered were 1st+2nd+3rd. For every set of sequences, all obscure sites were eliminated leaving 682 sites in the eventual data-set. MEGA 7 (Kumar et al., 2016) was used to do the evolutionary analysis. To construct the tree, we exported the aligned sequences to MEGA and converted them into Mega format. We then set the analysis parameters like bootstrapping values (in this case 1000 times) as well as appropriate substitutional model and left it to compute and finally saved the generated tree. All prototypical sequences were retained in a collective database (GenBank) for easy access by interested stakeholders.

RESULTS

Morphological identification of termites

In this study it was observed that termite mounds were present in all the types of plant life surveyed. Collectively, 54 active termitaria were documented. Of the total number, 17 were located in the herbaceous grassland vegetation, seven in the rain-fed trees, another seven mounds in the shrub savannah vegetation and six mounds forested in the vegetation. The disturbed land recorded 17 active termite mounds. It was noted that in the disturbed areas such as the area occupied by the institutions, there were more active mounds compared to farmlands, which had mounds that were either destroyed or inactive. Despite encountering few mounds in the rain-fed trees and shrub savanna vegetation, occurrence of inactive mounds was uncommon. In the herbaceous grassland vegetation type (the most dominant vegetation in the County), the active mounds were dominant.

Mound architectural characteristics

A comprehensive analysis of the mound characteristics revealed the presence of three types of mounds. These were regular and irregular conoid mounds with several

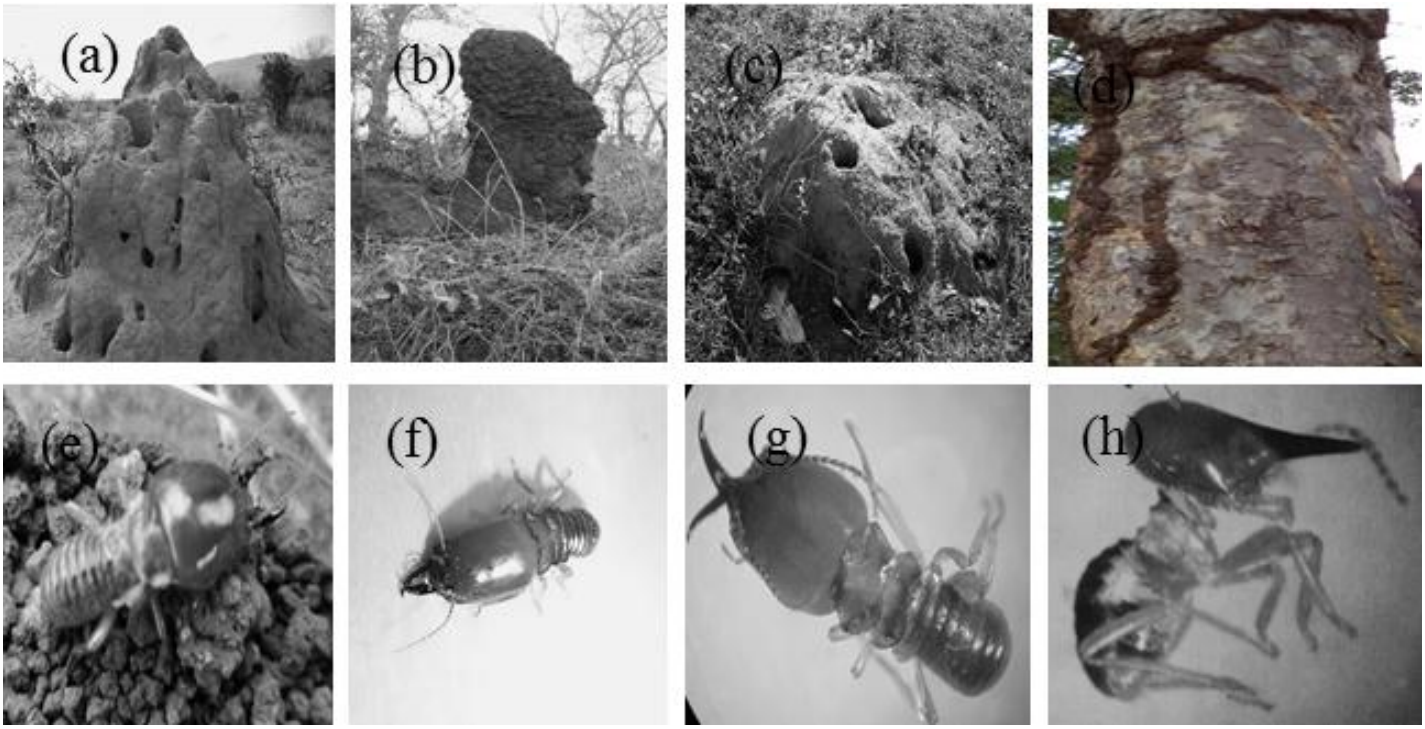

Figure 1. Representatives of termite mounds and termite species identified in Taita Taveta county Kenya. (a) and (b) are open and closed cone and dome shaped *Macrotermes* mounds, (c) slightly raised above the ground *Odontotermes* mound, (d) subterranean gallery of *Amitermes* species (e) and (f) rounded and oval shaped head capsule of *Macrotermes* species (g) Flattened semi rectangular *Odontotermes* head capsule and (h) single mandibular snouted head capsule of *Amitermes* species.

vents, elongated closed dome-shaped mounds and above ground mounds. Of these mounds, 32 were huge irregularly conoid with several vents. The elongated closed dome shaped mounds were 17, the slightly above ground mounds were three while the arboreal nests were two (Figure 1).

Termite identification

Termite samples identified from this study clustered into three groups based on the mound structures they construct and the observed termite characters noted, including the head capsule, antennae segments, mandibular features, pronotum, labrum, mesonotum and metanotum (Table 2).

Cluster analysis of morphological characters

There were five clusters distinguished as a result of examining the phenotypic characteristics of the termites when a dendrogram was generated (Cluster I, II, III, IV and V). Most samples (41 members) clustered together in Cluster I (Figure 2). The populations in this cluster were a mixture from the different vegetation types; DL (37%), RFT (15%), HG (24%), shrub savannah (20%) and forest (4%). Sample 12DL formed a cluster of its own in Cluster II. Cluster III comprised six members, namely 18RFT, 19FS, 26FS, 21HG, 32SV, and 38RFT. Furthermore, five samples (24SV, 25FS, 29FS, 51RFT, 28FS, and 27FS) formed Cluster IV whereas 27FS formed a singleton in Cluster V. Clustering of the termites based on morphological characters indicated that the soldier characters were not in any way dependent on vegetation type as some characters were shared by members of different vegetation types. Members sampled from the same plant life system also clustered separately, which is an indication of diversity among them. It also pointed to heterogeneity between members of the same vegetation and those of other vegetation types.

Molecular identification of termites

Nucleotide analysis, genetic distances and sequence divergence

The approximately 750 bp fragment of COII gene of the termite specimen amplified, suggests that there was no transfer of mitochondrial DNA to the nucleus which were

Table 2. Possible genus identity of termites collected from Taita Taveta County studied in 2013 and 2014.

Sample code	Collection site/vegetation	Mound characteristics	Soldier characteristics	Possible genus identification
IDL,3DL, 4DL	Disturbed land	Even and uneven conoid Shaped mounds	Head capsule has a fontanelle, Labrum with glassy tip, pronotum saddle shaped, mandibles fully developed, mandibles black with sabre shape, 17 antennae segments, large body size, dark brown or light, brown in colour, distended head capsule round or oval shaped, soldiers very aggressive.	*Macrotermes*
21HG, 34HG	Herbaceous Grassland			
30FS,	Exotic Forest	Mounds Ventilated		
14SV, 32SV	Shrub Savanna	Inert termitaria playing host to other creatures		
7RFT,17RFT, 42 RFT	Rain fed Trees	Conoid mounds		
31HG,41HG	Herbaceous Grassland	Unventilated secured mounds		
11SV, 24SV, 26SV	Shrub Savanna	Mounds a bit elevated With at least some vents	Present a characteristic bad odour, yellow body with a brown tinge, left mandible has a tiny tooth, long slender mandibles with slightly incurved tips, semi rectangular head capsule, light brown head capsule with few bristles on the periphery, saddle shape pronotum, reddish brown labrum with hawk shaped, sickle shaped mandibles, the mesonotum and metanotum broader than the pronotum.	*Odontotermes*
28FS, 29FS	Bura Exotic Forest.	Colonies found in degrading humus, others build subterranean galleries on live trees (don't build mounds)	Snouted head capsule, Small in size with total body length of 5 mm, Single mandible	*Amitermes*

sequenced. Stop codons were hardly present in majority of the samples. This is congruent with all amplified sequences depicting the nature of a functioning mtDNA cytochrome oxidase II arrangement of nucleotides. The parts omitted in some sequences were automatically fixed with the MEGA program applied before testing. The third codon set was weighted with A+T sequences at an average content of 63%. Figure 3 shows the nucleotide constitution of varied termite species.

We worked out the two-by-two genetic ranges between the sequences using Kimura two parameters (Kimura, 1980) presented in Table 2. Distance within species of the first set containing specimens 1DL, 3DL, 4DL, 21HG, 14SV. 34HG and 32SV haplotypes was 0.003 while that of group two comprising specimens 7RFT, 17RFT, 20RFT, 42RFT 41HG and 8SV was 0.004. Sequence of sample 28FS clustered together with *Amitermes conformis.* Interspecific distances among the four different clusters of the termites ranged from 0.04 between first and second clusters to 0.23 between third and fourth clusters (Table 3). Within the same plant life, there was low genetic divergence measure between species which ranged between 0.00 to 0.01 for the DL, 0.00 to 0.01 for RFT, 0.03 to 0.14 for SV samples, 0.22 for FS specimens and 0.00 to 0.04 for HG specimens. This same divergence was higher for species from disparate plant life systems. An example is the genetic range linking forest species and those from the RFT vegetation which fell between 0.19 and 0.21 (Table 3). COII Intercluster divergences were approximately 10-fold higher than intracluster divergences.

Phylogenetic analysis

UPGMA formula of evaluation used for inferring the revolutionary account on MEGA 7.0.2 revealed three main clusters, representing three different genera (*Macrotermes, Odontotermes* and *Amistermes*). One cluster (supported by a bootstrap value of 84%) had three samples indicated as 1DL [KT845957], 17RFT [KT845959] and 21HG [KT845960] on the phylogenetic tree, plus a close association to representatives of genus *Macrotermes* (Figure 4). These members (including *Macrotermes* species, *Macrotermes subhyalinus* and *Macrotermes michaelseni*) had a close taxonomic affiliation with ≥98% sequence similarity amongst them. Another cluster was related to *Odontotermes* species. *Amitermes* representatives assembled separately in a group with a strong bootstrap rate of 80% together with sample 28FS [KT845963]. The two *Amitermes* spp. (*Amitermes conformis* and *Amitermes obeuntis*) had 91% sequence similarity with sample 28FS [KT845963].

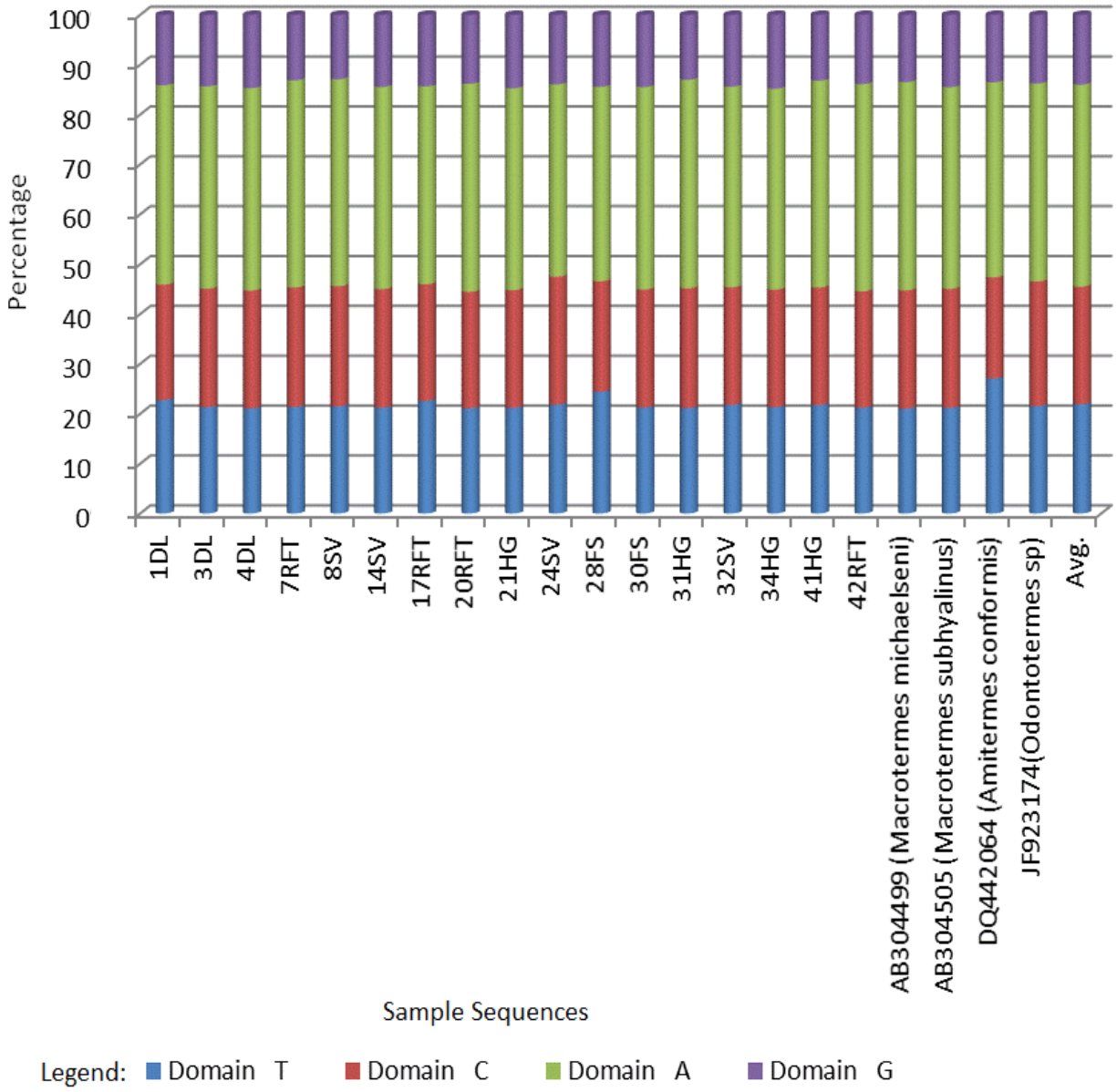

Figure 2. Nucleotide composition of COII gene in different species of termites collected from Taita Taveta County in 2013 and 2014. A= Adenine, C= Cytosine, T= Thymine and G= Guanine.

Termite taxa dispensation in the different vegetation types of the study site

Only a single termite species (*M. subhyalinus*) subjugated the fragmented land areas or DL. In the same way, the RFT land type comprised singly of the *M. michaelseni*. The herbaceous grassland vegetation type was inhabited by both *M. subhyalinus* and *M. michaelseni*. Notably, the shrub savannah vegetation type was rich species composition with three termite species; *M. subhyalinus, M. michaelseni* and an *Odontotermes* sp. Shockingly, the forest that is expected to be species-rich recorded only two termite species of genera; *Amitermes* and *Macrotermes.* The two species were *M. subhyalinus* and *A. conformis*. Overall, shrub savanna vegetation type was the richest termite species diversity (*M. subhyalinus, Odontotermes ceylonicus* and *M. michaelseni*) with a record of two different termite genera (*Microtermes* and *Odontotermes*). DL and RFT land systems were the lowest in species diversity. It was noted that termites of the genus *Macrotermes* had a high frequency of occurrence in all the land systems in the

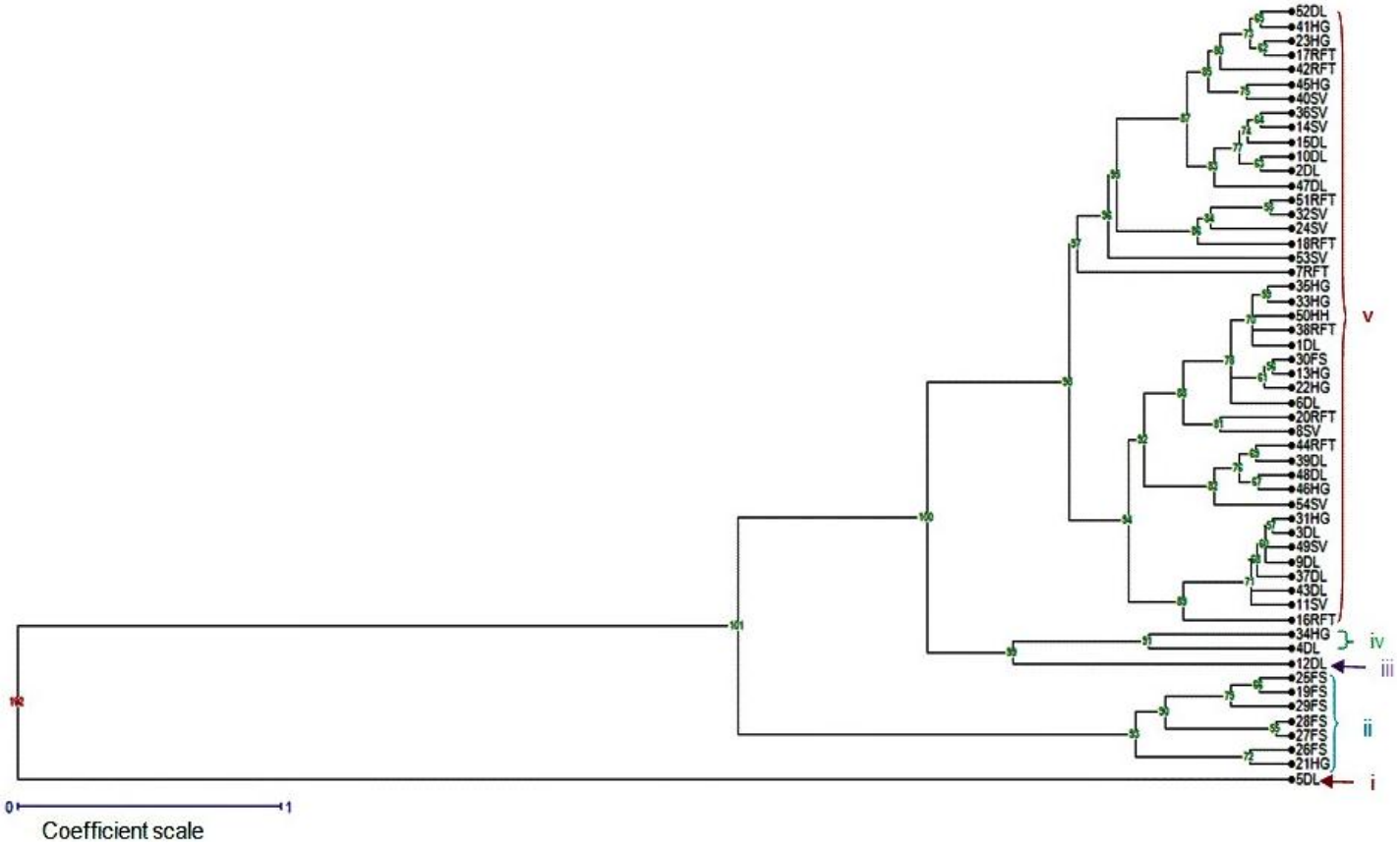

Figure 3. Cluster analysis based on morphological data with Euclidean distance measure. Numbers i-v marked in different colours are cluster grouping by morphology of the termite samples in 2013 and 2014.

study region while on the contrary, the other two genera *Odontotermes* and *Amitermes* were limited to SV and FS accordingly.

DISCUSSION

The mound structures variations observed, points to the existence of different termite species inhabiting the studied area. Although we did not classify the mounds on the basis of how different termite species feed, it is general knowledge that most soil feeders and fungus-growers are capable of constructing mounds (Makonde et al*.,* 2015); thus suggesting their presence. We observed two types of mound structures; conoid mound with external openings being analogous to the species *M. subhaylinus* and closed dome shaped mounds being analogous to the species *M. michaelseni.* This was supported by the fact that we had only collected those species from the respective mounds. Studies by Vesala et al. (2017) and Ocko et al. (2017) had reported that a termite species of *M. subhyalinus* is capable of constructing vented termitaria and *M. michaleseni* to construct closed dome shaped mounds. Not long ago, a similar finding in the same region was reported on the mound structure of *M. subhyalinus* and *M. jeanneli* (Vesala et al*.,* 2019). Another study reported a scenario where it was difficult to ascertain the true mound builder as termites of two different termite genera both inhabited the mound (Makonde et al*.,* 2013). More recently, Paejaroen et al. (2021) found other species in the genus *Globitermes* and *Microcerotermes* to build large epigeal mounds in the tropics of Thailand which suggests more species could be involved in the ultimate morphology of a mound. Termite samples identified from the study by morphology were ultimately placed in three genera; *Macrotermes, Odontotermes* and *Amitermes* all of the family Termitidae. Coincidentally, the termite colonies that were identified to genus level through morphological characterization using soldiers were phylogenetically affiliated with the three genera indicating that morphological differences among genera are great enough for phylogenetic analysis of termites at the genus level. However, for species-level classification, this

Table 3. Pair-wise Kimura-2- parameter genetic distances based on COII gene fragment in termite species identified in Taita Taveta County in 2013 and 2014.

S/N	Sample/Spp	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	1DL																					
2	3DL	0.00																				
3	4DL	0.01	0.00																			
4	7LT	0.08	0.04	0.04																		
5	8SV	0.07	0.03	0.03	0.00																	
6	14SV	0.01	0.00	0.00	0.04	0.04																
7	17RFT	0.07	0.05	0.06	0.01	0.01	0.05															
8	20RFT	0.04	0.03	0.03	0.00	0.00	0.03	0.02														
9	21HG	0.01	0.00	0.00	0.04	0.03	0.00	0.05	0.03													
10	24SV	0.19	0.15	0.15	0.14	0.14	0.15	0.15	0.15	0.15												
11	28FS	0.21	0.19	0.21	0.20	0.19	0.20	0.21	0.19	0.20	0.22											
12	30FS	0.00	0.00	0.00	0.04	0.03	0.00	0.06	0.03	0.00	0.15	0.21										
13	31HG	0.07	0.03	0.03	0.00	0.00	0.04	0.01	0.00	0.03	0.14	0.19	0.03									
14	32SV	0.04	0.00	0.00	0.03	0.03	0.00	0.05	0.03	0.00	0.15	0.21	0.00	0.03								
15	34HG	0.01	0.00	0.00	0.04	0.03	0.00	0.05	0.03	0.00	0.15	0.21	0.00	0.03	0.00							
16	41HG	0.07	0.04	0.03	0.00	0.00	0.04	0.01	0.00	0.04	0.14	0.19	0.04	0.00	0.03	0.04						
17	42RFT	0.04	0.03	0.03	0.00	0.00	0.03	0.02	0.00	0.03	0.15	0.19	0.03	0.00	0.04	0.03	0.00					
18	AB304499 (Mm)	0.03	0.03	0.03	0.00	0.00	0.03	0.01	0.00	0.03	0.15	0.18	0.03	0.00	0.03	0.03	0.00	0.00				
19	AB304505 (Ms)	0.01	0.00	0.00	0.04	0.03	0.00	0.04	0.03	0.00	0.15	0.20	0.00	0.03	0.00	0.00	0.04	0.03	0.03			
20	DQ442064 (Ac)	0.26	0.22	0.22	0.22	0.22	0.21	0.24	0.21	0.22	0.23	0.09	0.22	0.22	0.22	0.22	0.22	0.21	0.22	0.22		
21	JF923174 (O.sp)	0.20	0.18	0.19	0.16	0.16	0.19	0.18	0.17	0.19	0.08	0.22	0.18	0.16	0.19	0.19	0.17	0.17	0.17	0.19	0.23	

Mm= *Macrotermes michaelseni*, Ms= *Macrotermes subhyalinus*, Ac= *Amitermes conformis*, O.sp= *Odontotermes species*

approach might take time and definitely need specialized knowledge of character differences (Janowiecki, 2015).

Molecular characterization results indicated that COII sequences guaranteed the ability to allocate the termites into molecularly well-defined species. There was accurate disparity of most sequences into different clusters. In comparison to their predetermined morphospecies, there was a high level of consistency. The nodes directly defining the clusters presented 99% nodal support. Furthermore, the three clusters remained definite to the fact that such groups comprised well-defined COII lineages instead of dissipated sequence dissimilarity (Hajibabaei et al., 2006). For our study, sequence separation in COX II mtDNA within distinct groups (intraspecific) were smaller than separation among groups (interspecific), although they prevailed within COII sequence divergences from Genbank. This portion of the mtDNA has a general characteristic of showing wide interspecies, yet little intraspecies separation, elucidating that species regularly create well distinct groups on phylogenetic tree. The empirical levels of sequence separation associated with species delimitation studies and in particular bar-coding (Hebert et al*.,* 2003) support the finding of this study.

High Adenine Thiamine content of 63% in the studied sequences did not deviate from what mtDNA usually presents in insects (Crozier and Crozier, 1993) because it is a common characteristic of Cytochrome Oxidase II mtDNA

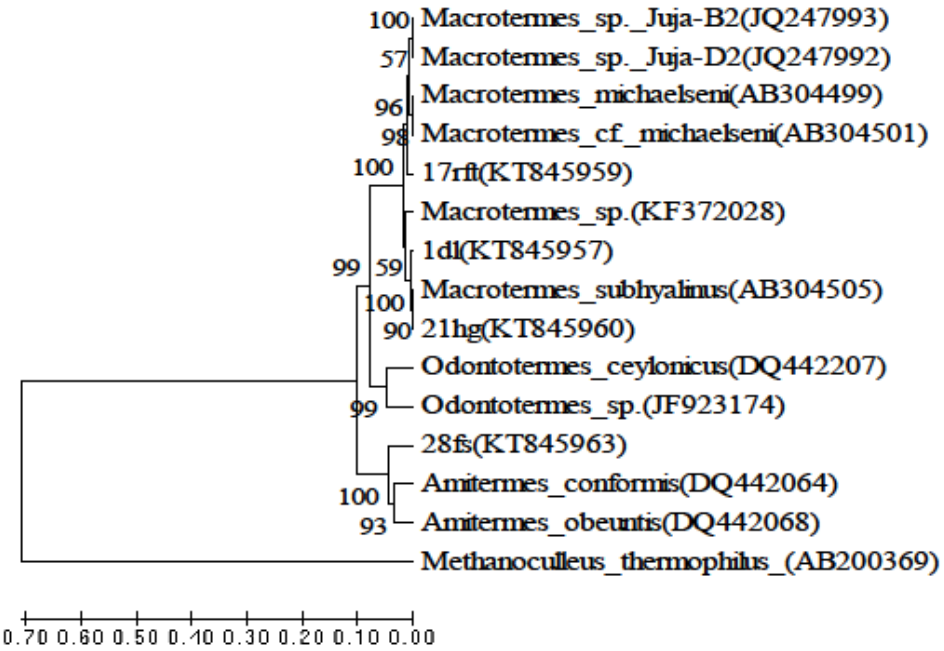

Figure 4. Phylogenetic analysis of COII gene in termites. Multiple sequence alignments were against a portion of the gene. A rooted phylogenetic tree was established with MEGA 7 by the UPGMA method with bootstrap values for 1,000 replicates shown at major nodes. The optimal tree with the sum of branch length = 1.77017127 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown above the branches.

region in arthropods. A+T partiality in such sequences is harmonious with information related to COII mitochondrial genes termite in the family Termitidae as well as some genera, that is, *Coptotermes* of the family Rhinotermitidae (Singla et al*.,* 2016; Yeap et al*.,* 2007). A report by Wahlberg et al. (2003) studying *Phyciodes*, family Nymphalidae, and order Lepidoptera also found A+T content from three varied gene portions in Nasuta subgroup of *Drosophila* in the range of 65-78%. In a different study by Hussin and Majid (2020), a lower value than that in our study was found (58.4%) and cited the use of incomplete sequences rather than complete COII gene region (which varies between insects in the range of 673 - 690 bp) as most probable reason for their findings. A study of genus *Coptotermes* in one of the Japanese forests (Tokuda et al*.,* 2012) also reported A+T bias and correlated it to the genetic structures and arrangement of cytochrome oxidase gene sequences. This propounds that AT alteration coercion as a consequence of preselection in order to incorporate amino acids encrypted by AT loaded lines accompanies sequence separation. The phylogenesis research using mtDNA COX II gene fragments therefore depicted the similarity between different termite species. The level of dissimilarity among them is very important in understanding their relatedness. There have however been congruous phylogenetic reconstructions in termites with single or combined mitochondrial markers and nuclear markers having no overlaps of intra- and intergroup sequence variability which avoids ambiguous definition of clusters (Hausberger et al*.,* 2011).

Our sequences from the DNA based characterisation are grouped into one major family Termitidae under the subfamilies *Macrotermitinae* and *Termitinae* and further to three main genera as in the morphological characterisation. From the genus *Macrotermes,* there was a clear separation into well supported two sub groups; *M. subhylinus* and *M. michaelseni* species. The grouping of these species was congruent with using conceptual approach for divergences occurring within the species and between species for termite delineation in *Termitidae*, going by the report of Osiemo et al*.* (2010). We did not however discover any obscure species as all the sequences clustering conjoined perfectly with morphospecies. The low sequence similarity between sample 28FS and Genebank samples may suggest a possibility of a new taxa, which still needs further detailed description using morphological, chemical and molecular properties. This is to say there was no sequences within the same morphospecies extensive branching nor clustering of distinct morphospecies. Other studies have analyzed morphospecies and found comparatively

greater diversity than in our studies e. g (Cassalla and Korb, 2019), hence it is completely hard to compare them with our findings. Osiemo et al. (2010), in their research on termite diversity assessment in Kakamega Forest (found in Kenya), had more or less similar findings. Their study however found that there was deep sequence separation in the phonologically unrecognized as well as identified morphologically different species in termites feeding on soil. It is for this reason that these were considered cryptical species and therefore placed together separately as groups of organisms with similar gene characteristics. The forest in question, unlike our study area, is large and diverse and is within the tropics hence their observation. Other authors who have successfully uncovered ambiguous convergence in overactive divergent groups like termites and nematodes have been reported (Bourguigno et al*.,* 2015).

Morphological characterisation using soldier morphology of termites in the current study was possible only to the genus level whereby three genera were identified. Identification based on DNA approach was possible to the species level and termite samples into three genera and four different species. Notably, before construction of the phylogenetic tree, there was a specimen which was presumably placed as *Nasutititermes* species, because of the external features it conferred. However, after phylogenetic analysis, it became apparent that the sample had closer genetic association with *A. conformis* and very distinct from *Nasutitermes* genus and thus was classified as a species of the genus *Amitermes*. Termite characters sometimes develop in response to their immediate environment for the sake of adaptation and so would present as a certain species. This is what leads to such morphological ambivalence (Hausberger et al*.,* 2011). Using both techniques, we were able to resolve the termite identification problem. Overall, molecular genetics have nearly endless possibilities for their applications to the study of termites.

Although many mounds were recorded in the herbaceous grassland vegetation, only one termite species from the genus *Macrotemes* was identified. The only possible explanation we could give for this observation was that the same colony was reestablished in different parts of the vegetation cover due to increase in their population. The many numbers of inactive mounds in the disturbed land was attributed to increased human activities which trigger deliberate destruction often in sites where agricultural activities were ongoing and also for fear that termites would cause damage. Notably, in sites such as the Taita Taveta University and Kenyatta High School (areas without farming practices), a few active mounds were recorded. Reduced species abundance in relation to land fragmentation is not a unique occurrence as it has been widely reported in Africa and other parts of the world (e.g. Ivory Coast: Coulibaly et al*.,* 2016; Vietnam: Neoh et al*.,* 2015; Panama: Basset et al*.,* 2017). This supports the speculation that it is a worldwide trend. For instance, recording only a single termite species in the disturbed sites was a clear indication that agricultural activities were rampant hence termite migration and consequently inactive mounds. Egan et al. (2021) also reported similar findings. The shrub savanna vegetation type had the highest termite diversity, demonstrating high termite activities in the vegetation type. The possible explanation for this is that most members from the genera *Macrotermes* and *Odontotermes* are general wood and litter feeders, hence the high activities in such environment. Hypothetically, dryland savannah has lower diversity in comparison to wet savannah (Luke et al*.,* 2014). Two termite species belonging to different genera were recorded in the forested vegetation. These were *Amitermes* and *Macrotermes.* The former, (though it can be found in a range of habitats) was only restricted to the forest. The explanation to this may be the forests or undisturbed areas support even the species that are or could be endangered as there are limited human disturbance. This however is dependent on whether the forest is protected or not as unprotected ones are subject to disturbance (Schyra et al., 2018). Moreover, it has been reported that *Amitermes* species has been reported to survive in cool and wet environments (Davies et al., 2014). In a West African study of termite species richness in protected and disturbed land of both savannah and forest ecosystems using COII gene, species richness decline was found in forest than in disturbed land whereas it was not the case for the savannah. However, collectively, the forest had more termite encounter rate as well as species number (Schyra et al*.,* 2019). In both ecosystems, there was low composition of termites in the disturbed sites. Another study (Jamil et al*.,* 2017) on the contrary, reported low species composition in the protected forests areas of Samusan Wildlife Sanctuary when compared to other forest studies elsewhere and they accredited their findings to intensive and extensive logging activities and loss of primary forest. In general, higher altitudes decrease heterogeneity of species of termites. This possibly is because at high altitudes there are reduced temperatures associated with rates of metabolism.

Conclusion

The results have shown that mound building termites of the three genera reported above have dominance over the studied regions of Taita Taveta county. The study demonstrates that agricultural activities affect termite activities and consequently the species abundances. Further, the study confirms that termite species identification based on polyphasic approach is more

concise and informative than using the morphological characters alone. The knowledge gathered from this study using the dataset will be essential in other studies aiming to identify Kenyan species of termites in terms of ubiquity as well as their organization. Such identifications have been proven to be hard when done using only the morphology based approach. Thus, the study underlines the value of employing the two methods; morphology based and DNA-based characterization techniques. These contributions provide a strong foundation for future work that is needed to better understand this important economic pest.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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