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Analysis of utilization of food resources by the African wood mouse *Hylomyscus denniae endorobae* (Rodentia: Muridae) from Ihururu Forest, Kenya

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ABSTRACT

Hylomyscus denniae endorobae **is a rodent important in ecosystems as predator, prey, seed disperser, plant pollinator, determinant of forest tree growth and structure as well as a contributor to biodiversity which subsequently plays a role in natural livelihood and national development. Fragmentation of tropical rain forest by humans continues to pose a serious threat to wildlife such as rodents, subjecting them to temperature fluctuations, starvation, and exposure to predators. Rapid habitat degradation and loss cause mass extinctions and sudden drops in biodiversity, resulting in the collapse of ecosystems leading to the spread of zoonotic diseases, food, water and wood fuel shortages. With habitat destruction, *H. d.endorobae* will get to human habitat, destroy stored seed crops and transmit diseases. Efficient utilization of food resources and ability to adapt to new food items as original food resources dwindle minimizes dependence of rodents on unpredictable seed crops and leaves of forest trees which are at a risk of extinction due to anthropogenic activities. The purpose of the study was to analyze utilization of food resources by *H. d. endorobae* to provide information for making informed decisions on conservation of the species. Diet comprised wheat, kale and a mixture of wheat, kale and locust (omnivore diet). Thirty male rodents weighing between 35- 50g from Ihururu Forest Nyeri County, Kenya (Appendix 1) dissected them, removed the gut, did morphometric measurements to obtain the average total gut length (TGL) and histological examinations to obtain the number and length of villi in different regions of the gut. I grouped nine other male rodents into three, caged individually and fed them on different diets. After six months, I dissected them, removed the gut and took morphometric measurements to obtain the average TGL. I did histological measurements to obtain the number and length of villi in different regions of the gut. Rodents fed on wheat diet had rugged and more blunted large intestinal and cecal villi compared to those fed on kale and omnivore diets. There was significant increase in the number of villi in the duodenum and caecum of rodents fed on wheat and in the colon of those fed on kale. Also, there was significant decrease ($p < 0.05$) in length of villi in all regions of the gut except in the caecum of rodents fed on omnivore diet. Natural habitats of *Hylomyscus denniae endorobae* should be conserved to prevent changes in gut morphology, which leads to changes in energetics and eventual erosion of the species.**

Key words: Diet, gut morphology, *Hylomyscus denniae endorobae*.

INTRODUCTION

Hylomyscus denniae endorobae is a rodent of family *Muridae* that inhabits tropical and sub-tropical moist mountain forests. Rodents are prey, predators, plant pollinators, determinants of tree plant structure among other roles. *H. d. endorobae* like other *murides* has a high mass specific metabolic rate making it susceptible to changes in nutrient and energy availability. Increased food intake to compensate for low- quality high fiber food is not always sufficient to maintain the energy balance since the quality of food changes with season. Small animals may use adaptations to maximize the amount of energy and nutrients from their low quality diets if their numbers are to remain stable. Phenotypic flexibility may occur in the gut due to change of food quality. Anthropogenic disturbances affect most forest ecosystems through loss of original habitats, reduction in habitat batch size and increasing isolation of habitat batches, which in turn affects nutrient resource availability to rodents. It is important to investigate how *H. d. endorobae* adapts to different food resources when natural diets dwindle in order to emphasize the need to maintain and conserve natural habitats to prevent erosion of the species. The rodents may also migrate to human habitats where they can transmit disease, destroy stored seed grains or die leading to a decrease in their population.

MATERIALS AND METHODS

Trapping and treatment of experimental animals

The rodents were trapped using Sherman live trap (Sherman, 2009) measuring 25 x 14x 8cm, during the month of June, 2011. Labeled traps were baited with about 5g of peanut butter on oat. Traps were set in the evening at 6.00 pm and opened at 6.00 am of each collection day. The animals were identified, placed in a ziploc bag, weighed on a Pesola spring balance (PESOLA, Switzerland) to the nearest 0.1g, sexed by

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observing the genitals and morphometric measurement taken using a graduated ruler. In total, thirty nine animals were trapped, thirty of which were used for field experiments and the remaining nine were carried to the laboratory where they were subjected to various treatments.

Thirty individuals of *Hylomyscus denniae endorobae* were euthanized using chloroform (Barry, 1998), and the entire digestive tract removed. The intact length of the gastrointestinal tract sections was determined to the nearest 0.1cm by gently straightening (without stretching) and laying flat each section of tissue along a metric ruler to obtain the total gut length (TGL). Gut sections were separated into stomach, duodenum, small intestine, caecum and large intestine. The sections were subjected to routine histological processes using varied concentrations of alcohol after which slides were made. For each intestinal tissue sample, cross sections measuring 2mm were prepared and staining done with hematoxylin and eosin. The sections were then examined under the light microscope at ×100 magnification for number and length of intestinal villi. Further, for each intestinal cross section, eight intact, well oriented crypt villus units were selected for examination. The microscopic examinations were conducted in triplicates (24 measurements for each sample section). Morphometric measurements were performed to obtain villi length. Villus length was measured from the tip to the villus-crypt junction. Villi bases were counted to determine the number of villi per millimeter of each section. The procedure was repeated twice for each section and the mean for each parameter determined.

Laboratory experiments comprised of three treatments with three replicates each. Nine male *H. d. endorobae* weighing between 35g and 50g were randomized into three groups of three individuals each and caged individually. The experimental animals were kept in an animal house at mean temperatures of 22^0C and fed on rodent pellets for eleven days to adjust prior to presentation of test foods which composed of wheat alone, kale alone and a mixture of wheat, kale and insect as a control. The animals were presented with 80g of test food that was replaced after every four days.

Morphological adaptability of the digestive tract

Experimental animals were fed for six months according to diet treatments, after which they were euthanized using chloroform (Barry, 1998), dissected as outlined above and histological examination of the gut done. Morphometric measurements were taken for each individual in the group and means obtained for the group. The experiment was replicated twice for each section.

Data analysis

SPSS (2014) analysis system was used to analyze data generated from the study. Analysis of variance (ANOVA) was used to compare the effect of diet on total gut length, and number of villi. Correlation coefficient and regression analysis were used to determine the relationship between diet and gut morphology.

RESULTS

Effect of diet on shape and number of villi

Villi in all gut segments of field collected animals had a normal slender shape with pointed tips and a thick mucosal layer (Plates 1 and 3) while villi of animals fed on wheat became rugged and more blunted, particularly those in the large intestine and caecum (Plates 2 and 4 respectively). The results showed no significant effect ($P > 0.05$) of diet on the number of villi in different regions of the gut. However, there was significant correlation ($P < 0.05$) between the number of villi in the duodenum and caecum of field collected animals and laboratory maintained animals fed on wheat, the large intestine and caecum of those fed on kale diets.

Effect of diet on total gut length

The mean total gut lengths for *Hylomyscus denniae endorobae* fed on wheat, kale and omnivore diets are shown in Figure 1. The results showed no statistically significant effect of diet on the total gut length ($p > 0.05$) between groups. However, numerical results showed a lower total gut length in animals fed on kale compared to those fed on wheat and omnivore diets while those fed on wheat and omnivore diets had numerically similar gut lengths. Also, those fed on kale and omnivore diet had statistically similar gut lengths (Figure 1).

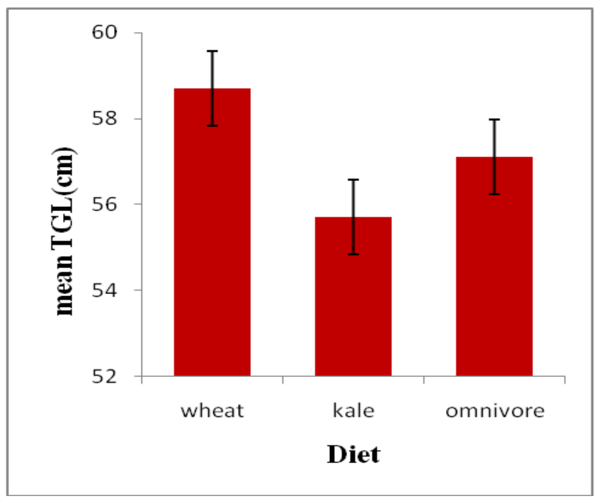

Figure 1. Mean total gut length (cm) of *Hylomyscus denniae endorobae* fed on wheat, kale and omnivore diets (n = 3 for each group).

Relationship between diet and length of villi in different regions of the gut of laboratory maintained *Hylomyscus denniae endorobae*

The results showed statistically significant correlation between diet and villi length (Fig.2) in the small intestine ($r = -0.832$, $p = 0.000$) and the large intestine ($r = -0.994$, $p = 0.000$) of animals fed on wheat, in the duodenum, small intestine, large intestine and caecum ($r = 0.289$, $p = 0.000$) of animals fed on kale and omnivore diets. Results showed significant effect of diet on the length of villi in the large intestine of animals fed on wheat ($F = 3.5308$, $p = 0.0465$), kale ($F = 16.327$, $p = 0.002$) and omnivore

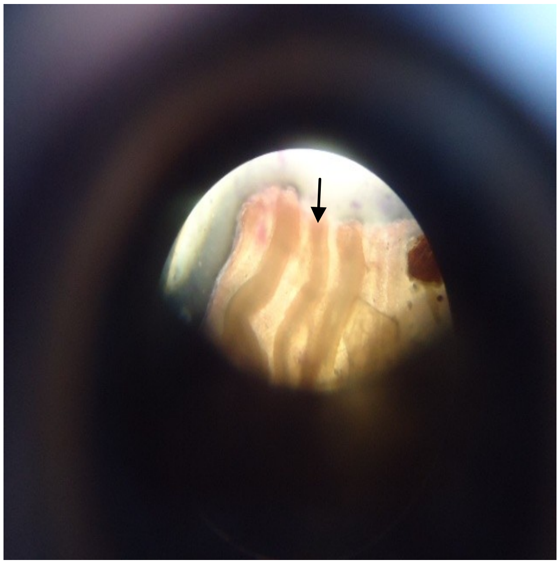

Plate 1. Large intestinal villi of field collected animals (mag x100) Arrow points at villus

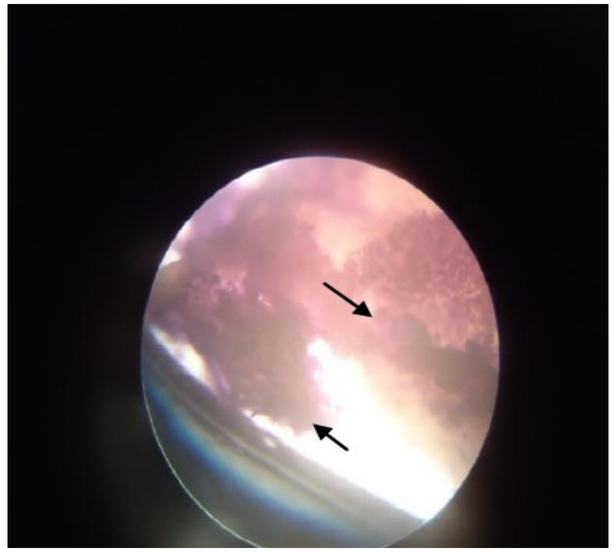

Plate 2. Large intestinal villi of animals fed on wheat (mag x100) Arrows point at villi

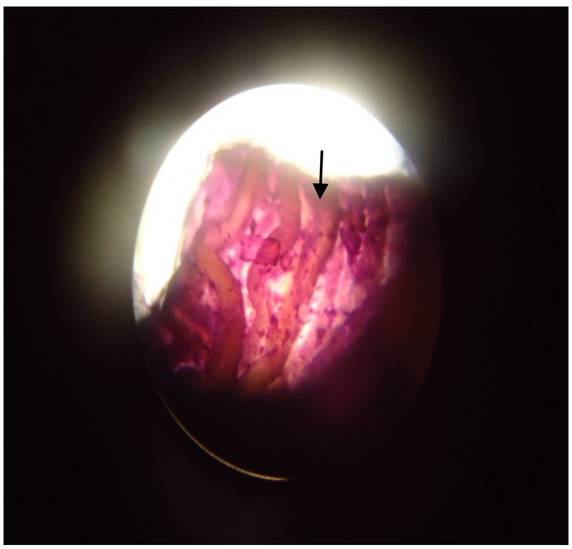

Plate 3. Cecal villi of field collected animals (mag x100) arrow points at villus

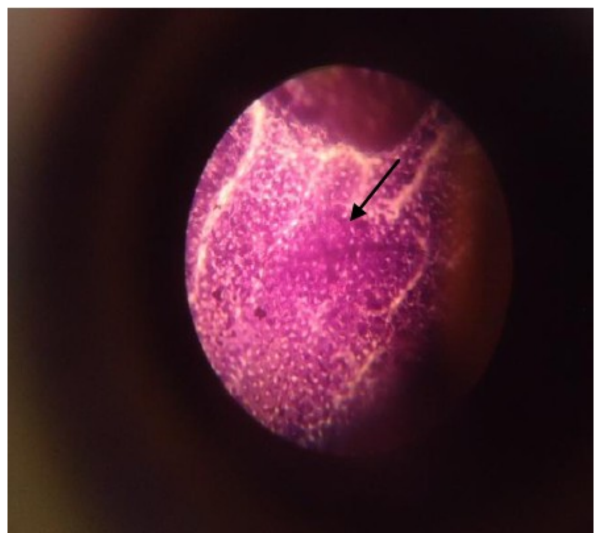

Plate 4. Cecal villi of animals fed on wheat (mag x100) Arrows point at villus

diet ($F = 4.082$, $p = 0.04918$). There was significant effect of diet on length of villi in the caecum of animals fed on kale diet ($F = 0.575$, $p = 0.0447$). This was the only region where increase in villi length occurred.

DISCUSSION

Effect of diet on shape and number of villi

The study showed that villi of wild *Hylomyscus denniae endorobae* fed on wheat protein became more blunted and broader than those fed on kale diet. This increases the surface area for absorption of nutrients. This result is in line with earlier findings that wheat protein makes rat villi to become more blunted and broader and that change is irreversible especially if the first encounter was in mature life (Dahlke *et al*., 2003). Wheat protein antigens and bacterial growth cause villi to atrophy, showing the protrusion to be more blunted and broader, with an increased surface area. If there is bacterial infection or other infections in the caecum, there may be changes of an immunological nature, a delayed immune reaction involving secretion of lg A or the production of antibodies, with the intestinal villi taking on a different morphological structure as a defense against foreign protein. Villi in the colon and caecum were shorter, broader and more rugged compared to villi in the proximal region of the gut. Intestinal villi of field collected animals were seen under the microscope as finger-like protrusions extending from the terminal web area of the cells with slightly blunted ends.

Increase in the number of villi increases the surface area for absorption, to compensate for the decrease in villi length due to diet (Dahlke *et al*., 2003). Growth of new villi being an energy demanding process would require the mice to increase their rate of food intake. If the food is not available, they would have to migrate to new habitats or die. Wild seed is rich in proteins and carbohydrates digested mainly in the foregut hence the small

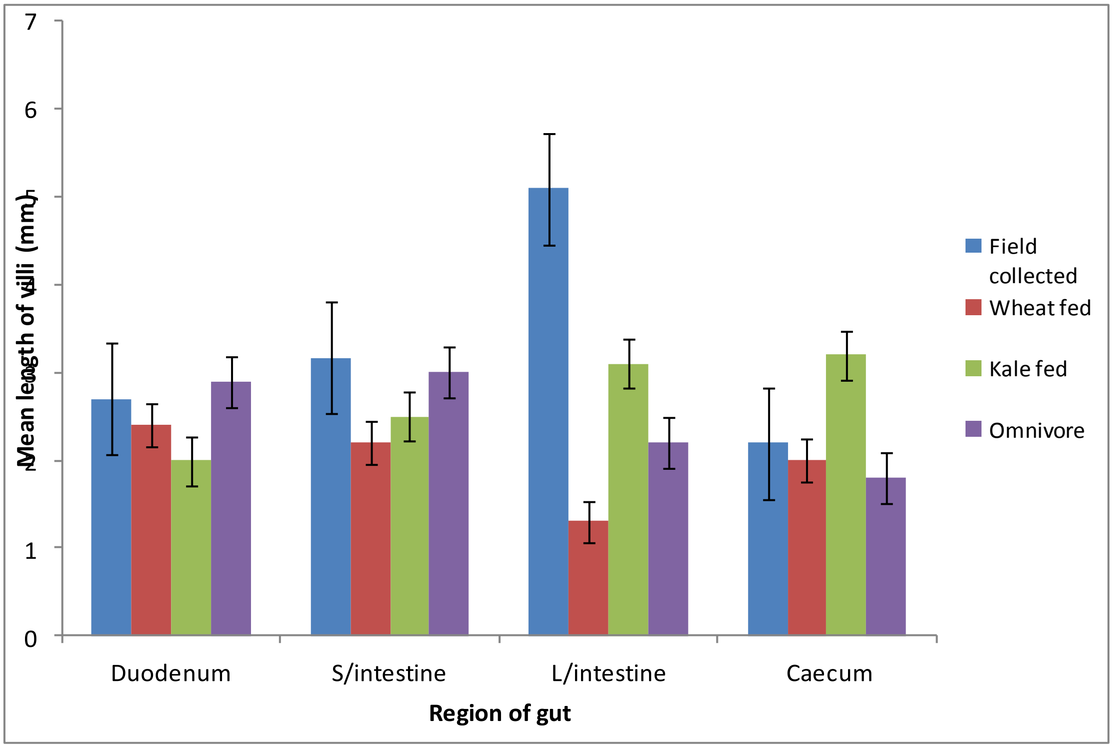

Figure 2: The mean length of villi (mm ± S.E) in different gut regions of field collected and laboratory maintained animals fed on different diets.

intestine had more villi for absorption compared to duodenum and large intestine.

The marked difference in shape and number of the distal intestinal villi could be attributed to the fact that protein does not stay as long in the proximal intestinal region as it does in the distal region, and by the time that part is reached the protein could have been broken down by the various intestinal enzymes. The effects could be due to not only the undigested protein but also to the various breakdown products, peptides and other potent chemicals resulting from digestion, some of which may be toxic and have detrimental effect on the villi (Dahlke *et al*., 2003).

Effect of diet on gut length

Animals fed on kale diet showed no significant change in gut length at colon and caecum level. This was not in line with the expected results of herbivorous rodents that have been reported to have a relatively longer colon and caecum, shorter proportion of small intestine and greater digestive tract capacity (Vorontsov, 2003). Strict herbivores such as *Microtus brandti* have longer large intestine and caecum compared to granivores and omnivores (Song and Wang, 2006). Omnivores and granivores have varied large intestine and caecum depending on the proportions of seed, vegetation and animal foods in their diets. The hindgut is more important for herbivores than omnivore and granivore rodents where it is used for food fermentation and acts as an indicator of food habits (Kerrin, 2010). *Hylomyscus denniae endorobae* is a granivore. These findings imply that *H. d. endorobae* may not survive if exposed to farm land habitats such as tea farms, where only plant leaf is available as feed. Herbivores rely upon a more continual flow of its low quality, high fiber diet throughout the anterior portion of the gut prior to retention, fermentation, and assimilation of nutrients in the caecum and colon (Kerrin, 2010). Cecal capacity is of greater importance than that of other gut sections in herbivorous rodents (Del velle and Busch, 2003). From the results on the total gut length, it appears that *H. d. endorobae* meets high energy demands by increasing feed intake and cecal villi length.

There were no clear trends in the relationship between wheat diet and mean gut length. Resistant starch and high fiber content in wheat delays intestinal transit time, enabling digestion and absorption to occur without changes in gut length. This is in line with earlier findings that omnivore rodents are more flexible than herbivore and granivore rodents (Browkoska, 1995). Increase in digestive organ size due to increase in diet fiber content has been found to occur in *Microtus branditi, Akodon azar*ae, *Microtus agrestis, Microtus pennsylvanicus and Meruxies ungulculatus* (Sagher *et al*., 1990; Derting and Bogue, 1993; Hammond and Wunder, 1993; Delvelle and Busch, 2003; Song and Wang, 2006). The study findings suggest that wheat diet has similar effects on gut length as the natural diet of *Hylomiscus denniae endorobae.* The results on gut flexibility are also in line with earlier findings that there are no differences in small intestine length between herbivores, omnivores and insectivores in wild rodents (Naya *et al.,* 2008). Wheat bran has insoluble polysaccharides which affect colonic fermentation and increase butyrate concentration. Resistant starch delays intestinal transit time and has little or only modest effects on fecal bulking. The present results imply increased feed intake and faster passage rates which limit utilization of nutrients (Wang *et al.,* 2000) which in turn lowers the performance of the species.

This study showed that *Hylomyscus deniae endorobae* fed on a mixture of wheat, kale and locust had the shortest large intestine length compared to those fed on wheat and kale. This could probably be due to the mice preferring wheat and locust to kale as was observed during the collection of food remains which had a larger proportion of kale compared to wheat and very little or no locust at all. Locusts have a higher amount of protein (10g per 20g of body weight) but very low carbohydrates compared to wheat (www. annecollins.com/sodium diet/ sodium grain-wheat.htm). Protein, lipid and soluble starch diet is normally digested in the foregut and midgut. In the natural habitat, insects are hard to find and wild seeds may be seasonally available resulting to *H. d. endorobae* consuming plant leaves. This is based on the fact that plant seeding and locust reproduction depend on climatic factors such as rainfall and sunlight which are seasonal (Nyeri meteorological report, 2011). Wheat bran and kale have non-soluble polysaccharides which are non-fermentable and cause an increase in colon and caecum dimensions. Their fermentation produces fatty acids which cause an increase in daily epithelial cell production 3-4 fold in rats (Wong *et al*., 2006). This was not observed probably due to high protein content (20.4 per 80g) in the omnivorous diet.

Influence of diet on length of villi in various regions of the gut

There was significant correlation between diet and length of villi in all regions of the gut. Diet caused a decrease in villi length with wheat diet causing the greatest decrease of 3.88mm in villi of the large intestine. However, unlike wheat and omnivore diets kale diet caused an increase of 1.0mm in cecal villi. This contrasts earlier findings that non-soluble polysaccharides in wheat generate short chain fatty acids during fermentation process which increases the daily epithelial cell production three to four fold in the intestine (colon-jejunum) of rats and that saturated fat diet or cholesterol enriched diets increase villi length in wild mice (Wong *et al*., 2006). Also, the results do not agree with the report that animals feeding on high fiber diet have longer small intestinal villi than those consuming low fiber diets in order to increase surface area for maximum absorption of nutrients (Kelly *et al*., 2012).

Wheat protein reduces height of villi in rats probably due to a first encounter phenomenon and might have immunological as well as morphological effects (Gu and Li, 2004). Probably wheat had low crude amino acids, the major macronutrients for the development of the morphological features of the villi and epithelial cells. Low protein, high fiber diet caused a reduction in mean villi length. An increase in villi length, epithelial thickness and crypt depth improves gut morphology by increasing the surface area for absorption (Hedemann *et al*., 2006; *Wikipedia,* 2009). A decrease in villi length reduces the surface area for absorption of nutrients in mice. This can lower utilization efficiency, leading to low reproductive efficiency thereby minimizing survival of the animals.

CONCLUSION

This study concludes that

i) Diet has no influence on total gut length (TGL) in *H. d. endorobae*. There was no change in total gut length for laboratory animals. The total gut length remained almost the same as that of field collected animals. This implies that the species may not cope with changes in food resource availability in fragmented natural habitats.

ii) Diet influences the number and length of villi. Wheat, kale and insect diets used in the study caused an increase in the number of villi in all gut regions and a decrease in villi lengths in all gut regions except for cecal villi length of animals fed on kale diet which increased. This increases energy demand of *H. d. endorobae* hence they may not survive in a habitat with limited food resources.

RECOMMENDATIONS

Wildlife conservationists such as the National Museums of Kenya and the Kenya Wildlife Services should formulate a policy to ban human settlement and small scale farming, logging and charcoal burning in Ihururu Forest. Protection of natural habitats of *Hylomscus denniae endorobae* will enable survival of the species. This will prevent species erosion, increase species diversity and improve ecosystem productivity.

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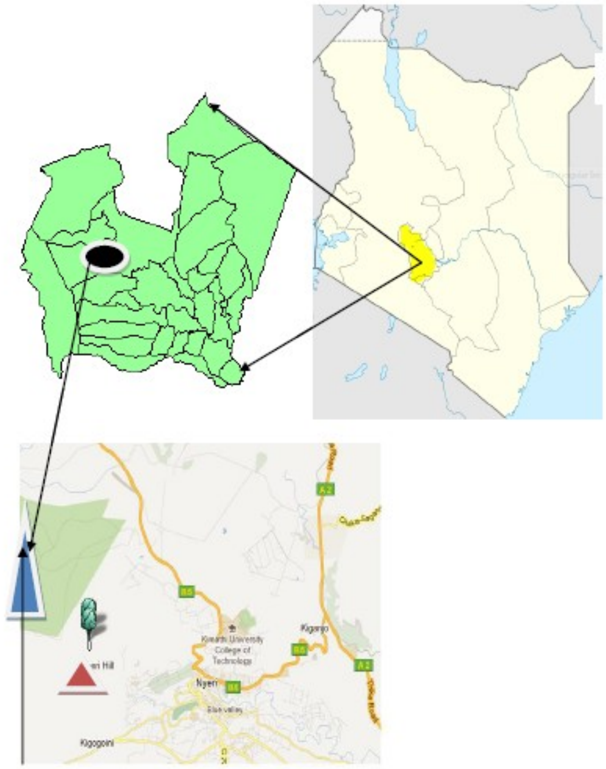

Appendix I. Map of Kenya showing location of Nyeri County (Adopted from Nyeri, Physical planning, 2011).

Appendix II. Photograph showing *Hylomyscus denniae endorobae*