MOLECULAR CHARACTERISATION OF HONEY BEES, *APIS MELLIFERA* SUBSPECIES IN EAST AFRICA

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Molecular characterisation of honey bees, *Apis mellifera* subspecies in East Africa

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DECLARATION

I, Harrison Gathenga Kibogo declare that this thesis is my original work and has not been presented to any other university for the award of a degree

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DEDICATION

This work is dedicated to my parents, late Mr Kibogo Mwangi and Mrs Hannah Wanjiru Kibogo who supported me in light and difficult moments

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ABBREVIATIONS AND ACRONYMS

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of molecular variance
ASF	Arabuko Sokoke Forest
AvSN	Average Sting Numbers
AvTS	Average Time for First Sting
bp	base pair
°C	Degree Celcius
CTAB	CetylTrimethyl Ammonium Bromide
DISPAN	Genetic Distance and Phylogenetic Analysis
DNA	Deoxyribonucleic acid
dNTPs	dexyNucleotide Tri Phosphates
FStat	F-Statistics
g	Gravity
GD	Genetic distance
GPS	Global positioning system
HGSC	Honey bee Genome Sequencing Consortium
HWE	Hardy-Weinberg Equilibrium
ICIPE	International Center of Insect Physiology and Ecology
IFAD	International Fund for Agricultural Development
JKUAT	Jomo Kenyatta University of Agriculture and Technology
MEGA	Molecular Evolutionary Genetic Analysis
MNA	Mean number of alleles
M	Molar
mM	milliMolar
MSA	Microsatellite Statistical Analysis-
NJ	Neighbour-joining method
NMK	National Museums of Kenya
OD	Optical Density
PCR	Polymerase Chain Reaction
PHYLIP	Phylogeny Inference Package

RAPD	Random Amplified Polymorphism of DNA
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic acid
rpm	Revolutions per minute
QTL	Quantitative Trait Locus
SD	Standard Deviation
SE	Standard Error
SSR	Simple Sequence Repeats
UPGMA	Unweighted Pair Group Method with Arithmetic Mean

ABSTRACT

Honey bee, *Apis mellifera* subspecies from East African populations were evaluated for phylogenetic relationships using mitochondrial markers. *Apis mellifera scutellata, A. m. litorea* and *A. m. monticola* were found to have a close genetic relationship and are largely indistinguishable. However, based on elevation three groups were documented; lowland, mid-altitude and highland bees. *Apis mellifera* populations from Kenya, Uganda, Tanzania and Madagascar out-group were also genotyped at 8 microsatellite loci. The average mean number of alleles and the average mean observed heterozygosity were high at 8.201±2.5035 and 0.781±0.00346 respectively, for all populations. Mean genetic diversity per locus and population was high at 0.783 for all populations indicating a high capacity for adaptation to harsh conditions such as drought, diseases and pests. A low overall mean population differentiation value of 0.056±0.04 signified moderate levels of structuring and genetic differentiation among populations. The probability values for genetic assignment of individual to populations were less than 0.5 indicating that the populations are not isolated and there is substantial hybridization. Results of clustering, admixture, genetic diversity attributed to variance and phylogenetics showed that the dynamics of the honey bee subspecies in East Africa is associated with a relatively stable population demographic structure, especially in unfragmented habitats, natural forests and mountainous regions. In these areas, the results suggest a lively demographic historical pattern of their existence characterized by recent evolutionary expansions and no bottlenecks. Association studies showed that on average colonies stored more nectar (69%) than pollen (31%). There is positive correlation between *sting* and *pln* (0.184458) suggesting that defensive colonies forage for nectar more than the less defensive ones. Candidate gene search identified three genes each associated with foraging (GB46589, GB44258, GB44259) and stinging behavior (GB48999, GB49000, GB55730). The knowledge of genetic relationship, admixture analysis and candidate gene identification amongst African honey bee subpecies is crucial for harnessing their economic important traits for selective breeding, conservation and productivity efforts.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Sub-Saharan Africa relies heavily on horticulture and agriculture sectors for its food production and income generation due to its favorable diverse climatic conditions (World Development Report, 2008, FAO *et al*., 2014). The sectors are dominated by resource-poor small scale holder farmers who practice subsistence farming as a source of income, daily food and employment (Chauvin *et al*., 2012; Joosten *et al*., 2015). However, both sectors are far below their full scale potential due to the increasing population pressure, food insecurity, declining levels of agricultural productivity, poor technologies and rapid natural resource degradation (Dercon & El Beyrouty, 2009; Adenkule *et al*., 2012). Rapid natural resource degradation is as a result of forest fragmentation into patches following clearance thereby interfering with biodiversity and especially economically important insects such as honey bees, *Apis mellifera* (Raina *et al*., 2009; Shaibi & Moritz, 2010). The honey bee population is affected by habitat destruction and human land use as well as poor technology of human beekeeping practice (Shaibi & Moritz, 2010).

Honey bees, *Apis mellifera* Linnaeus 1758 (Apini; Hymenoptera) are in the order Hymenoptera, which consists of approximately 130,000 recognized species of sawflies, parasitoids, hornets, wasps, ants and bees (Ruttner *et al*., 1978; Ruttner, 1988). The order consists of insects having two pairs of membranous wings and often lives in complex social groups (Ruttner, 1988, 1992). Most hymenopterans have some mode of defense, varying from stingers to mouthparts that can inflict painful bites (Ruttner, 1988). Usually, the ovipositor is specialized for stinging or piercing (Ruttner, 1988). They also exhibit haplodiploid sex determination, where males arise from unfertilized haploid eggs and females arise from fertilized diploid eggs (Beye *et al*., 2003). Many hymenopterans are important pollinators of flowering plants and consist of a large family known as Apidae that comprises honey bees,

stingless bees, carpenter bees, orchid bees, cuckoo bees, bumblebees, and various other less well-known groups (Ruttner *et al*., 1978). The honey bees, stingless bees, and bumblebees are colonial (eusocial), but they show notable differences in communication between workers and methods of nest construction (Ruttner, 1988). Of these, the honey bees play a key role in the human and natural world in terms of pollination and hive productivity (Oldroyd & Wongsiri, 2006; Allsopp *et al*., 2008; Ratcliffe *et al*., 2011). There are four different species of honey bees in the world; the Little honey bee (*Apis florea*) – which is native to southeast Asia, the Eastern honey bee (*Apis cerana*) – which is native to eastern Asia and is found as far north as Korea and Japan; the Giant honey bee (*Apis dorsata*) – which is native to southeast Asia and the Western honey bee (*Apis mellifera*) – which is native to Europe, Africa and western Asia (Daly *et al*., 1982; Oldroyd & Wongsiri, 2006; Rattanawannee *et al*., 2010). *Apis mellifera* is commonly known as the European, western or the common honey bee in different parts of the world and is largely domesticated for honey, pollen, beeswax, propolis, royal jelly and bee venom (Ratcliffe *et al*., 2011). It is the major agent for global ecology through pollination (Williams, 2002; Kajobe, 2006; Klein *et al*., 2007; Allsopp *et al*., 2008) and has been the main model of scientific social behavioral studies (Breed *et al*., 2004; Solignac *et al*, 2004; Beye *et al*., 2006; The Honey Bee Genome Sequencing Consortium, 2006).

The honey bee, *Apis mellifera* Linnaeus originated in eastern tropical Africa, spread to northern Europe and east into India and Tien Shan range in China due to evolutionary forces governed by natural and artificial selection (HGSC, 2006). Presently, honey bees live in all parts of the world except the extreme Polar Regions near the North Pole and South Pole.

The honey bee is a highly polytypic species represented globally by 29 recognized subspecies based on geographic variations from morphometric and molecular studies (Ruttner, 1988; Franck *et al*., 2000, 2001; Özdil *et al*., 2009). Behavior, color, size and anatomy can be quite different from one subspecies or even strain to another rendering their phylogeny enigmatic (Meixner *et al*., 2000; Franck *et al*., 2001; Raina & Kimbu, 2005). Based on morphometry, the subspecies are divided into three

distinct branches (lineages), a south and central African branch (A), a north African and west European branch (M) and a north Mediterranean branch (C) (Ruttner, 1988, 1992). The distribution frequency of mitochondrial DNA haplotypes also supports the three lineages.

The African honey bee (A lineage or branch) consists of 10 morphometrically described honey bee subspecies (Ruttner, 1988). These are; *Apis m. intermissa* of Tunisia and Algeria, *A. m. sahariensis* predominantly found in Magreb (Tunisia, Morocco, Egypt and Algeria), *A. m. lamarckii* of Egypt, *A. m. yemenitica* found in north and eastern African countries of Ethiopia, Somalia, Libya and Egypt, *A. m. monticola* found in Kenya and *A. m. litorea* in east Africa found in Kenya, Uganda, Tanzania, *A. m. unicolor* in Madagascar, *A. m. scutellata*, *A. m. capensis* in central and southern Africa and *A. m. adansonii* in western African countries of Ghana, Benin, Mali, Guinea Bissau, Ivory Coast, Nigeria, Senegal and Togo (Smith, 1961; El-Niweiri & Moritz, 2008). Based on mitochondrial DNA typing, more than thirteen haplotypes and their rearranged distribution for the A (African) lineage have been described (Garnery *et al*, 1992, 1995; Palmer *et al*., 2000; Franck *et al*., 2001) to include the O-lineage that refers to the north (Egypt) subspecies and Y-lineage that refers to subspecies in the east (Ethiopia) that includes *A. m. yeminitica* (Franck *et al*., 2001; El-Niweiri & Moritz, 2008). Moreover, new haplotypes for the East African subspecies have been recognized (Shi-Wei 2001) and a potential to discover more haplotypes and new lineages with more studies based on mitochondrial DNA analysis exists (Hepburn & Randloff, 1998; De la Rúa *et al.*, 1998; Smith, 1991; Garnery *et al.*, 1995; Franck *et al.*, 1998, 2001; Shi-Wei, 2001; El-Niweiri & Moritz, 2008). These studies are useful in understanding the honey bee phylogeny (Arias & Sheppard, 1996; Raffiudin & Crozier, 2007; Kekecoglu *et al*., 2009), genetic structure (Estoup *et al*., 1995; Franck *et al*., 2001; Tarpy & Nielsen, 2002; Solignac *et al*., 2004; Cánovas *et al*., 2011) and in linking evolutionary lineage to honey bee traits such as parasite and pathogen prevalence (Jara *et al*., 2012) and disease resistance (Runckel *et al*., 2011).

The benefits of honey bees to human existence cannot be overemphasized in terms of pollination, honey and beeswax production (Hepburn & Radloff, 1998; Raina *et al*., 2009; Ratcliffe *et al*., 2011). Honey bees account for 80% of all insect pollination (Morse & Calderone, 2000; Sung *et al*., 2006). Pollination is significant in horticulture and agriculture because fruiting is dependent on fertilization (Morse & Calderone, 2000; Ollerton *et al*., 2011). Without such pollination, there would be a significant decrease in the yield of fruits and vegetables, trees and biodiversity degradation (Kremen *et al*., 2002; Losey & Vaughan, 2006). Apart from facilitating pollination honey bees acts as a good indicator of beneficial resource management of an ecosystem among other benefits (Raina *et al.*, 2009). Presently, the honey bee is a model organism for studying human health issues such as immunity, allergic reaction, antibiotic resistance, development, mental health, longevity and diseases of the X chromosome (Page Jr *et al*., 2002; HGSC, 2006). In addition, biologists are interested in the honey bees' social instincts and behavioral traits (Page Jr *et al*., 2000; Page Jr & Eber, 2002; Crist, 2004; HGSC, 2006; Shruthi *et al*., 2009). Traits such as resistance to diseases and pests, survival to harsh tropical conditions, foraging and defensive behaviors are of agricultural value and they influence pollination and hive productivity either directly or indirectly (Hunt *et al*., 1998, 2003; Arechavaleta-Velasco & Hunt 2003; Paleolog, 2009; Shruthi *et al*., 2009; Oxley *et al*., 2010; Wray *et al*., 2011). For the African farmers to realize economic benefits through hive productivity and pollination, apiculture should be improved through proper management and use of specialized bees for production (CBI, 2006).

The African honey bee is highly defensive and much more aggressive towards humans and animals (Hepburn & Radloff, 1998; Hunt *et al*. 1998; Hunt *et al*. 2003; Schneider *et al.*, 2004; Hunt, 2007;). This defensive trait is exhibited by non-reproductive female worker bees and not by the male drones (Breed & Rogers, 1991; Lobo *et al*., 2003; Arechavaleta-Velasco & Hunt 2003). In addition, the African bee colonies have a greater emphasis on pollen collection (Page *et al.*, 2000; Quezada-Eu´an, 2000; Fewell & Bertram, 2002) and a more rapid conversion of pollen into brood (Schneider & McNally, 1993). Aggressiveness and pollen foraging phenotypes are determined by multifactorial traits controlled by complex contributions of

individual genetic makeup (Hunt *et al.*, 1995; Lobo *et al*., 2003; Arechavaleta-Velasco & Hunt 2003; Oldroyd & Thompson, 2007). These traits are exceptionally complex and are as a result of combinations of relatively small effects of DNA variations within a large number of unidentified polygenes known as quantitative trait loci (QTLs) (Hunt *et al*., 1998; Page Jr *et al.*, 2000; Lobo *et al*., 2003).

QTLs in honey bees are important because they display colony level traits that are as a result of an average behavior of many individual members of the colony (Hunt *et al.*, 1995; Rueppel *et al.*, 2004; Bourke, 2005; Oldroyd & Thompson, 2007). Colony level QTLs influencing nectar or pollen foraging behavior in honey bees have been identified through selective breeding and genetic linkage mapping (Hunt *et al.*, 1995; Page Jr. *et al.*, 2000). This means that variation in the genomic regions affects the amount of pollen stored in honey bee colonies and influences whether foragers will collect pollen or nectar. Additionally, five putative QTLs influencing colony stinging responses have been mapped (Hunt *et al*., 1998; Arechavaleta-Velasco *et al*., 2003; Review by Hunt *et al.*, 2007). Further, the honey bee genome has been sequenced and has thousands of expressed sequence tags (ESTs) and putative genes which need experimental evaluation (Whitfield *et al.*, 2002). Therefore, it is necessary to investigate putative genes in a bid to improve selection and breeding through molecular marker assisted selection (MAS) of the African honey bee. Many traits associated with economically valuable products such as honey, royal jelly, propolis and pollen are due to important traits that can only be measured at the colony level (Souza *et al*., 2002). Marker assisted selection will improve the efficiency and precision of conventional breeding leading to better adapted and healthier bees that can better fight off parasites, pathogens and pests. This will further enhance honey bees' phenotypic characteristics of agricultural value for optimal hive productivity and pollination. As a result, beekeepers will have a better income as they will market their honey and more farmers will be encouraged to practice beekeeping as an income generating project. Indirectly, this has the benefit of conserving indigenous forest biodiversity and enhancing pollination of their commercial crops leading to better yields.

1.2 Statement of the problem

Globally, there has been decline in managed honey bee population due to the little understood phenomenon of colony collapse disorder (CCD). Parasites (such as Varroa mites and Nosema microsporidia), pathogens, pesticide exposure, poor nutrition, reduced genetic diversity and management practices act synergistically to reduce honey bee populations (vanEngelsdorp *et al*., 2009). In Europe and North America where bees are exploited in large scale commercial operations, losses estimated to be 30% of their managed colonies every winter (vanEngelsdorp & Meixner, 2010). This corresponds to an estimated global loss of $63.6 billion of pollination services and 2.85% loss of the total value of world agriculture production for human consumption in the year 2005 alone, (Gallai *et al*., 2009). Such quantitative economic estimates are unavailable in East Africa as honey bees are not managed for commercial purposes. To meet the local and international market demands, East Africa relies largely on smallholder farmers (Raina *et al*., 2009; JAICAF Report, 2009) who carry out traditional beekeeping practice (Cleaver & Donovan, 1995; Jayne *et al*., 2003; Davis, 2006). The beekeepers are faced with a multitude of problems ranging from declining bee health due to diseases (Frazier, 2010; Kinati *et al*., 2012), pest and parasitic infestation (Martin *et al*., 2002; Davidson *et al*., 2003; Hood, 2004; El-Niweiri *et al*., 2008; Kinati *et al*., 2012), pesticides poisoning (Rortais *et al*., 2005; Hladun *et al*., 2012; Muli *et al*., 2014) to lack of proper skills and techniques (JAICAF report, 2009). Although there are efforts to improve beekeeping through better management, control of diseases and pests, better queen rearing methods through artificial insemination, selective bee breeding based on traits for hive productivity is still unachieved (JAICAF report, 2009). Therefore, the beekeepers should have access to high yielding bees as well as specialized bees selectively bred for different hive products in order to fully realize the hive benefits. This study was focused on identifying candidate genes for foraging and stinging traits that would be suitable for selection and breeding of high yielding honey bee stocks.

Efforts to improve apiculture based on the importation colonies and queens of non-native honey bees (*Apis mellifera*) (Hepburn & Radloff, 1998) has the negative effect of decimating the conserved genetic diversity of wild, endemic honey bee populations (El-Niweiri & Moritz, 2008) through diseases susceptibility, foreign gene introgression and reduced local food availability (Moritz *et al*., 2005; 2007). The introgression risk of introduced subspecies is particularly large because the mating of honey bees cannot be controlled. Previous attempts to introduce European honey bee stock into Africa have been unsuccessful (Hepburn & Radloff, 1998). However, East African honey bees are better adapted to harsh climatic conditions, parasites and pests and diseases that plague nearly all beekeepers of *A. mellifera* in other parts of the world (Frazier, 2010) and are adapted to tropical conditions. This is attributed to increased overall genetic diversity leading to healthier, hardier bees that can better fight off parasites, pathogens and pests (Whitefield & Crawford, 2006). The traits associated with the African honey bee are useful for hive productivity yet they have not been quantified and/or evaluated genetically, harnessed or exploited to improve apiculture productivity in feral or hived populations (Wray *et al*., 2011; Kinati *et al*., 2012).

The African honey bees are mostly hybrids of native populations (Meixner *et al*., 2000; Raina & Kimbu, 2005) and the extent of admixture is often genetically unknown (Baudry *et al*., 1998; Franck *et al*., 2001; Moritz *et al*., 2007; El-Niweiri & Moritz, 2008). Although certain subspecies were thought to belong to certain localities, recent studies reveal a lack of homogeneity probably due to hybridization, swarming, migration and introgression of new gene pools (El-Niweiri & Moritz, 2008). In order to realize the impact of apiculture, it is important to tap the available genetic diversity of the African honey bee populations in their distribution range (Ruttner, 1966, 1988; Fletcher, 1978; Schneider *et al*., 2004). The genetic diversity of the East African populations was studied using microsatellite markers.

East Africa would benefit from the mistakes made elsewhere by preventing problems made in managed honey bees for commercial purposes through the protection of honey bee populations. Before the states establish rules and restrictions, better

information for the primary stakeholders are needed. Beekeepers play a vital role. Supplied with selectively bred healthy stocks, they can help keep African honey bees healthy. The East African honey bee could be a major tool for researchers and beekeepers if they want to learn about conserving the western honey bee.

1.3 Justification

Honey bees' benefits to human existence cannot be overemphasized in terms of pollination and hive products. However, in the emerging challenges of climate change, land transformation, pests, parasites and diseases, pesticides, and unskilled beekeeping operations, there is need to consider selective breeding of African honey bees to augment conservation and increase hive productivity. African honey bees tend to have behavioral and genetic characteristics different from the European or American counterparts. For example, *A. m. scutellata* tends to be more aggressive; a trait which makes it more productive and resilient at their native range. This aggression trait can be manipulated and utilized for the continent's advantage (Raina *et al*., 2009) through selective breeding. The productivity of honey bee colonies does not depend primarily on individual physiological traits, like growth rate, but also on social traits, like foraging and defensiveness (Oldroyd & Thompson, 2007). This makes it easier to genetically improve honey bee stocks as African farmers are interested in colony level productivity of bee products.

Foraging and stinging traits are closely associated with nectar and pollen collection and subsequently honey production (Hunt *et al*., 2007). Quantitative trait loci (QTLs) for foraging (*pln*) and stinging (*sting*) behaviors have been identified for the commercial European honey bee (Hunt *et al*., 1998) but not yet characterized for the African honey bee. The study of QTLs has a high potential of revealing the genetic architecture of complex traits and identify candidate genes responsible for hive productivity (Phillips, 1999; Page *et al*., 2000; Hunt *et al*., 1998, 1999, 2003). The selected candidate genes can be utilized to genetically improve the honey bee for hive productivity traits. The essence of this study was to utilize microsatellite markers (Solignac *et al*., 2003) that are associated with foraging (*pln*) and stinging (*sting*) phenotypes in order to identified candidate genes suitable for selective

breeding. If successful, the improved honey bee stocks, yield more and can be utilized by farmers to improve their hive productivity and to generate more income.

African honey bees harbor the highest diversity in their entire distribution range (Whitfield *et al*., 2006). In Kenya alone, *A. m. litorea* hybridizes with *A. m. scutellata* in mid–altitude areas whereas *A. m. monticola* hybridizes with *A. m. scutellata* in high altitude areas during swarming and migration to regions of favorable conditions (Ruttner, 1988; Meixner *et al*., 1994; Raina & Kimbu, 2005). Due to hybridization and admixture, the genetic status of the Africa honey bee species in unknown. For the East African honey bees, population genetic structure is not yet exhaustive or outright conclusive. Therefore, the study focused on population genetic studies in order to understand the gene flow, genetic diversity and genetic structure of the available species. Also, the dissection between subspecies and lineages is largely unknown (Raina & Kimbu, 2005; Frazier, 2010). Assessment of the phylogeny status was carried out using mitochondrial DNA markers. Analyzing genetic diversity of *Apis mellifera* strains in East Africa could provide a theoretical foundation for the research on the conservation and reasonable utilization of honey bee populations. Overall these studies are important in ensuring that the honey bee stocks harboring high genetic diversity suitable for breeding are conserved in their native range.

1.4 Hypotheses

1.4.1 Main hypothesis

East African honey bee subspecies are homogeneous and show no dissection in colony-associated behavioral traits suitable for selective breeding.

1.4.2 Specific hypotheses

1. The East African honey bees exist as homogeneous, indistinguishable and closely related populations.
2. East African honey bees have a uniform gene pool characterized by low genetic diversity.
3. Gene flow is retricted within the East African subspecies and the gene pool does not change within and among populations.
4. Honey bee colonies do not show dissection in stinging and foraging behaviors.
5. Genes associated with foraging (*pln*) and stinging (*sting*) behavioral traits cannot be identified in colonies showing traits dissection.

1.5. Objectives of the study

1.5.1 General Objective

To determine the phylogenetic relationship, genetic diversity, population differentiation and traits dissection of the East African honey bees, *Apis mellifera* subspecies.

1.5.2 Specific Objectives

1. To determine the phylogenetic relationship of the East African *Apis mellifera* subspecies using mitochondrial DNA markers.
2. To assess the available genetic diversity for future conservation and reasonable utilization.
3. To determine the genetic differentiation, admixture and gene flow between honey bee populations of East Africa.

4. To document the stinging and foraging behavioral traits of selected honey bee colonies.
5. To identify genes associated with foraging (*pln*) and stinging (*sting*) behavioral traits for potential use in honey bee selection and breeding.

1.6 Research Questions

1. Do the East African honey bee populations display a close phylogenetic relationship?
2. How are alleles shared and how much genetic diversity exists in a population?
3. Do the honey bees exist as one large continous panmictic population or many small isolated subpopulations? How are the populations differentiated? Does migration and gene flow occur between different populations?
4. Do honey bee subspecies show colony level dissection in foraging and stinging behavior in their native range?
5. Which genes are associated with colony level foraging and stinging behavioral traits?

CHAPTER TWO

LITERATURE REVIEW

2.1 Honey bees' classification

The Honey bees are classified into Kingdom, Animalia: Phylum, Arthropoda: Class, Insecta: Order, Hymenoptera: Suborder, Apocrita: Superfamily, Apoidea: Family, Apidae: Subfamily, Apinae: Tribe, Apini: Genus, Apis: Species, *Apis mellifera* Linnaeus, 1758 (Ruttner, 1988). Honey bees diverged from Diptera and Lepidoptera 300 million years ago, whereas the last common ancestor with humans was 600 million years ago (Grimaldi & Engel, 2005). Honey bees are a subset of bees in the genus *Apis*, primarily distinguished by the production and storage of honey and the construction of perennial, colonial nests out of wax (Ruttner, 1988; Crane, 1999). Honey bees are the only extant members of the tribe Apini, all in the genus Apis. However the name honey bee in a broad sense refers to any insect of the tribe Apini (family Apidae, order Hymenoptera) that makes honey (Ruttner, 1988; Crane, 1999). Two species of honey bees, *A. mellifera* and *A. cerana*, are often maintained, fed, and transported by beekeepers (Ruttner, 1988). Substantial ecological and geographical variation exists among the honey bee populations, *Apis mellifera* of Africa but despite this variation, there are suggestions to regard these subspecies as ecotypes (A sub-specific group that is genetically adapted to a particular habitat, but can interbreed with other ecospecies of the same species without loss of fertility) (Ruttner, 1988; Arias & Sheppard, 2005; Whitfield *et al*., 2006).

2.2 Life history of the honey bee

Hymenoptera is one of 11 orders of holometabolous insects (Ruttner, 1988). All honey bees, *Apis mellifera* develop through a complete metamorphosis (Grimaldi & Engel, 2005). The life of a honey bee colony is perennial (Gary, 1963, 1992). The queen (female adult bee) lays over 1,500 eggs per day and lives for two to eight years (Ruttner, 1988; Grimaldi & Engel, 2005). The queen lays eggs singly in cells of the comb. The eggs that will eventually become queens are laid in a larger cell (Wilson,

1971). After three to four days, the egg hatches into a worm-like legless larva that resembles a tiny white sausage (Winston, 1987, 1992). The larva feeds voraciously and grows and molts through several stages within the cells each day for about four days. The larvae destined to develop into queen are fed on royal jelly only (Winston, 1987). The larva then goes into a resting stage, the pupa, which lasts for another few days in a capped cell until the bee emerges as an adult (Winston, 1987; Gary, 1992). Queens emerge from their cells in 16 days, workers in 21 days and drones in 24 days (Wilson, 1971; Winston, 1987). Only one queen is usually present in a hive (Winston, 1987). New virgin queens develop in enlarged cells through differential feeding of royal jelly by workers (Winston, 1987). When the existing queen ages or dies or the colony becomes very large a new queen is raised by the worker bees (Winston, 1987). Both the workers and the queen develop from fertilized eggs (egg + sperm) (Winston, 1987; Gary, 1992). After emergence, the virgin queen takes a nuptial flight to an area where hundreds to thousands of unrelated males (drones) from many colonies (Ruttner, 1988; Gary, 1992) congregate. The drones are the largest bees in the colony (Winston, 1987). The drone's head is much larger than that of either the queen's or the worker's. Drones are designed for mating only (Ruttner, 1966; Wilson, 1971). Drones take their first flights at about 8 days old and are sexually mature at 12 days old (Ruttner, 1966; Harrison, 1987). Their normal lifespan is 8 weeks or less (Wilson, 1971). Mating frequencies of up to 44 times per queen have been reported for *Apis mellifera* (Moritz *et al.*, 1995, 1996; Neumann & Moritz, 2000). After mating, the drone will often fall to the ground where he will die (Gary, 1963; Koeniger, 1970; Ruttner, 1966; Winston, 1992). Over 90 million sperm are deposited in the queen's oviducts though only about 7 million of the mixture is stored in the spermatheca (Palmer & Oldroyd, 2000). The sperms will be used, a few at a time, during the queen's life to fertilize her eggs (Moritz, 1986). The queen starts laying 2-4 days after mating and produces pheromones (chemical messengers) that inform the colony of her presence and inhibits queen formation (Crozier & Pamilo, 1996; Strassmann, 2001). The contribution of individual drones to this semen pool is not equal resulting in considerable differences in patriline frequencies of worker bees (Moritz, 1986). Furthermore, drones of one colony may out-compete others in mating efficiency (Berg *et al.*, 1997). Therefore, controlled artificial insemination

and selective breeding of a queen can incorporate the desired qualities and improve the bee colony (Kraus *et al*., 2003).

2.3 Haplodiploid sex determination in honey bees

In Hymenopterans (bees, ants, and wasps) and spider mites, coleopterans (bark beetles) and rotifers sex of the offspring is determined by the number of sets of chromosomes an individual receives (Crozier & Pamilo, 1996). An offspring formed from the union of a sperm and an egg develops as a female, and an unfertilized egg develops as a male (Boomsma & Ratnieks, 1996; Crozier & Pamilo, 1996; Boomsma, 2007). This means that the males have half the number of chromosomes that a female has, and are haploid while females are diploid (Boomsma, 2007; Hughes *et al*., 2008). Haplodiploid-induced asymmetries in relatedness between offspring and sisters have long been thought to be involved in the evolution or maintenance of eusociality in the Hymenoptera, but other life history traits also promote social evolution, and there are divergent perspectives on this issue at the present time (Page Jr & Fondrk, 1995; Page Jr & Erber, 2002; Seeley & Tarpy, 2007; Gove *et al*., 2009). Complementary sex determining (*csd*) gene locus in the honey bee *Apis mellifera* has been cloned and is thought to be the primary switch in the sex determination cascade of honey bees and possibly all Hymenoptera (Beye *et al*., 1994, 2003). Honey bee breeders and researchers endeavoring to develop genetically homogeneous lines should be able to avoid a very direct cost of inbreeding by selecting matings (even full-sibling matings) between individuals with distinct *csd* alleles. Screenings such as this could be used in the planning of germplasm banks used to maintain unique honey bee lineages (Evans *et al*., 2004).

2.4 Economic Importance of Honey bees

2.4.1 Honey bee products

Honey bee workers are important producers of honey, beeswax, royal jelly, pollen, bee venom and propolis which are commonly used in cosmetic industry and as

medicine (Bankova, 2005; Mutsaers *et al*., 2005; Sabatini *et al*., 2009; Ratcliffe *et al*., 2011).

Honey is composed of about 80% carbohydrates (~25 different sugars with fructose and glucose as the main components) and 15-20% water, minerals, vitamins and other trace elements (Mutsaers *et al*., 2005). The conversion of nectar into honey requires the physical removal of water and the addition to the nectar of the enzyme invertase included in the salivary glands of the bees (Mutsaers *et al*., 2005; Ratcliffe *et al*., 2011). This enzyme breaks down sucrose into glucose and fructose (Mutsaers *et al*., 2005). The honey flavor varies from sample to sample (Mutsaers *et al*., 2005). Also the honey color varies from almost black through to golden brown-yellow up to almost white depending on the amounts of phenolic compounds, flavones, and flavonols and antioxidant activity present which in turn depends on the area and different flowers the bees' visit (Pontis *et al*., 2014). Honey is a common home therapy against colds and infections of the throat (Jeffrey & Echazaretta, 1996), kills bacteria (Kwakman. *et al*., 2010) and has immune stimulatory effect (Majtan *et al*., 2006; Tonks *et al*., 2007; Majtan *et al*., 2010).

Beeswax is a natural wax made by young worker bees that secrete it as a liquid from wax glands on the underside of their abdomen (Mutsaers *et al*., 2005; Carroll, 2006). The composition of beeswax is complex and includes over 300 substances (Mutsaers *et al*., 2005). The melting temperature is between 61 and 65°C and smells nice due to over 50 different odours (Mutsaers *et al*., 2005). Beeswax is used in soaps, candles, ointments, batiks, shoe polish and in general cosmetics (Mutsaers *et al*., 2005). In sun creams, it improves the effect against UV rays. In lipsticks it contributes to the shiny effect and helps stabilize the colours (Mutsaers *et al*., 2005). It is also used as foundation sheet for Langstroth hives (Mutsaers *et al*., 2005; Carroll, 2006; Hattori *et al*., 2010).

Royal jelly is a special food which the larva feeds on to become a queen (Mutsaers *et al*., 2005; Carroll, 2006). It contains 60% water, proteins, lipids, sugars, minerals and vitamins (Maundu, 2004; Mutsaers *et al*., 2005; Sabatini *et al*., 2009). It is white to yellow in colour, tastes sour and its smell is strongly aromatic (Mutsaers *et al*., 2005;

Carroll, 2006). The acidic fraction Royalisin contains gamma globulins important in the immune system and 16% asparagin needed for tissue growth (Mutsaers *et al*., 2005; Sabatini *et al*., 2009). About half of the fat fraction is made up of 10-hydroxy-2-decanoic acid (10-HDA), which plays a role in growth, the hormonal system and the immune system (Mutsaers *et al*., 2005; Sabatini *et al*., 2009). Commercial production of royal jelly is uncommon as production requires special knowledge and equipment (Maundu, 2004; Mutsaers *et al*., 2005; Carroll, 2006). The biggest producer is China with a capacity of several dozens of tons (Zhenghua, 2011). Royal jelly is an ingredient in creams and lotions while bee venom is a treatment for arthritis and rheumatic diseases (Maundu, 2004; Majtan *et al*., 2006). Royal jelly is recommended for stomach, liver and digestion problems, high blood pressure, loss of appetite, weight loss, fatigue, listlessness, insomnia, pregnancy, menopause, old-age problems, convalescence and athletics (Maundu, 2004; Mutsaers *et al*., 2005; Carroll, 2006).

Pollen pellets are deposited in empty cells near the brood nest by the pollen-collecting workers (Mutsaers *et al*., 2005). Bee pollen consists of plant pollens collected by worker bees combined with plant nectar and bee saliva (Mutsaers *et al*., 2005). In the cells, the pollen undergoes a maturing process to what is commonly called bee-bread (a mixture of pollen and honey) (Mutsaers *et al*., 2005). Pollen is used as a vegetarian protein dietary supplement in either raw form, grains of powder, pastes, tablets or capsules while bee bread is an antibiotic and source of vitamins A, B12, C and E (Ratcliffe *et al*., 2011).

Specialized propolis (also known as bee-glue) collecting bees are rarely observed (Mutsaers *et al*., 2005). Propolis contains phenols, wax, essential oils and minerals, but the potent components of propolis are flavonoids, ferulic acid, resins, aromatic oils and carotenoids (Bankova, 2005; Mutsaers *et al*., 2005). Propolis differs throughout the world due to the different geographic region and plants used to form it (Bankova, 2005; Mutsaers *et al*., 2005). Above 30°C it is sticky and elastic and below it, it is hard (Mutsaers *et al*., 2005). Propolis is not stored in combs or elsewhere, but is removed from the corbiculae of the field bees and used in the hive

as needed to fill cracks or small openings especially the entrance and to embalm undesired invaders, such as dead mice (Mutsaers *et al*., 2005). A bee returning from foraging has to enter through the propolis channel to ensure that the bee is clean and free from harmful microorganisms (Mutsaers *et al*., 2005). One can expect a production of 50 to 100 grams of propolis per colony per year (Mutsaers *et al*., 2005). Propolis was traditionally used to embalm dead animals (ancient Egypt), and as a natural antibiotic in the treatment of many infections (Mutsaers *et al*., 2005). It is a constituent in cosmetics due to its dermatological regeneration and renovation properties (Lejeune, *et al.*, 1988) and in prevention of dental caries (Cherniack, 2010).

Honey bee venom is a yellowish liquid that tastes bitter and is found in the sting of the older worker bee (Mutsaers *et al*., 2005). It consists of proteins and lipid mixtures beside other compounds (Jang *et al*., 2009). Through a wire trap at the entrance to the hive the bee gets a slight electric shock and releases the venom through a foil into a container (Mutsaers *et al*., 2005). This is done mainly in Eastern Europe and USA. Traditionally Bee Venom Therapy (BVT) is used to treat diseases, such as arthritis and rheumatism (Abdel-Rahman *et al*., 2013), pain (Cherniack, 2010; Ram *et al*., 2014), multiple sclerosis (Castro *et al*., 2005; Wesselius *et al*., 2005), cancerous tumors, and skin diseases (Son *et al*., 2007).

The hive products earn a lot of income after exportation to industrialized countries such as Germany, Japan, USA, UK and Netherlands (Bradbear *et al.*, 2002; Market Analysis and Research, 2010). Honey, propolis, bee venom, beeswax and royal jelly have a ready market all over the world (Raina *et al*., 2009; Sabatini *et al*., 2009; Market Analysis and Research, 2010). The global trade in honey alone exceeded 400,000 tonnes in 2009 (Market Analysis and Research, 2010). China, USA, Canada, Brazil, Argentina, Uruguay, Chile and Vietnam are the major exporters globally and to European markets in particular (Market Analysis and Research, 2010). Well managed colonies in Canada yield 7g per harvest of royal jelly with an average yield of 300-500g within a three-month period, twice the amount yielded by an African honey bee (Market Analysis and Research, 2010). Ethiopia is the leading producer of

honey and other hive products in Africa (The World Market for Natural Honey, 2009). Market options for small-scale farmers include market-places, health food stores, roadside stands, agritourism sites, crafted stores or booths and selling to bulk honey packers (Raina *et al*., 2009). Despite the obvious benefits of honey bees in Africa, products such as honey, royal jelly, propolis and pollen are still in low supply and of low quality due to poor production skills and processing methods (Maundu, 2004; Raina *et al*., 2009). Hence, African farmers are yet to realize the economic potential of exporting their honey products (CBI, 2006; Raina *et al*., 2009).

2.4.2 Honey bees as pollinators of crop plants

Honey bees (*Apis mellifera*) are the most economically valuable pollinators of agricultural crops worldwide (Williams, 2002; Gordon & Davis, 2003; Potts *et al*., 2010). They are the only insect pollinators that can be controlled by man and therefore are valuable to him (Williams, 2002). They are the most efficient and the only dependable pollinators, because they visit flowers methodically to collect nectar and pollen and do not destroy the flower or the plant in the process (Williams, 2002). Only 15 percent of the crops that feed the world are serviced by domestic honey bees, while at least 80 percent are pollinated by wild bees and other pollinators (Gordon & Davis, 2003). Unfortunately, both wild bees and domestic honey bees are in decline (Kasina *et al*., 2009; Potts *et al*., 2010; Raina *et al*., 2011) probably due to the emerging challenges of climate change, land transformation, pests, parasites and diseases, use of pesticides and unskilled beekeeping operations.

Honey bee workers have an age-related socially-regulated division of labour: young worker bees act as ‘nurses’ caring for the brood, whereas older workers forage for nectar and pollen (Whitfield *et al*., 2003) away from the hive to feed the colony (Maundu, 2004; Kasina *et al*., 2009). The nectar is refined into honey, which is stored for times when nectar may be in short supply (Farrar, 1967; Faegri *et al*., 1971). As the field bees forage for nectar, periodically they stop to groom themselves and pollen sticks to the fuzzy hairs which cover their bodies and in special baskets on their hind legs, called corbicula (Farrar, 1967; Faegri *et al*., 1971; Mayfield *et al*., 2001). Some of this pollen rubs off on the next flower they visit, fertilizing the

flower and resulting in better fruit production (Faegri *et al*., 1971). The remaining pollen load is removed from their legs when they return to the hive and the housed bees store it in a special part of the comb (Faegri *et al*., 1971; Mayfield *et al*., 2001). The pollen provides protein and other essential nutrients for the bees (Farrar, 1967; Oldroyd & Thompson, 2007).

Pollen deposition and transfer occurs when the dorsum of a bee contacts anthers or stigmatic surfaces as it probes the perianth tube for nectar (Faegri *et al*., 1971; Mayfield *et al*., 2001). Pollination is said to occur when pollen grains (containing the male gametes) are transferred into the receptive female part of plants known as stigma (contained within the carpel), thereby enabling fertilization and sexual reproduction (Faegri *et al*., 1971; Mayfield *et al*., 2001). Although it is hard to estimate the total value of bee pollination in crop production (Gordon & Davis, 2003), in the United States alone honey bees accomplish 1/4 of the pollination needed for all fruit produced for human consumption which is an estimated $10 billion worth of work (Robinson *et al.*, 1989; Southwick & Southwick, 1992; American Bee Journal, 1993; Goulson, 2003). Some plants will not produce fruit at all without the help of honey bees (Morse & Calderone, 2000; Klein *et al*., 2007; Aizen & Harder, 2009). The fruits pollinated by honey bees include apples, almonds, citrus, cotton, soybeans, onions, broccoli, carrots, sunflower, water melons, among others (Klein *et al*., 2007). Large colonies are good at pollinating monoculture farms while solitary bees are better at pollinating more diverse plants over smaller areas (Morse & Calderone, 2000; Kasina *et al*., 2009; Otieno *et al*., 2010; Potts *et al*., 2010).

2.4.3 Role of beekeeping in biodiversity conservation

Beekeeping has an indirect potential of improving livelihoods by generating income to rural farmers and empowering women and youth by promoting effective use of forest resources and its biodiversity through participatory forest management initiatives (Kasina *et al*., 2009; Otieno *et al*., 2010; Raina *et al.*, 2009, 2011). Although production of honey and other bee related products is significant, honey bees' main value derives from their role as the most important managed pollinator of

horticultural and seed crops (Kasina *et al*., 2009). The primary aim of beekeepers in sub-Saharan Africa is for honey production and pollination is largely natural and unmanaged unlike in developed countries where commercial pollination of high-valued crops is practiced (Kasina *et al*., 2009; Karanja *et al*., 2010; Raina *et al*., 2011). Sub-Saharan Africa is yet to breed commercial queens for honey productivity or pollinator colonies (Raina *et al*., 2009). For example, in Kenya, large horticultural and coffee firms who practice beekeeping within their farms, do so without considering whether honey bees are effective pollinators of such crops (Kasina *et al*., 2009).

Small-scale farmers living adjacent to the forests often have to get incentives to practice beekeeping (Otieno *et al*., 2010; Raina *et al*., 2009). Using a two pronged approach to ecosystem restoration and poverty reduction it is possible to conserve the forests in buffer zone areas by practicing beekeeping through participatory forest management initiatives (Himberg *et al*., 2009; Sinclair *et al*., 2011). The farmers plant trees or plants that the bees frequently visit for nectar and pollen (Otieno *et al*., 2010; Sinclair *et al*., 2011). They also maintain the forest biodiversity by ensuring that trees beneficial to the forests are not cut down for firewood or any other use (Himberg *et al*., 2009; Raina *et al*., 2009, 2011). In return rural farmers harvest huge yields of honey which is sold as "organic honey" in markets abroad and locally (Norton-Griffiths & Southey, 1995; Muriithi & Kenyon, 2002). Biodiversity monitoring is done through socioeconomic monitoring, forests condition, tree regeneration and use, and honey bee insect indicators (Muriithi & Kenyon, 2002; Raina *et al*., 2011). Consequently, there is a need to protect the honey bees and the others pollinators because of their ecological importance (Norton-Griffiths & Southey, 1995; Himberg *et al*., 2009; Karanja *et al*., 2010; Raina *et al*., 2011; Sinclair *et al*., 2011).

2.4.4 Honey bee as a model of behavioral genetic studies

Honey bee is a "behavioral genetics" model revealing genes associated with behavioral variation and characteristics (Oldroyd & Thompson, 2007; Paleolog *et al*., 2011; Wray *et al*., 2011). These include dance language (Grüter & Farina, 2009),

defensive (Hunt, 2007), stinging (Lobo *et al*., 2003; Hunt *et al*., 1998, 1999), guarding (Hunt *et al*., 2003), foraging (Hunt *et al*., 1995; Rueppell *et al*., 2004; Rueppell, 2009), sucrose response (Scheiner & Page, 2004; Goode *et al*., 2006) and hygienic behavior (Spivak & Reuter, 2001; Pe´rez-Sato *et al*., 2009). The pursuit to understand the dynamic interaction between genotype, phenotype and the environment is still ongoing (Wray *et al*., 2011; Uzunov *et al*., 2014).

The honey bee is useful as a bioindicator of environment contamination and as a test organism in the assessment of the ecotoxicological effects of different xenobiotics and pesticides released in the environment (Decourtye *et al*., 2009; Hladun *et al*., 2012). Xenobiotics reach the aquatic and terrestrial ecosystems from discharges and leaks of industrial products, consumer waste and urban sewage, from farming and forestry run-off and accidental spills (Decourtye *et al*., 2009; Hladun *et al*., 2012).

Most behavioral studies require some level of breeding and honey bees are interesting as test organisms because they are easy to rear and manipulate (Dodologlu & Genc, 2003). However, there is prolonged generation time: at least eight weeks from mother colony establishment to daughter colony establishment thereby hindering the ability to inbreed or select recurrently and severe effects of inbreeding caused by the genetic load imposed by the complementary sex determining locus (Laidlaw Jr & Page Jr, 1997; Beye *et al.*, 2003; Cobey, 2007). The negative effects caused by inbreeding mean that the maintenance of mutants or selected lines is tedious, expensive and usually unsuccessful in the long term (Beye *et al.*, 2003; Cobey, 2007). Honey bee colonies are physically large and expensive to maintain, their propagation is seasonal, they are difficult to propagate in enclosures and in laboratory and because the workers sting, a large number of researchers and technicians who then prefer to avoid working with them (Laidlaw Jr & Page Jr, 1997; Beye *et al.*, 2003; Cobey, 2007).

2.5 The African Honey bee

Beekeepers in Europe and other developed world maintain *A. m. ligustica* and *A. m. carnica* or synthetic strains such as the buckfast bee which sting less and are

supposedly “superior” in honey production (Delaney *et al*., 2009). The African honey bee consists of 10 morphometrically described subspecies of *Apis mellifera* (Ruttner, 1988). The honey bee colonies can occupy almost any cavity of suitable size that is protected from the weather (Spivak *et al*., 1991; Hepburn *et al*., 1999). Favored sites are hollow trees, holes under rocks, termite nests, and cavities under floors and in the roofs and walls of buildings (Spivak *et al*., 1991; Hepburn *et al*., 1999). They are also capable of occupying boxes, electricity poles, drains, mail boxes, automobiles, discarded tires, and a wide assortment of other hollow places (Spivak *et al*., 1991). They frequently swarm to establish new nests (Spivak *et al*., 1991; Villanueva-G. & Roubik, 2004; Tarpy *et al*., 2010). The African honey bee is very aggressive though some tend to sting less than others and are more docile (Fletcher, 1978; Hunt *et al*., 1998; Hunt, 2007). They respond faster in greater numbers although each bee stings once (Fletcher, 1978; Hunt *et al*., 1998). However, it has been argued that with frequent handling they become used to being inspected and therefore are mildly aggressive (Fletcher, 1978; Raina, 2004; Hunt, 2007). However, African bees respond more rapidly and intensely than European bees and usually sting in larger numbers (Fletcher, 1978; Hunt, 2007). African bee and European bee venoms are chemically identical, but the African bees are a greater threat because they inflict injury from their numerous stings (Hunt, 2007; Ratcliffe *et al*., 2011). Bees normally attack first around the face and eyes (Fletcher, 1978). A bee smoker can be used to reduce defensive behavior (Fletcher, 1978; Raina, 2004).

Compared with European colonies, African colonies have a greater emphasis on pollen collection (Page *et al.*, 2000; Quezada-Eu´an, 2000; Fewell & Bertram, 2002), have a more rapid conversion of pollen into brood (Schneider & McNally, 1993), and devote two to four times as much comb area to brood rearing (Fletcher; 1978; McNally & Schneider, 1992; 1996; Carroll, 2006). This is an important attribute as the bees can be bred specially for pollination of cultivated crops (Carroll, 2006). African honey bee colonies still abscond readily, defend themselves vigorously, and build comb with the smaller-sized cells (Maundu, 2004; Carroll, 2006). The worker cells built by *A. m scutellata* bees are smaller than those of European bees and are commensurate with the smaller size of the bees (Carroll, 2006; Hunt, 2007). The

resulting higher growth rates allow for increased African swarm production (Schneider *et al.*, 2004). Honey bees swarm as part of the colony's reproductive process (Fletcher, 1978; Carroll, 2006). A honey bee queen is not able to survive on her own or start a new colony alone (Carroll, 2006). When an existing colony prepares to swarm, the bees produce a new queen (Hepburn & Radloff, 1998; Carroll, 2006). Then the old queen and about half of the worker bees will leave the parent colony to establish a new colony (Carroll, 2006; Ande *et al*., 2008). It is this group of bees that is called a swarm (Carroll, 2006). Although a swarm appears quite dangerous, honey bees are usually extremely gentle when swarming (Hepburn & Radloff, 1998; Carroll, 2006). When a swarm selects a nesting site, the bees begin building comb in which to store food and rear young (Carroll, 2006; Ande *et al*., 2008). Within two to four weeks, this colony of bees will have developed the defensive nature that the swarm lacks (Carroll, 2006).

They form elaborate nests called hives containing up to 20,000 individuals or more (Hepburn & Radloff, 1998; Ande *et al*., 2008). Since apiculture is not practiced intensively in Africa, there is unrestricted gene flow between the bees and human impact on genetic variation is low (Hepburn *et al*., 2004). The African honey bee is readily adapted to the tropical conditions. They have a high foraging efficiency compared to the introduced European worker bees that appear to have low foraging efficiency and vulnerable to pest attacks and other factors such as susceptibility to predation as compared to the African honey bee (Carroll, 2006). In East Africa, *A. m. monticola* is found in tropical mountain forests of altitude 2400-3100m above sea level with mean annual temperatures of 18.1°C. *A. m litorea* is found in thorn woodland and scrub between 0-500m altitude and 26.1°C mean annual temperature (Meixner *et al*., 1994; Raina & Kimbu, 2005; Carroll, 2006). Finally, *A. m. scutellata* is mainly found in thorn tree tall grass savanna and tropical semi-evergreen and deciduous forests between 500-2000m above sea level and 21.3°C mean annual temperatures (Meixner *et al*., 1994; Raina & Kimbu, 2005; Carroll, 2006). *A. m. unicolor* from Madagascar has 23.7°C mean annual temperature, exists between 0-1400m above sea level and in thorn tree tall grass savannas (Meixner *et al*., 1994; Raina & Kimbu, 2005; Carroll, 2006). Like the European counterparts the African

honey bees have a sophisticated method of communication (Schulz *et al*., 2002). They send out pheromone signals when the hive is under attack, help the queen find mates, and orient the foraging bees so they can return to their hive (Schulz *et al*., 2002; Hunt, 2007). Pheromones may act as alarm signals, provide trails to food sources, or attract mates (Schulz *et al*., 2002). The waggle dance, an elaborate series of movements by a worker bee, informs other bees where the best sources of food are located (Paleolog *et al*., 2011).

Tropical honey bees do not experience cold season and may forage throughout much of the year (McNally & Schneider, 1992; Carroll, 2006). The extended foraging season and emphasis on pollen collection may be associated with the high swarming rates and migrational movements of tropical honey bees (Fletcher, 1978; Raina, 2004; Carroll, 2006; Ande *et al*., 2008). The accumulation of large food reserves requires the construction of large amounts of comb, large colony population sizes, the collection of food over a large area of the environment, and an emphasis on nectar collection (Raina, 2004; Ande *et al*., 2008). The amassing of large food reserves may be less critical to colony survival and continuing availability of harvestable resources may favor high rates of swarming (Ande *et al*., 2008).

2.6 Apiculture in Africa

Beekeeping or apiculture is the art of managing bees in order to obtain hive products for medicine, cosmetics, food and as an income generating activity (Raina, 2004; Raina *et al*., 2009; Otieno *et al*., 2010). However, Africa's potential for hive productivity and export is not yet fully achieved (FLO, 2005; Raina *et al*., 2009; Otieno *et al*., 2010). Most African farmers keep bees alongside other farming activities and due to over-exploitation of the forest resources; there is a decreasing trend in the number of beehives kept in many areas (Otieno *et al*., 2010; Raina *et al*., 2011). Apiculture is practiced on a small scale using traditional beehives and is not perceived as a potential income-generating activity (Maundu, 2004; Carroll, 2006; Otieno *et al*., 2010; Raina *et al*., 2011). Many farmers are unaware of the pollination benefits associated with beekeeping and result to deforestation and exploitation of the plant sources of pollen and nectar for other uses such as construction and fuel

(Himberg *et al*., 2009; Raina *et al*., 2009, 2011). For the farmers who practice beekeeping the collection of honey often leads to the destruction of colonies and addition of impurities that includes wax and bee parts resulting in unhygienic honey which attracts only nominal rates from traders (Bradbear *et al.*, 2002; FLO, 2005; Otieno *et al*., 2010). Harvested and handled with care, African honey is of high quality, with unique attributes such as taste and colour and has high environmental credentials (Otieno *et al*., 2010). It is produced by indigenous honey bees thriving in natural environments, free from introduced diseases and predators that are a problem in the developed world (Bradbear *et al.*, 2002; Carroll, 2006; CBI, 2006; Otieno *et al*., 2010). This means that African honey has little risk of contamination by drug residues, a factor that could enhance greatly its quality and value on the world market if apiculture is managed properly (Carroll, 2006; CBI, 2006; Otieno *et al*., 2010). Better apicultural practices increase honey production, reduces bee loss and improves pollination efficacy (Himberg *et al*., 2009; JAICAF 2009; Otieno *et al*., 2010; Raina *et al*., 2011). Apiculture can be improved by training farmers on skilled modern beekeeping methods that enhance afforestation and planting of honey bee plants, protection of bee colonies, conservation of certain tree species, prevention of diseases, the risk of bush fires, improving the quality of hive products, identification and development of subspecies (Himberg *et al*., 2009; Otieno *et al*., 2010; Raina *et al*., 2011).

2.7 Declining bee health

Honey bees (*Apis mellifera*) are the most economically valuable pollinators of agricultural crops worldwide (vanEngelsdorp *et al*., 2008; 2009; 2010; Raina *et al.*, 2009, 2011; Sinclair *et al*., 2011). The Food and Agriculture Organization (FAO) of the United Nations (UN) estimates that out of some 100 crop species which provide 90% of food worldwide, 71 of these are bee-pollinated (FAO *et al*., 2014). However global bee health is declining (UNEP, 2010; Potts *et al*., 2010; vanEngelsdorp *et al*., 2009; 2010; 2011) and as a consequence, the earth is losing approximately one percent of its biodiversity annually due to habitat loss, pest invasion, pollution, over-

harvesting and disease (vanEngelsdorp *et al*., 2008; Klein *et al*., 2007; Gallai *et al*., 2009; Civantos *et al*., 2012).

Colony Collapse Disorder (CCD) is a disturbing phenomenon that is characterized by declining bee population in the world (Oldroyd, 2007; vanEngelsdorp *et al*., 2008, 2011; Potts *et al*., 2010). The effects of CCD are devastating with beekeepers losing more than half of their bees and parallel declines in the plants that rely on bees for pollination (Potts *et al*., 2010; vanEngelsdorp *et al*., 2011). It seems to affect bees from hives that are moved from place to place in order to pollinate crops (UNEP, 2010; Potts *et al*., 2010; vanEngelsdorp *et al*., 2009; 2010; 2011). Colony collapse disorder is thought to be caused by colonies infection by numerous pathogens, their interaction coupled with environmental stressors such as climate changes (Cox-Foster *et al*., 2007; Behrens *et al*., 2007; Watanabe, 2008; Giray *et al*., 2010; Soroker et al., 2010; Krupke *et al*., 2012). In those areas where CCD is prevalent, most queen bees or even honey bees have lost their genetic diversity which results in lower immune system and being more effected by disease and pests.

The use of pesticides has caused a sharp decline in the population and diversity of wild and solitary bees (Potts *et al*., 2010; Krupke *et al*., 2012). Systemic insecticides contaminate pollen, nectar and bee products (Rortais *et al*., 2005; Hladun *et al*., 2012). In Africa, honey bees are continuously being decimated by parasites or pests, drought, diseases and predators (Martin *et al*., 2002; Carroll, 2006; Dieteman *et al*., 2006; Narnok *et al*., 2007; Frazier *et al*., 2010; Gidey *et al*., 2012) that have enormously contributed to the declining bee health (Ricketts, 2004; Frazier, 2010; Karanja *et al*., 2010; Raina *et al*., 2011).

Although African honey bees appear to be better adapted to deal with the diseases and pests that afflict *A. mellifera* in other parts of the world (Frazier, 2010; Karanja *et al*., 2010; Otieno *et al*., 2010), it's unclear where the honey bee species is headed and exactly how the drop in population will affect food supply globally (Frazier, 2010). Although the drop in honey bee populations will not lead to sudden human race extinction, it will have a substantial effect on food if it continues (Karanja *et al*., 2010; Otieno *et al*., 2010; Raina *et al*., 2011).

2.8 Molecular markers

The use of molecular markers to study honey bees extends to allozyme polymorphism (Meixner *et al* ,1994), Random Amplified Polymorphic DNA (RAPD) (Sheppard & Smith, 2000), Restriction Fragment Length Polymorphism (RFLP), PCR-RFLP method, mitochondrial DNA polymorphism (Ozdil *et al*., 2009) and microsatellites (Franck *et al.,* 2001; Delaney *et al*., 2009). There are inherent advantages and disadvantages linked to each marker system. However, the choice of applying a particular marker depends on the research questions and the objectives of study.

Allozymes suffers from low polymorphism and low abundance in the genome. Hence they are unsuitable for genetic diversity studies of honey bees. RAPD analysis essentially scans part of the genome containing primers site close to one another that are located in an inverted orientation, although polymorphism is treated in a dominant fashion. RAPD technique is not a candidate marker for genetic diversity studies though Sheppard & Smith, (2000) used it to review the biogeography and interspecific phylogeny of *Apis mellifera*. It has also been used in the identification of three different breeds of honey bee *Apis mellifera* (Pozdnyakov *et al*., 2000) and to tell apart genetic differences between Africanized and European honey bees (Suazo *et al*., 1998).

RFLP variation is revealed by digesting the entire genome with restriction endonucleases. These enzymes are isolated from bacteria and cut DNA at a constant position within a specific recognition site that is typically 4 or 6 bases long. A battery of 4 (*Hinf*I) and 6-base (*Acc*I, *Ava*I, *Bcl*I, *Bgl*II, *Eco*RI, *Hinc*II, *Hind*II, *Hind*III, *Nde*I, *Pst*I, *Pvu*II, *Xba*I) recognition site restriction enzymes are used (Garnery *et al*., 1992; Arias *et al.,* 2006). RFLP surveys on honey bees supports the three major morphology-based lineages (M, A, and C) proposed by Ruttner (1988) and also reveals the existence of some subspecies-specific haplotypes. However, no single or composite set of restriction enzymes have proved to be diagnostic markers for subspecies identification. Although RFLPs, RAPDs and allozymes are useful markers for studies of both, small and larger geographical scales, low degree of

polymorphism, no detection of alleles and low reproducibility characterize their drawbacks. The RFLP method has been replaced by PCR-RFLP mainly to overcome the disadvantage that it requires relatively large amounts of non-degraded DNA. In both methods, haplotypes are resolved by employing restriction enzymes. However, unlike RFLPs, which survey the whole mitochondrial genome, PCR-RFLP variation is revealed within a specific PCR-amplified region. Several PCR-RFLP assays, spanning coding regions, have been developed and employed in identification of honey bee populations (Ivanova *et al.,* 2010; Stevanovic *et al.,* 2010). The PCR amplification of the non-coding region located between the $tRNA^{leu}$ and COII genes (COI-COII intergenic region) (Crozier *et al*., 1989), followed by digestion with the *Dra*I restriction enzyme, has been the most popular PCR-RFLP assay (Garnery *et al.,* 1993). This assay, which is commonly known as the *Dra*I test**,** has been widely used in honey bee maternal identification of honey bee populations.

Mitochondrial DNA (mtDNA) has been commonly used as a molecular genetic marker in vertebrates and invertebrates due to its characteristics, such as high-mutation rates, maternal inheritance, absence of recombination, and small molecular size (Brown *et al*., 1979; Moritz *et al.*, 1987; Harrison, 1989; Barni *et al*., 2007). Mitochondrial DNA has been applied to investigate the ancestors of individual colonies and to study gene flow. This includes studies such as the spread of African-derived honey bees from Brazil to other parts of America (Shepperd & Smitth, 2000). Analysis of mtDNA polymorphism has been applied in several animal studies, such as population dynamics, species and subspecies characterization, systematics, and phylogeny (Moritz *et al*., 1987; Patarnello *et al*., 1994; Weinlich *et al*., 2004). DNA microsatellites are a type of variable number of tandem repeats (VNTR), also known as simple sequence repeats (SSR) and short tandem repeats (STR). Microsatellites are segments of DNA with a sequence motif ranging from 1 to 6 bases, repeated from 4 to probably 100 or more times (Tautz, 1989). Honey bees and bumble bees were the first social insects for which DNA microsatellites were developed (Estoup *et al*., 1993). A valuable source for loci in honey bees was published by Solignac *et al*. (2003), including information on subspecies variation and possible amplification in three other species of *Apis mellifera*. For each

microsatellite locus, there is a specific set of primers is used for amplification with PCR. Microsatellites have shown to be the most popular genetic marker as the high degree of polymorphism is ideal to study small geographical scales of species. DNA microsatellites offer variation useful for inferring population differentiation, originating from a high mutation rate (Levinson & Gutman, 1987) during replication, by adding or removing repeat units compared to the original microsatellite element.

2.9 DNA Barcoding

A barcode is a digital tag having a series of stripes, which encodes information about the item to which it is attached when read by a machine (Savolainen *et al*., 2005; Kress & Erickson, 2008). The living organisms' genomes are analogous to barcodes as they contain adequate information on individual identity and group relationship and/or classification (Hebert *et al*., 2003; Savolainen *et al*., 2005; Kress & Erickson, 2008). The living organisms through selection are identical or near identical between members of a taxon but also vary between taxa in significant pieces of the genome (Savolainen *et al*., 2005; Kress *et al*., 2005). The variations within-taxon are not randomly distributed and cluster in less significant parts of the genome, especially in the third wobble bases of codons, and in intronic and intergenic DNA (Hebert *et al*., 2003; Kress & Erickson, 2008). These variations in DNA segments are very useful in identification and taxonomy because they have evolved over time thereby maintaining records of their profound pasts as well as indicating their more recent history (Crozier & Crozier, 1992; Garnery *et al*., 1992; Hurst & Jiggins, 2005). The evolutionary aspect distinguishes DNA barcodes from product barcodes as they encode data about relationships that is preserved through evolution in a stochastic manner, rather than being hard-coded for utility by a rational agent (Blaxter, 2004).

Animal DNA barcoding uses a standard 658bp region (the Folmer region) of cytochrome *c* oxidase 1 (COI) of the mitochondrial gene (Hebert *et al*., 2003; Blaxter, 2004). Mitochondrial DNA is widely used as a molecular genetic marker in vertebrates and invertebrates due to its characteristics such as high mutation rates, maternal inheritance, absence of recombination, and small molecular size (Moritz & Brown, 1987; Crozier & Crozier, 1992; Lemire, 2005). A COI marker amplifies a

gene fragment of approximately 650bp in size to act as a barcode to identify and delineate all animal life (Hebert *et al*., 2003; Stahls & Savolainen, 2008). Amplification utilizes universal primers for broad taxa; with the 5' end of COI as the focal region (Folmer *et al.,* 1994) but taxon specific primers are used in order to optimize PCR performance (Hebert *et al*., 2004; Penton *et al*., 2004) particularly with degraded material (Lambert *et al*., 2005; Frézal & Leblois, 2008). The obtained sequence is specific for a particular species and therefore used as a tag or 'barcode' in identification of organisms in the same species (Frézal & Leblois, 2008). DNA barcoding does not compete with taxonomy (Gregory, 2005). The first goal of DNA barcoding is to facilitate identification, particularly in cryptic and other organisms with complex morphology (Hebert *et al.,* 2003). The second goal is to facilitate storage, analysis and access to barcode data, the Barcode of Life Data System (BOLD-http://www.barcodinglife.org) was established as an informatics workbench (Hajibabaei *et al.,* 2005; Ratnasingham & Hebert, 2007). By choosing a standard DNA fragment, the efforts of multiple research groups can be coordinated, and they are able to construct a more comprehensive library of DNA sequences than would be possible if they worked independently (Caterino *et al.*, 2000). Although many aspects of DNA barcoding have been critiqued (Moritz & Cicero, 2004; Ebach & Holdrege, 2005; Will *et al*., 2005; Hickerson *et al*., 2006) there has been only limited discussion on the decision to use a specific 600bp fragment from the 5' end of COI as the DNA barcoding region (Erpenbeck *et al*., 2005; Packer *et al*., 2009).

2.10 Genetic Diversity Studies

Phenotypic differences in traits such as size, length and colour have been used to distinguish the honey bee subspecies (Ruttner, 1988; Crewe *et al.*, 1994; Radloff & Hepburn, 1997a, 1997b, 2000; Del Lama, 1999; Segura, 2000; Amssalu *et al.*, 2004; Hepburn *et al.*, 2004; Raina & Kimbu, 2005) but are unable to characterize them to subspecies level, and to give a good estimate of the genetic variation within and between species (Raina & Kimbu, 2005). Apart from RAPDs associated studies (Hunt & Page, 1995; Suazo *et al*., 1998), delineation of the honey bee subspecies has relied mainly on the analysis of the mitochondrial DNA molecule, especially the

region between the cytochrome oxidase, COI and COII genes with a variable 192–196bp sequence (Q) and the complete or partial deletion of 67bp sequence (Po) (Garnery *et al.*, 1993; De La Rua *et al*., 1998, 2001, 2006; Segura, 2000; Meixner *et al.*, 2000; Franck *et al.*, 2001; Susnik *et al.*, 2004; Delaney *et al*., 2009). The analysis of this region has led to discovery of new haplotypes (Franck *et al*., 2000; Palmer *et al*., 2000; Shi-Wei 2001; Özdil *et al*., 2009) and lineages (Palmer *et al*., 2000; Shaibi *et al*., 2009).

Additionally, microsatellite markers have been reported for honey bees (Estoup *et al.*, 1993, 1994, 1995; Hall, 1991; Oldroyd *et al.*, 1994; Rowe *et al.*, 1997; Solignac *et al*., 2004) and have been used along with mtDNA to study genetic structure of honey bee populations (Franck *et al.*, 2001; Susnik *et al.*, 2004; De La Rua *et al.*, 2003, 2006) although they show discordant patterns. Microsatellites are tandem repeats of short units of DNA (1-5bp) that occur with high frequency throughout the genomes of many organisms (Estoup *et al*., 1993, 1994; Rowe *et al.*, 1997; Solignac *et al*., 2004). They are polymorphic in nature and have an advantage of selective neutrality (Estoup *et al*., 1995; Rowe *et al.*, 1997; Solignac *et al*., 2004).

Genetic distances based on their linkage can be used to answer questions concerning population structure and divergence (Solignac *et al.*, 2004; De La Rua *et al.*, 2006, Insuan *et al.*, 2007, Stinchcombe & Hoekstra, 2007). The genetic distance between populations shows how genetically divergent two populations are from each other (Ji *et al*., 2011). When the genetic distance is large, the similarity is low and the time they diverged from each other is greater; whilst when the genetic distance is small, the genetic similarity is high and the time they diverged from each other is smaller (Radloff & Hepburn, 1998; Takezaki & Nei, 1996). Recently, microsatellites have become the marker of choice for ecological and evolutionary studies to answer questions of behavioral ecology, phylogeny, parentage and kinship and have revealed some nuclear introgression from one bee subspecies to another (Franck *et al.*, 1999; De La Rúa *et al*., 2003; Isuan *et al.*, 2007). Therefore, they qualify to be applied in molecular characterization of honey bee subspecies (Olio *et al.*, 2007).

2.11 Quantitative trait loci Analysis

Honey bees are highly social (eusocial) insects (Hunt *et al*., 2007; Oldroyd & Thompson, 2007; Hughes *et al*., 2008; Hranitz *et al*., 2009). They exhibit a wide variety of behavioral phenomena such as kin recognition, complex communication via the dance language, socially regulated division of labor, and large variety forms of learning (Page-Jr *et al*., 2000; Johnson *et al*., 2002; Lobo *et al*., 2003; Oldroyd & Thompson, 2007). These traits are quantitative in nature and are a result of genetic and environment interactions (Page-Jr *et al*., 2000; Oldroyd & Thompson, 2007). Behavioral traits are complex because the brain, which is the central mediator of behavior in most organisms is extraordinarily complex, integrating many developmental, physiological and biochemical systems (Page *et al.*, 1998; Page & Erber, 2002; Oldroyd & Thompson, 2007). Behaviors such as hygienic (Rothernbuhler 1964; Moritz 1988; Paleolog, 2009), defensive (Hunt *et al.* 1998; Lobo *et al* 2003), foraging (Hunt *et al.*, 1995, 2007), dance language (Boch, 1956, 1957; von Frisch, 1993), stinging (Hunt *et al.*, 1998), and tracheal mite resistance (Nasr *et al.*, 2001) have been studied. Bees carry pollen, nectar, or both, as well as propolis (plant resin) or water, back to the hive (Winston, 1987). Two major QTLs (*pln*1 and *pln*2) explain 59% of the variation in quantities of pollen stored by honey bee colonies of two selected strains (Lobo *et al*., 2003). These pollen hoarding QTLs influence response thresholds to sucrose of individual bees, confirming that allelic variation influences the behavior of individual bees in their society (Page Jr. *et al.*, 2002). Other QTLs such as stinging behavior (*sting*1, *sting*2) (Hunt *et al.*, 1998; Lobo *et al*., 2003) have been mapped and are known to influence bee stinging response. Honey bees exhibit defensive behavior near the nest but highly defensive bees may pursue a prey for considerable distances away from the nest (Hunt *et al* 1998; Guzman-Novoa *et al*., 2003; 2005; Hunt, 2007). This behavior is not thoroughly characterized in terms of correlated physiological and sensory traits (Hunt *et al*., 1998; Hunt *et al*., 2003; Hunt, 2007). The study of QTLs has a high potential of revealing the genetic architecture of complex traits and propose candidate genes for further study (Phillips, 1999; Page *et al*., 2000; Hunt *et al*., 1998; 1999; 2003). Additionally, the sequenced honey bee genome makes it easier to study

the annotated gene sequences for their expression and functional analyses through bioinformatics analyses of the genome (HGSC, 2006; Hunt, 2007; Oxlet *et al*., 2010). However, this should be followed by experimental evidence. With honey bees, many of the economically valuable products such as honey, royal jelly, propolis and pollen are influenced by polygenic traits which can be measured at the colony level (Breed *et al*., 2004; Scheiner, 2004; Oldroyd & Thompson, 2007). These traits are greatly influenced by the environment of the hive, both internal and external (Souza *et al.*, 2002; Scheiner, 2004; Oldroyd & Thompson, 2007). This study has focused on the identification of QTLs for the African honey bee showing dissection in foraging and stinging behaviors. This will help in marker selection of a honey bee subspecies with specialized hive productivity.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Basic methodology

3.1.1 Honey bee sampling sites

Feral worker honey bees were randomly collected from the East African countries of Kenya, Uganda, Tanzania and Madagascar. Honey bee samples from Madagascar were used for comparison purposes. Female worker honey bees' samples were collected from five representative biogeographical regions of Kenya namely; central and western highlands and eastern mid-altitude, coastal areas and north eastern semi arid regions. Samples were obtained at altitudes ranging from 5m to 2423m above sea level across the years. Identification of honey bees was done by a certified entomologist based on exterior morphology (e.g. size, abdominal banding pattern) who retained the voucher specimens in the Bee Health facilities of ICIPE (BH-ICIPE, Kenya). For population genetic studies, 288 samples from 57 colonies grouped into 14 populations (Table 1 below) were used in the study. A total of 80 individual COI gene sequences representing honey bee samples from Kenya were analysed. Of these 57.5% (46 samples) were *A. m. scutellata*, 37.5% (30 samples) were *A. m. litorea* and 5% (4 samples) were *A. m. monticola* subspecies. The subspecies were spread across the highland, mid altitude and low land coastal regions of Kenya and Tanzania. For Cytb-tRNA^{ser} sequences analysis, a total of 37 individual sequences representing populations from Eastern mid altitude areas, Western highland areas, Central highlands and Coastal regions were used. There were 12, *A. m. litorea*, 2, *A. m. monticola* and 22, *A. m. scutellata* spread across the regions and one, *A. m. ligustica* sequence from BLAST search. For COI-COII intergenic region, a total of 16 samples representing populations mainly from Eastern region with varying amplicon sizes and lineages were analyzed. There were 4, *A. m. litorea*, 1, *A. m. monticola* and 11, *A. m. scutellata* spread across the four eastern regions mid altitude areas and one, *A. m. ligustica* sequence from BLAST search. Mitochondrial large ribosomal RNA (lrRNA) and mitochondrial ATPase6-8 genes

were amplified but were not explored further with honey bee populations. Additionally, honey bees from Kenya colonies were evaluated for pollen and nectar foraging behavior. A total of 47 colonies (as shown in Appendices 3.1 & 3.2) were studied for stinging and foraging activities. In each colony five bees were collected genetic studies. Sixteen microsatellite markers were used for population structure assessment and association analysis. Global positioning system (GPS) coordinates were taken for each sampling location as shown in Figure 1.

3.1.2 Collection of honey bee specimens

Initially the hives were smoked slightly using a smoker to calm the bees. Adult worker honey bees were then collected by allowing individual bees from the hive entrance to enter into labeled 28 ml universal Bijou bottles placed upside down. The bottles containing bee samples were then instantly added ~15 ml of 95% ethanol to kill and preserve them. They were then transported to the laboratory for storage at 4°C before DNA isolation. The samples information was recorded according to the country, collection site, location and subspecies as shown in Table 1.

Figure 1: A map of East Africa and Madagascar showing sampling sites

Table 1: Sampling information for honey bees

Population	Site/location	No of samples	Region	Landscape	Collection date DD/MM/YYYY	GPS Location	Altitude
Malindi	Dabaso	5	Coast	Lowland	19/12/2007	S 3.339278 E 39.987472	5m
	Chambuko	5	Coast	Lowland	19/12/2007	S 3.372111 E 39.786028	142m
	Hewani	5	Coast	Low land	21/09/2007	S 2.2365 E 40.178111	13m
Taita Hills	Mituki	5	Coast	Highland	17/05/2007	S 3.424444 E 38.3425	1522m
	Ronge	5	Coast	Highland	15/09/2007	S 3.350139 E 38.417056	1284m
	Chawiya	5	Coast	Highland	14/10/2007	S 3.479 E 38.340722	1494m
Kasanga	Bidii	5	Eastern	Mid altitude	17/05/2007	S 0.760819 E 38.156194	1021m
	Kasanga	15	Eastern	Mid altitude	17/05/2007	S 0.774806 E 38.14925	929m
Tanga	Kwamtili	5	Tanzania	Lowland	21/09/2007	S 4.915694 E 38.72775	165m
	Sengoma	5	Tanzania	Lowland	14/10/2007	S 4.995917 E 38.760611	163m
	Semdoye	10	Tanzania	Lowland	30/06/2008	S 4.937056 E 38.711944	195m
Kathiani	Kathiani	15	Eastern	Mid altitude	12/04/2008	S 0.60755 E 38.023833	1009m
	Mikima	15	Eastern	Mid altitude	12/04/2008	S 0.549139 E 38.004	1051m
Mathiakani	Mathiakani1	15	Eastern	Lowland	12/04/2008	S 2.25 E 38.366667	573m
	Mathiakani2	5	Eastern	Lowland	26/04/2007	S 2.247125 E 38.384692	611m
Limuru	Loresho	15	Central	Highland	17/06/2008	S 1.028778 E 36.925889	1638m
	Kikuyu	10	Central	Highland	03/11/2008	S 1.236722 E 36.687917	1918m
Nairobi	ICIPE	5	Central	Highland	16/05/2008	S 1.224194 E 36.897333	1609m
	KARI	5	Central	Highland	16/05/2008	S 0.997778 E 37.078333	1715m
Kakamega	Isiekuti	15	Western	Highland	26/04/2008	N 0.248939 E 34.891464	1596m
	Makuchi	15	Western	Highland	26/04/2008	N 0.0666667 E 34.7666667	1696m
Aberdare	Nyahururu	15	Central	Highland	14/06/2008	N 0.035722 E 36.393142	2423m
	Gatundu	15	Central	Highland	10/10/2008	S 0.880506 E 36.775933	2107m
Isiolo	Iresamburu	10	Neastern	Arid land	01/10/2008	N 1.109278 E 38.809417	271m
Voi	Wusi	10	Coast	Lowland	30/06/2008	S 3.499583 E 38.377861	861m
Bulindi	Bulindi	15	Uganda	Highland	24/03/2006	N 1.455969 E 31.482136	1253m
	Rukooge	5	Uganda	Highland	27/03/2006	S 1.155778 E 30.158061	1776m
	Kikonoka	20	Uganda	Highland	25/03/2006	N 0.283333 E 31.6333	1214m
Madagascar	Tomatave	5	Madagascar	Lowland	01/06/2006	S 18.155697 E 49.322922	150m
	Morandava	5	Madagascar	Lowland	01/06/2006	S 18.965347 E 48.240281	800m
	Scoavenarina	3	Madagascar	High altitude	01/06/2006	S 19.166667 E 46.733056	1300m

3.1.3 Total DNA extraction

Total DNA were isolated from the entire bee without a head by a deproteinization method of proteinase K digestion using DNeasy® Blood and Tissue kit (Qiagen). Individual worker bees were sterilized in 5-10% bleach for 10 minutes to remove impurities and contaminants. They were then rinsed in sterilized double distilled water and dried at room temperature for one hour. This was then ground by hand using a polypropylene pestle in a 1.5 ml eppendorf (microcentrifuge) tube containing ATL lyses buffer (Qiagen). Ground samples were digested in proteinase K by incubating at 65^{o}C for between 30 minutes to 1 hour. The rest of the protocol followed Qiagen DNeasy® Blood and Tissue handbook protocol for tissue extraction (see Appendix 1 for protocols). The extracted DNA was suspended in 100 µl of AE elution buffer from the kit. An aliquot of 2µl was electrophoresed on a 1 % agarose gel to detect the yield, size and quality of extracted raw DNA. The remaining DNA stock DNA was stored at -20°C. For the old samples, a phenol chloroform protocol by Baruffi *et al*., (1995) was used to maximize the yield.

3.1.4 Selection of mitochondrial and microsatellite markers

COI (Barcode region) Cytb-tRNA^{ser} and COI-COII intergenic region of the mtDNA were selected for phylogenetic analysis of the East African honey bees. COI-COII intergenic region is suitable for delineation of honey bees' subspecies. Cytochrome *c* oxidase subunit 1 (COI) gene is the barcode region and carries both specific and systematic information of all organisms. Sequence data obtained from COI barcoding is able to identify closely related species in certain taxonomic groups and forms a standard for identifying members of the animal kingdom (Hebert *et al*., 2003; Hajibabaei *et al.*, 2007). Cytb-tRNA^{ser} was thought to be conserved but sufficiently variable to discriminate between honey bee species.

Microsatellite markers were obtained from the microsatellite database of the sequenced honey bee genome (http://tomato.bio.trinity.edu) which contains all primer note submissions to Molecular Ecology and Molecular Ecology Notes and Resources. Microsatellite loci selected for diversity analysis was in consideration of

the different linkage groups, polymorphism (>1 alleles), number of alleles, molecular size of the product, type of marker (dinucleotide or trinucleotide) and ease of PCR amplification using a standard protocol. Eight unlinked loci were selected from Solignac *et al*. (2003). The markers were fluorescently labelled with Ned (Yellow), 6-Fam (Blue), Pet (Red) and Vic (Green). The microsatellite marker information and PCR conditions are shown in Table 2.

For QTL association studies, six QTLs namely sting-1, sting-2, sting-3, pln-1, pln-2 and pln-3 with high LOD scores were considered based on Hunt *et al*., (2007). Sting-1 had the highest LOD score for colony stinging response and was also the only QTL associated with initiation of stinging at the individual-bee level. The sting-2 region contains two candidates for modulation of response to moving visual targets and alarm pheromone which are the primary stimuli that elicit stinging behavior. Sting-3 like sting-2, has genes with the potential to modulate sensitivity to visual and olfactory stimuli. Pln-1, pln-2 and pln-3 are QTLs based on colony pollen storage. For each QTL and based on available resources, two polymorphic microsatellite loci from the microsatellite database were selected by scanning the genome sequences linked to QTLs for repeat sequences using web-based tandem repeat finder and comparing with isolated markers by Solignac *et al*. (2003). Overall twelve microsatellite markers for QTL association analysis studies (genotype association with phenotypic characteristics) were selected. The primers were fluorescently labeled according to the size of the amplified fragments. The marker information and PCR conditions are shown in Table 2

Table 2: Primer sequence information for all markers

Marker	Region	Forward primer sequence (5'–3')	Reverse primer sequence (5'–3')	QTL	Size range (bp)	Ta (°C)	$MgCl_2$ (mM)	Reference
COI-COII	MtDNA	TCTATACCACGACGTTATTC	CCACAAATTTCTGAACATTGACC	--	>1000	60	2.0	Garnery, 1992
Cytb-$tRNA^{ser}$	MtDNA	TATGTACTACCATGAGGACAAATATC	GAAAATTTTATTTCTATATTATATTTTCA	--	800-900	48	2.0	Delarua *et al.*, 1998
lrRNA	MtDNA	CTATAGGGTCTTATCGTCCC	TTTTGTACCTTTTGTATCAGGGTT	--	650-900	55	2.5	
ATPase6-8	MtDNA	AAAAATTCCTCAAATAATAC	TTAATTTGATTCAGAGAAAT	--	750-900	40	2.5	
Folmer	MtDNA	GGTCAACAAATCATAAAGATATTG	TAAACTTCAGGGTGACCAAAAAATCA	--	600-700	45	1.5	Folmer *et al.*, 1994
AJ509690	LG13	Vic-GAACGTAAACAGAAATACGCG	GACATTGTGTGGGAGCGTG	--	140-170	50	1.5	
AJ509635	LG16	Ned-GATCGTGGAAACCGCGAC	CACGGCCTCGTAACGGTC	--	140-170	55	1.5	
AJ509381	LG12	Pet-GATTAGAGGCAGGAATTCGCA	CGCGAAACGGCTTACATTC	--	140-180	50	1.2	
AJ509656	LG9	Pet-CCGGTCTCTCGATATTTTTATC	AGCAATTGGCATCGATACAC	--	180-220	50	1.5	
AJ509390	LG1	Ned-CCATTCTTCCTCGATAACACG	AGGGCGTCAGGAAGGAAG	Pln-3	240-280	55	1.2	
AJ509721	LG2	6-Fam-GAATATGCCGCTGCCACC	TTTCGTTGCATCCGAGCG	Sting-3	150-190	55	1.2	
AJ509362	LG3	6-Fam-GCGAAAATTGCCGGGTTATA	TGCAACTTTATCGTTTCGACGT	Sting-2	140-170	50	1.5	
AJ509384	LG7	6-Fam-AAGGGTATCGCGGCGTAG	TTCGGTTTGACGAATGCG	Sting-1	270-300	50	1.5	
AJ509439	LG7	Vic-ACGGAGGGAAAATGGAGAG	GTTCGGTCACGTTAAACGG	Sting-1	110-140	50	1.5	
AJ509486	LG3	Pet-ATATCCCGGTGGCCACGT	TGTCGCCACGCATAACTCG	Sting-2	110-140	55	1.2	Solignac *et al* 2004
AJ509655	LG2	Vic-CGCCGATCTGGATGGAAC	CTTGCCAAGTTCACTGCACTG	Sting-3	160-180	50	1.5	
AJ509311	LG2	6-Fam-GTACACCAGAAGCGTCCCA	ACAGACTTGGGAGCATCGA	Pln-1	100-130	60	1.2	
AJ509387	LG2	Vic-CGCAACGCTTACTTACGG	AGCTCGAATCCAATTCGC	Pln-1	200-220	55	1.5	
AJ509441	LG1	Ned-GCTGCGGCCAACGCTAAC	GACACGGCTCGCGACCA	Pln-2	130-150	55	1.5	
AJ509729	LG1	Ned-GTCGGACGGTGTTCGGTC	AGAACAGGCGGAACGTGC	Pln-2	170-200	55	1.5	
AJ509307	LG1	Pet-CGGAAGCGTAAATAGAGAAG	AAATGGAAAGTAGATGTGCG	Pln-3	130-150	56	1.2	

QTL-Quantitative Trait Locus; Ta-Annealing Temperature

3.1.5 Amplification of mitochondrial DNA genes

Using 1 µL (~20ng) of the extracted DNA template, COI-COII intergenic region, Cytb-tRNA^{ser}, lrRNA, COI (Barcode region) and ATPase 6-8 gene of the mtDNA were amplified using a 9800 GeneAMP® PCR System (Applied Biosytems) machine. In each amplification reaction, a negative control sample containing all the PCR reagents minus the DNA was included. The PCR components were as follows; 1 × PCR buffer, 1.5-5.0 mM $MgCl_2$, 0.2 mM each dNTPs, 0.15 µM of each forward and reverse primer and 0.3 units of Taq DNA polymerase (Genscript) in a total volume of 25 µl. The reactions were subjected to an initial denaturation of 5 minutes at 94°C, followed by 35 cycles of 30 seconds at 94°C, 1 minute at appropriate annealing temperature for each primer as shown in Table 2, 1-minute extension at 72°C and a final extension step of 72°C for 5 minutes. The post-PCR samples were stored at −20°C before purification. Five microliters (µl) of the aliquot was electrophoresed in a 1.2% agarose gel containing ethidium bromide and prepared in 1 × TAE buffer to confirm the presence and size of the amplicon. The bands were visualized under an ultraviolet (UV) transilluminator (KODAK® Gel Logic 200).

3.1.6 Amplification of microsatellite markers

The PCR amplification for each sample was performed in a total reaction volume of 10 µl consisting of PCR buffer (10 × Buffer with $(NH_4)_2SO_4$, MBI Fermentas®), 1.0 µL (~20ng) template DNA solution, 200 µmol each dNTP, 0.8 µmol of each forward and reverse primer, 0.4 Units of Taq DNA polymerase (MBI Fermentas®). Magnesium chloride salt concentrations differed for each primer reaction. Touchdown PCR reaction at an initial denaturation at 95°C for 5 minutes, then 5 cycles at 94°C for 30 seconds, 30 seconds at 5°C above the primer specific annealing temperature and decreasing by 1°C per cycle and 72°C for 1 minute, then 30 cycles of denaturation at 95°C for 30 seconds, primer specific annealing temperature for 30 seconds and extension temperature of 72°C

for 1 minute. The last cycle was followed by a 10 minutes' extension step at 72°C. Two microliters were electrophoresed on a 2% agarose gel containing ethidium bromide and prepared in 1 × TAE buffer to confirm the presence, size, intensity and the quality of the amplicons. The bands were visualized under an ultraviolet transilluminator (KODAK® Gel Logic 200). The remaining PCR product were kept at –20°C before genotyping. An individual was declared null (not amplified at a locus) only after two or more amplification failures.

3.1.7 Microsatellite genotyping

The Gene Scan 500 LIZ internal size standard (orange in colour) (Applied Biosystems) was prepared by adding 10 µl of the standard to 1 ml of HiDi® formamide and mixed by pipetting and gentle vortexing. HiDi® formamide prevents reassociation of DNA strands after denaturation. From each sample mixture above, an aliquot of 1 µl consisting of four PCR products was taken and mixed with 9 µl standard/formamide mixture then vortexed gently. The PCR products were denatured at 95°C for 5 minutes and immediately placed on ice to prevent reassociation of the DNA. The mixture (standard/formamide/PCR products) was separated on a DNA automated sequencer (ABI PRISM® 3730*xl* DNA analyzer, Applied Biosystems). Applied Biosystems Incorporated 3730*xl* genetic analyzer was able to achieve reliable genotyping or sizing considering three conditions: spatial resolution, needed to separate microsatellite alleles differing in size by a single nucleotide; spectral resolution, needed to separate fluorescent dye colors from one another so that PCR products from loci labeled with different dyes could be resolved and consistent DNA sizing precision from run to run so that samples could be related to allelic ladders (internal-lane size standards) ran for calibration purposes. The 3rd order Least Squares Method which uses regression analysis to build a best-fit cubic function curve from the internal-lane size standards was used for size calling. The data points of the unknown fragments were compared to the size calling curve. The end result was an electropherogram with a series of peaks that represented different alleles according to the size, peak height and peak area detected by the software. The results of genotyping

data were collected by GeneMapper® software (version 3.7, Applied Biosystems) in excel format for statistical analysis.

3.2 Phylogenetic relationship analysis of honey bee subspecies

3.2.1 Purification of PCR products for sequencing

Amplicons after PCR using the following primers from barcode, COI-COII and Cytb-tRNA^{ser} intergenic regions were purified by sodium acetate and ethanol precipitation method. One-third volume of 3 molar sodium acetate, pH 5.2 and two volumes of cold absolute ethanol were added to each sample. They were then incubated at -70°C for 10 minutes or -20°C for 1 hour. The samples were centrifuged at 12000 × g for 15 minutes and supernatant discarded using a micropipette. The pellet was washed with 70% ethanol and centrifuged for 5 minutes at 12000 × g before discarding ethanol. The pellet was then dried for 1 hour at room temperature with tubes inverted on dry tissue paper. The pellet was then dissolved in 20 µl distilled water. Approximately 5 µl of the aliquot was electrophoresed in a 2 % agarose gel containing ethidium bromide and prepared in 1 × TAE buffer to confirm the presence and quality of purified amplicons. The bands were visualized under a UV transilluminator (KODAK® Gel Logic 200). The purified PCR product was kept at –20°C before sequencing.

3.2.2 Sequencing of purified PCR products

Direct cycle sequencing was done on an automated ABI 3730 DNA Analyzer (Applied Biosystems) using Dye-terminator method. Dye-terminator sequencing utilizes labeling of the chain terminator dideoxynucleotide triphosphates (ddNTPs), which permits sequencing in a single reaction. Therefore, dideoxyNTPs were modified by fluorescent labeling for detection in automated sequencing machines. These ddNTPs also lacked a 3'-OH group required for the formation of a phosphodiester nucleotides bond between two nucleotides, thus terminating DNA strand extension and resulting in DNA fragments of varying length. Each reaction contained a purified PCR product as the

template, a DNA primer for each amplified gene, DNA polymerase, normal deoxynucleotide phosphates (dNTPs), and modified nucleotides (dideoxyNTPs). The newly synthesized and labeled DNA fragments were separated by capillary electrophoresis. Bidirectional sequence reads using both forward and reverse primers were obtained for each PCR product. The DNA sequence and the associated electropherograms generated could be directly read off the computer. The generated electropherograms were assembled and edited using Chromas software (Copyright© 1998-2001, Technelysium Pty Ltd, Queensland, Australia, chromas@technelysium.com.au). The sequences were individually manually edited, verified for protein coding frame-shifts to avoid pseudogenes (Zhang & Hewitt, 1996). ClustalX version 2.1 (Larkin *et al*. 2007) was used for multiple sequence alignments. Quality of the alignment was further improved by doing manual editions using GeneDoc program (http://www.cris.com/~Ketchup/genedoc.shtml). Basic Local Alignment Search Tool (BLAST) (Altschul *et al*., 1997) using nucleotide query was used to search for highly similar sequences in the sequenced honey bee genome by specifying *Apis mellifera* in the organism name option or by searching in the *Apis mellifera* (honey bee) Nucleotide BLAST assembled genome. For comparative sequence analysis Molecular Evolutionary Genetics Analysis (MEGA version 6) (Tamura *et al*., 2013) was used. The genetic distances between the sequences were calculated and the resulting table of pairwise distances for the *Apis mellifera* subspecies generated. A bootstrap consensus tree from Neighbour-joining (NJ) method (Saitou & Nei, 1987) was chosen. The bootstrap consensus tree was inferred from 10000 replicates (Felsenstein, 1985) taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). COI mtDNA barcode sequences were submitted to the Barcode Of Life Database (BOLD; http://www.boldsystems.org/) GenBank accession numbers KP887018-KP887097. Phylogenetically, the sequences were analysed in MEGA using the standard neighbourjoining (NJ) method. Sequence divergences were calculated based on K2P distances (Tamura *et al*, 2004).

3.2.3 Delineation of honey bees using COI-COII intergenic sequences

The region between COI-COII genes contains the gene for $tRNA^{leu}$ and a nocoding sequence, the length of which varies between 200bp and 860bp. This sequence includes a combination of two components, P and Q (Cornuet & Garnery, 1991; Cornuet *et al*., 1991). The sequence P has two forms P and Po, which differ by an insertion/deletion sequence (P is 15bp shorter than Po). The other sequence Q is 191-196bp long and its 3' end (Q3) is almost identical to Po. The Po and Q segments are similar in approximately 79bp of their sequences. A lot of combinations have been observed in this non-coding sequence but nine are common; Q, PQ, PQQ, PQQQ, PQQQ, PoQ, PoQQ, PoQQQ and PoQQQQ. Q is characteristic of bees from lineage C. The four combinations with P (from PQ to PQQQQ) are characteristic of lineage M, and the latter three with Po (PoQ to PoQQQQ) are characteristic of A lineage (Garnery *et al*., 1992). In this study the lineage based on these classifications was evaluated.

3.3 Allelic proportions and genetic diversity of populations

3.3.1 Hardy Weinberg Equilibrium and linkage disequilibrium

A large population with constant gene and genotype frequencies is said to be in Hardy-Weinberg equilibrium (HWE) (Falconer & Mackay, 1996). The Hardy-Weinberg Equilibrium equation is $p^2 + 2pq + q^2 = 1$, where p is the frequency of dominant allele *A*, q is frequency of recessive allele *a*, $p + q$ always equals 1, pp or p^2 is probability of *AA* occurring, qq or q^2 is probability of *aa* occurring, $2pq$ is probability of *Aa* occurring (pq is probability of *Aa*, qp is probability of *aA*, so $2pq$ is probability of all heterozygotes *Aa*). These add up to 1 because they represent all possibilities. If the alleles at a set of loci are not randomly assorted in the next generation, but inherited together as a unit, then the population is said to be at linkage disequilibrium. If an assumption is made that genotypes in a population are in HWE proportions, then F_{IS} is the probability that two alleles in an individual are identical by descent relative to the subpopulation from which they are drawn. The Fisher's exact test for Hardy-Weinberg equilibrium, genotypic linkage disequilibrium between pairs of loci in each population (100 batches, 1000 iterations per batch) and genetic structure (genotypic differentiation) were computed with GENEPOP Version 4.0 (Raymond & Rousset, 1995; Rousset, 2008). Bonferroni correction was used for all tests involving multiple comparisons (Rice, 1989). The program performs exact tests for deviation from Hardy-Weinberg, linkage disequilibrium, population differentiation and isolation by distance (IBD). Linkage disequilibrium (LD) is the non-random association of alleles at two or more loci, not necessarily on the same chromosome and was measured by R-squared. Non-random associations between polymorphisms at different loci are measured by the degree of linkage disequilibrium. Numerically, it is the difference between observed and expected (assuming random distributions) allelic frequencies. The level of linkage disequilibrium is influenced by a number of factors including genetic linkage, selection, rate of recombination, rate of mutation, genetic drift, non-random mating, and population structure (Wallberg *et al*., 2014).

3.3.2 Genetic diversity analysis

Genetic diversity is an important index for estimating a population's long-term survival possibility (Frankel & Soule 1981), and is required for making biologically sound conservation plans (Friar *et al*., 2000). Common measurements of genetic diversity include average number of alleles per locus (observed and effective), heterozygosity (observed and expected), and the proportion of polymorphic loci (Frankel & Soule 1981).

The mean number of alleles (n_a) per locus, number of rare alleles (n_r) with frequencies less than 0.05, number of private alleles (n_p), frequency of private alleles (A_p), observed (H_O) and expected heterozygosities (H_E) (for all loci and locus by locus) were estimated using GENEPOP, version 4.0 software (Rousset, 2008). Mean number of alleles (n_a) is the average number of alleles observed in a population while the expected heterozygosity (H_E) is the proportion of heterozygotes observed in a population. The two are good indicators of genetic polymorphism within populations under study (Raymond & Rousset, 2000). Gene diversity (H_S), allelic richness (R_S) and the inbreeding index (F_{IS}) estimates were assessed by use of FSTAT 2.9.3.2 (Goudet, 2001). Measures of allelic richness were corrected for variation in sample size by using the rarefaction method incorporated in FSTAT program. Rarefaction is an infrequently used statistical method that produces unbiased estimates of allelic richness (Hurlbert, 1971; Leberg, 2002) due to sample size.

MicroSatellite Analysis (MSA) software, version 4.05 (Dieringer & Schotterer, 2003) was used to study the degree of genetic differentiation between pairs of populations, in terms of pairwise *F*st values (Weir & Cockerham, 1984). The statistical significance of each value was assessed by the comparison of the observed value with the values obtained in 10,000 matrix permutations. The frequency of null alleles was calculated using the Brookfield estimation (Brookfield 1996) in Micro-Checker 2.2.3 (Van Oosterhout *et al*. 2004). The Microsatellite Toolkit program was used to obtain

population parameters and the input files for Arlequin (Schneider et al., 1997), Microsat, Genepop, Fstat and Dispan (Ota, 1993) statistical packages. The Genepop file format was used in Bottleneck, WhichRun (Banks & Eichert, 2000) and GeneClass software programs.

3.3.3 Genetic bottleneck

A population bottleneck (or genetic bottleneck) is an evolutionary event in which a significant percentage of a population or species is killed or otherwise prevented from reproducing, and the population is reduced by 50% or more, often by several orders of magnitude. However, a slightly different sort of genetic bottleneck can occur if a small group becomes reproductively separated from the main population. This is called a founder effect. In honey bees, founder effect occurs when a new colony is started by a few members of the original population. This small population size means that the colony may have reduced genetic variation from the original population and a non-random sample of the genes in the original population. Populations that have experienced a recent size reduction exhibit a correlative reduction of the allele numbers and heterozygosities at polymorphic loci. In this case, allelic diversity is reduced faster than heterozygosity, i.e., the observed heterozygosity is larger than the expected heterozygosity. Allele frequency data were tested for heterozygosity excess or deficiency using the program BOTTLENECK (version 1.2.02; Cornuet & Luikart, 1996, 1997). Effective populations sizes (*N*e) and mutation was estimated following Chakraborty and Nei's (1982) method, which assumes that populations are in mutation-drift equilibrium for the loci under study. Two mutation models, considered appropriate for microsatellites (Piry *et al*., 1999), were applied: the strict Stepwise Mutation Model (SMM) and the Two Phase Model (TPM). The Two Phase Model is intermediate to the SMM and the infinite allele mutation (IAM) models. It considers that loci evolve under the 90% single step mutations and 10% multiple step mutations unlike the (IAM) model. To determine whether a population exhibits a significant number of loci with gene diversity excess the "Wilcoxon sign-rank test" (Luikart *et al*., 1997) was used. The

Wilcoxon test provides relatively high power and it can be used with as few as four polymorphic loci and any number of individuals (15-40 individuals and 10-15 polymorphic loci is recommended to achieve high power). A qualitative descriptor of the allele frequency distribution ("mode-shift" indicator) which discriminates bottlenecked populations from stable populations (Luikart *et al*., 1997).

3.4 Population differentiation, admixture and gene flow studies

3.4.1 Population differentiation by F-statistics

Honey bees naturally subdivide themselves into colonies by swarming thereby decreasing heterozygosity and generating genetic differentiation through genetic drift and natural selection. The genetic composition of a population shows the number of alleles shared by individuals and can be compared between populations to account for population genetic differentiation. The decline in heterozygosity due to honey bee subdivision within a population can be quantified using an index known as Wright's *F* statistic, also known as the fixation index. F_{ST} is a fixation index that was developed by Wright (1951) to account for inbreeding within populations. F_{ST} therefore measures inbreeding due to the correlation among alleles because they are found in the same population. F_{ST} (θ_{ST}) is the fixation index defined by the following equation;

$$\text{Fixation index} = F_{ST} = \frac{H_T - H_S}{H_T}$$

Fixation index, F_{ST} is a measure of the extent of genetic differentiation among subpopulations. It is the mean reduction in heterozygosity of a subpopulation (relative to the total population) due to genetic drift among subpopulations. H_T is the expected heterozygosity in random mating total population equal to $2pq$ and H_S is the mean observed heterozygosity within randomly mating subpopulations equal to $2p_iq_i$. F_{ST} can range from 0 (no genetic differentiation) to a theoretical maximum of 1 (complete differentiation with subpopulations fixed for alternative alleles). F_{ST} is the correlation

between two alleles chosen at random within a subpopulation relative to the alleles sampled at random from the total population. Thus, F_{ST} measures the heterozygote deficit relative to its expectation under Hardy-Weinberg equilibrium (Hartl & Clark 1997). The Nei's coefficient of gene differentiation, G_{ST} (Nei, 1977) is analogous to Wright's, F_{ST} in that it is a measure of relative differentiation among a finite number of subpopulations given by;

$$G_{ST} = \frac{D_{ST}}{H_T} = \frac{H_T - H_S}{H_T}$$

where, D_{ST} is the average gene diversity within and among populations. H_S is the Hardy–Weinberg's average subpopulation heterozygosity and H_T is the total population heterozygosity given by $H_T = 1 - \sum \bar{p}_i^2$ for any number of alleles, where p_i is the average frequency of allele i over subpopulations. G'_{ST} is a sample size independent estimator of G_{ST}. The estimation of heterozygosities, H_S in G_{ST} rely on allele frequencies only (Nei 1987); whereas that of F_{ST} accounts for the individual genotypes within subpopulations. Inbreeding coefficient, F_{IS} (where *I* stand for *I*ndividual and *S* for *S*ubpopulation), measures the extent of genetic inbreeding within subpopulations or departures from random mating within populations at each locus. Agreement between observed and expected genotypic frequencies can be conveniently expressed using Wright's coefficient of inbreeding equation given by;

$$F_{IS} = 1 - \frac{H_I}{H_S} = \frac{H_S - H_I}{H_S}$$

F_{IS} measures the mean reduction in heterozygosity of an individual due to non-random mating within a subpopulation. H_I is the mean observed heterozygosity per individual within subpopulations and H_S is the mean expected heterozygosity within randomly mating subpopulations equal to $2p_iq_i$. F_{IS} values can range from -1.0 (all individuals heterozygous) to $+1.0$ (no observed heterozygotes). Inbreeding coefficient is sometimes

simply referred to as F rather than F_{IS}. The overall fixation index estimator, F_{IT} is given by the equation;

$$\text{Overall Fixation Index} = F_{IT} = \frac{(H_T - H_I)}{H_T}$$

F_{IT} measures the mean reduction in heterozygosity of an individual relative to the total population. The relationship between the three F-statistics is;

$$(1 - F_{IT}) = (1 - F_{IS})(1 - F_{ST})$$

F_{IT} combines contributions from non-random mating within demes (F_{IS}) and effects of random drift among demes (F_{ST}). F_{ST}, F_{IS}, F_{IT}, H_S, H_T and H_O (observed heterozygosity) values were estimated using FSTAT 2.9.3 software following the method of Goudet, 2000. The programme also calculates Hamilton's (1971) relatedness, $relat = 2F_{ST}/(1 + F_{IT})$, calculated using an estimator strictly equivalent to Queller and Goodnight's (1989). This measure is the average relatedness of individuals within samples when compared to the whole data set. The statistical significance of each value was assessed by the comparison of the observed value with the values obtained in 10,000 matrix permutations.

3.4.2 Genetic distance and phylogenetic relationship

Genotypic data is multivariate in nature meaning that the frequency of each allele at each locus is usually different in each population. Genetic distance metrics summarizes the differences in an overall measure of differentiation for a pair of populations. At a given locus, the distance is 1 if both individuals have the same genotype, 0 if they have no allele in common and 0.5 otherwise. The standard genetic distance of Nei (1972, 1978), D_S and the chord distance, D_A (Nei *et al*., 1983), which have distinct properties, were used in the analysis. The Nei's standard genetic distance (D_S) is based on the proportion of shared alleles (Bowcock *et al*., 1994). A formula is available for obtaining

nearly unbiased estimates of D_S from genotypic data (Nei 1978). The D_A distance of Nei (Nei *et al*. 1983) is a modification of the original Cavalli-Sforza chord distance (1967). It assumes that genetic differences arise due to mutation and genetic drift and is known to give more reliable population trees for microsatellite DNA data. These two distances (D_A and D_S) were used to construct the NJ tree after 1000 bootstrap resampling of the allele frequency data. The PHYLIP computer package version 3.5c (Felsenstein, 1993, 2005) was used to give phylogenetic relationships of the honey bee populations using the distances to form a neighbourjoining (NJ) dendogram (Saitou & Nei, 1987). The dendogram was viewed using TREEVIEW programme (Page, 1996). The distance between two populations is the average of the distances between one individual from one population and one individual from the other populations. To calculate the proportion of variation, R^2, explained by the dendogram in the genetic distance matrix, the equation below was used;

$$R^2 = 1 - \sum \frac{(D_{ij} - d_{ij})}{(D_{ij} - \overline{D})^2}$$

where D_{ij} represent the observed genetic distance between populations *i* and *j*. *dij* represents the distance between populations *i* and *j* in the tree and $\overline{D}$ is the mean distance. If R^2 is near 1.0, the tree represents a good summary of the genetic relationships shown in the distance matrix. Treefit program (Kalinowski, 2009) was used to calculate these values.

3.4.3 Analysis of Molecular Variance (AMOVA)

To complete the clustering analysis, a locus by locus Analysis of Molecular Variance (AMOVA) was performed using (version 3.5.1.2) (Excoffier & Lischer, 2010). Analysis of molecular variance was employed to assess the component of genetic diversity attributable to five geographical regions of Kenya namely; north eastern, eastern, coast, central and western. AMOVA was also tested according to the altitude levels of Kenya

(lowland, 50-600m; mid altitude, 700-1200 and highland, 1200 and above) where honey bees show subspecies barrier. Lastly analysis of molecular variance was performed to assess the genetic variability attributable to the different subspecies of *A. m. unicolor*, *A. m. scutellata*, *A. m. monticola* and *A. m. litorea* as they were identified morphologically. Estimates of population divergence were computed using variance of computed distance matrix from different number of alleles. Significance for the AMOVA analysis was ascertained using 10,000 permutations.

3.4.4 Population and racial admixture

Two methods were used to infer the clustering of the 13 populations: Principal Coordinate Analysis (PCA) and a Bayesian clustering algorithm. For PCA, the allele frequency for each population was calculated using GENALEX programme (Peakall & Smouse, 2005). The relationships between populations were then performed by double centering the similarity matrix (SM), and computing eigenvectors using NTSYSpc software version 2.2 (Rohlf, 2005).

The Bayesian approach in Structure 2.2 (Pritchard *et al*. 2000a) uses the individual as the unit, assessing whether it belongs to one or more population groups or clusters (K), regardless of its geographical origin. To determine the most likely number of clusters underlying our samples, the data was conditioned on various values of K ranging from 1 to 10. The software was run under the admixture model with the option of correlated allele frequencies (the 'F model') (Falush *et al*., 2003): this option allows frequencies in the different populations to be similar, allowing for ongoing migration or shared ancestry. Simulations were run using the admixture model without prior population information (Pritchard *et al*. 2000a). Other default settings were: different values of F_{ST} for different subpopulations, prior F_{ST} mean 0.01 and standard deviation 0.05. The length of the initial burn-in period was set to 10000 iterations followed by a run of 10000 Markov chain Monte Carlo (MCMC) repetitions, replicated 10 times to ensure convergence on parameters and likelihood values. To identify the most likely number of

sub-populations (K) among individuals, ΔK was used, the maximum second order rate of change of posterior probability given the data, Pr(X/K), standardized by the standard deviation of Pr(X/K) (Evanno *et al*., 2005). The mean and standard deviation of Pr(X/K) and DK were calculated from 10 replicate analyses for each value of K. Output of Structure analyses were visualized using the Distruct software (Rosenberg, 2004).

Genotypic data obtained from unlinked markers may infer population structure in a model based clustering method. The model assumes *K* unknown populations each characterised by a set of allele frequencies at each locus. The optimum number of clusters (K) was determined by using the admixture model and testing for different possible numbers of populations (K between 1 and 10 populations were considered). Individuals in the sample were assigned to populations or jointly to two or more populations if their genotypes indicated that they are admixed (Pritchard *et al.*, 2000a). The assumption is that within populations, the loci are in Hardy-Weinberg equilibrium and linkage equilibrium.

3.4.5 Population Assignments and Exclusion

The program GENECLASS 2.0 (Piry *et al*., 2004) was used to assign or exclude reference populations as possible origins of individuals, on the basis of multilocus genotypes. The program calculates for each individual, the probability of belonging to each other, as a resident in the population where it was sampled and to the reference populations. The standard criterion for analysis described by Rannala and Mountain (1997), which applies Bayesian statistics for multilocus genotypes to compute assignment probabilities, was utilised. The Monte Carlo resampling method (Paetkau *et al*., 2004) aimed at the identification of accurate exclusion/inclusion critical values was used. The results were based on 10,000 individual simulated genotypes for each population and on a threshold probability value of 0.001.

3.5 Dissection of foraging and stinging behaviors in honey bees

3.5.1 Selection for pollen and nectar foraging behavior

Honey bees from Kenya were evaluated for pollen and nectar foraging behavior. A total of 47 colonies were studied for stinging and foraging activities. In each colony five bees were collected after study. The hive colony status was evaluated for the total number of honey bees, number of occupied frames, sealed brood (s), open brood (o), honey (n) and pollen (p) loads. Honey bee colonies with plenty of honey and/or pollen reserves are usually stabilized and little or no absconding took place. They tended to defend the colony and sting more. Depending on the area, the average estimated number of honey bees in each colony was approximately 20,000 honey bees. The number of frames occupied in each colony were between 8 and 10 (above 8 frames were acceptable for the experiment). The number of sealed and open brood loads was recorded. Colony-level trait for high and low pollen and nectar-hoarding behavior were phenotypically characterized by observing the amount of nectar and pollen loads stored in the combs and recording them. The names of the colonies used, recorded observations and calculations are found in Appendix 3.1.

3.5.2 Selection for stinging behavior

Stinging behavior was evaluated following the procedure of Collins *et al*. (1984). In each colony five bees were then collected for genetic studies. Each colony was stimulated with 0.30 ml of artificial alarm pheromone rubbed in a 5 cm diameter dark leather ball. This alarm pheromone was made up of equal proportions of lavender and jasmine oil. The ball was balanced at 20 cm in front of the colony and near the hive entrance for one minute. The balls were used only once for each colony and were not reused. Time at first sting (T1S) was recorded for each colony. After one-minute stinging time, the balls were immediately placed in individual bags to avoid stinging after the test timing. For each colony, observations were done for one hour. The total

number of stings (SN) for each experiment was registered for each colony. An interval of at least 3 days between successive stinging experiments was allowed on the colony to avoid an increase in the number of guard bees. The experiment was replicated 3 times. Depending on the average number of stings, a colony was classified as either high defensive or low defensive. The names of the colonies used, recorded average number of stings observed and time at first sting with subsequent calculations are found in Appendix 3.2.

3.5.3 Association between variables

The association between quantitative and qualitative variables was characterized using Torocor software version 1.0 (Hardy, 2009) which also performs tests of spatial autocorrelation using randomization. The four quantitative variables analysed were as follows; average sting number (AvSN), average time at first sting (AvTS), sealed-to-open brood (SOS) and nectar-pollen ratio (NPN). The two qualitative variables that were analysed included the sting (honey bees grouped either A-aggressive, or M-mild) and pln (honey bees grouped either N-nectar foraging, or P-pollen foraging). The basic statistics describing each quantitative variable (the mean, standard deviation, range, and proportion of missing data) and each qualitative variable (number of states, proportion of samples of each state) were calculated. Two matrices; Pearson's correlation coefficients between quantitative variables and Khi-square (χ^2) statistics of independence between qualitative variables were given. The intra-class correlation coefficient of each quantitative variable classified by each qualitative variable gave a third matrix. Bilateral tests were used for Pearson's correlation coefficients between quantitative variables, whereas unilateral tests were used for the χ^2 statistics and the intra-class correlation coefficients. Using randomization tests statistically significant values were marked by * ($P<0.05$ and at least 99 randomizations), ** ($P<0.01$ and at least 499 randomizations), or *** ($P<0.001$ and at least 4999 randomizations) and preceded by + or – to indicate whether the observed value was higher or smaller than the mean value after randomization. Tests of spatial autocorrelation were done using complete randomization

(5000 replicates) with null hypothesis (Ho) of no spatial autocorrelation between variables.

3.6 Identification of candidate genes

3.6.1 Marker polymorphism and population admixture

The software of Microsatellite Analyzer (MSA) (Dieringer & Schlötterer, 2003) was used to calculate allele number, allele frequency, gene diversity, polymorphism information content (PIC) and gene frequency. Rare alleles (with frequency<5%) in the population were treated as missing data for population structure, linkage disequilibrium analysis and association analysis (Breseghello & Sorrells, 2006).

Sixteen marker loci, distributed over the entire honey bee linkage groups, were used to assess the population structure of 235 honey bee samples using the model-based (Bayesian clustering) method implemented in the software of *Structure v2.3.3* (Pritchard *et al*. 2000a, 2000b). The number of subgroups (K) was set from 1 to 10 based on models characterized by admixture and correlated allele frequencies. For each K, ten runs were performed separately, 100,000 iterations and a burn-in period of 100,000 were carried out for each run. A value of *K* (number of clusters) was selected when the estimate of InPr(X|K) peaked in the range of 1 to 10 sub-populations.

3.6.2 Linkage disequilibrium

Trait Analysis by aSSociation, Evolution and Linkage (TASSEL) program, version 2.1 (Bradbury *et al*., 2007) was used to analyze the marker properties, Linkage Disequilibrium (LD), principal component (PC) matrix, hierarchical clustering, and Q+K mixed model. TASSEL allows for linkage disequilibrium statistics to be calculated and visualized graphically. Linkage disequilibrium was estimated by D', r^2 and P-values by setting 1000 permutations. D' is the standardized disequilibrium coefficient, a useful statistic for determining whether recombination or homoplasy has occurred between a

pair of alleles. The absolute value of D' is determined by dividing D by its maximum possible value, given the allele frequencies at the two loci.

$$D' = \frac{D}{D_{max}}$$

Where $D_{max} = \begin{cases} \max(-p_1q_1, -p_2q_2) \text{ when } D < 0 \\ \min(p_1q_2,\ p_2q_1) \text{ when } D > 0 \end{cases}$

The parameter D is the coefficient of linkage disequilibrium and is given by;

$$D = x_{11}x_{22} - x_{12}x_{21}$$

Where x_{11} and x_{22} are referred to as "coupling" gametes and x_{12} and x_{21} the "repulsion" gametes. The case of $D' = 1$ is known as complete LD. Values of $D' < 1$ indicate that the complete ancestral LD has been disrupted. The magnitude of values of $D' < 1$ has no clear interpretation. Estimates of D' are strongly inflated in small samples. Therefore, statistically significant values of D' that are near 1 provide a useful indication of minimal historical recombination, but intermediate values should not be used for comparisons of the strength of LD between studies, or to measure the extent of LD. A value of 0 for D' indicates that the examined loci are in fact independent of one another, while a value of 1 demonstrates complete dependency. The r^2 value represents the squared correlation coefficient between alleles at two loci, which is informative for evaluating the resolution of association approaches and summarizes both mutational and recombination history.

$$r = \frac{D}{\sqrt{p_1p_2q_1q_2}} \text{ or } r^2 = \frac{D^2}{p_1p_2q_1q_2}$$

The case when r^2=1 is referred to as perfect LD. For multiple alleles of the Kenyan honey bees, a weighted average of r^2 were calculated between paired loci rather than Lewontin's D'. This weighted average was determined by calculating r^2 for all possible

combinations of alleles, and then weighting them according to the allele's frequency. P-values were determined by permutations based on calculation of the proportion of permuted gamete distributions that are less probable than the observed gamete distribution under the null hypothesis of independence. The loci were considered to be in significant LD if $P < 0.001$.

3.6.3 Association between phenotypic variables

Basic statistics for quantitative variables nectar occupied combs (npn), sealed combs (sos), average sting number (AvSN) and average time in seconds for sting (AvTS) were determined by the program AutocorQ ver. 2.00. The spatial autocorrelation of each variable were tested using complete randomizations (5000 replicates), whereby the values of a variable are randomly shuffled among all samples. *I*(*d*), as well as the regression slopes *b*lin and *b*log, were recomputed for many randomized data sets to assess their distributions under the null hypothesis (*H*o) that there is no spatial structure. Bilateral tests were used for Moran's I while unilateral tests were employed for regression slopes. All sample pairs were considered to give Moran's *I* in three classes of 2, 4 and 8 km distances. A matrix of Pearson's correlation coefficients between quantitative variables was given showing variables that are positively correlated. Chi-square (χ^2) coefficient between variables showed statistically significant correlated variables.

3.6.4 Marker-Trait Association

Association between markers and traits was analyzed using a general linear model (GLM) and a mixed linear model (MLM) method in TASSEL version 2.1 (Bradbury *et al*., 2007). TASSEL is an open-source software package that uses genetic markers to evaluate associations with traits, identify evolutionary patterns, and analyze linkage disequilibrium. TASSEL requires three types of data primarily for the analysis (i) marker segregation data (ii) phenotype data and (iii) Ancestry coefficient data (Q

matrix). In order to identify if there was any structure/grouping in the population for association mapping analysis, a Bayesian phylogenetic method using STRUCTURE software (Pritchard *et al*., 2010) was used. This software was used to obtain an optimum population structure by determining a value for K and an estimate inferred ancestry (Q matrix) of individuals suitable for TASSEL analysis. The Q matrix of inferred ancestry coefficients of individuals across the subpopulations were combined to covariate in the association tests (GLM). GLM analysis accounts only for population structure in the association analysis. To reduce an elevated false-positive rate (spurious associations), the relative kinship matrix (K matrix) was estimated by SPAGEDi software (Hardy and Vekemans, 2002) which uses markers to develop an estimate of the identity by descent (IBD) relationship matrix. Kinship or relatedness is estimated by the "coefficient of relatedness", which is defined as the probability that the alleles of a locus chosen at random from two individuals are identical by descent. Kinship coefficients were used in association analysis (MLM) to refine the results. The significant marker-phenotype associations were declared by $P \leq 0.001$ and the magnitude of the QTL effects were evaluated by R^2-marker. P-values larger than 1e-3 or 0.001 were not considered.

3.6.5 Candidate genes search

Based on association mapping results, the candidate genes were determined. To identify putative genes that flank microsatellites, identified microsatellites were mapped on the *Apis mellifera* genome at www.beebase.org using the blastn tool. For each microsatellite, a genome browser on the website was used to determine upstream and downstream genes. Further, the putative names of the resulting genes were identified by a search on the non redundant protein database of national center for biotechnology information (NCBI) using blastp. Gene ontology was done using Blast2GO® (Cornesa *et al*., 2005) programme.

CHAPTER FOUR

RESULTS

4.1 Phylogenetic relationships of honey bee subspecies

4.1.1 Amplification of COI gene

Polymerase Chain Reaction amplification using DNA barcode primers (Folmer *et al.*, 1994) yielded a fragment size between 600 and 800bp on 2% agarose gel as shown in Figure 2. *Apis mellifera* samples from across 8 populations namely; lane 1-Eastern, lane 2-Coast, lane 3-Western, lane 4-Central, lane 5-North Eastern, lane 6-Tanzania, lane 7-Uganda and lane 8-Madagascar are shown. M is a 100bp DNA ladder (ABgene Inc®) used as a molecular size marker.The fragments appear to be of the same size for all representative samples used.

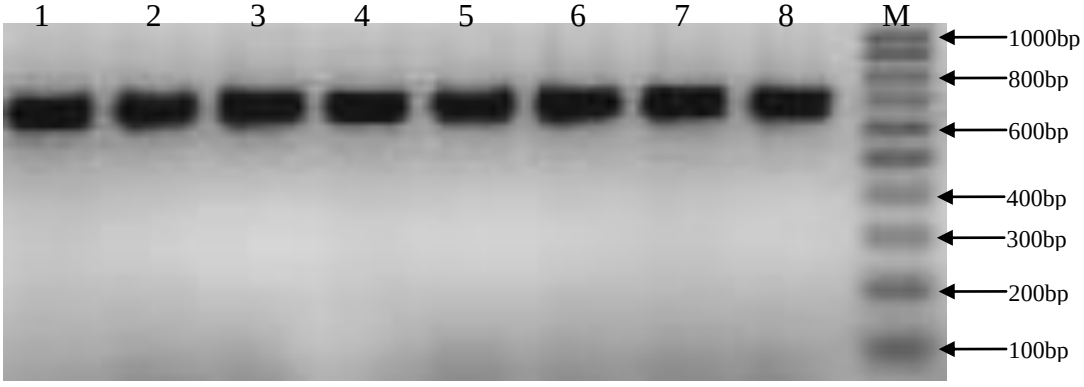

Figure 2: DNA barcoding (COI gene) analysis on agarose gel

4.1.2 COI sequence analysis

A total of 80 sequences analysed using ClustalX (version 2.1) and MEGA6 software. There were no deletions, insertions or stop codons observed in the sequences. COI gene sequences showed AT bias averaging 74% in agreement with *Apis mellifera* mitochondrial genome (Clary & Wolstenholme, 1985). The sequences are deposited in

the barcode of life database (BOLD; http://www.boldsystems.org/), where a project (Kenyan Honey Bees) was created. They are also deposited in the GenBank with accession numbers ranging from KP887018-KP887097. Similarity search on the nucleotide databases (www.ncbi.nlm.nih.gov) using the BLAST algorithm (Altschul *et al*., 1990) yielded a best hit of a 658bp sequence highly similar (98%) to *Apis mellifera ligustica* mitochondrial sequence consistent with the mtDNA sequences published by Crozier & Crozier, (1993). Similarity search in protein database using a translated nucleotide query (BLASTX) yielded a partial mitochondrion cytochrome oxidase subunit 1 from *Apis mellifera* with 86% sequence identity and 94% sequence similarity. An example of *Apis mellifera* COI nucleotide and a translated protein sequence are shown below. Other sequences used in this study are attached in Appendix 2.1.

>H002 *Apis mellifera*

GATCTTGTATATTATTCTAGCTTTATGATCTGGAATACTAGGATCATCAATGAGACTTA
TTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAATAATGATCAAATTTATAAT
ACAATTGTTACTAGCCATGCATTTCTAATAATTTTTTTTATAGTTATACCATTTTTAAT
TGGAGGATTTGGAAATTGGCTTATTCCTTTAATACTAGGATCACCTGATATAGCATTTC
CCCGAATAAATAATATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTA
AGAAATTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTATCAGC
ATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCTCTTCATATATCAG
GAATTTCCTCAATTATAGGATCATTAAATTTAATAGTTACAATTATAATAATAAAAAAT
TTTTCTATAAATTATGACCAAATTTCATTATTTCCATGATCAGTTTTTATTACAGCAAT
TTTATTAATTATATCACTACCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATC
GAAATTTTAATACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAA
CATTTATTT

COI sequence for a representative sample of the honey bee, *Apis mellifera* samples

>H002 *Apis mellifera*

ILYIILALWSGMLGSSMSLIIRMELSSPGSWINNDQIYNTIVTSHAFLMIFFMVMPFLI
GGFGNWLIPLMLGSPDMAFPRMNNISFWLLPPSLFMLLLSNLFYPSPGTGWTVYPPLSA
YLYHSSPSVDFAIFSLHMSGISSIMGSLNLMVTIMMMKNFSMNYDQISLFPWSVFITAI
LLIMSLPVLAGAITMLLFDRNFNTSFFDPMGGGDPILYQHLF

Partial mitochondrion cytochrome oxidase subunit 1 protein sequence from *Apis mellifera*

4.1.3 Sequence alignment of COI

Consensus sequences were aligned using ClustalX 2.1 (Larkin *et al*., 2007). Substitution or single point mutations in the form of transitions were mainly observed but only one transversion of the form A-C was observed out of a total of 80 sequences. No insertions/deletions (indels) and stop codons were observed in the sequences on multiple sequence alignment.

4.1.4 Phylogenetic relationships of COI gene

A Neighbour-joining bootstrap (10000 iterations) phylogenetic tree constructed using the MEGA6 software grouped the 80 samples representing Eastern, Coast and Tanga (Tanzania) regions into 4 major clusters namely; Northeast Coast, Eastern, Tanga, and Northwest Coast as shown in Figure 3. Cluster 1 had only samples from lowland areas of Hewani in Tana River which is located in Northeast Coast region. Cluster 2 had the largest number of samples representing mid altitude areas of Eastern region. Cluster 3 had samples representing lowland areas mainly of Tanga population located in coastal area of Tanzania near Indian Ocean and a few Eastern samples. Cluster 4 consisted of samples from highland regions of the Northwest Coast mainly from Taita Hills which borders Eastern region in the east and a few samples from the Eastern region.

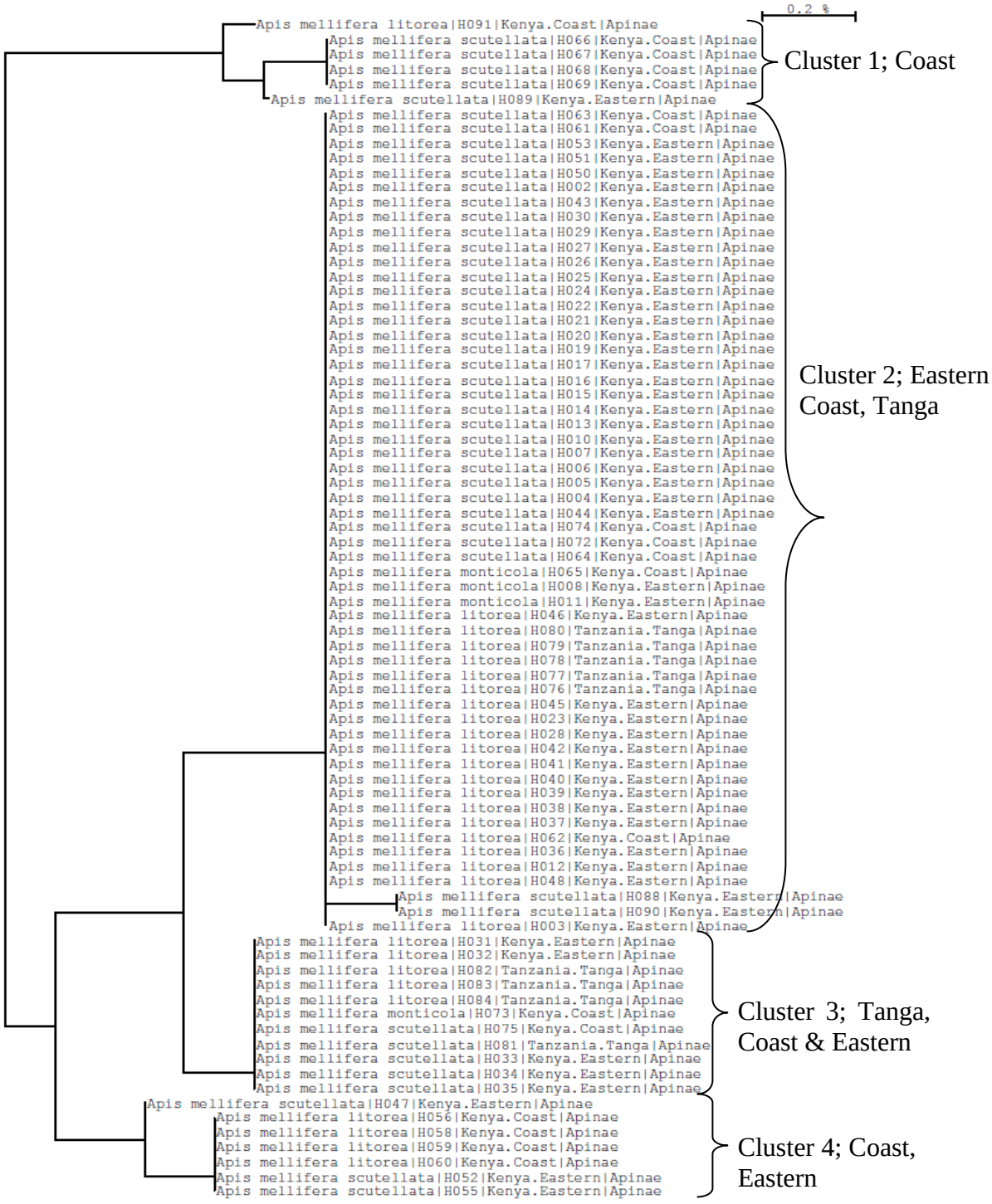

Figure 3: Neighbour-joining consensus tree from COI sequences

4.1.5 Amplification of Cytb-tRNA^{ser} intergenic region

PCR amplifications using Cytb-tRNA^{ser} primers yielded a fragment of size between 800-900bp as shown on 1.5% agarose gel represented in Figure 4. Honey bee samples from 8 populations namely; lane 1-Eastern, lane 2-Coast, lane 3-Western, lane 4-Central, lane 5-North Eastern, lane 6-Tanzania, lane 7-Uganda and lane 8-Madagascar are presented. M is a 100bp DNA ladder (ABgene Inc®) used as a molecular size standard. The eight fragments are of the same size for all representative samples used.

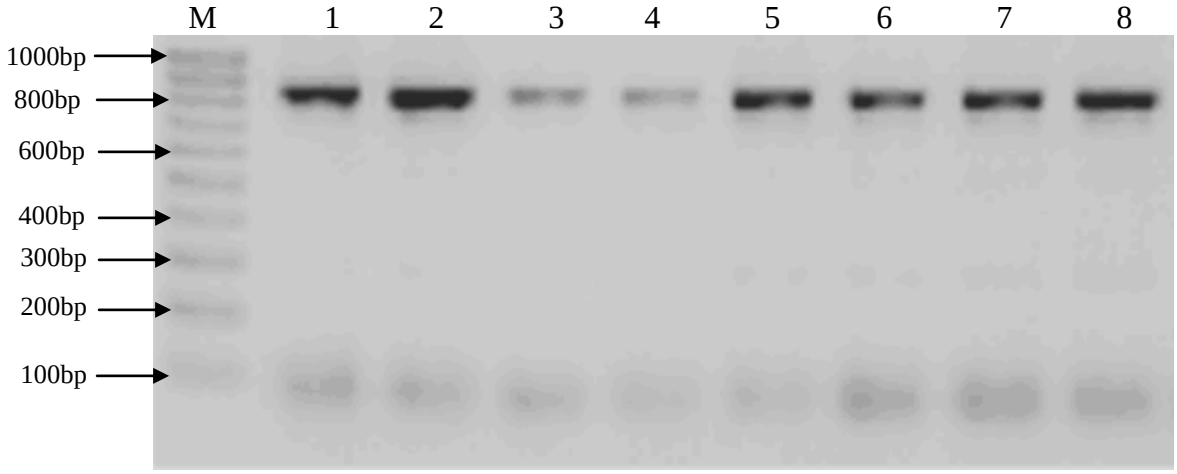

Figure 4: Cytb-tRNAser analysis on agarose gel

4.1.6 Sequence analysis of Cytb-tRNA^{ser} intergenic region

Similarity search for 37 sequences on NCBI (www.ncbi.nlm.nih.gov) nucleotide database for *Apis mellifera* genome using BLAST found a sequence of length 804bp similar to *Apis mellifera ligustica* mitochondrion complete genome which was confirmed from published mtDNA sequences by Crozier and Crozier (1993). Substitution or single point mutations in the form of transitions were observed in the alignment. Only one transversion of the form C-A was observed at the 106th position in the alignment. Two sample sequences from Hewani, Tana River (H69 & H70) and an *Apis mellifera ligustica* sequence had a base A at the position while 35 other sequences

had base C in the same position. A Multiple Sequence Alignment (MSA) of the Cytb-tRNA^{ser} intergenic sequences using ClustalX software is attached in Appendix 2.3.

4.1.7 Phylogenetic relationships of Cytb-tRNA^{ser} sequences

A neighbour-joining bootstrap (1000 iterations) consensus tree drawn from Cytb-tRNA^{ser} sequence data (MEGA6 by Tamura *et al*., 2013) is shown on Figure 5. Phylogenetic analysis revealed three clusters. Cluster 1 had a total of 18 samples representing mid-altitude areas; 13 were from eastern regions of Mumoni, Kathiani, Mituki ya Iveti, Kasanga, Mathiakani, 2 samples were from western (Isiekuti), 2 samples from Coast (Taita, Ronge Mwakalele) and one sample from Tanzania (Tanga, Sengoma). The second cluster had a total of 16 samples mainly representing high altitude areas; 9 samples from western (Makuchi & Isiekuti), 2 samples from Central (Nairobi & Thika), 2 samples from Tanzania (Tanga, Usambara mountains), and 3 samples from Eastern (Mathiakani & Mumoni). The third cluster had 2 samples from the Coast (Hewani, Tana River). This branch, as in BOLD clustering, separated from the others and may represent a different race. *A. m. ligustica* did not cluster with any of the three clusters.

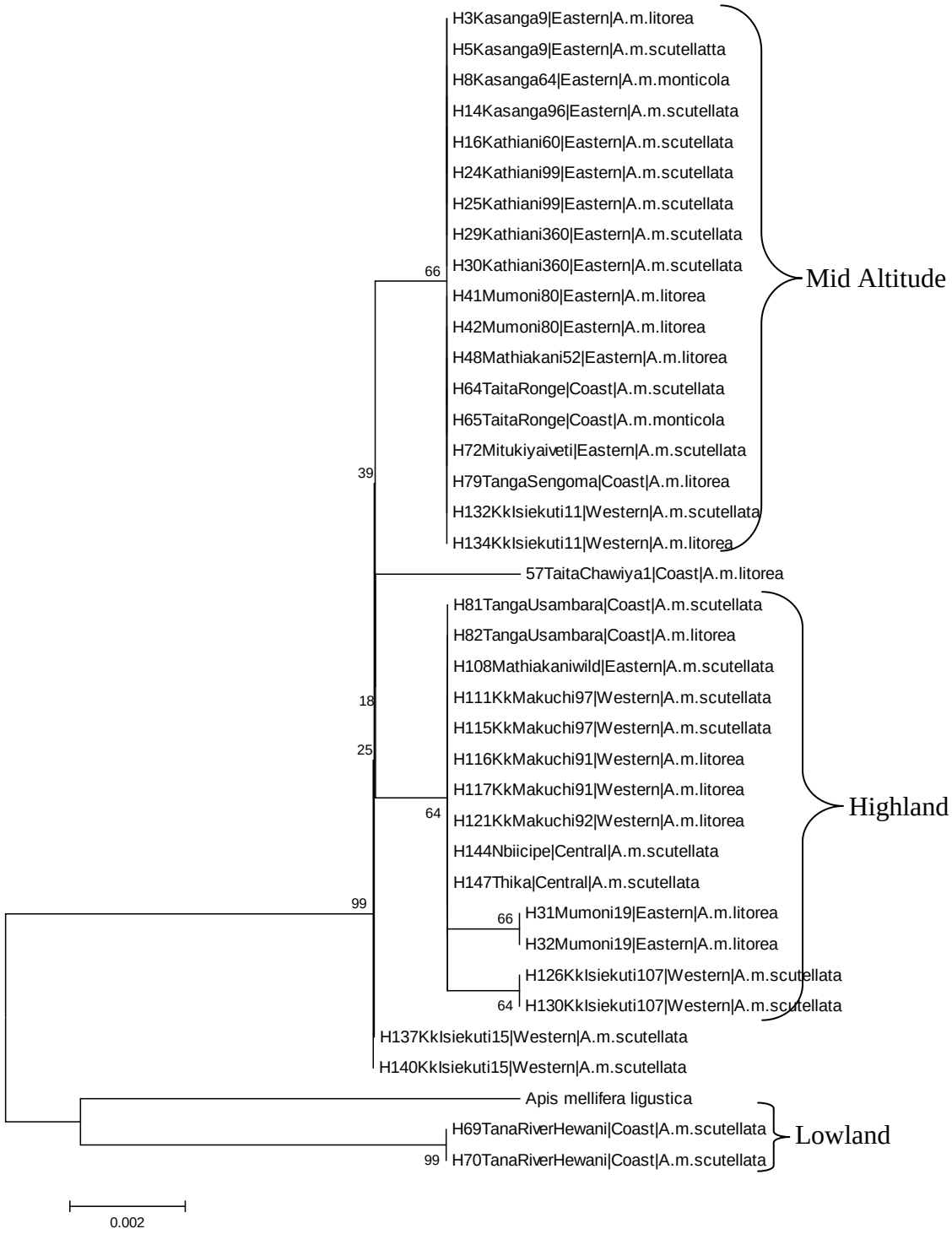

Figure 5: NJ consensus phylogenetic relationship from Cytb-tRNAser sequences

4.1.8 Amplification and sequence analysis of COI-COII intergenic region

Polymerase Chain Reaction amplification using COI-COII primers yielded varied fragment sizes above 1000bp. The variability of the COI-COII intergenic region was used to delineate honey bee subspecies and to discriminate among the *A. mellifera* subspecies. For the purpose of comparison, an 1124bp COI-COII region for *Apis mellifera ligustica* mitochondrion complete genome was retrieved from the database through BLAST search for high similar sequence on the NCBI nucleotide databases (www.ncbi.nlm.nih.gov). The sequence was used in the subsequent comparative analysis. All the 16 COI-COII intergenic sequences are presented in Appendix 2.3.

4.1.9 Multiple sequence alignment of COI-COII intergenic region

Substitution or single point mutations in the form of transitions were mainly observed (see Appendix 2.3). There was variation in the region between COI-COII genes arising from the non-coding sequence, the length of which varied between 200bp and 860bp besides the consistent gene sequence for $tRNA^{leu}$. For all the honey bees there exists a P sequence and a repeatable Q sequence that brings forth the assignment of lineages into mitotypes. A part of P sequence (approximately 79bp) is usually repeated in the Q sequence. The African honey bee sequences have Po and Q forms of an insertion/deletion sequence (Po for the African honey bees is approximately 15bp longer than P). This sequence was in agreement with a combination of two components, P and Q studied by Cornuet and Garnery (1991) and Cornuet *et al*. (1991).

4.1.10 COI-COII intergenic region haplotype and lineage determination

The Po and Q segments are shown below. They are similar in approximately 79bp of their sequences as shown in the bold typed bases of both sequences. The 16 sequences were analysed for the haplotypic differences. Only two mitotypes were found, A1 and A4 as shown on Table 3. More samples would need to be sequenced for more haplotypes to be discovered. *A. m. ligustica* was found to have a C1 mitotype sequence.

Po sequence

TTTAAAC**TTTTATTAAAATTAATAAATTAATATAAAATAAAACAAAATATAACAGAATA**
TATTTATTAAAATTTAATTTATTAAAA-86bp

Q sequence

TTCCCACTTAATTCATATTAATTTAAAAATAAATTAATAACAATTTTAATAAAATAAAT
AATTAATTTTATTTTTATATTGAATTTTTAATTCAATCTTAAAGATTTAATCT**TTTTAT**
TAAAATTAATAAATTAATATAAAATAAAACAAAATATAACAGAATATATTTATTAAAAT
TTAATTTATTAAAA-191bp

Table 3: Haplotypes information and lineage determination based on P and Po

Sample names	Region	Size (bp)	Haplotype	Lineage	Mitotype
H5Kasanga9	Eastern	515	PoQQ	A	A4
H8Kasanga64	Eastern	515	PoQQ	A	A4
H9Kasanga64	Eastern	515	PoQQ	A	A4
H14Kasanga96	Eastern	515	PoQQ	A	A4
H15Kasanga96	Eastern	515	PoQQ	A	A4
H16Kathiani60	Eastern	515	PoQQ	A	A4
H20Kathiani60	Eastern	516	PoQQ	A	A4
H24Kathiani99	Eastern	515	PoQQ	A	A4
H29Kathiani360	Eastern	515	PoQQ	A	A4
H30Kathiani360	Eastern	515	PoQQ	A	A4
H31Mumoni19	Eastern	324	PoQ	A	A1
H32Mumoni19	Eastern	324	PoQ	A	A1
H41Mumoni80	Eastern	515	PoQQ	A	A4
H48Mathiakani52	Eastern	515	PoQQ	A	A4
H55Mathiakani58	Eastern	324	PoQ	A	A1
H64TaitaRonge	Coast	324	PoQ	A	A1
A. m. ligustica	Europe	258	PQ	C	C1

4.1.11 COI-COII phylogenetic relationships and cluster analysis

A neighbour-joining bootstrap (1000 iterations) consensus tree derived from COI-COII intergenic sequences was drawn as shown on Figure 6. Phylogenetic analysis was conducted to understand the lineage relationships among the eastern region populations. Selection of Eastern region samples was based on the clustering of some of its samples in other regions of Coast, Western, Central and Tanga in the previous analyses and yielded two clusters. Cluster 1 consists of samples from mid-altitude areas of eastern region. These are 5 Kathiani samples, 5 Kasanga samples, 1 Mathiakani sample and 1 Mumoni sample forms. Cluster 2 consists of samples from high-altitude areas of eastern region. These are 1 Taita Hills (Ronge Mwakalele) population, 2 Mumoni populations, and 1 Mathiakani population. The two clusters are representative of mitotypes A4 and A1 respectively as shown in Table 3 above. The two clusters originate from the same branch and the bootstrap values for the two populations are low (less than 50) showing that the two clusters are closely related. *A. m. ligustica* has a C1 mitotype and thus is a distant relative of the other mitotypes.

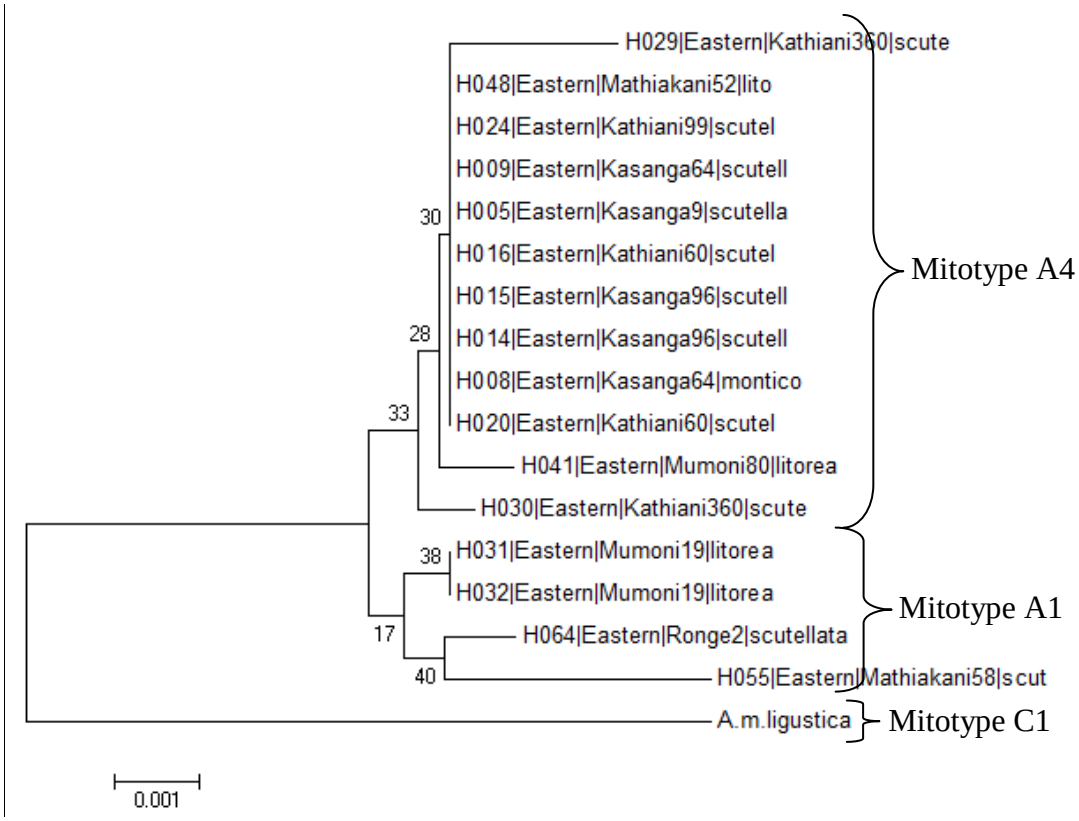

Figure 6: NJ consensus phylogenetic tree from COI-COII intergenic sequences

4.2 Allelic proportions and genetic diversity of honey bee subspecies

4.2.1 Hardy-Weinberg Equilibrium (HWE) using Fisher's exact test

The conformity to Hardy-Weinberg equilibrium for all 8 microsatellite loci and fourteen populations is presented in Table 4 with the exact *p*-values indicating the deviations from Hardy-Weinberg equilibrium. Forty (40) out of 112 (or 35.7 %) possible tests were in not Hardy Weinberg Equilibrium; these are more than what would be expected by chance alone. This means that one or more evolutionary forces of mutation, migration, non random mating, gentic drift and natural selection may have been active in the populations. All populations showed deviations from Hardy-Weinberg Equilibrium in at least one locus (see Table 4 below). The highest deviation at heterozygote deficiency was recorded in Bulindi population at 5 different loci (AJ509384, AJ509635, AJ509656, AJ509721 and AJ509390). The lowest deviation was from Isiolo population which had only one deviation arising from heterozygote deficiency at locus AJ509390. None of the locus deviated in all the populations due to heterozygote deficiency. AJ509390 and AJ509656 loci had the highest deviations in nine and eight populations respectively mainly characterised by a heterozygote deficiency at $p<0.001$. AJ509384 and AJ509362 loci did not have a heterozygote excess deviation in any of the populations. Tanga (2), Kakamega (2), Voi (1) and Madagascar (1) showed significant deviations from HWE with higher than expected heterozygosity (heterozygote excess). No population was observed to have deviated from HWE for all loci and no locus deviated from HWE for all populations.

Table 4: Fishers exact test on HWE for all populations and loci

Locus	Kas	Kat	Mat	Tai	Tan	Mal	Kak	Nai	Lim	Aber	Isi	Voi	Bul	Mdg
AJ509384	NS	NS	*1	*1	NS	NS	NS	NS	NS	NS	NS	NS	***1	**1
AJ509635	NS	NS	NS	*1	NS	NS	*2	NS	NS	NS	NS	NS	***1	NS
AJ509656	***1	**1	NS	NS	***1	*1	NS	NS	***1	NS	NS	*1	***1	*2
AJ509362	NS	NS	NS	NS	NS	*1	NS	NS	NS	*1	NS	NS	NS	NS
AJ509381	NS	NS	*1	NS	NS	NS	*2	NS	NS	NS	NS	*2	NS	*1
AJ509721	*1	**1	NS	NS	NS	NS	***1	NS	**1	NS	NS	NS	**1	***1
AJ509390	NS	NS	**1	NS	**2	NS	***1	***1	***1	***1	**1	*1	*1	NS
AJ509690	NS	*1	NS	NS	*2	NS	NS	*1	**1	NS	NS	NS	NS	NA

Kas, Kasanga; Kat, Kathiani; Mat, Mathiakani; Tai, Taita; Tan, Tanga; Mal, Malindi; Kak, Kakamega; Nai, Nairobi; Lim, Limuru; Aber, Aberdare; Isi, Isiolo; Bul, Bulindi; Mdg, Madagascar

NS, not significant, NA – Not applicable, *P<0.05, **P<0.01, ***P<0.001; 1 = heterozygote deficiency; 2 = heterozygote excess

4.2.2 Loci testing for linkage disequilibrium (LD)

The results of *p*-value for each locus pair across all populations (Fisher's method) of genotypic disequilibrium is shown in Table 5. Linkage disequilibrium is the non-random association of alleles at two or more loci that may or may not be on the same chromosome. Six (21.4%) out of 28 possible locus pair combinations across all populations were highly significant and in linkage disequilibrium. The pairs of loci involved are AJ509635 with AJ509384, AJ509635 with AJ509656, AJ509381 with AJ509656, AJ509390 with AJ509721, AJ509690 with AJ509384 and AJ509690 with AJ509390.

Table 5: Pairwise linkage for each locus across all populations

Locus	AJ509384	A509J635	A509J656	AJ509362	AJ509381	AJ509721	AJ509390	AJ509690
AJ509384	----							
AJ509635	***	----						
AJ509656	NS	***	----					
AJ509362	NS	NS	NS	----				
AJ509381	NS	NS	***	NS	----			
AJ509721	NS	NS	NS	NS	NS	----		
AJ509390	NS	NS	NS	NS	NS	***	----	
AJ509690	***	NS	NS	NS	NS	NS	***	----

(Fisher's method) (significance level=0.001). ***Significant linkage disequilibrium and NS-Not Significant

4.2.3 Allele counts per locus and population

From a total of 288 honey bee samples from 31 locations grouped into 14 populations and genotyped at 8 microsatellite loci, 145 alleles were detected as shown in Table 6. The 8 loci were all polymorphic in terms of allele number and allele size range but differed in their level of polymorphism. The number of alleles ranged from 12 to 32 per locus: AJ509384: 23 alleles (246 to 396); AJ509635: 18 alleles (137 to 175bp); AJ509656: 14 alleles (181 to 211bp); AJ509362: 32 alleles (121 to 181bp); AJ509381: 12 alleles (182 to 202bp); AJ509721: 20 alleles (135 to 179bp); AJ509390: 13 alleles (240 to 262bp) and AJ509690: 13 alleles (139 to 163bp). The most polymorphic marker was AJ509362 with 32 alleles and the least was AJ509381 with 12 alleles. Locus AJ509690 was monomorphic for the Madagascar population. On average, all the loci were highly polymorphic for all the populations. Among the East African populations, Isiolo and Voi populations had the lowest allele counts number at 44. The rest of the populations had alleles numbering more than 50 for all loci.

Table 6: Number of alleles per locus and population

Loci	Size range	Kas	Kat	Mathi	Tai	Tan	Mal	Kak	Nai	Lim	Aber	Isi	Voi	Bul	Mdg	Total
AJ509384	246 to 396	13	11	7	8	9	7	12	8	11	10	5	6	8	6	**23**
AJ509635	137 to 175	8	11	10	11	9	10	12	7	11	11	5	6	10	6	**18**
AJ509656	181 to 211	9	8	6	6	6	5	7	4	8	7	6	5	7	6	**14**
AJ509362	121 to 181	18	20	13	11	15	13	11	9	15	14	8	7	17	6	**32**
AJ509381	182 to 202	6	8	6	6	7	5	6	6	6	5	5	5	6	5	**12**
AJ509721	135 to 179	11	9	11	7	9	8	12	7	10	10	4	6	10	5	**20**
AJ509390	240 to 262	8	11	5	8	7	7	8	5	9	8	3	4	9	4	**13**
AJ509690	139 to 163	9	9	8	7	9	8	9	6	9	9	8	5	10	1	**13**
Total Alleles per population		**82**	**87**	**66**	**64**	**71**	**63**	**77**	**52**	**79**	**74**	**44**	**44**	**77**	**39**	**145**

Kas, Kasanga; Kat, Kathiani; Math, Mathiakani; Tai, Taita; Tan, Tanga; Mal, Malindi; Kak, Kakamega; Nai, Nairobi; Lim, Limuru; Aber, Aberdare; Isi, Isiolo; Bul, Bulindi; Mdg, Madagascar.

4.2.4 Mean Number of Alleles (MNA) and Heterozygosities

The sample size, mean expected heterozygosity (H_E), mean observed heterozygosity (H_O), mean number of alleles (MNA) and their standard deviations for populations are given in Table 7. The Mean Number of Alleles (MNA) ranged from 4.88±1.73 in Madagascar to 10.88±3.91 in Kathiani population for all individuals in the populations. The mean expected heterozygosity ranged from 0.561±0.0855 to 0.855±0.0197 in Madagascar and Kathiani populations respectively. The mean observed heterozygosity, H_O ranged from 0.452±0.0488 to 0.788±0.0457 in Madagascar and Voi populations respectively. The overall average mean number of alleles (MNA), expected heterozygosity (H_E) and observed heterozygosity (H_O) across populations were 8.201±2.5035, 0.781±0.0346 and 0.709±0.0376 respectively. Overall, the mean number of alleles (MNA), expected heterozygosity (H_E) and observed heterozygosity (H_O) across populations were relatively high; an indication of a high genetic diversity base among the honey bee subspecies from East Africa.

Table 7: Sample size, H_E, H_O, MNA and their SDs for each population

Population	Sample size	Loci typed	MNA±SD	H_O± SD	H_E± SD
Kathiani	30	8	10.88±3.91	0.779±0.0268	0.855±0.0197
Kasanga	25	8	10.25±3.77	0.740±0.0310	0.847±0.0219
Limuru	25	8	9.88±2.64	0.679±0.0331	0.833±0.0198
Bulindi	40	8	9.63±3.34	0.725±0.0250	0.792±0.0287
Kakamega	30	8	9.63±2.45	0.725±0.0288	0.829±0.0277
Aberdare	30	8	9.25±2.71	0.717±0.0291	0.780±0.0505
Tanga	20	8	8.88±2.75	0.769±0.0333	0.779±0.0401
Mathiakani	15	8	8.25±2.82	0.650±0.0435	0.782±0.0399
Taita	15	8	8.00±2.00	0.750±0.0395	0.804±0.0287
Malindi	15	8	7.88±2.64	0.733±0.0404	0.792±0.0312
Nairobi	10	8	6.50±1.60	0.675±0.0524	0.739±0.0419
Isiolo	10	8	5.50±1.77	0.738±0.0492	0.758±0.0353
Voi	10	8	5.50±0.93	0.788±0.0457	0.782±0.0129
Madagascar	13	8	4.88±1.73	0.452±0.0488	0.561±0.0855
Mean Total		**8**	**8.201±2.5035**	**0.709±0.0376**	**0.781±0.0346**

4.2.5 Genetic bottleneck

Tests for heterozygosities excess and deficiency were done under the Two Phase Mutation (TPM) model at $p<0.001$, based on 10000 simulation replicates (after Bonferroni corrections) and under the Wilcoxon rank test as shown in Table 8. The results for populations with samples less than 30 should be viewed with caution according to Cornuet & Luikart (1996). Under this model, Kathiani and Voi populations had the highest heterozygote deficit of 0.9961 and 0.9941 respectively. The lowest heterozygote deficit was noted in Madagascar (0.0117) population. Mode-shift graphical representation showed a Normal L-shaped distribution for all population groups as shown in Figure 7. Therefore, there is no evidence for a recent genetic bottleneck based on a mode shift, i.e. a paucity of rare alleles. Under the same model none of the populations examined exhibited a significant genetic bottleneck based on a two-tailed test for heterozygote excess and deficit (Cornuet & Luikart 1996).

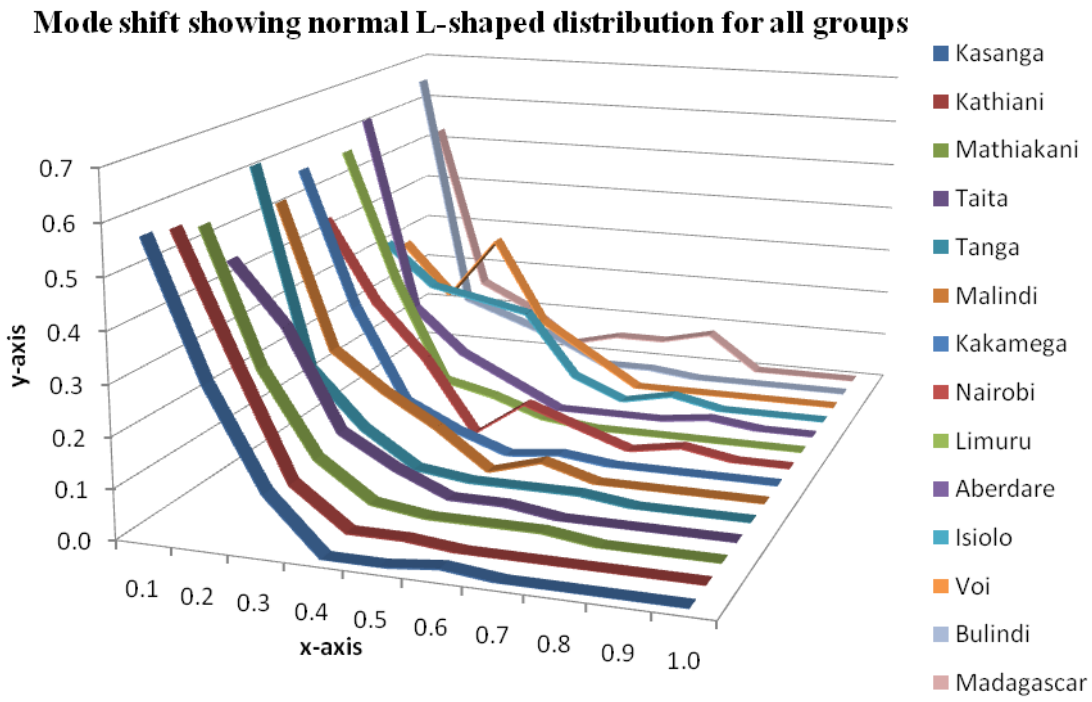

Figure 7: Mode-shift for Normal L-shaped distribution across 8 loci

Table 8: Probability values of genetic bottleneck

Population	Size	One tail H deficiency	One tail H excess	Two tailed
Kasanga	25	0.9023	0.1250	0.2500
Kathiani	30	**0.9961**	0.0059	0.0117
Mathiakani	15	0.2734	0.7695	0.5469
Taita	15	0.7695	0.2734	0.5469
Tanga	20	0.4219	0.6289	0.8438
Malindi	15	0.6797	0.3711	0.7422
Kakamega	30	0.9863	0.0195	0.0391
Nairobi	10	0.1563	0.8750	0.3125
Limuru	25	0.9023	0.1250	0.2500
Aberdare	30	0.9023	0.1250	0.2500
Isiolo	10	0.9863	0.0195	0.0391
Voi	10	**0.9941**	0.0098	0.0195
Bulindi	40	0.4727	0.5781	0.9453
Madagascar	13	0.0117	0.9922	0.0234

4.2.6 Genetic diversity per locus and population

Table 9 shows the genetic diversity in each population over the eight microsatellite loci typed from Fstat program. Genetic diversity among populations occurs if there are differences in allele and genotype frequencies between those populations. Genetic diversity is the basis for both natural evolutionary changes and artificial selection in breeding populations. Gene diversity is the proportion of polymorphic loci across the genome. It can be measured using several different metrics that are all based on allele frequencies in populations. These are F_{ST} and analogues, genetic distance, e.g., Nei's D, sequence divergence and allelic richness which strongly depends on the effective population size, past evolutionary history, sample size and the genetic marker system used. The average genetic diversity among populations ranged from 0.565 to 0.857 in Madagascar and Kathiani populations respectively. Four loci namely; AJ509362 at 0.936, AJ509381 at 0.836, AJ509390 at 0.882, and AJ509690 at 0.872 out 8 loci had the highest genetic diversity among loci contributing to the high genetic diversity observed in Kathiani population. Six loci namely; AJ509384 at 0.596, AJ509362 at 0.715, AJ509381 at 0.519, AJ509721 at 0.647 and AJ509690 at 0.0 out 8 loci had lower genetic diversity among loci compared to the overall mean of 0.783. Among loci the average genetic diversity ranged from 0.670 to 0.877 in AJ509656 and AJ509362 loci respectively. Locus AJ509656 had the lowest genetic diversity among populations with 9 out of 14 populations. Eleven out of 14 populations had the highest genetic diversity among populations and contributed significantly to high genetic diversity observed in locus AJ509362. Madagascar was monomorphic over locus AJ509690 with no value for genetic diversity at this locus. Mean genetic diversity per locus and population was generally high (0.783) in all populations over the 8 loci.

Table 9: Genetic diversity per locus and population

Locus	Kas	Kat	Mat	Tai	Tan	Mal	Kak	Nai	Lim	Aber	Isi	Voi	Buli	Mdg	Mean
AJ509384	0.878	0.874	0.810	0.821	0.866	0.824	0.876	0.806	0.882	0.864	0.778	0.806	0.868	0.596	**0.825**
AJ509635	0.842	0.875	0.848	0.914	0.821	0.876	0.887	0.744	0.848	0.849	0.800	0.772	0.856	0.763	**0.835**
AJ509656	0.737	0.747	0.583	0.636	0.591	0.640	0.656	0.656	0.793	0.454	0.806	0.744	0.627	0.705	**0.670**
AJ509362	0.931	0.936	0.900	0.869	0.900	0.893	0.890	0.894	0.923	0.905	0.856	0.806	0.853	0.715	**0.877**
AJ509381	0.795	0.836	0.688	0.783	0.830	0.755	0.785	0.783	0.798	0.714	0.711	0.722	0.741	0.519	**0.747**
AJ509721	0.886	0.833	0.895	0.812	0.654	0.714	0.868	0.800	0.748	0.822	0.728	0.822	0.758	0.647	**0.785**
AJ509390	0.864	0.882	0.726	0.824	0.714	0.798	0.851	0.733	0.840	0.839	0.567	0.789	0.810	0.577	**0.772**
AJ509690	0.864	0.872	0.845	0.786	0.854	0.852	0.830	0.522	0.856	0.798	0.828	0.789	0.833	0.000	**0.752**
Mean	**0.850**	**0.857**	**0.787**	**0.806**	**0.779**	**0.794**	**0.830**	**0.742**	**0.836**	**0.781**	**0.759**	**0.781**	**0.793**	**0.565**	**0.783**

Kas, Kasanga; Kat, Kathiani; Mat, Mathiakani; Tai, Taita; Tan, Tanga; Mal, Malindi; Kak, Kakamega; Nai, Nairobi; Lim, Limuru; Aber, Aberdare; Isi, Isiolo; Bul, Bulindi; Mdg, Madagascar

4.2.7 Allelic richness for all loci and populations

The allelic richness per locus and population is presented in Table 10. Measures of allelic richness were corrected for variation in sample size by standardization on the basis of smallest number of samples which was 10 using the rarefaction method (Leberg, 2002). The allelic richness ranged from 4.43 to 7.92 in Madagascar and the Kasanga populations respectively. The highest mean allelic richness was 12.08 recorded in the locus AJ509362 and contributed by 10 populations starting with the highest allelic richness at this locus as follows; Kathiani, 11.96; Kasanga, 11.66; Tanga, 10.75; Limuru, 10.68; Malindi, 10.60; Mathiakani, 10.42; Aberdare, 9.66; Bulindi, 9.62; Nairobi, 9.00 and Isiolo, 8.00 as shown in Table 10. The lowest allelic richness was 6.61 in the locus AJ509656 and contributed by 6 populations namely; Kathiani, 6.18; Taita, 4.97; Tanga, 4.89; Malindi, 4.56; Bulindi, 4.28 and Nairobi, 4.00 which had the lowest allelic richness within population as shown in Table 10. The highest allelic richness within populations was 11.96 observed in Kathiani at locus AJ509362 while the lowest was observed in Madagascar population which was monomorphic for the locus AJ509690. Isiolo population had the highest number of the lowest allelic richness per locus contributed by 4 out of 8 loci namely AJ509384, 5.00; AJ509635, 5.00; AJ509721, 4.00 and AJ509390, 4.00. The overall mean for all loci was 8.21 inferring a relatively high allelic richness for all the populations.

Table 10: Allelic richness on minimum sample size of 10 diploid individuals

Locus	Kas	Kat	Mat	Tai	Tan	Mal	Kak	Nai	Lim	Aber	Isi	Voi	Bul	Mdg	Total
AJ509384	9.40	7.90	6.20	6.86	7.63	6.27	8.40	8.00	8.68	7.87	5.00	6.00	7.27	5.08	**8.66**
AJ509635	6.87	8.06	8.42	9.49	7.26	8.52	8.94	7.00	7.55	7.62	5.00	6.00	7.71	5.71	**8.82**
AJ509656	7.06	6.18	5.19	4.97	4.89	4.56	5.81	4.00	6.59	4.51	6.00	5.00	4.28	5.31	**6.61**
AJ509362	11.66	11.96	10.42	8.87	10.75	10.60	8.75	9.00	10.68	9.66	8.00	7.00	9.62	5.49	**12.08**
AJ509381	5.26	6.32	5.23	5.83	6.14	4.79	5.18	6.00	5.79	4.14	5.00	5.00	4.96	4.49	**5.94**
AJ509721	8.35	6.99	9.32	6.29	6.50	6.46	8.94	7.00	6.25	7.38	4.00	6.00	6.82	4.49	**8.51**
AJ509390	7.26	8.28	4.79	7.11	5.25	6.22	6.78	5.00	7.17	6.81	3.00	4.00	6.22	3.91	**7.28**
AJ509690	7.52	7.36	6.96	5.99	7.55	7.35	7.01	6.00	7.60	6.55	8.00	5.00	7.76	1.00	**7.76**
Mean	**7.92**	**7.88**	**7.07**	**6.92**	**7.00**	**6.85**	**7.48**	**6.50**	**7.54**	**6.82**	**5.50**	**5.50**	**6.83**	**4.43**	**8.21**

Kas, Kasanga; Kat, Kathiani; Mat, Mathiakani; Tai, Taita; Tan, Tanga; Mal, Malindi; Kak, Kakamega; Nai, Nairobi; Lim, Limuru; Aber, Aberdare; Isi, Isiolo; Bul, Bulindi; Mdg, Madagascar

4.2.8 Locus by population estimate null alleles frequencies

Table 11 shows the null alleles estimates per locus and population estimated by FSTAT (version 2.9.3; Goudet, 1995). Frequency of null alleles was estimated with the Expectation Maximization (EM) algorithm presented in Dempster *et al*. (1977). Locus AJ509656 had the highest null allele estimates at 0.164. The highest contribution for the null alleles' estimates in locus AJ509656 was Voi population with 0.482 followed by Isiolo population at 0.394. Locus AJ509362 had the lowest null allele estimate at 0.010. Ten out of 14 populations in this locus had no null allele contribution and the rest had very low null allele estimate. Only locus AJ509381 matched this zero contribution in 10 populations but with slightly higher null allele estimates in the remaining four populations. Limuru population had the highest null allele estimate at 0.094. Seven out 8 loci had zero contribution to the null allele estimate for this population. The lowest null allele estimate was in Tanga population with 0.028. Except locus AJ509381 the rest of the loci contributed substantially to the overall null estimated observed in this population. Overall, the average null allele estimate for all the loci and populations was 0.060 contributed by 46 out of 112 loci and populations combinations. Three loci namely AJ509656, AJ509721 and AJ509390 had their null allele estimates above the average value at 0.164, 0.065 and 0.124 respectively. Seven populations namely Mathiakani, Kakamega, Nairobi, Limuru, Isiolo, Voi and Madagascar with their mean estimated null allele frequency values of 0.071, 0.081, 0.066, 0.094, 0.086, 0.079 and 0.075 respectively were above overall mean estimated null allele frequency value of 0.060. High frequency of microsatellite null alleles was expected in honey bees consistent with large effective population sizes found in other insects' orders such as Lepidoptera, (Meglecz *et al*., 2004; Diptera, (Lehmann *et al*., 1997) and Orthoptera, (Chapuis *et al*., 2005).

Table 11: Table of estimated null allele frequencies

Locus	Kas	Kat	Mat	Tai	Tan	Mal	Kak	Nai	Lim	Aber	Isi	Voi	Bul	Mdg	Average
AJ509384	0.000	0.000	0.124	0.159	0.000	0.046	0.000	0.000	0.058	0.000	0.000	0.000	0.094	0.141	**0.044**
AJ509635	0.000	0.000	0.000	0.049	0.000	0.000	0.016	0.000	0.002	0.000	0.000	0.000	0.142	0.003	**0.015**
AJ509656	0.297	0.167	0.079	0.000	0.222	0.065	0.153	0.090	0.266	0.000	0.394	0.482	0.087	0.000	**0.164**
AJ509362	0.000	0.000	0.000	0.000	0.000	0.030	0.000	0.000	0.003	0.075	0.000	0.000	0.000	0.038	**0.010**
AJ509381	0.054	0.029	0.182	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.180	**0.032**
AJ509721	0.060	0.108	0.005	0.000	0.000	0.111	0.185	0.000	0.116	0.017	0.021	0.000	0.054	0.235	**0.065**
AJ509390	0.036	0.000	0.177	0.074	0.000	0.018	0.259	0.341	0.228	0.141	0.269	0.149	0.046	0.000	**0.124**
AJ509690	0.000	0.097	0.000	0.000	0.000	0.000	0.034	0.094	0.081	0.000	0.000	0.000	0.000	0.001	**0.022**
Average	**0.056**	**0.050**	**0.071**	**0.035**	**0.028**	**0.034**	**0.081**	**0.066**	**0.094**	**0.029**	**0.086**	**0.079**	**0.053**	**0.075**	**0.060**

Kas, Kasanga; Kat, Kathiani; Mat, Mathiakani; Tai, Taita; Tan, Tanga; Mal, Malindi; Kak, Kakamega; Nai, Nairobi; Lim, Limuru; Aber, Aberdare; Isi, Isiolo; Bul, Bulindi; Mdg, Madagascar

4.2.9 Private allele frequencies

Private alleles are alleles that are found only in a single population among a broader collection of populations. The mean private allele frequency ranged from 0.130 in Bulindi population to 0.360 in Tanga population as shown in Table 12. Except locus AJ509390 which had zero contribution to the private alleles frequency observed in Tanga population, the other 7 loci contributed significantly to the high number of private allele frequency. Locus AJ509362 had the highest contribution at 1.319 which was the highest overall. Although Bulindi population had the lowest mean private allele frequency the contribution of locus AJ509381 in Voi population was high at 0.984 which raised the mean private allele frequency for this population to 0.190. This uneven high private allele frequency contribution was also observed in Isiolo population at loci AJ509656, AJ509362 and AJ509690 with frequency values of 0.672, 0.786 and 0.412 respectively. A similar trend was also observed in Mathiakani population where locus AJ509721 contibuted a high private allele frequency value of 0.821. The highest mean private allele frequency was observed in locus AJ509362 at 0.543 while the lowest frequency was observed at locus AJ509390 with 0.108. The mean private allele frequency observed in locus AJ509362 was consistently high for all the populations similar to that of the lowest locus AJ509390 which was consistently low for all populations. The overall mean private allele frequency was 0.243. Five loci with their mean private allele frequencies, AJ509635 at 0.204, AJ509656 at 0.170, AJ509381 at 0.220, AJ509390 at 0.108 and AJ509690 at 0.125 had their private alleles below the average value while 3 loci had their average mean private allele frequency well above the value. Five populations namely Kasanga, Kathiani, Tanga, Kakamega and Limuru had their mean private allele frequency values at 0.290, 0.300, 0.360, 0.340 and 0.310 respectively much higher than the overall average value of 0.243.

Table 12: Table of private allele frequencies

Locus	Kas	Kat	Mat	Tai	Tan	Mal	Kak	Nai	Lim	Aber	Isi	Voi	Bul	Mdg	Average
AJ509384	1.143	0.059	0.000	0.133	0.257	0.409	0.570	0.313	0.799	0.200	0.044	0.000	0.000	0.459	**0.313**
AJ509635	0.083	0.232	0.347	0.309	0.333	0.419	0.672	0.001	0.096	0.251	0.004	0.001	0.098	0.015	**0.204**
AJ509656	0.302	0.040	0.018	0.000	0.209	0.002	0.001	0.498	0.001	0.125	0.672	0.001	0.126	0.388	**0.170**
AJ509362	0.406	1.170	0.255	0.337	1.319	0.286	0.664	0.545	0.109	0.368	0.786	0.076	0.413	0.868	**0.543**
AJ509381	0.115	0.509	0.000	0.719	0.251	0.013	0.025	0.005	0.389	0.000	0.000	0.984	0.000	0.077	**0.220**
AJ509721	0.122	0.009	0.821	0.043	0.445	0.565	0.334	0.014	0.767	0.149	0.000	0.339	0.093	0.031	**0.267**
AJ509390	0.121	0.361	0.001	0.005	0.000	0.052	0.236	0.000	0.203	0.162	0.000	0.000	0.258	0.120	**0.108**
AJ509690	0.031	0.034	0.031	0.390	0.094	0.009	0.202	0.231	0.126	0.005	0.412	0.132	0.052	0.000	**0.125**
Average	**0.290**	**0.300**	**0.180**	**0.240**	**0.360**	**0.220**	**0.340**	**0.200**	**0.310**	**0.160**	**0.240**	**0.190**	**0.130**	**0.240**	**0.243**

Kas, Kasanga; Kat, Kathiani; Mat, Mathiakani; Tai, Taita; Tan, Tanga; Mal, Malindi; Kak, Kakamega; Nai, Nairobi; Lim, Limuru; Aber, Aberdare; Isi, Isiolo; Bul, Bulindi; Mdg, Madagascar

4.3 Population differentiation, admixture and gene flow

4.3.1 F-statistics

Table 13 shows the average Weir & Cockerham (1984) estimates of F-statistics namely, F_{IT}, F_{IS}, F_{ST}, G_{ST} (Nei's coefficient of gene differentiation) and Nei's estimation of heterozygosity after Jackknifing over all loci. The overall estimates for F_{ST} and the equivalent G_{ST} were 0.056 and 0.069 respectively. F_{ST} and the equivalent G_{ST} values were moderate for the eight loci analyzed though G_{ST} showed slightly higher values. All loci contributed significantly to the F_{ST} to this variation. The overall within population inbreeding estimate F_{IS} value was 0.101 but ranged from -0.004 (locus AJ509381) to 0.242 (locus AJ509390) among loci. This was consistent with Nei's overall estimate of G_{IS} at 0.096 but ranged from -0.004 to 0.279 for AJ509381 and AJ509390 loci respectively. The moderate low values are indicative of high heterozygosity hence genetic diversity within honey bee populations is high for all loci. The overall fixation index, F_{IT} value for all populations was 0.151 but ranged from 0.054 (locus AJ509381) to 0.284 (locus AJ509390). This indicates that a low rate of alleles fixation among populations. The average estimates of the observed (H_O), expected (H_S) and total (H_T) heterozygosities were 0.708, 0.784 and 0.841, respectively. Locus AJ509656 contributed the least to the mean observed heterozygosity (H_O) at 0.512 while AJ509362 contributed the most at 0.863. This is reflected in the other related parameters, H_S, H_T and D_{ST} with locus AJ509656 having least values of 0.669, 0.710 and 0.041 respectively. H'_T, D'_{ST}, and G'_{ST} are independent estimators of H_T, D_{ST} and G_{ST} respectively and their values differ only slightly.

Table 13: Estimation of F_{IT}, F_{ST} and F_{IS} and heterozygosity

Locus	F_{ST}	F_{IT}	F_{IS}	G_{IS}	H_O	H_S	H_T	H'_T	D_{ST}	D'_{ST}	G_{ST}	G'_{ST}
AJ509384	0.043*	0.131**	0.091**	0.079	0.761	0.826	0.877	0.881	0.051	0.055	0.058	0.062
AJ509635	0.037*	0.055*	0.019*	-0.001	0.837	0.836	0.877	0.881	0.041	0.044	0.047	0.050
AJ509656	0.052**	0.281**	0.242**	0.235	0.512	0.669	0.710	0.713	0.041	0.044	0.058	0.062
AJ509362	0.050**	0.082*	0.033*	0.016	0.863	0.878	0.935	0.939	0.057	0.062	0.061	0.066
AJ509381	0.057**	0.054*	-0.004$^{n.s}$	-0.004	0.751	0.748	0.808	0.813	0.060	0.065	0.075	0.080
AJ509721	0.075**	0.235**	0.172**	0.163	0.657	0.785	0.860	0.866	0.075	0.080	0.087	0.093
AJ509390	0.055**	0.284**	0.242**	0.279	0.556	0.772	0.843	0.848	0.071	0.076	0.084	0.090
AJ509690	0.056**	0.101**	0.048**	0.031	0.730	0.754	0.819	0.824	0.065	0.070	0.080	0.085
Overall	**0.056****	**0.151****	**0.101***	**0.096**	**0.708**	**0.784**	**0.841**	**0.846**	**0.058**	**0.062**	**0.069**	**0.073**

Statistical significance: *p<0.05, **p<0.01, ***p<0.001, $^{n.s.}$not significant after 10000 randomisations (after Bonferroni corrections)

4.3.2 Within-population inbreeding estimates, F_{IS}

Table 14 shows Weir and Cockerham within-population inbreeding estimates (F_{IS}) for each locus in 14 populations and their significance. The overall mean within-population inbreeding estimate was 0.096. Above this estimate were loci AJ509390, AJ509656 and AJ509721 with mean values of 0.285, 0.228, and 0.168 respectively. Five out fourteen populations namely; Kasanga (0.129), Mathiakani (0.174), Kakamega (0.127), Limuru (0.188) and Madagascar (0.201) F_{IS} estimate for all loci were higher than the overall 0.096. Locus AJ509390 had the highest F_{IS} value of 0.285 while AJ509635 was the lowest with F_{IS} value of -0.003. Of these, Madagascar population had the highest inbreeding index with a F_{IS} value of 0.201. Voi was the lowest inbred population with F_{IS} value of -0.008. Among the East African populations Limuru had the highest inbreeding index at 0.188 followed by Kasanga and Kakamega populations with 0.129 and 0.127 values respectively. The highest F_{IS} value was in Nairobi at 0.864 in the locus AJ509390 followed by Isiolo at 0.824 in the locus AJ509390. The lowest inbreeding index was in Voi population (-0.385) at locus AJ509381.

Table 14: Within population inbreeding estimates (F_{IS})

Locus	Kas	Kat	Mat	Tai	Tan	Mal	Kak	Nai	Lim	Aber	Isi	Voi	Bul	Mdg	Total
AJ509384	0.044	0.009	0.341	0.432	0.018	0.110	0.049	-0.117	0.183	-0.041	-0.286	-0.241	0.194	0.484	**0.084**
AJ509635	0.050	-0.105	-0.023	0.125	-0.035	-0.141	-0.127	-0.209	0.056	-0.021	0.000	0.094	0.299	-0.008	**-0.003**
AJ509656	0.620	0.241	-0.029	0.161	0.577	0.167	0.137	0.390	0.445	0.119	0.131	0.463	0.082	-0.309	**0.228**
AJ509362	0.055	0.003	0.111	-0.074	0.111	0.104	-0.012	-0.118	0.004	0.190	-0.169	-0.241	0.004	0.247	**0.015**
AJ509381	0.145	0.083	0.516	-0.021	0.037	-0.060	-0.146	-0.149	-0.003	-0.120	-0.266	-0.385	-0.080	0.556	**0.008**
AJ509721	0.142	0.280	0.032	-0.067	0.006	0.347	0.424	0.000	0.198	0.108	0.176	0.027	0.043	0.644	**0.168**
AJ509390	0.121	0.018	0.449	0.191	-0.330	0.081	0.569	0.864	0.476	0.325	0.824	0.366	0.105	-0.067	**0.285**
AJ509690	-0.065	0.235	0.054	-0.188	-0.171	0.062	0.117	0.234	0.173	0.081	0.034	-0.141	0.009	NA	**0.033**
Overall	**0.129**	**0.091**	**0.174**	**0.069**	**0.013**	**0.077**	**0.127**	**0.091**	**0.188**	**0.082**	**0.028**	**-0.008**	**0.086**	**0.201**	**0.096**

Statistics: significant at $p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, ns, not significant. NA means not applicable for monomorphic marker.

Kas, Kasanga; Kat, Kathiani; Mat, Mathiakani; Tai, Taita; Tan, Tanga; Mal, Malindi; Kak, Kakamega; Nai, Nairobi; Lim, Limuru; Aber, Aberdare; Isi, Isiolo; Bul, Bulindi; Mdg, Madagascar.

4.3.3 Pairwise F_{ST} differentiation

Table 15 shows Weir & Cockerham, (1984) pairwise F_{ST} values for population differentiation between 14 populations and their levels of significance. An F_{ST} value range between 0 and 0.1 show low differentiation, 0.1-0.25 shows moderate differentiation wheres the range above 0.25 show high differentiation. Most F_{ST} values were between 0 and 0.1 displaying low differentiation levels between populations. The highest F_{ST} value (0.2765) was between Isiolo and Madagascar populations. High pairwise F_{ST} values were from Madagascar and ranged from 0.1628 (against Kakamega) and 0.2765 (against Isiolo). Among the East African populations, Isiolo population was the most divergent with F_{ST} values ranging from 0.0585 (against Limuru population) to 0.1252 (against Nairobi population). Although most F_{ST} values were low they were relatively significant to allow for pairwise population differentiation between them. The lowest pairwise differentiation was between Kasanga and Kathiani populations having the F_{ST} value of 0.0012. Figure 8 show the Neighbour-joining (NJ) bootstrap (1000 iterations) phylogenetic tree representing the F_{ST} comparisons of allele frequencies for population differentiation graphically. An R^2 value for F_{ST} dendogram was 0.976. The East African populations separated from Madagascar outgroup population to form a major cluster with three subclusters based on altitude levels suggesting that the populations are closely related. Kathiani, Kasanga, Taita, Isiolo, Voi and Limuru forms subcluster 1 which is mid altitude subcluster. Mathiakani, Tanga, Malindi and Bulindi populations form subcluster 2 which is mainly a lowland coastal subcluster. Nairobi, Aberdare and Kakamega populations are highland populations in subcluster 3. Among the East African populations, the most differentiated are Isiolo, Voi and Limuru in that order as confirmed by the Neighbour-joining tree.

Table 15: Pairwise F_{ST} matrix for population differentiation between populations

	Kas	**Kat**	**Mat**	**Tai**	**Tan**	**Mal**	**Kak**	**Nai**	**Lim**	**Aber**	**Isi**	**Voi**	**Bul**	**Mdg**
Kas	-----	***	***	***	***	***	***	***	***	***	***	***	***	***
Kat	**0.0012**	-----	***	***	***	***	***	***	***	***	***	***	***	***
Mat	0.0211	0.0348	-----	***	***	***	***	***	***	***	***	***	***	***
Tai	0.0145	0.0293	0.0697	-----	***	***	***	***	***	***	***	***	***	***
Tan	0.0318	0.0376	0.0482	0.0609	-----	***	***	***	***	***	***	***	***	***
Mal	0.0351	0.0401	0.0625	0.0582	0.0689	-----	***	***	***	***	***	***	***	***
Kak	0.0169	0.0188	0.0485	0.0257	0.0289	0.0449	-----	***	***	***	***	***	***	***
Nai	0.0417	0.0525	0.0763	0.0646	0.0596	0.0768	0.0435	-----	***	***	***	***	***	***
Lim	0.0246	0.0262	0.0571	0.0281	0.044	0.0605	0.0251	0.0463	-----	***	***	***	***	***
Aber	0.0270	0.0286	0.0507	0.043	0.0385	0.0415	0.0084	0.0345	0.0439	-----	***	***	***	***
Isi	0.0647	0.0633	0.0900	0.0882	0.1022	0.1003	0.0801	0.1252	0.0585	0.1113	-----	***	***	***
Voi	0.0573	0.0611	0.0634	0.0803	0.0761	0.1032	0.0481	0.1051	0.0338	0.0771	0.0916	-----	***	***
Bul	0.0208	0.0354	0.0477	0.0389	0.0437	0.0381	0.0268	0.0534	0.0452	0.0238	0.0864	0.0823	-----	***
Mdg	0.1725	0.1642	0.2065	0.1788	0.2211	0.1919	0.1628	0.1742	0.1745	0.1654	**0.2765**	0.2276	0.1785	-----

F_{ST} values below the diagonal and their tests of significance above the diagonal. *** means the pairwise differentiation is significant at $p<0.001$.

Kas, Kasanga; Kat, Kathiani; Mat, Mathiakani; Tai, Taita; Tan, Tanga; Mal, Malindi; Kak, Kakamega; Nai, Nairobi; Lim, Limuru; Aber, Aberdare; Isi, Isiolo; Bul, Bulindi; Mdg, Madagascar

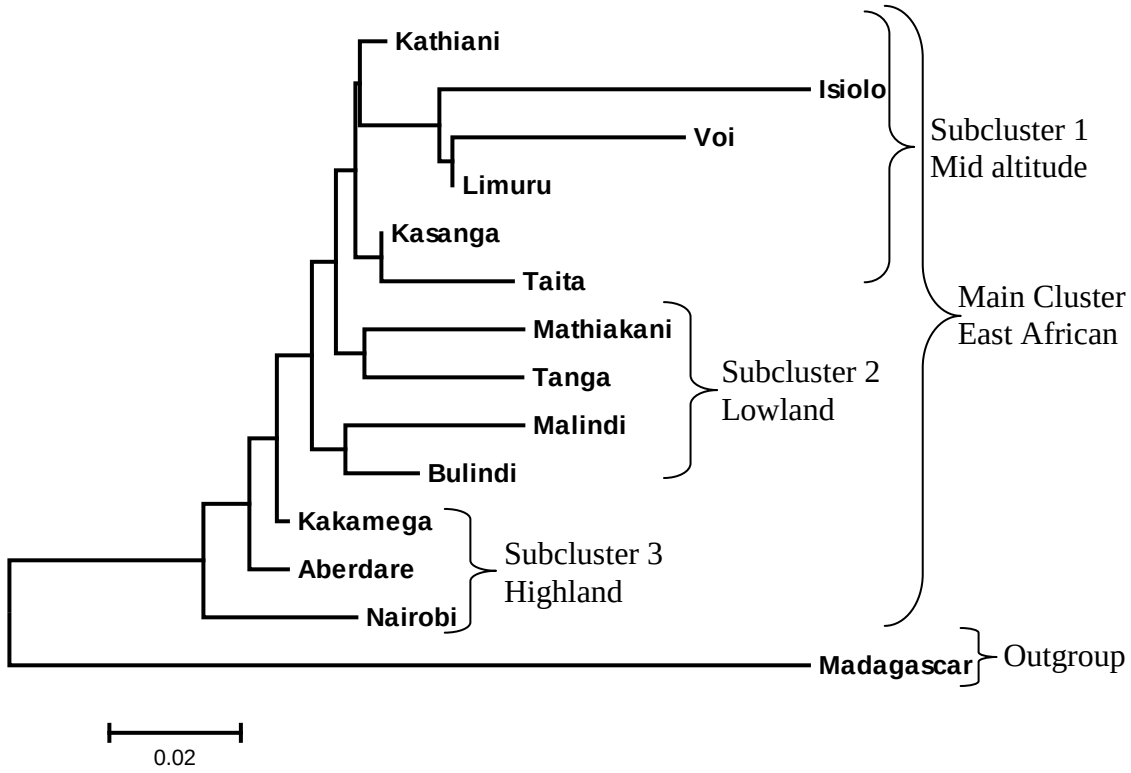

Figure 8: Fst Neighbour-joining phylogenetic tree between populations

4.3.4 Nei's standard genetic distances

Table 16 shows pairwise genetic distances (D_S) according to Nei (1978) for the 14 populations studied. The highest genetic distance is between Isiolo and Madagascar populations at 1.283 while the smallest pairwise distance is between Kathiani and Kasanga populations at 0.018. Among the East African populations, the highest pairwise genetic distance is between Isiolo and Nairobi at 0.572. The highest and lowest pairwise genetic distances are consistent with the previous pairwise F_{ST} genetic population differentiation results. The pairwise genetic distances found in Isiolo and Voi populations against the other populations are higher suggesting they are relatively genetically distinct. Overall the pairwise genetic distances were low but the small differences between populations allows for their separation significantly. The genetic distance NJ bootstrap (10,000 iterations) tree in Figure 9 shows the tree topology for the pairwise genetic distances, D_S. The proportion of variation, R^2, explained by the NJ phylogenetic trees for D_S is height at 0.926. In congruent with the F_{ST} tree topology the 14 populations separated out into subclusters based on altitude levels with a few inconsistencies from some populations. Kasanga, Kathiani, Mathiakani, and Tanga formed subcluster 1 mainly consisting of mid altitude bees. Taita, Limuru, Isiolo and Voi formed subcluster 2 which is both a Highland and a lowland subcluster suggesting some unknown relationship. Malindi, Bulindi, Kakamega, Aberdare and Nairobi populations form subcluster 3 which is mainly a highland subcluster. Nairobi, Aberdare and Kakamega populations separated out as a highland group in subcluster 3 consistent with F_{ST} tree topology. Isiolo, Voi and Limuru remained the most differentiated in that order.

Table 16: Matrix of Nei's standard genetic distances (Ds)

Population	Kas	Kat	Mat	Tai	Tan	Mal	Kak	Nai	Lim	Aber	Isio	Voi	Bul	Mdg
Kas	0.000													
Kat	**0.018**	0.000												
Mat	0.108	0.181	0.000											
Tai	0.082	0.165	0.362	0.000										
Tan	0.149	0.181	0.210	0.284	0.000									
Mal	0.190	0.222	0.306	0.293	0.321	0.000								
Kak	0.105	0.115	0.256	0.139	0.133	0.240	0.000							
Nai	0.185	0.243	0.334	0.277	0.230	0.333	0.192	0.000						
Lim	0.161	0.172	0.320	0.160	0.218	0.352	0.152	0.215	0.000					
Aber	0.121	0.127	0.224	0.193	0.157	0.181	0.035	0.132	0.214	0.000				
Isio	0.347	0.340	0.433	0.437	0.481	0.502	0.435	0.572	0.303	0.560	0.000			
Voi	0.330	0.363	0.301	0.419	0.346	0.568	0.254	0.474	0.182	0.364	0.413	0.000		
Bul	0.096	0.178	0.218	0.182	0.189	0.173	0.127	0.219	0.233	0.099	0.416	0.419	0.000	
Mdg	0.668	0.625	0.756	0.590	0.876	0.659	0.583	0.471	0.666	0.530	**1.283**	0.844	0.648	0.000

Kas, Kasanga; Kat, Kathiani; Mat, Mathiakani; Tai, Taita; Tan, Tanga; Mal, Malindi; Kak, Kakamega; Nai, Nairobi; Lim, Limuru; Aber, Aberdare; Isi, Isiolo; Bul, Bulindi; Mdg, Madagascar

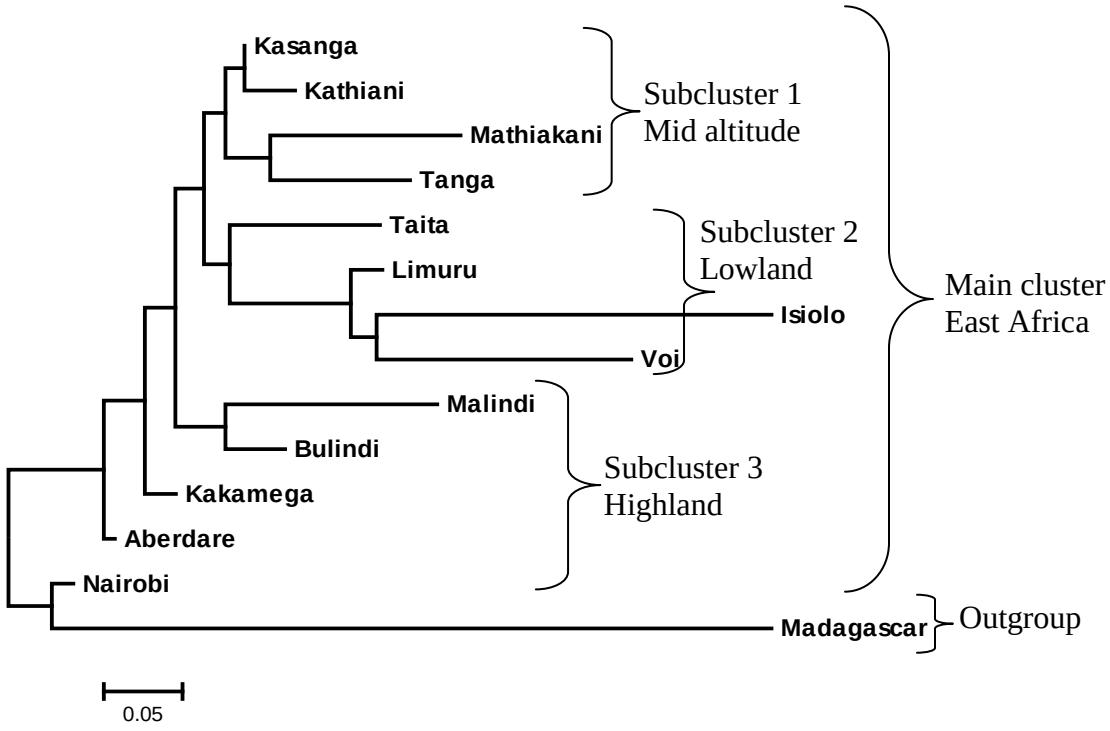

Figure 9: Ds Neighbour-joining phylogenetic tree between populations

4.3.5 Nei's chord distances

Table 17 shows D_A (Nei, 1987) pairwise relationships between 14 populations. The largest distance is between Madagascar and Isiolo populations in D_A pairwise distance relationships at 0.639 consistent with both D_S and F_{ST} population differentiations. The smallest distance is between Kathiani and Kasanga D_A pairwise distance relationships at 0.106. Among the east African populations, the pairwise genetic distances between Voi, Isiolo and other populations are higher compared to the rest of the populations suggesting they are genetically distant. Additionally, the pairwise genetic distance between Voi and Isiolo populations was the highest among east African populations at 0.433. Despite the low values of pairwise genetic distances the small differences between them allowed them to be separated from each other. The low values indicate that these subspecies are not differentiated enough and they may have had a common history of breeding practices. The genetic distance relationships were represented by a Neighbour-joining (NJ) (10,000 bootstrap) tree for the pairwise genetic distances D_A. The proportion of variation, R^2, explained by the NJ phylogenetic tree, D_A is 0.955. The neighbourjoining tree topology (Figure 10) showed three subclusters based on altitude levels and in consistent with pairwise F_{ST} and D_S trees. Limuru, Isiolo, Voi and Mathiakani populations formed subluster 1 consisting of lowland bees. Tanga, Nairobi, Kasanga and Kathiani populations formed subcluster 2 mainly consisting of mid altitude bees. Bulindi, Kakamega, Aberdare, Malindi and Taita populations formed a third subcluster 3 consisting of highland bees. Based on the tree topologies, most populations are closely related and share alleles which means they share a recent common ancestor. Madagascar outgroup population separated on its own and only shares a few alleles.

Table 17: Pairwise population matrix of Nei genetic distance (D_A)

Population	Kas	Kat	Mat	Tai	Tan	Mal	Kak	Nai	Lim	Aber	Isi	Voi	Bul	Mdg
Kas	0.000													
Kat	**0.106**	0.000												
Mat	0.204	0.210	0.000											
Tai	0.198	0.220	0.305	0.000										
Tan	0.185	0.209	0.246	0.283	0.000									
Mal	0.237	0.226	0.272	0.295	0.295	0.000								
Kak	0.169	0.186	0.257	0.211	0.221	0.277	0.000							
Nai	0.249	0.266	0.310	0.292	0.227	0.334	0.280	0.000						
Lim	0.190	0.187	0.245	0.208	0.233	0.273	0.209	0.257	0.000					
Aber	0.174	0.170	0.201	0.225	0.200	0.209	0.149	0.217	0.184	0.000				
Isi	0.316	0.314	0.330	0.352	0.333	0.418	0.369	0.376	0.285	0.371	0.000			
Voi	0.320	0.333	0.312	0.346	0.337	0.423	0.298	0.391	0.256	0.325	**0.433**	0.000		
Bul	0.132	0.171	0.206	0.198	0.198	0.214	0.152	0.237	0.199	0.128	0.340	0.310	0.000	
Mdg	0.438	0.423	0.463	0.410	0.524	0.457	0.431	0.442	0.380	0.408	**0.639**	0.522	0.435	0.000

Kas, Kasanga; Kat, Kathiani; Mat, Mathiakani; Tai, Taita; Tan, Tanga; Mal, Malindi; Kak, Kakamega; Nai, Nairobi; Lim, Limuru; Aber, Aberdare; Isi, Isiolo; Bul, Bulindi; Mdg, Madagascar

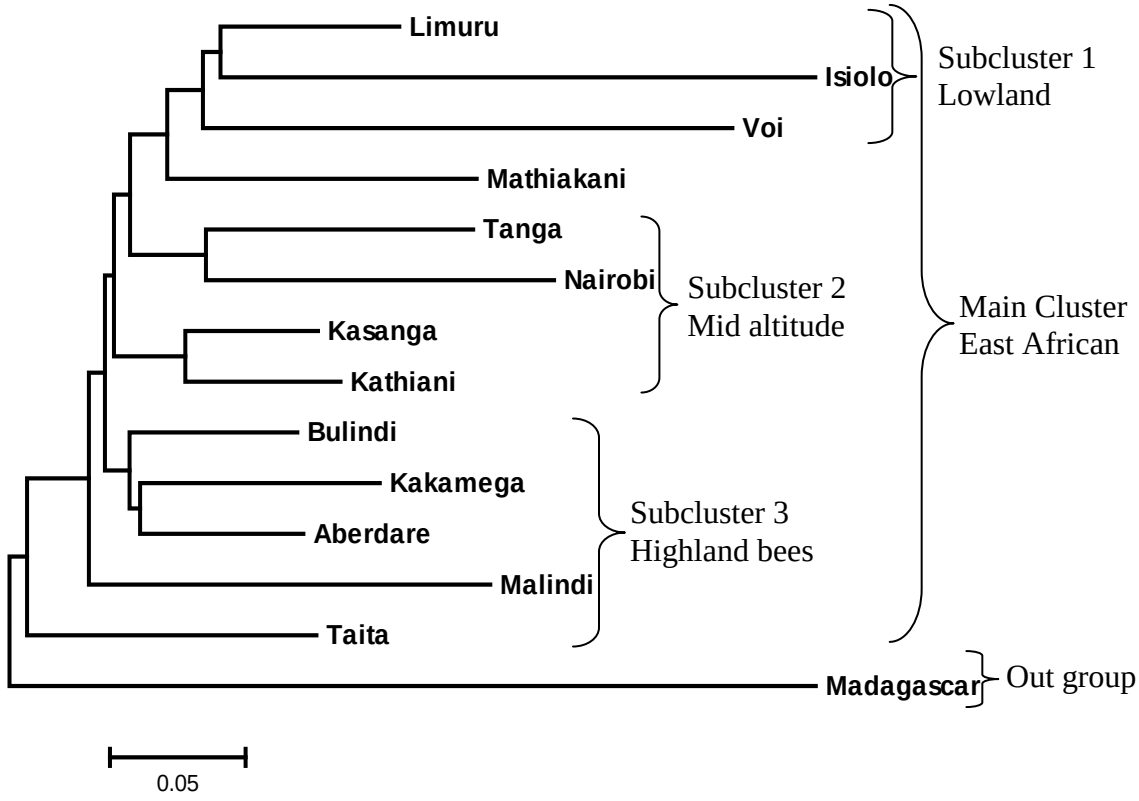

Figure 10: D_A Neighbour-joining 10,000 bootstrap phylogenetic tree

4.3.6 Population differentiation by AMOVA

Table 18 represents the genetic diversity attributed to variance between and within three different population groupings by Analysis of MOlecular VAriance (AMOVA). Within populations, a high percentage of variance ranging from 89.84% (between western and north eastern regions) to 97.65% (between western and central regions) was found among regions. A high percentage variance within populations was observed when populations were grouped according to altitude in the range of 95.12% (between lowland and highland) to 96.56% (between mid altitude and highland) and subspecies in the range of 84.27% (between monticolor and unicolor) to 98.65% (between litorea and monticola). A small percentage of variance lies among populations within groups ranging from 1.03% (between yementica vs monticola) in subspecies grouping to 7.94% (between coast vs north eastern) in regional grouping. The lowest percentage variance was found among groups and ranged from -3.85% (between Coast and Western) in regional grouping to 14.2% (between monticola and unicolor) in subspecies grouping. Among groups the highest variance was between Western versus North eastern region (0.22198) which accounted for approximately 7.13% of total variance and a *p*-value (0.33436±0.00446). The higher the *p*-value the lower the significance of differentiation among groups. This variance is not significant and shows there is small difference to allow for differentiation between the two populations. All the *p*-values for variance among groups are higher than a *p*-value of 0.05 suggesting the variance among groups is not significant. However, from variance values among groups, closely related groupd can be determined. When grouped according to the landscape, variance was lowest between lowland and mid-altitude at –0.00352 and a *p*-value of 0.40782±0.00504. Variance between highland and lowland areas was highest at 0.02353 with a *p*-value of 0.03317±0.00170. The *p*-value is significant suggesting that the altitude levels between highland and lowland is important in distinguishing between honey bees found in these areas. At the subspecies level, the highest variance among subspecies was between scutellata and unicolor (0.52940) and the lowest variance was between litorea and monticola (-0.00632). Hence *A. m. litorea* and *A. m. monticola* appear to be the most

related subspecies in terms of allele and genotype sharing. Among east Africa subspecies the highest variance was between *A. m. monticola* and *A. m. yementica* at 0.22721. *A. m. yementica* occupies north eastern Kenya especially areas that receive occasional drought and limited resources. The variance between *A. m. scutellata* and *A. m. litorea* was 0.00048 and between *A. m. scutellata* and *A. m. monticola* was 0.00244 indicating a difficulty in differentiating these subspecies by variance means. The variance between *A. m. unicolor* and the other subspecies was high accounting for 13.92% variance with *A. m. scutellata* and 14.2% with *A. m. monticola*. Hence the choice of *A. m. unicolor* subspecies as an outgroup species was applicable.

Table 18: Analysis of Molecular Variance by Arlequin

Groups		Among groups			Among populations within groups			Within populations		
		Va	**%age**	***P***	**Vb**	**%age**	***P***	**Vc**	**%age**	***P***
Regions	Eastern vs Coast	0.0019	0.06	0.19455±0.00380	0.13999	4.08	<0.00001	3.28597	95.86	<0.00001
	Eastern vs Western	0.02914	0.85	0.49515±0.00543	0.06268	1.83	0.00040±0.00019	3.33808	97.32	<0.00001
	Eastern vs Central	0.02545	0.75	0.10010±0.00287	0.10445	3.08	<0.00001	3.25912	96.17	<0.00001
	Coast vs Western	-0.13012	-3.85	1.00000±0.00000	0.27245	8.07	<0.00001	3.23578	95.79	<0.00001
	Coast vs Central	-0.02559	-0.77	0.69406±0.00450	0.20059	6.00	<0.00001	3.1677	94.76	<0.00001
	Western vs Central	-0.07326	-2.23	1.00000±0.00000	0.15065	4.58	<0.00001	3.21116	97.65	<0.00001
	Eastern vs Neastern	0.19028	5.34	0.24752±0.00423	0.06332	1.78	0.00109±0.00031	3.30959	92.88	<0.00001
	Western vs Neastern	0.22198	7.13	0.33436±0.00446	0.09404	3.02	0.01069±0.00102	2.79545	89.84	<0.00001
	Central vs Neastern	0.18196	5.23	0.25079±0.00437	0.15228	4.38	<0.00001	3.14596	90.40	<0.00001
	Coast vs Neastern	0.05118	1.47	0.24950±0.00420	0.27583	7.94	<0.00001	3.14688	90.59	<0.00001
Altitude	Lowland vs mid altitude	-0.00352	-0.10	0.40782±0.00504	0.14794	4.34	<0.00001	3.26524	95.76	<0.00001
	Lowland vs Highland	0.02353	0.70	0.03317±0.00170	0.13984	4.17	<0.00001	3.18769	95.12	<0.00001
	Midaltitude vs Highland	0.00762	0.23	0.23158±0.00468	0.10895	3.22	<0.00001	3.26864	96.56	<0.00001
Subspecies	scutellata vs yementica	0.20905	5.90	0.19653±0.00434	0.06813	1.92	<0.00001	3.26447	92.17	<0.00001
	scutellata vs unicolor	0.52940	13.92	0.20129±0.00376	0.06885	1.81	<0.00001	3.205	84.27	<0.00001
	scutellata vs litorea	0.00048	0.01	0.50911±0.00522	0.06610	1.97	<0.00001	3.28899	98.02	<0.00001
	scutellata vs monticola	0.00244	0.07	0.46277±0.00463	0.06388	1.90	<0.00001	3.29238	98.03	<0.00001
	yementica vs litorea	0.18922	5.36	0.24960±0.00393	0.06091	1.73	0.00119±0.00035	3.28014	92.91	<0.00001
	yementica vs monticola	0.22721	6.39	0.33574±0.00524	0.03655	1.03	0.19406±0.00414	3.29053	92.58	<0.00001
	litorea vs monticola	-0.00632	-0.19	0.60149±0.00437	0.05208	1.54	0.00208±0.00045	3.33454	98.65	<0.001
	monticola vs unicolor	0.51540	14.20	0.33158±0.00471	0.04270	1.18	0.19881±0.00388	3.07129	84.62	<0.00001

4.3.7 PCA analysis among populations

Figure 11 represents the principal coordinates analysis of the 14 honey bee populations from Nei's distance data standardization. Table 19 shows the associated eigenvalues by axis and sample eigenvectors. Coordinate 1 accounts for a low variation of 38.95% within the populations. The highest difference using coordinate 1 is between Madagascar and Isiolo populations with an eigen vector of 0.582 while the lowest is between Kakamega and Taita at 0.009. Kasanga and Kathiani are very close with a difference of 0.010. Using coordinate 2 accounts for 19.45% of the total variation. The highest difference is between Voi and Malindi populations at 0.165 while the lowest difference is between Kakamega and Taita at 0.000. Coodinate 3 explains 14.10% of the total variation which gives a cummulative variation by three coordinates of 73.39%. Approximately 26.61% of the total variation is explained by other coordinates. There are three clusters from PCA; Limuru, Isiolo, Voi and Kakamega consists of cluster 1 mainly lowland areas, Kasanga, Kathiani, Mathiakani and Tanga forms cluster 2 consisting of mid altitude areas while Taita, Malindi, Nairobi, Aberdares and Bulindi forms cluster 3 consisting of highland bees. Madagascar appears on its own coordinate. Overall the honey bee populations are closely related considering the amount of variation explained by 3 coordinates.

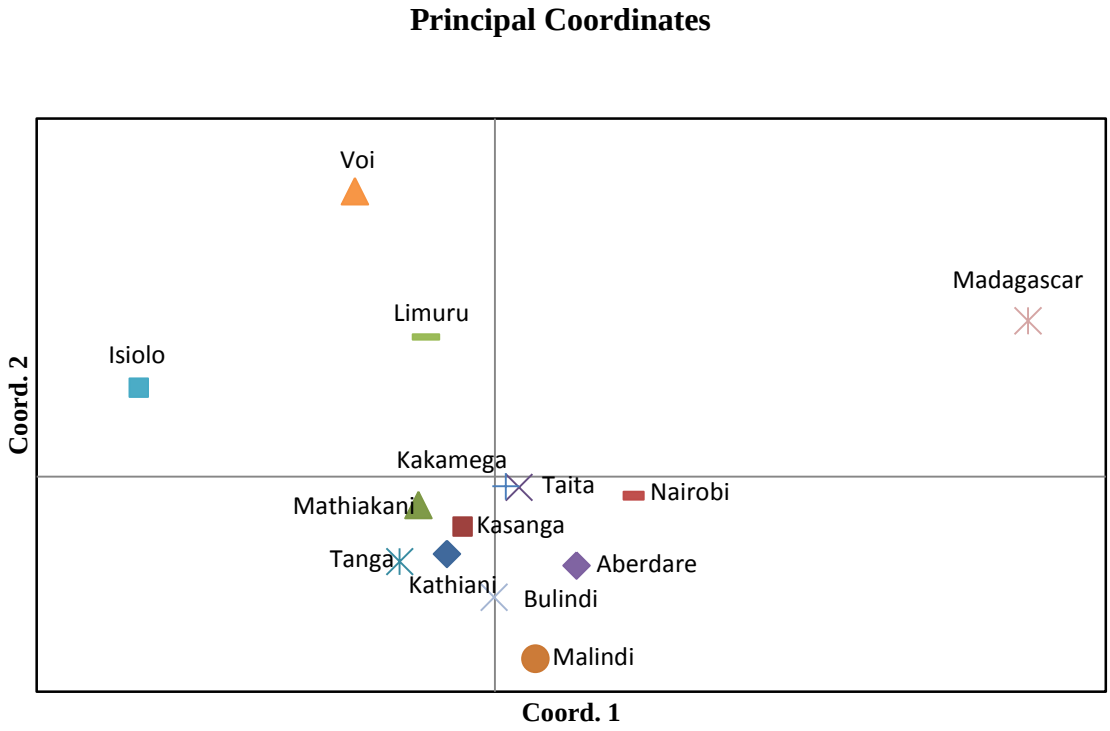

Figure 11: PCA via covariance matrix for sampling areas

Table 19: Eigenvalues by axis and sample eigenvectors

Axis No.	**1**	**2**	**3**	**4**	**5**	**6**
% variation	**39.85**	**19.45**	**14.10**			
EigenValue	**0.205**	**0.100**	**0.073**	**0.059**	**0.040**	**0.038**
Kasanga	-0.031	-0.054	-0.008	0.007	-0.043	0.071
Kathiani	-0.021	-0.035	-0.025	0.012	-0.038	0.068
Mathiakani	-0.050	-0.020	0.072	0.154	-0.002	0.067
Taita	0.016	-0.007	-0.076	-0.095	-0.098	0.036
Tanga	-0.062	-0.059	0.124	-0.038	0.027	0.010
Malindi	0.026	-0.127	-0.077	0.072	0.002	-0.108
Kakamega	0.007	-0.007	0.044	-0.056	-0.039	-0.046
Nairobi	0.084	-0.013	0.029	-0.066	0.139	0.039
Limuru	-0.045	0.098	-0.015	-0.078	0.011	-0.007
Aberdare	0.053	-0.062	0.060	-0.026	-0.002	-0.045
Isiolo	-0.233	0.062	-0.149	0.023	0.067	0.002
Voi	-0.092	0.199	0.088	0.039	-0.035	-0.060
Bulindi	-0.001	-0.084	-0.011	0.001	0.003	-0.039
Madagascar	0.349	0.109	-0.056	0.052	0.007	0.012

4.3.8 PCA analysis between altitude levels

Honey bees from Kenya were grouped on the basis of altitudes determined from GPS taken for the sampling sites. Figure 12 represents the principal coordinates analysis of the 3 levels of elevations from which honey bees were collected; highland (1200m and above), mid altitude (700-1200m) and lowland (50-400m above sea level) according to Nei's distance data standardization. Table 20 shows the associated eigenvalues by axis and sample eigenvectors. Coordinate 1 accounts for 58.58% variation among the elevations. The highest difference using coordinate 1 is between Highland and Lowland areas with an eigen vector of 0.098 while the lowest is between Highland and Mid altitude (0.022). Using coordinate 1 the difference between Mid altitude and Lowland areas had an eigen vector of 0.076. Coordinate 2 accounts for 41.42% of the total variation. The highest difference is between Highland and Mid altitude areas (0.085) while the lowest difference is between Highland and Lowland areas (0.026). Overall two coordinates explains absolute (100%) variation among the landscape areas.

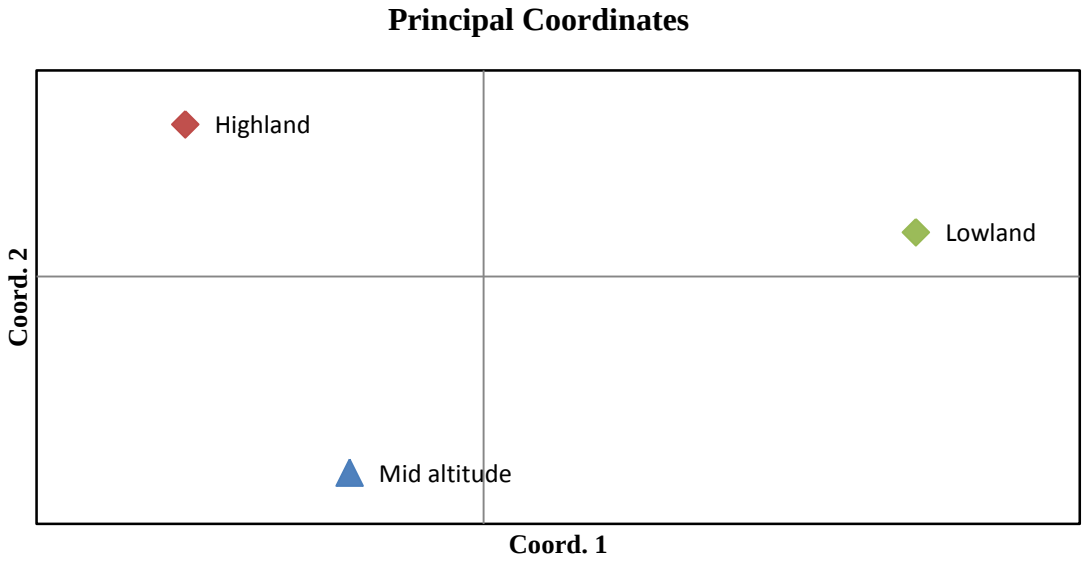

Figure 12: PCA via covariance matrix for elevation

Table 20: Eigenvalues by axis and elevation eigenvectors

Axis No.	1	2
% Variation	58.58	41.42
Cumulative %	58.58	100.00
EigenValue	0.005	0.004
Mid altitude	-0.018	-0.048
Highland	-0.040	0.037
Lowland	0.058	0.011

4.3.9 PCA analysis between honey bee subspecies

Figure 13 represents the principal coordinates analysis of the honey bee subspecies (scutellata, monticola, litorea, yementica and unicolor) using Nei's distance data standardization. Table 21 shows the associated eigenvalues by axis and sample eigenvectors. Coordinate 1 accounts for over 90% of variation within the honey bee subspecies. *A. m. litorea, A. m. scutellata* and *A. m. monticola* are very close while *A. m. yementica* and *A. m. unicolor* are distantly related. The highest difference using coordinate 1 is between *A. m. yementica* and *A. m. unicolor* (0.689) while the lowest is between *A. m. scutellata* and *A. m. monticola* (0.010). *A. m. scutellata* and *A. m. litorea* have a difference of 0.030. Using coordinate 2 the highest difference is between *A. m. yementica* and *A. m. litorea* (0.143) while the lowest difference is between *A. m. scutellata* and *A. m. monticola* (0.006) and *A. m. monticola* and *A. m. litorea* (0.006). The percentage variation accounted for by coordinate 2 is approximately 9.48%. Coodinate 3 does not explain much variation (0.30) and much variation is explained by the first two coordinates.

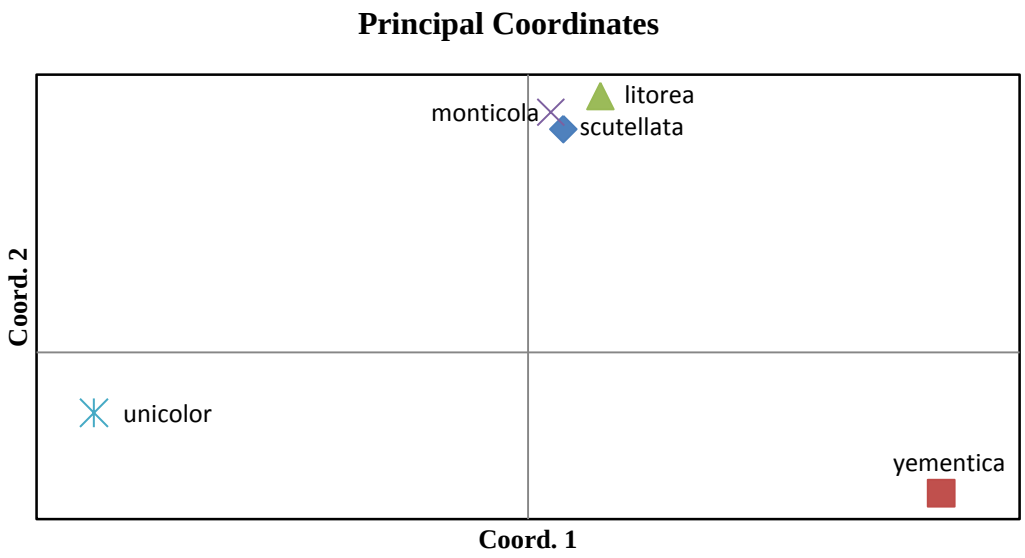

Figure 13: PCA via covariance matrix for *Apis mellifera* subspecies

Table 21: Eigenvalues by axis and subspecies eigenvectors

Axis No.	**1**	**2**	**3**
%age variation	90.22	9.48	0.30
EigenValue	0.242	0.025	0.001
scutellata	0.029	0.080	-0.020
yementica	0.336	-0.051	0.000
litorea	0.059	0.092	-0.001
monticola	0.019	0.086	0.020
unicolor	-0.353	-0.022	0.000

4.3.10 Population differentiation by admixture analysis

4.3.10.1 K Clusters determination

A plot of the mean and standard deviation of the posterior probabilities, lnPr(*X*|K), (Figure 14) among runs for each value of K, from 1–10 reached an asymptote at K=4, indicating that the true K value is 4. The rate of change of the likelihood [L(K)] distribution against K (Figure 15) showed the highest value of K at K=4 indicating four clusters. The absolute value of the 2nd order rate of change of the likelihood distribution (|L"(K)|) plot against K values (Figure 16) showed the highest peak at K=4 showing that the samples belongs to 4 clusters. The modal value for the distribution of ΔK against K (Figure 17) was at K=4 as well supporting four clusters in the honey bee populations studied.

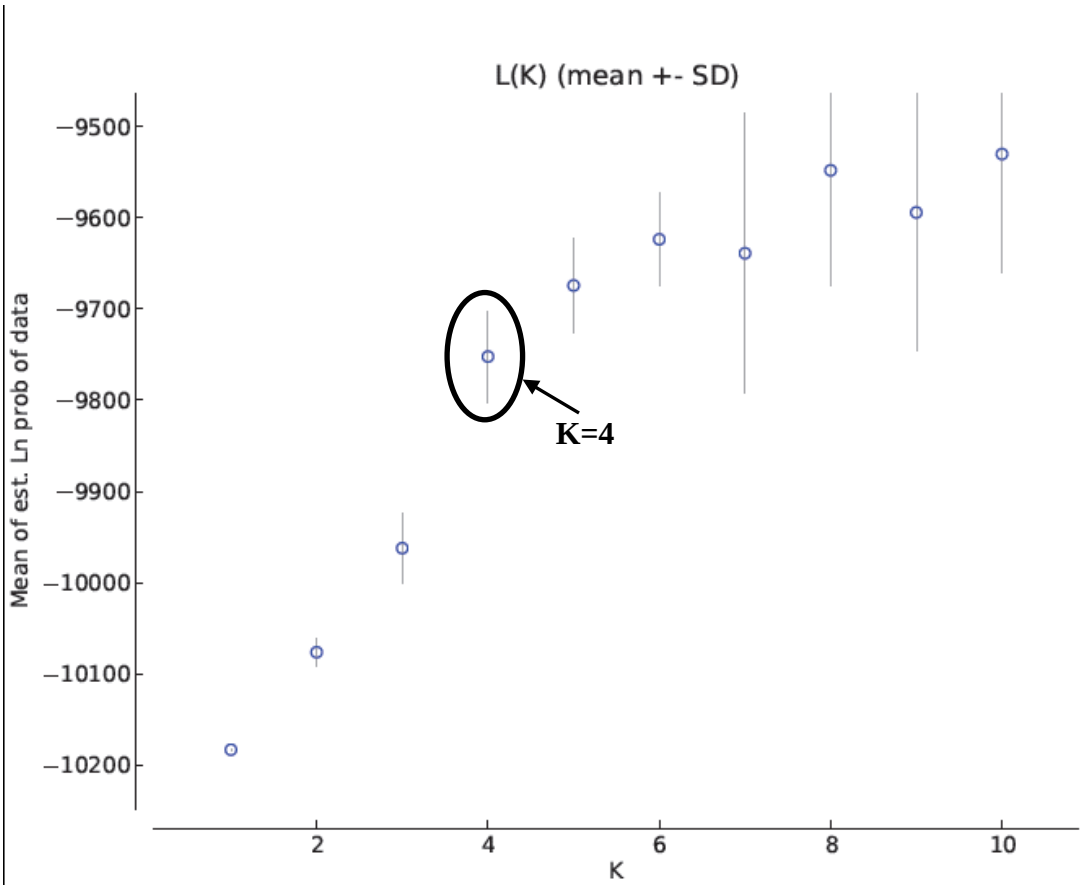

Figure 14: Mean of estimates of Ln probability of data against each value of K

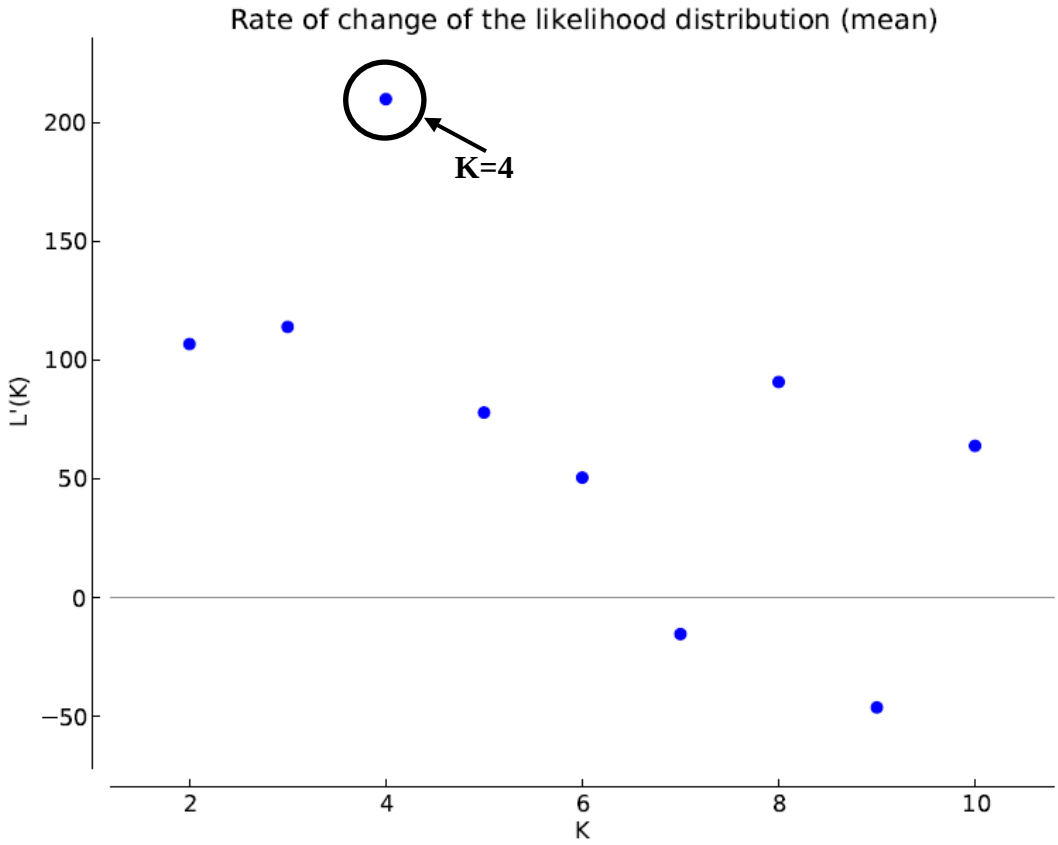

Figure 15: Rate of change of the likelihood distribution against K

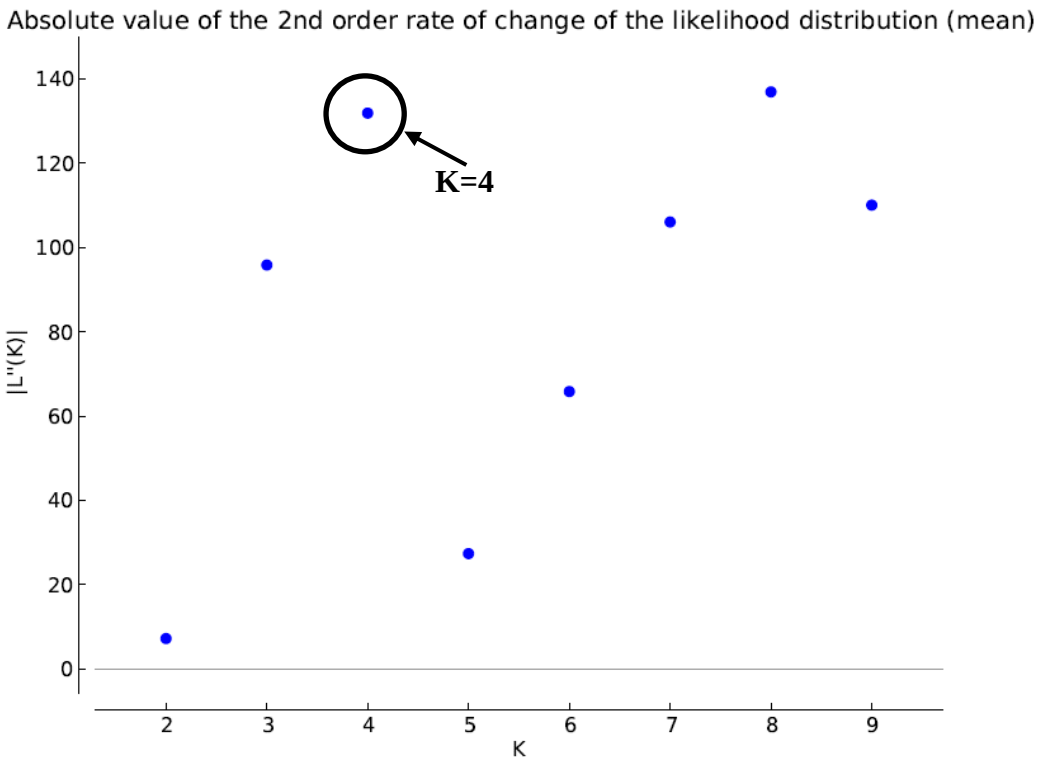

Figure 16: Plot of |L''(K)| distribution against K

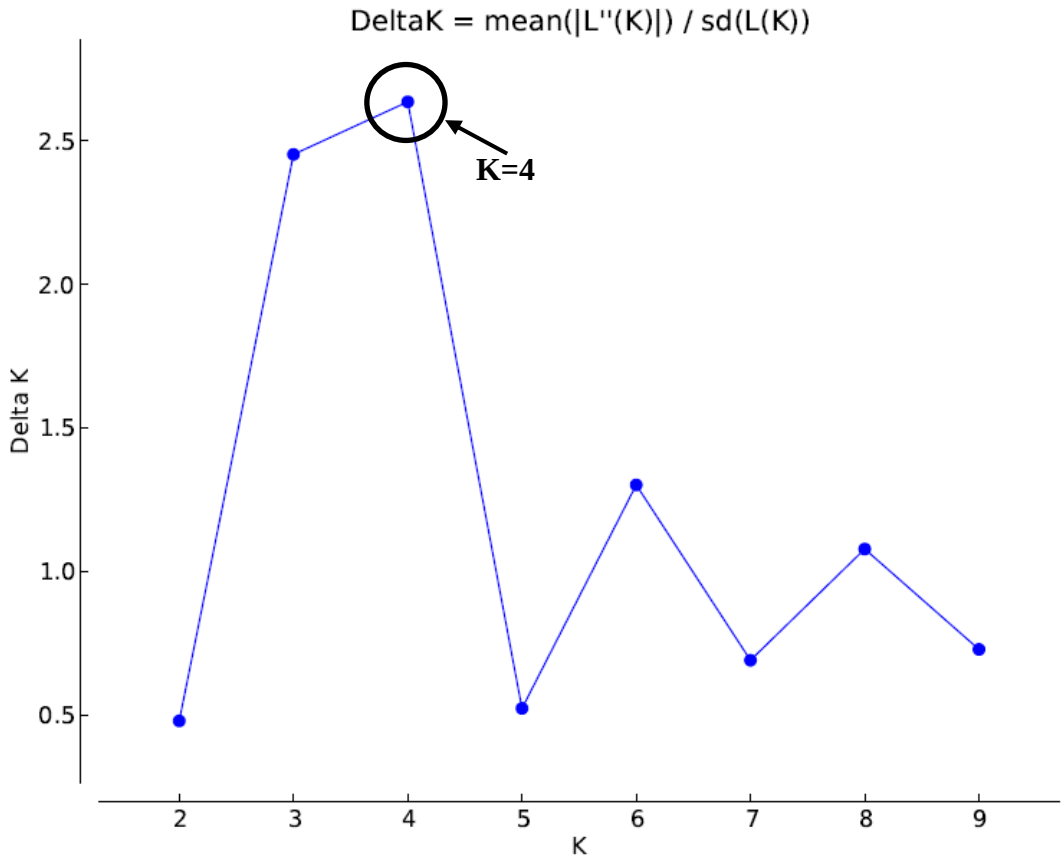

Figure 17: Plot of Delta K against K values

4.3.10.2 Cluster analysis from structure results

All estimates in the 4 clusters assumed admixture in the sampled genotypes from 14 populations and the highest contributions are in bold in Table 22. Cluster analysis placed all populations into four clusters with Tanga, Malindi, Kathiani, Kasanga and Kakamega populations forming cluster 1 (mid-altitude areas). Cluster 2 contains Aberdare, Mathiakani, Bulindi and Nairobi (highland areas) populations while a third cluster had only Madagascar outgroup population with 81.83% of its genotype represented. The fourth cluster was represented by Isiolo, Limuru, Voi and Taita (Lowland areas) populations consistent with results of Neighbour-joining phylogenetic trees drawn from Nei's D_S and D_A genetic distances. In cluster 1 Tanga population has the highest amount of its genotype at 55.84%, though it shares a significant amount of its genotype with cluster 2 and cluster 4 at 18.4% and 24.92%

respectively. Malindi, Kathiani, Kasanga and Kakamega populations have 49.33%, 46.77%, 46.61% and 39.51% of their genotypes in cluster 1 and 29.06%, 20.27%, 31% and 35.63% shared in cluster 2 and 30.23%, 21.02% and 22.29% shared in cluster 4 respectively. In cluster 2; Aberdare, Mathiakani, Bulindi and Nairobi populations their genotypes grouped together at 56.87%, 47.15%, 46.80% and 46.36% respectively but this cluster shares 25.34%, 18.55%, 35.39% and 35.04% with cluster 1 and 13.1%, 32.65%, 16.44%, 16.99% and 7.88% genotype with cluster 4 respectively. In cluster 4; Isiolo, Limuru, Voi and Taita populations' genotypes were 89.32%, 68.42%, 58.25% and 49.74% respectively. Taita and Voi populations had 32.89% and 35.75% genotypes in clusters 1 and 2 respectively. There appears to be a lot of genetic admixture between populations as illustrated in Figure 18 and Clusters 1, 2 and 4 can be considered to contain Mid altitude, Highland and Lowland bees respectively. However, the results of admixture analysis did not give a clear dissection of honey bee clusters according to regions or elevations. Clusters 1, 2 and 4 were further analysed to detect the number of subgroups in each cluster.

Table 22: Proportion of population membership in each of the four clusters

Populations	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Tanga	**0.5584**	0.1840	0.0083	0.2492
Malindi	**0.4933**	0.2906	0.0380	0.1781
Kathiani	**0.4677**	0.2027	0.0274	0.3023
Kasanga	**0.4661**	0.3100	0.0136	0.2102
Kakamega	**0.3951**	0.3563	0.0257	0.2229
Aberdare	0.2534	**0.5687**	0.0470	0.1310
Mathiakani	0.1855	**0.4715**	0.0165	0.3265
Bulindi	0.3539	**0.4680**	0.0137	0.1644
Nairobi	0.3504	**0.4636**	0.0161	0.1699
Madagascar	0.0368	0.0661	**0.8183**	0.0788
Isiolo	0.0685	0.0319	0.0064	**0.8932**
Limuru	0.1612	0.1019	0.0526	**0.6842**
Voi	0.0471	0.3575	0.0129	**0.5825**
Taita	0.3289	0.1327	0.0409	**0.4974**

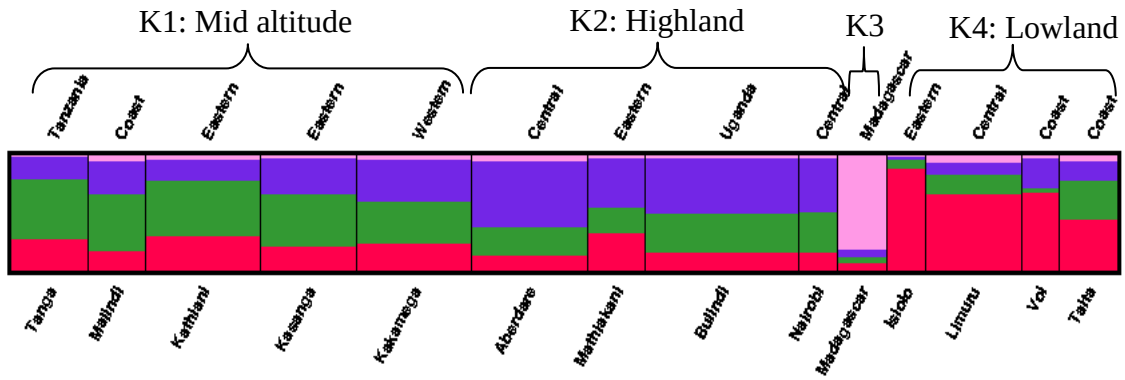

Figure 18: Graphical results from Structure at K=4

4.3.10.3 Subcluster analysis

Genetic structure within the first cluster detected a modal value for the distribution of ΔK at K=2 as in Table 23. The two subclusters correspond to Kasanga (57.2%), Kathiani (59.36%), Malindi (75%) on one hand and Tanga (55.24%), Kakamega (70.43%) populations in the other. However, the two clusters significantly share their genotypes in the range of 25% (Malindi) to 44.76% (Tanga). Kathiani and Kasanga population are grouped in one cluster and therefore appear to be closely related corroborating the results of F_{ST}, D_S and D_A genetic distances. In the second cluster shown in Table 24, a modal value for the distribution of ΔK separated this group into three subclusters (K=3). Mathiakani (54.74%) population separated to form subcluster 1, Nairobi (55.3%) and Aberdare (39.51%) populations formed subcluster 2 and Bulindi (40.61%) (Uganda) formed a third subcluster. Nairobi and Aberdare populations may probably be sharing genotypes due to gene flow arising from their close geographical proximity. In the fourth cluster above, Voi and Isiolo separated into their own populations while Taita and Limuru formed three subclusters. Taita and Limuru populations previously clustered in the phylogenetic tree from Nei's, D_S genetic distances. The modal value for the distribution of ΔK was found at K=5 as shown in Table 25. Notably, there is considerable genotypes sharing within clusters and it is difficult in some cases to define boundaries in the subclusters. No further substructuring was done.

Table 23: Cluster 1 subclustering, K=2

Population	Cluster 1	Cluster 2
Kasanga	0.4280	**0.5720**
Kathiani	0.4064	**0.5936**
Malindi	0.2500	**0.7500**
Tanga	**0.5524**	0.4476
Kakamega	**0.7043**	0.2957

Table 24: Cluster 2 subclustering, K=3

Population	Cluster 1	Cluster 2	Cluster 3
Mathiakani	**0.5474**	0.2093	0.2433
Nairobi	0.2575	**0.5530**	0.1895
Aberdare	0.3044	**0.3951**	0.3004
Bulindi	0.3353	0.2587	**0.4061**

Table 25: Cluster 3 subclustering, K=5

Population	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Taita	0.0170	0.2560	0.0624	0.2863	**0.3783**
Limuru	0.1014	0.2135	0.0696	**0.3527**	0.2627
Isiolo	0.0142	0.0376	**0.8264**	0.0933	0.0284
Voi	**0.4823**	0.3261	0.0392	0.0533	0.0991

4.3.11 Genetic assignment of individual to populations

The results of population self assignment tests obtained by Geneclass 2.0 (Piry *et al*., 2004) software using Bayesian statistics for the 14 populations are shown in Table 26. The diagonal values of the assignment matrix indicate the average probability with which individuals are assigned to their corresponding reference population. By converting the probability values to percentage, the self-assignment probability values were low and ranged from 19.9% to 45.6% for Mathiakani and Madagascar respectively. Three populations had a self assignment values less than that assigned to other populations; Mathiakani (19.9% self assignment), Nairobi (31.9%) and Bulindi (39.7%). Given that the program computes the probability that the multilocus genotype of each individual can be encountered in a given population, the probability of encountering Madagascan genotypes in other populations was low (last row) with all values less than 10%. The probability of encountering East African genotypes in Madagascar population is very low (last column) with values less than 0.04%. Voi and Isiolo populations had the lowest assignments from other populations ranging from 0.0% to 2.2%. However, 28.1% of Voi genotype was assigned to Limuru population and 17.5% of Isiolo population was assigned to Kasanga population. The highest assignment from other populations was found in Kasanga population ranging from 6.7% (Madagascar) to 40.7% (Bulindi). Kathiani population had a fair amount of genotype assigned to it ranging from 5.1% (Madagascar) to 30.8% (Nairobi). However, Kasanga shares 26.8% of its genotype significantly with Kathiani population and less than 10% genotype with other populations. The two populations seem to be the source populations as they share a considerable amount of their genotype with other populations.

Table 26: The genetic assignment tests using Bayesian statistics

Assigned	Kas	Kat	Mat	Tai	Tan	Mal	Kak	Nai	Lim	Aber	Isi	Voi	Bul	Mdg
Kas	**0.343**	0.268	0.084	0.063	0.057	0.041	0.077	0.037	0.063	0.056	0.003	0.004	0.056	0.000
Kat	0.262	**0.338**	0.035	0.049	0.033	0.030	0.070	0.027	0.106	0.071	0.001	0.022	0.034	0.001
Mat	0.219	0.151	**0.199**	0.039	0.045	0.036	0.077	0.044	0.095	0.100	0.008	0.008	0.055	0.001
Tai	0.184	0.187	0.012	**0.333**	0.017	0.027	0.163	0.038	0.193	0.044	0.003	0.004	0.049	0.002
Tan	0.231	0.192	0.036	0.057	**0.350**	0.020	0.103	0.063	0.074	0.091	0.007	0.012	0.084	0.001
Mal	0.172	0.193	0.050	0.034	0.025	**0.274**	0.043	0.037	0.086	0.054	0.001	0.001	0.048	0.002
Kak	0.190	0.106	0.025	0.088	0.040	0.025	**0.336**	0.026	0.136	0.086	0.004	0.013	0.094	0.001
Nai	0.345	0.308	0.118	0.112	0.207	0.047	0.154	**0.319**	0.274	0.213	0.008	0.005	0.189	0.004
Lim	0.158	0.126	0.027	0.068	0.038	0.010	0.030	0.035	**0.293**	0.049	0.006	0.007	0.012	0.002
Aber	0.287	0.254	0.117	0.092	0.107	0.084	0.256	0.113	0.182	**0.366**	0.001	0.012	0.170	0.002
Isi	0.175	0.091	0.060	0.032	0.049	0.014	0.022	0.029	0.144	0.008	**0.340**	0.004	0.014	0.000
Voi	0.135	0.158	0.061	0.101	0.046	0.005	0.051	0.058	0.281	0.062	0.010	**0.288**	0.065	0.000
Bul	0.407	0.264	0.097	0.116	0.087	0.087	0.218	0.052	0.149	0.201	0.021	0.007	**0.397**	0.001
Mdg	0.067	0.051	0.023	0.088	0.002	0.034	0.012	0.045	0.095	0.018	0.000	0.001	0.019	**0.456**

Kas, Kasanga; Kat, Kathiani; Mat, Mathiakani; Tai, Taita; Tan, Tanga; Mal, Malindi; Kak, Kakamega; Nai, Nairobi; Lim, Limuru; Aber, Aberdare; Isi, Isiolo; Bul, Bulindi; Mdg, Madagascar

4.4 Characterization of foraging and stinging behaviors

4.4. 1 Mean, range and standard deviation of phenotypic variables

The mean, standard deviation, range (minimum and maximum values), proportion of missing data for the four quantitative variables are shown in Table 27 below. The mean for npn is 0.69 indicating that 69% of the honey bee combs were occupied by the nectar as compared to 31% occupied by pollen in the 47 colonies studied. The mean for sos is 0.43 indicating that the sealed combs were only 43% compared to the 57% opened combs. The average time in seconds for sting (AvTS) was 11.8 secs for the populations studied showing that the honey bee colonies responded relatively fast to the aggression in reference to a standard of 25 stings (see Table 27). This is also consistent with the Average Sting Numbers (AvSN) recorded at 21.46 stings within the first one minute of the experiment. Overall the standard deviation indicates that for the quantitative variables the values were widespread within their ranges.

For qualitative variables, there were two dissection states in each of the two traits; stinging and foraging. For *sting* variable the honey bees were either aggressive (A) or mild (M) depending on the number of stings and response time of the first sting. For *pln* variable, the honey bees were either nectar foraging (N) or pollen foraging (P) depending on the nectar or pollen comb numbers recorded. The frequency of the aggressive state (A) was 0.5532 while for the mild bees (M) was 0.4468. Hence relatively more colonies were aggressive as compared to mild colonies. The frequency of the nectar foraging was 0.7447 while that of pollen foraging was 0.2553. This means that on average colonies foraged for and stored more honey than pollen in the hives.

Table 27: Basic statistics for quantitative and qualitative variables

		Mean	Standard deviation	Min value	Max value
Quantitative	npn	0.69	0.24	0.05	1.00
	sos	0.43	0.19	0.00	0.82
	AvTS	11.87	10.89	2.00	37.00
	AvSN	21.46	10.82	6.00	40.30
Qualitative	Aggressive	0.5532			
	Mild	0.4468			
	Nectar foragers	0.7447			
	Pollen foragers	0.2553			

4.4.2 Spatial autocorrelation analysis

The spatial autocorrelation of each variable using complete randomizations (5000 replicates), showed Moran's *I* and regression slope with distance for the average time in seconds for sting (AvTS), the average sting numbers (AvSN) and the sting qualitative variable (Table 28 below). The null hypothesis of no spatial structure was rejected for the three variables and hence there is significant spatial structure with the three variables at P<=0.001. Npn (nectar occupied combs), sos (sealed combs) and pln (foraging) did not show any significant spatial autocorrelation of these variables hence accepted the null hypothesis of spatial structure.

Table 28: Tests of spatial autocorrelation using complete randomization

Max dist of class:	**2**	**4**	**8**	
Number of pairs in class:	456	387	238	
Mean dist of class:	1.07233	2.903623	4.864842	
Variable		**Moran's I:**		**Slope with dist**
npn	0.015305	0.014298	-0.052572	-0.02779
sos	0.007167	0.010908	-0.031469	-0.00561
AvTS	0.255687 ***	-0.192880 **	-0.176255 *	-0.1506 ***
AvSN	0.210829 ***	-0.102929 *	-0.236574 **	-0.1428 ***
sting	0.197053 ***	-0.149965 *	-0.232435 **	-0.1401 ***
pln	0.169549	-0.195964 *	-0.104942	-0.08605

Coding: *** for P<=0.001 and >=4999 replicates; ** for P<=0.01 and >=499 replicates; * for P<=0.05 and >=99 replicates

4.4.3 Association between variables

A matrix of Pearson's correlation coefficients between quantitative variables is given in Table 29 below. There is a negative correlation between sealed brood and the nectar foraging (sos vs npn at -0.03051) variables. A negative correlation was also observed between sealed brood and the average time of the first sting (sos vs AvTS at -0.14329) quantitative variables. Average time of the first sting (AvTS) and average sting numbers (AvSN) showed a positive correlation with the nectar foraging variable. The Pearson correlation coefficient between average time of the first sting (AvTS) and average sting numbers (AvSN) was statistically significant and the mean observed value was much less compared to the mean permuted value.

The Chi-square (χ^2) coefficient between *sting* and *pln*, qualitative variables (from contingency tables) was 0.184458 showing that they are positively correlated although this was not statistically significant.

A matrix with significant intra-class correlation coefficients of quantitative variables classified by qualitative variables is given in Table 30. The coefficients for the average time of the first sting (AvTS) and average sting numbers (AvSN) was statistically significant for the *sting* qualitative variable. Quantitative variables sos and npn were negatively correlated with *sting* qualitative variable and no statistical significance was noted. The coefficient for the nectar foraging phenotype (npn) was statistically significant for the *pln* qualitative variable. However, sos, AvTS and AvSN were negatively correlated with *pln*. The interpretation of this is that when honey bees store more honey they sting more and time at first sting is less.

Table 29: Pearson's correlation coefficients between quantitative variables

	npn	sos	AvTS	AvSN	sting
npn	0.00000				----
sos	-0.03051	0.00000			----
AvTS	0.115781	-0.14329	0.00000		----
AvSN	0.035495	0.113362	-0.817063 -***	0.00000	----
pln	----	----	----	----	0.184458

Codes: *** for P<=0.001 and >=4999 replicates; ** for P<=0.01 and >=499 replicates; * for P<=0.05 and >=99 replicates (bilateral tests); + for observed value > mean permuted value; - for observed value < mean permuted value

Table 30: Intra-class correlation coefficients of quantitative variables

	sting	pln
npn	-0.04168	0.792202 ***
sos	-0.00543	-0.05871
AvTS	0.854592 ***	-0.01691
AvSN	0.901667 ***	-0.05699

Coding: *** for P<=0.001 and >=4999 replicates; ** for P<=0.01 and >=499 replicates; * for P<=0.05 and >=99 replicates

4.5 Candidate genes associated with foraging and stinging behaviors

4.5.1 Marker polymorphism

In total, a count of 235 alleles were recognized at 16 microsatellite markers among the 235 honey bee samples, and the number of alleles per locus ranged from 6 to 32 with an average number of alleles as 14.63. The unbiased heterozygosity was high at 0.74. The average polymorphic information content (PIC) value was 0.72 and ranged from 0.21~0.87 as shown in Table 31 below.

Table 31: Polymorphic statistical information of markers

Item	Information for all markers
Number of marker loci	16
Sample size	235
Variation of allele number	6 – 31
Average number of alleles	14.63
Unbiased heterozygosity	0.74
Variation in PIC	0.21 – 0.87
Average PIC	0.72

4.5.2 Linkage disequilibrium

The critical values, r^2, D' and P-values were obtained by analysis of 16 SSR markers. At the highly significant threshold of $r^2 \geq 0.1$, none of SSR marker pairs remained in perfect LD. The pairs of loci with significant P-values less than 0.001 ($P<0.001$) were 6.7% representing 8 out of 120 pairwise comparisons (Figure 19). These were as follows; AJ509635 vs AJ509439, AJ509655 vs AJ509656, AJ509390 vs AJ509655, AJ509690 vs AJ509387, AJ509390 vs AJ509384, AJ509390 vs AJ509635, AJ509690 vs AJ509655 and AJ509486 vs AJ509721. Figure 19 shows a graphical representation of the linkage disequilibrium statistics and the P-values showing the levels of significance. None of the pairs of loci showed complete linkage disequilibrium with $D'=1$. D' values ranged from 0 to 0.90 while r^2 values for all pairs of loci were below 0.01.

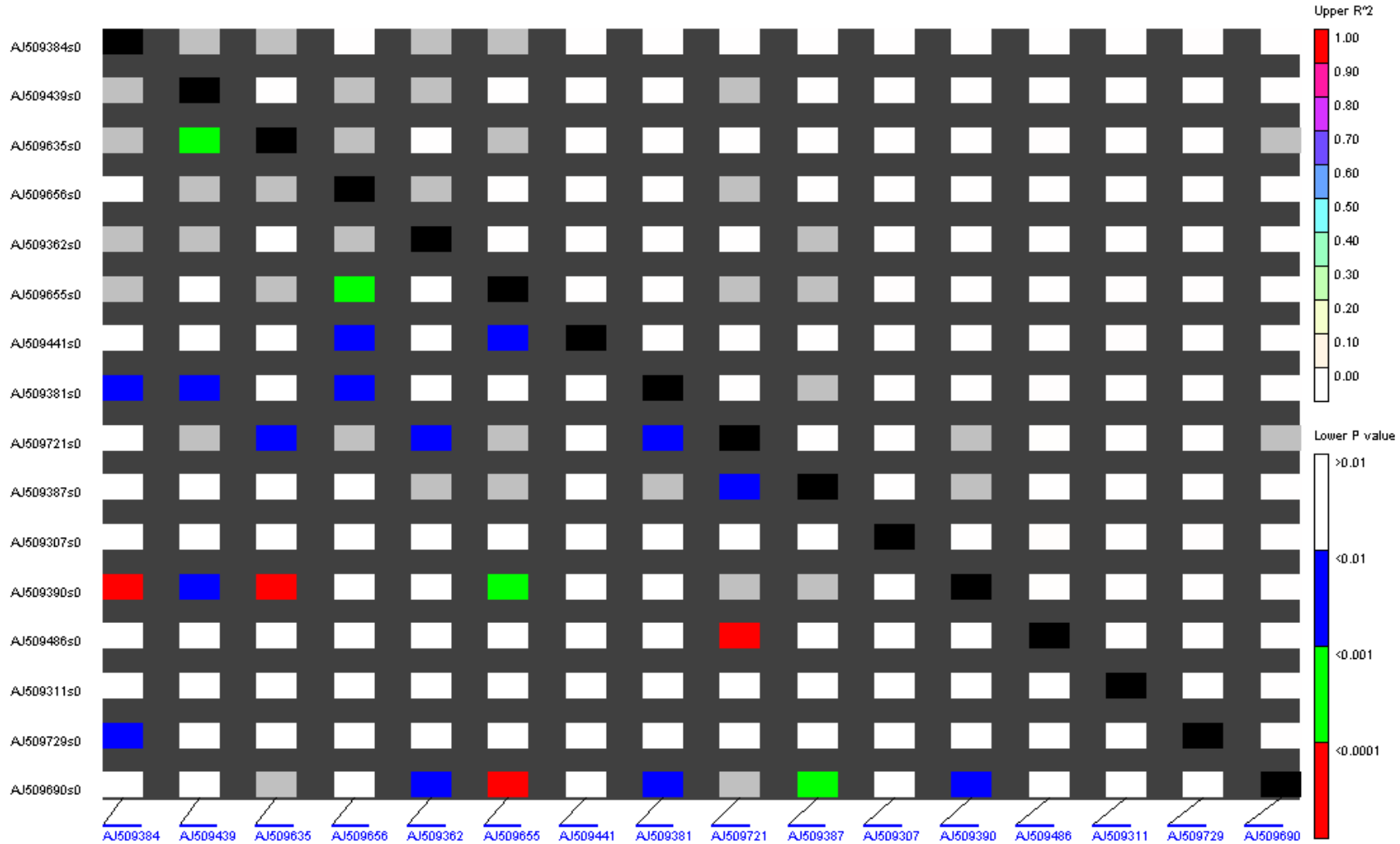

Figure 19: Graphic representation of linkage disequilibrium statistics

4.5.3 Determining the optimum population structure

To determine the optimum value for K, the simulation summary on the tree pane of STRUCTURE file was selected and saved in a text file. The values of K and LnP(D) were copied in excel worksheet and the average of LnP(D) against each K was determined across replications. The K at which LnP(D) plateaus at K=8 was taken as the optimum K as shown in Figure 20 below. A plot of the rate of change of the likelihood [L(K)] distribution against K (Figure 21) showed the highest value of K at K=8. The modal value for the distribution of ΔK against K (Figure 22) was at K=8 as well supporting eight clusters in the honey bee populations. K represents the optimum population structure and from this we estimated the inferred ancestry (Q matrix) of individuals.

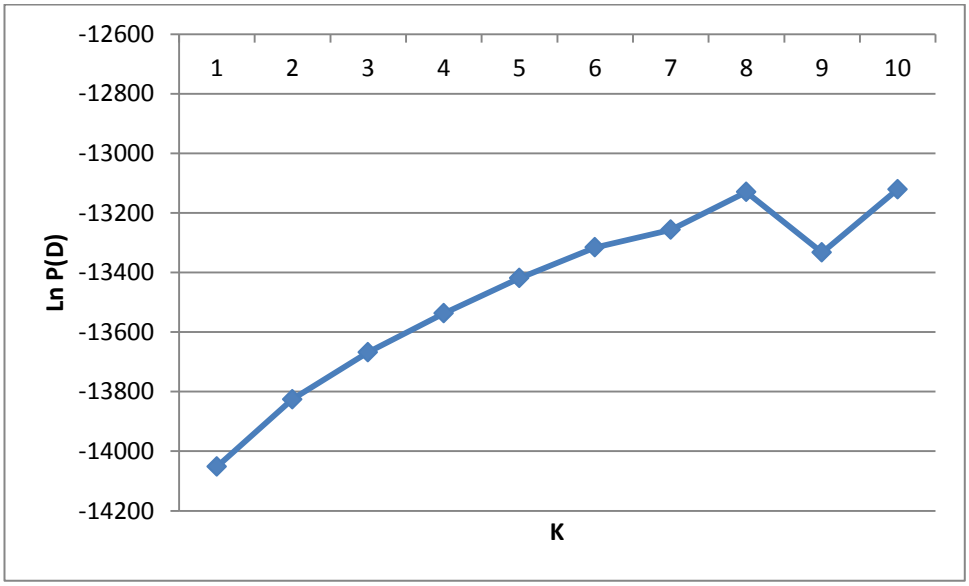

Figure 20: A plot of LnP(D) against each K obtained from simulation summary

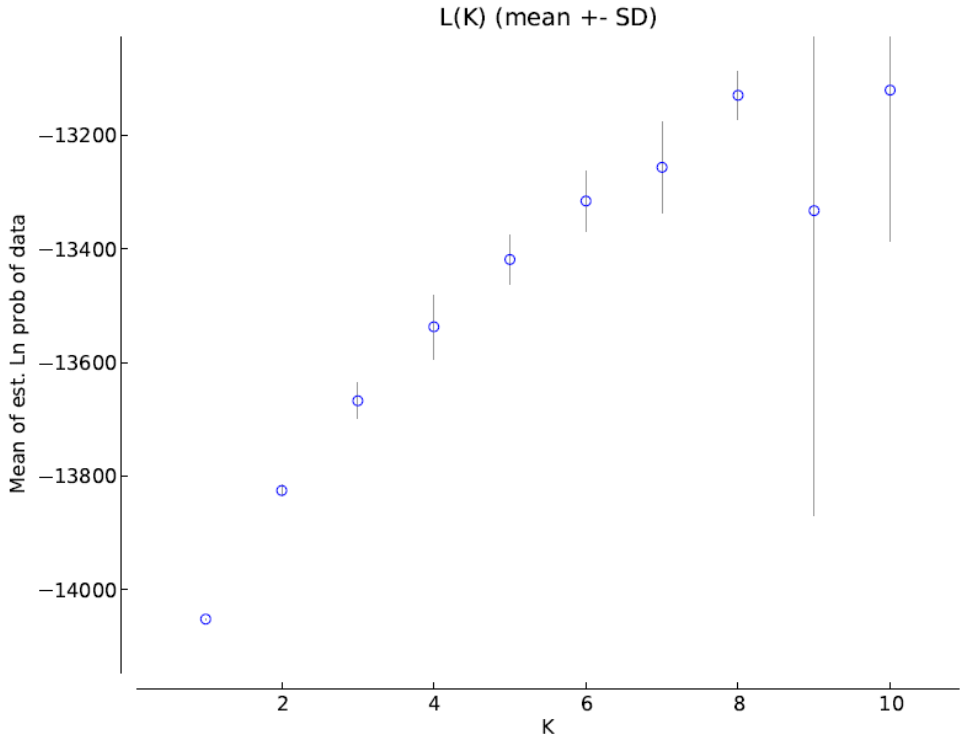

Figure 21: Mean of estimates of Ln probability of data for each value of K

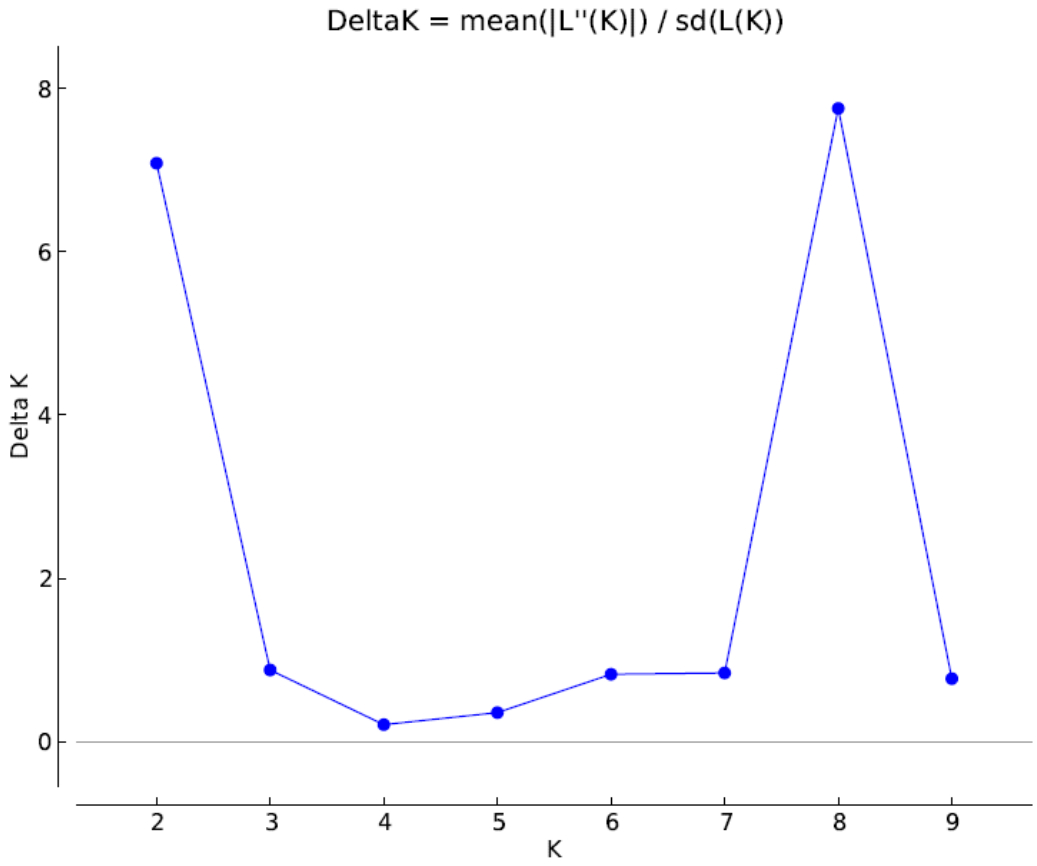

Figure 22: Plot of ΔK vs. K values

4.5.4. Identification of marker-phenotype association

The results of General Linear Model using TASSEL ver 2.1 programme are presented in Table 32. The table displays the F-statistics and p-values for the F-tests. In addition, it contains information about degrees of freedom, the error mean square for the model, R-square of the model, and R-square for the marker. The model R-square is the portion of total variation explained by the full model. The marker R-square is the portion of total variation explained by the marker but not by the other terms in the model. The #perm_Marker is the number of permutations run, pperm_Marker is a test of individual markers, and p-adj_Marker is the marker p-value adjusted for multiple tests. The p-adj_Marker value is a permutation test derived using a step-down MinP procedure and controls the family-wise error rate (FWER). For example, if only markers with p-adj values of 0.05 or less are accepted as significant, then the probability of rejecting a single true null hypothesis across the entire set of hypothesis is held to 0.05 or less. This test takes dependence between hypothesis into account and does not assume that hypotheses are independent as do other multiple test correction procedures.

Out of 16 markers tested four markers showed a significant correlation with the phenotypes and are associated with their respective phenotypes. Marker AJ509381 (LG12) was found to be strongly associated with three of the four phenotypes namely; the Average Sting Numbers (AvSN), Average Time of the first Sting (AvTS) and sealed brood (sos). Marker AJ509307 was significantly associated with Average Sting Number (AvSN). Finally, markers AJ509384 and AJ509721were significantly associated with nectar foraging (npn) phenotype.

Initially, selection of AJ509384 (LG7) was associated with sting-1 QTL, AJ509721 (LG2, position 195.8cM) was associated with sting-3 QTL, AJ509307 (LG1, Grp 1.24) was associated with pln-3 QTL and AJ509381 (LG12) was not associated with any of the QTLs but was found to have a correlation with all of the phenotypes.

Table 32: General Linear Model result file

Trait	Locus	df	F	p_value	#perm	p-perm	p-adj	df_Model	df_Error	MS_Error	Rsq_Model	Rsq
AvSN	**AJ509381**	29	2.4441	1.62e-04	5000	6.00e-04	2.00e-04	36	198	0.0426	0.3648	0.2274
AvSN	**AJ509307**	16	2.7619	4.81e-04	5000	8.00e-04	0.0022	23	211	0.0449	0.2868	0.1494
npn	**AJ509384**	61	1.9369	5.05e-04	5000	4.00e-04	0.0014	68	166	0.0254	0.4743	0.3742
npn	**AJ509721**	66	1.8524	9.19e-04	5000	1.00e-03	0.005	73	161	0.0255	0.4885	0.3884
sos	**AJ509381**	29	2.5143	1.00e-04	5000	2.00e-04	2.00e-04	36	198	87.2428	0.3672	0.233
AvTS	**AJ509381**	29	3.9478	3.94e-09	5000	2.00e-04	2.00e-04	36	198	75.5281	0.4451	0.3208

Where df = degrees of freedom, F = F-statistics, p_value = p values for the requested F tests, #perm = no of permutations run, p-perm = a test of individual markers, p-adj = marker p value adjusted for multiple tests, df_Model = degrees of freedom for the model, df_Error = standard error for the degrees of freedom, MS_Error = Error mean square of the model, Rsq = marker R-square, i.e. proportion of total variation explained by the marker, Rsq_Model= model R-square variation which is proportion of variation explained by the full model.

The results of Mixed Linear Model (MLM) analysis are shown on Table 33 below. Markers AJ509307, AJ509384 and AJ509721 identified in GLM analysis were found not to be associated with any of the four phenotypes when kinship data from SPAGeDi was included in the analysis showing that only marker AJ509381 had a significant correlation with all the four phenotypes observed in this experiment at a p-value less than 0.05. However, this marker was strongly associated with the Mean time of the first Sting (AvTS).

Table 33: Mixed Linear Model result file

Trait	Locus	df_Marker	F_Marker	p_Marker	lnLikelihood
AvSN	**AJ509381**	27	1.8208	0.0111	39.2977
npn	**AJ509381**	27	1.8921	0.0074	85.3007
sos	**AJ509381**	27	1.5547	0.0473	-7.01e+02
AvTS	**AJ509381**	27	2.7257	3.81e-05	-6.82e+02

4.5.5 Candidate gene identification

The results of marker effects on MLM analysis show four microsatellites namely AJ509381, AJ509384, AJ509721 and AJ509307 (Table 34) that were mapped on the *Apis mellifera* genome at www.beebase.org using the blastn tool. Blast2GO programme identified the functions of the upstream and downstream genes as shown in Table 35 below.

Table 34: Upstream and downstream candidate genes identification

Marker	**Upstream**	**Downstream**
AJ509381	GB55730: CUGBP Elav-like family member 4-like isoform X1	GB55732: Protein coding
AJ509384	GB44258: aryl hydrocarbon receptor nuclear translocator homolog isoform X5	GB44259: aryl hydrocarbon receptor nuclear translocator homolog isoform X4
AJ509721	GB46589: SAGA-associated factor 29 homolog	GB46588: amino acid ABC transporter substrate-binding protein
AJ509307	GB48999: helix-loop-helix protein 11	GB49000: TBC1 domain family member 13-like

Table 35: Description of gene id and the associated gene ontology

Name	**Description**	**Length (aa)**	**#Hits**	**min. eValue**	**mean Similarity**	**#GOs**	**Gene Ontology**
GB46589	saga-associated factor 29 homolog	293	20	0	86.45%	0	transcriptional regulation
GB55730	cugbp elav-like family member 4-like isoform x1	404	20	0	97.05%	0	Protein coding
GB48999	transcription factor ap-4	537	20	0	80.95%	14	Defense response
GB49000	tbc1 domain family member 13-like	396	20	0	87.75%	3	Regulation of Rab GTPase activity
GB44258	aryl hydrocarbon receptor nuclear translocator homolog isoform x1	78	14	2.10e-31	86.50%	10	Sequence-specific DNA binding
GB44259	aryl hydrocarbon receptor nuclear translocator homolog	614	20	0	93.95%	10	transcription factor activity

SAGA-Associated Factor 29 (SGF29) has a dual role in ER Stress Survival by coordination of both histone H3 acetylation and histone H3 lysine-4 trimethylation in

honey bees (Schram *et al*., 2013). Specifically, it recognizes and binds methylated 'Lys-4' of histone H3 (H3K4me), with a preference for trimethylated form (H3K4me3) in transcription and transcription regulation process of the nucleus. The beebase identifies saga-associated factor 29 homolog as GB46589 with a gene identification symbol LOC408702 found in *Apis mellifera*. This is a protein coding type gene also known as GB18746 and located in the linkage group 2 (LG2). A sequence length of 293 amino acids and approximately 20 hits were observed. The predicted associated function is similar to that of humans.

The sequence name GB46588 found on bee base did not match any associated gene sequence in the database and therefore no function was associated with it. GB55730 is a CUGBP elav-like family member 4-like isoform x1 which in humans has molecular function in nucleotide binding, translation repressor activity, nucleic acid binding, mRNA binding and BRE binding. This gene is a cellular component of nucleus, nucleolus, cytoplasm and the biological processes involved are alternative mRNA splicing, via spliceosome, regulation of alternative mRNA splicing, via spliceosome, mRNA splice site selection, germ cell development, embryo development, positive regulation of mRNA splicing, via spliceosome, negative regulation of excitatory postsynaptic membrane potential. The beebase identifies CUGBP elav-like family member 4-like as GB55730 with a gene identification symbol LOC10353 found in *Apis mellifera* as a protein coding gene also known as GB17926 located in chromosomal linkage group 12 (LG12; NC_007081.3). A sequence length of 404 amino acids and approximately 20 hits were observed. NCBI gene id records number 410353. The sequence name GB55732 found on bee base did not match any associated gene sequence in the database and therefore no function was associated with it.

The beebase identifies GB48999 as the helix-loop-helix protein 11 with transcription factor ap-4. A basic helix-loop-helix (bHLH) is a protein structural motif that characterizes a family of transcription factors. The motif is characterized by two α-helices connected by a loop. In general, transcription factors including this domain are dimeric each with one helix containing basic amino acid residues that facilitate

DNA binding. bHLH transcription factors are often important in development or cell activity that include; neurogenesis, myogenesis, hematopoiesis, sex determination and gut development. Approximately 51 bHLH transcription factors have been identified in honey bees. Transcription factor ap-4 of the bHLH in NCBI ID is 726729, Uniprot is H9KBF8 and is found in the chromosomal linkage group 1 (LG1). The sequence is 537 amino acids long and 20 hits were recorded. The alternative gene id is GB14420 with gene identification symbol LOC100679356.

The gene GB49000 is identified as tbc1 domain family member 13-like in the beebase which is a member of Rab family of proteins. Rab family of proteins is a member of the Ras superfamily of monomeric G proteins. There are approximately 70 different Rabs that have been identified in humans thus far. They are mostly involved in vesicle trafficking, defining the identity and routing of vesicles. Rab GTPases regulate many steps of membrane traffic, including vesicle formation, vesicle movement along actin and tubulin networks and membrane fusion. These processes make up the route through which cell surface proteins are trafficked from the Golgi to the plasma membrane and are recycled. In honey bees the gene identification symbol is LOC408604 which is a protein coding gene type with an alternative gene id as GB13802. The gene is located in the chromosomal linkage group 1 (LG1, NC_00707.3). Rab family of proteins are peripheral membrane proteins, anchored to a membrane via a lipid group covalently linked to an amino acid. Rabs are anchored via prenyl groups on two cysteines in the C-terminus.

GB44258 and GB44259 in beebase represent an aryl hydrocarbon receptor nuclear translocator homolog (ARNT) which is associated with ahr-1 (aryl hydrocarbon receptor-1) and has a role in cellular differentiation. It is located in the nucleus and functions in DNA binding in the biological processes of transcription and transcription regulation. The isoform has been chosen as the canonical sequence. The gene is required for pharyngeal development and is expressed in many cell types throughout development, including hypodermal cells, intestinal cells, pharyngeal cells, and neurons (Huang *et al*., 2004). It is expressed in every cell during embryo development. If impaired or unavailable, it leads to abnormal cell morphology in

developing neurons and arrested development at larval life stage due to its requirement in the pharynx and aggregation behavior is diminished in *C. elegans*.

ARNT homolog contains 1 bHLH (basic helix-loop-helix) domain, 1 PAC (PAS-associated C-terminal) domain and 2 PAS (PER-ARNT-SIM) domains. This gene is an ortholog of the Dmel/*tango* gene (shares 56% amino acid identity with *tango*) found in *Drosophila melanogaster*. *Tango* is a basic helix-loop-helix-PER-ARNT-SIM (bHLH-PAS) transcription factor that responds to hypoxia and is critical for the development of the fly neural midline and antennae. Other bHLH-PAS transcription factors act as heterodimers to sense light, temperature, oxygen, or endogenous hormones, and some have roles in regulating circadian rhythm (Roenneberg & Merrow 2003). ARNT has been associated with *sting*-1 QTL (Hunt *et al*., 2007) and is known to be diverged in the region important for activation of target genes, making it impossible to infer function (Sonnenfeld *et al*., 2005). *Sting*-1 QTL is responsible for the honey bee's individual guarding and stinging behavior (Hunt *et al*., 2007).

Aryl hydrocarbon receptor nuclear translocator has been shown to interact with HIF1A (Hypoxia inducible Factor A), SRC-1, AIP, SIM1, Aryl hydrocarbon receptor, EPAS1, SIM2 and Nuclear receptor coactivator 2. Due to these interactions it is involved in several important biological processes such as response to hypoxia, embryonic placenta development, positive regulation of endothelial proliferation, transcription (DNA dependent), positive regulation of vascular endothelial growth factor production, cell differentiation, positive regulation of vascular endothelial growth factor receptor signaling pathway, positive regulation of protein sumoylation, cellular response to stress, mRNA transcription from polymerase II promoter in response to oxidative stress, positive regulation of erythrocytes differentiation, positive regulation of glycolysis, positive regulation of transcription (DNA dependent), positive regulation of transcription from RNA polymerase II promoter, positive regulation of hormone biosynthetic process, regulation of transcription from polymerase II promoter in response to hypoxia and cellular response to hypoxia.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Phylogenetic relationships of honey bee subspecies

Mitochondrial DNA (mtDNA) is particularly suited for inferring phylogenetic relationships among the components of a species. Amplification and sequencing was done on COI, Cytb-tRNA^{ser} and COI-COII intergenic genes regions. Mitochondrial DNA sequences using COI gene, the COI-COII and the Cytb-tRNA^{ser} intergenic regions showed a relatively high level of diversity suitable for subspecies delineation and comparison along conventional geographical regions and elevations. DNA barcoding using COI sequences formed clusters based on elevations and regions. Based on altitude, the three clusters were Lowland areas (Tana River, North coast and Tanga, off Tanzania coast), Mid-altitude areas of Eastern region and Highland regions of Taita Hills. Geographically, of the four clusters formed, three had an Eastern haplotype detected suggesting an interaction between eastern honey bees with the neighbouring regions of Taita hills and Tanga. However, the three clusters show that the major region represented in each cluster has maintained its diversity even after interbreeding with subspecies from proximate regions. The Eastern honey bee which mainly consists of *A. m. scutellata* seems to be the migrating subspecies causing hybridization with other subspecies in Taita hills and Tanga regions. This means that hybridization, migration or swarming happens in search for nectar and pollen in times of abundance and scarcity in these regions. The fact that hybridization occurs is not unexpected because *A. m. scutellata* in Eastern region and *A. m. litorea* in Tanga and Kenyan Coast region are previously known to form hybrids (Meixner *et al*., 2000; Raina & Kimbu, 2005) in bordering regions and hybrid zones exist. Also according to COI, Hewani samples in cluster 1 seem to have diverged earlier and have conserved their genetic status. Less hydridization and interaction may be due to Tana River delta barrier that separates the eastern region from north coast regions.

Mitochondrial Cytb-tRNA^{ser} gene sequence cluster analysis identified honey bees according to altitude and regions yielding three clusters. Cluster 1 consisted of samples largely from Mid-altitude areas of Eastern region. The second cluster had populations sampled majorly from Highland areas of Western, Central and Eastern. The third cluster has samples from Lowland Coastal areas of Tana River, Hewani. These samples (Coast population) formed an own branch suggesting less interaction and hybridization with the Eastern counterparts which are separated by the Tana River delta. Tana River is a Lowland area located in the coastal area and is geographically distant from the Eastern region. These samples could have diverged earlier, maintaining their genetic status despite hybridization and unaffected by swarming and hybridization that occurs in the neighbourhood. Further analysis with more sampling is required to verify the genetic status of this population. The third cluster consisted of Highland bees found in Central and Western regions suggesting that despite hybridization with the eastern honey bees, they maintain a genetic pool identified with the high elevation.

Analysis of COI-COII intergenic region from Eastern region showed that the East African honey bees belong to the A lineage. The differences in length (presence/absence of a P sequence, number of repeated Q sequences, and possibility of small deletions) showed haplotypes A with two mitotypes, A1 and A4. *A. m. ligustica* was found to have C1 mitotype. Mumoni samples from Eastern region share haplotypes with Taita samples from Coast region though the two areas border each other in the south west. Mumoni and Taita haplotype sharing could be due to interaction in the bordering regions from hybridization, migration and swarming of bees.

The phylogenetic relationship of Cytb-tRNA^{ser} gene sequences is consistent with COI gene (barcode results) in terms elevation and regions. Both COI and Cytb-tRNA^{ser} phylogeny shows that *A. m scutellata* is widely distributed across the Kenyan geographical regions and forms hybrids easily except in some fragmented habitats like Tana River. Meixner *et al*., (2000) while studying Kenyan bees using mitochondrial DNA found that *A. m. scutellata* hybridizes easily with other

subspecies horizontally in areas similar in elevations and ambiance patterns. Due to climate change effects, *A. m. scutellata* has high potential to hybridize, migrate and swarm into new areas both vertically in areas above their savanna habitat elevations and horizontally in regions similar to their habitats.

5.1.2 Allelic proportions and Genetic diversity of honey bees

5.1.2.1 Fisher's exact test on Hardy-Weinberg Equilibrium (HWE)

All the loci showed significant deviations from Hardy Weinberg Equilibrium (HWE) ($p < 0.05$) but none of them deviated in all the populations due to heterozygote deficiency or excess. Deviations for heterozygote deficiency and excess were distributed within the 14 populations analyzed. Potential causes for such deviations in a given population include: (i) technical artifacts, such as the occurrence of null alleles, stuttering or large allele drop-out, (ii) the Wahlund effect, (iii) the selection of specific alleles and (iv) inbreeding effects (Hoarau *et al.*, 2002; Pereira *et al.*, 2009). Null alleles are not concentrated in one locus so their effects can be ruled out. Selection of specific alleles and inbreeding are not common with bees and the loci used. African honey bees (example, *A. m. scutellata*) are highly mobile insects which move to new places by swarming and seasonal migration over vast distances. They are also known to hybridize easily with other subspecies and females are polyandrous. Therefore, selection of specific alleles and inbreeding are not possible due to this lifestyle. The Wahlund effect refers to reduction of heterozygosity in a population caused by subpopulation structure. Honey bees populations diverge all the time due to swarming and migration to areas of abundant flora. Therefore, the Wahlund effect may play a role in the reduction of heterozygosity as populations diverge to establish new colonies.

5.1.2.2 Linkage disequilibrium

The level of linkage disequilibrium of two loci in a population is a sum of a number of factors that include evolutionary forces (such as random genetic drift, natural selection, mutation, and line origin), molecular forces such as historical

recombination events, and the population's inbreeding history such as historical effective population sizes, intensity and direction of artificial selection, population admixture, and mating patterns (Feng-Xing *et al.,* 2007). Many of these factors are unlikely to have contributed to the linkage disequilibrium in honey bees. Honey bees undergo swarming and migration in climatic and altitudinal variations to avoid inbreeding as a possible causative factor of linkage disequilibrium. Honey bee (*Apis mellifera*) linkage mapping shows that this species has an unusually high meiotic recombination rate (19 cM/Mb), in the order of 10-fold higher than that of *Drosophila* (1.59 cM/Mb) and other genetic model systems (Hunt & Page Jr. 1995, Solignac *et al*. 2004, Beye *et al*., 2006). Honey bee queens mate in flight with more than 10 drones from diverse genetic sources (Palmer *et al*., 2001, Tarpy *et al*., 2004). African honey bees form stable colonies containing up to 20,000 individuals or more (Hepburn & Radloff, 1998). Therefore, random genetic drift, inbreeding and population substructure (Wahlund effect) are unlikely sources of linkage disequilibrium in honey bees. There were no genetic bottlenecks and therefore the effect of random drift caused by small effective population sizes was not expected in the populations studied. African subspecies show significantly higher average heterozygosity and average number of alleles in agreement with larger effective population sizes compared to European subspecies (Estoup *et al*., 1995).

Geographic features and many other factors determine the distribution of honey bee subspecies in East Africa. Honey bees are forced to change their swarming patterns and drift to regions of abundance flora during dry spells. This may result into mixing of two or even three different races in one location and the production of hybrids, which differ from their pure parents in vigor and behavior (Raina & Kimbu, 2005). Therefore, the occurrence of genetic admixture, mutations and recombination events among loci is expected to account for the observed 21.4% linkage disequilibrium in the honey bee populations studied. The genetic admixture of the honey bee populations has been supported by the results of STRUCTURE and PCA which creates 4 clusters. Within these clusters, honey bees show sub-structuring. In consistent with other studies (Estoup *et al*., 1995, Franck *et al*., 2000), microsatellite loci in honey bees undergo high mutation rates and a large amount of genetic

variation (between twelve and 32 alleles per locus) were detected. When such new mutations occur in a finite population, linkage disequilibrium is created and the degree is dependent on the frequency of the allele that is haplotyped with the new mutation. As the copies of the mutant allele accumulate, the linkage disequilibrium between this locus and other loci depend on recombination rate, random drift, population admixture, and selection.

Alleles at neighbouring loci tend to be inherited together and remain closely associated even in a segregating population. However, recombination (a genetic process by which the combinations of alleles observed at different loci in two parental individuals become shuffled in offspring individuals) usually reduces the possibility that such neighbouring loci will remain linked. The honey bee recombination rates are usually high (Hunt & Page, 1995). In a large population under no selection as in most honey bee populations, the estimate of linkage disequilibrium decays at rate of $1-\theta$ under random mating, where θ is the recombination fraction. In these populations, recombination effects could have possibly resulted in the observed linkage disequilibrium due to the shuffling of the loci found in the parental population to create new combinations in the offsprings which might lead to novel linkage disequilibrium associations or severing already existing linkage disequilibrium associations.

5.1.2.3 Allele counts per locus and population

A high number of alleles (145) are detected with only eight microsatellite loci. Kathiani populations appears to be the most variable as it anchors the highest number of alleles (87) whereas the Madagascar populations had the least number of alleles at 39. The high range of alleles (from 12 to 32) in each locus shows that the honey bees are highly genetically variable. It is likely that the observed low alleles count and subsequent genetic variability of the Madagascar population could be attributed to its geographical isolation. Among the east African populations, Isiolo (North Eastern region) and Voi had an equal number of alleles (44) which can be attributed to their genetic variability. Isiolo is an arid area and its low allele counts number can be attributed to a relatively low level of gene flow with other subspecies in its

neighbourhood. The harsh environmental conditions may be responsible for its low variability. Morphological studies suggest that Isiolo population to belong to *A. m. yementica*. It is not clear why Voi population has low alleles count but low allele counts could also be attributed to low level of interaction with other subspecies in the vicinity. Honey bee subspecies typically exhibit reduced gene flow with others due to water, mountain or desert barriers and are called "geographical races", to reflect their adaptation to specific geographic areas (Ruttner, 1988). The Isiolo and Voi populations are isolated depicting an association with their fragmented regions and separates from other regions consistent with STRUCTURE results in this study. Hybridization in these races is not intense only influenced by the closely related honey bee individuals found in these regions. It is important to note that beekeeping practices in Isiolo is relatively new and uncommon and the communities living in these areas prefer a nomadic lifestyle.

5.1.2.4 Mean Number of Alleles (MNA) and Heterozygosity

After genotyping the fourteen honey bee populations across eight microsatellite loci, the mean number of alleles (MNA), expected and observed heterozygosities obtained were 8.201, 0.781 and 0.709 respectively, an indication of a high genetic diversity base among the honey bee subspecies. These values are consistent with previous reported microsatellite loci genetic diversity by Estoup *et al.,* (1995) for the African honey bee as compared to the other European subspecies. Only the Madagascar population had a low observed heterozygosity probably due to the presence of null alleles or the Wahlund effect caused by subpopulation structure considering that higher values of F_{IS} and rare alleles were observed in this population. Basically the number of alleles observed at a locus is an indication of genetic variability at that locus and this has a direct impact on differentiation within a species. Nei (1987) indicates that MNA is an appropriate measure of genetic variation in comparison to heterozygosity since it is expected to be larger when the extent of polymorphism is higher, while the heterozygosity is hardly affected by the low-frequency alleles. The mean allele number (allele diversity) therefore, provides a reasonable indicator of the levels of variability present within a species assuming that the population is in

mutation drift equilibrium (MacHugh *et al.*, 1998). With only eight loci the average allele diversity measure of 8.201 is a substantial level of genetic variability in the honey bee subspecies. Generally, the average heterozygosity and average number of alleles are significantly higher in African subspecies, in agreement with larger effective population sizes (Estoup *et al*., 1995) and rare likelihood of genetic drift. The higher number of alleles in the African honey bee may explain the inherent genetic capacity to tolerate or resist *Varroa destructor*, tracheal mites and contagious brood diseases which are well known (Guerra Jr *et al*., 2000) though global honey bee health is on the decline. There is preliminary evidence to suggest that selection and breeding would be an efficient and sustainable approach to deal with novel pathogens or group of pathogens, including those that may be involved in CCD (Oldroyd, 2007). Based on this fact a high priority should be given to selecting and breeding African honey bees that can remain healthy with minimal need for chemical inputs in the bee hive.

5.1.2.5 Genetic diversity per locus and population

Genetic diversity serves as a way for populations to adapt to changing environments. The more the allele's variation, the more likely the individuals are suited for certain environments. Those individuals are more likely to survive to produce offspring bearing that allele. The population will continue for more generations because of the success of these individuals (Robinson & Page 1989). In this study the Madagascar population had the least diversity among the populations while Kathiani had the highest genetic diversity. *Apis mellifera scutellata* which is known to be highly mobile, performs seasonal migration over extensive distances horizontally and vertically across mountainous and savanna areas (Meixner *et al*., 2000) is the dominant subspecies in this region. Therefore, the relatively higher diversity observed in the Kathiani population could be due to the improved *A. m. scutellata* from honey bee management and beekeeping practices in this area. Therefore, this region harbors the richest genetic diversity resulting from gene flow from hybridization and migration effects with other populations across savanna and high altitude areas. Honey bee colonies that are genetically diverse tend to form strong

colonies faster by swarming, are more resistant to pests and diseases, and are better suited to their environment and this has an effect on improved hive productivity (Mattila & Seeley, 2007; Mattila *et al*., 2008). Apart from Kathiani high genetic diversity was also observed in the other populations suggesting adaptability to their regions arising from swarming and migration patterns during dry spells between border regions. The relatively reduced genetic variability in the Madagascar populations could be attributed to the geographical isolation of the bee in the island and the ability of the bee to adapt to local conditions. Therefore, hybridization with other honey bees across the seas is minimal thus lowering the genetic diversity and variability as supported by the high value of F_{IS}.

5.1.2.6 Allelic richness

Although no reasonable sample size (*N*) will ensure detection of all alleles, the use of large sample sizes is encouraged because it will result in better estimates of allelic richness (*A*) than the use of small sample sizes. As expected loci such as AJ509381 and AJ509656 with lower polymorphism were less biased by differences in sample size than the estimates for more polymorphic loci such as AJ509362 and AJ509635 (Leberg, 2002). Measures of allelic richness were high for Kasanga (7.92), Kathiani (7.88), Limuru (7.54) and Kakamega (7.48) populations. However, the decrease of allelic richness in Madagascar (4.43) and Isiolo (North Eastern, 5.50) populations could be attributed to processes that differentially affect the allelic richness (diversity parameter), such as bottlenecks due to long-distance founding events, selection during population establishment, and decreased gene flow at low population densities (Leberg 2002). Honey bees from Madagascar and North Eastern (Isiolo) are geographically and climatically isolated respectively. The low allelic richness can be attributed decreased gene flow in their areas and therefore hybridization with other subspecies is restricted. The opposite is true for populations with higher allelic richness as hybridization freely occurs in these areas. Overall the allelic richness for the loci used in honey bees is high and high gene flow between populations is assumed.

5.1.2.7 Null Alleles

Deviations from Hardy-Weinberg proportions can suggest that the sample does not represent a panmictic population, or alternatively, it might indicate the presence of null alleles and/or aberrations in microsatellite gel scoring. Null alleles are non-amplified alleles that, when segregating with another allele, result in an apparent homozygote. For microsatellites, such null alleles can arise when mutations occur in the flanking regions, preventing one or both of the primers from binding (Pemberton *et al*., 1995; Jones *et al*., 1998; Holm *et al*., 2001). The null allele's occurrence is a result of low efficiency of the primer hybridization used to amplify some loci, due to point mutation in one or more annealing sites of these primers (Dakin & Avise, 2004). The effects of null alleles appear to be particularly clear when populations differ in null allele frequencies. However, even a good estimate of the null allele frequency does not salvage the locus, and without adjusted allele and genotype frequencies, a locus with null alleles remains useless for further population analyses. Null alleles may cause a locus to deviate from Hardy-Weinberg equilibrium and show a homozygous excess, particularly when their frequency is high (say 10% or more). When some loci are in Hardy-Weinberg equilibrium while other loci show clear disequilibrium, this is interpreted as evidence for random mating and panmixia. Loci AJ509656 (0.164) and AJ509390 (0.124) showed the highest estimate of null alleles consistent with Hardy Weinberg deviations. All populations showed a lower than 10% frequency of null alleles corroborating the evidence for a random mating and panmixia population. It also agrees with the fact that no deficiency observed in the mean observed heterozygosity in all the populations. Null allele's frequency estimates vary between 0.028 and 0.094 for Tanga and Limuru populations respectively. The deficiency of observed heterozygosity in Madagascar population could be related to a Wahlund effect that is caused by subpopulation structure; this especially considering that a higher value of F_{IS} (0.201) and a relatively high number of rare alleles (0.240) are observed in this population. The overall frequency was less than 10% for all loci and populations. Therefore, this serves as an evidence for random mating and panmixia in all populations.

5.1.2.8 Private Alleles

The number of unique alleles in a population depends heavily on sample size, and they can be difficult to interpret when sample sizes differ across populations. The rarefaction strategy (trimming unequal samples to the same standardized sample size, a number less than or equal to the smallest sample size across populations) to produce estimates that are comparable in different populations (Hurlbert, 1971; Petit *et al.*, 1998; Kalinowski, 2004, 2005) was used. Thus, although Bulindi population had a high number of samples (N=40) compared to the other populations, it had the lowest private allele frequency at 0.130. Perhaps Bulindi was sampled intensively compared to other regions with fewer samples but rarefaction was used to compensate for differences in sample size and number. Tanga population had the highest frequency of private alleles (0.360) while Kakamega population ranked second with a frequency of 0.340. From these estimates the range in the number of private alleles observed between populations is small ranging from 0.13 to 0.36. The number of unique alleles in a population (private allelic richness) is a simple measure of genetic distinctiveness. This shows that the populations sampled have few alleles private to individual populations which can be used to differentiate them. Private allele richness is also attributable to the loci used. Some loci had more private alleles to some population than others. For instance, Voi population at locus AJ509381 had a high private allele frequency at 0.984. This means that based on this locus alone Voi population appears genetically distinct from the other populations but close to Taita population with a frequency of 0.719 at the same locus. The two populations are from neighbouring localities.

5.1.2.9 Genetic bottleneck

There was no evidence of a recent population bottleneck based on a mode shift (paucity of rare alleles) although the method should be viewed with caution because the sample size for some populations was less than 30 (Cornuet & Luikart, 1996). Allele frequency distribution is established in order to see whether it is approximately L-shaped (as expected under mutation-drift equilibrium) or not (recent bottlenecks provoke a mode shift). A significant heterozygote deficit was detected in

all the populations except Madagascar under the Stepwise Mutation Model (SMM) suggesting the populations are expanding. Based on a two tailed test for heterozygote excess and deficit (Wilcoxon rank test, Cornuet & Luikart, 1996) no population exhibited a significant bottleneck.

5.1.3 Population differentiation, admixture and gene flow

5.1.3.1 Population genetic differentiation by F-statistics

The overall fixation index, F_{IT} value for all populations indicated a low rate of allele's fixation among populations. The overall F_{IS} is a measure of the extent of genetic inbreeding within subpopulations and ranges from -1 (all individuals heterozygous) to $+1$ (no observed heterozygotes). The average within population inbreeding over all loci, F_{IS} was 0.101 and its analogue G_{IS} was 0.096. This frequency lies closer to the middle and the heterozygosity observed is moderately high with low levels of inbreeding. Although bee health is on the decline, observed low viability, reduced fecundity, increased sterility, slower development and increased susceptibility to environmental stress in some cases may not be attributable to inbreeding depression because of the polyandrous queen mating system. The average estimates of heterozygosities for the observed (H_O), expected (H_S) and total (H_T) were relatively high at 0.708, 0.784 and 0.841, respectively confirming low levels of inbreeding. The fixation index, F_{ST} and its analogue G_{ST} (the coefficient of gene differentiation) were used to estimate the degree of population differentiation. An overall mean F_{ST} value was 0.056±0.04, implying moderate levels of structuring and genetic differentiation among populations. The values indicate that only 5.6% to 6.9% differentiation is ascribed to between population variability while 93.1% to 94.4% is within population variability. Therefore, within-breed diversity is actively maintained to enable honey bee stocks to adapt to future demands and conditions such as resistance to diseases and pests. G_{ST} is measure of the relative differentiation among subpopulation and loci values below 0.2 were observed; a clear indication that all markers had moderate to low discriminatory power between populations. African honey bees' events such as intermixing, hybridization, swarming and

migration reduces genetic divergence between populations. However genetic differences detected were biologically meaningful for population differentiation.

5.1.3.2 Within-population inbreeding estimates, F_{IS}

The within-population inbreeding estimates, F_{IS} for the east African populations ranged from –0.008 to 0.201 in Voi and Madagascar populations respectively (F_{IS} range from –1 to +1). This is relatively a good estimate of heterozygosity with low inbreeding for most of the populations. Madagascar honey bee population consists of one subspecies, *A. m. unicolor* in an island isolated in the sea. Therefore, while levels of inbreeding may not high there is a likely chance of encountering a homogeneous population. For the east African populations, the highest inbreeding was observed in Limuru population (0.188) followed by Mathiakani population (0.174) but overall the inbreeding effects are low. Limuru is a high altitude area and *A. m. monticola* genotype is expected to predominate but hybridization effects with *A. m. scutellata* from vertical migrations lower the inbreeding estimates. Mathiakani is located in a savanna region slightly out of contact with other commercially active beekeeping eastern regions of Kasanga and Kathiani but horizontal migrations are expected to reduce inbreeding effects. The low inbreeding estimate could be due to commercial beekeeping practice in these areas and there is a possibility of honey bee sharing genotypes with the wild bees of the same or different subspecies. This therefore means that there's high diversity and hence overall low values of F_{IS}. Overall the low levels of inbreeding and a high mean observed heterozygosity allows for population differentiation among populations studied.

5.1.3.3 Pairwise population differentiation by F_{ST}

F_{ST} is a fixation index parameter used to estimate the degree of population differentiation. Pairwise F_{ST} for all the fourteen honey bee populations studied showed significant differentiation between them. Kasanga and Kathiani populations had the lowest differentiation with a low level 0.0012. The two areas are actively involved in small scale beekeeping practice and they border each other in the eastern region. Therefore, the honey bee populations from the two areas largely share an

enormous number of alleles. The pairwise F_{ST} values evidently show that Madagascar population is differentiated from the East African populations. This is anticipated because Madagascar is an out-group population and may represent a different subspecies particularly *A. m. unicolor*. Among the East African populations pairwise F_{ST} values also evidently show Isiolo population was the most divergent against the other populations especially against Nairobi population (0.1252). Honey bee populations in the two areas are separated by a geographical barrier (Mount Kenya) and the climate patterns differ. Therefore, allele sharing between the two populations is uncommon and minimal. Similar outcome with other populations is expected because Isiolo is an isolated area facing drought challenges and a vast land with fewer floras. Swarming and migration out of this land is nominal taking into account the distance to be covered by already feeble bees. Isiolo (Northern region) is isolated climatically (semi arid area) from the rest of the populations in East Africa. Perhaps the genotype from this honey bee is adapted to these conditions for survival. *A. m. yementica* is found to dominate the Isiolo (Neastern region) population. The neighbourjoining (NJ) phylogenetic tree has a main cluster that covers all the East African populations with four subclusters. Madagascar population was the most differentiated followed by Isiolo, Voi and Limuru in that order as confirmed by the Neighbour-joining tree. An F_{ST} value lying in the range 0–0.05 indicates little genetic differentiation; a value between 0.05 and 0.15, moderate differentiation; a value between 0.15 and 0.25, great differentiation; and values above 0.25, very great genetic differentiation (Wright, 1978; Hartl & Clark, 1997). All F_{ST} values obtained were below 0.3 which is a clear indication that all markers had low discriminatory power between populations though there was some level of structuring within the subspecies. The low discriminatory power may indicate that these subspecies are not differentiated enough and they may have common history of breeding practices.

5.1.3.4 Genetic distances and phylogenetic relationships

Nei's (1978) and Nei's (1987) pairwise genetic distances (D_S) and (D_A) respectively showed the honey bee populations were highly variable presenting different topologies. Madagascar which was an out-group population in this study separated

from all the other East African populations consistent with pairwise F_{ST} values. The highest pairwise genetic distance was between Madagascar and Isiolo population for pairwise D_S and D_A and corroborates with pairwise F_{ST} values. A comparable observation to pairwise F_{ST} values was that Isiolo Voi and Limuru populations were the most differentiated from rest of the East African honey bee populations while Kasanga and Kathiani populations were the most closely related. The pairwise D_S and D_A Neighbour-joining trees supported this observation and were corroborated by the results of pairwise F_{ST} values and NJ tree. The pairwise Nei's chord distance, D_A Neighbour-joining tree clustered Bulindi, Kakamega and Aberdare populations together. Considering that the populations are from high altitude areas they may harbor some common genetic diversity that enables them to adapt to these regions of high altitude and cold climate. *A. m. monticola* is expected to dominate in high altitude areas though gene flow with other subspecies resulting into hybrids or mixed populations occurs. It is not clear why pairwise Nei's chord distance, D_A subclustered Nairobi and Tanga populations together. The pairwise Nei's standard genetic distance, D_S Neighbour-joining tree subclustered Kasanga, Kathiani, Mathiakani and Tanga suggesting that these populations may be sharing alleles probably from hybridization effects caused by swarming and migration within their feral regions. Presumably, the route of hybridization is through Mathiakani to Tanga from Kathiani and Kasanga as the two populations show a close relationship as they are on the same branch in the NJ tree. *A. m. scutellata* genotype predominates in these regions. The results of F_{ST}, D_S and D_A consistently groups Limuru, Isiolo and Voi populations into one cluster from shared alleles suggesting that these populations may be interacting in their native range. It is not clear how these populations share alleles. Overall the results of F_{ST}, D_S and D_A are consistent with those of mt DNA markers in grouping populations according the altitudes. Therefore, there exist conserved alleles prevalent in the native areas which have not been fragmented that enable separation of honey bees according to the elevations or altitude levels despite hybridization and swarming of bees. These results are congruent with the results of phylogenetic relationships based on COI and Cytb-tRNAser sequence analysis.

5.1.3.5 Analysis of Molecular Variance (AMOVA)

AMOVA was employed to assess the component of genetic diversity attributable to variance between and within three different population groupings namely; regions, altitude and subspecies. The highest genetic diversity from variance in the three groupings was ascribed to within populations' variation. These results are corroborating the results of F_{ST} and G_{ST}, which showed that a high percentage of genetic variation was within populations as opposed to among groups. Consequently, within-population diversity is sustained to enable the honey bee populations to adapt to future demands and conditions. A small part of the variability was ascribed to the among-population variability suggesting that the honey bee populations are not differentiated enough and they have a common history of interbreeding. This is predictable from *A. m. scutellata* migration and hybridization practices with other subspecies. Among groups less than 5% of genetic variance accounted for the molecular variance among East African populations. There was no significant variation between Central, Western and Coast populations and they lacked a strong regional structure to differentiate them. This means there is sharing of alleles between these regions and it is difficult to differentiate populations according to regions. North eastern population depicted a significant variance differentiating it from other regions. Eastern populations also varied significantly with Western, Coast and Central populations demonstrating that although hybridization occurs from savanna to adjacent high and low altitude areas and vice versa the subspecies found in these regions are adapted to their areas and still retains their genotypes to certain proportions. The results of variance attributed to groups according to altitude shows that lowland subspecies interacts more with mid altitude subspecies than highland and mid altitude subspecies. This is corroborated by among groups low variation from molecular variance (0.002) observed between Eastern and Coast regions while the molecular variance between Eastern and Central was approximately 12.5 times higher (0.025). The molecular variance between Eastern and Western was approximately 15 times higher suggesting that molecular variance increases as the distances with which honey bees have to travel. More analysis with more sampling to confirm these findings are recommended. Molecular variance between highland and

lowland subspecies was high indicating a low interaction and less intermixing between the subspecies. However mid altitude areas form an interphase between high and low altitude areas through which races intermix before they move upwards to high altitude and downwards to low altitude areas. With more migrations between high, mid and low altitude areas and with more commercial breeding activities within these areas a homogeneous population is expected to be established with time and a small regional genotype variance conserved will become rare. In terms of subspecies variance, *A. m. litorea* and *A. m. monticola* subspecies are the most related while *A. m. scutellata* is more related to *A. m. litorea* than to *A. m. monticola*. In the absence of *A. m. yementica* and *unicolor*, the three subspecies of *A. m. scutellata*, *A. m. litorea* and *A. m. monticola* are not significantly different from each other. The three subspecies interact at high, mid and low altitude regions of East Africa. *A. m. yementica* was found to more related to *A. m. litorea* that to *A. m. monticola* and *A. m. scutellata* respectively. *A. m. yementica* and *A. m. litorea* borders each other in the vast north eastern Kenya areas that receive occasional drought and limited resources and there may be gene flow between them. It is not clear why the invasive *A. m. scutellata* has not ventured into north eastern areas of Kenya. The adaptability to drought areas and migration patterns of this subspecies needs a further consideration in future studies. *A. m. unicolor* and *A. m. yementica* subspecies clearly separates from the other East Africa subspecies of *A. m. scutellata, A. m. monticola* and *A. m. litorea* as supported by the molecular variance. Taking into account that *A. m. yementica* is a Kenyan honey bee subspecies it is expected that observed molecular variance between this subspecies and other subspecies will reduce in over time leading to an establishment of a common gene pool.

5.1.3.6 PCA population differentiation by regions

Principal Component Analysis (PCA) results suggested that the honey bee populations can be separated into four clusters consistent with mtDNA data analysis, pairwise NJ trees drawn from F_{ST} and genetic distances D_S and D_A and STRUCTURE results. Typically, Madagascar population separated from the other populations due to its unique genotype comprising of *A. m. unicolor*. Limuru, Voi

and Isiolo populations clustered together suggesting they share a genotype. It is not clear why Limuru population falls on this axis. Mathiakani, Kasanga, Kathiani and Tanga clustered suggestive of an Eastern region genotype mainly consisting of *A. m. scutellata*. Taita, Aberdare, Nairobi, Bulindi, Malindi and Kakamega formed a cluster suggestive of a Highland regions genotype. According to PCA, Kakamega and Taita populations were the most closely related. The two areas are high altitude with mountainous habitats but other results in this study do not corroborate this finding. Probably there is a shared genotype adapted to these conditions. Although the first cluster populations are grouped together they differ significantly on the second axis indicating they retain a genotype which they don't share. The results of PCA are also supported by the results from pairwise F_{ST} relatedness and phylogenetic analysis using genetic distance methods. The consistent variation between Madagascar and other populations in this study is a reflection of different allele frequencies rather than unique alleles in Madagascar population.

5.1.3.7 PCA by altitude levels

Climatic variations, geographical features, altitude among other factors influence the migration and swarming patterns of the honey bees in East Africa especially Kenya (Raina & Kimbu, 2005). At an altitude above 1600m the full-black *A. m. monticola* is found; at an altitude between 600-1600m three-yellow banded *A. m. scutellata* is found while at an altitude below 600m, two-yellow banded *A. m. litorea* is found. Depending on the genotype honey bee subspecies adapt to high, mid, or low altitudes. From the analysis mid altitude bees forms a central genotype that interacts with high altitude bees and low altitude bees consistent with AMOVA results. High altitude bees seem to interact less with low altitude bees probably due to the mid altitude buffer resulting from geographical barriers and climatic conditions separating these areas. Since there is a clear separation based on altitude factor, there exist some conserved genotypes that uniquely show a dissection in the honey bee adaptations in terms of elevation levels. This does not rule out intermixing of the honey bee subspecies between the three altitude levels due to swarming and migrations.

5.1.3.8 PCA by known subspecies

PCA analysis of *A. m. scutellata*, *A. m. litorea* and *A. m. monticola* subspecies showed that these species are closely related while *A. m. yementica* and *A. m. unicolor* are distantly related to the three subspecies. *A. m. scutellata* is more related to *A. m. monticola* than *A. m. litorea* subspecies contradicting the results of AMOVA analysis. This drift is expected for closely related subspecies and is governed by the marker types and number, genotype and the geographic origin of the samples analyzed. At high altitudes *A. m. scutellata* is expected to hybridize more with *A. m. monticola* and in low altitudes *A. m. scutellata* is expected to hybridize more with *A. m. litorea* which is distributed along the coastal areas. The extent of hybridization largely depends on the season of migration and availability of flora. Markedly there exist very little differences between the three subspecies. On the other hand, *A. m. unicolor* and *A. m. yementica* do not share much genotype with the other three subspecies and are definitely distinct. However, *A. m. unicolor* seems to be more related to the three subspecies than *A. m. yementica*. The reason for this is not clear but *A. m. yementica* is suspiciously a migrant subspecies.

5.1.3.9 Structure results analysis

STRUCTURE results suggested that honey bee populations can be separated into four main clusters. A plot of the mean and standard deviation of the posterior probabilities, ln Pr (X|K) and the modal value for the distribution of ΔK against K (Evanno *et al*., 2005) confirmed that the true value of K is 4 (K = 4). Apart from Madagascar population which separated, the other populations are intermixed in three clusters and further sub-structuring was needed. The three clusters are supported by results of COI data, COI-COII and Cytb-tRNA^{ser} intergenic regions sequence analysis, pairwise F_{ST} and genetic distances D_S and D_A. Even with further substructuring of cluster 1, Kathiani Kasanga and Malindi populations appear to share a substantial percentage of their genotype suggesting they are closely related. Tanga and Kakamega clustered together in a second group suggesting that they share a significant genotype. Substructuring of cluster 2 shows Aberdare and Nairobi populations are closely related while Bulindi and Mathiakani are separated out into

their own groups. Aberdare and Nairobi regions border each other and migrations and hybridization activities are suspected in these areas. Substructuring of cluster 3 separates Isiolo and Voi populations to form distinct genotypes while Taita and Limuru are closely related. Taita and Limuru are high altitude areas forming a buffer for the high altitude genotype suspected to be from *A. m. monticola* subspecies. A review of structure results shows it was difficult to separate the populations into distinct clades as there is lot of intermixing between genotypes and populations.

5.1.3.10 Genetic assignment of individual to populations

The assignment test of Geneclass 2.0 (Piry *et al*., 2004) software is a direct method to ascertain population membership of individuals by Bayesian statistics. The low self assignment values imply that the populations studied consists of mixed genotypes from their neighbours suggesting admixture resulting from hybridization and migration activities. The self-assignment probability values were low (<50%) for all populations indicating that there is some introgression from contiguous areas. The probability of finding the East African genotype in Madagascar population is lower than the probability of finding the Madagascar genotype in East African populations suggesting that Madagascar genotype is conserved in the East African honey bee with the latter representing an older population. The probability of finding genotypes from other populations in Voi and Isiolo populations was also low as compared to assignments of their genotypes to other populations. This means the present genotype is as result of evolution through mutation, genetic drift and selection or may have been derived from migrants from adjacent regions. Kasanga and Kathiani populations were assigned a high amount of genotypes from other East African populations suggesting that they could be the source of migration of honey bee subspecies into other regions. This means that the two regions also harbor an affluent reservoir of genotypes from other regions with high genetic diversity suitable for its adaptability in savanna, high altitude lands and varying climatic conditions. Considering that the two regions consist mainly of *A. m. scutellata*, perhaps the two regions are the source populations of migrants and hybrid populations of this subspecies found in other regions. Mathiakani, Nairobi and Bulindi populations had a

self assignment values less than that assigned to Eastern populations (Kasanga and Kathiani). Mathiakani could have derived its honey bees from Kasanga. Apart from Malindi, Voi, Isiolo and Madagascar populations (<5%), Nairobi shares a fair amount (>11%) of its genotype with other East African populations. Nairobi is a cosmopolitan city and is central to high and low altitude areas of Kenya. Therefore, vertical migrations from low savanna to mountainous regions occur through this interphase forming a hybrid zone and retaining genotypes from both dissections. Malindi, Isiolo Voi and Mathiakani populations are separated by a significant distance from Nairobi and this may explain why these populations share very little genotype with Nairobi population. It is not clear why Bulindi population has much of its genotype assigned to Kasanga and Kathiani population though a substantial amount of its genotype is also found in Taita (11.6%), Kakamega (21.8%) Limuru (14.9%) and Aberdare (20.1%) high altitude populations consistent with clustering in observed in Nei's D_A genetic chord distances in the NJ tree. This suggests that while Bulindi honey bee has hybridized with *A. m. scutellata* in low altitude areas, it has conserved variability suitable for survival and adaptability in high altitude areas. Apart from Madagascar population the lowest assignments were observed in Isiolo and Voi populations. Isiolo is a semi arid area while Voi is an area between Coast and Eastern region bordering Tsavo National Park in east. From the study it is possible to hypothesize that although horizontal migrations occur in these areas there is less hybridization due to low beekeeping activities. A conclusion can be made that Isiolo, Voi and Madagascar outgroup population have separated from the other East African populations due to evolutionary forces acting on them.

5.1.4 Characterization of honey bee foraging and stinging behaviors

The average nectar stored in combs (npn) was 0.69±0.24 (see Table 27) which means that between 45% and 93% of the honey bee combs occupied by the nectar. The rest of the combs were either empty or had pollen (see Appendix 3.0). This suggests that on average honey bee colonies foraged for and stored more honey than pollen in the hives. Since honey bees seem to respond quickly to availability of floral resources, seasonal patterns may still appear (Hepburn & Radloff, 1998). The mean for sos is

0.43 indicating that the sealed combs were only 43% compared to the 57% opened combs. In tropical regions, floral resources are available throughout the year, but are not always sufficient to warrant colony growth and subsequent production of sexuals and swarming. Therefore, colonies of honey bees respond quickly to the changing environmental conditions rapidly by adjusting the brood size and numbers of sexuals produced. The average time in seconds for the first sting (AvTS) was 11.87secs (Table 27) for the populations studied showing that the honey bee colonies responded relatively fast to the aggression. This is also consistent with the average sting numbers (AvSN) recorded at 21.46 stings within the first one minute of the experiment and the frequency of the qualitative aggressive state (A) was 0.5532 as compared to the mild bees (M) was 0.4468 (This means that most African honey bees are aggressive compared to the mild type honey bees. The aggression is correlated with the need to protect the stored honey in the combs and the brood.

There is a negative correlation between sealed brood and the nectar foraging (sos vs npn at –0.03051) variables. It appears that honey bees leave combs open in readiness for the storage of honey. A negative correlation was also observed between sealed brood and the average time of the first sting (sos vs AvTS at –0.41329) quantitative variables. This means that honey bees do not find a need to defend empty combs. Average time of the first sting (AvTS) and average sting numbers (AvSN) showed a positive correlation with the nectar foraging variable showing that foraging bees respond faster to irritation from predators at the colony level. The Pearson correlation coefficient between *sting* and *pln*, qualitative variables (from contingency tables) was 0.184458 (see Table 29) showing that they are positively correlated although this was not statistically significant. This implies that African honey bees with a high guarding and stinging behavior also forage more for nectar.

5.1.5 Candidate genes identification based on foraging and stinging QTLs

The study of stinging and foraging behavioral aspects of feral African honey bees identified important associations between these phenotypes with the markers genotype and proposed candidate genes. A few markers were found to be in linkage disequilibrium (LD) suggesting they could have been coinherited. These pairs of loci

with significant LD (P<0.001), were 6.7% representing only 8 out of 120 pairwise comparisons. This is a small percentage of the overall marker combinations which can be explained by the rate of LD decay which is dependent on multiple factors, including the population size, the number of founding chromosomes in the population, and the number of generations for which the population has existed. A fairly constant rate of LD decay is expected due to honey bees swarming and hybridization behavior. In fact, colonies of *Apis mellifera* issue 4-5 swarms yearly (Bego, 1982; Roubik, 1989) and under favourable conditions up to twelve swarms per year have been reported (Winston, 1992). In honey bees, the old queen leaves the nest with about half of the bees. From that moment contact between the two halves is lost. The swarm has to find another nesting place that can be far away from the original colony. As such, different honey bees' subpopulations have different degrees and patterns of LD. The large population sizes found in bees explains why LD for honey bees has a small percentage. However, honey bees have genes that are in LD despite a high rate of recombination events associated with honey bees. High r^2 values indicate that two microsatellites convey similar information, as one allele of the first microsatellite is often observed with one allele of the second microsatellite, so only one of the two microsatellites needs to be genotyped to capture the allelic variation.

Out of 16 markers tested four markers showed a significant correlation with the phenotypes and are associated with their respective phenotypes. Marker AJ509381 (LG12) was found to be strongly associated with three of the four phenotypes namely; the Average Sting Number (AvSN), Average Time of the first Sting (AvTS) and sealed brood (sos). The gene associated with this marker is CUGBP Elav-like family member 4-like isoform which is characterized as protein coding gene in *Apis mellifera* (Kaplan & Linial, 2006). However, in humans it has molecular function in nucleotide binding, translation repressor activity, nucleic acid binding, mRNA binding and BRE binding. This gene is a cellular component of nucleus, nucleolus, cytoplasm and the biological processes involved are alternative mRNA splicing, via spliceosome, regulation of alternative mRNA splicing, via spliceosome, mRNA splice site selection, germ cell development, embryo development, positive

regulation of mRNA splicing, via spliceosome, negative regulation of excitatory postsynaptic membrane potential. It is not clear which role this gene plays in honey bee stinging or forgaing behavior. Marker AJ509307 was significantly associated with Average Sting Number (AvSN) which had two genes; tbc1 domain family member 13-like and transcription factor ap-4. TBC1 domain family member 13-like is a member of Rab family of proteins mostly involved in vesicle trafficking, defining the identity and routing of vesicles but no apparent role has been identified in honey bees. Transcription factors are often important in development or cell activity that include; neurogenesis, myogenesis, hematopoiesis, sex determination and gut development. These factors could have an overall effect of age of first foraging in honey bees. Finally, markers AJ509384 and AJ509721 were positively associated with nectar foraging (npn) phenotype and have aryl hydrocarbon receptor nuclear translocator homolog isoform and SAGA-associated factor 29 homolog associated with them respectively. Aryl hydrocarbon receptor nuclear translocator has been shown to interact with many other factors and is involved in many important biological processes such as response to hypoxia, embryonic placenta development, positive regulation of endothelial proliferation. Therefore, this gene maybe important not only to foraging but also to the stinging behavior observed in honey bees. SAGA-associated factor 29 homolog in *Apis mellifera* is predicted to be a component of Ada2/Gcn5/Ada3 transcription activator complex (or SAGA complex) involved in transcriptional regulation through association with methylated histone residue binding in chromatin remodelling. It promotes acetylation, eviction, and methylation of nucleosomes in transcribed coding (Mosesson *et al*., 2014). This analysis demonstrates that association mapping analysis can help identify the molecular markers significantly linked to traits of interest.

5.2 Conclusions

5.2.1 Phylogenetic relationships of honey bee subspecies

The Kenyan honey bees have been previously classified according to geographical location and ecological zone based on morphological data such as body color (abdominal yellow banding patterns) and size (Raina & Kimbu, 2005). Bees from distant mountains are more similar to each other than to their direct savanna neighbours (Meixner *et al*., 1994; 2000) but no distinct polymorphism has been able to separate the populations (Hepburn *et al*., 1999). According to Hepburn *et al*., (1999), *A. m. monticola* found on the mountain areas has been regarded as an ecotype of the surrounding savanna subspecies, *A. m scutellata* and *A. m litorea* subspecies, although subsequent studies separates them (Meixner *et al*., 2000; Raina & Kimbu 2005). The difficulty in morphological and molecular identification lies in the hybridization of the three honey bees' subspecies namely, *A. m scutellata*, *A. m litorea* and *A. m. monticola*. Hybridization occurs horizontally across savanna areas and vertically between savanna areas and the mountain bees (Meixner *et al*., 2000). Raina & Kimbu (2005) insinuated geographical races to describe bees based on elevation, bee size, and abdomen color and climate considerations. However current trends in climate change, hybridization effects, swarming and migration of bees has compromised the previous findings on geographical races but the extent in unknown. This was confirmed in the samples collected as it was not unusual to find the three subspecies *A. m scutellata*, *A. m litorea* and *A. m. monticola* intermixed in one colony. It is now more challenging to separate the subspecies based on morphological characters such as size and banding pattern.

Among the honey bee samples analysed, the Eastern region appears to be the epicenter of the honey bee activities because the branches formed from the results of COI gene (barcode), COI-COII and Cytb-tRNA^{ser} intergenic regions showed haplotypes sharing with the regions of East African populations except Tana River samples. Due to this sharing, the Eastern population suggests honey bees evolutionary ancestral source to the other East African populations. It appears that the Eastern population habors a rich reservoir of the African A haplotype and the

associated mitotypes due to its interaction with its neighbours. Therefore, the Eastern region is central to iteractive activities of the other East African honey bee subspecies due to its migration, hybridization and swarming events. Considering that this region lies in a savanna area and mainly consists of *A. m. scutellata* it is not unexpected as this subspecies is known to be highly mobile, easily absconds and often performs seasonal migrations over vast distances (Smith, 1961; Fletcher, 1978; Hepburn & Radloff, 1998).

Typically, it was possible to find different subspecies or hybrids in one colony. However, based on elevation, results of COI gene, COI-COII and Cytb-tRNA^{ser} intergenic regions suggest that resident haplotypes are still maintained in their native areas. Therefore, bees from mountainous areas are more related to each other than to their counterparts found in the vicinity lowland areas and vice versa. Notably a common genetic pool is shared in high altitude areas of Western and Central region and Taita hills. Similarly, such a genetic pool predominates in coastal Lowland and Mid-Altitude Eastern regions consistent with previously published results by Raina and Kimbu (2005). In unfragmented areas such as Tana River, the resident haplotypes are conserved. More of these areas should be sampled for further analysis. Overall the results of COI gene (barcode) were consistent with Cytb-tRNA^{ser} intergenic region analysis.

5.2.2 Genetic diversity of honey bees

The East African honey bee populations studied here display a great genetic variation which provides some advantages to improve the honey bee culture. The survival of local or native honey bees' populations' results from a number of traits commonly perceived as adaptive, many of which are related to reproductive, swarming and defensive behavior. From the results documented here, the mean number of alleles and heterozygosity levels observed in these subspecies and populations signify a high genetic diversity reservoir and an affluent genetic resource that may enable them to adapt to varying climatic conditions and altitude levels and to resist pests and diseases. Analyzing genetic diversity of *Apis mellifera* strains in East Africa could

provide a theoretical foundation for the research on the conservation and reasonable utilization of honey bee populations.

5.2.3 Population genetic differentiation of honey bee subspecies

East African honey bee populations were found to be genetically differentiated based on geographical proximity, elevation and different subspecies. The Eastern region of Kenya appears central to migration, swarming and hybridization activities in East Africa from population assignment results. The major subspecies occupying this region is *A. m. scutellata* which is a highly mobile, migrating and hybridizing insect. The results of clustering, admixture and phylogenetic showed that the dynamics of the honey bee subspecies is associated with a relatively stable population demographic structure, especially in unfragmented habitats, natural forests and mountainous regions. Isiolo and Voi populations present unfragmented natural areas with a relatively stable population demographic structure due to the climatic conditions and proximity to preserved national park. Increasing deforestation of mountain regions and climate change may have necessitated the movement of honey bees from their habitats leading to intermixing and hybridization in most of the areas studied. However, the knowledge of the genetic relationship and admixture among East African honey bee subspecies is crucial for harnessing economic important behavioral traits and in their conservation efforts. Overall the genetic profile of the considered populations suggests a close relationship between the regions and subspecies. The climate change effects, modern beekeeping methods, hybridization effects, swarming and migration are responsible for homogeneous honey bee populations observed in most geographical regions of Kenya.

5.2.4 Honey bee foraging and stinging behavioral traits

Although the African honeybee is very aggressive, some tend to sting less than others and are more docile. Aggressive strains respond faster in greater numbers although each bee stings once. In this study the dissection between these behaviors was detected in the colonies that were visited. For instance, for two colonies in the same locality, one colony was found to be highly aggressive than a secondone

showing there was a clear dissection in their defensive capability. More aggressive colonies make them more productive and resilient. The aggression trait can be manipulated and utilized for high honey productivity through selective breeding. In terms of foraging, some colonies were found to have a greater emphasis on pollen collection, and a more rapid conversion of pollen into brood while others collected nectar and had massive honey reserves. Studying of the foraging traits and the dynamics of honey bee hoarding and foraging activities may assist in selective breeding for pollen or nectar resources.

5.2.5 Candidate genes Identification

Many economically valuable honey bee products such as honey, royal jelly, propolis and pollen are influenced by polygenic traits which can be measured at the colony level. The role of the six genes discovered cannot be conclusively get discussed in this thesis. However, the identification of genes and analysis in this study demonstrated that association mapping analysis can help identify the genes that are significantly linked to the traits of interest which may further be investigated for their role in hive productivity or in pollination activities. Although this needs additional work, the candidate genes so identified may assist in marker selection of a honeybee line with specialized hive productivity or better pollinators of crops.

5.3 Recommendations

The use of mitochondrial markers to identify and to differentiate between East African honey bee subspecies was unable to resolve *Apis mellifera* to susbspecies level but had a better view when subspecies were grouped according to geographical elevations. COI gene (barcode), COI-COII and the Cytb-tRNA^{ser} intergenic regions phylogenetic results were congruent but did not sufficiently resolve the deep evolutionary relationships. Based on variability in the COI-COII intergenic region, presence of an African A haplotype and mitotypes A1 and A4 were confirmed. However due to the limited sample size and subsequent resources, only a few samples from Eastern region were analysed. The greatest limitation in resolving intra-specific categories in the East African subspecies were due to inadequate

sampling, inadequate resources, incompatible differences in sample sizes, sampling distance and range of geographical scales employed. The results reported here should be explored further by use of more mitochondrial markers such as the large ribosomal RNA (lrRNA), Cytb-tRNA^{ser} intergenic region, COI, ATPase 6-8 and COI-COII intergenic region genes using a larger sample size. Further studies focusing on more samples and extended sampling regions within Kenya and East Africa region is recommended for future tangible analysis of the A lineage. In unfragmented areas where low migrations and hydrization occur (e.g. Tana River and mountain regions) where resident haplotypes are conserved, more samples should be collected for further analysis. Use of samples from other sub Saharan African regions is recommended.

Microsatellite data confirmed that hybridization occurs between *A. m scutellata*, *A. m litorea*, and *A. m. monticola* subspecies horizontally (across savanna areas) and vertically (between savanna areas and the mountain bees) and it is intricate to differentiate the subspecies. Additionally, the East African subspecies have a rich reservoir of conserved genes and the genetic diversity is high. Therefore, within species diversity is vigorously maintained to enable honey bee to adapt to future challenges and demands. Conservation is important from a biodiversity perspective, where a priority should be laid on preserving the endemic races of honey bees in the East African region. More sampling of geographical regions covering unfragmented, lowland, coastal, savanna and mountain regions of East Africa is recommended in order to have a conclusive representation of the available genetic resources. Presently, only meager information is available on the genetic diversity of the East African honey bees. Seasonal migrations should also be evaluated to understand the dynamics of honey bee subspecies within East Africa. It is from this perspective that the East African honey bees can be protected from a number of negative effects derived from human practices (e.g., inappropriate use of pesticides and herbicides) leading to better management of apicultural practice.

Studying of the foraging traits and the dynamics of honey bee hoarding and foraging activities may assist in selective breeding for pollen or nectar resources. For instance,

African bees are more aggressive than the docile European honeybees which make them more productive and resilient. The aggression trait can be manipulated and utilized for the East African's advantage through selective breeding. Since African scientists are interested in discovering valuable traits in the African honey bee that other world honey bee populations lack, these traits could be selected-for to sustain their role as pollinators and hive producers. Identification of the genes in this study was crucial as a preliminary to realization of this objective. Better experimentation and sampling towards this goal is recommended. More traits should be characterized and using recent techniques more genes should be identified that are critical in selective breeding of a proficient honey bee that is able to survive infestation and diseases, facilitate pollination, has high hive productivity and continues to stay healthy and breed continuously.

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APPENDICES

Appendix 1: Protocols

Appendix 1.1: Insect DNA extraction protocol by Hunt and Page (1992)

The following procedure for obtaining insect DNA relies on a non-ionic detergent, hexadecyltrimethylammonium bromide (CTAB) to lyse cells and has been previouly used for RAPD analyses of bees (*Apis*, *Bombus*, *Frieseomelitta*, *Trigona*, *Scaptotrigona*, *Melipona*), wasps (*Vespid* species) ants (*Leptothorax*, *Pogonomyrmex*), beetles (*Tribolium*) and mites (*Varroa*).

Materials and Equipment: Polypropylene plastic pestles, Electric hand-held drill (mortar), Fluorometer (spectrophotometer), Microcentrifuge (such as Eppendorf 5415)

Solutions: Phenol/chloroform-phenol is pre-equilibrated to pH 7.4 and mixed 1:1 with chloroform (store at -20°C in 50 ml aliquots), Chloroform, 5-10% Bleach, Proteinase K (20 mg/ml in water, store at -20°C), 3M Sodium Acetate (pH 5.2), Ethanol (100% and 70%, keep at -20°C), Lyses solution (1% CTAB, 50mM Tris, pH 8, 10mM EDTA, 1.1M NaCl, autoclave or boil the solution for ten minutes and store at room temperature)

Honey bee samples: Samples of larvae or adult honey bees may be used for DNA extraction. Alcohol-preserved specimens may be used, by keeping the alcohol concentration high (about 95%). This is done by adding fresh alcohol after bees have soaked for several hours.

DNA extraction protocol

1. Thaw the phenol/chloroform for the day's extractions
2. Sterilize individual honey bees in 5-10% bleach to remove impurities and contaminants, then rinse in sterilized double distilled water and dry at room temperature for up to one hour
3. Put the bee in a 1.5ml microcentrifuge tube containing CTAB lyses solution. Use 200µl for adult workers and 350 µl for drones and queens
4. Add proteinase K to the lyses solution (2µl for adult worker bees and 5µl for the larger males, for a final concentration of about 100µg/ml).
5. Grind the bee quickly and thoroughly with the electric drill (or by hand) in the lysis solution. Use polypropylene plastic pestles that fit the microcentrifuge tubes. About 4 individuals can be processed at one time with a drill. Use a clean pestle for each bee, or rinse thoroughly between bees. Grind until the solution becomes viscous. Then immediately place samples in a 65°C water bath for 30 minutes

Note: CTAB and polysaccharides will come out of solution if salt concentration is too low (see Fang *et al*., 1992). CTAB can also form a complex with DNA and can be used to precipitate it (Murray & Thompson, 1980).

6. Extract the samples once by adding an equal volume of 1:1 phenol/chloroform to each tube. Invert all of the tubes about twenty times (very gently if relatively high molecular weight DNA is needed for southern blotting). Then, centrifuge at 12,000-17,000g for 10 min (full speed in a microcentrifuge)

   Caution: Phenol is both a strong oxidant and carcinogen, and can cause burns. Gloves should be worn and extractions should be done in a fume hood

7. Carefully draw off the aqueous (upper) phase and transfer this supernatant to a new, labeled tube. Avoid drawing up any of the interface. Discard the bottom phase with the old tube.

   Note: If the phenol is not properly equilibrated to a high pH (>7.0), DNA will be lost in the phenol/chloroform extraction. Proteinase K that is no longer active or insufficient grinding in Step 2 will also result in low yields

8. The DNA solution is then extracted in the same way with chloroform and centrifuged for 2-3 min at high speed. An additional phenol extraction will help remove more protein and polysaccharide, but is not necessary for good PCR results. Always finish with the chloroform extraction to remove excess phenol
9. DNA is precipitated by adding one-tenth volume of 3M sodium acetate and two volumes cold ethanol and incubating the samples for 10 min at -70°C
10. Centrifuge the samples for 20min at approximately 4000 - 6000 x g, and then remove all of the ethanol with a pipettor. The pellet is then washed with cold 70% ethanol and allowed to dry (tube inverted) for about 1-3 min to remove traces of ethanol. Do not let the pellet dry for too long or the DNA pellet might not dissolve (especially if it is high molecular weight DNA)
11. The DNA pellets are dissolved in 10 mM Tris buffer (pH 7.6) with 1 mM EDTA (TE). Use 50-100µl of TE per sample. After adding TE, heat the samples to 65°C in the water bath for 10 min and "flick" the tubes with your finger to help dissolve the DNA. Keep the tubes on ice or in a freezer
12. Quantify the DNA with a fluorometer or spectrophotometer, following the manufacturer's instructions. Always spin DNA samples at full speed for 2-3 minutes in a microcentrifuge prior to pipetting a sample for quantification or dilution. Centrifugation insures that any undissolved DNA is pelleted

    Caution: The dye used with the fluorometer is a potential mutagen because it binds to DNA

13. It is convenient to dilute all of the concentrated DNA stocks to 100ng/µl as they are quantified by adding cold TE. In addition to the concentrated stocks, prepare dilute aliquots for PCR (3ng/µl) by adding modified TE that contains only 0.3mM EDTA. The molecular weight of the DNA may be checked on an agarose

gel, if desired. If the genomic DNA is somewhat degraded, it may be desirable to prepare more concentrated stocks for PCR (e. g., 20ng/µl)

Appendix 1.2: DNA extraction using Qiagen® DNAeasy Blood and Tissue extraction kit

Preparation of honey bees

1. Female worker bees were collected from apiaries in vials containing absolute ethanol. Samples were bleached in 5% sodium hypochlorite solution and then rinsed thoroughly in double distilled water before drying on a paper towel for 30 minutes.

2. Place the entire bee in a 1.5ml eppendorf tube and add 180µl of buffer ATL.

3. Crush the insect using a polypropylene pestle

4. Add 20µl of Proteinase K. Mix by vortexing and incubate at 56°C until completely lysed. Normally 1-3hrs is enough or overnight incubation. For each 30 minutes, mix them by vortexing.

5. Add 200µl of buffer AL to the sample and mix thoroughly by vortexing. Then add 200µl of absolute or 96% ethanol and mix again thoroughly. Alternatively premix buffer AL and ethanol and add together

6. Pipet the mixture into a DNAeasy mini spin column in a 2ml collection tube

7. Centrifuge the mixture at ≥6000 x g (8000rpm) for 1 minute and discard the flow-through and collection tube

8. Place the spin column in a new collection tube and add 500µl buffer AW1. Centrifuge for 1min at ≥6000 X g (8000rpm) and discard the flow-through and collection tube

9. Place the spin column in a new 2ml collection tube and add 500µl buffer AW2. Centrifuge for 3 minutes at 20,000 x g (14,000rpm) and discard the flow-through and collection tube. The spin column is removed carefully to avoid contact with the flow-through.

10. Transfer the spin column to a new 1.5ml or 2ml microcentrifuge tube and add 200µl buffer AE for elution. Incubate the samples for 1 minute at room temperature and centrifuge for 1 minute at ≥6000 X g (8000rpm). This step may be repeated for maximum yield

Appendix 2: Sequences

Appendix 2.1: COI gene CLUSTAL 2.1 MSA

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H088|Eastern|Bidii|scutellata7     GATCTTGTATATTATTCTAGCTTTATGATCTGGAATACTAGGATCATCAA
H091|Coast|Chambuko|litorea8       GATCTTGTATATTATTCTAGCTTTATGATCTGGAATACTAGGATCATCAA
A.                                 GATCTTGTATATTATTCTAGCTTTATGATCTGGAATACTAGGATCATCAA
H052|Eastern|Mathiakani|scutel     GATCTTGTATATTATTCTAGCTTTATGATCTGGAATACTAGGATCATCAA
H055|Eastern|Mathiakani|scutel     GATCTTGTATATTATTCTAGCTTTATGATCTGGAATACTAGGATCATCAA
H056|Coast|Chawiya|litorea1        GATCTTGTATATTATTCTAGCTTTATGATCTGGAATACTAGGATCATCAA
H058|Coast|Chawiya|litorea1        GATCTTGTATATTATTCTAGCTTTATGATCTGGAATACTAGGATCATCAA
H059|Coast|Chawiya|litorea1        GATCTTGTATATTATTCTAGCTTTATGATCTGGAATACTAGGATCATCAA
H060|Coast|Chawiya|litorea1        GATCTTGTATATTATTCTAGCTTTATGATCTGGAATACTAGGATCATCAA
H036|Eastern|Mumoni|litorea12      GATCTTGTATATTATTCTAGCTTTATGATCTGGAATACTAGGATCATCAA
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H005|Eastern|Kasanga|scutellat    TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H012|Eastern|Kasanga|litorea96    TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
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H011|Eastern|Kasanga|monticola    TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H019|Eastern|Kathiani|scutella    TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H023|Eastern|Kathiani|litorea9    TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H022|Eastern|Kathiani|scutella    TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H021|Eastern|Kathiani|scutella    TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H024|Eastern|Kathiani|scutella    TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H025|Eastern|Kathiani|scutella    TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H027|Eastern|Kathiani|scutella    TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H028|Eastern|Kathiani|litorea3    TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H029|Eastern|Kathiani|scutella    TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
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H030|Eastern|Kathiani|scutella     TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H016|Eastern|Kathiani|scutella     TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H015|Eastern|Kasanga|scutellat     TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H008|Eastern|Kasanga|monticola     TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H064|Coast|Ronge|scutellata2       TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H065|Coast|Ronge|monticola2        TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H063|Coast|Ronge|scutellata2       TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H061|Coast|Ronge|scutellata2       TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H062|Coast|Ronge|litorea2          TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H072|Eastern|Mituki|scutellata     TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H074|Eastern|Mituki|scutellata     TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H080|Tanga|Sengoma|litorea5        TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H079|Tanga|Sengoma|litorea5        TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H078|Tanga|Sengoma|litorea5        TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H077|Tanga|Sengoma|litorea         TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H076|Tanga|Sengoma|litorea5        TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H069|Coast|Hewani|scutellata3      TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H068|Coast|Hewani|scutellata3      TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H067|Coast|Hewani|scutellata3      TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
H066|Coast|Hewani|scutellata3      TGAGACTTATTATTCGAATAGAATTAAGATCCCCAGGATCATGAATTAAT
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H088|Eastern|Bidii|scutellata7     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H091|Coast|Chambuko|litorea8       AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
A.                                 AATGATCAAATTTATAATACAATTGTTACTAGTCATGCATTCCTAATAAT
H052|Eastern|Mathiakani|scutel     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H055|Eastern|Mathiakani|scutel     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H056|Coast|Chawiya|litorea1        AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H058|Coast|Chawiya|litorea1        AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H059|Coast|Chawiya|litorea1        AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H060|Coast|Chawiya|litorea1        AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H036|Eastern|Mumoni|litorea12      AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H037|Eastern|Mumoni|litorea12      AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H038|Eastern|Mumoni|litorea12      AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H039|Eastern|Mumoni|litorea12      AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H040|Eastern|Mumoni|litorea12      AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H041|Eastern|Mumoni|litorea80      AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H042|Eastern|Mumoni|litorea80      AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H043|Eastern|Mumoni|scutellata     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H044|Eastern|Mumoni|scutellata     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H045|Eastern|Mumoni|litorea80      AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H046|Eastern|Mathiakani|litore     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H048|Eastern|Mathiakani|litore     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H050|Eastern|Mathiakani|scutel     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H051|Eastern|Mathiakani|scutel     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H026|Eastern|Kathiani|scutella     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H053|Eastern|Mathiakani|scutel     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H017|Eastern|Kathiani|scutella     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H020|Eastern|Kathiani|scutella     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H031|Eastern|Mumoni|litorea19      AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTCCTAATAAT
H032|Eastern|Mumoni|litorea19      AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTCCTAATAAT
H033|Eastern|Mumoni|scutellata     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTCCTAATAAT
H034|Eastern|Mumoni|scutellata     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTCCTAATAAT
H035|Eastern|Mumoni|scutellata     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTCCTAATAAT
H073|Eastern|Mituki|monticola4     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTCCTAATAAT
H075|Eastern|Mituki|scutellata     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTCCTAATAAT
H081|Tanga|Usambara|scutellata     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTCCTAATAAT
H082|Tanga|Usambara|litorea6       AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTCCTAATAAT
H083|Tanga|Usambara|litorea6       AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTCCTAATAAT
H084|Tanga|Usambara|litorea6       AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTCCTAATAAT
H013|Eastern|Kasanga|scutellat     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H002|Eastern|Kasanga|scutellat     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H004|Eastern|Kasanga|scutellat     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H005|Eastern|Kasanga|scutellat     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H012|Eastern|Kasanga|litorea96     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H006|Eastern|Kasanga|scutellat     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H007|Eastern|Kasanga|scutellat     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H010|Eastern|Kasanga|scutellat     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H014|Eastern|Kasanga|scutellat     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H011|Eastern|Kasanga|monticola     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H019|Eastern|Kathiani|scutella     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H023|Eastern|Kathiani|litorea9     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
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H022|Eastern|Kathiani|scutella     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H021|Eastern|Kathiani|scutella     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H024|Eastern|Kathiani|scutella     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H025|Eastern|Kathiani|scutella     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H027|Eastern|Kathiani|scutella     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H028|Eastern|Kathiani|litorea3     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H029|Eastern|Kathiani|scutella     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H030|Eastern|Kathiani|scutella     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H016|Eastern|Kathiani|scutella     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H015|Eastern|Kasanga|scutellat     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H008|Eastern|Kasanga|monticola     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H064|Coast|Ronge|scutellata2       AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H065|Coast|Ronge|monticola2        AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H063|Coast|Ronge|scutellata2       AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H061|Coast|Ronge|scutellata2       AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H062|Coast|Ronge|litorea2          AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H072|Eastern|Mituki|scutellata     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H074|Eastern|Mituki|scutellata     AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H080|Tanga|Sengoma|litorea5        AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H079|Tanga|Sengoma|litorea5        AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H078|Tanga|Sengoma|litorea5        AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H077|Tanga|Sengoma|litorea         AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H076|Tanga|Sengoma|litorea5        AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H069|Coast|Hewani|scutellata3      AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H068|Coast|Hewani|scutellata3      AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H067|Coast|Hewani|scutellata3      AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
H066|Coast|Hewani|scutellata3      AATGATCAAATTTATAATACAATTGTTACTAGCCATGCATTTCTAATAAT
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H088|Eastern|Bidii|scutellata7     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H091|Coast|Chambuko|litorea8       TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
A.                                 TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H052|Eastern|Mathiakani|scutel     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H055|Eastern|Mathiakani|scutel     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H056|Coast|Chawiya|litorea1        TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H058|Coast|Chawiya|litorea1        TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H059|Coast|Chawiya|litorea1        TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H060|Coast|Chawiya|litorea1        TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H036|Eastern|Mumoni|litorea12      TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H037|Eastern|Mumoni|litorea12      TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H038|Eastern|Mumoni|litorea12      TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H039|Eastern|Mumoni|litorea12      TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H040|Eastern|Mumoni|litorea12      TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H041|Eastern|Mumoni|litorea80      TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H042|Eastern|Mumoni|litorea80      TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H043|Eastern|Mumoni|scutellata     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H044|Eastern|Mumoni|scutellata     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H045|Eastern|Mumoni|litorea80      TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H046|Eastern|Mathiakani|litore     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H048|Eastern|Mathiakani|litore     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H050|Eastern|Mathiakani|scutel     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H051|Eastern|Mathiakani|scutel     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H026|Eastern|Kathiani|scutella     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H053|Eastern|Mathiakani|scutel     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H017|Eastern|Kathiani|scutella     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H020|Eastern|Kathiani|scutella     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H031|Eastern|Mumoni|litorea19      TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H032|Eastern|Mumoni|litorea19      TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H033|Eastern|Mumoni|scutellata     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H034|Eastern|Mumoni|scutellata     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H035|Eastern|Mumoni|scutellata     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H073|Eastern|Mituki|monticola4     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H075|Eastern|Mituki|scutellata     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H081|Tanga|Usambara|scutellata     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H082|Tanga|Usambara|litorea6       TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H083|Tanga|Usambara|litorea6       TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H084|Tanga|Usambara|litorea6       TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H013|Eastern|Kasanga|scutellat     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H002|Eastern|Kasanga|scutellat     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H004|Eastern|Kasanga|scutellat     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H005|Eastern|Kasanga|scutellat     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H012|Eastern|Kasanga|litorea96     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
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H006|Eastern|Kasanga|scutellat     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H007|Eastern|Kasanga|scutellat     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H010|Eastern|Kasanga|scutellat     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H014|Eastern|Kasanga|scutellat     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H011|Eastern|Kasanga|monticola     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H019|Eastern|Kathiani|scutella     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H023|Eastern|Kathiani|litorea9     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H022|Eastern|Kathiani|scutella     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H021|Eastern|Kathiani|scutella     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H024|Eastern|Kathiani|scutella     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H025|Eastern|Kathiani|scutella     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H027|Eastern|Kathiani|scutella     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H028|Eastern|Kathiani|litorea3     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H029|Eastern|Kathiani|scutella     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H030|Eastern|Kathiani|scutella     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H016|Eastern|Kathiani|scutella     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H015|Eastern|Kasanga|scutellat     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H008|Eastern|Kasanga|monticola     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H064|Coast|Ronge|scutellata2       TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H065|Coast|Ronge|monticola2        TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H063|Coast|Ronge|scutellata2       TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H061|Coast|Ronge|scutellata2       TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H062|Coast|Ronge|litorea2          TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H072|Eastern|Mituki|scutellata     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H074|Eastern|Mituki|scutellata     TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H080|Tanga|Sengoma|litorea5        TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H079|Tanga|Sengoma|litorea5        TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H078|Tanga|Sengoma|litorea5        TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
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H076|Tanga|Sengoma|litorea5        TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H069|Coast|Hewani|scutellata3      TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H068|Coast|Hewani|scutellata3      TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H067|Coast|Hewani|scutellata3      TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
H066|Coast|Hewani|scutellata3      TTTTTTTATAGTTATACCATTTTTAATTGGAGGATTTGGAAATTGGCTTA
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H088|Eastern|Bidii|scutellata7     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H091|Coast|Chambuko|litorea8       TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCTCGAATAAATAAT
A.                                 TTCCTTTAATACTAGGATCACCTGATATAGCATTCCCCCGAATAAATAAT
H052|Eastern|Mathiakani|scutel     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCTCGAATAAATAAT
H055|Eastern|Mathiakani|scutel     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCTCGAATAAATAAT
H056|Coast|Chawiya|litorea1        TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCTCGAATAAATAAT
H058|Coast|Chawiya|litorea1        TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCTCGAATAAATAAT
H059|Coast|Chawiya|litorea1        TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCTCGAATAAATAAT
H060|Coast|Chawiya|litorea1        TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCTCGAATAAATAAT
H036|Eastern|Mumoni|litorea12      TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H037|Eastern|Mumoni|litorea12      TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H038|Eastern|Mumoni|litorea12      TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H039|Eastern|Mumoni|litorea12      TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H040|Eastern|Mumoni|litorea12      TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H041|Eastern|Mumoni|litorea80      TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H042|Eastern|Mumoni|litorea80      TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H043|Eastern|Mumoni|scutellata     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H044|Eastern|Mumoni|scutellata     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H045|Eastern|Mumoni|litorea80      TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H046|Eastern|Mathiakani|litore     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H048|Eastern|Mathiakani|litore     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H050|Eastern|Mathiakani|scutel     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H051|Eastern|Mathiakani|scutel     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H026|Eastern|Kathiani|scutella     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H053|Eastern|Mathiakani|scutel     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H017|Eastern|Kathiani|scutella     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H020|Eastern|Kathiani|scutella     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H031|Eastern|Mumoni|litorea19      TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H032|Eastern|Mumoni|litorea19      TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H033|Eastern|Mumoni|scutellata     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H034|Eastern|Mumoni|scutellata     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H035|Eastern|Mumoni|scutellata     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H073|Eastern|Mituki|monticola4     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H075|Eastern|Mituki|scutellata     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H081|Tanga|Usambara|scutellata     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H082|Tanga|Usambara|litorea6       TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
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H083|Tanga|Usambara|litorea6      TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
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H013|Eastern|Kasanga|scutellat    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H002|Eastern|Kasanga|scutellat    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H004|Eastern|Kasanga|scutellat    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H005|Eastern|Kasanga|scutellat    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H012|Eastern|Kasanga|litorea96    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H006|Eastern|Kasanga|scutellat    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H007|Eastern|Kasanga|scutellat    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
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H014|Eastern|Kasanga|scutellat    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H011|Eastern|Kasanga|monticola    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H019|Eastern|Kathiani|scutella    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H023|Eastern|Kathiani|litorea9    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H022|Eastern|Kathiani|scutella    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
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H024|Eastern|Kathiani|scutella    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H025|Eastern|Kathiani|scutella    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H027|Eastern|Kathiani|scutella    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H028|Eastern|Kathiani|litorea3    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H029|Eastern|Kathiani|scutella    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H030|Eastern|Kathiani|scutella    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H016|Eastern|Kathiani|scutella    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H015|Eastern|Kasanga|scutellat    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H008|Eastern|Kasanga|monticola    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H064|Coast|Ronge|scutellata2      TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H065|Coast|Ronge|monticola2       TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H063|Coast|Ronge|scutellata2      TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
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H072|Eastern|Mituki|scutellata    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H074|Eastern|Mituki|scutellata    TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H080|Tanga|Sengoma|litorea5       TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H079|Tanga|Sengoma|litorea5       TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
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H076|Tanga|Sengoma|litorea5       TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCCCGAATAAATAAT
H069|Coast|Hewani|scutellata3     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCTCGAATAAATAAT
H068|Coast|Hewani|scutellata3     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCTCGAATAAATAAT
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H066|Coast|Hewani|scutellata3     TTCCTTTAATACTAGGATCACCTGATATAGCATTTCCTCGAATAAATAAT
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H091|Coast|Chambuko|litorea8      ATTAGATTTTGATTACTTCCTCCCTCATTATTTATACTTTTATTAAGAAA
A.                                ATTAGATTTTGATTACTTCCTCCCTCATTATTTATACTTTTATTAAGAAA
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H055|Eastern|Mathiakani|scutel    ATTAGATTTTGATTACTTCCTCCCTCATTATTTATACTTTTATTAAGAAA
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H037|Eastern|Mumoni|litorea12     ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
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H046|Eastern|Mathiakani|litore    ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
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H050|Eastern|Mathiakani|scutel    ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
H051|Eastern|Mathiakani|scutel    ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
H026|Eastern|Kathiani|scutella    ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
H053|Eastern|Mathiakani|scutel    ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
H017|Eastern|Kathiani|scutella    ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
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H031|Eastern|Mumoni|litorea19     ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
H032|Eastern|Mumoni|litorea19     ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
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H033|Eastern|Mumoni|scutellata     ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
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H073|Eastern|Mituki|monticola4     ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
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H005|Eastern|Kasanga|scutellat     ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
H012|Eastern|Kasanga|litorea96     ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
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H011|Eastern|Kasanga|monticola     ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
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H022|Eastern|Kathiani|scutella     ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
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H027|Eastern|Kathiani|scutella     ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
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H016|Eastern|Kathiani|scutella     ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
H015|Eastern|Kasanga|scutellat     ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
H008|Eastern|Kasanga|monticola     ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
H064|Coast|Ronge|scutellata2       ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
H065|Coast|Ronge|monticola2        ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
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H061|Coast|Ronge|scutellata2       ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
H062|Coast|Ronge|litorea2          ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
H072|Eastern|Mituki|scutellata     ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
H074|Eastern|Mituki|scutellata     ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
H080|Tanga|Sengoma|litorea5        ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
H079|Tanga|Sengoma|litorea5        ATTAGATTTTGATTACTTCCTCCCTCATTATTTACACTTTTATTAAGAAA
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H068|Coast|Hewani|scutellata3      ATTAGATTTTGATTACTTCCTCCCTCATTATTTATACTTTTATTAAGAAA
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H066|Coast|Hewani|scutellata3      ATTAGATTTTGATTACTTCCTCCCTCATTATTTATACTTTTATTAAGAAA
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H088|Eastern|Bidii|scutellata7     TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
H091|Coast|Chambuko|litorea8       TTTATTTTACCCAAGACCAGGAACTGGGTGAACAGTATATCCACCATTAT
A.                                 TTTATTTTATCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
H052|Eastern|Mathiakani|scutel     TTTATTTTATCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
H055|Eastern|Mathiakani|scutel     TTTATTTTATCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
H056|Coast|Chawiya|litorea1        TTTATTTTATCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
H058|Coast|Chawiya|litorea1        TTTATTTTATCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
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H036|Eastern|Mumoni|litorea12      TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
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H048|Eastern|Mathiakani|litore     TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
H050|Eastern|Mathiakani|scutel     TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
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H051|Eastern|Mathiakani|scutel     TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
H026|Eastern|Kathiani|scutella     TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
H053|Eastern|Mathiakani|scutel     TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
H017|Eastern|Kathiani|scutella     TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
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H035|Eastern|Mumoni|scutellata     TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
H073|Eastern|Mituki|monticola4     TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
H075|Eastern|Mituki|scutellata     TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
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H082|Tanga|Usambara|litorea6       TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
H083|Tanga|Usambara|litorea6       TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
H084|Tanga|Usambara|litorea6       TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
H013|Eastern|Kasanga|scutellat     TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
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H011|Eastern|Kasanga|monticola     TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
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H023|Eastern|Kathiani|litorea9     TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
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H021|Eastern|Kathiani|scutella     TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
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H008|Eastern|Kasanga|monticola     TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
H064|Coast|Ronge|scutellata2       TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
H065|Coast|Ronge|monticola2        TTTATTTTACCCAAGACCAGGAACTGGATGAACAGTATATCCACCATTAT
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H069|Coast|Hewani|scutellata3      TTTATTTTACCCAAGACCAGGAACTGGGTGAACAGTATATCCACCATTAT
H068|Coast|Hewani|scutellata3      TTTATTTTACCCAAGACCAGGAACTGGGTGAACAGTATATCCACCATTAT
H067|Coast|Hewani|scutellata3      TTTATTTTACCCAAGACCAGGAACTGGGTGAACAGTATATCCACCATTAT
H066|Coast|Hewani|scutellata3      TTTATTTTACCCAAGACCAGGAACTGGGTGAACAGTATATCCACCATTAT
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H088|Eastern|Bidii|scutellata7     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H091|Coast|Chambuko|litorea8       CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
A.                                 CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H052|Eastern|Mathiakani|scutel     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H055|Eastern|Mathiakani|scutel     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H056|Coast|Chawiya|litorea1        CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H058|Coast|Chawiya|litorea1        CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H059|Coast|Chawiya|litorea1        CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H060|Coast|Chawiya|litorea1        CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H036|Eastern|Mumoni|litorea12      CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H037|Eastern|Mumoni|litorea12      CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H038|Eastern|Mumoni|litorea12      CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H039|Eastern|Mumoni|litorea12      CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H040|Eastern|Mumoni|litorea12      CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H041|Eastern|Mumoni|litorea80      CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
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H042|Eastern|Mumoni|litorea80      CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H043|Eastern|Mumoni|scutellata     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H044|Eastern|Mumoni|scutellata     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H045|Eastern|Mumoni|litorea80      CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H046|Eastern|Mathiakani|litore     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H048|Eastern|Mathiakani|litore     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H050|Eastern|Mathiakani|scutel     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H051|Eastern|Mathiakani|scutel     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H026|Eastern|Kathiani|scutella     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H053|Eastern|Mathiakani|scutel     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H017|Eastern|Kathiani|scutella     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H020|Eastern|Kathiani|scutella     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H031|Eastern|Mumoni|litorea19      CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H032|Eastern|Mumoni|litorea19      CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H033|Eastern|Mumoni|scutellata     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H034|Eastern|Mumoni|scutellata     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H035|Eastern|Mumoni|scutellata     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H073|Eastern|Mituki|monticola4     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H075|Eastern|Mituki|scutellata     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H081|Tanga|Usambara|scutellata     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H082|Tanga|Usambara|litorea6       CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H083|Tanga|Usambara|litorea6       CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H084|Tanga|Usambara|litorea6       CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H013|Eastern|Kasanga|scutellat     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H002|Eastern|Kasanga|scutellat     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H004|Eastern|Kasanga|scutellat     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H005|Eastern|Kasanga|scutellat     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H012|Eastern|Kasanga|litorea96     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H006|Eastern|Kasanga|scutellat     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H007|Eastern|Kasanga|scutellat     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H010|Eastern|Kasanga|scutellat     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H014|Eastern|Kasanga|scutellat     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H011|Eastern|Kasanga|monticola     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H019|Eastern|Kathiani|scutella     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H023|Eastern|Kathiani|litorea9     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H022|Eastern|Kathiani|scutella     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H021|Eastern|Kathiani|scutella     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H024|Eastern|Kathiani|scutella     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H025|Eastern|Kathiani|scutella     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H027|Eastern|Kathiani|scutella     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H028|Eastern|Kathiani|litorea3     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H029|Eastern|Kathiani|scutella     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H030|Eastern|Kathiani|scutella     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H016|Eastern|Kathiani|scutella     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H015|Eastern|Kasanga|scutellat     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H008|Eastern|Kasanga|monticola     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H064|Coast|Ronge|scutellata2       CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H065|Coast|Ronge|monticola2        CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H063|Coast|Ronge|scutellata2       CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H061|Coast|Ronge|scutellata2       CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H062|Coast|Ronge|litorea2          CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H072|Eastern|Mituki|scutellata     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H074|Eastern|Mituki|scutellata     CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H080|Tanga|Sengoma|litorea5        CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H079|Tanga|Sengoma|litorea5        CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H078|Tanga|Sengoma|litorea5        CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H077|Tanga|Sengoma|litorea         CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H076|Tanga|Sengoma|litorea5        CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTACAATTTTTTCT
H069|Coast|Hewani|scutellata3      CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H068|Coast|Hewani|scutellata3      CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H067|Coast|Hewani|scutellata3      CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
H066|Coast|Hewani|scutellata3      CAGCATATTTATATCATTCTTCACCTTCAGTAGATTTTGCAATTTTTTCT
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H088|Eastern|Bidii|scutellata7     CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H091|Coast|Chambuko|litorea8       CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
A.                                 CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H052|Eastern|Mathiakani|scutel     CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H055|Eastern|Mathiakani|scutel     CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H056|Coast|Chawiya|litorea1        CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H058|Coast|Chawiya|litorea1        CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H059|Coast|Chawiya|litorea1        CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
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H060|Coast|Chawiya|litorea1       CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H036|Eastern|Mumoni|litorea12     CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H037|Eastern|Mumoni|litorea12     CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H038|Eastern|Mumoni|litorea12     CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H039|Eastern|Mumoni|litorea12     CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H040|Eastern|Mumoni|litorea12     CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H041|Eastern|Mumoni|litorea80     CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H042|Eastern|Mumoni|litorea80     CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H043|Eastern|Mumoni|scutellata    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H044|Eastern|Mumoni|scutellata    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H045|Eastern|Mumoni|litorea80     CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H046|Eastern|Mathiakani|litore    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H048|Eastern|Mathiakani|litore    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H050|Eastern|Mathiakani|scutel    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H051|Eastern|Mathiakani|scutel    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H026|Eastern|Kathiani|scutella    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H053|Eastern|Mathiakani|scutel    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H017|Eastern|Kathiani|scutella    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H020|Eastern|Kathiani|scutella    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H031|Eastern|Mumoni|litorea19     CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H032|Eastern|Mumoni|litorea19     CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H033|Eastern|Mumoni|scutellata    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H034|Eastern|Mumoni|scutellata    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H035|Eastern|Mumoni|scutellata    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H073|Eastern|Mituki|monticola4    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H075|Eastern|Mituki|scutellata    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H081|Tanga|Usambara|scutellata    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H082|Tanga|Usambara|litorea6      CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H083|Tanga|Usambara|litorea6      CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H084|Tanga|Usambara|litorea6      CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H013|Eastern|Kasanga|scutellat    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H002|Eastern|Kasanga|scutellat    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H004|Eastern|Kasanga|scutellat    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H005|Eastern|Kasanga|scutellat    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H012|Eastern|Kasanga|litorea96    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H006|Eastern|Kasanga|scutellat    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H007|Eastern|Kasanga|scutellat    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H010|Eastern|Kasanga|scutellat    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H014|Eastern|Kasanga|scutellat    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H011|Eastern|Kasanga|monticola    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H019|Eastern|Kathiani|scutella    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H023|Eastern|Kathiani|litorea9    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H022|Eastern|Kathiani|scutella    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H021|Eastern|Kathiani|scutella    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H024|Eastern|Kathiani|scutella    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H025|Eastern|Kathiani|scutella    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H027|Eastern|Kathiani|scutella    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H028|Eastern|Kathiani|litorea3    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H029|Eastern|Kathiani|scutella    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H030|Eastern|Kathiani|scutella    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H016|Eastern|Kathiani|scutella    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H015|Eastern|Kasanga|scutellat    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H008|Eastern|Kasanga|monticola    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H064|Coast|Ronge|scutellata2      CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H065|Coast|Ronge|monticola2       CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H063|Coast|Ronge|scutellata2      CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H061|Coast|Ronge|scutellata2      CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H062|Coast|Ronge|litorea2         CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H072|Eastern|Mituki|scutellata    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H074|Eastern|Mituki|scutellata    CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H080|Tanga|Sengoma|litorea5       CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H079|Tanga|Sengoma|litorea5       CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H078|Tanga|Sengoma|litorea5       CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H077|Tanga|Sengoma|litorea        CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H076|Tanga|Sengoma|litorea5       CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAATTTAATAGT
H069|Coast|Hewani|scutellata3     CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H068|Coast|Hewani|scutellata3     CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H067|Coast|Hewani|scutellata3     CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
H066|Coast|Hewani|scutellata3     CTTCATATATCAGGAATTTCCTCAATTATAGGATCATTAAACTTAATAGT
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H088|Eastern|Bidii|scutellata7    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
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H091|Coast|Chambuko|litorea8      TACAATTATAATAATAAAAAATTTTTCTATAAATTATGATCAAATTTCAT
A.                                TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H052|Eastern|Mathiakani|scutel    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H055|Eastern|Mathiakani|scutel    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H056|Coast|Chawiya|litorea1       TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H058|Coast|Chawiya|litorea1       TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H059|Coast|Chawiya|litorea1       TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H060|Coast|Chawiya|litorea1       TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H036|Eastern|Mumoni|litorea12     TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H037|Eastern|Mumoni|litorea12     TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H038|Eastern|Mumoni|litorea12     TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H039|Eastern|Mumoni|litorea12     TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H040|Eastern|Mumoni|litorea12     TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H041|Eastern|Mumoni|litorea80     TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H042|Eastern|Mumoni|litorea80     TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H043|Eastern|Mumoni|scutellata    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H044|Eastern|Mumoni|scutellata    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H045|Eastern|Mumoni|litorea80     TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H046|Eastern|Mathiakani|litore    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H048|Eastern|Mathiakani|litore    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H050|Eastern|Mathiakani|scutel    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H051|Eastern|Mathiakani|scutel    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H026|Eastern|Kathiani|scutella    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H053|Eastern|Mathiakani|scutel    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H017|Eastern|Kathiani|scutella    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H020|Eastern|Kathiani|scutella    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H031|Eastern|Mumoni|litorea19     TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H032|Eastern|Mumoni|litorea19     TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H033|Eastern|Mumoni|scutellata    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H034|Eastern|Mumoni|scutellata    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H035|Eastern|Mumoni|scutellata    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H073|Eastern|Mituki|monticola4    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H075|Eastern|Mituki|scutellata    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H081|Tanga|Usambara|scutellata    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H082|Tanga|Usambara|litorea6      TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H083|Tanga|Usambara|litorea6      TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H084|Tanga|Usambara|litorea6      TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H013|Eastern|Kasanga|scutellat    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H002|Eastern|Kasanga|scutellat    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H004|Eastern|Kasanga|scutellat    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H005|Eastern|Kasanga|scutellat    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H012|Eastern|Kasanga|litorea96    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H006|Eastern|Kasanga|scutellat    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H007|Eastern|Kasanga|scutellat    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H010|Eastern|Kasanga|scutellat    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H014|Eastern|Kasanga|scutellat    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H011|Eastern|Kasanga|monticola    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H019|Eastern|Kathiani|scutella    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H023|Eastern|Kathiani|litorea9    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H022|Eastern|Kathiani|scutella    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H021|Eastern|Kathiani|scutella    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H024|Eastern|Kathiani|scutella    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H025|Eastern|Kathiani|scutella    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H027|Eastern|Kathiani|scutella    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H028|Eastern|Kathiani|litorea3    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H029|Eastern|Kathiani|scutella    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H030|Eastern|Kathiani|scutella    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H016|Eastern|Kathiani|scutella    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H015|Eastern|Kasanga|scutellat    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H008|Eastern|Kasanga|monticola    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H064|Coast|Ronge|scutellata2      TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H065|Coast|Ronge|monticola2       TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H063|Coast|Ronge|scutellata2      TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H061|Coast|Ronge|scutellata2      TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H062|Coast|Ronge|litorea2         TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H072|Eastern|Mituki|scutellata    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H074|Eastern|Mituki|scutellata    TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H080|Tanga|Sengoma|litorea5       TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H079|Tanga|Sengoma|litorea5       TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H078|Tanga|Sengoma|litorea5       TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H077|Tanga|Sengoma|litorea        TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
H076|Tanga|Sengoma|litorea5       TACAATTATAATAATAAAAAATTTTTCTATAAATTATGACCAAATTTCAT
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H069|Coast|Hewani|scutellata3      TACAATTATAATAATAAAAAATTTTTCTATAAATTATGATCAAATTTCAT
H068|Coast|Hewani|scutellata3      TACAATTATAATAATAAAAAATTTTTCTATAAATTATGATCAAATTTCAT
H067|Coast|Hewani|scutellata3      TACAATTATAATAATAAAAAATTTTTCTATAAATTATGATCAAATTTCAT
H066|Coast|Hewani|scutellata3      TACAATTATAATAATAAAAAATTTTTCTATAAATTATGATCAAATTTCAT
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H088|Eastern|Bidii|scutellata7     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H091|Coast|Chambuko|litorea8       TATTTCCATGATCAGTTTTCATTACAGCAATTTTATTAATTATATCATTA
A.                                 TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCATTA
H052|Eastern|Mathiakani|scutel     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H055|Eastern|Mathiakani|scutel     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H056|Coast|Chawiya|litorea1        TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H058|Coast|Chawiya|litorea1        TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H059|Coast|Chawiya|litorea1        TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H060|Coast|Chawiya|litorea1        TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H036|Eastern|Mumoni|litorea12      TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H037|Eastern|Mumoni|litorea12      TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H038|Eastern|Mumoni|litorea12      TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H039|Eastern|Mumoni|litorea12      TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H040|Eastern|Mumoni|litorea12      TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H041|Eastern|Mumoni|litorea80      TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H042|Eastern|Mumoni|litorea80      TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H043|Eastern|Mumoni|scutellata     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H044|Eastern|Mumoni|scutellata     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H045|Eastern|Mumoni|litorea80      TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H046|Eastern|Mathiakani|litore     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H048|Eastern|Mathiakani|litore     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H050|Eastern|Mathiakani|scutel     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H051|Eastern|Mathiakani|scutel     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H026|Eastern|Kathiani|scutella     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H053|Eastern|Mathiakani|scutel     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H017|Eastern|Kathiani|scutella     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H020|Eastern|Kathiani|scutella     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H031|Eastern|Mumoni|litorea19      TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H032|Eastern|Mumoni|litorea19      TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H033|Eastern|Mumoni|scutellata     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H034|Eastern|Mumoni|scutellata     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H035|Eastern|Mumoni|scutellata     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H073|Eastern|Mituki|monticola4     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H075|Eastern|Mituki|scutellata     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H081|Tanga|Usambara|scutellata     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H082|Tanga|Usambara|litorea6       TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H083|Tanga|Usambara|litorea6       TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H084|Tanga|Usambara|litorea6       TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H013|Eastern|Kasanga|scutellat     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H002|Eastern|Kasanga|scutellat     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H004|Eastern|Kasanga|scutellat     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H005|Eastern|Kasanga|scutellat     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H012|Eastern|Kasanga|litorea96     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H006|Eastern|Kasanga|scutellat     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H007|Eastern|Kasanga|scutellat     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H010|Eastern|Kasanga|scutellat     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H014|Eastern|Kasanga|scutellat     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H011|Eastern|Kasanga|monticola     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H019|Eastern|Kathiani|scutella     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H023|Eastern|Kathiani|litorea9     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H022|Eastern|Kathiani|scutella     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H021|Eastern|Kathiani|scutella     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H024|Eastern|Kathiani|scutella     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H025|Eastern|Kathiani|scutella     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H027|Eastern|Kathiani|scutella     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H028|Eastern|Kathiani|litorea3     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H029|Eastern|Kathiani|scutella     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H030|Eastern|Kathiani|scutella     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H016|Eastern|Kathiani|scutella     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H015|Eastern|Kasanga|scutellat     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H008|Eastern|Kasanga|monticola     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H064|Coast|Ronge|scutellata2       TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H065|Coast|Ronge|monticola2        TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H063|Coast|Ronge|scutellata2       TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H061|Coast|Ronge|scutellata2       TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H062|Coast|Ronge|litorea2          TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
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H072|Eastern|Mituki|scutellata     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H074|Eastern|Mituki|scutellata     TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H080|Tanga|Sengoma|litorea5        TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H079|Tanga|Sengoma|litorea5        TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H078|Tanga|Sengoma|litorea5        TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H077|Tanga|Sengoma|litorea         TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H076|Tanga|Sengoma|litorea5        TATTTCCATGATCAGTTTTTATTACAGCAATTTTATTAATTATATCACTA
H069|Coast|Hewani|scutellata3      TATTTCCATGATCAGTTTTCATTACAGCAATTTTATTAATTATATCATTA
H068|Coast|Hewani|scutellata3      TATTTCCATGATCAGTTTTCATTACAGCAATTTTATTAATTATATCATTA
H067|Coast|Hewani|scutellata3      TATTTCCATGATCAGTTTTCATTACAGCAATTTTATTAATTATATCATTA
H066|Coast|Hewani|scutellata3      TATTTCCATGATCAGTTTTCATTACAGCAATTTTATTAATTATATCATTA
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H088|Eastern|Bidii|scutellata7     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H091|Coast|Chambuko|litorea8       CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
A.                                 CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H052|Eastern|Mathiakani|scutel     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H055|Eastern|Mathiakani|scutel     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H056|Coast|Chawiya|litorea1        CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H058|Coast|Chawiya|litorea1        CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H059|Coast|Chawiya|litorea1        CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H060|Coast|Chawiya|litorea1        CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H036|Eastern|Mumoni|litorea12      CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H037|Eastern|Mumoni|litorea12      CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H038|Eastern|Mumoni|litorea12      CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H039|Eastern|Mumoni|litorea12      CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H040|Eastern|Mumoni|litorea12      CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H041|Eastern|Mumoni|litorea80      CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H042|Eastern|Mumoni|litorea80      CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H043|Eastern|Mumoni|scutellata     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H044|Eastern|Mumoni|scutellata     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H045|Eastern|Mumoni|litorea80      CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H046|Eastern|Mathiakani|litore     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H048|Eastern|Mathiakani|litore     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H050|Eastern|Mathiakani|scutel     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H051|Eastern|Mathiakani|scutel     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H026|Eastern|Kathiani|scutella     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H053|Eastern|Mathiakani|scutel     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H017|Eastern|Kathiani|scutella     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H020|Eastern|Kathiani|scutella     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H031|Eastern|Mumoni|litorea19      CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H032|Eastern|Mumoni|litorea19      CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H033|Eastern|Mumoni|scutellata     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H034|Eastern|Mumoni|scutellata     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H035|Eastern|Mumoni|scutellata     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H073|Eastern|Mituki|monticola4     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H075|Eastern|Mituki|scutellata     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H081|Tanga|Usambara|scutellata     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H082|Tanga|Usambara|litorea6       CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H083|Tanga|Usambara|litorea6       CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H084|Tanga|Usambara|litorea6       CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H013|Eastern|Kasanga|scutellat     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H002|Eastern|Kasanga|scutellat     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H004|Eastern|Kasanga|scutellat     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H005|Eastern|Kasanga|scutellat     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H012|Eastern|Kasanga|litorea96     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H006|Eastern|Kasanga|scutellat     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H007|Eastern|Kasanga|scutellat     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H010|Eastern|Kasanga|scutellat     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H014|Eastern|Kasanga|scutellat     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H011|Eastern|Kasanga|monticola     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H019|Eastern|Kathiani|scutella     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H023|Eastern|Kathiani|litorea9     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H022|Eastern|Kathiani|scutella     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H021|Eastern|Kathiani|scutella     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H024|Eastern|Kathiani|scutella     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H025|Eastern|Kathiani|scutella     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H027|Eastern|Kathiani|scutella     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H028|Eastern|Kathiani|litorea3     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H029|Eastern|Kathiani|scutella     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H030|Eastern|Kathiani|scutella     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H016|Eastern|Kathiani|scutella     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
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H015|Eastern|Kasanga|scutellat     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H008|Eastern|Kasanga|monticola     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H064|Coast|Ronge|scutellata2       CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H065|Coast|Ronge|monticola2        CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H063|Coast|Ronge|scutellata2       CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H061|Coast|Ronge|scutellata2       CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H062|Coast|Ronge|litorea2          CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H072|Eastern|Mituki|scutellata     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H074|Eastern|Mituki|scutellata     CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H080|Tanga|Sengoma|litorea5        CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H079|Tanga|Sengoma|litorea5        CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H078|Tanga|Sengoma|litorea5        CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H077|Tanga|Sengoma|litorea         CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H076|Tanga|Sengoma|litorea5        CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H069|Coast|Hewani|scutellata3      CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H068|Coast|Hewani|scutellata3      CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H067|Coast|Hewani|scutellata3      CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
H066|Coast|Hewani|scutellata3      CCTGTATTAGCTGGAGCAATTACTATACTATTATTTGATCGAAATTTTAA
                                   **********************************************

H088|Eastern|Bidii|scutellata7     TACATCATTTTTTGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H091|Coast|Chambuko|litorea8       TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
A.                                 TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTCTTTATCAAC
H052|Eastern|Mathiakani|scutel     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H055|Eastern|Mathiakani|scutel     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H056|Coast|Chawiya|litorea1        TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H058|Coast|Chawiya|litorea1        TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H059|Coast|Chawiya|litorea1        TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H060|Coast|Chawiya|litorea1        TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H036|Eastern|Mumoni|litorea12      TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H037|Eastern|Mumoni|litorea12      TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H038|Eastern|Mumoni|litorea12      TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H039|Eastern|Mumoni|litorea12      TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H040|Eastern|Mumoni|litorea12      TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H041|Eastern|Mumoni|litorea80      TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H042|Eastern|Mumoni|litorea80      TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H043|Eastern|Mumoni|scutellata     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H044|Eastern|Mumoni|scutellata     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H045|Eastern|Mumoni|litorea80      TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H046|Eastern|Mathiakani|litore     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H048|Eastern|Mathiakani|litore     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H050|Eastern|Mathiakani|scutel     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H051|Eastern|Mathiakani|scutel     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H026|Eastern|Kathiani|scutella     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H053|Eastern|Mathiakani|scutel     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H017|Eastern|Kathiani|scutella     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H020|Eastern|Kathiani|scutella     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H031|Eastern|Mumoni|litorea19      TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H032|Eastern|Mumoni|litorea19      TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H033|Eastern|Mumoni|scutellata     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H034|Eastern|Mumoni|scutellata     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H035|Eastern|Mumoni|scutellata     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H073|Eastern|Mituki|monticola4     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H075|Eastern|Mituki|scutellata     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H081|Tanga|Usambara|scutellata     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H082|Tanga|Usambara|litorea6       TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H083|Tanga|Usambara|litorea6       TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H084|Tanga|Usambara|litorea6       TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H013|Eastern|Kasanga|scutellat     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H002|Eastern|Kasanga|scutellat     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H004|Eastern|Kasanga|scutellat     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H005|Eastern|Kasanga|scutellat     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H012|Eastern|Kasanga|litorea96     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H006|Eastern|Kasanga|scutellat     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H007|Eastern|Kasanga|scutellat     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H010|Eastern|Kasanga|scutellat     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H014|Eastern|Kasanga|scutellat     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H011|Eastern|Kasanga|monticola     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H019|Eastern|Kathiani|scutella     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H023|Eastern|Kathiani|litorea9     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H022|Eastern|Kathiani|scutella     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H021|Eastern|Kathiani|scutella     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
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H024|Eastern|Kathiani|scutella     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H025|Eastern|Kathiani|scutella     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H027|Eastern|Kathiani|scutella     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H028|Eastern|Kathiani|litorea3     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H029|Eastern|Kathiani|scutella     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H030|Eastern|Kathiani|scutella     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H016|Eastern|Kathiani|scutella     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H015|Eastern|Kasanga|scutellat     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H008|Eastern|Kasanga|monticola     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H064|Coast|Ronge|scutellata2       TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H065|Coast|Ronge|monticola2        TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H063|Coast|Ronge|scutellata2       TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H061|Coast|Ronge|scutellata2       TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H062|Coast|Ronge|litorea2          TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H072|Eastern|Mituki|scutellata     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H074|Eastern|Mituki|scutellata     TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H080|Tanga|Sengoma|litorea5        TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H079|Tanga|Sengoma|litorea5        TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H078|Tanga|Sengoma|litorea5        TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H077|Tanga|Sengoma|litorea         TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H076|Tanga|Sengoma|litorea5        TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H069|Coast|Hewani|scutellata3      TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H068|Coast|Hewani|scutellata3      TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H067|Coast|Hewani|scutellata3      TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
H066|Coast|Hewani|scutellata3      TACATCATTTTTCGATCCTATAGGAGGTGGAGATCCAATTTTATATCAAC
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H088|Eastern|Bidii|scutellata7     ATTTATTT
H091|Coast|Chambuko|litorea8       ATTTATTT
A.                                 ATTTATTT
H052|Eastern|Mathiakani|scutel     ATTTATTT
H055|Eastern|Mathiakani|scutel     ATTTATTT
H056|Coast|Chawiya|litorea1        ATTTATTT
H058|Coast|Chawiya|litorea1        ATTTATTT
H059|Coast|Chawiya|litorea1        ATTTATTT
H060|Coast|Chawiya|litorea1        ATTTATTT
H036|Eastern|Mumoni|litorea12      ATTTATTT
H037|Eastern|Mumoni|litorea12      ATTTATTT
H038|Eastern|Mumoni|litorea12      ATTTATTT
H039|Eastern|Mumoni|litorea12      ATTTATTT
H040|Eastern|Mumoni|litorea12      ATTTATTT
H041|Eastern|Mumoni|litorea80      ATTTATTT
H042|Eastern|Mumoni|litorea80      ATTTATTT
H043|Eastern|Mumoni|scutellata     ATTTATTT
H044|Eastern|Mumoni|scutellata     ATTTATTT
H045|Eastern|Mumoni|litorea80      ATTTATTT
H046|Eastern|Mathiakani|litore     ATTTATTT
H048|Eastern|Mathiakani|litore     ATTTATTT
H050|Eastern|Mathiakani|scutel     ATTTATTT
H051|Eastern|Mathiakani|scutel     ATTTATTT
H026|Eastern|Kathiani|scutella     ATTTATTT
H053|Eastern|Mathiakani|scutel     ATTTATTT
H017|Eastern|Kathiani|scutella     ATTTATTT
H020|Eastern|Kathiani|scutella     ATTTATTT
H031|Eastern|Mumoni|litorea19      ATTTATTT
H032|Eastern|Mumoni|litorea19      ATTTATTT
H033|Eastern|Mumoni|scutellata     ATTTATTT
H034|Eastern|Mumoni|scutellata     ATTTATTT
H035|Eastern|Mumoni|scutellata     ATTTATTT
H073|Eastern|Mituki|monticola4     ATTTATTT
H075|Eastern|Mituki|scutellata     ATTTATTT
H081|Tanga|Usambara|scutellata     ATTTATTT
H082|Tanga|Usambara|litorea6       ATTTATTT
H083|Tanga|Usambara|litorea6       ATTTATTT
H084|Tanga|Usambara|litorea6       ATTTATTT
H013|Eastern|Kasanga|scutellat     ATTTATTT
H002|Eastern|Kasanga|scutellat     ATTTATTT
H004|Eastern|Kasanga|scutellat     ATTTATTT
H005|Eastern|Kasanga|scutellat     ATTTATTT
H012|Eastern|Kasanga|litorea96     ATTTATTT
H006|Eastern|Kasanga|scutellat     ATTTATTT
H007|Eastern|Kasanga|scutellat     ATTTATTT
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H010|Eastern|Kasanga|scutellat     ATTTATTT
H014|Eastern|Kasanga|scutellat     ATTTATTT
H011|Eastern|Kasanga|monticola     ATTTATTT
H019|Eastern|Kathiani|scutella     ATTTATTT
H023|Eastern|Kathiani|litorea9     ATTTATTT
H022|Eastern|Kathiani|scutella     ATTTATTT
H021|Eastern|Kathiani|scutella     ATTTATTT
H024|Eastern|Kathiani|scutella     ATTTATTT
H025|Eastern|Kathiani|scutella     ATTTATTT
H027|Eastern|Kathiani|scutella     ATTTATTT
H028|Eastern|Kathiani|litorea3     ATTTATTT
H029|Eastern|Kathiani|scutella     ATTTATTT
H030|Eastern|Kathiani|scutella     ATTTATTT
H016|Eastern|Kathiani|scutella     ATTTATTT
H015|Eastern|Kasanga|scutellat     ATTTATTT
H008|Eastern|Kasanga|monticola     ATTTATTT
H064|Coast|Ronge|scutellata2       ATTTATTT
H065|Coast|Ronge|monticola2        ATTTATTT
H063|Coast|Ronge|scutellata2       ATTTATTT
H061|Coast|Ronge|scutellata2       ATTTATTT
H062|Coast|Ronge|litorea2          ATTTATTT
H072|Eastern|Mituki|scutellata     ATTTATTT
H074|Eastern|Mituki|scutellata     ATTTATTT
H080|Tanga|Sengoma|litorea5        ATTTATTT
H079|Tanga|Sengoma|litorea5        ATTTATTT
H078|Tanga|Sengoma|litorea5        ATTTATTT
H077|Tanga|Sengoma|litorea         ATTTATTT
H076|Tanga|Sengoma|litorea5        ATTTATTT
H069|Coast|Hewani|scutellata3      ATTTATTT
H068|Coast|Hewani|scutellata3      ATTTATTT
H067|Coast|Hewani|scutellata3      ATTTATTT
H066|Coast|Hewani|scutellata3      ATTTATTT
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Appendix 2.2: Cytb-tRNAser CLUSTAL 2.1 MSA

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H144Nbiicipe|Central|A.m.scute     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H147Thika|Central|A.m.scutella     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H3Kasanga9|Eastern|A.m.litorea     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H5Kasanga9|Eastern|A.m.scutell     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H8Kasanga64|Eastern|A.m.montic     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H14Kasanga96|Eastern|A.m.scute     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H16Kathiani60|Eastern|A.m.scut     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H24Kathiani99|Eastern|A.m.scut     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H25Kathiani99|Eastern|A.m.scut     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H29Kathiani360|Eastern|A.m.scu     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H30Kathiani360|Eastern|A.m.scu     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H31Mumoni19|Eastern|A.m.litore     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H32Mumoni19|Eastern|A.m.litore     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H41Mumoni80|Eastern|A.m.litore     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H42Mumoni80|Eastern|A.m.litore     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H48Mathiakani52|Eastern|A.m.li     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
57TaitaChawiya1|Coast|A.m.lito     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H64TaitaRonge|Coast|A.m.scutel     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H65TaitaRonge|Coast|A.m.montic     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H69TanaRiverHewani|Coast|A.m.s     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H70TanaRiverHewani|Coast|A.m.s     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H72Mitukiyaiveti|Eastern|A.m.s     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H79TangaSengoma|Coast|A.m.lito     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H81TangaUsambara|Coast|A.m.scu     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H82TangaUsambara|Coast|A.m.lit     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H108Mathiakaniwild|Eastern|A.m     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H111KkMakuchi97|Western|A.m.sc     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H115KkMakuchi97|Western|A.m.sc     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H116KkMakuchi91|Western|A.m.li     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H117KkMakuchi91|Western|A.m.li     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H121KkMakuchi92|Western|A.m.li     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H126KkIsiekuti107|Western|A.m.     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H130KkIsiekuti107|Western|A.m.     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H132KkIsiekuti11|Western|A.m.s     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H134KkIsiekuti11|Western|A.m.l     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H137KkIsiekuti15|Western|A.m.s     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
H140KkIsiekuti15|Western|A.m.s     ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
Apis_mellifera_ligustica           ATATTGAGGTGCAACAGTTATTACTAATCTTTTATCAGCAATTCCTTATA
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H144Nbiicipe|Central|A.m.scute     TTGGTGATACAATTGTATTATGAATCTGAGGTGGGTTCTCAATTAATAAT
H147Thika|Central|A.m.scutella     TTGGTGATACAATTGTATTATGAATCTGAGGTGGGTTCTCAATTAATAAT
H3Kasanga9|Eastern|A.m.litorea     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
H5Kasanga9|Eastern|A.m.scutell     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
H8Kasanga64|Eastern|A.m.montic     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
H14Kasanga96|Eastern|A.m.scute     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
H16Kathiani60|Eastern|A.m.scut     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
H24Kathiani99|Eastern|A.m.scut     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
H25Kathiani99|Eastern|A.m.scut     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
H29Kathiani360|Eastern|A.m.scu     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
H30Kathiani360|Eastern|A.m.scu     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
H31Mumoni19|Eastern|A.m.litore     TTGGTGATACAATTGTATTATGAATCTGAGGTGGGTTCTCAATTAATAAT
H32Mumoni19|Eastern|A.m.litore     TTGGTGATACAATTGTATTATGAATCTGAGGTGGGTTCTCAATTAATAAT
H41Mumoni80|Eastern|A.m.litore     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
H42Mumoni80|Eastern|A.m.litore     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
H48Mathiakani52|Eastern|A.m.li     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
57TaitaChawiya1|Coast|A.m.lito     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
H64TaitaRonge|Coast|A.m.scutel     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
H65TaitaRonge|Coast|A.m.montic     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
H69TanaRiverHewani|Coast|A.m.s     TTGGTGATACAATTGTATTGTGAATTTGAGGTGGATTCTCAATTAATAAT
H70TanaRiverHewani|Coast|A.m.s     TTGGTGATACAATTGTATTGTGAATTTGAGGTGGATTCTCAATTAATAAT
H72Mitukiyaiveti|Eastern|A.m.s     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
H79TangaSengoma|Coast|A.m.lito     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
H81TangaUsambara|Coast|A.m.scu     TTGGTGATACAATTGTATTATGAATCTGAGGTGGGTTCTCAATTAATAAT
H82TangaUsambara|Coast|A.m.lit     TTGGTGATACAATTGTATTATGAATCTGAGGTGGGTTCTCAATTAATAAT
H108Mathiakaniwild|Eastern|A.m     TTGGTGATACAATTGTATTATGAATCTGAGGTGGGTTCTCAATTAATAAT
H111KkMakuchi97|Western|A.m.sc     TTGGTGATACAATTGTATTATGAATCTGAGGTGGGTTCTCAATTAATAAT
H115KkMakuchi97|Western|A.m.sc     TTGGTGATACAATTGTATTATGAATCTGAGGTGGGTTCTCAATTAATAAT
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H116KkMakuchi91|Western|A.m.li     TTGGTGATACAATTGTATTATGAATCTGAGGTGGGTTCTCAATTAATAAT
H117KkMakuchi91|Western|A.m.li     TTGGTGATACAATTGTATTATGAATCTGAGGTGGGTTCTCAATTAATAAT
H121KkMakuchi92|Western|A.m.li     TTGGTGATACAATTGTATTATGAATCTGAGGTGGGTTCTCAATTAATAAT
H126KkIsiekuti107|Western|A.m.     TTGGTGATACAATTGTATTATGAATCTGAGGTGGGTTCTCAATTAATAAT
H130KkIsiekuti107|Western|A.m.     TTGGTGATACAATTGTATTATGAATCTGAGGTGGGTTCTCAATTAATAAT
H132KkIsiekuti11|Western|A.m.s     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
H134KkIsiekuti11|Western|A.m.l     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
H137KkIsiekuti15|Western|A.m.s     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
H140KkIsiekuti15|Western|A.m.s     TTGGTGATACAATTGTATTATGAATCTGAGGTGGATTCTCAATTAATAAT
Apis_mellifera_ligustica           TTGGTGATACAATTGTATTATGAATTTGAGGTGGATTTTCAATTAATAAT
                                   ******************.***** ********.** ************

H144Nbiicipe|Central|A.m.scute     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H147Thika|Central|A.m.scutella     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H3Kasanga9|Eastern|A.m.litorea     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H5Kasanga9|Eastern|A.m.scutell     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H8Kasanga64|Eastern|A.m.montic     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H14Kasanga96|Eastern|A.m.scute     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H16Kathiani60|Eastern|A.m.scut     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H24Kathiani99|Eastern|A.m.scut     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H25Kathiani99|Eastern|A.m.scut     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H29Kathiani360|Eastern|A.m.scu     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H30Kathiani360|Eastern|A.m.scu     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H31Mumoni19|Eastern|A.m.litore     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H32Mumoni19|Eastern|A.m.litore     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H41Mumoni80|Eastern|A.m.litore     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H42Mumoni80|Eastern|A.m.litore     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H48Mathiakani52|Eastern|A.m.li     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
57TaitaChawiya1|Coast|A.m.lito     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H64TaitaRonge|Coast|A.m.scutel     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H65TaitaRonge|Coast|A.m.montic     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H69TanaRiverHewani|Coast|A.m.s     GCTACATTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H70TanaRiverHewani|Coast|A.m.s     GCTACATTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H72Mitukiyaiveti|Eastern|A.m.s     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H79TangaSengoma|Coast|A.m.lito     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H81TangaUsambara|Coast|A.m.scu     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H82TangaUsambara|Coast|A.m.lit     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H108Mathiakaniwild|Eastern|A.m     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H111KkMakuchi97|Western|A.m.sc     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H115KkMakuchi97|Western|A.m.sc     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H116KkMakuchi91|Western|A.m.li     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H117KkMakuchi91|Western|A.m.li     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H121KkMakuchi92|Western|A.m.li     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H126KkIsiekuti107|Western|A.m.     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H130KkIsiekuti107|Western|A.m.     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H132KkIsiekuti11|Western|A.m.s     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H134KkIsiekuti11|Western|A.m.l     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H137KkIsiekuti15|Western|A.m.s     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
H140KkIsiekuti15|Western|A.m.s     GCTACCTTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
Apis_mellifera_ligustica           GCTACATTAAATCGATTTTTTTCTTTACATTTTATTTTACCATTATTAAT
                                   ******.*****************************************

H144Nbiicipe|Central|A.m.scute     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H147Thika|Central|A.m.scutella     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H3Kasanga9|Eastern|A.m.litorea     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H5Kasanga9|Eastern|A.m.scutell     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H8Kasanga64|Eastern|A.m.montic     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H14Kasanga96|Eastern|A.m.scute     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H16Kathiani60|Eastern|A.m.scut     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H24Kathiani99|Eastern|A.m.scut     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H25Kathiani99|Eastern|A.m.scut     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H29Kathiani360|Eastern|A.m.scu     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H30Kathiani360|Eastern|A.m.scu     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H31Mumoni19|Eastern|A.m.litore     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H32Mumoni19|Eastern|A.m.litore     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H41Mumoni80|Eastern|A.m.litore     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H42Mumoni80|Eastern|A.m.litore     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H48Mathiakani52|Eastern|A.m.li     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
57TaitaChawiya1|Coast|A.m.lito     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H64TaitaRonge|Coast|A.m.scutel     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H65TaitaRonge|Coast|A.m.montic     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H69TanaRiverHewani|Coast|A.m.s     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
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H70TanaRiverHewani|Coast|A.m.s     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H72Mitukiyaiveti|Eastern|A.m.s     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H79TangaSengoma|Coast|A.m.lito     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H81TangaUsambara|Coast|A.m.scu     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H82TangaUsambara|Coast|A.m.lit     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H108Mathiakaniwild|Eastern|A.m     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H111KkMakuchi97|Western|A.m.sc     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H115KkMakuchi97|Western|A.m.sc     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H116KkMakuchi91|Western|A.m.li     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H117KkMakuchi91|Western|A.m.li     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H121KkMakuchi92|Western|A.m.li     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H126KkIsiekuti107|Western|A.m.     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H130KkIsiekuti107|Western|A.m.     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H132KkIsiekuti11|Western|A.m.s     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H134KkIsiekuti11|Western|A.m.l     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H137KkIsiekuti15|Western|A.m.s     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
H140KkIsiekuti15|Western|A.m.s     TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
Apis_mellifera_ligustica           TTTATTTATAGTTATTCTTCATTTATTTGCCTTACATTTAACTGGATCAT
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H144Nbiicipe|Central|A.m.scute     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H147Thika|Central|A.m.scutella     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H3Kasanga9|Eastern|A.m.litorea     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H5Kasanga9|Eastern|A.m.scutell     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H8Kasanga64|Eastern|A.m.montic     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H14Kasanga96|Eastern|A.m.scute     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H16Kathiani60|Eastern|A.m.scut     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H24Kathiani99|Eastern|A.m.scut     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H25Kathiani99|Eastern|A.m.scut     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H29Kathiani360|Eastern|A.m.scu     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H30Kathiani360|Eastern|A.m.scu     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H31Mumoni19|Eastern|A.m.litore     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H32Mumoni19|Eastern|A.m.litore     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H41Mumoni80|Eastern|A.m.litore     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H42Mumoni80|Eastern|A.m.litore     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H48Mathiakani52|Eastern|A.m.li     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
57TaitaChawiya1|Coast|A.m.lito     TTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H64TaitaRonge|Coast|A.m.scutel     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H65TaitaRonge|Coast|A.m.montic     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H69TanaRiverHewani|Coast|A.m.s     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H70TanaRiverHewani|Coast|A.m.s     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H72Mitukiyaiveti|Eastern|A.m.s     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H79TangaSengoma|Coast|A.m.lito     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H81TangaUsambara|Coast|A.m.scu     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H82TangaUsambara|Coast|A.m.lit     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H108Mathiakaniwild|Eastern|A.m     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H111KkMakuchi97|Western|A.m.sc     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H115KkMakuchi97|Western|A.m.sc     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H116KkMakuchi91|Western|A.m.li     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H117KkMakuchi91|Western|A.m.li     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H121KkMakuchi92|Western|A.m.li     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H126KkIsiekuti107|Western|A.m.     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H130KkIsiekuti107|Western|A.m.     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H132KkIsiekuti11|Western|A.m.s     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H134KkIsiekuti11|Western|A.m.l     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H137KkIsiekuti15|Western|A.m.s     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
H140KkIsiekuti15|Western|A.m.s     CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
Apis_mellifera_ligustica           CTAATCCTCTTGGATCAAATTTTAATAATTATAAAATTTCATTTCATCCA
                                    **************************************************

H144Nbiicipe|Central|A.m.scute     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H147Thika|Central|A.m.scutella     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H3Kasanga9|Eastern|A.m.litorea     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H5Kasanga9|Eastern|A.m.scutell     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H8Kasanga64|Eastern|A.m.montic     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H14Kasanga96|Eastern|A.m.scute     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H16Kathiani60|Eastern|A.m.scut     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H24Kathiani99|Eastern|A.m.scut     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H25Kathiani99|Eastern|A.m.scut     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H29Kathiani360|Eastern|A.m.scu     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H30Kathiani360|Eastern|A.m.scu     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H31Mumoni19|Eastern|A.m.litore     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
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H32Mumoni19|Eastern|A.m.litore     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H41Mumoni80|Eastern|A.m.litore     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H42Mumoni80|Eastern|A.m.litore     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H48Mathiakani52|Eastern|A.m.li     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
57TaitaChawiya1|Coast|A.m.lito     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H64TaitaRonge|Coast|A.m.scutel     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H65TaitaRonge|Coast|A.m.montic     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H69TanaRiverHewani|Coast|A.m.s     TATTTTTCAATTAAAGATCTTTTAGGATTTTATATCATCTTATTTATTTT
H70TanaRiverHewani|Coast|A.m.s     TATTTTTCAATTAAAGATCTTTTAGGATTTTATATCATCTTATTTATTTT
H72Mitukiyaiveti|Eastern|A.m.s     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H79TangaSengoma|Coast|A.m.lito     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H81TangaUsambara|Coast|A.m.scu     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H82TangaUsambara|Coast|A.m.lit     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H108Mathiakaniwild|Eastern|A.m     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H111KkMakuchi97|Western|A.m.sc     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H115KkMakuchi97|Western|A.m.sc     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H116KkMakuchi91|Western|A.m.li     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H117KkMakuchi91|Western|A.m.li     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H121KkMakuchi92|Western|A.m.li     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H126KkIsiekuti107|Western|A.m.     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H130KkIsiekuti107|Western|A.m.     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H132KkIsiekuti11|Western|A.m.s     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H134KkIsiekuti11|Western|A.m.l     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H137KkIsiekuti15|Western|A.m.s     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
H140KkIsiekuti15|Western|A.m.s     TATTTTTCAATTAAAGACCTTTTAGGATTTTATATTATCTTATTTATCTT
Apis_mellifera_ligustica           TATTTTTCAATTAAAGATCTTTTAGGATTTTATATCATCTTATTTATCTT
                                   ********** ********** ********** **

H144Nbiicipe|Central|A.m.scute     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H147Thika|Central|A.m.scutella     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H3Kasanga9|Eastern|A.m.litorea     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H5Kasanga9|Eastern|A.m.scutell     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H8Kasanga64|Eastern|A.m.montic     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H14Kasanga96|Eastern|A.m.scute     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H16Kathiani60|Eastern|A.m.scut     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H24Kathiani99|Eastern|A.m.scut     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H25Kathiani99|Eastern|A.m.scut     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H29Kathiani360|Eastern|A.m.scu     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H30Kathiani360|Eastern|A.m.scu     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H31Mumoni19|Eastern|A.m.litore     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H32Mumoni19|Eastern|A.m.litore     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H41Mumoni80|Eastern|A.m.litore     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H42Mumoni80|Eastern|A.m.litore     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H48Mathiakani52|Eastern|A.m.li     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
57TaitaChawiya1|Coast|A.m.lito     TATATTCATTAATTTTCAATTTCCATATCATTTAAGAGATCCAGATAATT
H64TaitaRonge|Coast|A.m.scutel     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H65TaitaRonge|Coast|A.m.montic     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H69TanaRiverHewani|Coast|A.m.s     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H70TanaRiverHewani|Coast|A.m.s     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H72Mitukiyaiveti|Eastern|A.m.s     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H79TangaSengoma|Coast|A.m.lito     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H81TangaUsambara|Coast|A.m.scu     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H82TangaUsambara|Coast|A.m.lit     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H108Mathiakaniwild|Eastern|A.m     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H111KkMakuchi97|Western|A.m.sc     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H115KkMakuchi97|Western|A.m.sc     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H116KkMakuchi91|Western|A.m.li     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H117KkMakuchi91|Western|A.m.li     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H121KkMakuchi92|Western|A.m.li     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H126KkIsiekuti107|Western|A.m.     TATATTCATTAACTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H130KkIsiekuti107|Western|A.m.     TATATTCATTAACTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H132KkIsiekuti11|Western|A.m.s     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H134KkIsiekuti11|Western|A.m.l     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H137KkIsiekuti15|Western|A.m.s     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
H140KkIsiekuti15|Western|A.m.s     TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGATAATT
Apis_mellifera_ligustica           TATATTCATTAATTTTCAATTTCCATATCATTTAGGAGATCCAGACAATT
                                   ********** ********************.********** ****

H144Nbiicipe|Central|A.m.scute     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H147Thika|Central|A.m.scutella     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H3Kasanga9|Eastern|A.m.litorea     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H5Kasanga9|Eastern|A.m.scutell     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
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H8Kasanga64|Eastern|A.m.montic     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H14Kasanga96|Eastern|A.m.scute     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H16Kathiani60|Eastern|A.m.scut     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H24Kathiani99|Eastern|A.m.scut     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H25Kathiani99|Eastern|A.m.scut     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H29Kathiani360|Eastern|A.m.scu     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H30Kathiani360|Eastern|A.m.scu     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H31Mumoni19|Eastern|A.m.litore     TTAAAATTGCAAATCCAATAAATACTCCGACTCATATTAAACCTGAATGA
H32Mumoni19|Eastern|A.m.litore     TTAAAATTGCAAATCCAATAAATACTCCGACTCATATTAAACCTGAATGA
H41Mumoni80|Eastern|A.m.litore     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H42Mumoni80|Eastern|A.m.litore     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H48Mathiakani52|Eastern|A.m.li     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
57TaitaChawiya1|Coast|A.m.lito     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H64TaitaRonge|Coast|A.m.scutel     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H65TaitaRonge|Coast|A.m.montic     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H69TanaRiverHewani|Coast|A.m.s     TTAAAATTGCAAATCCAATAAATACTCCAACTCACATTAAACCTGAATGA
H70TanaRiverHewani|Coast|A.m.s     TTAAAATTGCAAATCCAATAAATACTCCAACTCACATTAAACCTGAATGA
H72Mitukiyaiveti|Eastern|A.m.s     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H79TangaSengoma|Coast|A.m.lito     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H81TangaUsambara|Coast|A.m.scu     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H82TangaUsambara|Coast|A.m.lit     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H108Mathiakaniwild|Eastern|A.m     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H111KkMakuchi97|Western|A.m.sc     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H115KkMakuchi97|Western|A.m.sc     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H116KkMakuchi91|Western|A.m.li     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H117KkMakuchi91|Western|A.m.li     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H121KkMakuchi92|Western|A.m.li     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H126KkIsiekuti107|Western|A.m.     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H130KkIsiekuti107|Western|A.m.     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H132KkIsiekuti11|Western|A.m.s     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H134KkIsiekuti11|Western|A.m.l     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H137KkIsiekuti15|Western|A.m.s     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
H140KkIsiekuti15|Western|A.m.s     TTAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
Apis_mellifera_ligustica           TCAAAATTGCAAATCCAATAAATACTCCAACTCATATTAAACCTGAATGA
                                   * **************************.***** ***************

H144Nbiicipe|Central|A.m.scute     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H147Thika|Central|A.m.scutella     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H3Kasanga9|Eastern|A.m.litorea     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H5Kasanga9|Eastern|A.m.scutell     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H8Kasanga64|Eastern|A.m.montic     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H14Kasanga96|Eastern|A.m.scute     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H16Kathiani60|Eastern|A.m.scut     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H24Kathiani99|Eastern|A.m.scut     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H25Kathiani99|Eastern|A.m.scut     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H29Kathiani360|Eastern|A.m.scu     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H30Kathiani360|Eastern|A.m.scu     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H31Mumoni19|Eastern|A.m.litore     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H32Mumoni19|Eastern|A.m.litore     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H41Mumoni80|Eastern|A.m.litore     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H42Mumoni80|Eastern|A.m.litore     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H48Mathiakani52|Eastern|A.m.li     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
57TaitaChawiya1|Coast|A.m.lito     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H64TaitaRonge|Coast|A.m.scutel     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H65TaitaRonge|Coast|A.m.montic     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H69TanaRiverHewani|Coast|A.m.s     TATTTCCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H70TanaRiverHewani|Coast|A.m.s     TATTTCCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H72Mitukiyaiveti|Eastern|A.m.s     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H79TangaSengoma|Coast|A.m.lito     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H81TangaUsambara|Coast|A.m.scu     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H82TangaUsambara|Coast|A.m.lit     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H108Mathiakaniwild|Eastern|A.m     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H111KkMakuchi97|Western|A.m.sc     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H115KkMakuchi97|Western|A.m.sc     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H116KkMakuchi91|Western|A.m.li     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H117KkMakuchi91|Western|A.m.li     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H121KkMakuchi92|Western|A.m.li     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H126KkIsiekuti107|Western|A.m.     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H130KkIsiekuti107|Western|A.m.     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H132KkIsiekuti11|Western|A.m.s     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H134KkIsiekuti11|Western|A.m.l     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
H137KkIsiekuti15|Western|A.m.s     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
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H140KkIsiekuti15|Western|A.m.s     TATTTTCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
Apis_mellifera_ligustica           TATTTCCTATTTGCATATTCAATTTTACGAGCAATTCCTAATAAATTAGG
                                   ***** ****************************************

H144Nbiicipe|Central|A.m.scute     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H147Thika|Central|A.m.scutella     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H3Kasanga9|Eastern|A.m.litorea     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H5Kasanga9|Eastern|A.m.scutell     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H8Kasanga64|Eastern|A.m.montic     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H14Kasanga96|Eastern|A.m.scute     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H16Kathiani60|Eastern|A.m.scut     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H24Kathiani99|Eastern|A.m.scut     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H25Kathiani99|Eastern|A.m.scut     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H29Kathiani360|Eastern|A.m.scu     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H30Kathiani360|Eastern|A.m.scu     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H31Mumoni19|Eastern|A.m.litore     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H32Mumoni19|Eastern|A.m.litore     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H41Mumoni80|Eastern|A.m.litore     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H42Mumoni80|Eastern|A.m.litore     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H48Mathiakani52|Eastern|A.m.li     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
57TaitaChawiya1|Coast|A.m.lito     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H64TaitaRonge|Coast|A.m.scutel     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H65TaitaRonge|Coast|A.m.montic     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H69TanaRiverHewani|Coast|A.m.s     AGGTGTAATTGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H70TanaRiverHewani|Coast|A.m.s     AGGTGTAATTGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H72Mitukiyaiveti|Eastern|A.m.s     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H79TangaSengoma|Coast|A.m.lito     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H81TangaUsambara|Coast|A.m.scu     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H82TangaUsambara|Coast|A.m.lit     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H108Mathiakaniwild|Eastern|A.m     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H111KkMakuchi97|Western|A.m.sc     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H115KkMakuchi97|Western|A.m.sc     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H116KkMakuchi91|Western|A.m.li     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H117KkMakuchi91|Western|A.m.li     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H121KkMakuchi92|Western|A.m.li     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H126KkIsiekuti107|Western|A.m.     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H130KkIsiekuti107|Western|A.m.     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H132KkIsiekuti11|Western|A.m.s     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H134KkIsiekuti11|Western|A.m.l     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H137KkIsiekuti15|Western|A.m.s     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
H140KkIsiekuti15|Western|A.m.s     AGGTGTAATCGGACTAGTAATATCAATTCTTATTCTTTATATTATAATTT
Apis_mellifera_ligustica           AGGTGTAATCGGATTAGTAATATCAATTCTTATTCTTTATATTATAATTT
                                   ********* *** ***********************************

H144Nbiicipe|Central|A.m.scute     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H147Thika|Central|A.m.scutella     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H3Kasanga9|Eastern|A.m.litorea     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H5Kasanga9|Eastern|A.m.scutell     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H8Kasanga64|Eastern|A.m.montic     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H14Kasanga96|Eastern|A.m.scute     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H16Kathiani60|Eastern|A.m.scut     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H24Kathiani99|Eastern|A.m.scut     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H25Kathiani99|Eastern|A.m.scut     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H29Kathiani360|Eastern|A.m.scu     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H30Kathiani360|Eastern|A.m.scu     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H31Mumoni19|Eastern|A.m.litore     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H32Mumoni19|Eastern|A.m.litore     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H41Mumoni80|Eastern|A.m.litore     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H42Mumoni80|Eastern|A.m.litore     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H48Mathiakani52|Eastern|A.m.li     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
57TaitaChawiya1|Coast|A.m.lito     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H64TaitaRonge|Coast|A.m.scutel     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H65TaitaRonge|Coast|A.m.montic     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H69TanaRiverHewani|Coast|A.m.s     TTTATAATAATAAAATAATAAACAATAAATTTAATATATTAAATAAAATT
H70TanaRiverHewani|Coast|A.m.s     TTTATAATAATAAAATAATAAACAATAAATTTAATATATTAAATAAAATT
H72Mitukiyaiveti|Eastern|A.m.s     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H79TangaSengoma|Coast|A.m.lito     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H81TangaUsambara|Coast|A.m.scu     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H82TangaUsambara|Coast|A.m.lit     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H108Mathiakaniwild|Eastern|A.m     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H111KkMakuchi97|Western|A.m.sc     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H115KkMakuchi97|Western|A.m.sc     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
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H116KkMakuchi91|Western|A.m.li     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H117KkMakuchi91|Western|A.m.li     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H121KkMakuchi92|Western|A.m.li     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H126KkIsiekuti107|Western|A.m.     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H130KkIsiekuti107|Western|A.m.     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H132KkIsiekuti11|Western|A.m.s     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H134KkIsiekuti11|Western|A.m.l     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H137KkIsiekuti15|Western|A.m.s     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
H140KkIsiekuti15|Western|A.m.s     TTTATAATAATAAAATAATAAATAATAAATTTAATATATTAAATAAAATT
Apis_mellifera_ligustica           TTTATAATAATAAAATAATAAACAATAAATTTAATATATTAAATAAAATT
                                   ********************** **************************

H144Nbiicipe|Central|A.m.scute     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H147Thika|Central|A.m.scutella     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H3Kasanga9|Eastern|A.m.litorea     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H5Kasanga9|Eastern|A.m.scutell     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H8Kasanga64|Eastern|A.m.montic     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H14Kasanga96|Eastern|A.m.scute     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H16Kathiani60|Eastern|A.m.scut     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H24Kathiani99|Eastern|A.m.scut     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H25Kathiani99|Eastern|A.m.scut     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H29Kathiani360|Eastern|A.m.scu     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H30Kathiani360|Eastern|A.m.scu     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H31Mumoni19|Eastern|A.m.litore     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H32Mumoni19|Eastern|A.m.litore     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H41Mumoni80|Eastern|A.m.litore     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H42Mumoni80|Eastern|A.m.litore     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H48Mathiakani52|Eastern|A.m.li     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
57TaitaChawiya1|Coast|A.m.lito     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H64TaitaRonge|Coast|A.m.scutel     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H65TaitaRonge|Coast|A.m.montic     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H69TanaRiverHewani|Coast|A.m.s     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H70TanaRiverHewani|Coast|A.m.s     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H72Mitukiyaiveti|Eastern|A.m.s     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H79TangaSengoma|Coast|A.m.lito     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H81TangaUsambara|Coast|A.m.scu     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H82TangaUsambara|Coast|A.m.lit     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H108Mathiakaniwild|Eastern|A.m     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H111KkMakuchi97|Western|A.m.sc     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H115KkMakuchi97|Western|A.m.sc     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H116KkMakuchi91|Western|A.m.li     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H117KkMakuchi91|Western|A.m.li     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H121KkMakuchi92|Western|A.m.li     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H126KkIsiekuti107|Western|A.m.     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H130KkIsiekuti107|Western|A.m.     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H132KkIsiekuti11|Western|A.m.s     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H134KkIsiekuti11|Western|A.m.l     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H137KkIsiekuti15|Western|A.m.s     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
H140KkIsiekuti15|Western|A.m.s     TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
Apis_mellifera_ligustica           TATTATTGAATATTTATTAATAACTTCATTTTATTAACATGATTAGGTAA
                                   **************************************************

H144Nbiicipe|Central|A.m.scute     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H147Thika|Central|A.m.scutella     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H3Kasanga9|Eastern|A.m.litorea     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H5Kasanga9|Eastern|A.m.scutell     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H8Kasanga64|Eastern|A.m.montic     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H14Kasanga96|Eastern|A.m.scute     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H16Kathiani60|Eastern|A.m.scut     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H24Kathiani99|Eastern|A.m.scut     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H25Kathiani99|Eastern|A.m.scut     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H29Kathiani360|Eastern|A.m.scu     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H30Kathiani360|Eastern|A.m.scu     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H31Mumoni19|Eastern|A.m.litore     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H32Mumoni19|Eastern|A.m.litore     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H41Mumoni80|Eastern|A.m.litore     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H42Mumoni80|Eastern|A.m.litore     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H48Mathiakani52|Eastern|A.m.li     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
57TaitaChawiya1|Coast|A.m.lito     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H64TaitaRonge|Coast|A.m.scutel     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H65TaitaRonge|Coast|A.m.montic     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H69TanaRiverHewani|Coast|A.m.s     ACAATTGATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
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H70TanaRiverHewani|Coast|A.m.s     ACAATTGATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H72Mitukiyaiveti|Eastern|A.m.s     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H79TangaSengoma|Coast|A.m.lito     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H81TangaUsambara|Coast|A.m.scu     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H82TangaUsambara|Coast|A.m.lit     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H108Mathiakaniwild|Eastern|A.m     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H111KkMakuchi97|Western|A.m.sc     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H115KkMakuchi97|Western|A.m.sc     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H116KkMakuchi91|Western|A.m.li     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H117KkMakuchi91|Western|A.m.li     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H121KkMakuchi92|Western|A.m.li     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H126KkIsiekuti107|Western|A.m.     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H130KkIsiekuti107|Western|A.m.     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H132KkIsiekuti11|Western|A.m.s     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H134KkIsiekuti11|Western|A.m.l     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H137KkIsiekuti15|Western|A.m.s     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
H140KkIsiekuti15|Western|A.m.s     ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
Apis_mellifera_ligustica           ACAATTAATTGAATATCCATTTACTAATATTAATATATTATTTACAACAA
                                   ******.****************************************

H144Nbiicipe|Central|A.m.scute     CATATTTCTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
H147Thika|Central|A.m.scutella     CATATTTCTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
H3Kasanga9|Eastern|A.m.litorea     CATATTTCTTATATTTTTTCCTAAATTTCTATTTAAGAAAATTATGAGAT
H5Kasanga9|Eastern|A.m.scutell     CATATTTCTTATATTTTTTCCTAAATTTCTATTTAAGAAAATTATGAGAT
H8Kasanga64|Eastern|A.m.montic     CATATTTCTTATATTTTTTCCTAAATTTCTATTTAAGAAAATTATGAGAT
H14Kasanga96|Eastern|A.m.scute     CATATTTCTTATATTTTTTCCTAAATTTCTATTTAAGAAAATTATGAGAT
H16Kathiani60|Eastern|A.m.scut     CATATTTCTTATATTTTTTCCTAAATTTCTATTTAAGAAAATTATGAGAT
H24Kathiani99|Eastern|A.m.scut     CATATTTCTTATATTTTTTCCTAAATTTCTATTTAAGAAAATTATGAGAT
H25Kathiani99|Eastern|A.m.scut     CATATTTCTTATATTTTTTCCTAAATTTCTATTTAAGAAAATTATGAGAT
H29Kathiani360|Eastern|A.m.scu     CATATTTCTTATATTTTTTCCTAAATTTCTATTTAAGAAAATTATGAGAT
H30Kathiani360|Eastern|A.m.scu     CATATTTCTTATATTTTTTCCTAAATTTCTATTTAAGAAAATTATGAGAT
H31Mumoni19|Eastern|A.m.litore     CATATTTCTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
H32Mumoni19|Eastern|A.m.litore     CATATTTCTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
H41Mumoni80|Eastern|A.m.litore     CATATTTCTTATATTTTTTCCTAAATTTCTATTTAAGAAAATTATGAGAT
H42Mumoni80|Eastern|A.m.litore     CATATTTCTTATATTTTTTCCTAAATTTCTATTTAAGAAAATTATGAGAT
H48Mathiakani52|Eastern|A.m.li     CATATTTCTTATATTTTTTCCTAAATTTCTATTTAAGAAAATTATGAGAT
57TaitaChawiya1|Coast|A.m.lito     CATATTTCTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
H64TaitaRonge|Coast|A.m.scutel     CATATTTCTTATATTTTTTCCTAAATTTCTATTTAAGAAAATTATGAGAT
H65TaitaRonge|Coast|A.m.montic     CATATTTCTTATATTTTTTCCTAAATTTCTATTTAAGAAAATTATGAGAT
H69TanaRiverHewani|Coast|A.m.s     CATATTTCTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
H70TanaRiverHewani|Coast|A.m.s     CATATTTCTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
H72Mitukiyaiveti|Eastern|A.m.s     CATATTTCTTATATTTTTTCCTAAATTTCTATTTAAGAAAATTATGAGAT
H79TangaSengoma|Coast|A.m.lito     CATATTTCTTATATTTTTTCCTAAATTTCTATTTAAGAAAATTATGAGAT
H81TangaUsambara|Coast|A.m.scu     CATATTTCTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
H82TangaUsambara|Coast|A.m.lit     CATATTTCTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
H108Mathiakaniwild|Eastern|A.m     CATATTTCTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
H111KkMakuchi97|Western|A.m.sc     CATATTTCTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
H115KkMakuchi97|Western|A.m.sc     CATATTTCTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
H116KkMakuchi91|Western|A.m.li     CATATTTCTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
H117KkMakuchi91|Western|A.m.li     CATATTTCTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
H121KkMakuchi92|Western|A.m.li     CATATTTCTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
H126KkIsiekuti107|Western|A.m.     CATATTTCTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
H130KkIsiekuti107|Western|A.m.     CATATTTCTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
H132KkIsiekuti11|Western|A.m.s     CATATTTCTTATATTTTTTCCTAAATTTCTATTTAAGAAAATTATGAGAT
H134KkIsiekuti11|Western|A.m.l     CATATTTCTTATATTTTTTCCTAAATTTCTATTTAAGAAAATTATGAGAT
H137KkIsiekuti15|Western|A.m.s     CATATTTCTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
H140KkIsiekuti15|Western|A.m.s     CATATTTCTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
Apis_mellifera_ligustica           CATATTTTTTATATTTTTTCTTAAATTTCTATTTAAGAAAATTATGAGAT
                                   ******* ************ ****************************

H144Nbiicipe|Central|A.m.scute     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H147Thika|Central|A.m.scutella     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H3Kasanga9|Eastern|A.m.litorea     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H5Kasanga9|Eastern|A.m.scutell     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H8Kasanga64|Eastern|A.m.montic     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H14Kasanga96|Eastern|A.m.scute     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H16Kathiani60|Eastern|A.m.scut     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H24Kathiani99|Eastern|A.m.scut     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H25Kathiani99|Eastern|A.m.scut     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H29Kathiani360|Eastern|A.m.scu     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H30Kathiani360|Eastern|A.m.scu     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H31Mumoni19|Eastern|A.m.litore     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
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H32Mumoni19|Eastern|A.m.litore     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H41Mumoni80|Eastern|A.m.litore     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H42Mumoni80|Eastern|A.m.litore     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H48Mathiakani52|Eastern|A.m.li     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
57TaitaChawiya1|Coast|A.m.lito     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H64TaitaRonge|Coast|A.m.scutel     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H65TaitaRonge|Coast|A.m.montic     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H69TanaRiverHewani|Coast|A.m.s     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H70TanaRiverHewani|Coast|A.m.s     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H72Mitukiyaiveti|Eastern|A.m.s     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H79TangaSengoma|Coast|A.m.lito     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H81TangaUsambara|Coast|A.m.scu     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H82TangaUsambara|Coast|A.m.lit     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H108Mathiakaniwild|Eastern|A.m     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H111KkMakuchi97|Western|A.m.sc     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H115KkMakuchi97|Western|A.m.sc     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H116KkMakuchi91|Western|A.m.li     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H117KkMakuchi91|Western|A.m.li     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H121KkMakuchi92|Western|A.m.li     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H126KkIsiekuti107|Western|A.m.     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H130KkIsiekuti107|Western|A.m.     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H132KkIsiekuti11|Western|A.m.s     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H134KkIsiekuti11|Western|A.m.l     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H137KkIsiekuti15|Western|A.m.s     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
H140KkIsiekuti15|Western|A.m.s     AATTTAATTTGAAATTCACCATCAAATTAAATTAAATAAAAAATTAATTT
Apis_mellifera_ligustica           AATTTAATTTGAAATTCACCATTAAATTAAATTAAATAAAAAATTAATTT
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H144Nbiicipe|Central|A.m.scute     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H147Thika|Central|A.m.scutella     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H3Kasanga9|Eastern|A.m.litorea     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H5Kasanga9|Eastern|A.m.scutell     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H8Kasanga64|Eastern|A.m.montic     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H14Kasanga96|Eastern|A.m.scute     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H16Kathiani60|Eastern|A.m.scut     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H24Kathiani99|Eastern|A.m.scut     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H25Kathiani99|Eastern|A.m.scut     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H29Kathiani360|Eastern|A.m.scu     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H30Kathiani360|Eastern|A.m.scu     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H31Mumoni19|Eastern|A.m.litore     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H32Mumoni19|Eastern|A.m.litore     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H41Mumoni80|Eastern|A.m.litore     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H42Mumoni80|Eastern|A.m.litore     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H48Mathiakani52|Eastern|A.m.li     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
57TaitaChawiya1|Coast|A.m.lito     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H64TaitaRonge|Coast|A.m.scutel     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H65TaitaRonge|Coast|A.m.montic     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H69TanaRiverHewani|Coast|A.m.s     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H70TanaRiverHewani|Coast|A.m.s     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H72Mitukiyaiveti|Eastern|A.m.s     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H79TangaSengoma|Coast|A.m.lito     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H81TangaUsambara|Coast|A.m.scu     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H82TangaUsambara|Coast|A.m.lit     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H108Mathiakaniwild|Eastern|A.m     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H111KkMakuchi97|Western|A.m.sc     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H115KkMakuchi97|Western|A.m.sc     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H116KkMakuchi91|Western|A.m.li     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H117KkMakuchi91|Western|A.m.li     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H121KkMakuchi92|Western|A.m.li     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H126KkIsiekuti107|Western|A.m.     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H130KkIsiekuti107|Western|A.m.     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H132KkIsiekuti11|Western|A.m.s     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H134KkIsiekuti11|Western|A.m.l     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H137KkIsiekuti15|Western|A.m.s     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
H140KkIsiekuti15|Western|A.m.s     TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
Apis_mellifera_ligustica           TTTTAAAATCAATTTTTAAATTTTAAGTTAATGAACTTGAATAAGTATAT
                                   **************************************************

H144Nbiicipe|Central|A.m.scute     ATTT
H147Thika|Central|A.m.scutella     ATTT
H3Kasanga9|Eastern|A.m.litorea     ATTT
H5Kasanga9|Eastern|A.m.scutell     ATTT
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H8Kasanga64|Eastern|A.m.montic     ATTT
H14Kasanga96|Eastern|A.m.scute     ATTT
H16Kathiani60|Eastern|A.m.scut     ATTT
H24Kathiani99|Eastern|A.m.scut     ATTT
H25Kathiani99|Eastern|A.m.scut     ATTT
H29Kathiani360|Eastern|A.m.scu     ATTT
H30Kathiani360|Eastern|A.m.scu     ATTT
H31Mumoni19|Eastern|A.m.litore     ATTT
H32Mumoni19|Eastern|A.m.litore     ATTT
H41Mumoni80|Eastern|A.m.litore     ATTT
H42Mumoni80|Eastern|A.m.litore     ATTT
H48Mathiakani52|Eastern|A.m.li     ATTT
57TaitaChawiya1|Coast|A.m.lito     ATTT
H64TaitaRonge|Coast|A.m.scutel     ATTT
H65TaitaRonge|Coast|A.m.montic     ATTT
H69TanaRiverHewani|Coast|A.m.s     ATTT
H70TanaRiverHewani|Coast|A.m.s     ATTT
H72Mitukiyaiveti|Eastern|A.m.s     ATTT
H79TangaSengoma|Coast|A.m.lito     ATTT
H81TangaUsambara|Coast|A.m.scu     ATTT
H82TangaUsambara|Coast|A.m.lit     ATTT
H108Mathiakaniwild|Eastern|A.m     ATTT
H111KkMakuchi97|Western|A.m.sc     ATTT
H115KkMakuchi97|Western|A.m.sc     ATTT
H116KkMakuchi91|Western|A.m.li     ATTT
H117KkMakuchi91|Western|A.m.li     ATTT
H121KkMakuchi92|Western|A.m.li     ATTT
H126KkIsiekuti107|Western|A.m.     ATTT
H130KkIsiekuti107|Western|A.m.     ATTT
H132KkIsiekuti11|Western|A.m.s     ATTT
H134KkIsiekuti11|Western|A.m.l     ATTT
H137KkIsiekuti15|Western|A.m.s     ATTT
H140KkIsiekuti15|Western|A.m.s     ATTT
Apis_mellifera_ligustica           ATTT
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Appendix 2.3: COI-COII CLUSTAL 2.1 MSA

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H020|Eastern|Kathiani60|scutel     -GTTGAATTCAATTTCATCTATAGGATCAATAATTTCATTAAATAGAATA
H064|Eastern|Ronge2|scutellata     -GTTGAATTCAATTTCATCAATAGGATCAATAATTTCATTAAATAGAATA
H015|Eastern|Kasanga96|scutell     -GTTGAATTCAATTTCATCTATAGGATCAATAATTTCATTAAATAGAATA
H032|Eastern|Mumoni19|litorea      -GTTGAATTCAATTTCATCAATAGGATCAATAATTTCATTAAATAGAATA
H014|Eastern|Kasanga96|scutell     -GTTGAATTCAATTTCATCTATAGGATCAATAATTTCATTAAATAGAATA
H031|Eastern|Mumoni19|litorea      -GTTGAATTCAATTTCATCAATAGGATCAATAATTTCATTAAATAGAATA
H008|Eastern|Kasanga64|montico     -GTTGAATTCAATTTCATCTATAGGATCAATAATTTCATTAAATAGAATA
H016|Eastern|Kathiani60|scutel     -GTTGAATTCAATTTCATCTATAGGATCAATAATTTCATTAAATAGAATA
H005|Eastern|Kasanga9|scutella     -GTTGAATTCAATTTCATCTATAGGATCAATAATTTCATTAAATAGAATA
H048|Eastern|Mathiakani52|lito     -GTTGAATTCAATTTCATCTATAGGATCAATAATTTCATTAAATAGAATA
H055|Eastern|Mathiakani58|scut     -GTTGAATTCAATTTCATCTATAGGATCAATAATTTCATTAAATAGAATA
H024|Eastern|Kathiani99|scutel     -GTTGAATTCAATTTCATCTATAGGATCAATAATTTCATTAAATAGAATA
H009|Eastern|Kasanga64|scutell     -GTTGAATTCAATTTCATCTATAGGATCAATAATTTCATTAAATAGAATA
H029|Eastern|Kathiani360|scute     -GTTGAATTCAATTTCATCTATAG-GACAATAATTTCAT-----------
H030|Eastern|Kathiani360|scute     -GTTGAATTCAATTTCATCAATAG-GACAATAATTTCAT-----------
H041|Eastern|Mumoni80|litorea      -GTTGAATTCAATTTCATCTATAGGATCAATAATTTCATTAAATAGAATA
A.m.ligustica                      GTTGAAATTCAATTTCATCTATAGGATCAATAATTTCATTAAATAGAATA
                                     * .**************:**** .:************

H020|Eastern|Kathiani60|scutel     ATTTTTTTAATTTTTATTATTTTAGAAAGATTAATTTCTAAACGAATATT
H064|Eastern|Ronge2|scutellata     ATTTTTTTAATTTTTATTATTTTAGAAAGATTAATTTCTAAACGAATATT
H015|Eastern|Kasanga96|scutell     ATTTTTTTAATTTTTATTATTTTAGAAAGATTAATTTCTAAACGAATATT
H032|Eastern|Mumoni19|litorea      ATTTTTTTAATTTTTATTATTTTAGAAAGATTAATTTCTAAACGAATATT
H014|Eastern|Kasanga96|scutell     ATTTTTTTAATTTTTATTATTTTAGAAAGATTAATTTCTAAACGAATATT
H031|Eastern|Mumoni19|litorea      ATTTTTTTAATTTTTATTATTTTAGAAAGATTAATTTCTAAACGAATATT
H008|Eastern|Kasanga64|montico     ATTTTTTTAATTTTTATTATTTTAGAAAGATTAATTTCTAAACGAATATT
H016|Eastern|Kathiani60|scutel     ATTTTTTTAATTTTTATTATTTTAGAAAGATTAATTTCTAAACGAATATT
H005|Eastern|Kasanga9|scutella     ATTTTTTTAATTTTTATTATTTTAGAAAGATTAATTTCTAAACGAATATT
H048|Eastern|Mathiakani52|lito     ATTTTTTTAATTTTTATTATTTTAGAAAGATTAATTTCTAAACGAATATT
H055|Eastern|Mathiakani58|scut     ATTTTTTTAATGGGATTATTTTAGAAAGATTAATTTCTAAACGAATATT
H024|Eastern|Kathiani99|scutel     ATTTTTTTAATTTTTATTATTTTAGAAAGATTAATTTCTAAACGAATATT
H009|Eastern|Kasanga64|scutell     ATTTTTTTAATTTTTATTATTTTAGAAAGATTAATTTCTAAACGAATATT
H029|Eastern|Kathiani360|scute     -----------TTTTATTATTTTAGAAAGATTAATTTCTAAACGAATATT
H030|Eastern|Kathiani360|scute     -----------TTTTATTATTTTAGAAAGATTAATTTCTAAACGAATATT
H041|Eastern|Mumoni80|litorea      ATTTTTTTAATTTTTATTATTTTAGAAAGATTAATTTCTAAACGAATATT
A.m.ligustica                      ATTTTTTTAATTTTTATTATTTTAGAAAGATTAATTTCTAAACGAATATT
                                             *   **********************************

H020|Eastern|Kathiani60|scutel     ATTATTTAAATTCAACCAATCATCACTTGAATGATTAAATTTTTTACCAC
H064|Eastern|Ronge2|scutellata     ATTATTTAAATTCAACCAATCATCACTTGAATGATTAAATTTTTTACCAC
H015|Eastern|Kasanga96|scutell     ATTATTTAAATTCAACCAATCATCACTTGAATGATTAAATTTTTTACCAC
H032|Eastern|Mumoni19|litorea      ATTATTTAAATTCAACCAATCATCACTTGAATGATTAAATTTTTTACCAC
H014|Eastern|Kasanga96|scutell     ATTATTTAAATTCAACCAATCATCACTTGAATGATTAAATTTTTTACCAC
H031|Eastern|Mumoni19|litorea      ATTATTTAAATTCAACCAATCATCACTTGAATGATTAAATTTTTTACCAC
H008|Eastern|Kasanga64|montico     ATTATTTAAATTCAACCAATCATCACTTGAATGATTAAATTTTTTACCAC
H016|Eastern|Kathiani60|scutel     ATTATTTAAATTCAACCAATCATCACTTGAATGATTAAATTTTTTACCAC
H005|Eastern|Kasanga9|scutella     ATTATTTAAATTCAACCAATCATCACTTGAATGATTAAATTTTTTACCAC
H048|Eastern|Mathiakani52|lito     ATTATTTAAATTCAACCAATCATCACTTGAATGATTAAATTTTTTACCAC
H055|Eastern|Mathiakani58|scut     ATTATTTAAATTCAACCAATCATCACTTGAATGATTAAATTTTTTACCAC
H024|Eastern|Kathiani99|scutel     ATTATTTAAATTCAACCAATCATCACTTGAATGATTAAATTTTTTACCAC
H009|Eastern|Kasanga64|scutell     ATTATTTAAATTCAACCAATCATCACTTGAATGATTAAATTTTTTACCAC
H029|Eastern|Kathiani360|scute     ATTATTTAAATTCAACCGATCATCGCTTGAATGATTAAATTTTTTACCAC
H030|Eastern|Kathiani360|scute     ATTATTTAAATTCAACCAATCATCACTTGAATGATTAAATTTTTTACCAC
H041|Eastern|Mumoni80|litorea      ATTATTTAAATTCAACCAATCATCACTTGAATGATTAAATTTTTTACCAC
A.m.ligustica                      ATTATTTAAATTCAACCAATCATCACTTGAATGATTAAATTTTTTACCAC
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H020|Eastern|Kathiani60|scutel     CTTTAGATCATTCACATTTAGAAATTCCATTATTAATTAAAAATTTAAAT
H064|Eastern|Ronge2|scutellata     CTTTAGATCATTCACATTTAGAAATTCCATTATTAATTAAAAATTTAAAT
H015|Eastern|Kasanga96|scutell     CTTTAGATCATTCACATTTAGAAATTCCATTATTAATTAAAAATTTAAAT
H032|Eastern|Mumoni19|litorea      CTTTAGATCATTCACATTTAGAAATTCCATTATTAATTAAAAATTTAAAT
H014|Eastern|Kasanga96|scutell     CTTTAGATCATTCACATTTAGAAATTCCATTATTAATTAAAAATTTAAAT
H031|Eastern|Mumoni19|litorea      CTTTAGATCATTCACATTTAGAAATTCCATTATTAATTAAAAATTTAAAT
H008|Eastern|Kasanga64|montico     CTTTAGATCATTCACATTTAGAAATTCCATTATTAATTAAAAATTTAAAT
H016|Eastern|Kathiani60|scutel     CTTTAGATCATTCACATTTAGAAATTCCATTATTAATTAAAAATTTAAAT
H005|Eastern|Kasanga9|scutella     CTTTAGATCATTCACATTTAGAAATTCCATTATTAATTAAAAATTTAAAT
H048|Eastern|Mathiakani52|lito     CTTTAGATCATTCACATTTAGAAATTCCATTATTAATTAAAAATTTAAAT
H055|Eastern|Mathiakani58|scut     CTTTAGATCATTCACATTTAGAAATTCCATTATTAATTAAAAATTTAAAT
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H024|Eastern|Kathiani99|scutel     CTTTAGATCATTCACATTTAGAAATTCCATTATTAATTAAAAATTTAAAT
H009|Eastern|Kasanga64|scutell     CTTTAGATCATTCACATTTAGAAATTCCATTATTAATTAAAAATTTAAAT
H029|Eastern|Kathiani360|scute     CTTTAGATCATTCACATTTAGAAATTCCATTATTAATTAAAAATTTAAAT
H030|Eastern|Kathiani360|scute     CTTTAGATCATTCACATTTAGAAATTCCATTATTAATTAAAAATTTAAAT
H041|Eastern|Mumoni80|litorea      CTTTAGATCATTCACATTTAGAAATTCCATTATTAATTAAAAATTTAAAT
A.m.ligustica                      CTCTAGATCATTCACATTTAGAAATTCCATTATTAATTAAAAATTTAAAT
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H020|Eastern|Kathiani60|scutel     TTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAA
H064|Eastern|Ronge2|scutellata     TTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAA
H015|Eastern|Kasanga96|scutell     TTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAA
H032|Eastern|Mumoni19|litorea      TTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAA
H014|Eastern|Kasanga96|scutell     TTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAA
H031|Eastern|Mumoni19|litorea      TTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAA
H008|Eastern|Kasanga64|montico     TTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAA
H016|Eastern|Kathiani60|scutel     TTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAA
H005|Eastern|Kasanga9|scutella     TTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAA
H048|Eastern|Mathiakani52|lito     TTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAA
H055|Eastern|Mathiakani58|scut     TTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAA
H024|Eastern|Kathiani99|scutel     TTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAA
H009|Eastern|Kasanga64|scutell     TTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAA
H029|Eastern|Kathiani360|scute     TTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAA
H030|Eastern|Kathiani360|scute     TTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAA
H041|Eastern|Mumoni80|litorea      TTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAA
A.m.ligustica                      TTAAAATCAATTTTAATTAAATTTTAATATGGCAGAATAAGTGCATTGAA
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H020|Eastern|Kathiani60|scutel     CTTAAGATTCAAATATAAAGTATTTTTAAACTTTTATTAAAATTAATAAA
H064|Eastern|Ronge2|scutellata     CTTAAGATTCAAATATAAAGTATTTTTAAACTTTTATTAAAATTAATAAA
H015|Eastern|Kasanga96|scutell     CTTAAGATTCAAATATAAAGTATTTTTAAACTTTTATTAAAATTAATAAA
H032|Eastern|Mumoni19|litorea      CTTAAGATTCAAATATAAAGTATTTTTAAACTTTTATTAAAATTAATAAA
H014|Eastern|Kasanga96|scutell     CTTAAGATTCAAATATAAAGTATTTTTAAACTTTTATTAAAATTAATAAA
H031|Eastern|Mumoni19|litorea      CTTAAGATTCAAATATAAAGTATTTTTAAACTTTTATTAAAATTAATAAA
H008|Eastern|Kasanga64|montico     CTTAAGATTCAAATATAAAGTATTTTTAAACTTTTATTAAAATTAATAAA
H016|Eastern|Kathiani60|scutel     CTTAAGATTCAAATATAAAGTATTTTTAAACTTTTATTAAAATTAATAAA
H005|Eastern|Kasanga9|scutella     CTTAAGATTCAAATATAAAGTATTTTTAAACTTTTATTAAAATTAATAAA
H048|Eastern|Mathiakani52|lito     CTTAAGATTCAAATATAAAGTATTTTTAAACTTTTATTAAAATTAATAAA
H055|Eastern|Mathiakani58|scut     CTTAAGATTCAAATATAAAGTATTTTTAAACTTTTATTAAAATTAATAAA
H024|Eastern|Kathiani99|scutel     CTTAAGATTCAAATATAAAGTATTTTTAAACTTTTATTAAAATTAATAAA
H009|Eastern|Kasanga64|scutell     CTTAAGATTCAAATATAAAGTATTTTTAAACTTTTATTAAAATTAATAAA
H029|Eastern|Kathiani360|scute     CTTAAGATTCAAATATAAAGTATTTTTAAACTTTTATTAAAATTAATAAA
H030|Eastern|Kathiani360|scute     CTTAAGATTCAAATATAAAGTATTTTTAAACTTTTATTAAAATTAATAAA
H041|Eastern|Mumoni80|litorea      CTTAAGATTCAAATATAAAGTATTTTTAAACTTTTATTAAAATTAATAAA
A.m.ligustica                      CTTAAGATTCAAATATAAAGTATTTTTAAACTTTTATTAAAAT-------
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H020|Eastern|Kathiani60|scutel     TTAATATAAAATAAAACAAAATATAACAG----------------------
H064|Eastern|Ronge2|scutellata     TTAATATAAAATAAAACAAAATATAACAG----------------------
H015|Eastern|Kasanga96|scutell     TTAATATAAAATAAAACAAAATATAACAGAATATATTTATTAAAATTTAA
H032|Eastern|Mumoni19|litorea      TTAATATAAAATAAAACAAAATATAACAG----------------------
H014|Eastern|Kasanga96|scutell     TTAATATAAAATAAAACAAAATATAACAGAATATATTTATTAAAATTTAA
H031|Eastern|Mumoni19|litorea      TTAATATAAAATAAAACAAAATATAACAG----------------------
H008|Eastern|Kasanga64|montico     TTAATATAAAATAAAACAAAATATAACAGAATATATTTATTAAAATTTAA
H016|Eastern|Kathiani60|scutel     TTAATATAAAATAAAACAAAATATAACAGAATATATTTATTAAAATTTAA
H005|Eastern|Kasanga9|scutella     TTAATATAAAATAAAACAAAATATAACAGAATATATTTATTAAAATTTAA
H048|Eastern|Mathiakani52|lito     TTAATATAAAATAAAACAAAATATAACAGAATATATTTATTAAAATTTAA
H055|Eastern|Mathiakani58|scut     TTAATATAAAATAAAACAAAATATAACA-----------------------
H024|Eastern|Kathiani99|scutel     TTAATATAAAATAAAACAAAATATAACAGAATATATTTATTAAAATTTAA
H009|Eastern|Kasanga64|scutell     TTAATATAAAATAAAACAAAATATAACAGAATATATTTATTAAAATTTAA
H029|Eastern|Kathiani360|scute     TTAATATAAAATAAAACAAAATATAACAGAATATATTTATTAAAATTTAA
H030|Eastern|Kathiani360|scute     TTAATATAAAATAAAACAAAATATAACAGAATATATTTATTAAAATTTAA
H041|Eastern|Mumoni80|litorea      TTAATATAAAATAAAACAAAATATAACAGAATATATTTATTAAAATTTAA
A.m.ligustica                      --------------------------------------------------

H020|Eastern|Kathiani60|scutel     --------------------------------------------------
H064|Eastern|Ronge2|scutellata     --------------------------------------------------
H015|Eastern|Kasanga96|scutell     TTTATTAAAATTCCCACTTAATTCATATTAATTTAAAAATAAATTAATAA
H032|Eastern|Mumoni19|litorea      --------------------------------------------------
H014|Eastern|Kasanga96|scutell     TTTATTAAAATTCCCACTTAATTCATATTAATTTAAAAATAAATTAATAA
H031|Eastern|Mumoni19|litorea      --------------------------------------------------
H008|Eastern|Kasanga64|montico     TTTATTAAAATTCCCACTTAATTCATATTAATTTAAAAATAAATTAATAA
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H016|Eastern|Kathiani60|scutel     TTTATTAAAATTCCCACTTAATTCATATTAATTTAAAAATAAATTAATAA
H005|Eastern|Kasanga9|scutella     TTTATTAAAATTCCCACTTAATTCATATTAATTTAAAAATAAATTAATAA
H048|Eastern|Mathiakani52|lito     TTTATTAAAATTCCCACTTAATTCATATTAATTTAAAAATAAATTAATAG
H055|Eastern|Mathiakani58|scut     --------------------------------------------------
H024|Eastern|Kathiani99|scutel     TTTATTAAAATTCCCACTTGATTCATATTAATTTAAAAATAAATTAATAA
H009|Eastern|Kasanga64|scutell     TTTATTAAAATTCCCACTTGATTCATATTAATTTAAAAATAAATTAATAA
H029|Eastern|Kathiani360|scute     TTTATTAAAATTCCCACTTAATTCATATTAATTTAAAAATAAATTAATAA
H030|Eastern|Kathiani360|scute     TTTATTAAAATTCCCACTTAATTCATATTAATTTAAAAATAAATTAATAA
H041|Eastern|Mumoni80|litorea      TTTATTAAAATTCCCACTTGATTCATATTAATTTAAAAATAAATTAATAA
A.m.ligustica                      --------------------------------------------------

H020|Eastern|Kathiani60|scutel     --------------------------------------------------
H064|Eastern|Ronge2|scutellata     --------------------------------------------------
H015|Eastern|Kasanga96|scutell     CAATTTTAATAAAATAAATAATTAATTTTATTTTTATATTGAATTTTTAA
H032|Eastern|Mumoni19|litorea      --------------------------------------------------
H014|Eastern|Kasanga96|scutell     CAATTTTAATAAAATAAATAATTAATTTTATTTTTATATTGAATTTTTAA
H031|Eastern|Mumoni19|litorea      --------------------------------------------------
H008|Eastern|Kasanga64|montico     CAATTTTAATAAAATAAATAATTAATTTTATTTTTATATTGAATTTTTAA
H016|Eastern|Kathiani60|scutel     CAATTTTAATAAAATAAATAATTAATTTTATTTTTATATTGAATTTTTAA
H005|Eastern|Kasanga9|scutella     CAATTTTAATAAAATAAATAATTAATTTTATTTTTATATTGAATTTTTAA
H048|Eastern|Mathiakani52|lito     CAATTTTAATAAAATAAATAATTAATTTTATTTTTAGATTGAATTTTTAA
H055|Eastern|Mathiakani58|scut     --------------------------------------------------
H024|Eastern|Kathiani99|scutel     CAATTTTAATAAAATAAATAATTAATTTTATTTTTATATTGAATTTTTAA
H009|Eastern|Kasanga64|scutell     CAATTTTAATAAAATAAATAATTAATTTTATTTTTATATTGAATTTTTAA
H029|Eastern|Kathiani360|scute     CAATTTTAATAAAATAAATAATTAATTTTATTTTTATATTGAATTTTTAA
H030|Eastern|Kathiani360|scute     CAATTTTAATAAAATAAATAATTAATTTTATTTTTATATTGAATTTTTAA
H041|Eastern|Mumoni80|litorea      CAATTTTAATAAAATAAATAATTAATTTTATTTTTATATTGAATTTTTAA
A.m.ligustica                      --------------------------------------------------

H020|Eastern|Kathiani60|scutel     --------------------------------------------------
H064|Eastern|Ronge2|scutellata     --------------------------------------------------
H015|Eastern|Kasanga96|scutell     TTCAATCTTAAAGATTTAATCTTTTTATTAAAATTAATAAATTAATATAA
H032|Eastern|Mumoni19|litorea      --------------------------------------------------
H014|Eastern|Kasanga96|scutell     TTCAATCTTAAAGATTTAATCTTTTTATTAAAATTAATAAATTAATATAA
H031|Eastern|Mumoni19|litorea      --------------------------------------------------
H008|Eastern|Kasanga64|montico     TTCAATCTTAAAGATTTAATCTTTTTATTAAAATTAATAAATTAATATAA
H016|Eastern|Kathiani60|scutel     TTCAATCTTAAAGATTTAATCTTTTTATTAAAATTAATAAATTAATATAA
H005|Eastern|Kasanga9|scutella     TTCAATCTTAAAGATTTAATCTTTTTATTAAAATTAATAAATTAATATAA
H048|Eastern|Mathiakani52|lito     TTCAATCTTAAAGATTTAATCTTTTTATTAAAATTAATAAATTAATATAA
H055|Eastern|Mathiakani58|scut     --------------------------------------------------
H024|Eastern|Kathiani99|scutel     TTCAATCTTAAAGATTTAATCTTTTTATTAAAATTAATAAATTAATATAA
H009|Eastern|Kasanga64|scutell     TTCAATCTTAAAGATTTAATCTTTTTATTAAAATTAATAAATTAATATAA
H029|Eastern|Kathiani360|scute     TTCAATCTTAAAGATTTAATCTTTTTATTAAAATTAATAAATTAATATAA
H030|Eastern|Kathiani360|scute     TTCAATCTTAAAGATTTAATCTTTTTATTAAAATTAATAAATTAATATAA
H041|Eastern|Mumoni80|litorea      TTCAATCTTAAAGATTTAATCTTTTTATTAAAATTAATAAATTAATATAA
A.m.ligustica                      --------------------------------------------------

H020|Eastern|Kathiani60|scutel     --------------------AATATATTTATTAAAATTTAATTTATTAAA
H064|Eastern|Ronge2|scutellata     --------------------AATATATTTATTAAAATTTAATTTATTAAA
H015|Eastern|Kasanga96|scutell     AATAAAACAAAATATAACAGAATATATTTATTAAAATTTAATTTATTAAA
H032|Eastern|Mumoni19|litorea      --------------------AATATATTTATTAAAATTTAATTTATTAAA
H014|Eastern|Kasanga96|scutell     AATAAAACAAAATATAACAGAATATATTTATTAAAATTTAATTTATTAAA
H031|Eastern|Mumoni19|litorea      --------------------AATATATTTATTAAAATTTAATTTATTAAA
H008|Eastern|Kasanga64|montico     AATAAAACAAAATATAACAGAATATATTTATTAAAATTTAATTTATTAAA
H016|Eastern|Kathiani60|scutel     AATAAAACAAAATATAACAGAATATATTTATTAAAATTTAATTTATTAAA
H005|Eastern|Kasanga9|scutella     AATAAAACAAAATATAACAGAATATATTTATTAAAATTTAATTTATTAAA
H048|Eastern|Mathiakani52|lito     AATAAAACAAAATATAACAGAATATATTTATTAAAATTTAATTTATTAAA
H055|Eastern|Mathiakani58|scut     --------------------AAATATATTTATTAAAATTTAATTTATTAAA
H024|Eastern|Kathiani99|scutel     AATAAAACAAAATATAACAGAATATATTTATTAAAATTTAATTTATTAAA
H009|Eastern|Kasanga64|scutell     AATAAAACAAAATATAACAGAATATATTTATTAAAATTTAATTTATTAAA
H029|Eastern|Kathiani360|scute     AATAAAACAAAATATAACAGAATATATTTATTAAAATTTAATTTATTAAA
H030|Eastern|Kathiani360|scute     AATAAAACAAAATATAACAGAATATATTTATTAAAATTTAATTTATTAAA
H041|Eastern|Mumoni80|litorea      AATAAAACAAAATATAACAGAATATATTTATTAAAATTTAATTTATTAAA
A.m.ligustica                      --------------------------------------------------

H020|Eastern|Kathiani60|scutel     ATTCCCCACTGAATTCATATTAATTTAAAAATAAATTAATAACAATTTT-
H064|Eastern|Ronge2|scutellata     ATTCCCCACTGAATTCATATTAATTTAAAAATAAATTAATAACAATTTT-
H015|Eastern|Kasanga96|scutell     ATTCCCCACTTAATTCATATTAATTTAAAAATAAATTAATAACAATTTT-
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H032|Eastern|Mumoni19|litorea     ATTCCCCACTTAATTCATATTAATTTAAAAATAAATTAATAACAATTTT-
H014|Eastern|Kasanga96|scutell    ATTCCCCACTTAATTCATATTAATTTAAAAATAAATTAATAACAATTTT-
H031|Eastern|Mumoni19|litorea     ATTCCCCACTTAATTCATATTAATTTAAAAATAAATTAATAACAATTTT-
H008|Eastern|Kasanga64|montico    ATTCCCCACTTAATTCATATTAATTTAAAAATAAATTAATAACAATTTT-
H016|Eastern|Kathiani60|scutel    ATTCCCCACTTAATTCATATTAATTTAAAAATAAATTAATAACAATTTT-
H005|Eastern|Kasanga9|scutella    ATTCCCCACTTAATTCATATTAATTTAAAAATAAATTAATAACAATTTT-
H048|Eastern|Mathiakani52|lito    ATTCCCCACTTAATTCATATTAATTTAAAAATAAATTAATAACAATTTT-
H055|Eastern|Mathiakani58|scut    ATTCCCCACTGAATTCATATTAATTTAAAAATAAATTAATAACAATTTT-
H024|Eastern|Kathiani99|scutel    ATTCCCCACTTAATTCATATTAATTTAAAAATAAATTAATAACAATTTT-
H009|Eastern|Kasanga64|scutell    ATTCCCCACTTAATTCATATTAATTTAAAAATAAATTAATAACAATTTT-
H029|Eastern|Kathiani360|scute    ATTCCCCACTTAATTCATATTAATTTAAAAATAAATTAATAACAATTTT-
H030|Eastern|Kathiani360|scute    ATTCCCCACTTAATTCATATTAATTTAAAAATAAATTAATAACAATTTT-
H041|Eastern|Mumoni80|litorea     ATTCCCCACTGAATTCATATTAATTTAAAAATAAATTAATAACAATTTT-
A.m.ligustica                     -TTCCCCACTTAATTCATATTAATTTAAAAATAAATTAATAACAATTTTT
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H020|Eastern|Kathiani60|scutel    AATAAAATAAATAATTAATTTTATTTTTATATTGAATTTTTAATTCAATC
H064|Eastern|Ronge2|scutellata    AATAAAATAAATAATTAATTTTATTTTTATATT-----------------
H015|Eastern|Kasanga96|scutell    AATAAAATAAATAATTAATTTTATTTTTATATT-----------------
H032|Eastern|Mumoni19|litorea     AATAAAATAAATAATTAATTTTATTTTTATATT-----------------
H014|Eastern|Kasanga96|scutell    AATAAAATAAATAATTAATTTTATTTTTATATT-----------------
H031|Eastern|Mumoni19|litorea     AATAAAATAAATAATTAATTTTATTTTTATATT-----------------
H008|Eastern|Kasanga64|montico    AATAAAATAAATAATTAATTTTATTTTTATATT-----------------
H016|Eastern|Kathiani60|scutel    AATAAAATAAATAATTAATTTTATTTTTATATT-----------------
H005|Eastern|Kasanga9|scutella    AATAAAATAAATAATTAATTTTATTTTTATATT-----------------
H048|Eastern|Mathiakani52|lito    AATAAAATAAATAATTAATTTTATTTTTATATT-----------------
H055|Eastern|Mathiakani58|scut    AATAAAATAAATAATTAATTTTATTTTTATATT-----------------
H024|Eastern|Kathiani99|scutel    AATAAAATAAATAATTAATTTTATTTTTATATT-----------------
H009|Eastern|Kasanga64|scutell    AATAAAATAAATAATTAATTTTATTTTTATATT-----------------
H029|Eastern|Kathiani360|scute    AATAAAATAAATAATTAATTTTATTTTTATATT-----------------
H030|Eastern|Kathiani360|scute    AATAAAATAAATAATTAATTTTATTTTTATATT-----------------
H041|Eastern|Mumoni80|litorea     AATAAAATAAATAATTAATTTTATTTTTATATT-----------------
A.m.ligustica                     AATAAAATAAATAATTAATTTTATTTTTATATT-----------------
                                  **********************************

H020|Eastern|Kathiani60|scutel    TTAAAGATTTAATCTTTTTATTAAAATTAATAAATTAATATAAAATAAAA
H064|Eastern|Ronge2|scutellata    -------------------------------------------------
H015|Eastern|Kasanga96|scutell    -------------------------------------------------
H032|Eastern|Mumoni19|litorea     -------------------------------------------------
H014|Eastern|Kasanga96|scutell    -------------------------------------------------
H031|Eastern|Mumoni19|litorea     -------------------------------------------------
H008|Eastern|Kasanga64|montico    -------------------------------------------------
H016|Eastern|Kathiani60|scutel    -------------------------------------------------
H005|Eastern|Kasanga9|scutella    -------------------------------------------------
H048|Eastern|Mathiakani52|lito    -------------------------------------------------
H055|Eastern|Mathiakani58|scut    -------------------------------------------------
H024|Eastern|Kathiani99|scutel    -------------------------------------------------
H009|Eastern|Kasanga64|scutell    -------------------------------------------------
H029|Eastern|Kathiani360|scute    -------------------------------------------------
H030|Eastern|Kathiani360|scute    -------------------------------------------------
H041|Eastern|Mumoni80|litorea     -------------------------------------------------
A.m.ligustica                     -------------------------------------------------

H020|Eastern|Kathiani60|scutel    CAAAATATAACAGAATATATTTATTAAAATTTAATTTATTAAAATTCCCC
H064|Eastern|Ronge2|scutellata    -------------------------------------------------
H015|Eastern|Kasanga96|scutell    -------------------------------------------------
H032|Eastern|Mumoni19|litorea     -------------------------------------------------
H014|Eastern|Kasanga96|scutell    -------------------------------------------------
H031|Eastern|Mumoni19|litorea     -------------------------------------------------
H008|Eastern|Kasanga64|montico    -------------------------------------------------
H016|Eastern|Kathiani60|scutel    -------------------------------------------------
H005|Eastern|Kasanga9|scutella    -------------------------------------------------
H048|Eastern|Mathiakani52|lito    -------------------------------------------------
H055|Eastern|Mathiakani58|scut    -------------------------------------------------
H024|Eastern|Kathiani99|scutel    -------------------------------------------------
H009|Eastern|Kasanga64|scutell    -------------------------------------------------
H029|Eastern|Kathiani360|scute    -------------------------------------------------
H030|Eastern|Kathiani360|scute    -------------------------------------------------
H041|Eastern|Mumoni80|litorea     -------------------------------------------------
A.m.ligustica                     -------------------------------------------------
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H020|Eastern|Kathiani60|scutel     ACTTAATTCATATTAATTTAAAAATAAATTAATAACAATTTTAATAAAAT
H064|Eastern|Ronge2|scutellata     --------------------------------------------------
H015|Eastern|Kasanga96|scutell     --------------------------------------------------
H032|Eastern|Mumoni19|litorea      --------------------------------------------------
H014|Eastern|Kasanga96|scutell     --------------------------------------------------
H031|Eastern|Mumoni19|litorea      --------------------------------------------------
H008|Eastern|Kasanga64|montico     --------------------------------------------------
H016|Eastern|Kathiani60|scutel     --------------------------------------------------
H005|Eastern|Kasanga9|scutella     --------------------------------------------------
H048|Eastern|Mathiakani52|lito     --------------------------------------------------
H055|Eastern|Mathiakani58|scut     --------------------------------------------------
H024|Eastern|Kathiani99|scutel     --------------------------------------------------
H009|Eastern|Kasanga64|scutell     --------------------------------------------------
H029|Eastern|Kathiani360|scute     --------------------------------------------------
H030|Eastern|Kathiani360|scute     --------------------------------------------------
H041|Eastern|Mumoni80|litorea      --------------------------------------------------
A.m.ligustica                      --------------------------------------------------

H020|Eastern|Kathiani60|scutel     AAATAATTAATTTTATTTTTATATTGAATTTTTAATTCAATCTTAAAGAT
H064|Eastern|Ronge2|scutellata     -------------------------GAATTTTTAATTCAATCTTAAAGAT
H015|Eastern|Kasanga96|scutell     -------------------------GAATTTTTAATTCAATCTTAAAGAT
H032|Eastern|Mumoni19|litorea      -------------------------GAATTTTTAATTCAATCTTAAAGAT
H014|Eastern|Kasanga96|scutell     -------------------------GAATTTTTAATTCAATCTTAAAGAT
H031|Eastern|Mumoni19|litorea      -------------------------GAATTTTTAATTCAATCTTAAAGAT
H008|Eastern|Kasanga64|montico     -------------------------GAATTTTTAATTCAATCTTAAAGAT
H016|Eastern|Kathiani60|scutel     -------------------------GAATTTTTAATTCAATCTTAAAGAT
H005|Eastern|Kasanga9|scutella     -------------------------GAATTTTTAATTCAATCTTAAAGAT
H048|Eastern|Mathiakani52|lito     -------------------------GAATTTTTAATTCAATCTTAAAGAT
H055|Eastern|Mathiakani58|scut     -------------------------GAATTTTTAATTCAATCTTAAAGAT
H024|Eastern|Kathiani99|scutel     -------------------------GAATTTTTAATTCAATCTTAAAGAT
H009|Eastern|Kasanga64|scutell     -------------------------GAATTTTTAATTCAATCTTAAAGAT
H029|Eastern|Kathiani360|scute     -------------------------GAATTTTTAATTCAATCTTAAAGAT
H030|Eastern|Kathiani360|scute     -------------------------GAATTTTTAATTCAATCTTAAAGAT
H041|Eastern|Mumoni80|litorea      -------------------------GAATTTTTAATTCAATCTTAAAGAT
A.m.ligustica                      -------------------------GAATTTTAAATTCAATCTTAAAGAT
                                                            *******:*****************

H020|Eastern|Kathiani60|scutel     TTAATCTTTTT-ATTAAAATTAATAAATTAATATAAAATAAAACAAAATA
H064|Eastern|Ronge2|scutellata     TTAATCTTTTT-ATTAAAATTAATAAATTAATATAAAATAAAACAAAATA
H015|Eastern|Kasanga96|scutell     TTAATCTTTTT-ATTAAAATTAATAAATTAATATAAAATAAAACAAAATA
H032|Eastern|Mumoni19|litorea      TTAATCTTTTT-ATTAAAATTAATAAATTAATATAAAATAAAACAAAATA
H014|Eastern|Kasanga96|scutell     TTAATCTTTTT-ATTAAAATTAATAAATTAATATAAAATAAAACAAAATA
H031|Eastern|Mumoni19|litorea      TTAATCTTTTT-ATTAAAATTAATAAATTAATATAAAATAAAACAAAATA
H008|Eastern|Kasanga64|montico     TTAATCTTTTT-ATTAAAATTAATAAATTAATATAAAATAAAACAAAATA
H016|Eastern|Kathiani60|scutel     TTAATCTTTTT-ATTAAAATTAATAAATTAATATAAAATAAAACAAAATA
H005|Eastern|Kasanga9|scutella     TTAATCTTTTT-ATTAAAATTAATAAATTAATATAAAATAAAACAAAATA
H048|Eastern|Mathiakani52|lito     TTAATCTTTTTTATTAAAATTAATAAATTAATATAAAATAAAACAAAATA
H055|Eastern|Mathiakani58|scut     TTAATCTTTTT-ATTAAAATTAATAAATTAATATAAAATAAAACAAAATA
H024|Eastern|Kathiani99|scutel     TTAATCTTTTT-ATTAAAATTAATAAATTAATATAAAATAAAACAAAATA
H009|Eastern|Kasanga64|scutell     TTAATCTTTTT-ATTAAAATTAATAAATTAATATAAAATAAAACAAAATA
H029|Eastern|Kathiani360|scute     TTAATCTTTTT-ATTAAAATTAATAAATTAATATAAAATAAAACAAAATA
H030|Eastern|Kathiani360|scute     TTAATCTTTTT-ATTAAAATTAATAAATTAATATAAAATAAAACAAAATA
H041|Eastern|Mumoni80|litorea      TTAATCTTTTT-ATTAAAATTAATAAATTAATATAAAATAAAACAAAATA
A.m.ligustica                      TTAATCTTTTT-ATTAAAATTAATAAATTAATATAAAATAAAACAAAATA
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H020|Eastern|Kathiani60|scutel     TAACAGAATATATTTATTAAAATTTAATTTATTAAAATTTCCACATGATT
H064|Eastern|Ronge2|scutellata     TAACAGAATATATTTATTAAAATTTAATTTATTAAAATTTCCACATGATT
H015|Eastern|Kasanga96|scutell     TAACAGAATATATTTATTAAAATTTAATTTATTAAAATTTCCACATGATT
H032|Eastern|Mumoni19|litorea      TAACAGAATATATTTATTAAAATTTAATTTATTAAAATTTCCACATGATT
H014|Eastern|Kasanga96|scutell     TAACAGAATATATTTATTAAAATTTAATTTATTAAAATTTCCACATGATT
H031|Eastern|Mumoni19|litorea      TAACAGAATATATTTATTAAAATTTAATTTATTAAAATTTCCACATGATT
H008|Eastern|Kasanga64|montico     TAACAGAATATATTTATTAAAATTTAATTTATTAAAATTTCCACATGATT
H016|Eastern|Kathiani60|scutel     TAACAGAATATATTTATTAAAATTTAATTTATTAAAATTTCCACATGATT
H005|Eastern|Kasanga9|scutella     TAACAGAATATATTTATTAAAATTTAATTTATTAAAATTTCCACATGATT
H048|Eastern|Mathiakani52|lito     TAACAGAATATATTTATTAAAATTTAATTTATTAAAATTTCCACATGATT
H055|Eastern|Mathiakani58|scut     TAACAGAATATATTTATTAAAATTTAATTTATTAAAATTTCCACATGATT
H024|Eastern|Kathiani99|scutel     TAACAGAATATATTTATTAAAATTTAATTTATTAAAATTTCCACATGATT
H009|Eastern|Kasanga64|scutell     TAACAGAATATATTTATTAAAATTTAATTTATTAAAATTTCCACATGATT
H029|Eastern|Kathiani360|scute     TAACAGAATATATTTATTAAAATTTAATTTATTAAAATTTCCACATGATT
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H030|Eastern|Kathiani360|scute     TAACAGAATATATTTATTAAAATTTAATTTATTAAAATTTCCACATGATT
H041|Eastern|Mumoni80|litorea      TAACAGAATATATTTATTAAAATTTAATTTATTAAAATTTCCACATGATT
A.m.ligustica                      TAACAGAATATATTTATTAAAATTTAATTTATTAAAATTTCCACATGATT
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H020|Eastern|Kathiani60|scutel     CATATTTATATTTCAAGAATCAAATTCATATTATGCTGATAATTTAATTT
H064|Eastern|Ronge2|scutellata     CATATTTATATTTCAAGAATCAAATTCATATTATGCTGATAATTTAATTT
H015|Eastern|Kasanga96|scutell     CATATTTATATTTCAAGAATCAAATTCATATTATGCTGATAATTTAATTT
H032|Eastern|Mumoni19|litorea      CATATTTATATTTCAAGAATCAAATTCATATTATGCTGATAATTTAATTT
H014|Eastern|Kasanga96|scutell     CATATTTATATTTCAAGAATCAAATTCATATTATGCTGATAATTTAATTT
H031|Eastern|Mumoni19|litorea      CATATTTATATTTCAAGAATCAAATTCATATTATGCTGATAATTTAATTT
H008|Eastern|Kasanga64|montico     CATATTTATATTTCAAGAATCAAATTCATATTATGCTGATAATTTAATTT
H016|Eastern|Kathiani60|scutel     CATATTTATATTTCAAGAATCAAATTCATATTATGCTGATAATTTAATTT
H005|Eastern|Kasanga9|scutella     CATATTTATATTTCAAGAATCAAATTCATATTATGCTGATAATTTAATTT
H048|Eastern|Mathiakani52|lito     CATATTTATATTTCAAGAATCAAATTCATATTATGCTGATAATTTAATTT
H055|Eastern|Mathiakani58|scut     CATATTTATATTTCAAGAATCAAATTCATATTATGCTGATAATTTAATTT
H024|Eastern|Kathiani99|scutel     CATATTTATATTTCAAGAATCAAATTCATATTATGCTGATAATTTAATTT
H009|Eastern|Kasanga64|scutell     CATATTTATATTTCAAGAATCAAATTCATATTATGCTGATAATTTAATTT
H029|Eastern|Kathiani360|scute     CATATTTATATTTCAAGAATCAAATTCATATTATGCTGATAATTTAATTT
H030|Eastern|Kathiani360|scute     CATATTTATATTTCAAGAATCAAATTCATATTATGCTGATAATTTAATTT
H041|Eastern|Mumoni80|litorea      CATATTTATATTTCAAGAATCAAATTCATATTATGCTGATAATTTAATTT
A.m.ligustica                      TATATTTATATTTCAAGAATCAAATTCATATTATGCTGATAATTTAATTT
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H020|Eastern|Kathiani60|scutel     CATTTCATAATATAGTTATAATAATCATTATTATAATTTCAACATTAACT
H064|Eastern|Ronge2|scutellata     CATTTCATAATATAGTTATAATAATCATTATTATAATTTCAACATTAACT
H015|Eastern|Kasanga96|scutell     CATTTCATAATATAGTTATAATAATCATTATTATAATTTCAACATTAACT
H032|Eastern|Mumoni19|litorea      CATTTCATAATATAGTTATAATAATCATTATTATAATTTCAACATTAACT
H014|Eastern|Kasanga96|scutell     CATTTCATAATATAGTTATAATAATCATTATTATAATTTCAACATTAACT
H031|Eastern|Mumoni19|litorea      CATTTCATAATATAGTTATAATAATCATTATTATAATTTCAACATTAACT
H008|Eastern|Kasanga64|montico     CATTTCATAATATAGTTATAATAATCATTATTATAATTTCAACATTAACT
H016|Eastern|Kathiani60|scutel     CATTTCATAATATAGTTATAATAATCATTATTATAATTTCAACATTAACT
H005|Eastern|Kasanga9|scutella     CATTTCATAATATAGTTATAATAATCATTATTATAATTTCAACATTAACT
H048|Eastern|Mathiakani52|lito     CATTTCATAATATAGTTATAATAATCATTATTATAATTTCAACATTAACT
H055|Eastern|Mathiakani58|scut     CATTTCATAATATAGTTATAATAATCATTATTATAATTTCAACATTAACT
H024|Eastern|Kathiani99|scutel     CATTTCATAATATAGTTATAATAATCATTATTATAATTTCAACATTAACT
H009|Eastern|Kasanga64|scutell     CATTTCATAATATAGTTATAATAATCATTATTATAATTTCAACATTAACT
H029|Eastern|Kathiani360|scute     CATTTCATAATATAGTTATAATAATCATTATTATAATTTCAACATTAACT
H030|Eastern|Kathiani360|scute     CATTTCATAATATAGTTATAATAATCATTATTATAATTTCAACATTAACT
H041|Eastern|Mumoni80|litorea      CATTTCATAATATAGTTATAATAATCATTATTATAATTTCAACATTAACT
A.m.ligustica                      CATTTCATAATATAGTTATAATAATTATTATTATAATTTCAACATTAACT
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H020|Eastern|Kathiani60|scutel     GTATATATTATTTTAGATTTATTTATAAATAAATTTTCAAATTTATTTTT
H064|Eastern|Ronge2|scutellata     GTATATATTATTTTAGATTTATTTATAAATAAATTTTCAAATTTATTTTT
H015|Eastern|Kasanga96|scutell     GTATATATTATTTTAGATTTATTTATAAATAAATTTTCAAATTTATTTTT
H032|Eastern|Mumoni19|litorea      GTATATATTATTTTAGATTTATTTATAAATAAATTTTCAAATTTATTTTT
H014|Eastern|Kasanga96|scutell     GTATATATTATTTTAGATTTATTTATAAATAAATTTTCAAATTTATTTTT
H031|Eastern|Mumoni19|litorea      GTATATATTATTTTAGATTTATTTATAAATAAATTTTCAAATTTATTTTT
H008|Eastern|Kasanga64|montico     GTATATATTATTTTAGATTTATTTATAAATAAATTTTCAAATTTATTTTT
H016|Eastern|Kathiani60|scutel     GTATATATTATTTTAGATTTATTTATAAATAAATTTTCAAATTTATTTTT
H005|Eastern|Kasanga9|scutella     GTATATATTATTTTAGATTTATTTATAAATAAATTTTCAAATTTATTTTT
H048|Eastern|Mathiakani52|lito     GTATATATTATTTTAGATTTATTTATAAATAAATTTTCAAATTTATTTTT
H055|Eastern|Mathiakani58|scut     GTATATATTATTTTAGATTTATTTATAAATAAATTTTCAAATTTATTTTT
H024|Eastern|Kathiani99|scutel     GTATATATTATTTTAGATTTATTTATAAATAAATTTTCAAATTTATTTTT
H009|Eastern|Kasanga64|scutell     GTATATATTATTTTAGATTTATTTATAAATAAATTTTCAAATTTATTTTT
H029|Eastern|Kathiani360|scute     GTATATATTATTTTAGATTTATTTATAAATAAATTTTCAAATTTATTTTT
H030|Eastern|Kathiani360|scute     GTATATATTATTTTAGATTTATTTATAAATAAATTTTCAAATTTATTTTT
H041|Eastern|Mumoni80|litorea      GTATATATTATTTTAGATTTATTTATAAATAAATTTTCAAATTTATTTTT
A.m.ligustica                      GTATATATTATTTTAGATTTATTTATAAACAAATTCTCAAATTTATTTTT
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H020|Eastern|Kathiani60|scutel     ATTAAAAAATCATAATATTGAAATTATTTGAACAGTTATTCCAATTATTA
H064|Eastern|Ronge2|scutellata     ATTAAAAAATCATAATATTGAAATTATTTGAACAGTTATTCCAATTATTA
H015|Eastern|Kasanga96|scutell     ATTAAAAAATCATAATATTGAAATTATTTGAACAGTTATTCCAATTATTA
H032|Eastern|Mumoni19|litorea      ATTAAAAAATCATAATATTGAAATTATTTGAACAGTTATTCCAATTATTA
H014|Eastern|Kasanga96|scutell     ATTAAAAAATCATAATATTGAAATTATTTGAACAGTTATTCCAATTATTA
H031|Eastern|Mumoni19|litorea      ATTAAAAAATCATAATATTGAAATTATTTGAACAGTTATTCCAATTATTA
H008|Eastern|Kasanga64|montico     ATTAAAAAATCATAATATTGAAATTATTTGAACAGTTATTCCAATTATTA
H016|Eastern|Kathiani60|scutel     ATTAAAAAATCATAATATTGAAATTATTTGAACAGTTATTCCAATTATTA
H005|Eastern|Kasanga9|scutella     ATTAAAAAATCATAATATTGAAATTATTTGAACAGTTATTCCAATTATTA
H048|Eastern|Mathiakani52|lito     ATTAAAAAATCATAATATTGAAATTATTTGAACAGTTATTCCAATTATTA
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H055|Eastern|Mathiakani58|scut     ATTAAAAAAATCATAATATTGAAATTATTTGAACAGTTATTCCAATTATTA
H024|Eastern|Kathiani99|scutel     ATTAAAAAAATCATAATATTGAAATTATTTGAACAGTTATTCCAATTATTA
H009|Eastern|Kasanga64|scutell     ATTAAAAAAATCATAATATTGAAATTATTTGAACAGTTATTCCAATTATTA
H029|Eastern|Kathiani360|scute     ATTAAAAAAATCATAATATTGAAATTATTTGAACAGTTATTCCAATTATTA
H030|Eastern|Kathiani360|scute     ATTAAAAAAATCATAATATTGAAATTATTTGAACAGTTATTCCAATTATTA
H041|Eastern|Mumoni80|litorea      ATTAAAAAAATCATAATATTGAAATTATTTGAACAGTTATTCCAATTATTA
A.m.ligustica                      ATTAAAAAAATCATAATATTGAAATTATTTGAACAATTATTCCAATTATTA
                                   **********************************.***************

H020|Eastern|Kathiani60|scutel     TTCTATTAATTATTTGTTTTCCATCATTAAAAATTTTATATTTAATTGAT
H064|Eastern|Ronge2|scutellata     TTCTATTAATTATTTGTTTTCCATCATTAAAAATTTTATATTTAATTGAT
H015|Eastern|Kasanga96|scutell     TTCTATTAATTATTTGTTTTCCATCATTAAAAATTTTATATTTAATTGAT
H032|Eastern|Mumoni19|litorea      TTCTATTAATTATTTGTTTTCCATCATTAAAAATTTTATATTTAATTGAT
H014|Eastern|Kasanga96|scutell     TTCTATTAATTATTTGTTTTCCATCATTAAAAATTTTATATTTAATTGAT
H031|Eastern|Mumoni19|litorea      TTCTATTAATTATTTGTTTTCCATCATTAAAAATTTTATATTTAATTGAT
H008|Eastern|Kasanga64|montico     TTCTATTAATTATTTGTTTTCCATCATTAAAAATTTTATATTTAATTGAT
H016|Eastern|Kathiani60|scutel     TTCTATTAATTATTTGTTTTCCATCATTAAAAATTTTATATTTAATTGAT
H005|Eastern|Kasanga9|scutella     TTCTATTAATTATTTGTTTTCCATCATTAAAAATTTTATATTTAATTGAT
H048|Eastern|Mathiakani52|lito     TTCTATTAATTATTTGTTTTCCATCATTAAAAATTTTATATTTAATTGAT
H055|Eastern|Mathiakani58|scut     TTCTATTAATTATTTGTTTTCCATCATTAAAAATTTTATATTTAATTGAT
H024|Eastern|Kathiani99|scutel     TTCTATTAATTATTTGTTTTCCATCATTAAAAATTTTATATTTAATTGAT
H009|Eastern|Kasanga64|scutell     TTCTATTAATTATTTGTTTTCCATCATTAAAAATTTTATATTTAATTGAT
H029|Eastern|Kathiani360|scute     TTCTATTAATTATTTGTTTTCCATCATTAAAAATTTTATATTTAATTGAT
H030|Eastern|Kathiani360|scute     TTCTATTAATTATTTGTTTTCCATCATTAAAAATTTTATATTTAATTGAT
H041|Eastern|Mumoni80|litorea      TTCTATTAATTATTTGTTTTCCATCATTAAAAATTTTATATTTAATTGAT
A.m.ligustica                      TTCTATTAATTATTTGTTTTCCATCATTAAAAATTTTATATTTAATTGAT
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H020|Eastern|Kathiani60|scutel     GAAATTGTAAATCCTTTTTTTTCAATTAAATCAATTGGTCATCAATGATA
H064|Eastern|Ronge2|scutellata     GAAATTGTAAATCCTTTTTTTTCAATTAAATCAATTGGTCATCAATGATA
H015|Eastern|Kasanga96|scutell     GAAATTGTAAATCCTTTTTTTTCAATTAAATCAATTGGTCATCAATGATA
H032|Eastern|Mumoni19|litorea      GAAATTGTAAATCCTTTTTTTTCAATTAAATCAATTGGTCATCAATGATA
H014|Eastern|Kasanga96|scutell     GAAATTGTAAATCCTTTTTTTTCAATTAAATCAATTGGTCATCAATGATA
H031|Eastern|Mumoni19|litorea      GAAATTGTAAATCCTTTTTTTTCAATTAAATCAATTGGTCATCAATGATA
H008|Eastern|Kasanga64|montico     GAAATTGTAAATCCTTTTTTTTCAATTAAATCAATTGGTCATCAATGATA
H016|Eastern|Kathiani60|scutel     GAAATTGTAAATCCTTTTTTTTCAATTAAATCAATTGGTCATCAATGATA
H005|Eastern|Kasanga9|scutella     GAAATTGTAAATCCTTTTTTTTCAATTAAATCAATTGGTCATCAATGATA
H048|Eastern|Mathiakani52|lito     GAAATTGTAAATCCTTTTTTTTCAATTAAATCAATTGGTCATCAATGATA
H055|Eastern|Mathiakani58|scut     GAAATTGTAAATCCTTTTTTTTCAATTAAATCAATTGGTCATCAATGATA
H024|Eastern|Kathiani99|scutel     GAAATTGTAAATCCTTTTTTTTCAATTAAATCAATTGGTCATCAATGATA
H009|Eastern|Kasanga64|scutell     GAAATTGTAAATCCTTTTTTTTCAATTAAATCAATTGGTCATCAATGATA
H029|Eastern|Kathiani360|scute     GAAATTGTAAATCCTTTTTTTTCAATTAAATCAATTGGTCATCAATGATA
H030|Eastern|Kathiani360|scute     GAAATTGTAAATCCTTTTTTTTCAATTAAATCAATTGGTCATCAATGATA
H041|Eastern|Mumoni80|litorea      GAAATTGTAAATCCTTTTTTTTCAATTAAATCAATTGGTCATCAATGATA
A.m.ligustica                      GAAATTGTAAATCCTTTTTTTTCAATTAAATCAATTGGTCATCAATGATA
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H020|Eastern|Kathiani60|scutel     TTGATCATATGAATATCCAGAATTTAATAATATTGAATTTGATTCATACA
H064|Eastern|Ronge2|scutellata     TTGATCATATGAATATCCAGAATTTAATAATATTGAATTTGATTCATATA
H015|Eastern|Kasanga96|scutell     TTGATCATATGAATATCCAGAATTTAATAATATTGAATTTGATTCATACA
H032|Eastern|Mumoni19|litorea      TTGATCATATGAATATCCAGAATTTAATAATATTGAATTTGATTCATATA
H014|Eastern|Kasanga96|scutell     TTGATCATATGAATATCCAGAATTTAATAATATTGAATTTGATTCATACA
H031|Eastern|Mumoni19|litorea      TTGATCATATGAATATCCAGAATTTAATAATATTGAATTTGATTCATATA
H008|Eastern|Kasanga64|montico     TTGATCATATGAATATCCAGAATTTAATAATATTGAATTTGATTCATACA
H016|Eastern|Kathiani60|scutel     TTGATCATATGAATATCCAGAATTTAATAATATTGAATTTGATTCATACA
H005|Eastern|Kasanga9|scutella     TTGATCATATGAATATCCAGAATTTAATAATATTGAATTTGATTCATACA
H048|Eastern|Mathiakani52|lito     TTGATCATATGAATATCCAGAATTTAATAATATTGAATTTGATTCATACA
H055|Eastern|Mathiakani58|scut     TTGATCATATGAATATCCAGAATTTAATAATATTGAATTTGATTCATATA
H024|Eastern|Kathiani99|scutel     TTGATCATATGAATATCCAGAATTTAATAATATTGAATTTGATTCATACA
H009|Eastern|Kasanga64|scutell     TTGATCATATGAATATCCAGAATTTAATAATATTGAATTTGATTCATACA
H029|Eastern|Kathiani360|scute     TTGATCATATGAATATCCAGAATTTAATAATATTGAATTTGATTCATACA
H030|Eastern|Kathiani360|scute     TTGATCATATGAATATCCAGAATTTAATAATATTGAATTTGATTCATACA
H041|Eastern|Mumoni80|litorea      TTGATCATATGAATATCCAGAATTTAATAATATTGAATTTGATTCATACA
A.m.ligustica                      TTGATCATATGAATATCCAGAATTTAATAATATTGAATTTGATTCATATA
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H020|Eastern|Kathiani60|scutel     TACTAAATTATAATAATTTAAATCAATTTCGTTTACTAGAAACTGATAAT
H064|Eastern|Ronge2|scutellata     TACTAAATTATAATAATTTAAATCAATTTCGTTTACTAGAAACTGATAAT
H015|Eastern|Kasanga96|scutell     TACTAAATTATAATAATTTAAATCAATTTCGTTTACTAGAAACTGATAAT
H032|Eastern|Mumoni19|litorea      TACTAAATTATAATAATTTAAATCAATTTCGTTTACTAGAAACTGATAAT
H014|Eastern|Kasanga96|scutell     TACTAAATTATAATAATTTAAATCAATTTCGTTTACTAGAAACTGATAAT
H031|Eastern|Mumoni19|litorea      TACTAAATTATAATAATTTAAATCAATTTCGTTTACTAGAAACTGATAAT
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H008|Eastern|Kasanga64|montico     TACTAAATTATAATAATTTAAATCAATTTCGTTTACTAGAAACTGATAAT
H016|Eastern|Kathiani60|scutel     TACTAAATTATAATAATTTAAATCAATTTCGTTTACTAGAAACTGATAAT
H005|Eastern|Kasanga9|scutella     TACTAAATTATAATAATTTAAATCAATTTCGTTTACTAGAAACTGATAAT
H048|Eastern|Mathiakani52|lito     TACTAAATTATAATAATTTAAATCAATTTCGTTTACTAGAAACTGATAAT
H055|Eastern|Mathiakani58|scut     TACTAAATTATAATAATTTAAATCAATTTCGTTTACTAGAAACTGATAAT
H024|Eastern|Kathiani99|scutel     TACTAAATTATAATAATTTAAATCAATTTCGTTTACTAGAAACTGATAAT
H009|Eastern|Kasanga64|scutell     TACTAAATTATAATAATTTAAATCAATTTCGTTTACTAGAAACTGATAAT
H029|Eastern|Kathiani360|scute     TACTAAATTATAATAATTTAAATCAATTTCGTTTACTAGAAACTGATAAT
H030|Eastern|Kathiani360|scute     TACTAAATTATAATAATTTAAATCAATTTCGTTTACTAGAAACTGATAAT
H041|Eastern|Mumoni80|litorea      TACTAAATTATAATAATTTAAATCAATTTCGTTTACTAGAAACTGATAAT
A.m.ligustica                      TACTAAATTATAATAATTTAAACCAATTTCGTTTACTAGAAACTGATAAT
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H020|Eastern|Kathiani60|scutel     CGAATAGTAATTCCAATAAAAATTCCACTACGTTTAATTACAACATCAAC
H064|Eastern|Ronge2|scutellata     CGAATAGTAATTCCAATAAAAATTCCACTACGTTTAATTACAACATCAAC
H015|Eastern|Kasanga96|scutell     CGAATAGTAATTCCAATAAAAATTCCACTACGTTTAATTACAACATCAAC
H032|Eastern|Mumoni19|litorea      CGAATAGTAATTCCAATAAAAATTCCACTACGTTTAATTACAACATCAAC
H014|Eastern|Kasanga96|scutell     CGAATAGTAATTCCAATAAAAATTCCACTACGTTTAATTACAACATCAAC
H031|Eastern|Mumoni19|litorea      CGAATAGTAATTCCAATAAAAATTCCACTACGTTTAATTACAACATCAAC
H008|Eastern|Kasanga64|montico     CGAATAGTAATTCCAATAAAAATTCCACTACGTTTAATTACAACATCAAC
H016|Eastern|Kathiani60|scutel     CGAATAGTAATTCCAATAAAAATTCCACTACGTTTAATTACAACATCAAC
H005|Eastern|Kasanga9|scutella     CGAATAGTAATTCCAATAAAAATTCCACTACGTTTAATTACAACATCAAC
H048|Eastern|Mathiakani52|lito     CGAATAGTAATTCCAATAAAAATTCCACTACGTTTAATTACAACATCAAC
H055|Eastern|Mathiakani58|scut     CGAATAGTAATTCCAATAAAAATTCCACTACGTTTAATTACAACATCAAC
H024|Eastern|Kathiani99|scutel     CGAATAGTAATTCCAATAAAAATTCCACTACGTTTAATTACAACATCAAC
H009|Eastern|Kasanga64|scutell     CGAATAGTAATTCCAATAAAAATTCCACTACGTTTAATTACAACATCAAC
H029|Eastern|Kathiani360|scute     CGAATAGTAATTCCAATAAAAATTCCACTACGTTTAATTACAACATCAAC
H030|Eastern|Kathiani360|scute     CGAATAGTAATTCCAATAAAAATTCCACTACGTTTAATTACAACATCAAC
H041|Eastern|Mumoni80|litorea      CGAATAGTAATTCCAATAAAAATTCCACTACGTTTAATTACAACATCAAC
A.m.ligustica                      CGAATAGTAATTCCAATAAAAATCCCACTACGTTTAATTACAACATCAAC
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H020|Eastern|Kathiani60|scutel     AGATGTAATTCATTCATGAACAGTTCCATCTTTAGGTATTAAAGTCGATG
H064|Eastern|Ronge2|scutellata     AGATGTAATTCATTCATGAACAGTTCCATCTTTAGGTATTAAAGTCGATG
H015|Eastern|Kasanga96|scutell     AGATGTAATTCATTCATGAACAGTTCCATCTTTAGGTATTAAAGTCGATG
H032|Eastern|Mumoni19|litorea      AGATGTAATTCATTCATGAACAGTTCCATCTTTAGGTATTAAAGTCGATG
H014|Eastern|Kasanga96|scutell     AGATGTAATTCATTCATGAACAGTTCCATCTTTAGGTATTAAAGTCGATG
H031|Eastern|Mumoni19|litorea      AGATGTAATTCATTCATGAACAGTTCCATCTTTAGGTATTAAAGTCGATG
H008|Eastern|Kasanga64|montico     AGATGTAATTCATTCATGAACAGTTCCATCTTTAGGTATTAAAGTCGATG
H016|Eastern|Kathiani60|scutel     AGATGTAATTCATTCATGAACAGTTCCATCTTTAGGTATTAAAGTCGATG
H005|Eastern|Kasanga9|scutella     AGATGTAATTCATTCATGAACAGTTCCATCTTTAGGTATTAAAGTCGATG
H048|Eastern|Mathiakani52|lito     AGATGTAATTCATTCATGAACAGTTCCATCTTTAGGTATTAAAGTCGATG
H055|Eastern|Mathiakani58|scut     AGATGTAATTCATTCATGAACAGTTCCATCTTTAGGTATTAAAGTCGATG
H024|Eastern|Kathiani99|scutel     AGATGTAATTCATTCATGAACAGTTCCATCTTTAGGTATTAAAGTCGATG
H009|Eastern|Kasanga64|scutell     AGATGTAATTCATTCATGAACAGTTCCATCTTTAGGTATTAAAGTCGATG
H029|Eastern|Kathiani360|scute     AGATGTAATTCATTCATGAACAGTTCCATCTTTAGGTATTAAAGTCGATG
H030|Eastern|Kathiani360|scute     AGATGTAATTCATTCATGAACAGTTCCATCTTTAGGTATTAAAGTCGATG
H041|Eastern|Mumoni80|litorea      AGATGTAATTCATTCATGAACAGTTCCATCTTTAGGTATTAAAGTCGATG
A.m.ligustica                      AGATGTAATTCATTCATGAACAGTTCCATCCTTAGGTATTAAAGTTGATG
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H020|Eastern|Kathiani60|scutel     CAGTCCCAGGACGAATTAATCAATTAAATTTAATTAGAAAA
H064|Eastern|Ronge2|scutellata     CAGTCCCAGGACGAATTAATCAATTAAATTTAATTAGAAAA
H015|Eastern|Kasanga96|scutell     CAGTCCCAGGACGAATTAATCAATTAAATTTAATTAGAAAA
H032|Eastern|Mumoni19|litorea      CAGTCCCAGGACGAATTAATCAATTAAATTTAATTAGAAAA
H014|Eastern|Kasanga96|scutell     CAGTCCCAGGACGAATTAATCAATTAAATTTAATTAGAAAA
H031|Eastern|Mumoni19|litorea      CAGTCCCAGGACGAATTAATCAATTAAATTTAATTAGAAAA
H008|Eastern|Kasanga64|montico     CAGTCCCAGGACGAATTAATCAATTAAATTTAATTAGAAAA
H016|Eastern|Kathiani60|scutel     CAGTCCCAGGACGAATTAATCAATTAAATTTAATTAGAAAA
H005|Eastern|Kasanga9|scutella     CAGTCCCAGGACGAATTAATCAATTAAATTTAATTAGAAAA
H048|Eastern|Mathiakani52|lito     CAGTCCCAGGACGAATTAATCAATTAAATTTAATTAGAAAA
H055|Eastern|Mathiakani58|scut     CAGTCCCAGGACGAATTAATCAATTAAATTTAATTAGAAAA
H024|Eastern|Kathiani99|scutel     CAGTCCCAGGACGAATTAATCAATTAAATTTAATTAGAAAA
H009|Eastern|Kasanga64|scutell     CAGTCCCAGGACGAATTAATCAATTAAATTTAATTAGAAAA
H029|Eastern|Kathiani360|scute     CAGTCCCAGGACGAATTAATCAATTAAATTTAATTAGAAAA
H030|Eastern|Kathiani360|scute     CAGTCCCAGGACGAATTAATCAATTAAATTTAATTAGAAAA
H041|Eastern|Mumoni80|litorea      CAGTCCCAGGACGAATTAATCAATTAAATTTAATTAGAAAA
A.m.ligustica                      CAGTTCCAGGACGAATTAATCAATTAAATTTAATTAGAAAA
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Appendix 3: Phenotypic Data

Appendix 3.1: Nectar/pollen foraging phenotype data

Colony	No	ID	TF	OF	SB	OB	Pollen	Nectar	N / P	N/P+N ratio
Kasanga	H1-H5	9	10	8	2.5	2.5	1	3	N	0.75
Kasanga	H6-H10	64	10	9	2	2	1	4	N	0.80
Kasanga	H11-H15	96	10	10	3	2	2	3	N	0.60
Kasanga	H101-H105	0	10	8	3	1	3	1	P	0.25
Kathiani	H16-H20	60	10	8	1	3	2.5	1.5	P	0.31
Kathiani	H21-H25	99	10	9	1	3	3	2	P	0.40
Kathiani	H26-H30	360	10	9	1	2.5	0.5	5	N	0.91
Mikima	H31-H35	19	10	10	1.5	1	3.5	4	N	0.53
Mikima	H36-H40	12	10	8	1.5	1.5	2	3	N	0.60
Mikima	H41-H45	80	10	9	1	2.5	0.5	5	P	0.91
Mathiakani	H46-H50	52	10	9	2.5	2.5	0	4	N	1.00
Mathiakani	H51-H55	58	10	9	3	2	0	4	N	1.00
Mathiakani	H106-H110	55	10	9	2	3	1	3	N	0.75
Chawiya	H56-H60	1	10	10	3	1	0	5	N	1.00
Ronge	H61-H65	2	10	10	3.5	4.5	0.25	1.25	N	0.83
Hewani	H66-H70	1	10	8	1	2.5	0.5	3	N	0.86
Mituki	H71-H75	1	10	9	2	2.5	0.5	3	N	0.86
Sengoma	H76-H80	1	10	9	2	2.5	0.5	3	N	0.86
Usambara	H81-H85	1	10	10	3	1.5	1.5	4	N	0.73
Bidii	H86-H90	1	10	8	0	0.5	2.5	5	N	0.67
Chambuko	H91-H95	1	10	8	0	0	0.25	7.75	N	0.97
Dabaso	H96-H100	1	10	8	0.5	2	0.5	5	N	0.91
Makuchi	H111-H115	97	10	9	3	2	3	1	P	0.25
Makuchi	H116-H120	91	10	9	3	1.5	2.5	2	P	0.44
Makuchi	H121-H125	92	10	9	3	1.5	2.5	2	P	0.44
Isiekuti	H126-H130	107	10	10	2.5	2.5	1	4	N	0.80
Isiekuti	H131-H135	11	10	10	2.5	2.5	2	3	N	0.60
Isiekuti	H136-H140	15	10	10	2	3	3	2	P	0.40
Nairobi	H141-H145	1	10	8	2	2	1	3	N	0.75
Thika	H146-H150	149	10	8	1	3	1	3	N	0.75
Kikuyu	H151-H155	4	10	9	3.5	3	0.5	2	N	0.80
Kikuyu	H156-H160	6	10	9	1	3	1	4	N	0.80
Gatundu	H161-H165	1	10	9	1	5	0	3	N	1.00
Gatundu	H166-H170	2	10	9	1	2	1	5	N	0.83
Gatundu	H171-H175	3	10	9	3	1	1	4	N	0.80

Appendix 3.1: Nectar/pollen foraging phenotype data continued

Colony	No	ID	TF	OF	SB	OB	Pollen	Nectar	N/P	N/P+N ratio
Loresho	H176-H180	1	10	8	1	1	1	5	N	0.83
Loresho	H181-H185	2	10	9	1	3	2	3	N	0.60
Loresho	H186-H190	3	10	10	1	2	2	5	N	0.71
Nyahururu	H191-H195	52	10	8	1	2	2	3	N	0.60
Nyahururu	H196-H200	64	10	9	2	2	1	4	N	0.80
Nyahururu	H201-H205	97	10	8	2	2.75	0.25	3	N	0.92
Iresamburu	H206-H210	1	10	8	1	2	4	1	P	0.25
Iresamburu	H211-H215	2	10	8	1	1	4	2	P	0.50
Semdoye	H216-H220	5	10	10	3	3	1	3	N	0.75
Semdoye	H221-H225	7	10	10	4.5	1	0.5	4	N	0.89
Voi	H226-H230	3	10	9	1.5	3.5	3	1	P	0.25
Voi	H231-H235	10	10	9	1	2.75	5	0.25	P	0.05

Key

ID = Sample Identification
TF = Total number of frames
OF = Occupied number of frames
SB = Sealed number of broods
OB = Open number of broods
N/P = Nectar or Pollen
N/P+N ratio = Proportion of Nectar to (Pollen+Nectar) ratio

Appendix 3.2: Sting phenotype data

Colony	No	ID	T1S(s)	SN1	T2S	SN2	T3S	SN3	AvTS	AvSN	Status
Kasanga	H1-H5	9	5	31	6	38	3	29	**4.6**	**32.6**	A
Kasanga	H6-H10	64	3	19	4	33	5	27	**4.0**	**26.3**	A
Kasanga	H11-H15	96	5	22	7	39	1	41	**4.3**	**34.0**	A
Kasanga	H101-H105	0	5	23	5	31	5	33	**5.0**	**29.0**	A
Kathiani	H16-H20	60	15	10	10	12	9	14	**11.3**	**12.0**	M
Kathiani	H21-H25	99	16	14	11	12	13	7	**13.3**	**11.0**	M
Kathiani	H26-H30	360	20	12	21	16	15	13	**18.6**	**13.6**	M
Mikima	H31-H35	19	5	29	2	17	3	29	**3.3**	**25.0**	A
Mikima	H36-H40	12	2	25	7	51	3	43	**4.0**	**39.6**	A
Mikima	H41-H45	80	5	28	3	45	4	43	**4.0**	**38.6**	A
Mathiakani	H46-H50	52	4	33	2	43	4	33	**3.3**	**36.3**	A
Mathiakani	H51-H55	58	4	35	1	39	5	23	**3.3**	**32.3**	A
Mathiakani	H106-H110	55	3	37	6	41	5	43	**4.6**	**40.3**	A
Chawiya	H56-H60	1	5	29	3	37	3	21	**3.6**	**29.0**	A
Ronge	H61-H65	2	1	30	2	33	3	29	**2.0**	**30.6**	A
Hewani	H66-H70	1	13	15	12	17	18	13	**14.3**	**15.0**	M
Mituki	H71-H75	1	1	23	4	25	5	21	**3.3**	**23.0**	A
Sengoma	H76-H80	1	2	29	3	36	5	31	**3.3**	**32.0**	A
Usambara	H81-H85	1	1	19	2	17	3	18	**2.0**	**18.0**	A
Bidii	H86-H90	1	3	23	4	25	2	21	**3.0**	**23.0**	A
Chambuko	H91-H95	1	3	26	1	29	2	31	**2.0**	**28.6**	A
Dabaso	H96-H100	1	1	27	2	35	3	19	**2.0**	**27.0**	A
Makuchi	H111-H115	97	13	12	33	11	29	7	**25.0**	**10.0**	M
Makuchi	H116-H120	91	13	15	15	12	9	13	**12.3**	**13.3**	M
Makuchi	H121-H125	92	12	13	23	8	17	10	**17.3**	**10.3**	M
Isiekuti	H126-H130	107	5	14	2	38	1	23	**2.6**	**25.0**	A
Isiekuti	H131-H135	11	5	18	5	19	3	32	**4.3**	**23.0**	A
Isiekuti	H136-H140	15	11	12	13	13	10	12	**11.3**	**12.3**	M
Nairobi	H141-H145	1	12	13	23	5	36	7	**23.6**	**8.3**	M
Thika	H146-H150	149	14	18	27	15	46	5	**29**	**12.6**	M
Kikuyu	H151-H155	4	20	14	41	12	45	7	**35.3**	**11.0**	M
Kikuyu	H156-H160	6	21	13	43	10	47	6	**37.0**	**10.0**	M
Gatundu	H161-H165	1	25	13	33	12	38	5	**32.0**	**10.0**	M
Gatundu	H166-H170	2	12	15	28	11	32	12	**24.0**	**12.6**	M
Gatundu	H171-H175	3	22	15	12	13	15	9	**16.3**	**12.3**	M
Loresho	H176-H180	1	14	14	26	12	27	6	**22.3**	**10.6**	M
Loresho	H181-H185	2	15	13	8	8	23	14	**15.3**	**11.6**	M
Loresho	H186-H190	3	17	11	30	4	35	3	**27.3**	**6.0**	M

Appendix 3.2: Sting phenotype data continued

Colony	No	ID	T1S(s)	SN1	T2S	SN2	T3S	SN3	AvTS	AvSN	Status
Nyahururu	H191-H195	52	19	13	21	5	23	4	**21.0**	**7.3**	M
Nyahururu	H196-H200	64	21	12	24	2	39	5	**28.0**	**6.3**	M
Nyahururu	H201-H205	97	16	12	41	5	43	4	**33.3**	**7.0**	M
Iresamburu	H206-H210	1	5	22	1	39	5	39	**3.6**	**33.3**	A
Iresamburu	H211-H215	2	5	29	5	41	3	37	**4.3**	**35.6**	A
Semdoye	H216-H220	5	5	45	2	33	3	34	**3.3**	**37.3**	A
Semdoye	H221-H225	7	5	34	1	23	2	26	**2.6**	**27.6**	A
Voi	H226-H230	3	5	23	3	34	5	31	**4.3**	**29.3**	A
Voi	H231-235	10	5	22	2	23	3	42	**3.3**	**29.0**	A

T1S = Time taken to make the first sting in the 1st trial
T2S = Time taken to make the first sting in the 2nd trial
T3S = Time taken to make the first sting in the 3rd trial
SN1 = Number of stings made in the 1st trial
SN2 = Number of stings made in the 2nd trial
SN3 = Number of stings made in the 3rd trial
AvTS = Average time in seconds for the first sting to take place
AvSN = Average Number of stings in the 3 trials
A = Aggressive
M = Mild