Genetic characterization and distribution of termites in Taita Taveta County, Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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To my late parents Mr. and Mrs. Timon Agutu, my dear husband Everton, my children Hillary, Keith, Nina and Everton Junior, my uncle Charles and to my sisters Carolyn and Emily. I love you all!

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LIST OF ABBREVIATIONS

BLASTN	Basic Local Alignment Search tool
COI	Cytochrome Oxidase subunit one
COII	Cytochrome Oxidase subunit two
DNA	Deoxyribonucleic Acid
dNTPs	Deoxy ribonucleotide Triphosphates
EDTA	Ethylene Diamine Tetra-acetic acid
gDNA	Genomic Deoxyribonucleic Acid
GPS	Global Positioning System
HCA	Hierarchical cluster analysis
IBR	Institute of Biotechnology Research
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KWS	Kenya Wildlife Service
mtDNA	Mitochondrial Deoxyribonucleic Acid
MEGA	Molecular Evolutionary Genomic Analysis
NACOSTI	National Commission for Science, Technology and Innovation
NCBI	National center for Biotechnology Information

NEMA	National Environmental Management Authority
NUMTs	Nuclear DNA sequences originating from mitochondrial DNA sequences
PCR	Polymerase Chain Reaction
rDNA	Ribosomal Deoxyribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
SDS	Sodium Dodecyl Sulfate
Taq	*Thermus aquaticus*
TE	Tris EDTA
UPGMA	Unweighted pair-group method using arithmetic averages

ABSTRACT

Termites have colonized tropical ecosystems with both ecological and economic impact but their diversity remains ununderstood. This study aimed to assess the diversity of termite communities in Taita Taveta County, a threatened biodiversity hotspot in Kenya using morphological and molecular approaches. Termites (soldiers) were collected from different vegetation types within the County using descriptive survey design. The termites were observed under a dissecting microscope and morphological characters noted were transformed into quasi-characters then used for the construction of a dendrogram. Mitochondrial cytochrome oxidase II (COII) gene region was amplified from DNA extracted from termite samples, sequenced and analyzed. Phylogenetic tree construction was performed using the newly obtained sequences and closely taxonomically affiliated sequences retrieved from the nucleotide sequence database (GenBank). The phylogenetic analysis results were confirmed based on nucleotide composition and pairwise genetic distance measures. Three termite genera *Macrotermes, Amitermes* and *Odontotermes* were differentiated by morphological characterization. Molecular characterization clustered the termites into the three genera and into their respective species using relative percentage sequence similarity with the Genebank sequences. The termite species clustered with *M. M. michaelseni, A. conformis* and *O. ceylonicus. Macrotermes* species were ubiquitous whereas the *Amitermes* and *Odontotermes* were restricted to the forests and shrub savannah respectively. The study noted possibility of two new species and recommends further

investigation using series of data sets to enrich the knowledge of termite diversity and distribution in Taita Taveta County and in Kenya.

CHAPTER ONE

INTRODUCTION

1.1 General introduction

Termites are important species in tropical and subtropical ecosystems with a unique feature of colony caste system (Lefebvre, Chaline, Limousin, Dupont & Bagneres, 2008). They are known both as ecosystem engineers contributing to a wide array of ecosystem processes such as even distribution of soil nutrients, improved air and water flow as well as improved soil structure (Fall *et al.,* 2007). They are also known as pests causing damage to natural vegetation, crops and manmade structures (Ulyshen, Diehl &Jeremic, 2016; Jouquet, Dauber, Lagorlof, Lavelle & Lepage, 2006). They habour numerous microbial populations in their gut system with which they have developed a symbiotic relationship (Ohkuma & Brune, 2011). These microbes aid in the cellulotic feeding and negatively affect property and vegetation.

1.2 Importance of termites

Termites are economically significant by being pests to plants, forest and human structures (Pranesh & Harini, 2014). Over 80% of the subterranean termites are considered to be among the destructive pests (Su & Scheffran, 2000). Termite damage to buildings and structures is both costly and irreversible. The mound structures constructed by termites not only affect cultivation processes but also aggravate termite infestation of plants and vegetation (Ibrahim & Adebote, 2012; Piper, 2007).

Apart from being pestiferous, cumulative activities of termites and ants contribute significantly to soil modification and water infiltration. Termite species in the larger family *Termitidae* belonging to the genus *Macrotermes* are especially known to be ecosystem engineers (Joquet, Traore, Choosai, Hartman & Bignell, 2011). Their activities largely contribute to a variety of ecosystem processes such as decomposition, distribution of natural resources which also increases diversity of microbes, plants and animals (Jessen, 2014). Though termite mound structures make cultivation difficult, they act as habitats to other animals (Hyodo *et al.,* 2003). Termite mound soil, termite cultivated mushrooms as well as some termite species are consumed by animals including man in some African countries for nutritional or other benefits depending on indigenous belief systems (Kelemu *et al.,* 2015; Srivastava, Dwivedi & Pandey, 2011; Sileshi *et al.,* 2009). Moreover termites and their symbionts have potential functional genes that can produce enzymes with industrial applications (Matsui, Tokuda & Shinzato, 2009).

1.3 Distribution of termites

Termites are found in all the continents except antarctica. They inhabit approximately 75% of the Earth's land surface and are distributed between latitudes 45°N and 45°S (Ngugi, 2008). They are widely distributed throughout the tropics with the highest abundance and species diversity in the tropical rain forests of Africa, South America and Southeast Asia. This diversity further extends into the temperate zones (Kemabonta & Balogun, 2014; Ravan, 2010). Termites that feed on wood, litter and grass are denser in

savannas and grasslands whereas soil and humus feeders are more abundant in the rainforests. The tropical species are more established in the subtropics while the subtropical species have expanded their range into the temperate areas (Grace, 2006).

1.4 Diversity of termites and methods used in termite identification

Limited data on diversity and distribution of termites in Africa is widely reported (Mugerwa, Mpairwe, Zziwa, Sawaans & Peden, 2014; Ahmed *et al.,* 2011). Termite diversity and abundance is higher in lowland tropical forests than in arid and semi-arid regions (Boga, Moise & Mauricette, 2015). The generic richness of termites is highest and lowest in African and Neotropical rainforests, respectively (Jones & Eggleton, 2011). Several studies have shown that termite species richness may be drastically reduced as a result of habitat disturbance (Davies *et al.,* 2014).

For a long time termite biodiversity studies has relied on morphological description and analyses where species identification is based on the caste system provided by the termites (Wang *et al.,* 2009). The process has been made possible by the presence of conspicuous morphological characters possessed by the soldier castes in termites (Engel, Grimaldi & Krishna, 2009). Depending on the termite group under study the choice of morphological characters used for characterization and identification can vary. When using this method identification beyond the family level is difficult especially when alates and workers are being investigated. This is because the workers are morphologically similar thereby presenting inadequate taxonomic characters for species identification (Osiemo, 2009). Moreover best character for worker identification is the

gut which has to be dissected and the process is tasking. The alates on the other hand can only be collected during certain seasons of the year and are barely found in a collection. They are also only useful for certain groups of termites such as the soldierless termites (Austin, Szalanski & Cabrera, 2004). Soldiers are easily identified with their defensive mandibles or head characters (Wang *et al.*, 2009). The distinct colours of the tibia and wing membranes of different species have been indicated as useful identification characters in soldier caste (Nutting, 1990).

Classification based on morphological description is a complex undertaking. When morphological identification is haphazardly done it may lead to misidentification and underestimation of biodiversity (Harper, Gile, James, Carpenter & Keeling, 2009). Moreover external features are influenced by changes in the environment which might never affect the genetic makeup of an organism. Limitations in the use morphological characterization of species include; both phenotypic plasticity and genetic variability in characters employed for species recognition which can lead to incorrect identifications. Second, the traditional approach overlooks morphologically cryptic taxa that are common in many groups. Third, since morphological keys are often effective only for a particular life stage or gender, many individuals cannot be identified. Finally, the use of keys often demands such a high level of expertise that misdiagnoses are common (Bahder, 2009).

Chemical characterization of termites is another method often used. It involves the analysis of cuticular hydrocarbons and termite feces to identify the termite species. This

is because the cuticle covering the outside surface of the termites also lines the inside of the rectum where the fecal pellets are formed and stored. Cuticular hydrocarbons are species specific (Haverty, Woodrow, Nelson & Kenneth, 2005). The hydrocarbon composition from termite species is compared with the fecal pellets. The advantage of this method is that termite taxa can be identified without the diagnostic castes. The limitation is that some of the hydrocarbons are unstable and can degrade easily when not carefully handled.

The use of molecular techniques to identify species has recently advanced understanding of the relationship among organisms at various levels of taxonomy (Mandal, Chhakchhuak, Gurusubraniana & Kumar, 2014). Typically, this involves the use of the polymerase chain reaction (PCR) to amplify short sections of DNA, which are then characterized using the sequencing technology (Armstrong & Ball, 2005). Molecular techniques can overcome some of the shortcomings posed by morphological identification and have been adopted for the purposes of termite identification and inferring phylogenies (Szalanski *et al.*, 2004). This technique uses nucleotide sequence similarity to identify species from genomic DNA therefore identification can be achieved regardless of the encountered caste (Monaghan, Balke, Pons & Jibia, 2006).

Molecular phylogenetics as well as DNA barcoding are more informative and more reliable than morphological phylogenetics when carefully carried out. If poorly conducted, they can also lead to misidentification just as the phylogenetic studies based on morphological characters. Therefore, adequate and proper taxon sampling as well as

sufficient genetic loci should be used (Husein, Kamble & Stone, 2006). Use of mitochondrial DNA (mtDNA) sequences is efficient and a successful tool for species identification across invertebrate groups. The mtDNA can also be used to determine their phylogenetic relationships as their analyses are more accurate even with little or very old DNA samples (Garrick, Collins, Yi, Dyer & Hyseni, 2015; Melton, Holland & Holland, 2012).

1.5 DNA barcoding and Cytochrome oxidase II [COII] gene

DNA barcoding is a method that uses short mtDNA sequences to group unknown individuals. Sequences of already defined taxonomic organisms present in the public database system are used for comparison with newly isolated nucleotide sequences to determine similarity. DNA barcoding was proposed by Hebert, Ratnasingham & de Waard (2003). It was later identified to be useful in identifying and classifying unknown specimens. Identification by mtDNA is fully dependent on DNA rather than morphological characters. Computer programs aid statistical analyses on DNA sequences to resolve challenges with tree production (Bahder, 2009; Monaghan *et al.*, 2006).

Mitochondrial genes evolve comparatively faster than nuclear genes making them suitable markers for analysing relatively close relationship (Yeap, Othman, Lee & Lee, 2007). These genes have been widely used for phylogenetic reconstructions. Mitochondrial DNA is easy to isolate and amplify (Mandal *et al.,* 2014). In most cases mtDNA is uniparentaly inherited and encodes genes that originate by vertical descent

from a single gene of the last common ancestor. This allows comparison among distant taxa (Yeap *et al.,* 2007). In phylogenetic studies of insects like termites, the most frequently sequenced mitochondrial genes are cytochrome oxidase I (COI), COII, 16S rDNA and 12S rDNA (Ye *et al.,* 2004). Of these, COII has been sequenced over the widest range of taxa, so that homologous sequences are available for nearly all orders (Garrick *et al.,* 2015). The COII gene contains both highly conserved and variable regions and consequently used to estimate phylogenetic relationships among arthropods (Garrick *et al.,* 2015). Barcoding of specimen has also used the region for species identification (Singla, Goyal, Sobti & Sharma, 2015; Osiemo, 2009). Other DNA markers like 12S 16S and 18SrRNA have also been used (Ye *et al.,* 2004; Singla, Sharma, Sobti, Sodhi & Kumari, 2013) but Cytochrome oxidase subunit two (COII) is a well-known and widely used gene in phylogenetic studies of termites with many sequences available in the Genbank (Garrick *et al.,* 2015).

Due to the limited data on diversity and distribution of termites, phylogenetic analyses which can lead to species level identification are needed so that termite inventories can be established. Current knowledge also points that molecular characterization based on the COII gene sequences indicate promising success in allocating the termites into their distinct groups (Osiemo, 2009). This study was therefore conducted to identify termite species in Taita Taveta County, Kenya using both morphological and molecular characterization. Taita Taveta County is a typical tropical savanna and a biodiversity hotspot in Kenya whose biodiversity is perceived to be threatened by human activities

(Dansk Ornitologisk Forening [DOF], 2015-2017). Identification of termite species in the county will form a baseline for planning and management of termites.

1.6 Statement of the problem

Despite the advances in the use of DNA molecular markers such techniques have not been fully utilised in termite diversity studies in Kenya. Termites being pests of economic importance, more species still need to be unveiled in Kenya and Africa. Taita Taveta County in Kenya is a tropical savanna and one of the known threatened biodiversity hotspots in the region (DOF, 2015-2017). However, very little has been done to document the species diversity using molecular techniques and their distribution in the county. Existence of few termite taxonomic experts in Africa coupled with inadequate identification facilities contribute to underestimation of termite species richness. Most sampling strategies are not always exhaustive and often target the majority worker caste whose external features are uniform and difficult to tell apart (Osiemo, 2009).

1.7 Justification of the study

Albeit considerable termite identification work has been done in some parts of Africa, a large number of termite species remain unknown. Many groups of African termites still need accurate identification and status revision (Uys, 2002). Few studies have been conducted on the diversity and distribution of termites in Kenya. Studies on termites have focused on their gut symbionts and their effects on the soil properties. Studies on termite genetic diversity and distribution using both morphological and molecular

methods are thus needed to understand their diversity, phylogeny and contribution to the ecosystem (Osiemo, 2009). The findings from Taita Taveta could form a basis for identification of termite species in the savanna. This study may further be used to establish termite management strategies as well as understand their biotechnological and industrial potential. This study therefore aimed to assess the diversity of termite communities within the tropical savanna region of Taita Taveta County, Kenya using both morphological and molecular analyses.

1.8 Null hypotheses

1. Taita Taveta County does not harbour a high termite diversity.
2. There is no difference in morphological and molecular methods in the identification of termites
3. There is no difference in the phylogeny of termites species in Taita Taveta County
4. There is no difference in the distribution of termites in various habitats in Taita Taveta County, Kenya

1.9 Objectives

1.9.1 General objective

To determine species diversity and distribution of termite species in Taita Taveta County.

1.9.2 Specific objectives

1. To characterize and identify termite species in Taita Taveta County.

2. To compare the identification of termites species in Taita Taveta County using morphological and molecular techniques
3. To compare the phylogenetic relationships of the termite species in Taita Taveta County, Kenya.
4. To determine the distribution of termite species in the various habitats in Taita Taveta County, Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Biology and taxonomy of termites

2.1.1 Classification of termites

The taxonomic classification of termites according to Inward *et al.* (2007) is given as;

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Subclass: Pterygota

Infraclass: Neoptera

Superorder: Dictyoptera

Order: Blattodea

Infraorder: Isoptera

Previously termites were grouped together with cockroaches and mantids in the family Termitidae of the order Blattodea (Inward, Vogler & Eggleton, 2007). Later, the order isoptera was established for termites. Conventionally the Isoptera are divided into lower and higher termites (Zhu *et al.*, 2012). More than 3500 termite species have been identified to date. These species belong to 281 genera, fifteen subfamilies and seven families (Grohmann, Oldeland, Stoyan & Linsemair, 2010). The seven subfamilies are arranged in a phylogenetic sequence (Table 2.1). Three families; *Mastotermitidae*, *Kalotermitidae* and *Hodotermitidae* are the lower termites.

Table 2. 1: Outline of Termite Classification (Engel *et al.*, 2009)

Family	Subfamily	Genera
Mastotermitidae		*Mastotermes darwiniensis*
Kalotermitidae		*Kalotermes*
Hodotermitidae	Carinatermitinaea	*Carinatermes*
	Lutetiatermitinaea	*Lutetiatermes*
	Hodotermitinae	*Hodotermes*
Termopsidae	Cretatermitinaea	*Cretatermes*
	Porotermitinae	*Porotermes*
	Stolotermitinae	*Stolotermes*
	Termopsinae	*Termopsis*
Rhinotermitidae	Archeorhinotermitinaea	*Archeorhinotermes*
	Coptotermitinae	*Coptotermes*
	Heterotermitinae	*Heterotermes*
	Prorhinotermitinae	*Prorhinntermes*
	Psammotermitinae	*Psammotermes*
	Stylotermitinae	*Stylotermes*
	Termitogetoninae	*Termitogeton*
	Rhinotermitinae	*Rhinotermes*
Serritermitidae		*Serritermes serrifer*
Termitidae	Apicotermitinae	*Apicotermes*
	Foraminitermitinae	*Foraminitermes*
	Sphaerotermitinae	*Sphaerotermes*
	Macrotermitinae	*Macrotermes*
	Nasutitermitinae	*Nasutitermes*
	Syntermitinae	*Syntermes*
	Termitinae	*Termes*

The lower termites mainly feed on dry or dump and decaying wood and do not construct definite nests but rather live in colonies in sound or dead wood (Mohammed, Abiodun &

Jibia, 2014) *Termopsidae*, *Rhinotermitidae*, *Serritermitidae* and *Termitidae* are the higher termites. The higher termites can feed on wood, exclusively on soil while others cultivate and consume cellulolytic fungi. They are also the most evolved as well as the most divergent group (Mohammed *et al.*, 2014). The *Termitidae*, which include mound-building and subterranean termites, is the largest family consisting of about 70% of described termite species (Engel *et al.*, 2009). This family alone comprises more than 664 species. There are four known subfamilies under this family and they are listed as *Apicotermitinae* having 70 species (Kanwal, Acharya, Ramesh & Reddy, 2011), *Termitinae* with 272 African species (Eggleton, 2000). *Macrotermitinae* has 165 African fungus growing termites (Eggleton, 1999) and *Nasutitermitinae* has 56 species (Mahaney *et al.*, 1999). The total number of species in the above subfamilies may surpass 90% of the world's known termite species. Africa has the highest termite species composition as result of its favorable environmental conditions. East Africa region alone has species that may exceed 177 (Ahmed *et al.*, 2011).

2.2 Classification of termites based on feeding and habitat preferences

Termites are divided into three major feeding groups. Each group is characterised by its own degradation process. The three groups are; soil-feeding, humus-feeding and wood-feeders (Kudo, 2009). Soil-feeding termites are important components of the soil macrofauna in rain forest and savanna ecosystems (Jones & Eggleton, 2011). The soil-feeding together with humus-feeding species have been found to thrive well on nitrogen-rich soil organic matter (Brune & Ohkuma, 2011). Feeding experiments with artificial humic substances have shown that soil-feeding termites are able to mobilize and digest

the peptidic components of soil organic matter. In return peptides, amino sugars and microbial biomass become abundant hence acting as dietary resource for humivorous termites (Ji & Brune, 2006). Most humivorous species are from the genus *Cubitermes*. The common species is *Cubitermes orthognathus.* Species from other genera include; *Termes propinquus, T. comis* and *Dicuspiditermes makhamensis* (Hyodo *et al.*, 2003).

The fungus-cultivating termites are found throughout the tropics, in rain forests and savannas, but are ecologically more dominant in tropical savannas (Aanen & Eggleton, 2005). This group of termites contributes substantially to ecosystem processes such as decomposition and bioturbation (Schuurman, 2005). The fungus-cultivating termites cultivate their fungal symbionts in monocultures on sponge like fungus combs within the nest (Aanen *et al.*, 2009). Species belonging to this group are mound builders of the genus *Macrotermes* like *M. jeanneli, M. bellicosus,* and *M. Michaelseni* (Osiemo, 2009). Wood-feeders feed on wood excavating galleries in larger items of woody litter, which may become colony centres (Swift & Bignell, 2001). Damp wood termites live and feed on very moist wood especially stumps and logs of trees (Turgbor, 2009). Dry wood termites belong to the Family *Kalotermitidae* and do not require contact with moisture or soil in order to survive. They nest entirely in timber above ground (Turgbor, 2009). Some examples of wood-feeding termites include some members of the genus *Nasutitermes* like *N. corniger, N. ephratae* and *N. macrocephalus* (Vasconcellos & Moura, 2010).

Another classification system of termites is based on their habitat types. They can be earth-dwelling (below ground or epigeal nests), wood-dwelling or arboreal (nest on trees) termites. Many types of earth-dwelling termites exist, such as the subterranean termites and mound building termites. The wood-dwelling termites on the other hand, restrict themselves to wood. Wood-dwelling termites are further classified as either dry wood termites which attack dry wood or damp wood termites attacking damp wood usually decaying wood (Kofoid, 1934).

2.2.1 Termite habitats

Termites are prone to predation by natural enemies like ants and other rodents (Fayle *et al.,* 2015). They therefore build different nesting types which they use for protection. One of the most sophisticated nest type built by termites are the earthen mounds. Members of the genus *Macrotermes* build the most sophisticated mounds (Brandl *et al.,* 2007). Different termite species build different mound structures, with geographical differences in the shape and size (Peters, King & Wylie,1996). In some instances, the same species can build mounds of varying shapes and structures while in some cases it may not be the case. (Makonde *et al.,* 2013). Mound structures constructed by termites exhibit a wide variation depending on the species type, genus type, soil type and even the geographical region of sampling (Plate 2.1).

Plate 2. 1: Images of existing recorded mound structures across the globe (Turner and Soar, 2008).

Key: (a, b and f) mounds sampled in Africa, mound sampled (c and d) mound sampled in Australia, (e) mound sampled in china.

2.3 Caste system of termites

A characteristic feature of termites is the caste system and reproductive division of labour among the various groups (Lefebvre *et al*., 2008). They live in large colonies and a colony consists of reproductive forms, sterile workers, soldiers and immature individuals (Noirot, 1992). There are two types of the reproductive forms; primary and supplementary. The primary reproductive forms are the king and queen which produce and disperse eggs by colonizing flights. Workers construct the distinctive shelter tubes and collect food to feed the young and other members of the colony. This fending is the cause of all the damages on crops, buildings and structures (Vasconcellos, Araujo, Moura & Bandeira, 2007). Soldier termites are responsible for guarding the colony through their defence mechanisms such as snapping, slashing or biting (Boomsma, Baer & Heinze, 2005). Their heads and mandibles are modified to protect the colony. They

can also release chemicals that cause irritation or toxicity to the enemy in question (Prestwich, 1984).

2.4 Emerging challenges in termite taxonomy

Phylogeny based on morphological identification is ambiguous and unreliable (Singla *et al.,* 2015). Some of the problems encountered with this method are biasness towards certain groups which present conspicuous features or which can be easily collected and limited morphological characters presented by some groups such as soldierless termites. Analyses that concentrate on only certain biogeographic groups and lack of rigorous cladistics have also contributed to ambiguities (Hausberger, Kimpel, van Neer & Korb, 2011; Eggleton, 2000). Other limitations include; outdated description of characters, inaccessibility to certain regions for sample collection, poor collection and survey strategies. In some cases description of ambiguous groups have created challenges in phylogenetic analyses. For instance the worker mandibles of the family *Termitdae* have produced inconsistencies in phylogenetic studies (Eggleton & Tayasu, 2001). In the subfamily *Apicotermitinae* where there is complete absence of the soldiers the description of species has been difficult. *Nasutitermes* of the subfamily *Nasutitermitinae* in Southeast Asia and Madagascar have been found to show a striking similarity in feeding strategies and mandibular structure. Generally castes like workers, pseudogates and immature reproductives in some genera lack specialized morphology of the mandibles for identification (Hausberger *et al.,* 2011). Since morphological methods present ambiguity complementary methods are now used alongside the morphological identification (Austin *et al.,* 2004).

2.5 Morphological identification of termites

All termite field research relies on information on the identity of the species in question. This makes termite identification key in termite studies (Lee, Forschler & Tracie, 2005). There are published keys to differentiate groups and families of termites. Some of the keys are regional whereas some are international (Husberger *et al.*, 2011). Lee *et al.* (2005) morphologically studied three termite subfamilies *Macrotermitinae, Termitinae* and *Nasutitermitinae.* They described four new species namely: *Microtermes gilvus, Macrotermes malaccensis, Dicupiditermes nemorosus* and *Microcerotermes crassus*. Wang *et al.* (2009) identified four different species from the genus *Reticulitermes* using morphological characters. In Thailand a systematic key based on morphological character was developed for the purposes of termite identification in Thailand. Soldier termites were used in the study to describe 37 genera of termites (Sornuwat, Vongkaloung & Takematsu, 2004). Some studies for instance have used the morphology of the gular plate to differentiate species (Husein *et al.,* 2006). However the use of the gular plate ratio was only proven to be viable on two Nebraskan soldier species, *Reticulitermes flavipes* and *Reticulitermes tibialis* (Husein *et al.,* 2006). Scanning electron microscopy was used to differentiate mechanosensory hairs among castes of the damp wood termite, *Hodotermopsis sjostedti* (Yuki, Shegeyuki & Toru, 2007). Another morphological phylogeny of termites was conducted by Engel *et al*. (2009). In their study 108 morphological characters were used to identify 76 species including 38 fossil taxa. The same study resulted in a revised classification scheme including the elevation of four new families three of which are extant. Morphological

analysis has been used recently to distinguish five common Indian *Odontotermes* species (Pranesh & Harini, 2014).

2.6 Molecular identification of termites

Preference in the use of mtDNA to infer phylogenetic relationships and evolutionary changes in termites has been documented (Mandal *et al.,* 2014). Garrick *et al.* (2015) identified five different species of the genus *Reticulitermes* using PCR based amplification of a section of mtDNA COII gene in the southern United States. These species could not be earlier identified by morphological characteristics. In India studies have reported the use mitochondrial DNA for termite identification (Singla *et al.,* 2013; Singla *et al.,* 2015; Murthy *et al.,* 2015). Elsewhere in Chile the *Reticulitermes* were identified using molecular techniques by analysis of three different mitochondrial genes in addition to soldier morphology (Su, Ye, Ripa, Scheffran & Giblin-Davis, 2006). DNA barcoding was used to study the population of moth and butterfly species in Papua New Guinea. In Kakamega Forest of Western Kenya termite species were identified using mtDNA COII gene. Osiemo, (2009) identified 22 termite species by molecular species delimitation six of which were morphologically cryptic and could not be earlier identified by morphology. Using mtDNA COII gene Makonde *et al.* (2013) identified four species of *Odontotermes* one species of *Macrotermes* form Juja Kenya and two species of *Microtermes* from Kajiado Kenya. These shows that termite identification worldwide relies on mtDNA markers and can therefore be adopted for more studies on termites.

Even though most African research on termites has concentrated on indigenous knowledge for identification some countries have embraced modern molecular tools coupled with traditional methods. In a West African savanna phylogenetic community approach was employed to study termite communities using molecular tools among which included COII gene. The study identified 20 different species from the family Termitidae (Hausberger *et al.,* 2011). Recently in Gabon Fayle *et al.* (2015) used DNA sequences of COII gene to detect termites from the guts of an ant species by separating them into molecular operational taxonomic units (MOTUS). In Kenya, Makonde *et al*. (2013) used COII gene sequences to confirm the identity of termites in Juja that had preliminary been assigned to specific genera by morphology and to further identify the specific species. The approach was found to be accurate for species identification as it unveiled many species in the three genera; *Macrotermes, Odontotermes* and *Microtermes*.

2.7. Distribution modelling and its significance in ecological research

Over the past decades, there has been increasing interest in species distribution modelling. Two situations that have contributed to this interest are the growing need to acquire information on the geographical distribution of biodiversity and need for new and improved techniques together with suitable data to address the information (Scott *et al.,* 2002). In order to come up with a suitable distribution model, species occurrence and environmental variables thought to have influence on their habitats need to be

investigated. It is necessary to model species distribution for the purpose of understanding the relationship between species and its biotic and abiotic environment and to test ecological hypotheses about species distribution and ranges. The greatest focus of ecology is to determine how pattern and scale influence the distribution and abundance of organisms (Scott *et al.,* 2002). Phylogenetic and population genetic studies also provide baseline data that is required for assignment of individuals to their geographic source of origin. Diversity and distribution of termites in a particular area is relevant as it necessitates effective planning on termite management practices (Menandro, 2013). Moreover, knowledge of distribution of termite taxa and other soil dwellers like earthworms helps to determine how and to what extent their diversity and activities are affected by land use and management practices (Ayuke *et al.,* 2011).

2.8 Status of termite identification and distribution in Kenya

Over 177 termite species have been described in East Africa and Kenya records a considerable number of these species (Ahmed *et al.*, 2011). Limited work on termite identification has been done in Kenya yet the few studies show potential in termite species richness. Species commonly associated with mound building in Kenya are from the family *Termitidae* (Osiemo, 2009). In 1984 Wanyonyi, Darlington and Bagine reported complete lack of information on taxonomy and distribution of termites in East Africa. A study conducted in Kisii District of south western Kenya later identified *Macrotermes michaelseni, Cubitermes ugandensis, Cubitermes testaceus* and species from other genera like *Pseudocanthotermes, Basidentitermes* and *Microcerotermes* (Kooyman, 1987). Ayuke *et al.* (2009) recorded *Odontotermes* species and species from

the family *Rhinotermitidae* species in Taita hills and Embu. A more elaborate study on identification of termites in Kakamega rainforest in western Kenya recorded 22 different species from different genera (Osiemo, 2009). A recent study on nutritional value of termites in western Kenya identified four different species of termites. They were members from two genera; *Macrotermes* and *Pseudacanthotermes* (Nyukuri, Mwale, Kirui, Chemgoyi & Koskei, 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

Termites were collected from unrestricted areas of four sub-counties of Taita Taveta County. Taita Taveta is located approximately 200 km northwest of the coastal city of Mombasa and 360 km southeast of Nairobi. It covers 17,084.1 km^2 and is within a tropical savanna with diverse vegetation. The study covered herbaceous grassland/ HG (Mwatate Sub-County Latitude: -3° 29′ 59.99″ S, Longitude: 38° 22′ 59.99″ E), exotic forests/ FS (Wundanyi Sub-County Latitude: -3° 24′ 6.95″ Longitude 38° 21′ 50.47″), shrub savanna/SV (Voi Sub-Sounty Latitude: -3° 23′ 45.78″ S, Longitude: 38° 33′ 21.92″ E), rain fed trees/ RFT (Taveta Sub-County Latitude: -3° 39′ 63.20″ S, Longitude: 37° 67′ 36.20 E″) vegetation and disturbed areas (DL) all over of the (Figure 3.1).

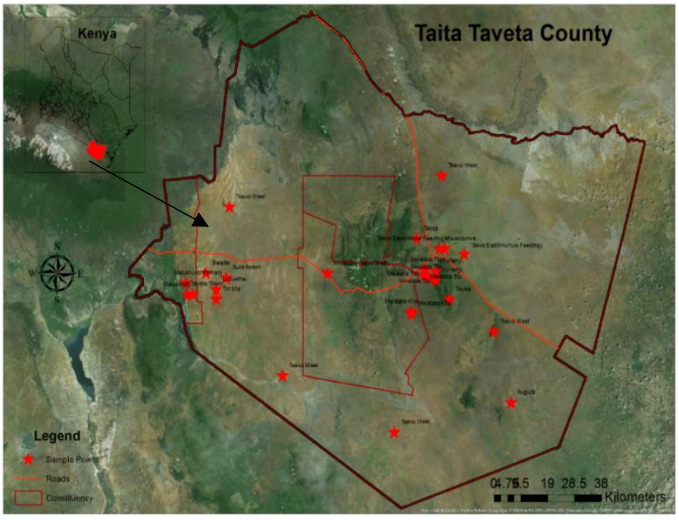

Figure 3. 1 Sampling points of termite specimens from Taita Taveta County in July 2013 and February 2014 on aerial map of the County (Developed on ArcGIS 10.2.1).

Global positioning system (GPS) coordinates of the sampling sites were taken using mobile friendly Gamin software and recorded on excel sheet (Table 3.1). The figures were exported to ArcGIS 10.2.1 software and used to generate the aerial map of the sampling sites

Table 3. 1 GPS coordinates of termite sampling locations in Taita Taveta County in July 2013 and February 2014

Sub-County	Sample site	Vegetation type	Latitude	Longitude
Mwatate	TTUC	DL	3° 25′ 17.24″ S	38° 30′ 13.15″ E
	TTUC	DL	3° 25′ 10.21″ S	38° 30′ 07.98″ E
	TTUC	HG	3° 25′ 10.21″ S	38° 30′ 07.98″ E
	TTUC	DL	3° 25′ 10.22″ S	38° 30′ 07.75″ E
	TTUC	DL	3° 25′ 09.10″ S	38° 30′ 07.11″ E
	KHS	DL	3° 22′ 28.81″ S	38° 22′ 40.40″ E
	Mwaktau	SV	3° 25′ 13.28″S	38° 10′ 19.94″ E
	Konenyi	RFT	3° 22′ 28.81″ S	38° 27′ 38.39″ E
	Bura forest	FS	3° 30′ 30.24″ S	38° 22′ 41.01″ E
	Mwambirwa	FS	3° 21′ 11.36″ S	38° 25′ 54.36″ E
Voi	Konenyi	RFT	3° 20′ 07.08″ S	38° 29′ 42.02″ E
	Tausa	RFT	3° 20′ 06.59″ S	38° 29′ 41.94″ E
	Tausa	RFT	3° 20′ 07.08″ S	38° 29′ 41.02″ E
	Tsavo east	SV	3° 20′ 07.08″ S	38° 29′ 42.02″ E
	Tsavo east	SV	3° 22′ 28.81″ S	38° 27′ 39.38″ E
	Buguta	DL	3° 40′ 59.35″ S	38° 29′ 41.94″ E
Taveta	Taveta town	DL	3° 23′ 47.54″ S	37° 40′ 42.60″ E
	Tsavo west	SV	3° 21′ 59.60″ S	37° 42′ 24.42″ E
	Malukilorit Pri.	DL	3° 21′ 47.51″ S	37° 42′ 22.99″ E
	Timbila	RFT	3° 23′ 52.49″ S	37° 43′ 05.09″ E
	Luduwhai	RFT	3° 25′ 13.28″ S	38° 10′ 09.94″ E
	Salaita	SV	3° 25′ 13.28″ S	37° 45′ 51.02″ E

Wundanyi	Werugha	DL	3° 23′ 13.28″ S	38° 21′ 59.72″ E
	Wumingu	RFT	3° 23′ 51.02″ E	38° 24′ 58.02″ E
	Mgange	SV	3° 45′ 51.02″ E	38° 27′ 59.92″ E

3.2 Termite collection

A reconnaisance was conducted during the month of June, 2013 to identify vegetation types and to establish presence of termite mounds in the study site. Actual sampling was done in July, 2013 and February, 2014. A total of 54 active termite mounds from across the five different vegetation selected. Five data collection assistants were recruited into the study. They were accompanied to the identified vegetation types, one vegetation type at a time and instructed to dig out termite mounds at most 100 metres apart and pick only soldier termites of the same colony per mound. These termites were then put into separate sterile falcon tubes containing absolute ethanol. From every data collection assistant, two falcon tubes with termites from one vegetation type were randomly picked. In additional four mounds were sampled during the training of the data collection assistants and were included in the study. Each tube represented a mound. A sum total of 10 termite mounds were sampled per vegetation type. There were five vegetation types making a total of 50 samples from the entire study site. The first 30 samples were collected in the first season in July 2013 while the last 24 samples were collected in January 2014. Descriptive survey design was used in this study. The active termite mounds were identified by the foraging activities of termites.

Termite mounds were dug to a depth of one and half feet or up to where a colony nest was found. From each colony, 20 major soldiers were picked using a pair of forceps.

All samples were placed in a cool box and transported to the Institute for Biotechnology Research (IBR) laboratory at the Jomo Kenyatta University of Agriculture and Technology (JKUAT) where they were stored at -20°C for pending morphological and molecular characterization.

3.3 Morphological characterization

3.3.1 Mound identification

Termite mound features including; shape and presence or absence of ventilations, were recorded and photographed during field sampling. The mound characteristics observed were compared with the ones published by Roonwal, (1977).

3.3.2 Termite identification

Soldier characters used in the identification of termites in the present study were outlined in Figure 3.2. Characters description of the parameters taken were provided on Table 3.2.

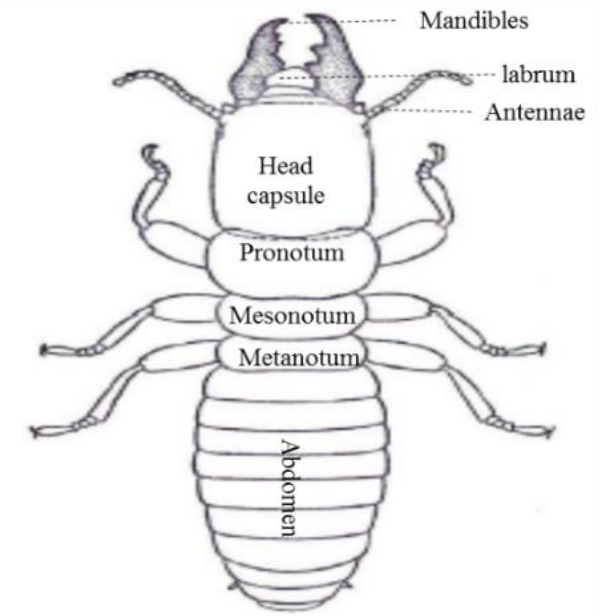

Figure 3.2: Morphological characteristics of soldier termites used in characterization of termites collected from Taita Taveta County in 2013 and 2014 (Wang *et al.*, 2009).

Table 3. 2: Morphological characters used in identification of termites from Taita Taveta County in 2013 and 2014

Character	Description
Length of head with mandibles	Length from posterior margin of head capsule to tip of mandibles
Width of head	The width of the head at its widest point
Labral width	Width of intact labrum at its widest point
Body colour	The general body pigmentation
Antennae segment	The number of characters present on each antennae
Total length	Length from tip of mandibles to tip of abdomen along the midline.
Shape of pronotum	-
Pronotal width	Width of pronotum at its widest point.

Morphological characterization of soldier termites was conducted with the aid of a dissecting microscope. The width and length of the soldier heads, total length, width and length of the pronotum, width and length of the mesonotum and metanotum were recorded. Other morphological characters noted were; number of antennae segments, head characteristics *viz*, size and shape of head, mandibular features, shape of the pronotum and fungus farming ability. A clear centimeter ruler was used to measure the soldier head properties. These measurements were taken from four soldier termites per mound and the mean obtained. The mean values were recorded for each character and combined to identify the samples up to the genus level. Identification up to the genus level was based on work by Sornuwat *et al.* (2004) and Wijerathna and Dias, (2012). Photographs of representative mound structures were taken using 5x zoom 16.1 MEGA PIXEL Sony lens digital camera. Identifications was confirmed using records from the National Museum of Kenya. Reference samples were deposited at the Entomology Department of the National Museums of Kenya for archiving purposes.

3.4 Molecular characterization

3.4.1 Genomic DNA extraction

Total DNA was extracted from soldier termite heads. This is because the head does not harbor microbes like the gut which could present a different source of DNA. The method applied was the phenol: chloroform extraction method described by Sambrook, Fritsch and Maniatis (1989) with minor modifications. Five heads of soldier termites were

sterilized and homogenized in 200 µl of TE (Tris EDTA pH 8) using a mortar and pestle. To break down the tissues, 500 µl of lysis buffer (400 µl of TE and 100 µl of 5% SDS) was added to the homogenate followed by 10 µl of Proteinase K. The mixture was incubated at 65°C for one hour. A mixture of 120 µl of phenol: chloroform: isoamyl alcohol (25: 24: 1) was added and the tubes were then vortexed for 30 seconds, and then centrifuged for 10 minutes at 10,000Xg. The upper aqueous layer was carefully transferred to a fresh sterile Eppendorf tube, without disturbing the protein layer at the interphase. In order to precipitate DNA 500 µl of isopropanol was added to this aqueous layer and stored at -4°C overnight and then centrifuged at 12,000Xg for 10 minutes. The supernatant was discarded and the remaining pellet was washed using 70 % ethanol and rinsed using double distilled water. The washed pellet was air-dried for 30 minutes and eluted in 40 µl of TE buffer to retain its stability and avoid possible enzymatic reactions. The extracted gDNA was stored at a temperature of -20°C to avoid degradation for subsequent use (Sambrook *et al.,* 1989).

3.4.2 DNA quality determination

Agarose gel electrophoresis was used to check for the presence of genomic DNA by running the extracted DNA on 0.8% agarose gel stained with ethidium bromide. The concentration of genomic DNA was determined by the optical density/absorbance which was generated automatically using a spectrophotometer. The quantification was done by mixing 5 µl of DNA with 495 µl of double distilled water in a microfuge tube. A blank cuvette containing double distilled water was used to standardize the spectrophotometer. Freshly extracted DNA for each sample was diluted with distilled water and placed into

a 10mm ultraviolet-transparent cuvette and loaded into the spectrophotometer one sample at a time. The absorbance readings were performed at 260nm (A_{260}). At 260nm DNA absorbs light most strongly allowing the spectrophotometer to automatically generate and estimate concentration of the DNA solution.

3.4.3 Amplification and sequencing of COII gene

The purified gDNA was used as a template for PCR amplification of the mtCOII gene fragment. A set of primers, A-tLeu (5'-CAG ATA AGT GCA TTG GAT TT-3') for the forward direction and B-tLys (5'-GTT TAA GAG ACC AGT ACT TG-3') for the reverse direction were used (Miura, Maekawa, Kitade, Abe & Matsumoto, 1998). A reaction mixture of 25 µl consisted 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 (5U/ µl), 1 µl of each forward and reverse primer sequences, (5 Pico moles) 0.5 µl of DNA, and 15.75 µl of PCR water. A control reaction tube was set up with all the reagents except the template. The thermal cycling program were set as follows; initial denaturation at 95^{o} C for five minutes, denaturation at 95^{o} C for 30 seconds, annealing at 52^{o} C for 45 seconds, and extension at 72^{o} C for 1 minute in an automated PCR machine. This was repeated 30 times, followed by five minutes of extension at 72^{o} C.

The PCR products were confirmed by gel electrophoresis using 1.5% agarose in 1x TAE buffer stained with ethidium bromide and visualized under ultraviolet light. The PCR products were purified using the QIAquick PCR purification Kit protocol (Qiagen, Germany) according to manufacturer's instructions. The purified products were stored

at -20^{o}C before they were sent for sequencing. In preparation to send them, the purified PCR products were placed in PCR tubes tightly warpped with parafilm. They were then put in a small sterile plastic bag. The sterile plastic bag was again wrapped in a sterile alluminium foil. The samples were then put inside a small envelope and sent to the commercial sequencing service provider using an international express mail services provider, DHL. Sequencing of the purified PCR products was done by Macrogen Netherlands, a commercial service provider (http://www.macrogen.com). Cycle sequencing was used where purified products of the first PCR cycle acted as templates. Both the forward and reverse sequences were used as the template in separate reactions in which only the forward or reverse primer is used. The same template was reused for as many cycles as programmed, (usually 25 cycles). The product, a mixture of DNA of various lengths was achieved by adding specially labeled bases called dye terminators. These terminators were randomly incorporated in the second cycle to terminate the sequence. Thus, fragments of every size were generated. The products were purified to remove unincorporated dye terminators, and the length of each was determined using capillary electrophoresis. The sequence data result files were sent back via email in four different formats; PDF, ABI, Text document and FASTA for easy access and also to enable analysis.

3.5 Data analyses

3.5.1 Morphological data analyses

The mound structures, shapes and size recorded were compared with the ones published by (Roonwal, 1977). The termite characters used for characterization were also compared with the work of Manzoor (2006) and Sornuwat *et al.* (2004) and placed into possible identification levels. The raw morphological data obtained were standardized to zero mean and unit variance so as to avoid the effect due to difference in scale. The resulting coefficients were used to calculate relationships among the termite populations with cluster analysis using hierarchical cluster analysis (HCA) with UPGMA (unweighted pair-group method using arithmetic averages). Pairwise distances for the all the sample (coefficient similarity values) were computed. The samples with the smallest pairwise distances were determined. The process was repeated for subsequent pairs of samples with smallest pairwise distance. These distances were then clustered continually. The resulting values were plotted to produce a rooted dendrogram with node values using Euclidean dissimilarity measure in DARwin v.6.1 software.

3.5.2 Phylogenetic analyses

Sequences of the termites were manually edited in CLC main workbench version 7.2.6 and checked for presence of artefacts or chimeric structures using the Mallard software (Ashelford, Chuzhanova, Fry, Jones & Weightman, 2006). A search for similar sequences using BLASTN was performed and sequence alignment was performed using the CLUSTAL Omega program (http://www.clustal.org). A Phylogenetic tree was

drawn based on distance neighbour-joining method in Geneious v.9.0.2. (Kearse *et al.*, 2012). Evolutionary distances were computed using the Kimura two Parameter (Kimura, 1980). To obtain support values for the branches, bootstrapping (Felsenstein, 1985) was conducted with 1000 replicates. All sites, including gaps in the sequence alignment, were excluded pairwise in the phylogenetic analysis. Using the resultant neighbour-joining tree, each termite sequence was assigned to the respective taxonomic group. Pairwise genetic distances among the sequences were calculated using Kimura two parameter (Kimura, 1980).

3.5.3 Distribution data analyses

The number of both mounds and termite species in the various vegetation zones were recorded on excel and subjected to R statistical analysis in format delimited (CSV). Diversity indices (Richness, Shannon and inverted Simpson) were calculated using vegan package version1.16-32 in R programming language (Vegan, 2016). The species richness of termites was determined by enumerating the number of species counted in the every vegetation type. Shannon diversity indices stipulate that values close to one are indicative of high species diversity whereas values close to zero are suggestive of low species diversity. The equation for Simpson's and Shannon diversity indices are given as:

Simpson's diversity

$D = \Sigma (n / N)^2$	$D = \frac{\Sigma\, n(n-1)}{N(N-1)}$
n = the total number of organisms of a particular species **N = the total number of organisms of all species**	

The value of **D** ranges between 0 and 1

Shannon diversity

$$H = \sum_{i=1} - (P_i * \ln P_i)$$

Where:

H = the Shannon diversity index

P_i = fraction of the entire population made up of species i

S = numbers of species encountered

$\sum$ = sum from species 1 to species S

CHAPTER FOUR

RESULTS

4.1 Morphological identification of termites

4.1.1 Abundance of termite mounds in the study area

The study noted that termite mounds occurred in all the vegetation zones sampled. A total of 86 termite mounds were recorded. Among these, 54 (62.7%) were active while 32 (37.3%) were inactive. Seventeen of the active mounds were located in the disturbed areas of the study site, 13 were in the herbaceous grassland vegetation, nine were in the rain-fed trees, and other nine were from the shrub savanna and six from the forests. Greatest number of termite mounds were recorded in disturbed land. However, in the disturbed land under farming no termite mounds were recorded. Mounds were recorded on inhabited places like homes and institutions. Forests situated at higher altitudes recorded few mounds as well (Table 4.1).

Table 4. 1 Number of active and inactive termite mounds recorded from different vegetation types in Taita Taveta County in 2013 and 2014

Vegetation type	Active mounds	Inactive mounds	Total (%)
Disturbed land	17	12	33.72
Herbaceous grassland	13	9	25.58
Shrub savannah	9	7	18.60
Rain-fed trees	9	2	12.79
Forests	6	2	9.31
Total	**54**	**32**	**100**

4.1.2 Features of the mound structures in the study sites

A detailed study of the mound structures identified three types of mound structures. There were cone-shaped mounds with more than one opening. Some of these were irregularly shaped while others were regularly shaped. The second type were elongated closed dome-shaped mounds that stood singly or in some instances were accompanied by emerging baby mounds. The third type were ground level mounds that were only slightly raised above the ground and were rarely recorded. Arboreal nests were also recorded in the forest vegetation type. The cone-shaped mounds with more than one opening were common in the study site just as much as elongated closed dome-shaped mounds that stood singly or had emerging baby mounds. Apart from the mound structures, the study also noted other termite nests. There were subterranean galleries on living or dead trees whereas some colonies formed on decomposing humus (Plate 4.1).

Plate 4. 1: Mound structures identified in Taita Taveta County in 2013 and 2014.

Key: (a) and (b); Regularly and irregularly cone-shaped *Macrotermes* mounds, (c) and (d); Closed dome-shaped *Macrotermes* mounds standing singly or accompanied with baby mounds, (e) and (f); Slightly raised above ground *Odontotermes* mounds, (g) and (h); Other termite nests.

4.2 Termite identification

Three different groups of termites were identified in the study using phenotypic characteristics such as head capsule, mandible features, antennae characters, pronotum, mesonotum and metanotum.

4.2.1 Group one termites

The characterization of this group was based on the soldier caste. They were associated with two different types of epigeous mounds. One was closed dome-shaped mound and produced samples 7RFT, 8SV, 17RFT, 31HG and 41HG. The other type was cone-shaped mounds with ventilations and produced samples1DL, 3DL, 4D, 21HG, 34HG, 14SV 30FS and 31SV. These samples had saddle shaped pronotum with a distinct anterior lobe with either a deep or shallow curve in the midline. Average length of head with mandibles was between 6mm and 8mm. The head capsule was brown in color and conspicuously distended. They had strong fully developed and sabre shaped mandibles black in color. They had a labrum with a hyaline/glassy tip. Their antennae segments were composed of 17 characters. The members of this group were aggressive and large with an actual size of between 18mm to 20mm. The body color of these termites ranged from light brown to dark brown. Some were black on the abdomen and brown on the head capsule (Plate 4.2).

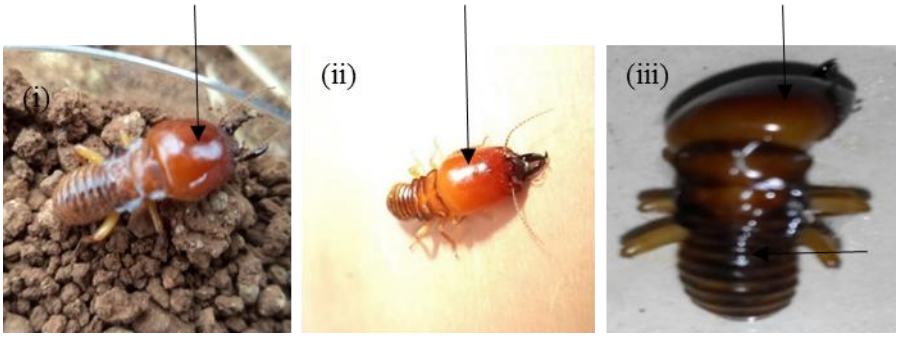

Plate 4. 2: Group one termites identified by morphology in Taita Taveta County in 2013 and 2014. (i) Round shaped head capsule, (ii) Oval-shaped head capsule and light bodied and (iii) Dark bodied termite species with distended head capsule.

4.2.2 Group two termites

Group two termites were collected from mounds that were not conspicuous but slightly raised above the ground. Their characterization was based on the soldier caste as well. They presented a characteristic bad odour, a feature that distinguished them from the other groups. Their head capsule was light brown and presented few bristles on the periphery. The labrum was reddish brown; antennae was light brown with brown tinge at the base. The antennae segments had 16 characters second nearly twice as long as third and fourth characters combined. The rest of the body was yellow with a brownish tinge. The mesonotum and metanotum were broader than the pronotum. Their head capsule was broadly oval almost semi rectangular. They had long slender mandibles whose tips were slightly incurved. The left mandible had a tooth. They possessed hawk shaped labrum. Their pronotum was saddle shaped but not as pronounced as in the members of group one (Plate 4.3).

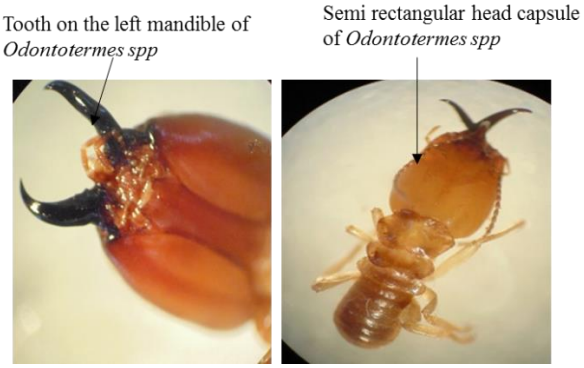

Plate 4. 3: Features of group two termites collected from Taita Taveta County in 2013 and 2014.

4.2.3 Group three termites

Group three termites consisted of soldiers of *Amitermes* characterized by a snouted head capsule and a single mandible each with a single tooth of various shapes on their inner margins. Species in this genus were subterranean and did not build mounds. They made small colonies in the ground, living trees or in decomposing organic matter (Plate 4.4).

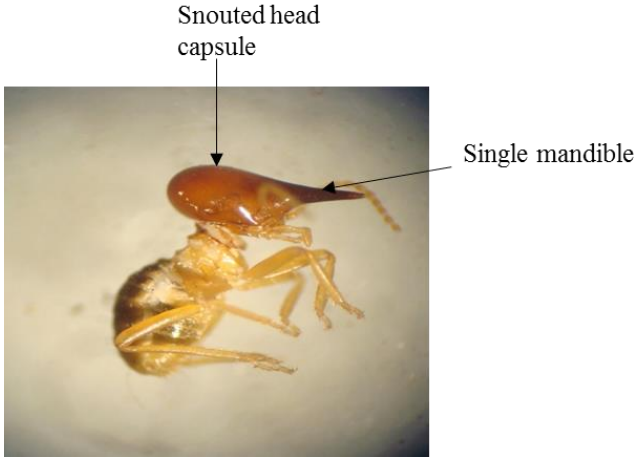

Plate 4. 4: Group three termites collected from a living tree in Bura forest and Mwambirwa Forest of Taita Taveta County in 2013 and 2014

4.2.4 Structural difference in the shape of pronotum in three termite groups from Taita Taveta County

There was no structural difference in the shapes of pronotum of different termite samples when observed with the naked eye. However, under the dissecting microscope a saddle shaped pronotum was observed in group one and group two termites. The shape was regular in samples belonging to group one and irregular in samples belonging to group two. The samples collected from dome-shaped and cone shaped mounds differed only on the depth of the curvature. The midline curvature was deep in some samples of the group and shallow in some. The samples collected from the forest arboreal nests were tiny hence telling the shape of the pronotum was not possible (Plate 4.5). Raw data of all morphological characters' measurement from all the samples under study in Taita Taveta County were provided on Appendix 4.1.

Plate 4. 5: Shapes of pronotum of termite groups collected from Taita Taveta County in 2013 and 2014

4.2.5 Possible identification of termite groups using the morphological characters

The groups were assigned to respective possible genera as summarized in Table 4.2.

4.2.6 Cluster analysis of morphological characters of termites studied

Clustering method of classification grouped the termite species into five main clusters, i, ii, iii, iv and v. Cluster i comprised one individual (5DL) which separated from the rest of the species indicating a wide morphological distance. Cluster ii consist of 21HG from the herbaceous grassland. Similarly, 26FS, 27FS, 28 FS, 29FS, 19FS and 25FS associated with Bura exotic forest origin were grouped in this cluster. Cluster iii contained one individual 12DL which distanced itself from members of cluster iv. Cluster iv grouped 4DL and 34HG together with soldiers associated with aggressiveness. Most samples clustered together in cluster v (43 members) with a number of sub clusters. The 43 members in this cluster were from across the various vegetation types under study covering 79.6% proportion of the total population. Disturbed land (DL) had 37%, rain fed trees (RFT) 15%, herbaceous grassland 24%, shrub savanna 20% and forest 4% (Figure 4.1). The members shared many characters suggesting a common evolutionary history. Clearly, clustering of these organisms based on morphological characters not only revealed a high genetic diversity between members of the same and different habitats but also showed a close relationship between members of different vegetation zones (Appendix 4.2).

Table 4. 2 Possible identity of termites collected from Taita Taveta County studied in 2013 and 2014

Sample codes	Collection site/vegetation	Mound characteristics	Soldier characteristics	Possible Identity
IDL,3DL, 4DL	Disturbed areas	Regular and irregular cone shapes Presence of ventilations Inactive mounds shelter for other reptiles	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			
14SV, 32SV	Shrub savanna			
7RFT,17RFT, 42 RFT,	Rain fed trees	Dome shaped mounds No ventilations/closed Some accompanied with baby mounds		
8SV	Shrub savanna			
31HG,41HG	Herbaceous grassland			
11SV, 24SV, 49SV	Shrub savanna	Slightly raised above ground Have few openings	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	Decomposing humus and subterranean galleries on living trees	Snouted head capsule Small in size with total body length of 5mm Single mandible	Amitermes

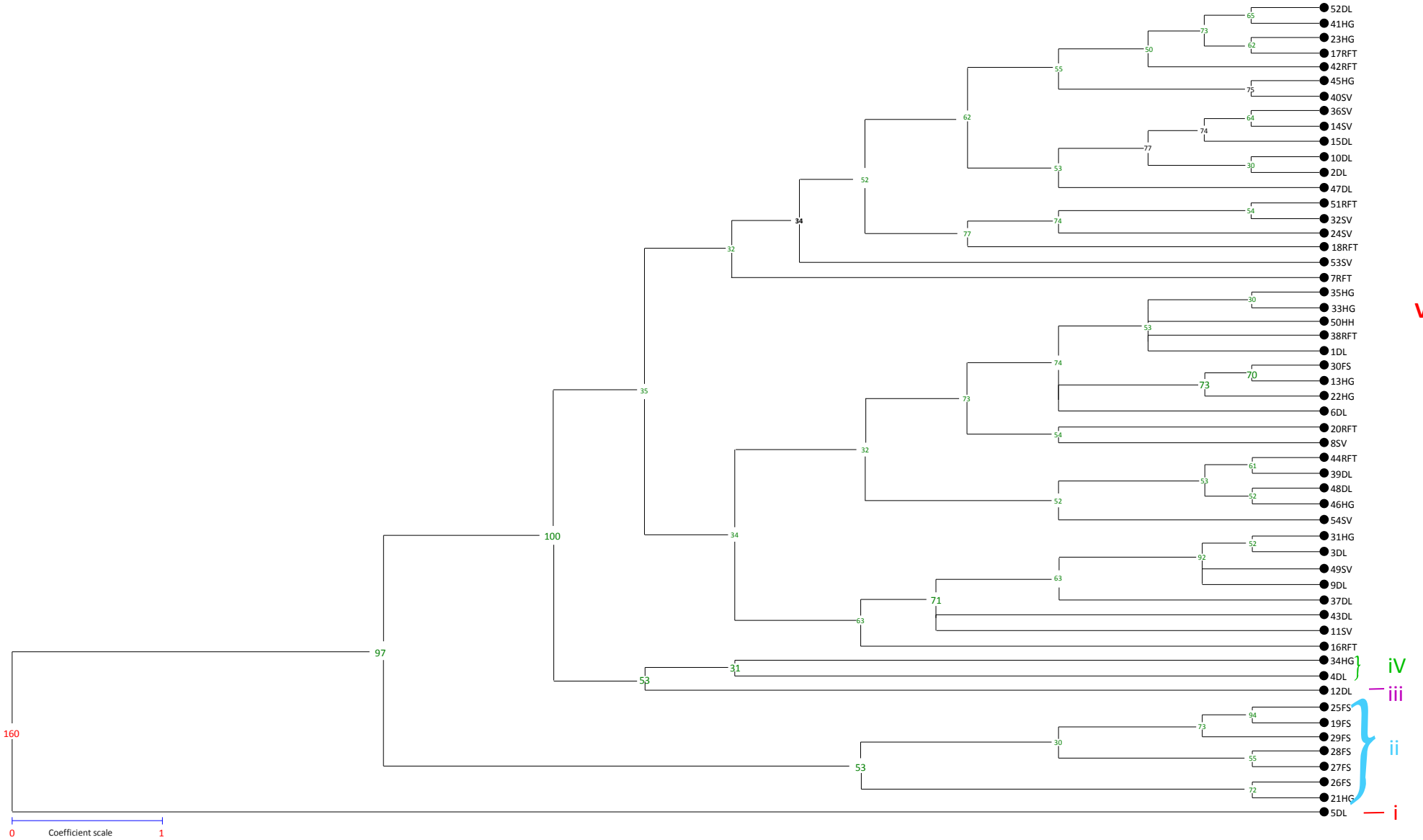

Figure 4. 1: Cluster analysis of morphological characters of termites identified in Taita Taveta County in 2013 and 2014.

4.3 Molecular characterization

4.3.1 Quantification and qualification of isolated genomic DNA

Good quality DNA is expected to have A_{260}/A_{280} ratio of 1.7-2.0. Although some isolations produced a highly fluoresced band without any smears, it was not the case for all times. Eventually the genomic DNA obtained from the termites' heads achieved the desired quality suitable for further analysis. DNA concentration on spectrophotometer readings were between spectrums of 230nm to 320 nm. Readings of 1.8 and 0.7 with A_{260}/A_{280} ratio were obtained from DNA of all the samples in this study.

4.3.2. PCR amplification of COII gene

All the termite samples produced PCR products of approximately 750 bp from the amplification of COII gene region of the termite specimens (Plate 4.6).

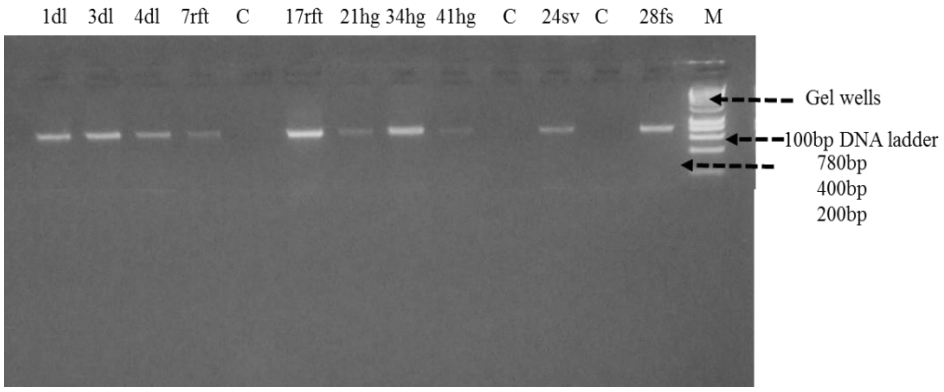

Plate 4. 6 PCR amplicon of mtDNA COII gene from termite samples in Taita Taveta County in 2013 and 2014 on a 1.5% agarose gel.

Key: Lanes 1dl through to 28fs are bands for representative termite samples from each vegetation zone. A 100bp (M) DNA ladder was used as a molecular size marker and a control experiments (C) was loaded in between samples.

4.3.3 Nucleotide analysis of termites from Taita Taveta County

The amplified fragments produced sequences of the same length (Nuclear DNA sequences originating from mitochondrial DNA sequences) were not sequenced. Only a few sequences had stop codons, which is consistent with all amplified sequences being functional mitochondrial COII sequences. The gap were encountered in some sequences and were edited with the Geneious programme used for analysis. Sequences were heavily AT biased (especially in the third codon position). The average A+T content was 63% (Table 4.3). Nucleotide composition in different termite species is shown in Figure 3.2.

Table 4. 3 Sequence statistics for 31 termite specimens from Taita Taveta County sampled in 2013 and 2014.

	Nucleotide frequencies			
Domain	**T**	**C**	**A**	**G**
1st position	13	26	54	6
2nd position	17	23	38	22
3rd position	36	21	29	14
Average	**22**	**24**	**41**	**14**

The re wer e 31 sequences which were successfully amplified out of which 14 haplotypes were omitted. Four of the 17 remaining sequences were used in the final analysis. Eight representative sequences were deposited in the public nucleotide sequence database (GenBank) under the following accession numbers; KT845956, KT845957, KT845958, KT845959, KT845960, KT845961, KT845962 and KT845963.

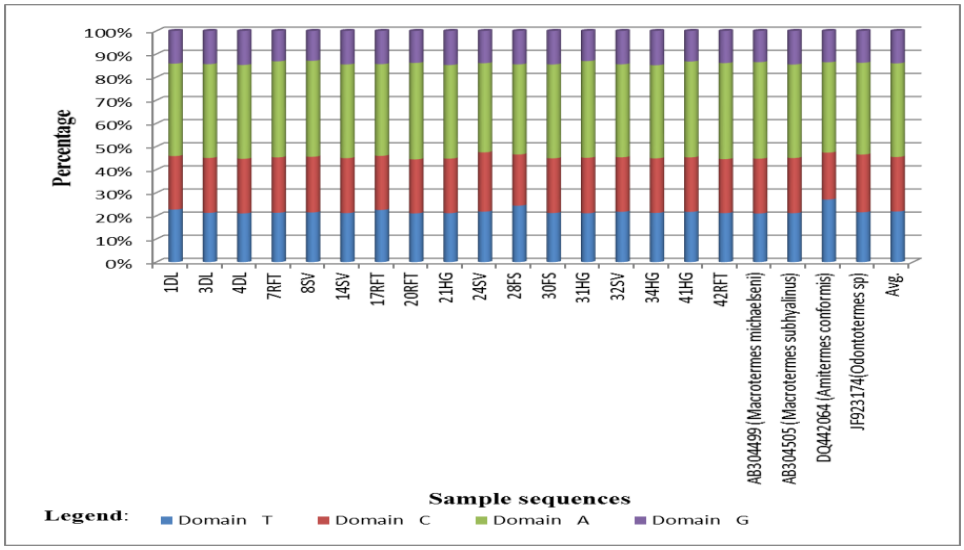

Figure 4. 2: Nucleotide composition of COII gene in different species of termites collected from Taita Taveta County in 2013 and 2014. A= Adenine, C= Cytosine, T= Thymine and G= Guanine

All sequence samples obtained showed that the termites obtained from Taita Taveta belonged to *Termitidae* family of order Isoptera. The termites belonged to two subfamilies; *Macrotermitinae* and *Termitinae.* (Table 4.4).

Table 4. 4 Termite families, subfamilies, genera and species collected from Taita Taveta County in 2013 and 2014

Family	Subfamily	Genus	Species
Termitidae	Macrotermitinae	Macrotermes	*Macrotermes subhyalinus* *Macrotermes michaelseni*
Termitidae	Macrotermitinae	Odontotermes	*Odontotermes ceylonicus*
	Termitinae	Amitermes	*Amitermes conformis*

4.3.4 Genetic distances and sequence divergence between termites from Taita Taveta County

Intraspecific distance within the first group comprising of 1DL, 3DL, 4DL, 21HG, 14SV. 34HG and 32SV haplotypes was 0.003 while that of second group with haplotypes 7RFT, 17RFT, 20RFT, 42RFT 41HG and 8SV was 0.004. The values was obtained by calculating the pairwise genetic distances within the groups and getting the average of all the pairs. There was only one sequence 24SV which clustered closely with *Odontotermes spp* with a BLASTN percentage similarity of 92%. Sample 28FS also clustered closely with *Amitermes conformis* BLAST percentage similarity of 92%. Termite groups ranged from 0.04 between group one and group two to 0.23 group three and group four. Genetic distance of species from the same vegetation was low ranging between 0.00 to 0.01 for the disturbed land, 0.00 to 0.01 for the rain fed trees, 0.03 to 0.14 for the shrub savanna samples, 0.22 for the forest samples and 0.00 to 0.04 for the herbaceous grassland samples. Genetic distance of the species from different vegetation zones was always higher than that of species within a vegetation type. For instance the genetic distance between a species from the forest and those from the rain fed trees ranged from 0.19 to 0.21.

Pairwise genetic distances among the sequences calculated by Kimura two parameter (Kimura 1980) were shown in Table 4.5.

Table 4. 5: Pairwise Kimura two parameter genetic distances based on COII gene fragments in termites collected from 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	0.00																				
2	3DL	0.01	0.00																			
3	4DL	0.01	0.02	0.00																		
4	7LT	0.08	0.04	0.04	0.00																	
5	8SV	0.07	0.03	0.03	0.04	0.00																
6	14SV	0.01	0.04	0.01	0.04	0.04	0.00															
7	17RFT	0.07	0.05	0.06	0.01	0.01	0.05	0.00														
8	20RFT	0.04	0.03	0.03	0.01	0.03	0.03	0.02	0.00													
9	21HG	0.01	0.02	0.02	0.04	0.03	0.01	0.05	0.03	0.00												
10	24SV	0.19	0.15	0.15	0.14	0.14	0.15	0.15	0.15	0.15	0.00											
11	28FS	0.21	0.19	0.21	0.20	0.19	0.20	0.21	0.19	0.20	0.22	0.00										
12	30FS	0.01	0.03	0.02	0.04	0.03	0.04	0.06	0.03	0.02	0.15	0.21	0.00									
13	31HG	0.07	0.03	0.03	0.01	0.02	0.04	0.01	0.03	0.03	0.14	0.19	0.03	0.00								
14	32SV	0.04	0.03	0.05	0.03	0.03	0.03	0.05	0.03	0.02	0.15	0.21	0.01	0.03	0.00							
15	34HG	0.01	0.02	0.05	0.04	0.03	0.01	0.05	0.03	0.02	0.15	0.21	0.02	0.03	0.00	0.00						
16	41HG	0.07	0.04	0.03	0.04	0.03	0.04	0.01	0.07	0.04	0.14	0.19	0.04	0.04	0.03	0.04	0.00					
17	42RFT	0.04	0.03	0.03	0.03	0.01	0.03	0.02	0.04	0.03	0.15	0.19	0.03	0.03	0.04	0.03	0.02	0.00				
18	AB304499 (Mm)	0.03	0.03	0.03	0.02	0.03	0.03	0.01	0.04	0.03	0.15	0.18	0.03	0.07	0.03	0.03	0.15	0.02	0.00			
19	AB304505 (Ms)	0.01	0.02	0.01	0.04	0.03	0.04	0.04	0.02	0.02	0.15	0.02	0.02	0.03	0.03	0.01	0.04	0.03	0.03	0.00		
20	DQ442064 (Ac)	0.26	0.22	0.22	0.22	0.22	0.21	0.24	0.09	0.22	0.23	0.09	0.22	0.22	0.22	0.22	0.22	0.21	0.22	0.22	0.00	
21	JF923174 (O.sp)	0.20	0.18	0.19	0.16	0.16	0.19	0.18	0.14	0.19	0.08	0.22	0.18	0.16	0.19	0.19	0.17	0.17	0.17	0.19	0.23	0

Key: S/N= Sample number, Mm= *Macrotermes michaelseni,* Ms= *Macrotermes subhyalinus* Ac= *Amitermes conformis,* O. sp= *Odontotermes species*

Genetic divergences between sequences making up different COII clusters were 10-fold higher than divergences within a cluster (Table 4.6).

Table 4. 6: Estimates of evolutionary sequence divergence over sequence pairs between cluster groups of termites collected from Taita Taveta County in 2013 and 2014

S/N	Cluster/spp	1	2	3	4
1	*Macrotermes subhyalinus*				
2	*Macrotermes michaelseni*	0.04			
3	*Odontotermes spp*	0.21	0.21		
4	*Amitermes conformis*	0.19	0.17	0.23	

4.3.5 Phylogenetic analysis of termites collected from Taita Taveta County

Phylogenetic analysis based on distance neighbor-joining method in Geneious 9.0.2 revealed three main termite clusters. Cluster one comprised members of the genus *Macrotermes*. Two samples indicated as 1DL and 17 RFT on the phylogenetic tree were affiliated with *Macrotermes subhyalinus* and *Macrotermes michaelseni*, respectively. *Macrotermes Sp. Juja_D2*, *Macrotermes Sp. Juja _B2*, *Macrotermes Michaelseni*, *Macrotermes c.f Michaelseni*, *Macrotermes sp. CD-2014* and *Macrotermes subhaylinus* of this cluster were retrieved from the GenBank. Cluster two was affiliated with members from the genus *Odontotermes*. One sample, indicated as 24SV (shrub savanna vegetation) formed a cluster together with two other *Odontotermes* species indicated as *Odontotermes ceylonicus* and *Odontotermes sp. 1BH-2011* were retrieved from the GenBank. Cluster three was associated with members of the genus *Amitermes* with one sample indicated as 28FS (from forest vegetation) forming the cluster together with

other two *Amitermes* species indicated as *Amitermes conformis* and *Amitermes obeuntis* were retrieved from the GenBank. The members that formed the cluster belonging to the *Macrotermes* revealed a close taxonomic affiliation amongst them. They were separated into two sub-clusters with high percentage similarity (≥98%) to two *Macrotermes* species; *Macrotermes subhylinus* and *Macrotermes michaelseni* (Figure 4.3).

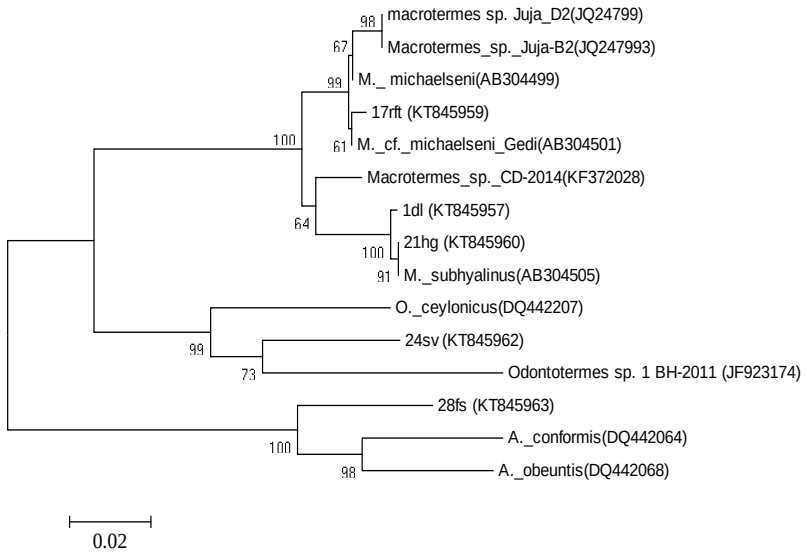

Figure 4. 3: Phylogenetic analysis of COII gene in termites. Multiple sequence alignments were against a portion of the gene. An unrooted phylogenetic tree was then established with Geneious 9.0.2 by the Neighbor-joining method with bootstrap values for 1,000 replicates shown at major nodes. Kimura two parameter genetic distances based on COII gene fragment in termite species were conducted using Geneious 9.0.2.

Two sequences clustered separately; one with very close similarity to *Odontotermes ceylonicus* with BLASTN Percentage similarity of (92%) while the other was similar to *Amitermes conformis* (92%). Samples 24SV and 28FS like all the other samples were

not identified to species using morphological characters. Phylogeny analyses of 750 DNA nucleotide revealed a strong neighbour-joining node (92%) separating them from the species they clustered with.

4.4 Geographical and genetic distance comparison

In the present study GPS coordinates acted as the geographical distances. The locations of the different vegetation types were more than a kilometer apart. The genetic distances of the samples in different vegetation types were provide in appendix 4.2. Using Kimura-two parameter the pairwise genetic distances were provided on Table 4.5. The smallest divergence was between populations of *Odontotermes* and *Amitermes* as well as between Macrotermes and *Odontotermes* and *Amitermes* but there was no divergence between population of *Macrotermes subhaylinus* and *Macrotermes michaelseni*. This means the two populations are more related. The mtDNA sequence generated provide a correlation of the phenotype/race of the samples investigated. Clearly they share a common phylogeographic pattern suggesting that comprehensive data from more species distributed across these systems should be gathered.

4.5 Comparison between morphological and molecular analyses

The morphological characterization placed the termite samples into the Family *Termitidae,* based on the fact that they were mound builders as well as fungus cultivators. Further classification based on mound structures, mandibular feature and head capsule identified three genera of termites. These were; *Macrotermes, Odontotermes* and *Amitermes*. Classification to the species level using morphological

characterization could not be achieved. However, through molecular characterization four different termite species namely; *Macrotermes subhyalinus*, *Macrotermes michaelseni*, *Amitermes spp* and *Odontotermes spp* were identified.

4.6 Distribution of termite mounds in Taita Taveta County

The three types of mound structures identified in this study were distributed in the study region as illustrated in Figure 4.4.

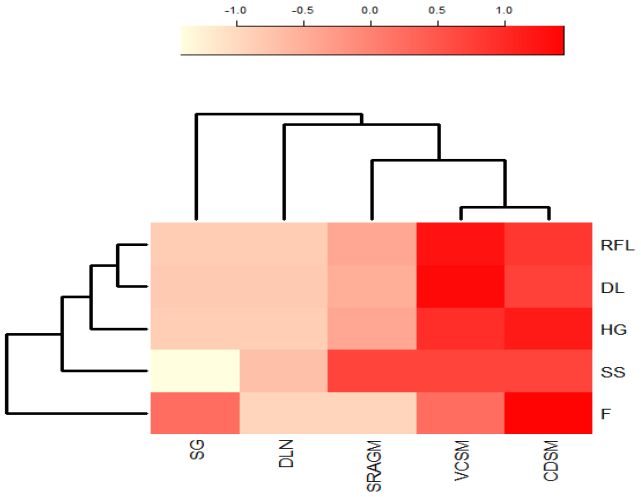

Figure 4. 4: Abundance of termite mounds in different vegetation types within Taita Taveta County

4.7 Distribution of termite species in Taita Taveta County

The study noted that disturbed land areas were dominated by the genus *Macrotermes* and a single species *Macrotermes subhyalinus.* Similarly, rain fed trees vegetation zones was dominated with a single species, *Macrotemes michaelseni*. Herbaceous grassland had two different termite species namely *Macrotermes subhyalinus* and

Macrotermes michaelseni. Likewise, shrub savanna vegetation zone had two termite species (*Macrotermes subhyalinus* and *Macrotermes michaelseni*) and an additional single species from the genus *Odontotermes*. Forests vegetation recorded two different termite species from two genera; *Amitermes conformis* and *Macrotermes subhyalinus.* Out of all the vegetation types the shrub savanna vegetation was the richest in terms of termite species with a record of three different species *Macrotermes subhyalinus, Odontotermes spp* and *Macrotermes michaelseni*. It was closely followed by forest and herbaceous grassland vegetation, respectively. Disturbed land and rain fed trees vegetation had the lowest number of termite species. The genus *Macrotermes* was noted to be ubiquitous in all the vegetation types whereas *Odontotermes* and *Amitermes* were restricted to the shrub savanna and forest respectively (Figure 4.5).

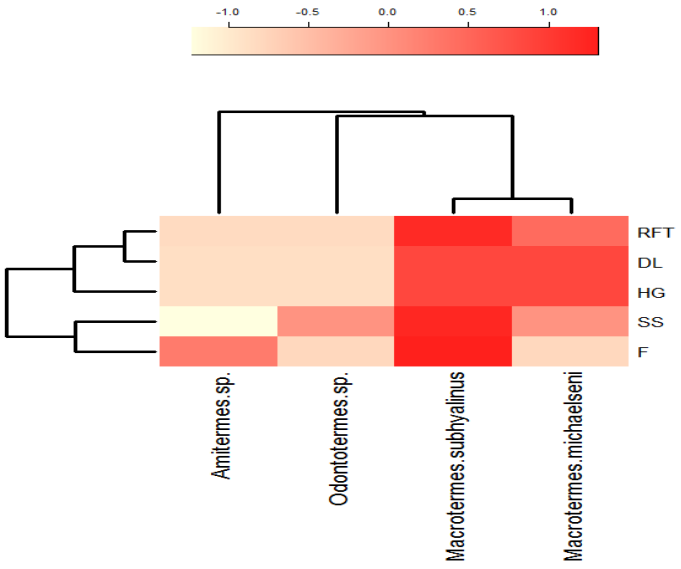

Figure 4. 5: The hierarchical clustering of the abundant taxa for termites in various vegetation types within Taita Taveta County based on Bray-Curtis dissimilarity

4.8 Species richness, diversity and abundance of termites in Taita Taveta County

The values obtained from the study show the study site was rich in species (Table 4.7).

Table 4. 7: Species richness, abundance and diversity of termites collected from Taita Taveta County in 2013 and 2014

Vegetation type	Richness	Abundance	Shannon diversity	Inverse Simpson
Forest	3	4	1.040	2.67
Herbaceous grassland	3	19	0.955	2.42
Shrub savanna	3	9	1.099	3.00
Rain fed trees	3	10	0.943	2.38
Disturbed land	3	13	0.898	2.25

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

On the basis of morphology, termites collected in this study from Taita Taveta County belonged to the major family *Termitidae* and three genera; *Macrotermes, Odontotermes* and *Amitermes.* The abundance of mounds in the study area suggested mound building and possible fungus cultivating activity. The three genera identified in this study had earlier been listed as prominent mound builders in related studies of termites. Meyer, Braack, Biggs and Ebersohn (1999) reported the three genera together with *Cubitermes* and *Trinivitermes* as prominent mound builders from his study in the Krugler National park, South Africa. The mound structures identified in the study were regular or irregular cone-shaped mounds with several ventilations, single closed dome-shaped mounds or in some cases accompanied with emerging baby mounds and slightly above ground mounds. The variation in the structures was an indication that more than one type of species were present in the study sites. There have been instances where two or more termite genera have been found to exist in a single mound making it hard to know the exact mound builder (Makonde *et al.,* 2013).

Previous studies have not agreed on which species build which type of mound structures (Debelo & Degaga, 2014). The present study found *Macrotermes subhaylinus* to be associated with the cone-shaped mound structure with external openings whereas *Macrotermes michaelseni* were associated with closed dome-shaped mounds as these

were the only species collected from these mounds. These results were consistent with the findings by Brandl *et al*. (2007) that reported a Kenyan species of *M. subhyalinus* to build mounds with many open ventilation chimneys. Similar consistency was noted with the findings of a study in Ethiopia that also reported the two species to build different mound structures (Debelo & Degaga, 2014). Hitherto, Darlington (1988) had also reported that *Macrotermes michaelseni* build closed and dome shaped mounds. This finding however contrasts a study in Uganda in which *Macrotermes subhyalinus* were found to build closed dome shaped mounds. This therefore remains a debatable issue as it seems reports on the structures give mixed and confusing argument (Nyeko & Olubayo, 2005).

The fungus farming ability of the termites identified from this study was based on the findings of a parallel study in the same study area. The parallel study focused on the isolation and characterization of fungi from the very mounds that the samples in this study were obtained. The study recorded more than eight species of *Termitomyces* of which two were reported as novel species (Kuja, 2015). Elsewhere in Kakamega forest these species had been found to be fungus cultivators (Osiemo, 2009). The two incidences offer support that indeed the termite species identified were fungus cultivators. Taita Taveta County being a semi-arid savanna harbors a range of fungus farming termites. The dominance of fungus cultivating termites has been widely reported in Africa (Davies *et al.*, 2014).

In addition to mound characteristics which immediately placed the termites into respective genera the soldier characteristics presented more supportive features. *Macrotermes* species had the features similar to those presented in other studies (Manzoor, 2009; Sornuwat *et al.*, 2004; Wang *et al.*, 2009). Although keys based on regional collections may slightly vary for specimens from other regions due to geographical variations and environmental influence, the samples in this study matched well with existing keys enabling identification to the genus level (Ye *et al*., 2004; Scheffrahn & Su, 1994). Moreover the characteristics of this genus are conspicuous due to their large body size and have been widely published (Dahisjo *et al.*, 2014). Members of this genus presented similar morphological features of the head capsule and mandibular features making it difficult to distinguish the species thereof. The genus *Odontotermes* was easily identified by a characteristic bad smell associated with them and their slender mandibles. This characteristic has been previously reported (Pranesh & Harini, 2014). The genus *Amitermes* if not for the fact that they were collected from the exotic forests which were wetter and in which they thrive well the morphological characters they presented could have misidentified them as *Nasutitermes*. This is because of their small size, single mandible and snouted head capsule which better describe *Nasutitermes* (Luke, Fayle, Eggleton, Turner & Davies, 2014; Engel, 2011).

Attaining right sized fragments from PCR amplification of COII gene in all the samples suggests that NUMTs were not sequenced in the samples under investigation. When NUMTs are sequenced they alter the size of the amplified fragment. This also supports earlier reports on the efficiency of the use of specific primers for this gene in termite

identification studies. Most sequences clustered into well differentiated groups, most of which showed congruence with their predefined morphospecies. Majority of nodes immediately defining the clusters showed high levels of nodal support (99%). The three clusters remained distinct indicating that such groups included distinct COII lineages rather than scattered sequence variation (Hajibabaei, Janzen, Burns, Hallwachs & Hebert, 2006). In this study, sequence divergence in COII mitochondrial DNA within distinct clusters (intraspecific) were usually much lower than divergence between clusters (interspecific), which were often higher, but remained within COII sequence divergences from Genbank. This result is a general agreement with empirical levels of divergence found between species delimitation studies especially barcoding (Hebert *et al.*, 2003).

There was high A+T content in the studied sequences consistent with what is expected in insect mitochondrial DNA (Crozier & Crozier, 1993). This is a general feature of COII mitochondrial DNA region in arthropods. Similar observations were made by Wahlberg, Weingartner and Nylin (2003) who sequenced 140 individuals of genus *Phyciodes* belonging to family Nymphalidae of order Lepidoptera. The A+T content for the fragments of genes COI, 16S and 12S in Nasuta subgroup of *Drosophila* was 65.30, 77.90 and 75.20 respectively (Nagaraja *et al.*, 2004). The genetic structures and arrangement of cytochrome sequences of *Coptotermes formosanus* populations collected from the three isolated islands in the Ryukyu Archipelago forest of Japan also revealed A+T bias (Tokuda, Isagawa & Sugio, 2012). The results of this study concur with the earlier studies. The phylogenetic analysis based on mitochondrial COII gene sequences,

thus mirrored the homology between different termite species and the degree of difference among them provides information about their relationship. The region of the COII gene sequenced has been used in various studies to infer phylogenetic relationships among, between and within termite families and species (Lim & Forschler, 2012).

All the sequenced samples belonged to the family *Termitidae* and two subfamilies *Macrotermitinae* and *Termitinae.* The dominance of this family and the subfamily Macrotermitinae in the natural ecosystem like this study region has been widely reported (Kemabonta & Balogun, 2014) Like in morphological characterization, the three main clusters revealed by molecular characterization of termites belonged to three genera *Macrotermes, Odontotermes* and *Amitermes.* The genus *Macrotermes* separated into well supported two sub groups belonging to two species; *Macrotermes subhylinus* and *Macrotermes michaelseni.* This species clustering was consistent with using theoretical thresholds for intraspecific and interspecific sequence divergences for termite delimitation within family *Termitidae*, as described by Osiemo (2009).

In the present study, no cryptic species were found as all the sequences clustering collaborated well with morphospecies. Hence neither sequences from same morphospecies showed deep divergence nor were different morphospecies clustered together. Osiemo (2009) while conducting a termite diversity assessment in Kakamega forest Kenya, recorded similar results. In Kakamega forest deeper sequence divergences of both morphologically unidentified and known morphospecies among soil feeders

which were subsequently considered as cryptic species and hence allocated different Molecular Operational Taxonomic Units (MOTUs) were reported. The observation by Osiemo (2009) can be attributed to heterogeneity of the tropical forest habitat and the large size of sampling area. This study was focussed on the mounds whose internal conditions remain constantly regulated. Advances in molecular techniques has enabled several authors to uncover cryptic speciation in hyper diverse groups such as termites, ants and nematodes (Kanzaki, Ragsdale, Hermann, Mayer & Sommer, 2012; Puillandre *et al.*, 2012; Hausberger *et al.*, 2011).

In this study species identification through molecular analysis was possible to the species level whereas morphology only attained identification up to generic level. Identification using soldier morphology yielded three genera while three genera and four species were identified by molecular characterization. However before construction of the phylogenetic tree there was suspicion that one sample had been identified as *Nasutititermes* species due to morphological characters presented. Sample 28FS was almost misidentified as *Nasutitermes* spp. because it represented features commonly used for identification of *Nasutitermes* like the tiny size as well as single mandible. The morphological and molecular characters thus suggested that samples 28 FS and 24SV were not members of any species group in this study. Consequently, with the strong percentage bootstrap support of 100% there could be a possibility of them being new taxa in the two genera. This possibility can be explored with further description with series of morphological, chemical and molecular properties where morphological characters should be more in number and there should be analysis of cuticular

hydrocarbon which is considered informative. However, after tree reconstruction it became clear that sample 28 FS had close genetic relationship to *Amitermes conformis* and was distinct from *Nasutitermes* species. This is because it clustered closely with a GenBank sequence of *A. conformis*. Such morphological ambiguities have been experienced as characters used in identification are often too variable and dependent on geographical region (Hausberger *et al.,* 2011). Using the two techniques hence results to close to accurate identification of species.

The possibility of two new taxa reported in this study suggests that cryptic species exist in this region and particularly in the forest and shrub savanna vegetation which recorded these findings. It also suggests that phenotypic characters and the DNA database species- specific COII sequences employed in this study were insufficient to identify the two samples to their respective species. Ayuke *et al.* (2009) reported many counts of termites of the family *Termitidae* in Taita hills and recommended further identification. This study has reported four different termite species with two that still need further investigation. New species' description is a whole new task which requires rigorous classification based on combined approaches of morphology, biochemical and molecular methods (Lee *et al.,* 2005).

Mwatate Sub-County majorly covered by herbaceous grassland vegetation recorded the greatest number of termite mounds. However, only termite species of the Genus *Macrotemes* were identified from this vegetation. No valid reason could be established for this finding. Disturbed land areas were also rich in mound abundance but most of

the mounds were inactive or had been destroyed. The few active mounds were recorded in the institutions *viz* Taita Taveta University College and Kenyatta High School where no form of agricultural activity took place. Cultivation practices have been reported to have negative impact on termite abundance (Nyeko & Olubayo, 2005). A single species from the same genus recorded in the disturbed land areas implied that cultivation activities had led to destruction of termite mounds hence driving away termite communities. Other studies in East Africa that have reported cultivation and farming activities to affect termite occurrence are found in a report by Mugerwa *et al.* (2014). In a related study by Luke et *al.* (2014), fewer termite feeding groups were found in the disturbed sites of their study area.

Rain fed trees vegetation apart from being averagely abundant with termite mounds, produced a single species. Samples from this vegetation however clustered together unlike the samples from the other vegetation types that cut across different clusters. This suggests a possible effect of the surrounding environment. Shrub savanna vegetation recorded two genera and three species. This was the highest number of species per vegetation type. High termite counts in Taita shrub land have been reported in a study by Ayuke *et al*. (2009) among other insect species. Dryland savanna ideally is not rich in species hence low diversity is expected compared to a wetter savanna (Luke *et al.,* 2014). Two genera and two species were recorded from the forest. Contrary to the findings of Osiemo (2009) that recorded 22 species from Kakamega forest which is a tropical rain forest, the forest in this study region were exotic and had few termite species. The genus *Amitermes* was restricted to this vegetation. This is probably

because the forests are not subjected to a lot of human disturbance therefore the more vulnerable species are likely to survive (Osiemo, 2009). Moreover *Amitermes* species thrive well in wetter places with lower temperatures (Davies *et al.*, 2014.)

5.2 Conclusions

1. Taita Taveta County is dominated by mound building (Fungus cultivating) termites of three genera *Macrotermes*, *Odontotermes* and *Amitermes* with *Macrotermes* being ubiquitous in all the vegetation types while *Amitermes* was restricted to the exotic forests and *Odontotermes* restricted to the shrub savanna vegetation. The null hypotheses were thus rejected.
2. It was noted that identification of termites through morphological characterization was possible only to the genus level whereas phylogenetic analysis was more informative and necessitated identification of termites to species level. DNA barcoding was found to be important for assigning the termite specimens into their respective taxa and for biodiversity determination.
3. The samples under study were all positive for the target gene (COII gene) with rightsized fragments produced from amplification of DNA specimens. These results have therefore confirmed the reliability of COII gene for termite identification.
4. This study unveils possible existence of two new or undescribed species of termites in the region. One from *Odontotermes* and another from *Amitermes.*

The morphological characters and molecular data sets used could not sufficiently discriminate them as new species.

5.3 Recommendations

This study yielded some observations that are notable and worthy of further investigation.

1. The study recommends establishment of written information on knowledge and keys of morphological characters on a number of groups of termites in the region and in Kenya. Lack of morphological identification keys specific to Kenyan species calls for expertise experience to avail keys for termite species identification.
2. It would be necessary to use more than one molecular marker in future studies to conduct a more robust phylogenetic study of termites. Use of the combined markers enables not only identification and population structure but also allows discrimination between closely related and distantly related taxa.
3. In future distribution of termites should be investigated in relation to other factors as habitat disturbance and environmental factors.
4. Investigation of the two possible new taxa is recommended with use of combined data sets such as cuticular hydrocarbon phenotypes, morphology of alates and soldiers of the same colony and genetics which necessitate accurate identification as well as species discrimination.

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APPENDICES