

**MOLECULAR CHARACTERIZATION AND SOME ENVIRONMENTAL
FACTORS INFLUENCING DISTRIBUTION OF THE ENDANGERED AND
ENDEMIC *Gulella taitensis* IN TAITA HILLS, KENYA**

MWAURA NJERI ANN, (BSc)

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DECLARATION

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

Mwaura Njeri Ann, (BSc)

Reg. No. I56/10628/2006

Signature.....

Date.....

We confirm that the work reported in this thesis was carried out by the candidate under our supervision.

Prof. Douglas Ndiritu

Department of Plant and Microbial Science

Kenyatta University

P.O Box 43844- 00100, Nairobi

Signature.....

Date.....

Dr. Charles N. Lange

Department of Invertebrate Zoology, National Museums of Kenya

P. O. Box 40658-00100, Nairobi

Signature.....

Date.....

Dr. Kem Githui

Department of Molecular Genetics, National Museums of Kenya

P. O. Box 40658-00100, Nairobi

Signature.....

Date.....

DEDICATION

I dedicate this work to my daughter Hope Gathoni, nephews Nduba, Berac, Neem and, my Niece Wairimu, my husband Francis and all my other family members.

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ACRONYMS AND ABBREVIATIONS

DNA	- Deoxyribonucleic Acid
EDTA	- Ethylene Diamine Tetra-Acetate
GPS	- Global Positioning System
IUCN	- International Union for Conservation of Nature
PCR	- The Polymerase Chain Reaction
R.P.M	- Rotations Per Minute
SDS	- Sodium Dodecyl Sulphate
STE	- Sodium Tris EDTA
TE	-Tris EDTA

ABSTRACT

Gulella taitensis is a land snail of the family Streptaxidae and genus *Gulella*. It is endemic to Taita hills and categorized as endangered on the IUCN Red List. The species is threatened by habitat loss and disturbance due to human activities. Two people sampled snails at four sampling plots using standard timed direct search for one hour. Soil samples were collected from four different points within these sampling plots, and its pH, calcium and electrical conductivity obtained using standard soil chemical analysis methods. Litter depth, litter cover, canopy cover, log and tree density were assessed. Distribution of *G. taitensis* varied significantly between all the forest fragments. Most of the environmental variables investigated recorded a positive association with the *G. taitensis* densities. Calcium is significant for the snail's survival and has a significant correlation with litter cover, canopy cover and log density and these influences the distribution between the forest fragments. Calcium, canopy cover, log density and tree density form the best combination of environmental variables (model) that influence the distribution and abundance of *G. taitensis*. Polymerase Chain Reaction of DNA extracted from these snails using microsatellite primers showed there is genetic diversity within this species. We predict that there could be genetic variations within this species since the phylogenetic tree showed two major clades and samples from the same forest fragment clustered at different clades while others clustered closely together within the same clade. There is a possibility of the presence of a hybrid or sub-species within this species. This requires more specific primers to be used to confirm it as genetic work of this genus is scanty.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The Eastern Arc mountains harbour an unusually high proportion of rare and endemic animals and plants, and together with the coastal forests of Kenya and Tanzania, are recognised as one of the world's 25 biodiversity hot-spots (Myers *et al.*, 2000). The Taita Hills, together with Sagala and Kasigau, form the northernmost part of the Eastern Arc mountains, and are the sole representatives of this ancient geological formation within the Kenyan political boundaries.

Land snails are molluscs found on all continents and they occur virtually everywhere. They live primarily in the upper leaf litter of forests, old fields, and wetlands, but also in more disturbed habitats such as active gardens and fields, riverbanks, suburbs, and even cities. Terrestrial molluscs generally reside in patchy habitats likely to promote geographical structuring and have been considered as ideal organisms for studies of ecological genetics over a large range of spatial scales (Goodacre, 2002). Although land snails are generally an inconspicuous part of the forests, and may go completely unnoticed to the people working in the forests, they are without a doubt both ecologically and evolutionarily significant. Many land snails are leaf litter dwelling, and by helping to break down decomposing material, they are likely a vital part of nutrient cycling in a healthy forest. Some land snail species may also indirectly influence the diversity of fungi and lichens in an area through their grazing activities.

The terrestrial mollusks feed upon a wide variety of organic material, mainly green or dead herbaceous plants, rotting wood and fungi, bark and algae, but they also consume empty snail shells, sap, animal scats and carcasses, and even rasp limestone

rock or cement. Land snails do not move far over their lifetime, so they can be excellent indicators of site history and site conditions. Because shelled land snails have a high calcium demand, they are sensitive to calcium availability due to soils and plants. Site moisture and past land clearing or fire also strongly influence snail populations.

The survival of some snails within many of the East African forests is uncertain due to high molluscan endemism. These forests have been highly manipulated due to an increasing demand for agricultural land and natural resource extraction occasioned by the expanding human population. Emberton *et al.* (1997) suggests that there is an amazing wealth of unknown snail diversity among the East African forests due to under-exploration. *Gulella taitensis* (Verdcourt; 2006) is a land snail of the family Streptaxidae and genus *Gulella* (appendix 1) It is endemic to Taita hills and categorized as endangered on the IUCN Red List (IUCN, 2008).

The species has a total extent of occurrence as 319.5 km² that is threatened by habitat loss and disturbance due to human activities. The species preferences include the most intact forest areas characterized by deep forest floor litter, dense moss growing on dead fallen wood or tree trunks, high percentage canopy cover, huge trees, and dead fallen wood. The number of known localities was five where *Gulella taitensis* is more frequently encountered during previous surveys but we recorded its presence in all the ten forest fragments covered in this study. Large numbers are recorded in the smallest, most vulnerable forest fragments while in the large and well-protected fragments only few individuals were recorded. The species exists at very low population densities. There is a need to conserve the smallest forest fragments, however, these are less valuable for bird and mammal conservation. It is estimated that habitat lost since 1990

is 40–80%. This ecosystem like many others worldwide is threatened by extinction due to the dynamics of increased population, socio- politics and economics. Avise (2004) recommended that conservation and management decisions must be made using the best, most up-to-date taxonomic information available to overcome contradiction between molecular and morphometric systematics.

1.2 Problem statement and justification

Since the declaration of *G. taitensis* as endangered several years ago, no efforts have been initiated to study the environmental factors influencing its distribution. Information that is very vital for promoting the species conservation is lacking yet many of the Taita forest fragments seem to be on the brink of extinction and are perhaps the species hotspots. Further preliminary observation suggested that the species might be sensitive to habitat changes. With the current conservation concerns facing Taita hills forest, a detailed environmental study of the species is vital in order to gather information on the potential impact of the ongoing habitat changes on the species population size. Further, molecular characterization of the species is useful to register the species genetic composition for documentation and confirmation of taxonomy so that such information does not disappear should the species become extinct with the continuing habitat changes.

1.3 Research questions

The study was carried out to try and answer the following questions;

- i) Is *Gulella taitensis* molecular characteristics uniform for populations from various forest fragments in Taita Hills?
- ii) Is *Gulella taitensis* evenly distributed among the Taita Hills forest fragments?

iii) Is the distribution of *G. taitensis* influenced by some environmental factors?

1.4 Hypotheses

- i. Genetic relationships of *Gulella taitensis* are not uniform for all the populations from the various fragments of Taita hills forest.
- ii. *Gulella taitensis* is not evenly distributed among the Taita Hills forest fragments.
- iii. The distribution of *Gulella taitensis* is not influenced by the environmental factors.

1.5 Objectives

1.5.1 General objective

To examine the genetic diversity and the distribution of the *Gulella taitensis* in Taita hills, Kenya.

1.5.2 Specific objectives

- i. To assess the genetic diversity of *Gulella taitensis* within and between the different forest fragments
- ii. To determine the distribution of *Gulella taitensis* within the Taita hills forest fragments.
- iii. To determine some environmental factors that influences the distribution of *Gulella taitensis* in Taita Hills.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Land snails in East Africa

The East African region (Kenya, Uganda and Tanzania), is very rich in land snails with over one thousand and sixty three species currently documented (Verdcourt, 2006) and probably many more undescribed (Tattersfield *et al.*, 1998). In comparison, there are only two hundred and seventy nine species present in Northwest Europe, which has a larger area than Kenya (Kerney, 1999). East African region has more species than the whole of southern Australia (Smith, 1985). About 83% of these species are forest dwellers (Verdcourt, 1972), occurring within the regions' tropical rain forests, central highlands, Eastern Arc Mountains and coastal forests (Tattersfield, 1996; Lange, 1999; Lange, 2000) where they show high levels of endemism (Tattersfield, 1998; Emberton *et al.*, 1997). However, these forests comprise only about 2-3% of the total East Africa land area (Sayer *et al.*, 1992) and are further rapidly declining due to human activities (Wass, 1995; Tsingalia, 1990; Brooks *et al.*, 1998; Matiku *et al.*, 1998).

Minimal research has been carried out on the molluscan biodiversity and conservation in the region as compared to other taxa. Tattersfield (1996) notes that though much has been reported about the East African terrestrial malacofauna, there has been little investigation on molluscan assemblages of different habitats. Consequently, there is an amazing wealth of unknown snail diversity among the East African forests due to under-exploration (Emberton *et al.*, 1997).

In view of the conservation concern facing the East African forests, high molluscan endemism reflected within many of these forests and low commitment to regional molluscan research and conservation, the survival of some of these snails is uncertain.

Currently, about 54 snail species are listed in the Red List of IUCN (IUCN, 2006). Majority of the other species are in the data deficient category (IUCN, 2006). Among them is *Gulella taitensis*, endemic to Taita hills of South-Eastern Kenya that is recognized endangered mainly because it is thought to have very small geographical ranges in habitats, which have been severely depleted over the recent years (Brooks *et al.*, 1998; IUCN, 2008; Lens *et al.*, 1999).

2.2 Taita hills forest

The Taita Hills (3°15'–3°30'S, 38°15'–38°30'E) are located in south-Eastern Kenya about 25 km west of Voi. They cover an area of about 250 km² and rise abruptly from the surrounding Tsavo plains at an altitude of 600-700 m, to a series of ridges with Vuria, the highest point, at 2228 m. Taita hills forest comprise the northern-most extension of the Eastern Arc range in South-East Kenya (Lange, 2006). The Taita hills form the first large inland barrier for moisture-laden clouds, which come in from coast, and the precipitation from tapped moisture creates almost permanently humid conditions in the hilltops forests. It is a densely populated region of high agricultural potential. These forests have been highly manipulated because of an increasing demand for agricultural land and natural resource exploitation by the expanding human population. The remaining indigenous forest in the Taita Hills is highly fragmented and despite the threat of their destruction, they support high levels of regionally rare and endemic plant and animal species. Among these are three land snail species; *Thapsia buraensis*, *Ziingis radiolata* and *Gulella taitensis* endemic to Taita Hills. The study concentrated on *G. taitensis*.

2.3 Land snail and forest calcium

Land snail shells are made mostly of calcium carbonate with a protein outer coating. Many kinds of wildlife obtain the nutrient calcium by consuming live land snails or their empty shells. Calcium plays a variety of roles in the body of land snails and slugs, including fluid regulation, cell wall function, muscle contraction, shell and egg formation. Shelled animals use a large amount of calcium in forming their shell structure. The shell-building organ, the mantle, develops a pH gradient to create a small electric current to move calcium ions into place.

Land snails obtain calcium from their environment in a variety of ways, depending upon their autecology. They eat live and decaying leaves and wood, fungi and algae on wood and rocks, sap, animal scats and carcasses, nematodes, and other snails. They can be found rasping old or unoccupied snails' shells, bones and antlers, rock particles or larger stones and outcrops, and the soil that they regularly consume contains calcium. In captivity, they consume lime and paper. Land snails also absorb calcium directly through the sole of their foot (Kado, 1960).

Calcium availability in forest environments generally is positively correlated with the number and species richness of land snails (Hotopp, 2002). However, at the greatest levels of site calcium, snail numbers may actually be slightly reduced (Valovirta, 1968). Acid precipitation can reduce the amount of calcium in forest soils, and this in turn can depress snail numbers as much as 80% on sensitive sites (Wäreborn, 1992). Conversely, land snail numbers respond positively to the addition of calcium (Johannessen and Solhøy, 2001).

Calcium in forest soils can come from below, from the breakdown of bedrock and other parent material that contains calcium, but it also comes from above, in the form of plant debris, though plants have also obtained their calcium from soil and rock via roots. Plants use calcium for nutrient and water translocation, cell division and cell wall formation. The amount of calcium in tree leaves and other litter varies, so forest tree species composition influences soil calcium (Boettcher and Kalisz, 1990; Vesterdahl and Rauland-Rasmussen, 1998).

Soil profiles may exhibit declining or increasing calcium with depth, depending upon the calcium content of bedrock and leaf litter. For our purposes, the important issue is the amount of calcium available to land snails at the upper soil horizons where they live. Soil horizon effects may also be interrupted, by calcium-rich limestone outcrops or limestone scree that can make large quantities of this nutrient available to land snails (and other animals), or by decaying logs of certain species such as sugar maple (*Acer saccharum*) that appear to support large numbers of snails.

Snails are eaten by herptiles including turtles and salamanders; by mammals including shrews, mice, squirrels, and deer (probably accidentally); and by birds including thrushes, ruffed grouse and wild turkey (Martin et al., 1951). Snail availability may be critical to calcium provisioning for some of these animals. Changes in snail numbers can have ripple effects through an ecosystem, as demonstrated for the great tit (*Parus major*) in the Netherlands (Graveland, 1996; Graveland et al. 1994), where, reduction in soil calcium due to acid rain resulted in fewer snails, which caused eggshell thinning and reduced reproductive success for the birds.

2.4 Land snail diet and behavior

Most land snail species are herbivorous or omnivorous, with only a few mainly predatory species. In snail diets, plants are the dominant food item, then fungi, animal matter and soil. Even within a species, snail diets vary widely, as they take advantage of the foods that are available within crawling distance. Unlike most invertebrate herbivores, which are specialist feeders, such as insects, land snails are generalists, sampling and assessing a large variety of food items in their path (Speiser 2001).

Land snails will often be active at night and during damp weather because crawling requires mucus, which is mostly water, and damp weather minimizes water evaporation. Once active, snails find food by using the chemoreceptors on their four tentacles (Atkinson, 2003). When food is reached, a snail will touch it with mouth and foot, then begin rasping with the radula in its mouth (Mackenstedt and Märkel, 2001). Saliva aids digestion in land snails, and muscular contractions move food along the esophagus as in people (Dimitriadis, 2001). Digestive juices begin to break down food items here and as they move into the gastric pouch. Connected to the gastric pouch is the large digestive gland that serves to absorb food, excrete waste, and regulate body chemistry.

From the gastric pouch, waste enters the intestine and rectum on its way out of the body. Land snails excrete the undigested parts of their food from the anal pore, located in the mantle, at the edge of the shell in shelled species. Snail excrement may appear as a tiny folded rope. Microscopic examination of its contents can reveal what a snail has been eating, but most of what is known is from observed feeding behavior.

Land snails may eat herbaceous plant leaves or stems; rotting herbaceous plants, leaves, wood or bark, including the fungi that live within these items; fungal fruiting

bodies such as mushrooms or conchs; and coatings of fungi or algae on rock or bark (Grime and Blythe, 1969; Mason, 1970; Hanley et al., 1995).

Snails are also found eating animal scats and carcasses; nematodes; old shells of other snails; or snail eggs, shells, and flesh. In the Pennsylvania woods large snails such as the toothed globe (*Mesodon zaletus*) might be found upon white-tailed deer scats, while the gray-foot lancetooth (*Haplotrema concavum*) hunts and consumes live snails and slugs.

Snails also ingest organic and inorganic soil and rock particles. Consumption of calcium-bearing minerals provides the nutrient that snails need to build their shells, which are mostly calcium carbonate with a protein outer coating, the periostracum. Land snails can ingest environmental contaminants and hold, or sequester those contaminants in their tissues, (Dallinger and Wieser, 1984), which makes snails useful indicators of pollution. Snails that are tiny usually live near or very near on their food – a drifted pile of leaf litter, or a rotten log. Others may move short distances from cover to food at night. Snails are often found at the base of a plant or tree upon which they feed at night or in damp weather.

2.5 Economic importance of land snails

Land snails include one of the most important groups of invertebrates in terrestrial ecosystem. These terrestrial mollusks feed upon a wide variety of organic material, mainly green or dead herbaceous plants, rotting wood and fungi, bark and algae, but they also consume empty snail shells, sap, animal scats and carcasses, and even rasp limestone rock or cement. Carnivorous snail species attack nematodes and other snails. Land snails in turn are eaten by a variety of invertebrate and vertebrate predators. Predators include invertebrates such as parasitic mites, nematodes and flies;

beetle larvae, beetles and millipedes; and other snails. Cyathine beetles have specialized bodies for preying upon land snails. Fireflies are a well-known insect whose larvae consume snails. Vertebrate predators of snails include herptiles such as salamanders and turtles; shrews, mice and other small mammals; and birds, especially ground-foragers such as thrushes, grouse, and turkey.

With regard to ecosystem function, shelled land snails are important in calcium cycling. They glean calcium from their food, concentrate it in their shells that are made mainly from calcium carbonate, and pass it up the food chain as they are consumed by predators. Snails as well can generally be categorized as decomposers, though they play only a small role compared to other decomposition organisms. Many people also consider some snails as a delicacy such as *Helix garden* snail. The turban snail of Australia is used in making pearly shirt buttons. Some snails also are disease vectors while others are bioindicators (Lange, 2003). Land snails do not move far over their lifetime, so they can be excellent indicators of site history and site conditions. Because shelled land snails have a high calcium demand, they are sensitive to calcium availability due to soils and plants. Site moisture and past land clearing or fire also strongly influence snail populations. They can also be indicators of pollution, as they uptake environmental toxins such as cadmium.

Human use of land snails as food ranges from Native American consumption of *Oreo helix* species snails in the western states, to fine dining upon *Helix* species snails served as escargot in restaurants. Medical uses include the production of an anti-agglutinin from the albumin glands of *Helix aspersa*.

Land snails can also have negative interactions with other organisms. Snails are intermediate hosts to a variety of mammalian parasites. The cervid brain worm

Parelaphostrongylus tenuis is carried by deer and can severely limit moose and caribou populations (Pybus *et al.*, 1992). However, the most serious ecosystem and agricultural impacts due to land snails are often related to non-native pest populations. For example, the introduced European white garden snail *Theba pisana* that can damage ornamental and citrus plants has been the subject of eradication programs in California.

2.6 Molecular systematics

A range of genetic techniques is available for the study of population genetics. The selection of the DNA marker used to study the genetic structure of a population is dependent on the genetic variation detected by that particular marker (Wilson *et al.*, 2000). In recent years, DNA-based studies involving PCR amplification of nuclear and mitochondrial DNA, followed by estimation of genetic divergences from sequence data have been used to discriminate between closely related species (Bourdy *et al.*, 2003) with regard to their taxonomic and phylogenetic classification. Mitochondrial DNA exhibits considerable variation among individuals both within and between populations and therefore provides useful insights into the population structure, geographic variation and phylogenetic relationships at several levels of the evolutionary hierarchy (Avisé *et al.*, 1987).

Several researchers have applied a range of this technology to study the biology of snails. Vidigal *et al.*, (1996) using Low Stringency Polymerase Chain Reaction (LS-PCR) showed that LS-PCR analysis is appropriate for distinguishing *B. glabrata* from *B. tenagophila*. Further, studies with populations of *B. straminea* using the same methodology suggests that this species is even more variable than *B. glabrata* and *B. tenagophila*. The technique requires a small amount of DNA and can be applied to

juvenile snails. It is useful in resolving problems of identification of potential intermediate snail hosts in situations where the classical taxonomy methodology is inconclusive. PCR analyses based on combined 12S-16S (~1,900bp) and COI (~658bp) revealed that introduced populations of *Pomaceae canaliculata* are genetically distinct from those of *Pomaceae insularum*, based on mtDNA sequences though these species have similar shell morphologies (Rawlings *et al.*, 2007). They also found considerable intraspecific variation and well supported phylogenetic structure in *Pomaceae insularum*.

Markus *et al.* (2006) found that contrary to DNA-taxonomy, shell morphology was not suitable for delimiting and recognizing species in *Radix* and proposed DNA-based taxonomy as a reliable, comparable, and objective means for species identification in biological research. Morphometric analysis showed that shell shape was unsuitable to define homogeneous, recognizable entities, because the variation was continuous, while the Molecularly defined Operational Taxonomic Units (MOTU), inferred from mitochondrial Cytochrome Oxidase I (COI) sequence variation, proved to be congruent with biological species inferred from geographic distribution patterns, congruence with nuclear markers and crossing experiments. Ngereza (2006) using spearman's rank correlation analysis demonstrated a significant positive association between both species richness and abundance and the abundance of both litter and dead fallen wood in land snail faunas in primary and secondary forests and in forestry plantations in the East Usambara mountains.

2.6.1 Amplification of microsatellites

Microsatellites are polymorphic loci present in nuclear and organellar DNA that consist of repeating units of 1-6 base pairs in length. The repetitive nature of these sequences causes the rapid accumulation of mutations, which allows their use as

highly variable molecular markers. They are typically neutral, co-dominant and are used as molecular markers which have wide-ranging applications in the field of genetics, including kinship and population studies. Such markers find applications in all kinds of fundamental and applied studies within fields such as ecology, forensics and research on genetic diseases. Microsatellites can also be used in the study of gene dosage (looking for duplications or deletions of a particular genetic region), (Freeland, 2005).

The use of microsatellites for instance in ecology and forensics depends on their tremendous variability, which allows them to differentiate between very closely related individuals. Microsatellites have been shown to accumulate mutations at rates between 10^{-2} (*E. coli*) and 6×10^{-6} (*Drosophila*) events per locus per generation (Hancock, 1999). It has been commonly accepted that the most important mechanism for mutations in microsatellites is what is called strand-slippage mispairing. Microsatellites can be amplified for identification by the polymerase chain reaction (PCR) process, using the unique sequences of flanking regions as primers. DNA is repeatedly denatured at a high temperature to separate the double strand, cooled to allow annealing of primers and the extension of nucleotide sequences through the microsatellite. During DNA replication, the template strand and the newly synthesized (nascent) strand can become dissociated as DNA-polymerase enzymes add new bases. Normally this is not a problem, because there is only one way in which the strands can re-anneal. However in stretches of repetitive sequences there are numerous ways in which the strands can re-anneal, which can result in looping out of a number of bases in one strand. These looped out bases cause the two strands to be somewhat mis-aligned, which causes DNA-polymerase to insert or delete a number of repeats in the nascent strand. Usually the difference is only one repeat, but sometimes the

change in repeat number is larger. This process results in production of enough DNA to be visible on agarose or acrylamide gels; only small amounts of DNA are needed for amplification as thermo cycling in this manner creates an exponential increase in the replicated segment (Levinson and Gutman, 1987)

One rare example of a microsatellite is a (CA) n repeat, where n is variable between alleles. These markers often present high levels of inter- and intra-specific polymorphism, particularly when tandem repeats number one hundred or greater (Jarne and Lagoda, 1996). The repeated sequence is often simple, consisting of two, three or four nucleotides (di-, tri-, and tetra nucleotide repeats respectively), and can be repeated 10 to 100 times. CA nucleotide repeats are very frequent in human and other genomes, and are present every few thousand base pairs. As there are often many alleles present at a microsatellite locus, genotypes within pedigrees are often fully informative, in that the progenitor of a particular allele can often be identified. In this way, microsatellites are ideal for determining paternity, population genetic studies and recombination mapping. It is also, the only molecular marker to provide clues about which alleles are more closely related (Kashi and Soller 1999).

Microsatellites owe their variability to an increased rate of mutation compared to other neutral regions of DNA. These high rates of mutation can be explained most frequently by slipped strand mispairing (slippage) during DNA replication on a single DNA strand. Mutation may also occur during recombination during meiosis (Hancock, 1999). Some errors in slippage are rectified by proofreading mechanisms within the nucleus, but some mutations can escape repair. The size of the repeat unit, the number of repeats and the presence of variant repeats are all factors, as well as the frequency of transcription in the area of the DNA repeat. Interruption of

microsatellites, perhaps due to mutation, can result in reduced polymorphism. However, this same mechanism can occasionally lead to incorrect amplification of microsatellites; if slippage occurs early on during PCR, microsatellites of incorrect lengths can be amplified.

Microsatellites are widely used in studies of population genetic structure due to their often high levels of intraspecific variability, (Estoup *et al.*, 1995) Microsatellites are tandem repeats of short nucleotide sequence motifs. They have been enthusiastically adopted in the past decade for linkage and population genetic studies because their high polymorphism (Litt and Luty 1989 and Tautz 1989) is believed to resolve population structure better than allozymes (Blouin *et al.*,1996; Estoup *et al.* 1996; Jarne and Lagoda 1996).

CHAPTER THREE

3.0 MATERIALS AND METHODS

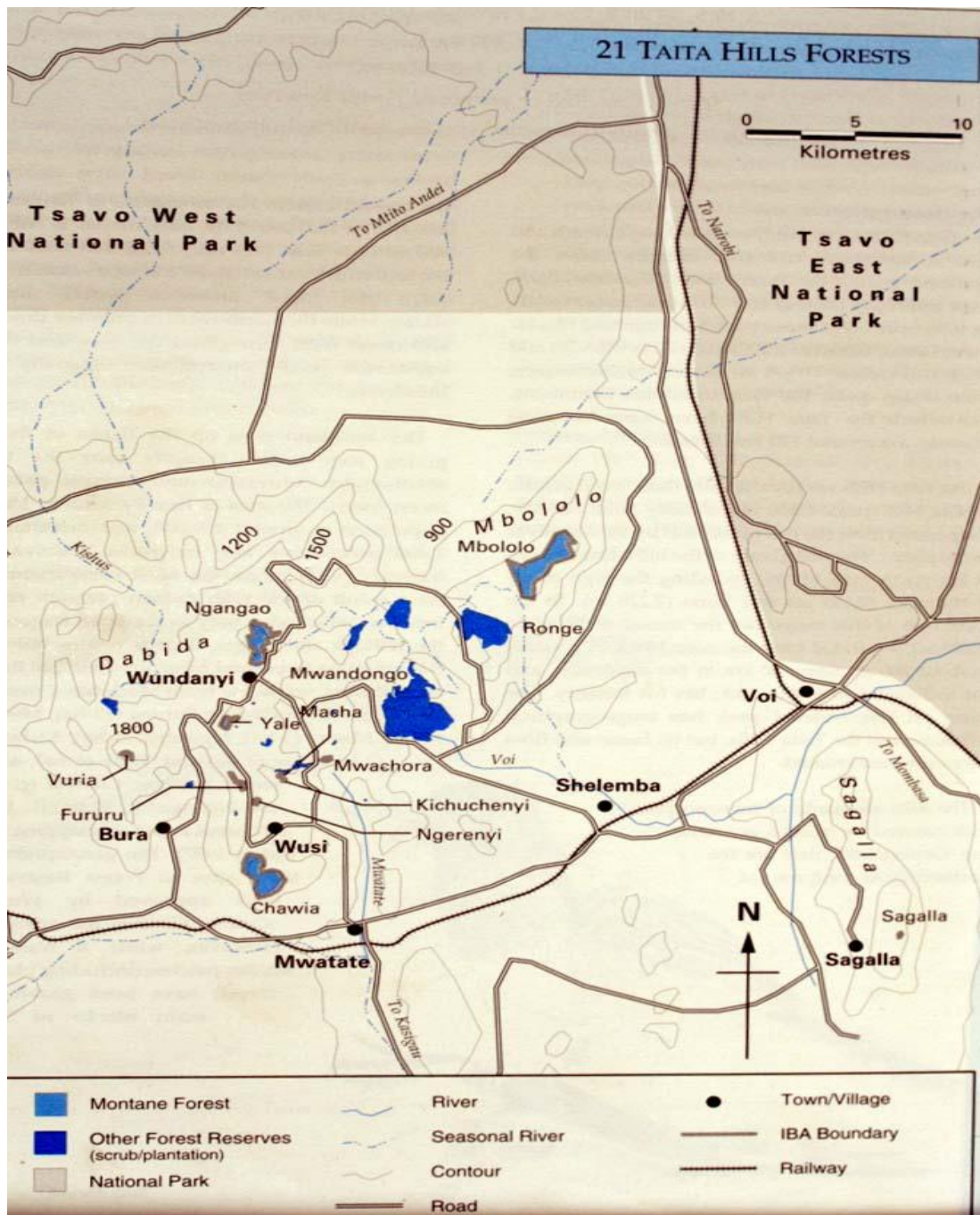
3.1 The study area

The Taita Hills are located to the Southeastern part of Kenya in Taita District, Coast Province ($3^{\circ}15' - 3^{\circ}30'S$, $38^{\circ}15' - 38^{\circ}30'E$) (fig. 1) The forest hills rise abruptly from the surrounding plain of altitude 600 – 700 meters, to form a series of ridges or massifs reaching a maximum altitude of 2228 meters at the Vuria peak. These massifs represent the north-most extension of the Eastern Arc range and are the only representative of this geological formation within Kenyan political boundaries (Lens and De Meyer, 1997).

The climate of the Taita hills is under the influence of the inter-tropical convergence zone, within a bimodal rainfall pattern. Of great significance to the hills are the moisture-laden clouds, which blow in from the coast, to which the hills accounts for the almost permanently humid conditions observed in the forest. The mean rainfall at Wundanyi, situated almost at the centre of the massifs, is 1329 mm per year (Brooks *et al.*, 1998) and is among the highest in Kenya. Geologically, Taita hills are mainly soft metamorphic rocks overlain by quartzite cap (Beentje, 1987).

The entire forest ecosystem is comprised of several isolated forest fragments located at the hills peaks. The largest is Mbololo forest with area of about 200 ha of moist forest at an altitude of 1800 meters to 2209 meters (Brooks *et al.*, 1998). To the south of Mbololo, lies a series of ten other relatively small fragments collectively known as Dabida by the locals. These fragments and their approximate sizes include Ngangao (92 ha), Chawia (50 ha), Fururu (4 ha), Vuria (2 ha), Yale (2 ha), Ndiwenyi (1.5 ha), Kichuchenyi (2 ha), Wundanyi (2 ha), Macha (2 ha) and Mwachora (1.5 ha), (fig3.1).

Figure 3. 1 Study area



The map shows the position of the forest fragments in Taita Taveta district.

3.2 Methods

3.2.1 Site selection

A preliminary survey was carried out to determine the structure of the various forest fragments the various unique habitats such as least disturbed sites, areas disturbed by human and livestock as well as areas with any unique characteristic. This general overview allowed generation of information on the association of specific environmental variables and the species and aided in the identification of sampling sites. Four sampling plots measuring 40 x 40 meters were designed at random per forest fragment. The geographical position of study sites identified were determined with aid of a GPS and recorded. The GPS positions were vital for tracing the identified sampling areas during the actual snail sampling.

3.2.2 Sampling *Gulella taitensis*

Snails were sampled at the plots selected during preliminary surveys using standard timed direct search (Tattersfield, 1996; Emberton *et al.*, 1997; Lange, 2003). Live snails and dead shells were searched and recorded for a period of one hour by two individuals. During the search, all potential snail micro-sites (base of big trees, under dead fallen wood, within forest floor litter, within moss, under leaves surfaces of low growing vegetation) were thoroughly investigated and any snail sighted recorded. The species is very distinct morphologically from many others and thus it was easy to distinguish it among many other forest snails. A biopsy from the foot was cut from selected live snail for molecular work. The foot is mainly tough muscular tissues that snail endo-parasites cannot penetrate easily and thus recommended source of quality land snail DNA.

3.2.3 Preservation of snail for DNA extraction

The tissue cut from the foot was preserved by freezing in liquid nitrogen while in the field. In the laboratory they were stored in freezers (-20°C). Freshly preserved tissues give non-degraded DNA good for molecular analysis.

3.2.4 DNA extraction

Out of the 207 live snails recorded, 88 of them have been used in molecular work. DNA was extracted from the preserved snail tissues using a modified Cetyltrimethylammonium bromide (CTAB) method (Winnepenninckx *et al.*, 1993). A macerated tissue from the foot of the snail was put in a 1.5 ml eppendorf tube with 300 µl of 2X CTAB buffer and then incubated in water bath at 60 °C for 30 minutes. The mixture was then cleaned by adding 300 µl of chloroform-isoamyl alcohol (24:1) and mixed for 2 minutes. This was followed by centrifuging at 15,000g for 10 minutes. The supernatant was aspirated to a clean eppendorff and chloroform-isoamyl alcohol extraction repeated three times.

The supernatant from the third chloroform-isoamyl alcohol extraction was aspirated into a clean eppendorff tube and 600 µl of ice-cold absolute ethanol (EtOH) and 25 µl of 3M Sodium acetate (NaOAc) was added to precipitate the DNA. It was mixed by inversion for five minutes and then centrifuged at 15,000g for 10 min. The ethanol was poured off and the pellet rinsed in 600 µl of 70% EtOH, Centrifuge at 15,000g for 5 minutes. The ethanol was poured off and the pellet dried in an open water bath at 60 °C for five minutes. The pellet was re-dissolved in 50 µl of 1X TE, placed in a water bath at 60°C for 30 minutes (to get all DNA into solution) and then preserved at -20°C. The presence and quality of DNA was tested using electrophoresis

3.2.5 Gel electrophoresis

3.2.5.1 Agarose gel electrophoreses

The agarose gel is convenient for separating DNA fragments of up to 20 kb and is regularly employed to determine the yield and purity of a DNA extraction or PCR reaction and for the fractionation of different sizes of DNA molecules (Maniatis *et al.*, 1982). Agarose gel is a complex network of polymeric molecules. At neutral pH, the sugar-phosphate backbone of a DNA molecule is negatively charged. Under an electric field, the DNA will migrate through the gel towards the anode. The rate of migration is usually dependent on molecular weight, voltage, the conformity of the DNA as well as the porosity of the gel (percentage component).

DNA electrophoresis is an analytical technique used to separate DNA fragments by size and net electric charge. An electric field forces the fragments to migrate through a gel. DNA molecules normally migrate from negative to positive potential due to the net negative charge of the phosphate backbone of the DNA chain. At the scale of the length of DNA molecules, the gel looks much like a random, intricate network. Longer molecules migrate more slowly because they are more easily 'trapped' in the network.

The presence of DNA was tested using a 1% agarose gel submerged in electrophoresis buffer in a horizontal tank. 1x TAE (tris acetate EDTA) buffer was used to prepare the gel as well as the running buffer. Ethidium bromide was included in the gel matrix to enable fluorescent visualization of the DNA fragment under UV light. 5µl of the resuspended DNA was mixed with 1µl of tracking dye and loaded into sample wells on the gel. It was then run under 80 volts for one hour and then

observed under ultra violet light and a photograph taken. A 123bp size marker was electrophoresed alongside the DNA samples.

3.2.5.2 Acrylamide gel electrophoresis

Acrylamide gel was used for analyzing the amplified microsatellites since its able to separate bands which differ by only one base pair. An 8% acrylamide gel was prepared by adding 4ml of 40% acrylamide, 2ml of 6X TBE (Tris Borate EDTA) buffer, 100µl of ammonium per sulphate and 10µl of TEMED and distilled water added to make a final volume of 20ml. This was mixed and poured on casting plates assembled vertically on a casting stand. Once the gel polymerized it was fixed into the running tank and 5 µl of the amplified sample mixed with 1µl of the loading dye were loaded each per well. The samples were allowed to run at 80volts for One hour and then the gel was stained using ethidium bromide (5 µl of ethidium in 250 ml of water) for about fifteen minutes. This was followed by distaining using distilled water and then observed under UV light and a photograph taken.

3.2.6 PCR

The Polymerase Chain Reaction (PCR) of the DNA was performed using nine microsatellite primers in a thermocycler pre-set PCR machine (Cheng *et al.*, 1994). The primers used were: mang-TAATAATAA, mang-ACCACCACC, cal-TTCCTTCCTTCC, cal-GGATTTGG, cal-AATTTTTTAA, Nap-CCTCCTCCT, mteu-ATAAATAA, piB-GGGGGGGG, mang -GGAGGAGGAGGA

The PCR components for this locus were 1.0 mM MgCl₂, 0.05 mM dNTPs, 1.0 μM PCR primer, 0.025 U/μl *Taq* (Promega), and 50 ng template DNA. Temperature and cycling parameters were denaturing at 94°C for 30 s, annealing at 45°C for 60s, and extension at 72°C for 60 s, repeated for 35 cycles on a tetrad thermocycler. Replicate samples were amplified under same conditions for consistency of results to rule out errors of mispriming. The products were run on 8% acrylamide gels photographs taken and bands scoring done using cross checker software.

3.2.7 Determination of environmental factors

Soil samples were collected from four different points within the sampling plot. It was dried under shade and its pH, calcium and conductivity obtained using the standard soil chemical analysis at Kenya Agricultural Research Institute, Nairobi. The raw data for all the environmental factors assed are shown in appendix 5.

3.2.8 Litter depth and percentage cover

Litter depth in each quadrant was determined by measuring the accumulated depth of the vegetation debris (mainly leaves and small twigs) using a uniform calibrated stick with a pointed penetrating tip. The stick was pushed through the debris until it touched hard ground and the depth taken in centimeters (Ngereza, 2006). Five litter depth records were taken randomly using this protocol within the quadrant and mean value calculated. The percentage litter cover was determined by placing a quadrant measuring 50 x 50 cm on the litter depth determination point and visually estimating the percentage litter cover within the quadrant area. Five estimates were made and mean value calculated.

3.2.9 Percentage canopy cover

The forest canopy cover was estimated visually using the following range of categories: (1) 0 – 20 %, (2) 21 – 40%, (3) 41 – 60%, (4) 61 –80%, (5)81 – 100 %, (Lange, 2003). By observing the interlocking pattern of the main forest canopy and also the amount of sunlight reaching the forest floor, a judgment was arrived on the range of forest canopy cover.

3.2.10 Trees density, dead fallen wood density

The density of trees was determined by enumerating all trees of diameter at breast height (dbh) greater than 0.2 meters and 1.5m from the ground. Dead fallen wood (logs) density was determined by enumerating all dead fallen wood with diameter at one point exceeding 0.2m and more than 2m in length.

3.3 Data analysis

The total number of individuals recorded was used as an indicator of the species population size in each fragment and entire Taita forest. Graphical presentation was used to demonstrate the species distribution within different forest fragments. The Pearson rank correlation was used to assess the effects of environmental factors on the distribution of the species.

Regression analysis was carried out to assess the best model that influences the distribution of *G. taitensis*.

The Cross Checker software was used to score the size of the bands obtained from acrylamide gel electrophoresis relative to their migration distance from the wells.

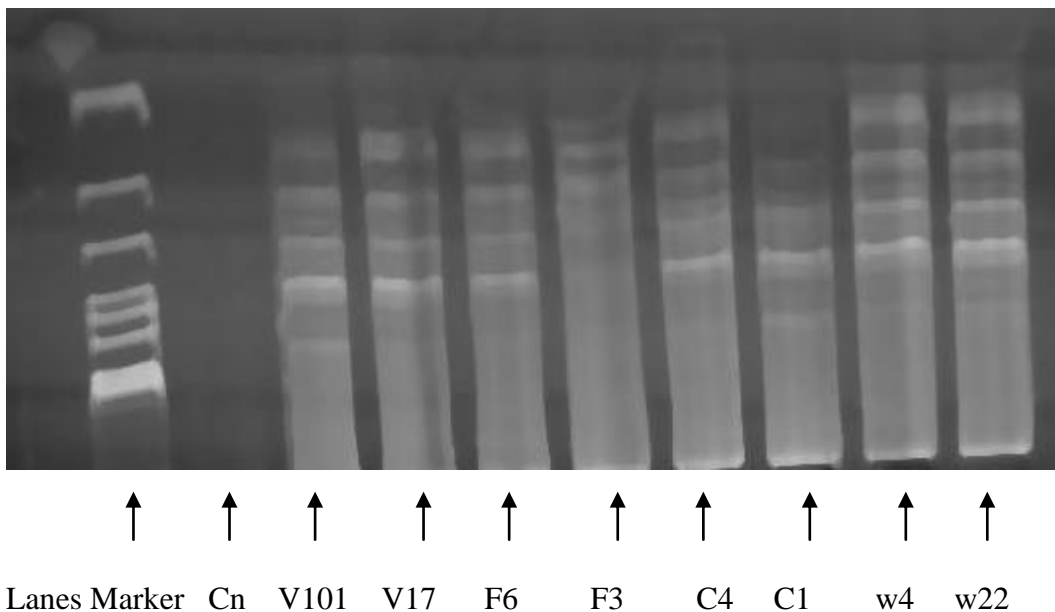
CHAPTER FOUR

4.0 RESULTS

4.1 Genetic diversity of *G. taitensis*

The snails did not show any difference at DNA level. Microsatellite amplification showed possibility of a rich heterozygous population of *G. taitensis* both between and within forest fragments. Different banding patterns were observed after electrophoreses using acrylamide gels (Fig 4.1a, 4.1b). The bands were scored using cross checker software (Stam and Van Ooijen, 1995) for variation analysis. The checker output data, was used in parsimony analysis to derive the phylogenetic tree (fig 4.2) using the past software. Clustering also revealed heterozygosity within this species (fig 4.31).

Figure 4. 1a) A gel representation of microsatellite amplification showing bands from different forest fragments

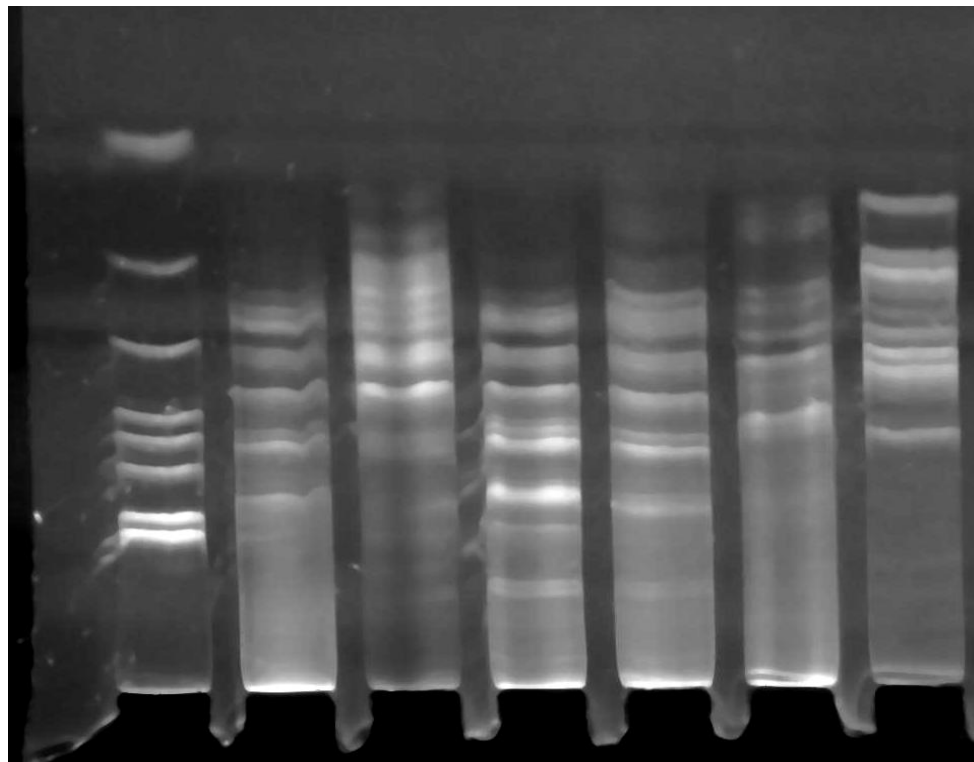


Legend:

-Marker-100 base pair marker,
 -V17, 16-samples from Vuria,
 -C 4, 1-samples from Chawia,

-Cn-control sample
 -F6, 3- samples from Fururu,
 W4,22- samples from Wundanyi.

Figure 4. 1b) A sample acrylamide gel showing the banding pattern of *G. taitensis*



↑ ↑ ↑ ↑ ↑ ↑ ↑
Lanes 100bpm F6 Nd1 Ng302 Ng205 Ng106 V208

Legend:

V – Represents sample from Vuria fragment

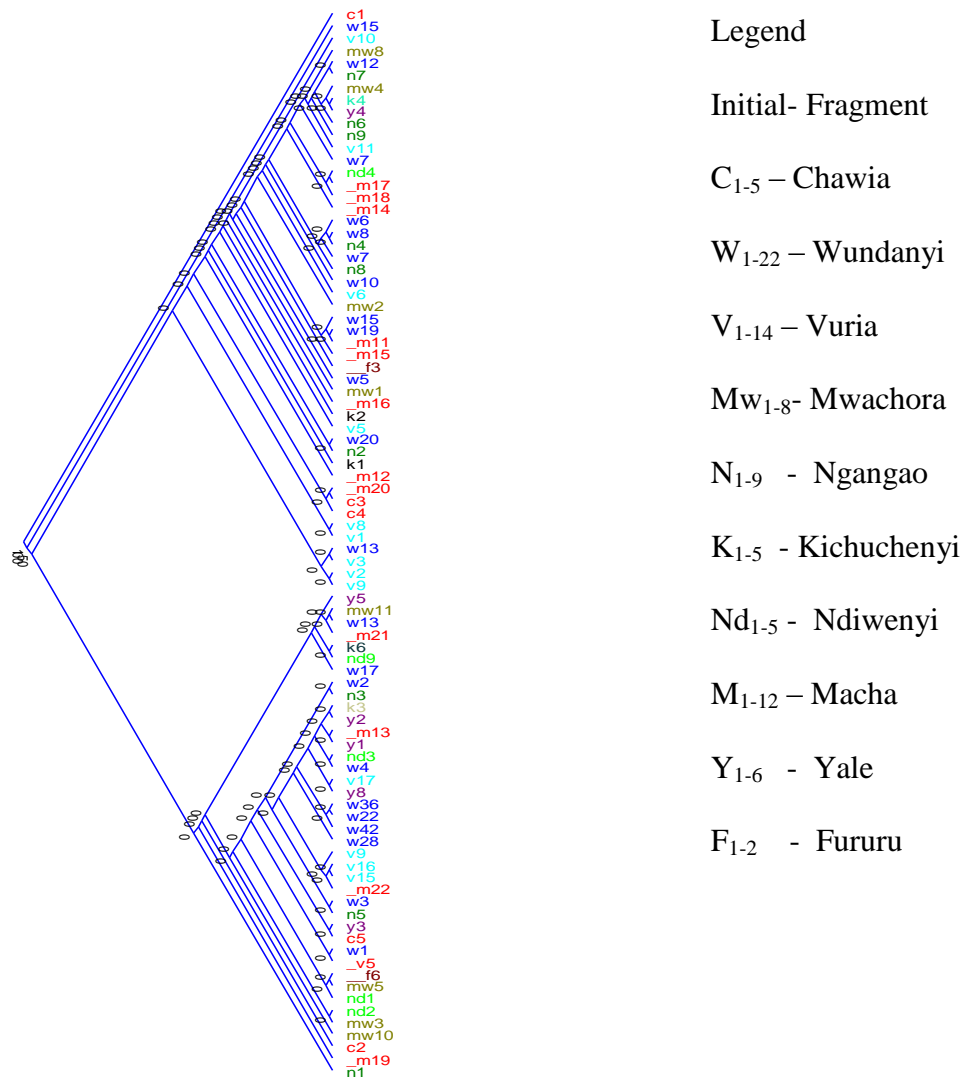
Ng – Represents samples from Ngangao fragment

Nd – Represents samples from Ndiwenyi fragment

F – Represents samples from Fururu fragment

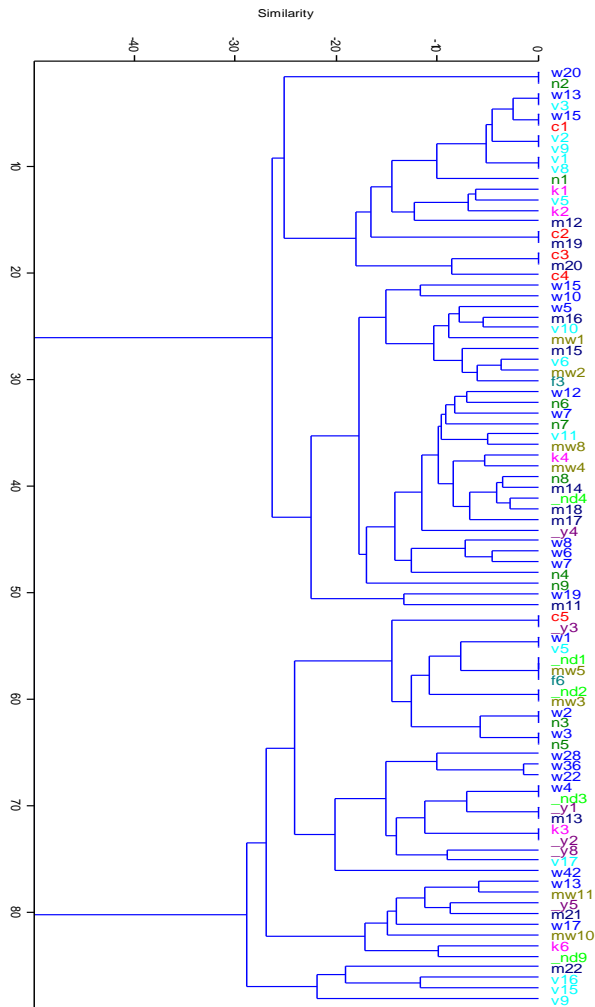
100bpm - 100 base pair molecular marker

Figure 4. 2 Phylogenetic tree showing clustering of samples of *Gulella taitensis* collected from the forest fragments



The figure above shows samples from the same fragment clustering at different clades for instance c1 and c2 while other samples from the same fragment clustered closely together in the same clade for instance v9, v16 and v15.

Figure 4.3 Clustering of *Gulella taitensis* collected from the ten fragments based on banding matrix



Legend

Initial - Fragment

W₁₋₂₂ – Wundanyi

N₁₋₉ – Ngangao

V₁₋₁₄ – Vuria

C₁₋₅ – Chawia

Mw₁₋₈ - Mwachora

K₁₋₅ – Kichuchenyi

M₁₋₁₂ – Macha

F₁₋₂ – Fururu

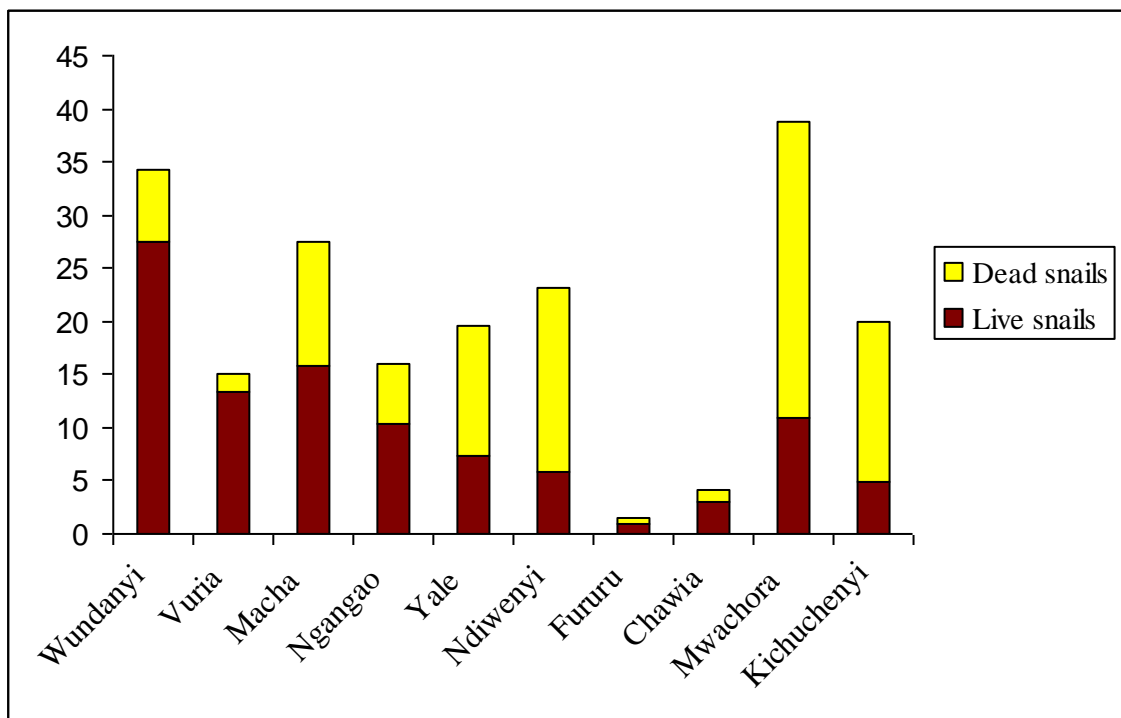
Nd₁₋₅ – Ndiwenyi

Y₁₋₆ - Yale

4.2 Distribution of *G. taitensis*

Among the ten fragments surveyed, four fragments namely Chawia, Ngangao, Vuria and Wundanyi are protected under the forest department while the rest six; Macha, Mwachora, Kichuchenyi, Fururu, Yale and Ndiwenyi are unprotected. The status of these fragments are shown in appendix 2 and 3. The number of *G. taitensis* recorded in the ten forest fragments were not the same. The number of live and dead snails differed in all the forest fragments (Fig 4.4). Descriptive statistics were used to test whether *G. taitensis* is distributed uniformly within the ten fragments.

Figure 4.4 Bar graphs showing total number of live and dead snails per fragment



The statistics (table 4.1) showed that the values for both kurtosis and skewness were not zero. This indicates that the distribution is not normal and this was as well seen in the histograms (fig 4.5a).

Table 4. 1 Distribution matrix of the live snails collected from the ten forest fragments

Live snails		
N	Valid	10
	Missing	0
Mean		20.3
Std. Error of Mean		4.946491
Median		18
Mode		2.00 ^a
Std. Deviation		15.64218
Variance		244.6778
Skewness		1.326033
Std. Error of Skewness		0.687043
Kurtosis		2.28062
Std. Error of Kurtosis		1.334249
Range		54
a-Multiple modes exist. The smallest value is shown		

Figure 4.5 a) Histogram of the number of live snails collected from the ten forest fragments

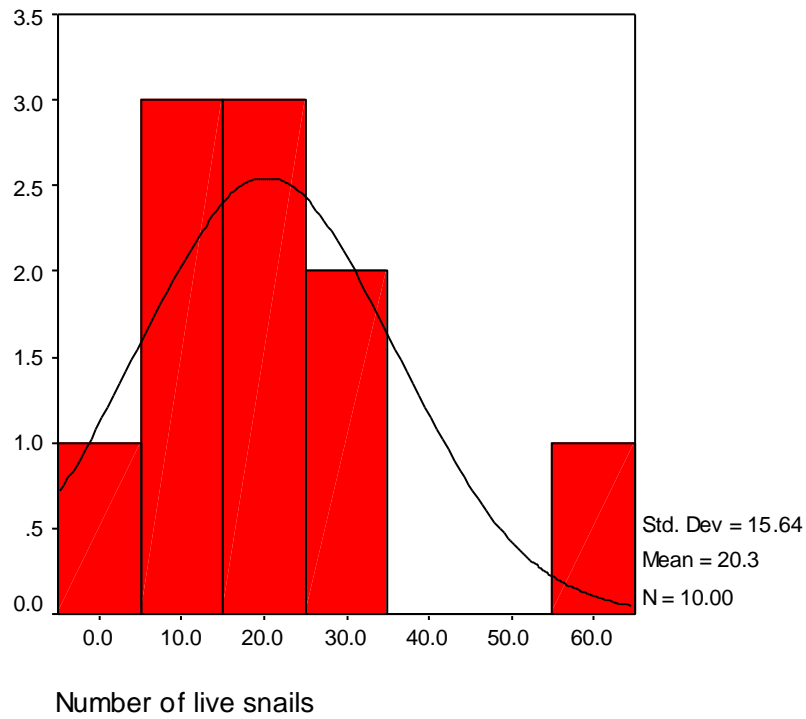


Figure 4.5 b) Histogram of the number of live snails collected from the four fragments protected under the forest department

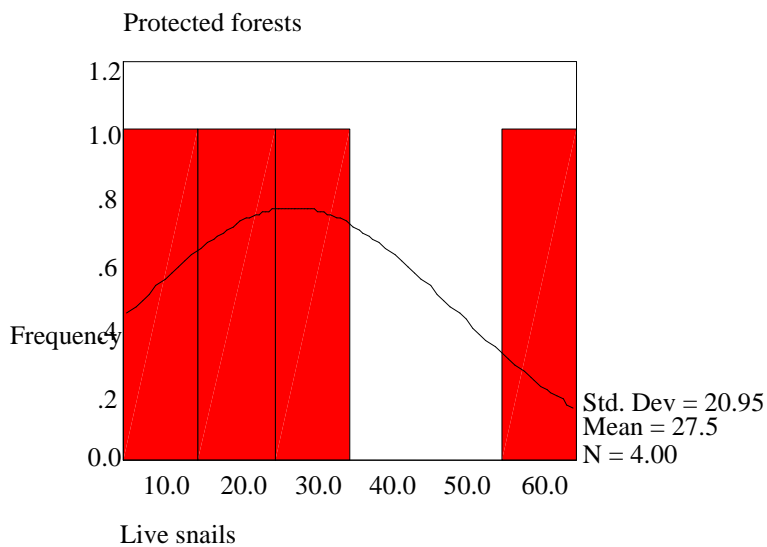
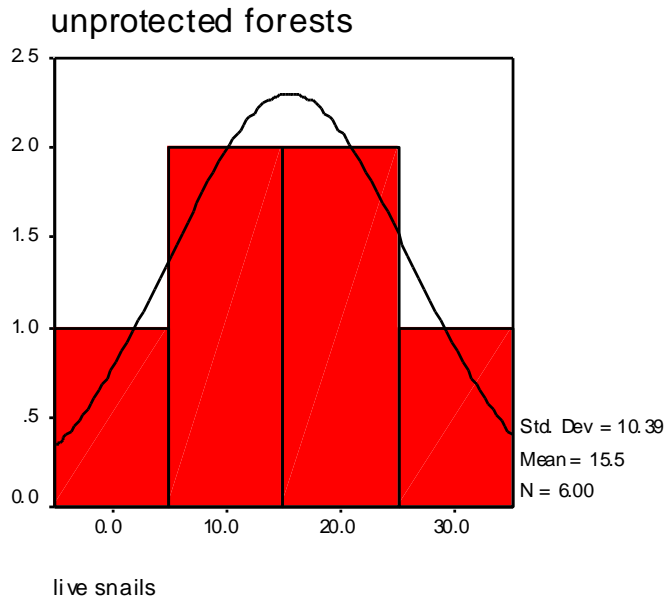


Figure 4.5 c) Histogram of the number of live snails collected from the six unprotected forest fragments.

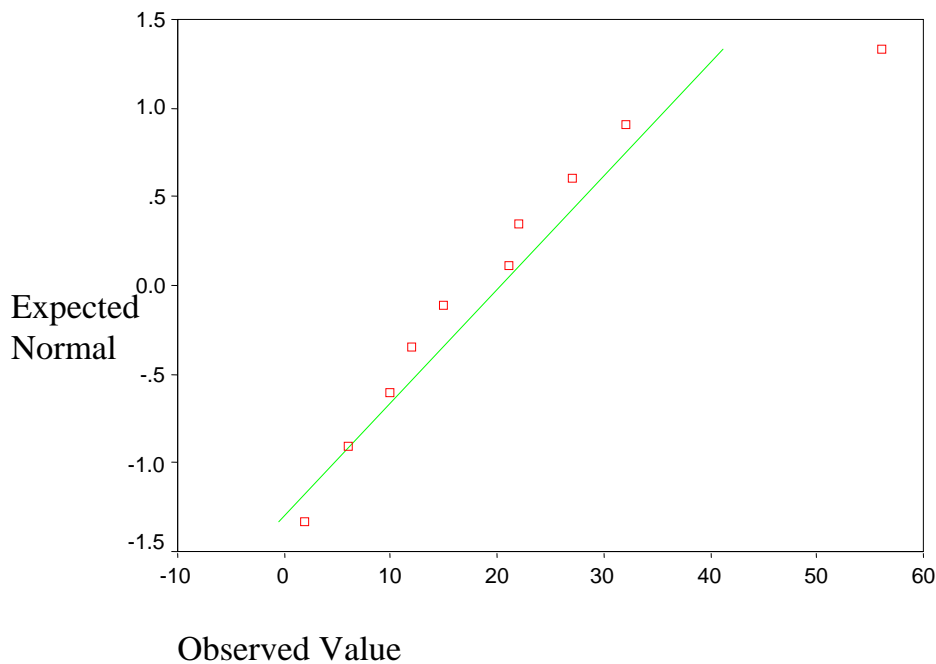


The unprotected forest fragments have a normal distribution where as in the protected fragments, the distribution is not normal.

Thus, the Kolmogorov-Smirnov and Shapiro-Wilk tests (Lilliefors, 1967) were used to see whether the distribution is significantly different from a normal distribution (Table 4.2). Normal Q-Q plots for the same showed some deviation from the expected straight line for a normal distribution.

Table 4. 2 Tests of normality

Live snails	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
	.157	10	.200*	.904	10	.301
* This is a lower bound of the true significance. a Lilliefors Significance Correction						

Figure 4.6 Normal Q-Q Plot of Live snails

The plot shows some deviation from the expected normal distribution.

4.3 Correlation analysis of environmental variables with snail

Pearson correlation analysis was carried out to test whether there was significant correlation between the environmental variables and the number of snails collected as well as whether the variables had significant correlation amongst themselves. The results indicate that there was no correlation of all the environment variables to live snails except for soil calcium, which was significant at 0.05 level, (Table 4.3).

Results further indicate that there was significant correlation between tree and log density, canopy cover and soil calcium to the number of dead snails recorded between the forest fragments at 0.001 level while litter depth and litter cover were significant at 0.05 level. Electrical conductivity and soil pH had no correlation with dead snails.

Calcium had no correlation with soil pH, soil Electrical conductivity (EC), and tree density. On the other hand, it had significant correlation with litter cover, canopy cover and log density. Soil Calcium and litter depth had a negative correlation with dead snails. Both electrical conductivity and soil pH had no correlation with all the other environmental factors, (Table 4.3).

Table 4.3 Pearson correlation matrix of environmental parameters with dead and live snails

	Ca	pH	EC	Litter depth	Litter cover	Canopy cover	Log density	tree density	live snails	dead snails
Calcium	1									
pH	.22	1								
EC	-0.059	.217	1							
Litter depth	.391*	.073	-	1						
Litter cover	.410**	.000	-	.608**	1					
canopy cover	.443**	.105	-	.677**	.717**	1				
Log density	.406**	.036	-	.455**	.719**	.614**	1			
Tree density	.166	.086	-	.549**	.570**	.645**	.708**	1		
Live snails	.392*	.058	-	.150	.211	.242	.054	.143	1	
dead snails	-	-	-	-.341*	.396*	.506**	.530**	.520**	.013	1

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

n=40

Ca- calcium, EC- Electrical conductivity

4.4 Regression analysis

Regression analysis was carried out to determine the best model that influences the distribution of *G. taitensis* in Taita hills forest. The results (R squared) of the environmental variables with the number of live and dead snails regression analysis are shown in table 4.4 below. The table presents results of simple and multiple regression model of each determined environmental variable with live and dead snails.

Table 4.4 R² values of simple and multiple regressions analysis of environmental variables and live and dead snails

(N) Variables	R ² values (LS)	R ² values (DS)	Variables in the model
1	0.003	0.0002	Ph
1	0.024	0.004	Electrical conductivity
1	0.023	0.117	Litter depth
1	0.044	0.157	% litter cover
1	0.154	0.165	Calcium
1	0.058	0.256	% canopy cover
1	0.02	0.27	Tree density
1	0.003	0.281	Log density
2	0.161	0.298	Calcium, canopy cover
3	0.193	0.357	Calcium, canopy cover , log density
4	0.224	0.392	Calcium, canopy cover , log density Tree density

The analysis indicates that soil calcium that accounts for 15.4% is the principal factor that influences the number of live snails. Percentage canopy cover and litter cover have minimal influence. Percentage canopy cover, tree density and log density are major factors that influence dead snails' abundance. They account for between 25.6-28.1% of the existence of dead snails.

Four variables namely calcium, canopy cover, log density and tree density form the best combination of environmental variables that influence the distribution and abundance of live and dead snails. They account for 22.4 and 39.2% respectively. (Table 4.4)

CHAPTER FIVE

5.0 DISCUSSION

5.1 Molecular characteristics of *G. taitensis*

Electrophoreses of microsatellite amplification indicates genetic diversity of *Gulella taitensis* within and between fragments. Snails from the same forest fragments showed different banding patterns when amplified with the same primer. These differences were as well reflected in the phylogeny tree that indicates that the snail population could be richly heterogeneous and have two main clades. This was as well confirmed by clustering matrix (fig. 4.3). Samples from same fragment are clustering at different sub-clades while few others from the same fragment cluster closely together. This could have resulted from sampling juveniles within the vicinity of their parents thus having same banding patterns and clustering together. At the same time there could have been different adaptation response after fragmentation thus being reflected in their genetic diversity since genetic variations are usually related to the environment conditions. These results could not conclusively confirm the taxonomy as no genetic data was available for comparison

5.2 Distribution of *G. taitensis* in Taita hills

The total number of individuals recorded was used as an indicator of the species population size in each fragments and entire Taita forest. This study recorded a total of 207 live *G. taitensis* snails from the ten forest fragments covered. The presence of live snails across all the fragments is crucial compared to a survey done by Lange (2006) which recorded a total of 37 live snails from five out of the nine fragments sampled. The highest abundance of live snails was recorded in one of the smallest fragments wundanyi followed by Macha and Vuria. This concurs with Lange (2006) findings. The study established that the snail distribution frequency is not normal

based on kurtosis and skewness but has a lower bound of the true significance to a normal distribution using the K-S tests. This shows that the deviation from the normal is not highly significant as seen in the Q-Q normal plots (Fig 4.6).

The distribution seems to differ and could be depending on the status of the environmental variables surveyed in each fragment since the same test for protected and unprotected fragments separately indicated a normal distribution for the unprotected fragments while the distribution in the protected fragments was not normal. Among the ten fragments covered Wundanyi, Ngangao, Vuria and Chawia are protected under forest department while the rest are not. The status of the environmental variables surveyed and human activity could have resulted to low average live snails recorded as compared to high number of dead snails per block. Snails that are tiny usually live near or very near their food – a drifted pile of leaf litter, or a rotten log. Others may move short distances from cover to food at night.

Log densities have an inverse relation with dead snails with an exception where we have exotic tree introduced in fragments like Ngangao. Vuria had the highest average number of log density, which could have contributed to less mean number of dead snails, which was also recorded in Chawia. The introduction of exotic trees in Ngangao to replace the indigenous trees contributed to tree density being high and could be one of the factors leading to high number of dead snails recorded in this fragment, since the canopy cover, litter cover and litter depth are influenced by species of the trees. *G. taitensis* seems to be sensitive to the status of the fragments as high number of dead snails were recorded in the unprotected fragments (fig. 4.4) compared to the protected fragments. The amount of calcium in tree leaves and other litter varies, so forest species composition influences soil calcium (Boettcher and Kalisz, 1990; Vesterdahl and Rauland-Rasmussen, 1998). This could have resulted to

more dead snails recorded in Ngangao fragment which has high number of introduced exotic trees.

5.3 Influence of environmental factors on distribution of *G. taitensis*

Soil Calcium had a positive correlation with the number of live snails recorded in this study. As calcium increased the number of snails increased. Soil Calcium had an inverse relation with the number of dead snails (as calcium decreased the number of dead snails increased). This concurs with Hotopp (2002) findings that calcium availability in forest environments generally is positively correlated with the number and species richness of land snails. Because shelled land snails have a high calcium demand, they are sensitive to calcium availability due to soils and plants. However, at the greatest levels of site calcium, snail numbers may actually be slightly reduced as seen in Vuria fragment (Valovirta, 1968).

Other environmental factors (litter depth, litter cover, canopy cover and log density) have positive correlation with soil calcium (table 4.3). These factors are essential for the abundance and survival of *G. taitensis* since they form the source of calcium in forests. Snails feed on both live and decaying leaves and wood that in turn contains calcium, as well as absorbs some through their foot (Kado, 1960). Calcium availability to snail determines the quality of shells as well as egg laying essential for their reproduction. This could explain why there were more dead than live snails in the unprotected forests as the sources of soil calcium have been interfered with thus reducing the amount and as well exposing the snails to their predators.

In this study soil pH did not show any direct relationship with both live and dead *G. taitensis* population. These findings concur with Ngereza (2006) findings in a survey carried in East Usambara mountains Tanzania which showed no correlation between the soil pH and snail abundance and species richness but could be dependent on other factors that contribute to soil pH for instance calcium.

There was a positive correlation between dead snails and litter cover, canopy cover, log density and tree density and a negative correlation with calcium and litter depth. Litter cover, canopy cover, log density and tree density also had significant positive correlation at 0.001 level with litter depth, canopy cover, log density and tree density with litter cover, log density and tree density with canopy cover as well as tree density with log density (table 4.3).

A stepwise multiple regression to determine the main factors that influenced the population and distribution of *G. taitensis* also concurred with the correlation analysis results. Soil calcium ($r^2=0.154$) and log density ($r^2=0.281$) were the main factors that contributed to the observed variability in number of live and dead snails recorded between the forest fragments. Further, the environmental variables showing significant positive relationship with both soil calcium and log density were selected by regression as the best combination of environmental factors influencing population and distribution of *G. taitensis* in Taita hills forest (table 4.4). Soil calcium, canopy cover, log density and tree density formed the best combination of a regression model that best accounts for these variations. This implies that the distribution and population of *G. taitensis* is influenced by a combination of environmental factors rather than one variable.

The findings in this study that environmental variables contribute to distribution and population of land snails have been reported in other studies elsewhere. Johannessen and Solhøy, (2001) reported that land snail numbers respond positively to the addition of calcium. Calcium plays a variety of roles in the body of land snails, including parts in fluid regulation, cell wall function, muscle contraction, and egg laying, and of course, the shelled animals use a large amount of calcium in forming their shell structure. Deficit of calcium leads to severe disturbances like difficulties to produce shells (Voelker 1959), reduced egg viability leading to low reproduction.

Lange (1996) reported that dead fallen wood (log density) microhabitats were highly preferred by forest snails in Kakamega tropical rain forest in Kenya. Logs provide land snails protective cover from predation, moist habitats during drought and food sources and for many species an area to lay eggs.

In Sweden a study of clearcutting in boreal forest found a long-term increase in calcium and the number of most land snails (Ström, 2004), despite an initial decline. Owen (1969) found that fragmentation of canopy cover led to the decline in the densities of the *Limicola mratensiana* in Kagugube forest, Uganda. Walden (1981) attributed decline in molluscan diversity in Scandinavian woodlands to tree cutting and other mechanical encroachments that subsequently lead to changes in sun (increased isolation) and wind exposure.

Emberton *et al.* (1997) found that snail diversity and abundance were strongly correlated with leaf litter abundance. Ngereza (2006) as well reported a significant positive correlation between percentage litter cover and dead fallen wood density (log density) with snail diversity and abundance in East Usambara mountains, Tanzania.

Based on this study and previous investigations, it is noteworthy to conclude that destruction of forest floor litter, removal of dead fallen wood, fragmentation of forest canopy cover, clearance of large sized trees and decline in soil calcium substantially lowers terrestrial malacofauna distribution and abundance. This study suggests that the valuable factors investigated accounts for 22.4 and 39.2% of the observed distribution and abundance of live and dead *G. taitensis* respectively. The remaining 77.6% and 60.8% of the variability not accounted for by these factors could be attributed to effects of human activities, predation competition as well as sampling design error. Predators include invertebrates such as parasitic mites, nematodes and flies; beetle larvae, beetles and millipedes; and other snails. Cychine beetles have specialized bodies for preying upon land snails. Fireflies are a well-known insect whose larvae consume snails. Vertebrate predators of snails and slugs include herptiles such as salamanders and turtles; shrews, mice and other small mammals; and birds, especially ground-foragers such as thrushes, grouse, and turkey. Most of these predators are present in Taita hills forest but their relationship with snails is unknown. Liu (1994) found that predation and interspecific competition for food are among the major causes of variability in Hong Kong limpets (*Callana grata*). Variability due to sampling error is almost negligible since an experienced malacologist was involved during sampling however more successive investigations are important to assess sampling efforts to observed availability.

The microsatellite findings cannot be conclusive since there lacks molecular data for the whole genus to base comparison with. The variations observed could have resulted from adaptations to environment after fragmentation as well as taxonomic errors based on morphology. Amplification errors were avoided through replication of same sample DNA under same conditions.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study established that *G. taitensis* has different molecular characteristics within and between the forest fragments. Microsatellite amplification revealed different number of bands of different molecular weight even between samples collected from the same forest fragments. Parsimony analysis also showed two major clusters with other sub-clusters but samples from same fragment could not form a clade but were rather scattered in other sub-clusters. This indicates possibility of having sub-species and or hybrids within this species. Alternatively, the adaptation of the snails to the fragmented and disturbed remnants of this forest could have resulted to these changes. McCauley, (1993) and Frankham *et al*, (2002) reported that habitat fragmentation may have significant effects on the genetic structure of subdivided populations because physical barriers are likely to impede gene flow. However, despite the reality of habitat patchiness and environmental instability, this study showed no clear evidence concerning the potential effects of fragmentation and human disturbances on the distribution of genetic variability.

A total of 207 live snails were recorded. The distribution of *G. taitensis* within the Taita hills fragments varied. Live and dead snails recorded in the ten forest fragments mostly depended on the status of the fragment whether protected or not as well as the value of the environmental factors. Indigenous and exotic trees also contributed to some extent to the number of snails recorded. The highest number of snails recorded were from the smallest forest fragments. This concurs with Lange (2006) findings who also recoded high number of *G. taitensis* from the smallest fragments.

This study has demonstrated that Taita Hills forest deserve protection for *Gulella taitensis* conservation to be achieved. A conservation-related intervention in the Taita Hills is urgent because only a small area of the forest fragments remains yet the environmental factors studied showed influence on distribution of *G. taitensis*. Soil Calcium in forests is dependent on other factors like canopy cover, log density, litter depth, litter cover and tree density. This study established that calcium contributes up to 15.4% to the distribution of *G. taitensis* and has a significant positive correlation with litter depth, litter cover, canopy cover and log density. The quality of these factors in terms of density contribute to soil calcium thus influencing distribution together with other factors not established in this study that could also be influencing the distribution for instance predators and competition for food. The significant positive correlation between the environmental factors studied also indicates that a combination rather than one factor influence the distribution of *G. taitensis*.

6.2 Recommendations

- 1) More research on the genetics of the genus need to be studied in order to get data to establish whether there are sub-species and hybrids within *G. taitensis*.
- 2) Molecular characterization of other snails need to be studied to establish close relatives of *G. taitensis*, as well as establish more specific primers for this genus.
- 3) Effect of fragmentation on the observed varied banding patterns need to be studied.
- 4) The indigenous remnants of Taita hills need urgent conservation plan that will balance between the human population pressure and conservation of the endemic and endangered species that exist only in this forest in the whole world.

5) Because of their importance, the Taita Hills urgently need to be conserved, and restoration programs are needed for the existing forest gaps that should be replanted with indigenous trees. Interventions will need to address local threats to biodiversity, but new policies and economic interventions will also be necessary. Enlightening the community on the need to conserve the forest and involving them in creating corridors to join the remaining fragment is vital in promoting biodiversity conservation. One approach that will be necessary to address the threats posed by poor local people will be to raise their income levels, in order to alleviate poverty and to reduce the need for the current destruction of natural resource practices.

CHAPTER SEVEN

7.0 REFERENCE

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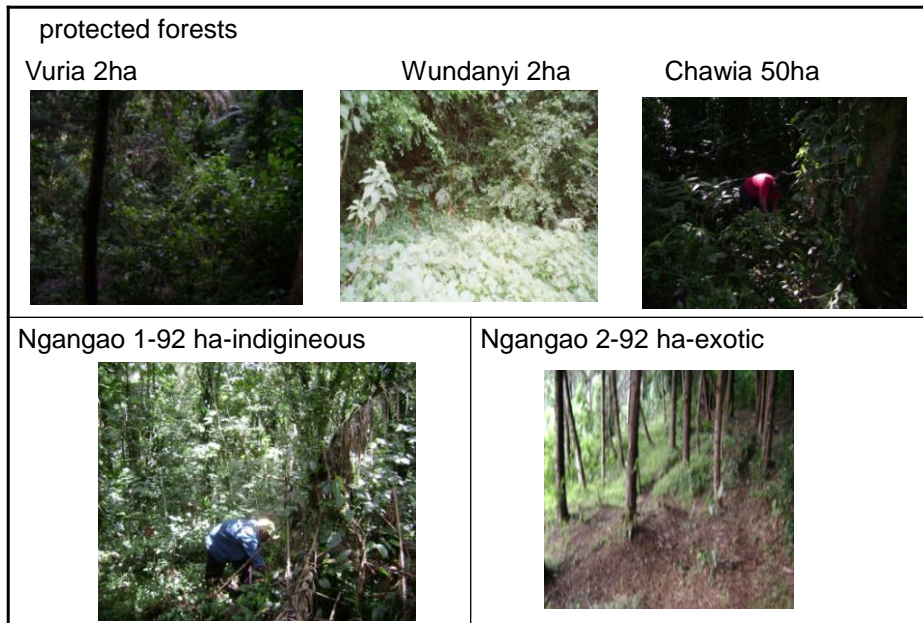
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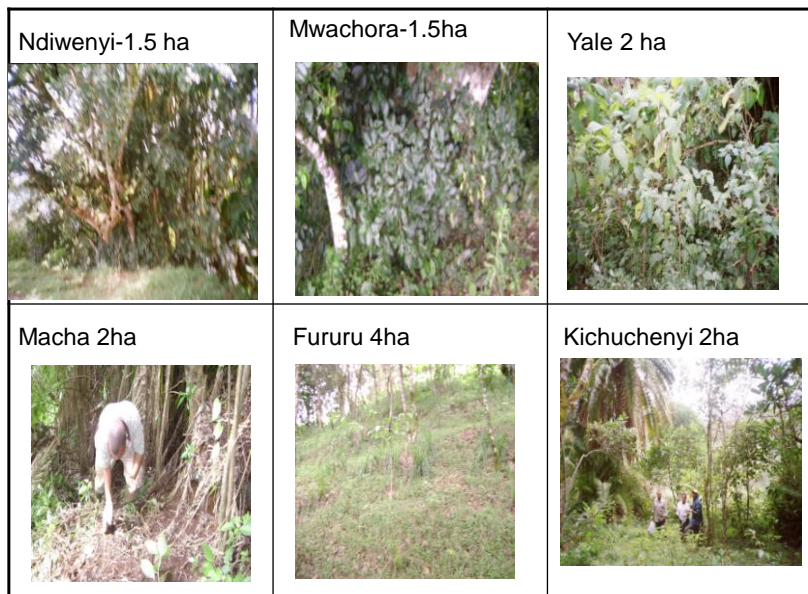
CHAPTER EIGHT**8.0 Appendices****Appendix 1: *Gulella taitensis* photo**

The first diagram shows a *Gulella taitensis* shell taken on a lab bench after it has been cleaned while the second one was the first sample collected in Chawia forest during the sampling. The snail has teeth like structures that make it easier for it to be recognized in the field.

Appendix 2: Photos showing the status of the four protected forest fragments surveyed



The protected forests have a good interlocking forest cover except where exotic trees were introduced in Ngangao 2 which has no forest cover thus having high number of dead snails and no live snails.

Appendix 3- Photos showing the status of the six unprotected fragments.

The fragments have little or no forest and litter cover and human activity including grazing, tree cutting and soil erosion were evident in these fragments. These fragments had few or no log densities and low tree densities

Appendix 4: Table showing mean raw data for the environmental factors recorded in these forest fragments

Fragment	Calcium	pH	Electrical conductivity	Litter depth	Litter cover	Canopy cover	Log density	Tree density	Live snails	Dead snails
Chawia	3.73	4.93	0.60	11.00	70.00	85.00	5.50	78.25	2	1
Vuria	5.98	4.71	0.54	6.88	85.00	85.00	11.00	56.00	7	1
Wundanyi	5.05	5.24	0.37	6.88	85.00	70.00	1.00	59.50	14	3
Ngangao	2.53	4.85	0.37	6.88	65.00	70.00	10.50	100.75	5	3
Average	4.32	4.93	0.47	7.91	76.25	77.50	7.00	73.63	7	2
Macha	2.63	4.41	0.57	3.75	45.00	40.00	1.25	31.25	8	5
Yale	2.03	3.99	0.46	4.00	50.00	50.00	3.00	59.75	4	6
Ndiwenyi	1.83	4.46	0.53	3.75	55.00	60.00	0.75	38.50	3	8
Fururu	2.80	4.38	0.56	2.25	35.00	50.00	2.00	49.25	1	0
Kichucheni	2.48	5.47	1.26	3.63	45.00	35.00	0.00	15.50	3	7
Mwachora	2.89	4.81	0.44	4.25	35.00	40.00	0.25	25.00	6	13
average	2.44	4.59	0.63	3.61	44.17	45.83	1.21	36.54	4	6

Appendix 5: A table showing the raw data collected from all the sampling plots

forest 1	site	calcium	pH	EC	litter depth	%litter cover	%canopy cover	log density	tree density	H.L.S.E	no of live snails	no of dead snails
macha	block 1	3.8	4	0.6	2	2	2	0	38	1	8	4
	block 2	2.6	5.28	0.6	1	2	1	0	15	1	3	5
	block 3	2.2	4.12	0.55	2	2	3	2	30	1	9	6
	block 4	1.9	4.25	0.51	1	3	2	3	42	1	12	6
Forest 2	block 1	3.8	4	0.23	3	4	4	8	54	2	6	0
Vuria	block 2	6.7	5.07	0.65	2	4	4	12	49	2	4	1
	block 3	6.5	4.52	0.55	3	5	5	9	63	2	10	2
	block 4	6.9	5.23	0.72	3	4	4	15	58	2	7	0
Forest 3	block 1	5.7	5.79	0.45	2	2	3	0	41	2	14	3
Wundanyi	block 2	3.4	4.41	0.45	3	3	4	0	58	2	15	4
	block 3	4.8	4.75	0.2	3	3	4	3	79	2	18	1
	block 4	6.3	6	0.36	3	2	3	1	60	2	9	4
Forest 4	block 1	3.8	5.54	0.55	2	2	2	0	18	1	2	6
kichuchenyi	block 2	2.2	5.57	0.24	3	2	2	0	21	1	3	7
	block 3	2	5.67	4	1	2	1	0	8	1	2	7
	block 4	1.9	5.11	0.23	2	3	2	0	15	1	3	7
Forest 5	block 1	3.2	4.56	0.3	3	4	4	12	116	2	10	2
ngangao	block 2	2.6	6.16	0.4	2	3	4	8	82	2	4	0
	block 3	2	4.67	0.4	3	3	3	12	108	2	7	2
	block 4	2.3	4	0.37	3	3	3	10	97	2	0	6
Forest 6	block 1	1.2	4.13	0.16	2	2	3	4	52	1	2	7
yale	block 2	1.2	4	0.27	2	2	2	0	41	1	2	6
	block 3	2.8	3.92	0.7	1	3	2	2	68	1	4	5
	block 4	2.92	3.89	0.7	3	3	3	6	78	1	7	4
Forest 7	block 1	3.2	3.54	0.5	4	3	3	2	38	1	2	6
ndiwenyi	block 2	1.2	4.47	0.55	5	3	2	0	43	1	2	9
	block 3	2	4.49	0.5	4	3	3	0	32	1	5	9
	block 4	0.9	5.35	0.57	2	2	4	1	41	1	3	7
forest 8	block 1	3.4	5.1	0.55	3	2	3	2	61	1	1	0
fururu	block 2	2.6	3.41	0.3	2	2	2	3	42	1	0	0
	block 3	2.8	4.56	0.7	1	1	2	1	50	1	1	0
	block 4	2.4	4.44	0.69	3	2	3	2	44	1	0	1
Forest 9	block 1	4.6	3.61	0.5	4	3	4	5	43	2	2	0

chawia	block 2	3.2	3.91	0.6	5	3	4	2	52	2	0	1
	block 3	3.2	5.71	0.6	5	4	4	6	98	2	2	0
	block 4	3.9	6.5	0.69	4	4	5	9	120	2	2	1
forest 10	block 1	2.6	4.57	0.4	2	2	2	0	16	1	7	12
mwachora	block 2	4	4.84	0.6	2	2	2	0	27	1	7	16
	block 3	3	4.78	0.26	1	1	1	1	21	1	3	8
	block 4	1.95	5.03	0.49	3	2	3	0	36	1	5	14

H.L.S. E. – represents human livestock and soil erosion and 1 represents presence and 2 absence and this was evident in all the unprotected fragments.