POPULATION ECOLOGY OF *Beamys hindei* AND *Rhynchocyon petersi* IN SELECTED COASTAL FORESTS OF TANZANIA

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EXTENDED ABSTRACT

This thesis is prepared according to "Publishable manuscripts" format of the Sokoine University of Agriculture. The study on the population ecology *vis-à-vis*: distribution, population dynamics, space use and behavior, genetic diversity and structure of *Beamys hindei* and *Rhynchocyon petersi* was conducted from 2010 to 2014 in selected coastal forests (Kwamsisi, Gendagenda, Madete, Mbulizaga, Askari and Zaraninge) within and around Saadani National Park, Tanzania. Distribution of *B. hindei* was assessed using Sherman traps while for *R. petersi* camera traps were used. Capture mark recapture was used for population dynamics of *B. hindei* in Zaraninge forest. Radio telemetry together with camera traps was used to determine home range and activity patterns for *R. petersi* in Zaraninge forest. Standard genetic methods were used for development of microsatellites and determining genetic diversity and structure of *R. petersi* in the Zaraninge, Mbulizaga, Askari and Gendagenda. Results on distribution indicate *R. petersi* was found in all forests while *B. hindei* was found in some forest and absent in some other forests. Population fluctuation, demographic patterns of *B. hindei* in Zaraninge forest, showed a mean survival rate of 0.72 (range 0.65-078) with a recruitment of 1 animal/28 days and a litter size of 2.8 indicating a low abundance with stable population. The average home range of *R. petersi* from radio telemetry was 2.6 ha. Activity pattern differed between seasons with more activity observed on dry season (F =9.75, df 1, 28, p = 0.004. Eight polymorphic microsatellites using next generation sequencing technology based on eighteen *R. petersi* were genotyped. Developed eight microsatellites were used to determine diversity and structure of *R. petersi*. Results indicate extant *R. petersi* have descended from a single population of high effective size (Ne) with no forest-distinctive signal. In contrast, *B. hindei* was more genetically structured. It recommends further investigation if the ‘population robustness’ of *R. petersi* in the fragmented coastal forest applies to other parts of its range, notably in the East Arc Mountain forests. Conservation status of *R. petersi* should remain as vulnerable while *B. hindei* should be reviewed to vulnerable. Conservation program are required to safe guard these species

DECLARATION

I. Christopher Andrew Sabuni do hereby declare to the Senate of Sokoine University of Agriculture that, this thesis is my own original work and that it has neither been submitted nor concurrently submitted for a degree award in any other institution.

29/09/2016

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CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Coastal Forests

Eastern African coastal forests are located within the Swahili regional centre of endemism and Swahili-Maputaland regional transition zone in eastern Africa, between 1°North and 25°South, and 34°- 41° East (Burgess *et al.*,1998). It is part of the Zanzibar Inhambane coastal forest mosaic first defined by White (1976) as a distinct area of plant endemism (35% of the larger woody plants were found to be endemic).

The coastal forest strip stretches from Southern Somalia through Kenya and Tanzania, to Southern Mozambique (Fig.1) (CEPF, 2005). These forests are generally found inland from the coast of the Indian Ocean about 200- 300 km (Moll and White, 1978). Different types of vegetation are present throughout this belt, lying within a mosaic of savanna, grassland habitats and wetland areas.

The typical vegetation formation type is the Eastern African Coastal dry forests, which according to White (1983) are described as a habitat of semi evergreen or evergreen undifferentiated dry forest. Variants of these forests that share features are treated as transitional formation and are known as the Eastern African Scrub forest and the Eastern African Coastal *Brachystegia* Forests (Clarke *et al.*, 2000). In addition, the forest is composed of a high heterogeneity of flora like herbs, grasses, shrubs (Clarke, 2000; Clarke and Robertson, 2000; Lowe and Clarke, 2000). The Coastal forests are particularly dependent on rainfall (average of 900-1400 mm/year) with two rainy seasons (Clarke, 2000). Other complex interactions play a major role in species composition of these forests such as rock types, landscape topography, soil composition and hydrology (Lowe and Clarke, 2000; Clarke and Burgess 2000; Burgess *et al.*, 2000 a).

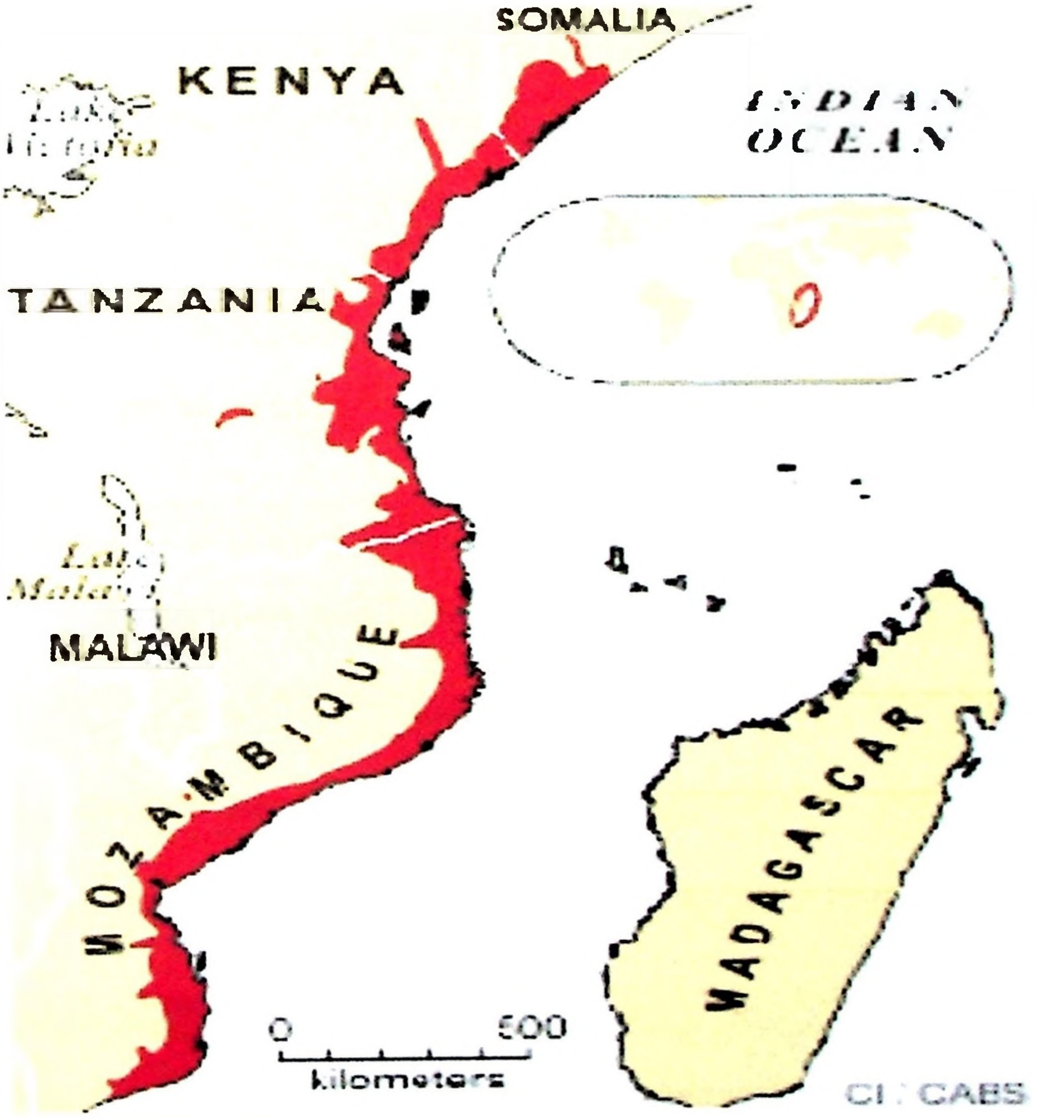

Figure 1.1: Eastern African Coastal Forest. Red strip indicates the delimination of the Coastal forests (CEPF, 2005).

Globally, Coastal forests are regarded as one of the 25 biodiversity hot spots and center of endemism for a number of species. For example, the endemic species cited in Burgess *et al.* (1998) include plants (550), mammals (6), birds (9), frogs (2), butterflies (79), snails (>86) and millipedes (>20).

In Tanzania there are 66 coastal forest patches covering an area of about 700 km^2 found at elevation between 0-500 m and even up to 1100 m above sea level (Burgess *et al.*, 2000 b). Most of coastal forest endemics have a narrow distributional range, often exhibiting single site endemism or with scattered or disjunct distribution patterns (Bloesch andKlötzli, 2002). The patches are interpreted as a 'vanishing refuge' with the endemic species gradually becoming more and more relict (and presumably extinct) due historically to climatic desiccation and more to anthropogenic activities (Burgess *et al.*, 1998). The species under this study the Black and rufous sengi, *Rhynchocyon petersi* and lesser pouched rat, *Beamys hindei* (Appendix 1 and 2) are among the endemic small mammals found in these forests.

1.2 General Information on *Rhynchocyon petersi*

The Black and Rufous sengi (elephant-shrew) *Rhynchocyon petersi* Bocage, 1880, is one of the four known giant sengis from the subfamily Rhynchocyoninae which belong to the supercohort Afrotheria grouping together aardvark (Orycteropodidae), tenrecs (tenrecidae), golden-moles (Chrysochloridae), elephants (Elephantidae), hyraxes (Procaviidae) and sea-cows (Dugongidae) (Springer *et al.*, 1997; Dumbacher *etal.*, 2012). The four giant sengi *Rhynchocyon* species includes; *R. petersi*, *R.cirnei*, *R. chrysopygus* and *R. udzungwensis* all are considered to be threatened due to habitat loss. The study species are endemic to East Africa, distributed in the Eastern

Arc Mountains and coastal forests of Tanzania and Kenya (Burgess *et al.*, 2000 b; Rathbun andButynski, 2008).

Studies on *R. petersi* have been limited to presence and absence and little is known on its ecology in these forests (Corbet and Hanks, 1968; Coster and Ribble, 2005). Its life is considered to share similar characteristics to other giant sengis that; they are all diurnal, feed on forest floor litter using their long nose as probe to search for prey such as beetle, ants and other invertebrates, while their long tongue is used to flick food items into their mouth (Rathbun, 1979). On sheltering, all species build and use leaf nests on the floor. They are found in altitudes ranging from sea level to about 2300 m (Coster and Ribble, 2005).

A detailed study on the ecology of sengis has been carried out only on *R. chrysopygus*, a species which is restricted only in the coastal forest of Kenya (Rathbun, 1979). The conservation status of the four giant sengis is of interest in terms of protecting Africa's unique biodiversity. However, various factors contributing to loss of habitat such as agriculture, logging etc., have been pointed out to be a threat to their survival (Hanna and Anderson, 1994; Nicoll and Rathbun, 1990). *Rhynchocyon petersi* is categorized as vulnerable (IUCN, 2015).

1.3 General Information on *Beamys hindei*

The lesser pouched rat *Beamys hindei* Thomas, 1909, is related to other two cricetid rodents, the giant pouched rat (*Cricetomys* spp) and the pouched mouse (*Saccostomus* spp) all belong to Nesomyidae family under sub-family

Cricetomyinae. The three species share similar characteristics as nocturnal, possess large cheek pouches and dig burrows in which they store food and eat at leisure (Kingdon, 1997). *Beamys hindei* is known to occur from the coastal forest of southeastern Kenya, throughout eastern and southern highlands of Tanzania and west to Udzungwa Mountains (FitzGibbon *et al.*, 1995) and south to Southern Malawi (Ansell and Dowsett, 1988) and west to northern Zambia (Ansell, 1978).

Only one detailed study on the ecology of *B. hindei* on distribution, population dynamics and habitat use has been done in Arabuko-Sokoke forest on the coast of Kenya (FitzGibbon *et al.*, 1995). The species feeds on seeds and fruits, inhabiting mainly forests and moist woodland, restricted to sand soils where they build complex large burrows (Hanney and Morris, 1962; Kingdon, 1997). These burrows are thought to provide protection from predators, reduce daily season variation in ambient temperatures, and as store for cached food (Ellison, 1993). The species has been recorded to exist in patchy distribution which contributes to it being considered as a rare species (Kingdon, 1974) which is in conformity to its status as "vulnerable" species (IUCN, 1990).

Knowledge on the distribution of lesser pouched rats has increased in recent years as a result of wider surveys on small mammals, in particular rodents, in different biotopes (Christensen, 1987; Clarke and Dickson, 1995; FitzGibbon *et al.*, 1995; 2005; Stanley *et al.*, 1998; Stanley *et al.*, 2005; Kiwia, 2006; Stanley *et al.*, 2007). Currently *B. hindei* is categorised with IUCN as "least concerned" (Howell *et al*, 2008). However, in these surveys, the lesser pouched rats were always found in low densities. Despite the knowledge on distribution little is known about the ecology of *B. hindei* in Tanzania.

1.4 Justification

Coastal forests within and around Saadani National Park (SANAPA) exists as disjointed patches varying in sizes, community structure and species composition (Bloesch and Klötzli, 2004). The most available studies of fauna in these forests have been limited mostly on the presence and absence or the distribution of the species including the study species (Burgess *et al.*, 2000 a; Kiwia, 2006; Sabuni, 2008), and hence there is no complete study on the ecology of *Rhynchocyon petersi* and *Beamys hindei* in the coastal forests of Tanzania.

Conservation of the species needs not only the inventory of the species but also how individual species exists and interacts in that particular environment. Therefore to understand how individual species exists in these forests, two small mammal species *Rhynchocyon petersi* and *Beamys hindei* were selected to investigate detailed aspects of their ecology.

The two species were selected in this study due to the fact that: (1) The species though found elsewhere (FitzGibbon and Rathbun, 1994; FitzGibbon *et al.*, 1995) both are relatively confined to coastal forests. (2) As non flying small mammals, they are limited in their long distance movements and can be expected to be affected by the quality of matrix of that they need to cross between patches. (3) Both species belong to different trophic levels (*R. petersi* is insectivorous where as *B. hindei* is granivorous and frugivorous) which increases the generality of observations. (4) *Rhynchocyon petersi* is categorized as "vulnerable" (IUCN, 2014) while *B. hindei* is categorized as "least concerned" (IUCN, 2014). However, the population trend is decreasing (Howell *et al.*, 2008) which call for determining the status of these species in these forests. It is therefore anticipated that the ecological data generated from this study will give insight in understanding the current status on how these species exist in their environments. In addition these data will be used as baseline for similar studies in other habitats, and for developing mitigation measures on the conservation of the study species.

1.5 Objectives

1.5.1 Overall objective

The general objective of the study is to assess the status of *Rhynchocyon petersi* and *Beamys hindei* in selected coastal forests in and around Saadani National Park.

1.5.2 Specific objectives

The specific objectives of the present study are to:

1. Determine distribution and habitat preference of study species in coastal forest.
2. Estimate the population density and survival of study species in coastal forests in relation to patch size and quality.
3. Study ecological behaviour related to space use and movements of the study species in the coastal forests.
4. Investigate genetic diversity of the study species in the coastal forest.

1.5.3 Hypothesis

1.5.3.1 Null hypothesis

Population status of the study species is indepent on coastal forest characteristics.

1.5.3.2 Alternative hypothesis

Population status of study species is dependent on coastal forest characteristics

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CHAPTER TWO

2.0 PAPER 1: Distribution and ecology of lesser pouched rat, Beamys hindei, in Tanzanian coastal forests

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Abstract

The lesser pouched rat, *Beamys hindei*, is a small rodent that is patchily distributed in the Eastern Arc Mountains and coastal forests in East Africa. The ecology of this species and its current distribution in coastal forests is not well known. Therefore, we conducted a study in selected coastal forests to assess the current distribution of the species and to investigate the population ecology in terms of abundance fluctuations and demographic patterns. Assessments of the species distribution were conducted in 5 forests through trapping with Sherman live traps. Data on ecology were obtained from monthly capture–mark–recapture studies conducted for 5 consecutive nights per month in two 1 ha grids set in Zaraninge Forest over a 2-year period. The results indicate the presence of *B. hindei* in 3 forests where it was not previously recorded. The population abundance estimates ranged from 1 to 40 animals per month, with high numbers recorded during rainy seasons. Reproduction patterns and sex ratios did not differ between months. Survival estimates were not influenced by season, and recruitment was low, with growth rate estimates of 1 animal per month. These estimates suggest a stable population of *B. hindei* in Zaraninge Forest. Further studies are recommended to establish the home range, diet and burrowing behavior of the species in coastal forests in East Africa.

Key words: abundance, *Beamys hindei*, coastal forests, recruitment, survival.

INTRODUCTION

Tanzanian coastal forests form part of the eastern Africa coastal forests and are synonymous with the forests of White's (1983) Zanzibar-Inhambane regional mosaic, stretching as a strip from Southern Somalia through Kenya and Tanzania to Southern Mozambique (Clarke 2000). Most of the forests are small and highly fragmented, consisting of many (over 250) separate forest patches, varying in shape and structure, with their sizes ranging from 1 to 50 km2 (Burgess *et al.* 2000; Bloesch & Klötzl 2004). Most of these forests are isolated from each other. Isolation can vary from less than 1 km, to several tens of kilometers, with the vegetation matrix between forests being a mixture composed of farmland, savannah woodland and thickets (Burgess *et al.* 2000).

The forests are generally described as dry coastal forests placed on well-drained soil and the dominant tree species include those from the genera *Scorodophloeus*, *Cynometra*, *Julbernardia*, *Hymnaea*, *Berlinia*, *Albizia*, *Millettia* and *Newtonia*. The forests are found at elevations between 0 and 1000 m, with many of them situated between 100 and 300 m above sea level. Coastal forests are generally complex in their formation and are influenced by various factors, including rock types, landscape topography, soil composition and hydrology (Burgess *et al.* 2000; Clarke 2000;

Clarke & Burgess 2000; Lowe & Clarke 2000). Despite their variable sizes, coastal forests are of global conservation importance because they support a very diverse flora and fauna, of which some species are endemic or near endemic (Rodgers 2000), including the current study species, the lesser pouched rat, *Beamys hindei* (Thomas, 1909).

Beamys hindei is a a small rodent in the subfamily Cricetomyinae together with 2 other genera *Cricetomys* and *Saccostomus*. It is known from the coast of Kenya and Tanzania, and inland from southwestern Tanzania, Malawi and northeastern Zambia (Ansell & Ansell 1973). The species weight ranges from 55 to 150 g, with head and body length ranging between 87 and 130 mm and tail length between 100 and 155 mm (Kingdon 1974). Coloration is grayish or grayish brown above, and white below. It is distinguished externally by the naked, white-tipped tail of medium length. The underside of the tail is dark on the proximal quarter and whitish for the rest of its length. The tail is not noticeably scaly, but only the basal centimeter is hairy. Like the other 2 genera *Cricetomys* and *Saccostomus*, *Beamys* has cheek pouches.

*Beamys hindei*have been found to occur in the forest and moist woodland from sea level to 2100 m. However, FitzGibbon *et al.* (1995) found *B.hindei*to be widespread along the coast and to be common in some localities, although they were unable to find it at many potential coastal and inland sites. The patchy distribution of the lesser pouched rats meant that it was considered a rare species (Kingdon 1974), resulting in it being categorized as "Vulnerable" (IUCN 1990). However, knowledge on the distribution of *B. hindei*has increased as a result of wider surveys on small mammals, in particular rodents, in different biotopes (Christensen 1987; Clarke & Dickson 1995; FitzGibbon *et al.* 1995; Stanley *et al.* 1998, 2005, 2007; Fadda *et al.* 2001; Kiwia 2006; Sabuni 2008); this has resulted in the species being recategorized as of "Least Concern (LC)" in *IUCN Red List* following assessment by Howel *et al.* (2008).

Apart from its distribution records, little is known about the ecology of *B. hindei* in Tanzanian coastal forests. Previous records on the ecology of *B. hindei* in the coastal forests are limited to the study by FitzGibbon *et al.* (1995) in Arabuko Sokoke Kenya. In that study, factors contributing to the distribution and population dynamics of *B. hindei* were studied.

Therefore, in this study we examined: (a) the current status on distribution (presence and absence) of *B. hindei* in selected Tanzanian coastal forests where there are no previous records of *B. hindei*; and (b) the ecology of *B. hindei*, taking into consideration the abundance, reproduction pattern, recruitment and survival rates of the species. We hypothesized that *B.hindei* are distributed in all forests assed, sex abundance and sex ratio would be higher in favor of males and that survival would be higher for females than males.

MATERIALS AND METHODS

Study area

The major component of this study on the ecology of *B. hindei* was carried out from August 2010 to July 2012 in Zaraninge, a dry evergreen coastal forest along the eastern coast of Tanzania. The forest is situated at 6°04′–6°13′S and 38°35′–38°42′E, 15 km inland from the Indian Ocean on the South West of Saadani National Park (SANAPA) (Fig. 1). The forest is within the Saadani National Park and occupies an area of approximately 20 km2 (Blosch & Klötzli 2004; Kiwia 2006).

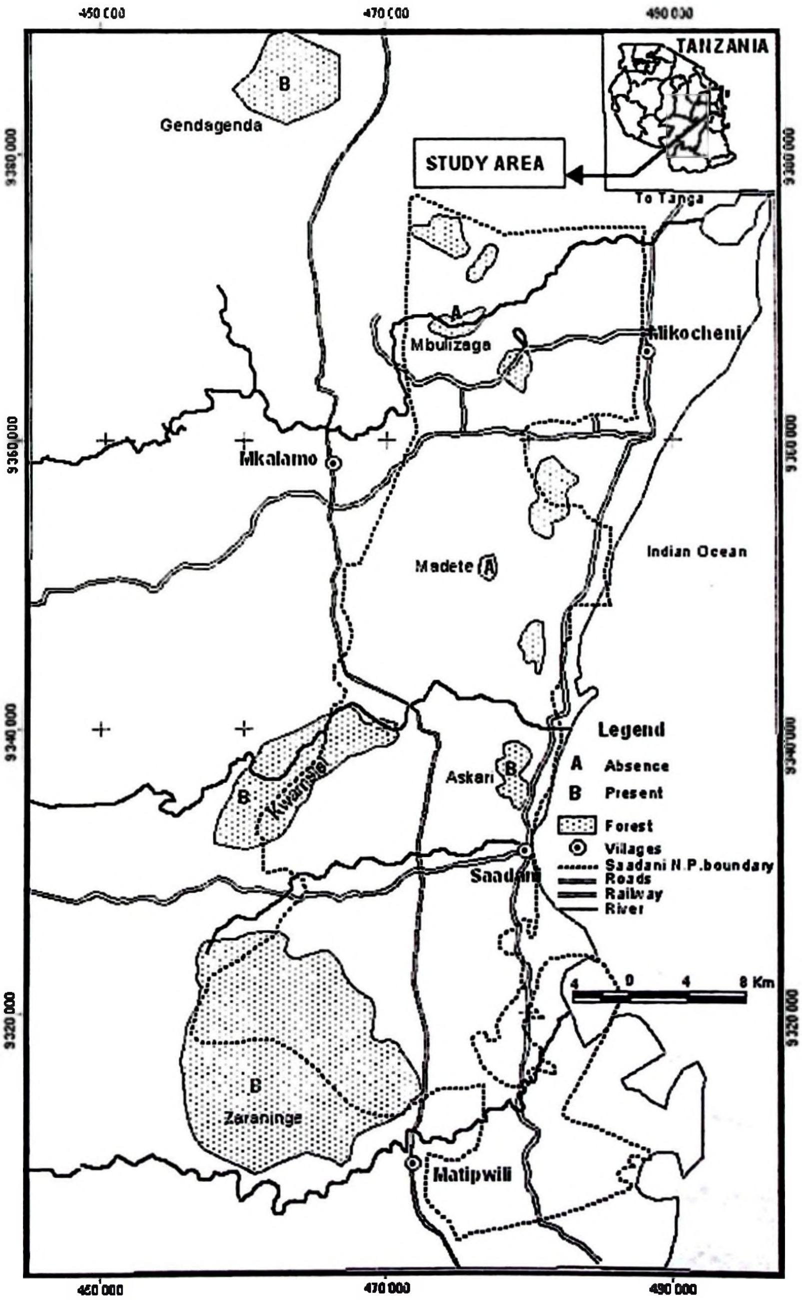

Figure 2.1 ***Beamys hindei*** **distribution in coastal forest patches (A, absent; B, present)**

The primary forest rises from 100 to 300 m above sea level between the lower Wami and Mligaji rivers with the dominant tree species including *Manilkara sulcuta* Dubard, 1915, *Cynometra webberi* Baker, 1942, *Diospyro sverrucosa* Gürke, 1898, *Baphia kirkii* Baker 1871, *Scorodophloeus fischeri* (Taub.) Léonard, 1951, *Bombax rhodognaphalon* Schum, 1895 and *Haplocoelopsis africana* Davies, 1997 (Clarke &Dickson 1995). The forest is surrounded by mixed vegetation of wooded grassland, small patches of evergreen forests and thickets.

The rainfall pattern of the area is bimodal, receiving short rains between October and December and long rains between March and June, although localized ranges between 900 and 1400 mm (Clarke 2000). The temperature is typical of equatorial coastal forest regions of East Africa, with an annual maximum temperature of 29.7°C and a minimum of 21.9°C.

Distribution of the lesser pouched rat was assessed in 5 forests patches where there were no previous records of *Beamys*. The forests included Kwamsisi, Gendagenda, Askari, Madete and Mbulizaga. Kwamsisi borders SANAPA on the northwest, with only a small part of the forest managed by the park authorities.

Kwamsisi Forest is situated on top of hills, with discontinuous patches separated by thickets and mixed grass woodlands. It occupies approximately 10.5 km^{2}of dry evergreen forest, with an even canopy varying from 8 to 20 m high, dominated by *Diospyros kabuyeana* White, 1988, *Lecaniodiscus fraxinifolius* Baker, 1868, *Combretum schumannii* Engler, 1894, *Scorodophloeus fischeri* (Taub.) Léonard, 1951, and *Manilkara sulcate*, Dubard, 1915. Other tree species include *Afzelia quanzensis* Welwitsch, 1859, *Premna chrysoclada* Gürke, 1903 and *Tarenna nigrescens*, Hiern, 1877. The ground is covered with shrub and leaf litter and sparse grass.

Gendagenda Forest is located in the north, outside SANAPA, and is exclusively managed by Gendagenda village. The forest occupies an area of approximately 14 km^2, with canopy ranging from 10 to 16 m in height and dominance shifting between *Craibia brevicaudata* (Vatke) Dunn, 1911, *Diospyros kabuyeana*, *Lecaniodiscus fraxinifolius*, *Combretum schumannii*, *Scorodophloeus fischeri*, *Manilkara sulcate*, *Afzelia quanzensis*, *Premna chrysoclada*, *Tarenna nigrescens*, *Rhoicissus revoilii* Planchon, 1887, *Toddaliopsis sansibarensis* Engler, 1895, *Balanites maughamii* Sprague, 1913, *Polysphaeria multiflora* Hiern, 1877, *Spirostachys africana* Sonder, 1950, *Croton pseudopulchellus* Pax, 1904, *Vepris eugeniifolia* (Engler) Verdoorn, 1895, and *Rawsonia lucida* Harv and Sond, 1860. The ground is covered with shrubs and leaf litter.

Askari Forest is small, approximately 1.2 km^2. Located within the eastern part of SANAPA, the vegetation includes green thickets of woodlands and sparse tree cover, with canopy ranging from 8 to 12 m in height; the ground is sparsely covered by shrubs and leaf litter, and is dominated by grass and open ground. Elephants are commonly found in this forest.

Madete Forest occupies an area of approximately 0.5 km^2within SANAPA. The vegetation is dominated by sparse green thickets of woodland, with sparse canopy trees ranging between 8 and 12 m in height; the ground is patchily covered with grass and a large part of the forest is dominated by open ground.

Mbulizaga Forest occupies an area of approximately 1 km^2in the northern part of SANAPA; vegetation is dominated by sparse green thickets of woodlands mixed with sparse canopy trees ranging between 8 and 12 m in height; the ground is covered with sparse leaf litter, and dominated by grass and open ground.

Distribution of *Beamys hindei*

To determine the current status of the distribution of *B. hindei*, we conducted trapping in 5 forest patches (Kwamsisi, Gendagenda, Askari, Madete and Mbulizaga) at different times during the study period. In each forest, Sherman traps (H.B. Sherman Traps, Tallahassee, FL, USA), baited with ripe banana, were set in 10 trap lines, each consisting of 20 traps: a total of 200 traps per forest patch. Trap lines and trap stations were 10 m apart. Traps were moved to new sites within a forest a minimum distance of 100 m after 5 nights of trapping. A trap night is defined as 1 trap baited and set to catch a small mammal for a 24-h period, whether anything was caught or not by morning (Nicolas & Colyn 2006). Trappings in Kwamsisi and Gendagenda Forests were carried out in September 2010, January, February, March and September 2011 and January and February 2012, while in Askari forest trapping was carried out in May and June 2012 and in Madete and Mbulizaga Forests trapping were carried out in August and September 2012.

Capture–mark–recapture

To estimate demographic parameters, a capture– mark–recapture (CMR) study was conducted in Zaraninge Forest from August 2010 to July 2012. Two permanent trapping grids of 100 × 100 m each were established, located approximately 1000 m apart. Each grid consisted of 10 parallel lines spaced at 10 m apart and with 10 trapping stations per line, also 10 m apart (a total of 100 trapping stations per grid). One Sherman LFA live trap (8 × 9 × 23 cm) was placed at each trapping station, and all were set for 5 consecutive nights of the first week for every month. Traps were baited with ripe banana and were set at dusk and checked at dawn.

Animal processing

All newly captured individuals, including species of rodents other than *B. hindei*, were anaesthetized briefly using isoflurane or diethyl ether to immobilize them. Each individual was identified by a unique number code by toe-clipping. Data recorded included species identification, date of capture, sex, reproductive condition and weight. Males were recorded as reproductively active when the testes were scrotal and inactive when they were non-scrotal. Females with a perforate vagina, or that were pregnant or lactating were recorded as reproductively active, while those with a closed vagina were recorded as inactive. After recording these data all individuals were released at the trapping station where they were captured.

Data analysis

Demographic parameters for *B. hindei* were determined only from CMR data generated in the 2 grids in Zaraninge. Population abundance was estimated from the

CMR data using the program CAPTURE with the jackknife estimator that was developed to take into account the individual heterogeneity in capture probability (White *et al.* 1982; Rexstad & Burnham 1992).

The proportion of reproductively active and inactive individuals in both grids was established for each trapping month. The sex ratio was determined in the 2 grids; in this study the sex ratio was defined, following Mulungu *et al.* (2013), as the proportion of the total population in the community is female-biased. Individuals were categorized as juveniles or adults based on their weight (Keesing 1998), which is linked with reproductive status. An animal was considered an adult if it weighed more than the median weight of the 10 smallest animals of each sex that were reproductively active at first capture (males ≥49 g; females ≥46 g); those below were considered to be sub-adults.

Data were subjected to a repeated measures test (rANOVA), where grid trapping points were fixed and months were random (SAS 1990). Comparisons of mean values for significant differences were done by means separation tests using Tukey's test method. Weights of males and female animals were compared for significant differences.

Survival and recruitment rates were estimated from the individual monthly capture histories built from the CMR records. The history indicates whether the individual was present or not present in a particular month (e.g. 011010100), where "0" indicates that an animal was not encountered in that particular month and "1" indicates that it was encountered at least once during a consecutive 5-day trapping session (Pollock 1982). We also subjected our data to a goodness of fit (GOF) test to check for transient and trap dependence. GOF statistics were calculated using the program U-CARE v.2.2.5 (Choquet *et al.* 2005). Gender (male and females), grid (2 grids) and season (3 classes: dry season [July to September and January to February], wet season short rains [October–December] and wet season long rains [March–June]) were included as explanatory variables. Model selection was performed based on corrected Akaike information criterion (AICc) values, with the lowest value indicating a more parsimonious model in terms of fit and amount of parameters consumed.

Recruitment analysis was performed on the reverse capture histories (Pradel 1996). We opted to simultaneously model 3 parameters: survival, recruitment and recapture rates. Grid and gender were included as explanatory variables. The realized population growth rate was derived analytically.

RESULTS

Distribution of lesser pouched rats in coastal forest patches

The distribution of lesser pouched rat and species composition is presented in Table 1. With the exception of Zaraninge, where records of *Beamys* are available (Kiwia 2006), lesser pouched rats were captured in 3 out of 5 coastal forests patches: Kwamsisi ($N = 17$), with a trapping success of 0.11% (a trap success [T] is defined as the number of individuals caught per 100 TN) (Nicolas & Colyn 2006), Gendagenda ($N = 11$), with a trapping success of 0.07%, and Askari ($N = 2$), with a trapping success of 0.01%. No rodent was captured in Madete or Mbulizaga.

Table 2.1: Species composition and distribution of small mammals in Coastal forests

Species	Zaraninge	Kwamsisi	Gendagenda	Askari	Mbulizaga	Madete
Beamys hindei	(158) 26 400†	(17) 15 000†	(11) 15 000†	(2) 12 000†	12 000	12 000
Grammomys spp.	(141) 26 400†	(21) 15 000†	(2)15 000†	(1)12 000†	12 000	12 000
Graphiurus spp.	(72) 26 400†	(1) 15 000†	(1)15 000†	12 000	12 000	12 000
Crocidura spp.	(1) 26 400†	15 000	15 000	12 000	12 000	12 000
Mastomys natalensis	(2) 26 400†	(12) 15 000†	15 000	12 000	12 000	12 000
Paraxerus spp.	(4) 26 400†	15 000	15 000	12 000	12 000	12 000
Rattus rattus	26 400	15 000	(1)15 000†	12 000	12 000	12 000
Tatera spp.	26 400	(4) 15 000†	15 000	12 000	12 000	12 000
Acomys spp.	26 400	(12) 15 000†	(1) 15 000†	12 000	12 000	12 000
Galago spp.	(8) 26 400†	15 000	15 000	12 000	12 000	12 000

†The numbers in parentheses represent the total number of individuals captured and the numbers not in parentheses represent trapping nights.

Ecological data

Population abundance

A total of 158 individuals of lesser pouched rats were captured in Zaraninge Forest, with a trapping effort of 26 400 trap nights. The trapping success was approximately 0.6% in the 2 grids; other species of rodent and small mammals captured in Zaraninge are presented in Table 1. The estimated population abundance during the study period ranged from 1 to 40 individuals per month in the 2 grids (Fig. 2); however, no significance different in abundance was observed between months (F18,23= 16.46, P = 0.83). Likewise, monthly trappings did not reveal any significant differences in numbers between females and males (Female F11,37= 4.08, P = 0.18; Males F11,37 = 3.43, P = 0.47) (Fig. 3). There were some relatively highly pronounced abundance fluctuations, particularly in November and December 2010, January, March, April and May 2011, and March 2012 (Fig. 2).

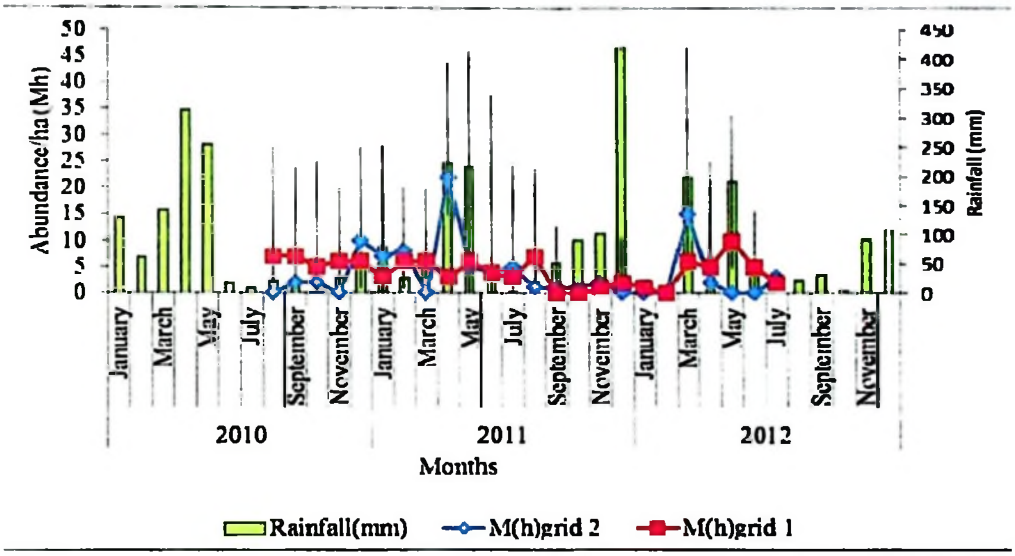

Figure 2.2: Estimated monthly abundance (Mh) with 95% confidence limits (number of animals/ha).

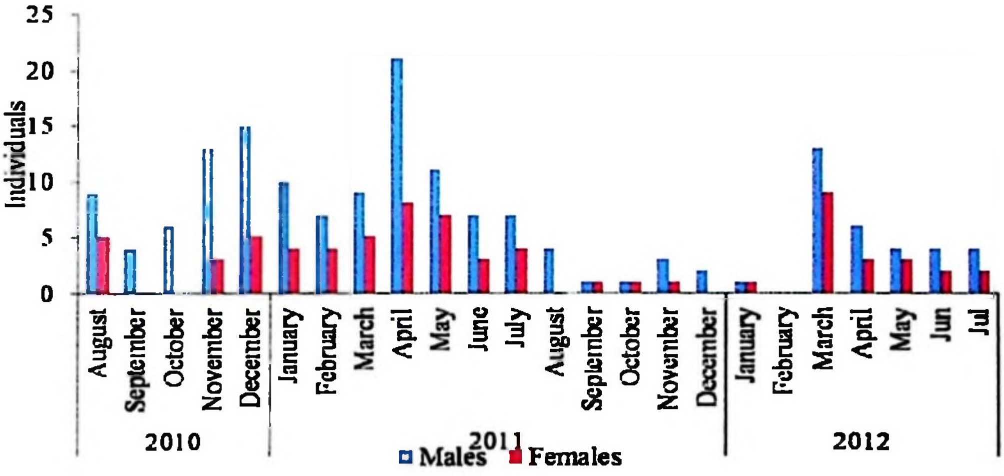

Figure 2.3: Individual trapped *Beamys hindei* males and females pooled from 2 grids.

Reproduction

The number of individuals males and females which were sexually active did not show any significant differences between months (male: *F*11,37= 2.49, *P* = 0.32; females: *F*11,37 = 0.19, *P* = 0.06). However, compared to females, males were sexually active in all months except May and July 2012 (Fig. 4a,b). Females were sexually active in December 2010, March and May 2011 and March and July 2012

(Fig. 4b). Both sexes were sexually active in December 2010 and March 2011 and 2012. These are months that receive short and long rains, respectively (Fig. 2). There were no significant differences between the numbers of females and males of *B. hindei*captured during the study period ($F11,35= 0.65$; $P = 0.77$). However, more females were captured in August 2010, January, February, March, May, September and October 2011, and March to June 2012 (Fig. 5).

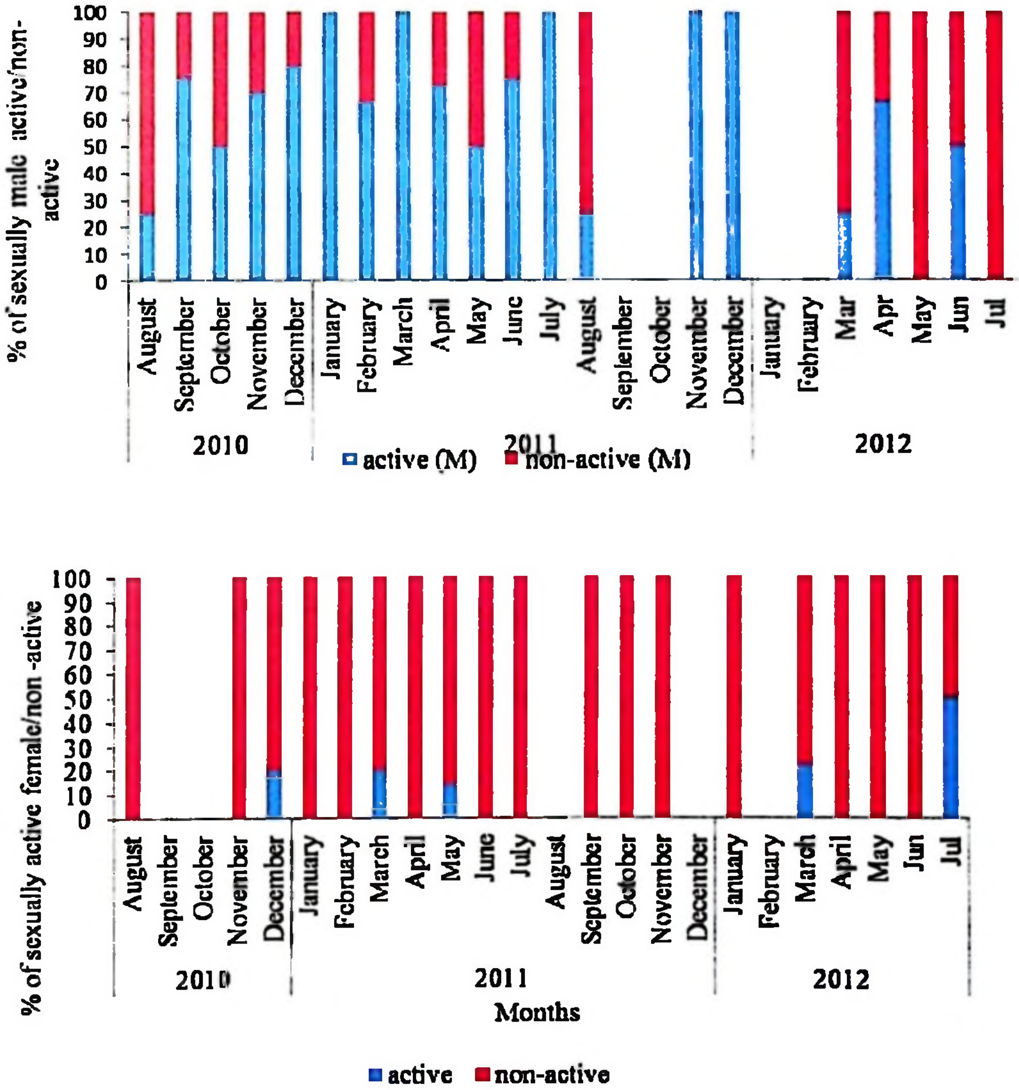

Figure 2.4(a): Percentage of reproductively active and inactive male lesser pouched rats in 2 grids and (b) percentage of reproductively active and inactive females *Beamys hindei* in 2 grids

Survival and recruitment

The goodness of fit test was performed on 4 single state datasets separated for gender and grid. The results indicated no trap dependence (global test for trap dependence: $N(0,1) = -1.298$, df = 50, $P = 0.194$), nor an excess of transients (global test for transients: $N(0,1) = 1.2417$, df = 50, P 1-sided = 0.1072). The 4 separate levels of grid * gender showed no significant differences either for transients or trap dependence.

Given that the data do not support a full-time dependent model we proceed with a full model having gender*grid interactions in both survival and recapture. Our results suggest the average of 28-day survival rates of the lesser pouched rat to be 0.72[0.65–0.78], taken from model (ϕ(.)p(.)) (Table 2). Furthermore, the selection procedure indicates that our CMR data support differences in survival probabilities between gender and grids (AICc weights = 0.45; see Fig. 6). The model favored a constant recapture probability, but differences in recapture based on grid and gender were supported by an AICc weight of, respectively, 21% and 16%. Female survival in grid 1 seemed to be lower compared to the other survival rates of males and females (ϕ(g)p(.)) (Fig. 6); additionally, season was included as a covariate. Table 3 demonstrates that the most parsimonious model from before remains, and, indeed, the data do not seem to support differences in seasonality (Table 4), although seasonal differences in survival rates might exist. Recruitment was modeled with gender and grid as covariates (Table 3). The most parsimonious model had constant rates in all 3 parameters (AICc weight = 0.28), while a difference in grids in both survival and recruitment became a close second (AICc weight = 0.25). From the constant model, the 28-day survival rate estimate was 0.72 [0.65–0.78], while the recruitment rate was 0.29 [0.23–0.36]. The realized population growth rate estimate was 1.014 [0.99–1.037].

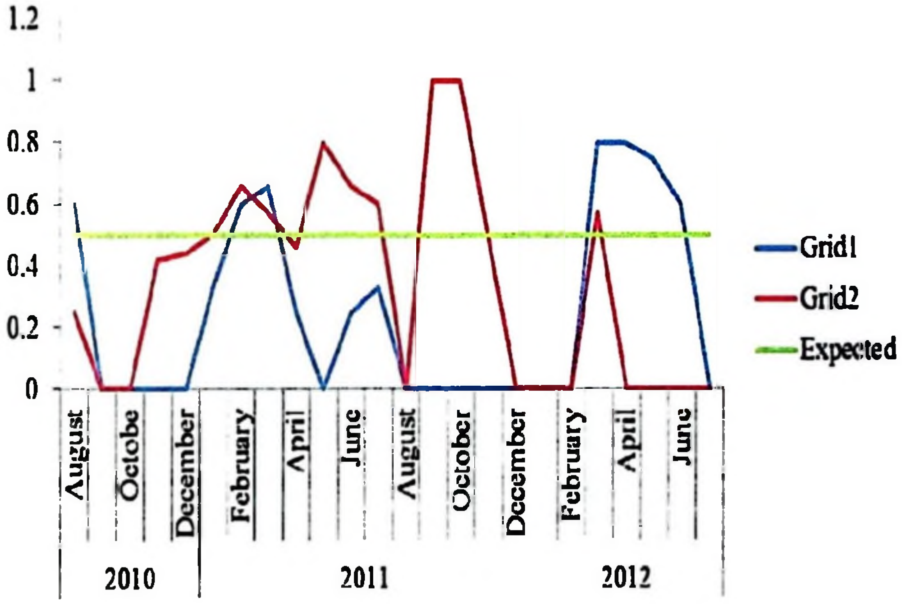

Figure 2.5: Sex ratio distribution of female in the 2-ha grids in Zaraninge Forest.

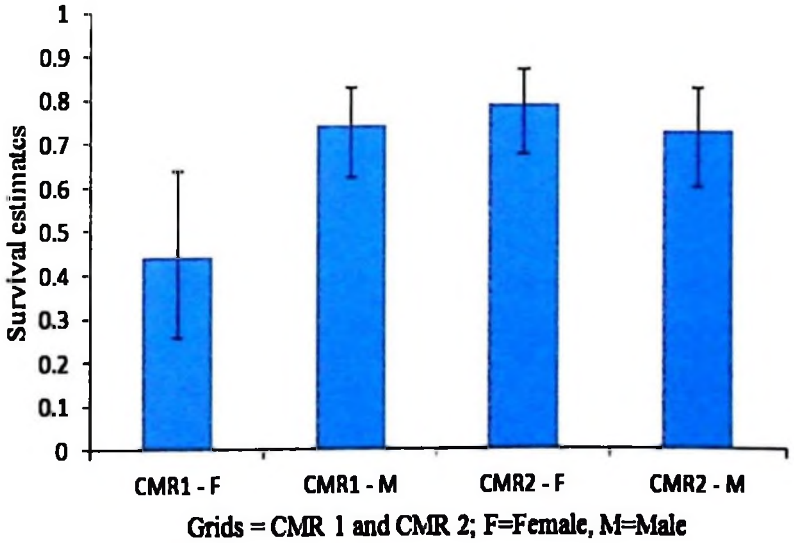

Figure 2.6: Survival estimates of lesser pouched rat in the two 1-ha grids (±SE).

Table 2.2: Overview of the results of different survival models and their AICc values and weights

Model	AICc	Delta AICc	AICc weights	Model likelihood	NP	Deviance
{ φ(g) p(.)}	434.1610	0.0000	0.45126	1.00000	5	358.2541
{ φ(g) p(grid)}	435.7391	1.5781	0.20500	0.45430	6	357.6863
{ φ(g) p(sex)}	436.2566	2.0956	0.15826	0.35070	6	358.2038
{ φ(grid) p(.)}	438.1240	3.9630	0.06221	0.13790	3	366.4330
{ φ(.) p(.)}	438.9760	4.8150	0.04063	0.09000	2	369.3560
{ φ(.) p(g)}	439.1064	4.9454	0.03807	0.08440	5	363.1994
{ φ(g) p(g)}	439.9032	5.7422	0.02556	0.05660	8	357.4806
{ φ(sex) p(.)}	440.4947	6.3337	0.01901	0.04210	3	368.8037

With sex: female and male and grid: CMR1 and CMR2, g = sex*grid: cmr1-F, cmr1-M, cmr2-F, cmr2-M; for constant estimates (i.e. same over all groups). AICs. Akaike Information Criterion

Table 2.3: Different recruitment models

Model	AICc	Delta AICc	AICc weights	Model likelihood	NP	Deviance
{φ (.) p(.) f(.) }	1067.8396	0.0000	0.27680	1.00000	3	510.0253
{ φ (grid) p(.) f(grid)}	1068.0414	0.2018	0.25023	0.90400	5	506.0126
{ φ (g) p(.) f(g) }	1068.8399	1.0003	0.16786	0.60640	9	498.0752
{ φ (sex) p(.) f(sex) }	1070.4145	2.5749	0.07639	0.27600	5	508.3857
{φ (g) p(.) f(.) }	1070.5845	2.7449	0.07016	0.25350	6	506.4106
{φ (.) p(g) f(.) }	1070.7285	2.8889	0.06529	0.23590	6	506.5547
{ φ (g) p(g) f(g) }	1072.5235	4.6839	0.02661	0.09610	12	494.9237
{ φ (grid) p(g) f(grid)}	1073.1003	5.2607	0.01994	0.07200	8	504.5590
{ φ (.) p(.) f(g) }	1073,1525	5.3129	0.01943	0.07020	6	508.9786
{ φ (sex) p(g) f(sex) }	1074.2026	6.3630	0.01149	0.04150	8	505.6612
{ φ (g) p(g) f(.) }	1074.4320	6.5924	0.01025	0.03700	9	503.6672
{ φ (.) p(g) f(g) }	1075.6655	7.8259	0.00553	0.02000	9	504.9007

NP, number of parameters; AICc, Akaike Information Criterion

Table 2.4: Different fitted models with respect to seasons

Model	AICc	Delta AICc	AICc weights	Model likelihood	NP	Deviance
{ φ(g) p(.)}	4.282933	0.0000	0.27113	100.000	5	3.523.863
{ φ(season+sex) p(.)	4.290137	0.7204	0.18912	0.69750	7	3.487.892
{ φ(g) p(sex) }	4.300899	17.966	0.11042	0.40730	6	3.520.370
{ φ(g) p(grid) }	4.304016	21.083	0.09449	0.34850	6	3.523.488
{ φ(g) p(season) }	4.307684	24.751	0.07865	0.29010	7	3.505.439
{ φ(season+g) p(.)}	4.313049	30.116	0.06015	0.22180	13	3.374.801
{ φ(grid) p(.)}	4.318369	35.436	0.04610	0.17000	3	3.601.459
φ(season+grid) p(.)}	4.320423	37.490	0.04160	0.15340	7	3.518.178
{ φ(g) p(g) }	4.323629	40.696	0.03544	0.13070	8	3.499.403
{ φ(season) p(.)}	4.325363	42.430	0.03250	0.11990	4	3.587.498
{ φ(.) p(.)}	4.335831	52.898	0.01925	0.07100	2	3.639.631
{ φ(sex) p(.)}	4.344782	61.849	0.01231	0.04540	3	3.627.872
{ φ(.) p(g) }	4.351398	68.465	0.00884	0.03260	5	3.592.328

DISCUSSION

There are few reports on the distributions of *B. hindei* in Tanzania (Hubbard 1970; FitzGibbon *et al.* 1995; Stanley *et al.* 1998; Stanley *et al.* 2005; Kiwia 2006; Stanley 2007). The species was thought to be absent in many coastal forests, but in the current study we report the occurrence of lesser pouched rats in Kwamsisi, Askari and Gendagenda Forests, sites, where there were no previous records of *Beamys*; this extends the number of localities with distributions of the species in Tanzania. The occurrence and distribution of the lesser pouched rat is thought to depend on factors such as the structure of the vegetation, soil and availability of suitable edible plants (Delany 1986; FitzGibbon *et al.* 1995; Clarke 2000; Clarke & Burgess 2000; Lowe *et al.* 2000). The less than suitable conditions could explain the absence of *B. hindei* in Mbulizaga and Madete Forests; these factors could contribute to the differences in abundance of the species between the study forests. The presence of the lesser pouched rat in Askari and Kwamsisi Forests within Saadani National Park enhances the conservation and protection of the species *in situ* compared to the forests outside the park, as many of the forest patches outside National park are subjected to environmental degradation due to the increase in anthropogenic activities that are threat to the survival of the lesser pouched rats, as observed in this study in the Gendagenda Forest which is managed by the village community.

No previous studies have estimated the population abundance (density) of the lesser pouched rats in coastal forests of Tanzania. However, when the established density is compared to the study by FitzGibbon *et al.* (1995) in Arabuko Sokoke on the same species, for both forests, the species show a stable population, although low abundance. This is thought to be a characteristic of forest-adapted species compared to widely distributed species inhabiting savannah habitats. Makundi *et al.* (2006), for example, report that rodent species inhabiting Magamba Forest Reserve in north-east Tanzania maintained a relatively low but stable population. Cultivated fields surrounding these forests were inhabited by species that exhibited high population density fluctuations between seasons and years (Makundi *et al.* 2007).

Individual females were sexually active during short and long rains, while males remained sexually active almost throughout the year. Seasonality in breeding is a common occurrence in rodents and has been reported in African murid rodent species by several authors (Delany 1986; Tellford 1989; Leirs *et al.* 1992; Wirminghaus & Perrin 1993; Leirs 1995; Makundi 1995; Keesing 1998; Lima *et al.* 2003; Massawe *et al.* 2007,2011; Makundi *et al.* 2009).

Variations in numbers between females and males are common in many species of rodents (Murua *et al.* 1986). These variations are attributed to various factors: for example, differences in spacing behavior, immune response and predation pressure (Sluydts *et al.* 2009). In this study, however, there were no significant differences in the numbers of females and males, although relatively more females than males were captured during the rain seasons. The same has been reported by Mulungu *et al.* (2013) on *Mastomys natalensis* Smith, 1834 in irrigated fields. The trigger factors for this are not yet known; it probably could be attributed to females wandering more outside their burrows to encounter males to mate. This is supported by observations by Hubbard (1970) that in captivity female lesser pouched rats initiate a courtship with males when they are in estrous.

Transience can be defined as a process of an animal entering and leaving its place of residence. Although this phenomenon exists among rodents, most individuals seem to stick to the same site or habitat with minimal dispersal (Perret *et al.* 2003). In this study, most individuals were captured and recaptured more than once, thus indicating low transience even among different sexes. Low transience could imply that the habitat had more than enough of the basic resources required for survival or it could be attached to burrowing behavior (Hanney & Morris 1968), with animals coming out only to search for food and mates.

Survival estimates of lesser pouched rats during the study period did not show any significant differences between sexes. Our original hypothesis that the survival would be higher for females than males was not supported by our data. The fact that there were no seasonal variations in survival is in line with the observation that the population of lesser pouched rats in the dry evergreen coastal forest is stable. The rate of recruitment in the population of the lesser pouched rats seems to be low compared to that of populations of savannah species of rodents such as the pouched mouse *Saccostomus mearnsi* Heller, 1910 (Keesing 1998) and *M. natalensis* (Leirs 1992). For example, in this study recruitment was approximately 1 animal for every month compared to the related savanna species *S. mearnsi*, at around 13.3–18.3/month (Keesing 1998). The relatively low recruitment estimates for the lesser pouched rats could be attributed to lower litter size (2.8 off spring/litter) (Egoscue 1972) compared to other savanna species such as *M. natalensis* (Leirs *et al.* 1994).

In conclusion, our results support the finding of FitzGibbon *et al.* (1995) that despite the low abundance of *B. hindei* observed, the species tends to have a stable population throughout the year, a characteristic reported for other forest inhabiting species (Makundi *et al.* 2006). The patchy distribution of the species could be attributed to its low abundance, which is influenced by low recruitment and survival rates and probably microhabitat heterogeneity, which may influence shelter and food availability. For example, the vegetation structure observed in Kwamsisi and Gendagenda is relatively similar to that in Zaranginge, which could probably offer a favorable environment for *B. hindei* survival compared to Madete and Mbulizaga. The sex ratio and sexual activity results reveal that reproduction takes places mainly during rainy seasons, the time when sexually active females were observed to be higher; this is thought probably to be the time of high abundance of food (Odhiambo *et al.* 2008; Makundi *et al.* 2011). To complement this study, further studies are required to determine the home range, diet and burrowing behavior of *B. hindei*, which are all important for determining the species distribution in coastal forests and other related habitats and for conservation of the species.

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CHAPTER THREE

3.0 PAPER 2: Space use, burrowing behavior and diet of the lesser pouched rat (*Beamyshindei*) in Zaraninge coastal forest, Tanzania.

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ABSTRACT

The lesser pouched rat *Beamys hindei* is a small rodent that occurs in forests and moist woodlands habitats in Eastern Africa. Although its distribution is quite well documented, hardly anything is known about its ecology. The aim of this study was to determine the home range, movement patterns, burrowing behavior and diet of *B. hindei* in Zaraninge coastal forest, Tanzania, where the species is abundant. Data on home ranges and movement were obtained from monthly capture-mark-recapture studies conducted in 2 1-ha grids over a period of 24 months. Home range overlap and visitation rates were calculated as proxies for territoriality. In addition, burrows were located by tracking individuals using telemetry. Mean home range sizes for *B. hindei* males and females were 2329 ± 321 m^2 and 1497 ± 320 m^2, respectively. Home ranges of adults were larger than those of subadults (adult =2160 ± 349 m^2, subadult = 854 ± 208 m^2), but female adults had the smallest home ranges. Mean daily distance moved by *B. hindei* within home ranges was 7.1± 7.1m (male = 6.4 ±1.3 m, female 7.9 ±3.7 m). Burrow placement was associated with the presence of

shrubs, herbs and grass and leaf litter in loamy sand soil. Burrow structures are large, linked to the large body size of the animals. Stomach analyses indicate a wide range of food types is used by *B. hindei* (plant materials, grains/seeds, fruits, invertebrates). Results suggest that space use of lesser poached rat is influenced by dietary behavior, physical soil properties and vegetation. This study support lesser pouched rat as burrow user, food storing behavior makes an animal to spend more times in burrows.
Key words: *Beamys hindei*, home range, telemetry, capture-mark-recapture.

3.1 Introduction

The lesser or long tailed pouched rat, *Beamys hindei* (Thomas, 1909) is a small rodent that is found mainly in coastal and Eastern Arc forests and moist woodlands (FitzGibbon *et al.*, 1995; Hubbard, 1970; Hanney and Morris, 1962) in eastern Africa. The term "pouched" refers to the internal, thin walled cheek sack used for collecting and transporting food (Hubbard, 1970), which is a common characteristic of species of the related genera *Beamys*, *Saccostomus* and *Cricetomys* (Kingdon, 1974).

Beamys hindei used to be considered a rare species (Kingdon, 1974) and hence was categorized as "vulnerable" (IUCN, 1990) due to its patchy distribution and collections from few localities (FitzGibbon *et al*, 1995; Kingdon, 1974). However, the knowledge on its distribution has recently increased as a result of wider surveys of small mammals in different biotopes (Sabuni, 2008; Stanley *et al.*, 2007; Kiwia, 2006; Stanley *et al.*, 2005; Stanley *et al.*, 1998; Clarke and Dickson, 1995; FitzGibbon *et al.*, 1995; Christensen, 1987). As these surveys showed a much wider distribution than previously thought, its IUCN status has changed; it was classified in 2004 as "Near Threatened" and in 2008 as "Least Concern" (Howell *et al.*, 2008).

Despite its status and the knowledge on its distribution (Stanley *et al.*, 2007; Clarke and Dickson, 1995; FitzGibbon *et al.*, 1995; Ansel and Dowsell, 1988; Ansell, 1978; Hubard, 1970) relatively little is known about ecological aspects of *B. hindei* such as space use, burrowing behavior and diet (FitzGibbon *et al.*, 1995; Hubbard, 1970; Hanney and Morris, 1962).

Distributions of animals can be influenced by space use, behavior and diet (Bjorneraas *et al.*, 2012; Stapp and Horne, 1997; Tufto *et al.*, 1996). Space use of mammals can be influenced by various factors such as resource availability, population density, and mating behavior (Said and Servant, 2005; Waterman, 2007; Adler, 2011). An individual's home range is, according to Burt (1943) an area that is traversed by the individual in its normal activities for food gathering, mating and caring of young. Various studies have been carried out to investigate factors that influence the home range of animals, for example, areas with high availability of food have shown a decrease in home range sizes (Rodel and Stubble, 2006; Said *et al.*, 2005; Mace *et al.*, 1983), while body size, sex and age are known to influence the area over which an animal ranges (Eiris and Barreto, 2009; Begg *et al.*, 2005; Mysterudi *et al.*, 2001; Mikesic and Drickamer, 1992; Harestad and Bunnel, 1979).

Important features of a home range are the shelters that an animal uses daily. For many burrowing species shelters are provided by constructing burrows that can be elaborate structures (Reichman and Smith, 1990). Burrows offer many benefits, and can function as protection from adverse environment such as hot/cold temperature and from predators, and as food storage and areas for nesting young (Kenagy, 1973).

As soil type is important for burrow construction, soil type will determine burrow placement (Reichman and Smith, 1990). Advantages and disadvantages of soil type for burrow construction have been explained by Best (1973), Miller (1964), Kennerly (1964). Many burrowing mammals cache food in their burrows, and the amount of stored food as well as the storage time varies between species (Smith and Reichman, 1984). The advantage of caching is the ability to control food availability in a protected environment (Reichman and Fay, 1983). Egoscue (1972), studying *B. hindei* in captivity, found it to have a habit of making burrows, to feed on a variety of foodstuffs and to have a habit of collecting and hiding food. Field observations on the closely related species *Beamys major* (Hanney and Morris, 1962) found burrows which had structures used for food storage and nesting. Seeds and fruits of various types of trees and grass were found stored in the nesting chamber of *B. major*, the types of food that have been also reported by Kingdon (2003). In the present study we investigated (1) home ranges and movements; (2) burrowing behavior and (3) diet of *B. hindei*in a Coastal forest in Tanzania.

3.2 Materials and Methods

3.2.1 Study Area

The study was conducted in Zaraninge forest situated at $6^0 04'$ - 6^0 13'S and $38^0 35'$ - $38^0 42'$E, 15 km inland from the Indian Ocean on the South West part of Saadani National Park (SANAPA) occupies an area of about 20 km^2 (Kiwia, 2006). The vegetation formation type is the Eastern African Coastal dry forest, an evergreen dry forest. The detailed vegetation composition of the forest is described by Mligo *et al.* (2009). The rainfall pattern of the area is bimodal receiving short rains between

October and December and long rains between March and June although localized showers can occur almost every month. Mean annual rainfall ranges between 900 and 1400 mm (Clarke, 2000). The temperature is typical of equatorial coastal forest regions of East Africa with an annual maximum temperature of 29.7^{0}C and a minimum of 21.9^{0}C (SANAPA, 2005).

3.2.2 Capturing Rodents

A CMR-study of *B. hindei* was carried out from August 2010 to July 2012. Two permanent trapping grids of 100 × 100 m were established, located about 1000 m apart. Each grid consisted of 10 parallel lines of 10 trapping stations, spaced 10 m apart (i.e. a total of 100 trapping stations per grid). One Sherman LFA live trap (8 × 9 × 23 cm; H.B. Sherman Traps Inc., Tallahassee, FL) was placed at each trapping station, and all traps were set monthly for five consecutive nights. Traps were baited by smearing ripe banana in the trap just to provide smell for attracting the animal, set at dusk and checked at dawn.

All newly captured individuals were anesthetized briefly using isoflurane or diethyl ether to immobilize them, and each individual was made identifiable using a unique number code by toe-clipping. Date of capture, species, sex, and weight were recorded during each trapping session. Males were considered to be sexually active when the testes were scrotal and females when the vagina was perforated, when visibly pregnant and/or when lactating. This trapping effort resulted in174 captures of 95 individuals in 26,400 trap nights.

3.2.3 Home range size, overlap and movement

Home ranges were estimated based on trap locations from the CMR data, using the minimum convex polygon (MCP) method with a 5 m inclusive boundary strip (Stickel, 1954). More sophisticated probabilistic methods were not possible due to the limited number of individual recaptures. Only animals that were captured 4 or more times were used for analyses, as these were more likely to be resident animals. This resulted in a sample size of 23 individuals (8 males and 5 females in grid 1, and 4 males and 6 females in grid 2) for which home ranges could be estimated. As a measure of territoriality, the overlap between home ranges was calculated for each individual as the percentage of that individual's home range that overlapped with home ranges of all other individuals that were alive simultaneously. As a measure of inter-individual contact, the number of individuals captured within an individual's home range was calculated (including individuals that were recaptured less than 4 times), hereafter referred to as "home range visitation". As a more precise measure of individual movement within home ranges, a movement rate was estimated as the distance between consecutive trap locations divided by the number of days between trapping events, resulting in a "travel speed" expressed in m/day.

The effects of sex, reproductive status and body weight (estimated as the average weight of an individual during its entire trapping period) were tested using ANOVA. Data manipulation, statistical testing and plotting was done in "R" version 3.0.1 (R Development Core Team, 2012), using packages adehabitatHR (Calenge, 2006), gpclib (Peng and Murta, 2012), maptools (Lewin-Koh and Bivand, 2012), lme4 (Bates *et al.*, 2012) and Matrix (Bates and Maechler, 2012). In the figures, mean whiskers denote standard errors. P values below 0.05 were considered to indicate statistical significance.

3.2.4 Burrow location, morphology and characteristics

Burrows were located by monitoring the animal after being released from the trap during CMR or by using telemetry tracking. Sixteen individuals weighing ≥ 50 gram were fitted with a radio transmitter collar tag Model M1520 weighing 2.2 g made by Advanced Telemetry System, Inc.. Tracking was done using a hand-held three element Yagi antenna model F 152 – 3FB10451 with a receiver model R410 made by Advance Telemetry Systems, Inc.. and localization was done using a GPS Garmin 60. After collar fitting the animal was released and followed to its burrow, and subsequent tracking sessions were carried out mainly during the night from 17:00 to 24:00 and 03:00 to 06.00 hours to confirm the individual's burrows where all collared individual remains in the same burrow previously identified. This was confirmed also during the day, where all animals remained inside their respective burrows.

To determine the soil type characteristics, soil profiles were dug from 12 excavated burrows which were located based on telemetry data, and at four random places spaced about 300 m in the study area where burrows were absent. The proportions of sand, loam and clay of the horizons were determined using a Buoyocous hydrometer method (Dewis and Freitas, 1970). A glass electrode pH-meter was used to measure soil acidity. Organic matter was analyzed through the loss of ignition method (Dewis and Freitas, 1970).

Vegetation was recorded at 12 burrows identified using telemetry and 20 identified by observing the animal after release from the trap, with vegetation variables sampled in a 5 m radius from the burrow entry. In addition, 41 locations without burrows were randomly selected by throwing a stone from the first point of each grid, after which a 5 m radius centered on the stone was sampled. Vegetation variables were estimated as the coverage percentage of different vegetation types; stems (ST) (< 5 cm diameter at 1 m), seedlings (SD), shrubs (SH) herbs and grass (HG), climbers (CL) and leaf litter (LT). Trees were counted at each point in 2 categories; trees with a diameter at breast height (DBH) of 5-10 cm and those greater than 10 cm. Stepwise regression backward elimination (SAS. 1990) was used to determine the possible associations of vegetation types with burrow placement.

3.2.5 Diet

Ten trapped individuals were sacrificed with an anestheticum overdose, and stomachs were removed and preserved in 70% ethanol. Food composition of preserved stomachs was determined using a binocular stereoscope (×25 and/or ×50 magnifications) following Smith *et al* (2002). Six food categories were used: plant materials, grains/seeds, fruits, invertebrates, hair and other (non-identified foods due to digestive process). Data analysis was performed following Smith *et al.* (2002) focusing on the percentage contribution (PC; the percentage of the total number of stomachs in which a particular food item has been detected), average percentage volume (PV; the contribution of each item to the volume of a particular stomach's content) and the combination of PV and PC was used to calculate an importance value (IV = PV × PC/100) for each dietary item (Cooper and Skinner, 1978). The relative importance (RI) of each food item was calculated as 100 × IV/$\sum$IV.

3.3 Results

3.3.1 Home range

Home range size of males (n=12) were larger than those of females (n=11) (male = 2329 ± 321 m^2, female = 1497 ± 320 m^2; F(1,19) = 5.907, p= 0.025). Body weight correlated positively with home range size (slope = 26.5 ± 15.8m^2, F(1,19) = 6.804, p = 0.017). Home ranges of adults (n=13) were significantly larger than those of subadults (n= 10) (Adult = 2160 ± 349 m^2, subadult = 854 ± 208 m^2; F(1,29) = 6.721, p = 0.016). There was a significant interaction between breeding status and sex, where female adults have much smaller home ranges than those of other categories (F(1,29) = 5.634, p=0.026) (Fig.7).

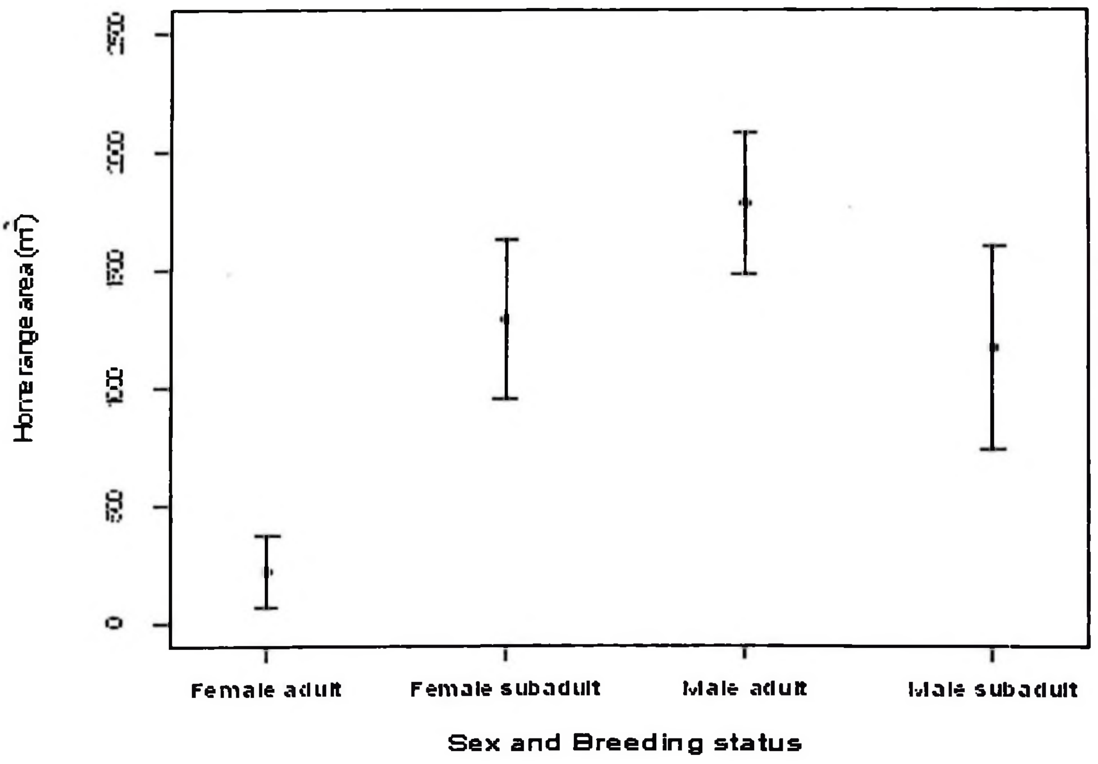

Figure 3.1: Home range size against sex and breeding status.

3.3.2 Home range overlap

No statistically significant differences in home range overlap between different demographic categories were detected, neither, no significant correlation was noticed for body weight (F (1, 19) = 0.485, P=0.495). However, we did observe a trend towards lower degrees of overlap in males (Table 4).

Table 3.1: Percentage overlap (mean ± standard error) between categories for sex and age class

Category	Overlap category	
	Male	**Female**
Male and females by all others (regardless breeding status)	71 ± 9%	76 ± 11%
Males and females by females (regardless breeding status)	33 ± 10%	50 ± 10%
Males and females by males (regardless breeding status)	56 ± 9%	70 ± 11%
Males and females by adults (regardless sex)	33 ± 12%	34 ± 24%
Male and females adults by subadults (regardless sex)	46 ± 15%	71 ± 24%
Male and female adults by subadults (regardless sex)	46 ± 11%	71 ± 24 %
Male and female subadults by adults (regardless sex)	45 ± 15%	47 ± 11%
Male and female subadults by subadults (regardless sex division)	30 ± 13%	60 ± 11%
Male and female by adults by males	31 ± 12%	34 ± 24%
Subadults by female subadults	30 ± 13%	47 ± 10%
Subadults by male subadults	0.5 ± 0.4%	36 ± 13%
	Adul	**Subadults**
Adults and subadults by adults (regardless sex)	61 ± 9'	56 ± 11%
Adults and subadults by subadults (regardless sex)	52 ± 10'	47 ± 9%

3.3.3 Home range visitation

Number of visits for males and females home ranges did not differ significantly (males = 8.0±1.5 visits, females = 6.9±1.5 visits; F(1,20) = 0.367, p=0.552), nor did visits to adults and subadults by other subadults (adults = 2.5±0.7 visits; subadults = 2.5±0.5 visits; female adult = 0.75±0.5 visits; female subadults = 2.9±0.7 visits; male adult = 3.2±0.8 visits; male subadult = 1.6±0.4 visits; F(1,26) = 0.03, p = 0.849). There was a significant difference in the number of visits to adult and subadult home ranges (adults = 5.3±0.9 visits; subadults = 1.4±0.6 visits; female adults = 3.8±1.8 visits; female subadults = 1.8±0.8 visits; male adults = 5.9±1.0 visits; male subadults = 0.4±0.4 visits; F(1,26) = 12.2, p = 0.002). Overall, we observed a positive correlation between body weight and number of visits (slope = 0.24, F (1,20) = 9.37, p = 0.006).

3.4 Movement

The overall moement of*B. hindei* within home ranges was 7.1 ± 7.1 m/day, where males had 6.4±1.3m/day and females 7.9±3.7m/day, with no significant difference between sexes (F (1,21) = 0.162, p = 0.691). Movements of adults (8.1±2.1m/day) were not different from those of subadults (4.5±2.6m/day; F(1,27) = 1.126, p = 0.298).

3.4.1 Movement behavior and burrow morphology characteristics

We observed that, to many animals for new and recaptured individuals after being released from traps their remained calm without rushing. After about 2 minutes their started moving slowly to the nearest climber, tree stem or branch (hereafter referred to as connectors), and quickly climbed it. No individual was seen moving straight to its burrow without using connectors. While moving to its burrow from tree to tree via connecting branches and lianas, it sometimes made an occasional stop for a minute or so, possibly to orient itself. Subadults were observed to wander in the branches of trees for a couple of minutes before starting to move using connectors. We were not always able to follow the subadults until they reached their burrow. When animals were just collared, their movements were initially very slow compared to non-collared individuals, and they often spent more time on the tree/climbers (up to one hour) before descending down into their burrows, this behavior was not observed on subsequent captures. The species was observed to be a good climber (as described in Kingdon (2003), ascending trees up to about 20 m high that means it is able to collect fruits and fresh leaves despite the height of the tree.

Out of 32 identified burrows, 12 were excavated; it was not possible to excavate all burrows because many were placed under big trees. Excavated burrows were typically made up of joint sections structures including an entry opening (EO), a single vertical entrance shaft (VES), a plug, a primary horizontal shaft (PHS), a nest and storage chamber (NC) and a secondary shaft (SS) connecting to a latrine (Figure 2). The lengths of all these sections were measured in cm, principal component analysis (PCA) using software (STATISTICA 12.SatSoft. Inc. 1984-2014) was used to identify which variables are important to both sexes. Burrow measurements did not differ between sexes (Figure 3), however weight of the animal could be the contributing factor of burrow size structure (Table 2).

Nests were observed to consist of mixed piles of dry and fresh green broad leaves lined in the chamber, fresh fruits, empty seed pods and husks. Feces were clearly placed separately in the latrine chamber at the distant end of the secondary shaft. In seven nesting chambers, earwigs (*Hemimerus* sp.) were found.

3.4.2 Burrow placement and vegetation

There was an association to some of vegetation variables with burrow's placements. These were shrubs, herbs and grass, and leaf litter, which predict the presence of burrows placements of *B. hindei* (Y= 0.56711- 0.00922SH - 0.0077HG + 0.01045LT).

3.4.3 Burrow soil characteristics

The soil in which burrows were present was loamy sand with pH ranging from 5.29 - 5.34 slightly lower than control (5.39 – 5. 61). Organic matter content ranged from 0.7 to 1.3% for shallow and deep burrows, respectively, while the range in the control sites was 0.8-1.0%. Sand- loam-clay content was 76%-4%-6% in shallow burrows, 87%-12%-13 in deep burrows, while sand, loam and clay content ranges in control sites were 77.2-84.4%, 4-22% and 6.6-12.6%, respectively.

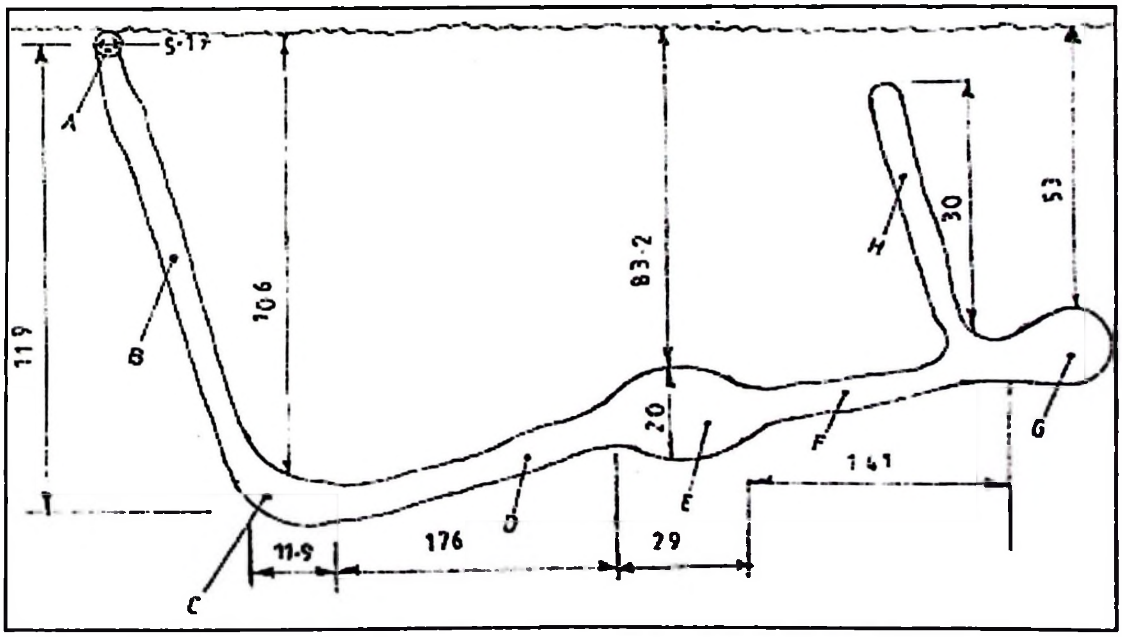

Figure 3.2: A sketched diagram of a typical burrow of a female adult *B. hindei* all dimension in centimeters. SCALE 1:3

A= Entrance opening (EO); B = Vertical entrance shaft from surface (VES); C =Earth plug; D = Primary horizontal shaft (PHS); E = Nesting and storage chamber (NC); F = Secondary horizontal shaft (SHS); G = Latrine chamber; H = Vertical tunnel extending to within a few centimeters from surface.

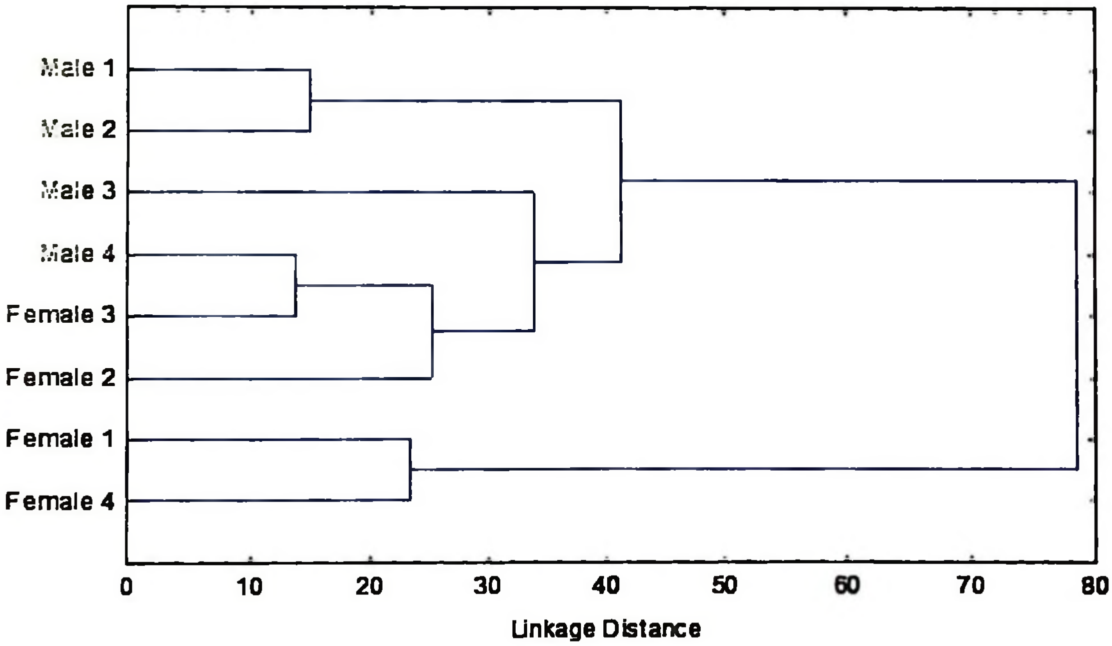

Figure 3.3: Tree diagram representing the similarity of burrow characteristics for 8 *Beamys hindei*.

Table 3.2: *Beamys hindei*'s burrow structure measurements (cm)Entrance opening (EO); Vertical entrance shaft (VES); Primary horizontal shaft (PHS); Nesting and storage chamber (NC); Secondary horizontal shaft (SHS)

SEX	WT(g)	EO	VES	PHS	NC	SSHS
Male 1	48	5.5	26	72	8.7	52
Male2	52	5.5	36	72	13	62
Male 3	55	5.6	27	100	20	90
Male 4	90	5.3	67	132	20	102
Female1	6	5.1	119	176	20.5	141
Female2	56	5.5	58	112	24	85
Female3	51	5.2	74	130	26	92
Female4	70	5.2	132	193	22.6	132

3.4.4 Diet

Plant materials (roots, leaves and stems) were dominant in *B. hindei*'s stomachs, with an average percent volume of (30.5 ± 9.38). Likewise, plant materials had a high relative importance value (51.75) (Table 2). A large percentage volume of food material (57.5 ± 9.41) was unidentified possibly due to digestive processes which were categorized as 'other'.

Table 3.3: Percentage contribution (PC), average percent volume (PV), importance value (IV) and the relative importance value (RI) of food items found in the stomachs (n=10) of *Beamys hindei*.

Food items	PC	PV (%) (mean ± SE)	IV	RI
Plants material	70	30.5 ± 9.38	20	27.87
Grain/seeds	10	0.5 ± 0.5	0.05	0.06
Fruits	30	3.5 ± 2.11	1.05	1.37
Invertebrates	30	8.0 ± 4.23	2.4	3.13
Hair	0	0	0	0
Others	90	57.5 ± 9.41	51.75	67.55

3.5 Discussion

3.5.1 Home range and overlap

This study found home range of male *B. hindei* in Zaraninge coastal forestto be larger than those of females. Although there have been no previous studies on the home range of *B. hindei*, the patterns observed in this study are similar to those seen for the East African Pouched Mouse *Saccostomus mearnsi* (Keesing, 1998). The average home range size for male *S. mearnsi* was about 0.21 ha and 0.06 ha for females, while in our study male *B. hindei* home ranges were 0.23 ha and those of females 0.15 ha (0.02 ha for female adults). These two species belong to the same subfamily Cricetomyinae but occupy different habitats and different ranges (Keesing, 1998; Kingdon, 2003). Various factors can contribute to larger male home ranges, as pointed out by Ostfeld (1990), male movement can be strongly influenced by the location of potential mates, while female space use is more likely to be determined by resources (food, protection) and rearing of offspring. Our study also found adults to have larger home ranges than subadults. This difference could probably be due to the fact that subadults take time to establish their home ranges and thus stays close to their burrows. As observed in this study, subadults moved more slowly after being released from the trap at the trapping location than did adults. possibly due to searching and locating a new habitat for food and burrow placements. while adults animal were actively moving using connectivity with a clear identification to their burrow. Adult females had much smaller home range than other demographic categories. The most likely explanation for this would be that female adults stay close to their burrows in order to protect their young (Borremans *et al.*, 2013; Kolb, 1985).

Home range overlap was significantlynot affected by demographic category. These results are insufficient to support the territory behavior of *B. hindei* for both sexes if it exists. The pairing behavior of *B. hindei* under captivity (Egoscue, 1972) shows a male to accept attack from non-receptive females, while females in estrus showed interest in males, initiating courtship display to males. This habit could probably explain the possible overlaps of males into female's home ranges and vice versa.

3.5.2 Movement

Despite the smaller home ranges, female movement tended to be longer than that of males, although statistically no significant difference was observed. One possible explanation for this could be that females have to move more to find extra food to feed themselves and the young in the nests. As observed by Egoscue (1972), under captivity, youngs were able to make their own excursion from the nest when they have attained a weight of about 40 g, usually after one month. Many of subadults captured in our study weighed between 30 to 40 g, which could explain the longer movements of females, and could also mean that female movement decreases again when their young become independent.

3.5.3 Burrow morphology and soil characteristics

Burrow morphology of *B. hindei* structural was similar to that described by Hanney and Morris (1962) on *B. major* having store and nesting chamber separated from latrine (Figure 2). An insect of the genus *Hemimerus*, a common ectoparasite known to be associated with the two rodent genera *Beamys* and *Cricetomys* (Nakata and Mata, 1974) was found in nesting chambers, the same hasbeen reported by Hanney and Morris, (1962). The elaborate burrow architecture used by *B. hindei* justify that the species spends most of its time in the burrow due to the presence of pile food stuffs and hips of feces found in the latrine chamber and it becomes out mainly for food searching and collections and probably for mating. The vertical entrance of the burrow is joined with the primary horizontal shaft at an angle of about 80^0 with a plug just before the angle which could possibly facilitate protection against predators such as small genets or snakes (Smith, 1967; Hickman, 1973).

3.5.4 Burrow placement and habitat association

Burrows were found deeply constructed underground, with important parts of the burrow (nesting and storage chamber, primary and secondary shaft) constructed in loamy sand. Although loamy sand soil is dominated by sand particles, it has smaller pores than pure sandy soils, which makes it ideal for the construction of permanent burrows that still allow air penetration (oxygen) and less rain seepage. Some vegetation types (shrubs, herbs, grass and leaf litter) were found to have an association for burrow placement, most likely for protection reasons (Kenagy, 1973). FitzGibbon *et al.* (1995) observed *B. hindei* to prefer areas with a high density of shrubs. The same trend was observed also for *Mastomy natalensis* to prefer densely covered sites (Leirs *et al.*, 1996).

3.5.5 Diet

The diet of *B. hindei* was found to contain a wide range of food varieties (Table 1) which were also seen in Egoscue (1972) and on related species *B. major* (Hanney and Morris, 1962). Non-identified food 'other' materials constituted the highest proportion of the diet, which could be contributed due to the trapping method employed in this study (CMR) where the animal is recovered live and spends a long time in the trap while digestion continued, a processwhich could hamper the identification of food items. Other studies (Mulungu *et al.*, 2013; Mulungu *et al.*, 2011) minimize the problem of continue digestion by employing snap traps where an animal is instantly killed and digestion stops. However, the method is non selective which could have killed non targeted species of study. Of the identified food items, plant material (stem, roots, and leaves) was the dominant food type, which supports the findings of Hanney and Morris (1962) on related species *B. major*. This trend of diet behavior is reported also by Mulungu, *et al.* (2014) in *Mastomys natalensis* where vegetative plant materials contributed significantly in the diet composition in the stomachs of *M. natalensis* found in irrigated environment where green vegetation are present through ought the year. Invertebrates contributed markedly to the diet of *B. hindei* which could be an important part of the diet as a source for protein (Monadjem, 1998). Grains/seeds and fruits occurred in relatively smaller quantities in stomachs. However, fruits and seeds were dominant food types found in burrows, these can probably be linked to contribute on the large percentage volume obtained on non-identified food items since foods such as fruits are easily digested (Witmer and Van Soest (1982). The species being a good climber as observed in this study is able to collect fresh leaves and culling of fresh fruits from trees as we found seeds and fruits in nesting chamber. Large leaves collected were used for nest making to keep the residents dry and warm in burrows (Kolb, 1985); collected thin young leaves and herbs were presumably used for diet (Hanney and Morris, 1962). Results suggest that space use of lesser poached rat is influenced by dietary behavior, physical soil properties and vegetation. This study support lesser pouched rat as burrow user, food storing behavior makes an animal to spend more times in burrows.

3.6 Acknowledgements

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CHAPTER FOUR

4.0 PAPER 3: Distribution, home range and activity patterns of black and rufous sengi *Rhynchocyon petersi* in Tanzanian coastal forests

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Abstract

A study on the distribution, home range and activity patterns of *Rhynchocyon petersi* was conducted in selected coastal forest of Tanzania between March 2011 and July 2013. Camera trapping and Radio telemetry were used for data collection. *Rhynchocyon petersi* were found to be distributed in all six forests assessed. Camera trap rate was used as proxy for response to vegetation variables where there was no significant differences between forests sites ($F_{5,72} = 1.84$, $P = 0.115$). No difference was observed between females and males on nest sizes. Results from tracking showed generally that *R. petersi* prefer shrubs vegetation for nests placements. However, females preferred areas with dense shrubs ($P<0.0001$) where males preference were areas with leaf litter ($P<0.0001$). Average home ranges for *R. petersi* was 2.64 ± 0.31 ha with no difference between sexes. There was a significant difference on activity patterns between seasons where more activity was observed during dry season (F =9.75, df 1, 28, p = 0.004). No differences on activity was observed between time and season (F = 0.02, df 1,28, p = 0.89) and between times morning and afternoon (AM/PM) (F = 3.76, df 1, 28, p =0.06). The study suggests that, the existence of *R. petersi* in all forests is associated probably with a combination of essential variables for survival present in all forests. Using home range results it is now possible to estimate the number of individual *R. petersi* which may occupy a particular forest and that information could be linked to conservation of the *R. petersi* in coastal forest and related habitats.

Key words *Rhynchocyon petersi*, habitat, behavior, home range, activities.

Introduction

Coastal forests are biological treasure house to many of large diversity species of flora and fauna with a range of exceptional levels of endemism (e.g. Burgess *et al*., 2000). However, like many other forest habitats, coastal forests have continued to decrease in their sizes due to the increase in human demands on land use for cultivating, logging and clearing land for other developments activities which are considered to be a threat to the survival of these forests including their fauna (Myers, 1988).

Among the fauna found in the coastal forests includes the elephant shrews or sengis which belong to Macroscelididae, a single family known to be endemic to Africa (Corbet and Hanks, 1968).The family have 18 extant species with well-defined sub families: the soft furred sengis (Macroscelidinae) with three genera and 14 species and the giant sengis (Rhynchocyoninae) with a single genus with four species (Ruthbun, 2009). The four giant sengis includes the checkered sengi, *Rhynchocyon* *cirnei*, the golden-rumped sengi *Rhynchocyn chrysopygus* known to be endemic only to coastal forest of Kenya (Corbet, 1971), the grey faced sengi *Rhynchocyon udzungwensis* (Rovero *et al*., 2008) endemic to Udzungwa Mountain forests and the black-and-rufous sengi *Rhynchocyon petersi* Bocage 1880 the species of current study.

All the giant sengi share similar life histories in that they are small shy animals (weighing 450-700 g), diurnal insectivores, found in lowland and montane forests and dense woodlands (Rathbun, 1984) with altitude ranging from sea level to about 2,500 m above sea level (Rovero *et al.*, 2013). They construct nests of dead and leaves on the forests in which they spend night and give birth. The dimensions of nests based on the study on *R. chrysopygus* (Rathbun, 1979), are about one meter wide, with a body sized bowl of 20 cm long, 15 cm wide and 10 cm deep, and that each can maintain up to ten nests (FitzGibbon and Rathbun, 1994).

All the species of giant sengis are currently of conservation concerned (IUCN, 2015) thus knowledge on the factors determining their distributions, home range and their activities patterns are important for establishing their conservation plans. Key factors that have limited the studies on distributions, home range, activity patterns are thought to be due to the difficulties involved in the whole process of catching sengis and the difficulty of working in dense forests (Rathbun, 2009, Sabuni, 2011, Rovero *et al.*, 2013).

Studies of giant sengis in particular *R. petrsi* are rare and have been mainly based on abundance estimation on the detection and nests counts of which their results have been referenced to that of *R. chryopygus* (Coster and Ribble, 2005; Hana and Anderson, 1994; Rathbun, 1994). However, in recent years there have been the increase in the use of camera traps on the study of sengis (Rovero *et al.*, 2008; Adanje *et al.*, 2010) which has resulted in opening avenue for gathering more quantitative information such as distribution and habitat association (Bowkett *et al.*, 2008). Similarly, camera have been in use for studying the secretive species such as carnivores (Pettorelli *et al.*, 2009; Msuha *et al.*, 2012) and also in estimating abundance (Noss *et al.*, 2003; Karanth *et al.*, 2004; Boris *et al.*, 2006; Jackson *et al.*, 2006; Wang and Mackdonald, 2009).

Complement to that, radio telemetry has been in place for gathering a wide range of information on a particular animal species including distribution, home range, habitat use and activity patterns including social behavior (e.g. Pierre, 1977; Betrolino *et al.*, 2003; Wong *et al.*, 2004; Rathbun and Rathbun, 2006; Marianne and Sandra, 2006). The information on home range, as an area that is traversed by individual for shelter, mating and essential basic needs for survival (Burt, 1949) is important since it gives an insight to how much area is occupied by individual animal.

The objectives of the current study was to utilize both camera tapping and radio telemetry methods to determine: (1) the current status on the distribution of *Rhynchocyon petersi* in selected coastal forests with vegetation association (2) home range and activities patterns including time budget and nesting behavior in terms of their placements and vegetation association.

MATERIALS AND METHODS

Study area

The study on the distribution of *Rhynchocyon petersi* was conducted in six coastal forests which are found in and around Saadani National Park (SANAPA. The park which is located between $05^0 59$S and $38^0 45$'E, is one of the 16 Tanzania National Park bordering Indian Ocean. One of the objectives of establishing SANAPA was to conserve a rich vegetation fauna including the coastal forests.

The Park encompasses Zaraninge forest, Askari, Mbulizaga, Madete and Kwamsisi forest while Gendagenda forest is outside the national park located in the north and is exclusively managed by village community (Sabuni *et al*., 2015). The forests are characterized by evergreen dry forests, exists in disjuncture patterns which are separated by matrices of mixed vegetation of grass woodlands, small patches of green thickets, miyombo woodlands and farm lands. Studies on the determination of home range and activity patterns including budget time and nesting behavior was conducted only in Zaraninge forest where previous information of presence of *R. petersi* was available and for the concentration of data based on difficulty of catching sengi (Hana and Anderson, 1994; Kiwia, 2006; Sabuni *et al*., 2011).

Methods

Distribution of *Rhynchocyon petersi*

Distribution patterns of *R. petersi* were conducted in the six forests using camera traps-survey between March 2011 and July 2013. Ten camera traps (Reconyx Hyper fire HC 500 (Reconyx, Inc) semi – covert infrared emitters) were set spaced at about

200 – 500 meters apart through the study forests. The study assumed that the position of points in setting camera is random with respect to sengi's habitat use. However, around the specific camera locations, specific signs were surveyed before setting the camera to maximize photographic capture rate, this included identification of a distinct path which was assumed to be used by sengi. At the camera view, the area was cleared by cutting some of small tree and shrubs so that camera is not blocked while taking photos.

Each station consisted of one camera tighten to a trunk of tree about 50 cm above the ground depending on the terrain. Each camera was programed to take 3 pictures per trigger and a picture interval of one second between pictures with no delay between triggers (Rovero *et al.*, 2013). Cameras were active continuously (24 hours a day) and left at each site for at least 15 days before moved to other site. On the last day after removing camera, vegetation variables were measured at each camera trap site deployed. Vegetation assessmentprotocol as developedby Bowkett *et al.* (2008) was followed with slight modifications based on the site and study species and study of Coster and Ribble (2005) and Fitzgibbon (1994). Variables measured were vegetation cover *vis-à-vis* proportional percentage cover of leaf litter, shrubs, herbs and grass, seedlings, stems, logs. Number of large and medium trees (DBH 10 (≤ 31.4 cm) and DBH 5 (≥ 15.7<31.4 cm)) within the radius of 10 m from camera traps were measure and counted. Species and number of small plant stems (<5cm diameter at ≥1 m height) were counted in four 3 × 3 m plots set randomly within 10 m around the camera traps. Proportional percentage ground cover (shrub, herbs and grass, stem, leaf litter, seedlings, logs) was estimated visually in four sub plots of 1 ×

1 m set at the corners of each 3 × 3 m plots within a radius of 10 m from the tree with camera trap. Percentage canopy cover was estimated visually as the amount of sky visible overhead at the camera trap tree.

Trapping *R. petersi* for determination of home range, activity patterns and nest placements

Trapping was conducted between 10 October to 18 December 2011, 10 March to 21 April 2012 and 10 July to 17 October 2012. Live individuals were captured using three types of traps wire mesh traps, fish nets and camera traps. 20 single door non collapsible wire-mesh traps (Tomahawk, WI 54487, USA model 102, 13×13×40.5 cm) and 25 medium collapsible single door wire mesh (Havahart Animal Trap, UK. Size 20 × 61×20 cm) were set in the distinct paths made by the four-toed sengi *Petrodromus tetraductylus* following (Rathbun, 1979) and in paths which we thought are made or used by *R. petersi* and or small antelopes. Traps were left at each site at least for three weeks before moving them to other sites; no bait was used during trapping since it has been observed to have no positive influence on catching the *R. petersi* (Sabuni *et al.*, 2011). Traps were inspected three times per day (morning, midday, and just before sun set).

Fish nets made of multi-filament nylon specified as 21OD/2/6 with a 7.6 cm stretch mesh and 66 cm deep varying in length of 50 and 100 meter were bought from fishnets shops supplied in white colour. Nets were later dyed by dipping into green colour emulsion and let them to dry with the aim of camouflaging into vegetation colour.

Fifty nets were laid following (Rathbun, 1979), in a narrow transect line of about 100 m, grass and small vegetation were cut and removed in a line where nets were to be set. Each net was prepared by stringing with a manila rope in a line through the mesh at the top and bottom so that it could be anchored and hung such that about 1/3 of the net was loose on the floor of the forest and supported by sapling stakes pushed down into the ground forming a vertical panel about 1 meter. This was done purposely such that when an animal moves through the net it can move freely without obstacles until it entangles itself. Traps were checked three times per day (morning, midday, and just before sun set).

For all animals captured, we recorded individual's body weight and sex. Individuals were fitted with radio transmitter M1610 made from Advanced Telemetry System (ATS) that weighed 3.5 g. The animal was released immediately at the point where it was trapped and the coordinates recorded by Global Position System (GPS) Gamin 60. Collared animals were tracked on foot and located using a receiver model R410 and a 3-element hand held Yagi antenna model F152-3FB made from ATS. Locations were recorded multiple times per day alternating the time of tracking for each individual. For each fix, the animal was located within 10 to 20 meters and as close as one meter at the nest using signal strength. After all nests of individual *R. petersi* were established the animal was trapped at the nest and removed the collar.

Data analysis

Distributions of *R. petersi* were ascertained based on presence of photo image in the camera at each site. A camera-trap rate was calculated following Bowkett *et al*

(2007) as the number of photographs of a species divided by the number of trap-days per site. Trap days were computed as the number of 24 hours periods from the deployment of camera until when the camera was retrieved. Photographs image were counted at interval of one hour as a single events, for those images where the same species appeared more than once within one hour were accounted as single event. Camera trap rates were used as response variables to vegetation and other habitat variables. Generalized linear model (SAS 1990) was used to describe the relationship between camera trap rates and vegetation variables for *R. petrsi*, similarly the analysis was applied for nest placements.

Software RANGE 8 (Kenward, 2009) was used to calculate home range sizes using Kernel contours at 95 % and an area where animal was mostly found at 50 %. Home range overlap was expressed as the percentage of overlap of *R. petersi*'s home range with the home range of all other *R. petersi* which were found to interact with each other.

Activity pattern was taken using time and date when animal was sighted during the whole period of tracking. A total of sightings were pooled together from all tracked animal on time bases each at one hour interval. Similarly, daily activity budget was estimated according to what an individual was doing when sighted which were; foraging, running, pair, moving and resting. Animal was considered foraging when was sighted walking with its mouth trunk pointed down and or when it was seen standing and scooping on leaf litters. Running was considered when an animal was actually seen running, when two animals were seen walking closely together sometime one in front and one behind and or side by side was considered as a pair.

Moving was an activities which was recorded when animal was not physically seen during tracking due to the vegetation cover but when approached the strength of the receiver signal increased and when further approached the signal strength decreased indicating that the animal was moving. In few sighting the animal was seen calmly resting under the thicket or under a fallen tree sheds and in the open of leaf litter and this activity was referred as "resting". On few instance the animal assumed to rest in the nest when it stays for approximate 1 to 2 hours. During radio telemetry tracking we were able also to identify and count number of each individual's nests.

Results

Distribution of *Rhynchocyon petersi*

Two hundred and fourteen independent photographs of *R. petersi* were recorded from all the six coastal forests with lowest events recorded in Madete forest (Table 4.1). Camera trap rates did not differ between forests sites ($F_{5,72} = 1.84$. $P = 0.115$), Camera trap rates were positively affected by herbs and grass, shrubs, leaf litter, seedling and leaf litter depth and negatively affected by stem, bare soil, canopy, logs and density of large and medium tree (Table 4. 2).

Table 4.1: Sampling detail and camera trap rate (number of captures) for *R. petersi* in six forests

Forest	Number of cameras and events	Trap-days	Trap rate
Askari	10 (11)	3600	0.028
Gendegenda	10 (32)	3600	0.084
Kwamsisi	10 (23)	3600	0.059
Madete	10 (2)	3600	0.004
Mbulizaga	10 (33)	3600	0.088
Zaraninge	27 (113)	9720	0.297

Number in the bracket are events number outside are number of camera trapped per forest.

Table 4.2: Variables from partial correlation coefficient of *Rhynchocyon petersi* as measured by camera trap rate in the Askari, Gendagenda, Kwamsisi, Madete, Mbulizaga and Zaraninge forests

Variables	Trap rate	P
Percent of stem	- 0.229130	0.0512
Percent of herbs and grass	0.028339	0.8119
Percent of shrubs	0.168856	0.1533
Percent of bare soil	- 0.156278	0.1867
Percent leaf-litter	0.015045	0.8995
Percent of seedling	0.015045	0.8995
Percent of leaf litter depth	0.147488	0.2131
Percent of canopy	- 0.108633	0.3603
Percent of logs	- 0.055600	0.2427
DBH 5 ($\geq$ 15.7<31.4 cm) density	-0.176273	0.1358
DBH 10 ($\leq$ 31.4 cm) density	-0.084232	0.4786

Trapped *R. petersi*

A total of 19 individuals (nine males and ten females) fitted with radio-collars of these, four were caught by wire mesh traps out of 2,880 trap days with a trapping success of (0.001/2,880 trap days) and 15 individuals in the fish nets out of 3,200 trap days with a trapping success (0.0046/3,200 trap days). All animals were caught during the day time; recaptured individuals were found only in fishnets traps and not in wire mesh traps. Out of 19 animals trapped, RP 8 was pregnant and RP 16 was sub adult female, their data were excluded in determining the average body weight of adult as well as for home range determination. Two animals RP 15 and 17 were preyed shortly after being released their data are excluded in home range estimation. The average body weight for *R. petersi* (n= 17) was 451.94 ± 6.8 g where males (n=10) was (469.33 ± 7.72 g) and females (n=7) (432.3 ± 5.67 g) with significance different between sexes (t-test, t = 3.79, df 18, p = 0.00).

Ranges areas and overlap

A female RP 8 which was pregnant and RP 16 a subadult female, their data were excluded in the determination of home range area. An average home range for *R.*

petersi (n=14) by kernel estimation in Zaraninge forest was 2.64 ± 0.31 ha. When sex was examined, range areas for male (n = 8) was (2.65 ± 1.29 ha) and female (n = 6) (2.62 ± 1.49 ha) with no significant difference between sexes (t-test, t = 0.04, df 8, p = 0.48). Home range forindividual *R. petersi* is presented in (Table 4.3)There was no correlation of body weigh to home range size (correlation = 0.45, p = 0.082). Amale RP 18 and female RP 2 had their home ranges approximately twice larger than the average mean home range observed (5.72 and 4.54 ha respectively). The kernel densities (Figure 4. 1) indicate the RP 2 had two main centers of activities presented with 50% of fixes referred as core area, while RP 18 tend to expand its home range but with a single core area.

Overlap was observed only on four animals RP 6 and 7, and RP 9 and 10 (Fig. 4.1). Females had high percentage overlaps compared to males (Table 4.4). No overlap was seen on two males (RP 3 and RP 4) (Fig 4.1 e). Based on average home range (2.64) and on the observed overlap of 26 - 89% (average 57.3%) in home ranges of the four neighboring paired animals (Table 4. 4), we estimated an average of "exclusive" home range of 1.54 ha. Assuming that *R. petersi* occupy Zaraninge forest evenly, and completely in this fashion, population density at this site is estimated to be 1.1 ha (SE= 0.31 ha).

Table 4.3: Estimated home range of *Rhynchocyon petersi* (RP) in Zaraninge forest

Id	Sex	Radio locations	Weight	Kernel 95% (ha)	MCP 100% (ha)
RP1	Female	129	449	2.93	4.55
RP2	Female	95	450	5.27	5.85
RP3	Male	112	490	1.95	2.69
RP4	Male	77	450	2.55	3.97
RP5	Female	83	430	1.446	1.47
RP6	Female	108	422	0.99	1.37
RP7	Male	170	468	1.96	3.59
RP8	Female	pr	498	pr	
RP9	Female	95	438	1.49	1.43
RP10	Male	154	488	2.17	2.08
RP11	Female	54	442	3.07	3.33
RP12	Male	130	468	1.78	2.16
RP13	Male	212	488	3.72	4.88
RP14	Female	130	403	1.56	2.11
RP15	Female	p	422	p	
RP16	Female	sb	340	sb	sb
RP17	Male	p	416	p	
RP18	Male	160	490	4.54	10.01
RP19	Male	129	469	2.59	3.65

p = preyed, sb = sub adult (got lost) and pr = pregnant

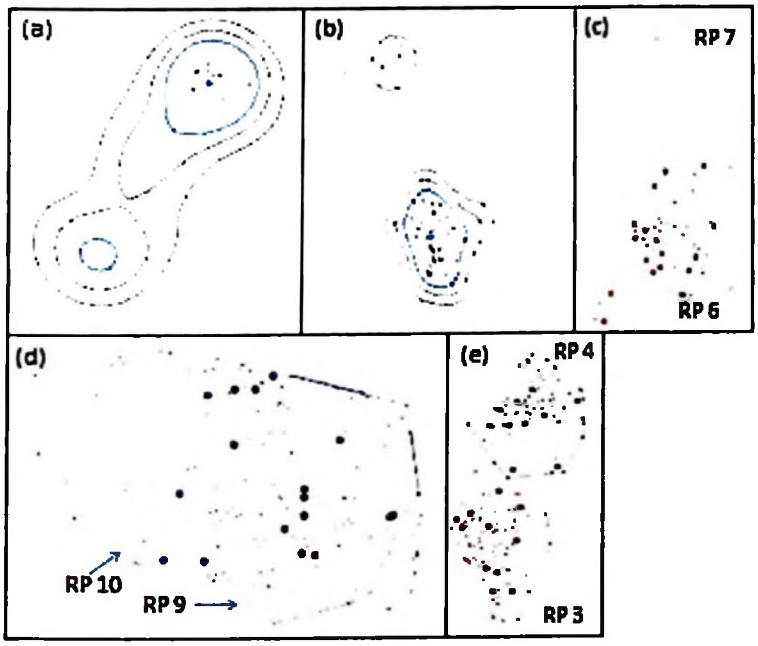

Figure 4.1: Example of Kernel home range for RP 2 (a) and RP 18 (b) and MCP for pair RP 6 and 7 (c), and RP 9 and 10 (d), and the male RP 3 and 4. The outer circle for figure (a and b) represent 95% fixes and the inner circle represents 50% fixes.

Table 4.4: Percentage overlaps of the two pair of *R. petersi*.

Overlap %	AGE	SEX	RP 6 (m^2)	RP 7 (m^2)
RP 6	adult	female	100	52.16
RP 7	adult	male	26.44	100
			RP 9 (m^2)	RP 10 (m^2)
RP 9	adult	female	100	89.22
RP 10	adult	male	61.35	100

Activity patterns

Both radio tracking and camera traps were used to determine the activity patterns of *R. petersi*. A total of 671 sightings were observed from radio telemetry. males were sighted 415 and females 256. No any significance differences were observed on: Sex (F = 2.54, df = 1, 26, p = 0.12), Time (F = 0.01, df = 1. 26. p = 0.92) and Sex interaction with time (F = 0.00, df = 1, 26, p = 0.97).

Thirty camera trap stations set during rainy season (March to May, 2011) and the dry season (June to August, 2012) (Fig. 4.3) three camera stations did not work. A total of 106 *R. petersi* photographs over 404 camera days were recorded during rainy season and 259 *R. petersi* photographs over 450 camera days during dry season, representing a capture percentage of 0.26 and 0.56 per camera day for rainy and dry season respectively. A photograph in the camera was considered independent when there was at least one hour interval between them (Bowkett *et al.*, 2007). There was a significant difference on activity patterns between seasons where more activity was observed during dry season (F =9.75, df 1, 28, p = 0.004). No significance differences was observed between time and season (F = 0.02, df 1,28, p = 0.89) and between times morning and afternoon (AM/PM) (F = 3.76, df 1, 28, p =0.06).

Results from both radio telemetry and camera traps had some similarities on *R. petersi* activity patterns. In both *R. petersi* were out as early as 5.00 AM and the activity stopped just after sunset 7.00 – 8.00 PM. Activities were more pronounced during mid-morning hours. The high activities peak from radio telemetry were observed around 10.00 – 11.00 AM and from 5.00 – 6.00 PM while on camera traps activity peaks were 7.00 – 8.00 AM for both wet and dry seasons (Figure 4.2 a and b).

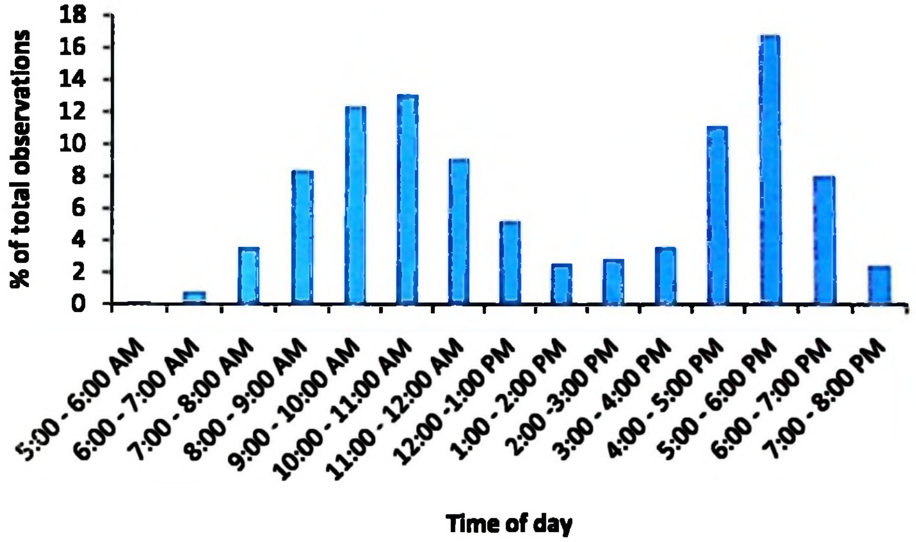

Figure 4.2 (a): ***Rhynchocyon petersi*** **activity patterns in Zaraninge forest (% of sightings per hour) during radiotracking.**

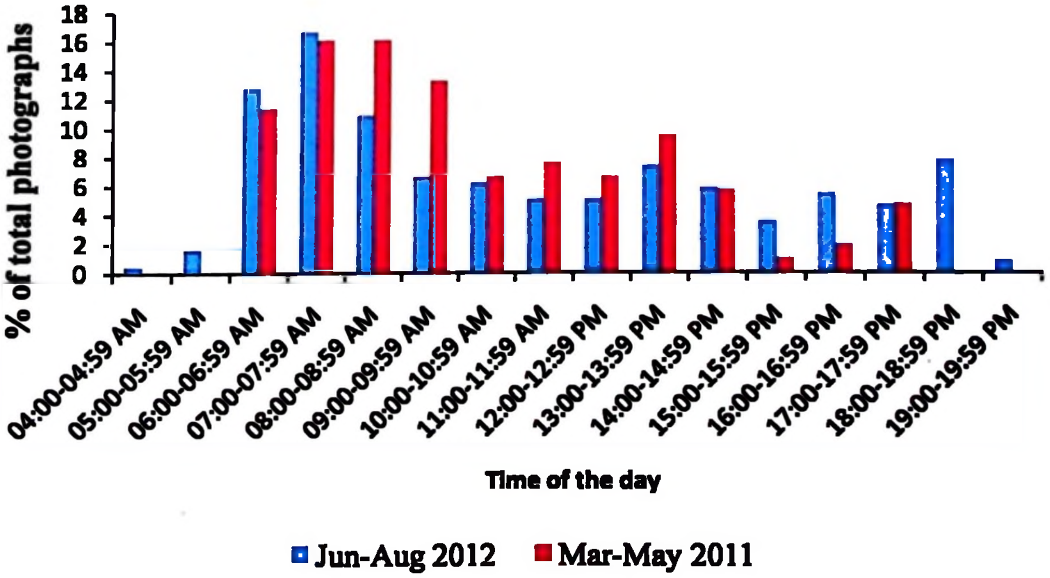

Figure 4.2 (b): ***Rhynchocyon petersi*** **activity patterns in Zaraninge forest according to camera traps (% of photographs per hour)**

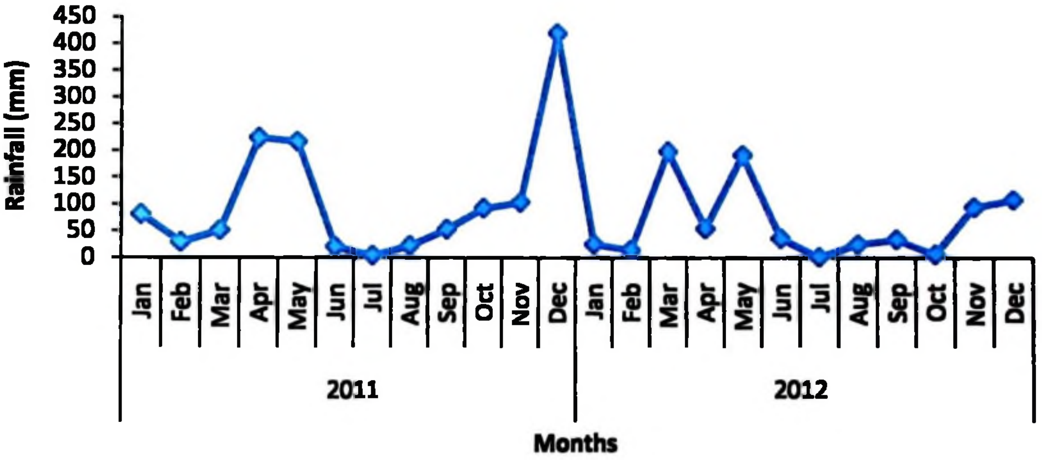

Figure 4.3: Rainfall patterns in Zaraninge forest.

Daily activity budget of *R. petersi*

Foraging constituted high percentage of the total time budget used by *R. petersi* (Figure 4.4). When sex is examine, there was significance difference on moving between males with mean of 48.33% and females 17.0 ($F = 5.08$, df 1, 14, $p = 0.04$). No significance difference were observed between sexes on foraging, run, rest and pair respectively; ($F = 0.46$, df=1,14, $p = 51$; $F = 0.17$, df = 1, 14, $p = 0.69$; $F = 0.29$, df = 1, 14, $p = 0.59$; and $F = 0.01$, df = 1, 14, $p = 0.92$).

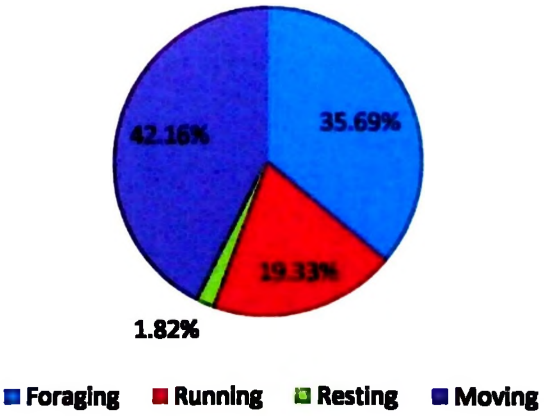

Figure 4.4: Activity budget estimates for *R. petersi* in Zaraninge forest according to radio telemetry

Nests placements and behavior

Nests recorded from each individual ranged from 9-12 for males (n=7) and 11-13 for females (n=7). Nests were made of excavate bout shape hollow with inside lined with tree leaves semi dry and on top was a construction as an architect of leaf litter mound with invisible of two opening where animal uses to get in and out systematically. There was no significant different on *R. petersi* nests placements to many of vegetation variables assessed. however, shrubs and leaf litter were the only vegetation variables where nests placement was preferred (Table 4.5a and b). When compared between sexes. no significant different was observed on sizes of nest's hollow (depth and width) which was 21.28±1.28: this was similar to vegetation variables assessed. Significant different was observed on shrubs and leaf litter to have contribution on nest placements where females preferred shrubs (23.0±1.68) and males preferred leaf litter (53.94±2.21) (Table 4. 6 a and b).

Table 4.5 (a): Variables from generalized liner models for nest placements of *R. petersi*SV=source of variation; df=degree of freedom; DBH5= diameter at breast height of tree (≥ 15.7<31.4 cm); DBH10 = diameter at breast height of tree (≤ 31.4 cm); LOG=logs; STE=stem; H/G=herbs and grass; SRB=shrubs; SDL=seedlings; LT=leaf litter; CAN=canopy

SV	df	Mean square								
		DBH5	DBH10	LOGS	STE	H/GR	SRB	SDL	LT	CAN
Indiv	49	5.21	3.66	8.23	28.68	4.78	68.10	11.02	151.24	415.81
Position	1	64.0	47.6	0.01	10.24	23.04	2480.0***	196.0	2540.16***	225.0
Error	49	3.75	5.36	8.17	23.13	3.91	82.41	14.89	139.83	115.81
Total	99									

(b) Nest placements

Variable	Position		LSD.
	Nest	No nest	
DB5	3.04±0.27	4.64±0.27	NS
DB10	3.22±0.32	4.6±0.32	NS
LOG	5.38±0.40	5.4±0.40	NS
STE	12.84±0.68	13.48±0.68	NS
HGR	3.58±0.27	4.54±0.27	NS
SRB	21.28±1.28	11.32±1.28	9.90
SDL	9.60±0.54	6.80±0.54	NS
LIT	48.18±1.67	58.26±1.67	10.08
CAN	62.0±1.62	65.0±1.62	NS

Table 4.6 (a): Variables from generalized linear models for nest placements of *R. petersi* and sexes. SV=source of variation; df=degree of freedom; DEP=depth of nest hollow; WD=width of nest hollow; DB5= diameter at breast height of tree (≥ 15.7<31.4 cm); DB10 = diameter at breast height of tree (≤ 31.4 cm); LOG=logs; STE=stem; HGR=herbs and grass; SRB=shrubs; SDL=seedlings; LT=leaf litter; PLC= nest placement position (under tree or not); CAN=canopy

		Mean square											
SV	df	DEP	WD	DB5	DB10	LOG	STE	HGR	SRB	SDL	LIT	PLC	CAN
Indiv.	6	3.45	1.5	3.98	5.25	5.42	67.55	7.74	149.96	22.19	244.73	1.45	85.71
Sex	1	3.05	1.42	1.42	3.65	18.51	21.72	2.05	1064.7**	30.22	1444.6**	2.26	142.85
Error	62	2.13	1.94	2.96	4.76	7.43	21.02	5.02	99.41	24.26	171.99	0.31	310.92
Total	69												

Table 4.6 (b): Size of burrows and nests placements between female and males

Variable	Mean Sex		
	Female	Male	LSD$_{50}$
DEP	12.05±0.24	12.0±0.24	NS
WD	20.45±0.23	20.74±0.23	NS
DB5	3.17±0.29	3.45±0.29	NS
DB10	3.00±0.36	3.45±0.36	NS
LOG	5.34±0.46	4.31±0.46	NS
STE	13.62±0.77	12.51±0.77	NS
HGR	3.88±0.37	3.54±0.37	NS
SRB	23.0±1.68	15.2±1.68	7.8
SDL	9.0±0.83	10.3±0.83	NS
LIT	44.85±2.21	53.94±2.21	9.09
PLC	1.3±0.09	1.5±0.09	NS
CAN	59.8±2.98	62.7±2.98	NS

Discussion

The question that black-and rufous sengi with other related species of giant sengis carry different levels of conservation categories (IUCN, 2015) calls for attention on the determination to their current status in distribution in the coastal forests and in other related ent habitats.

Our current studies using camera traps show promising distribution that. the species is found in all the six forests assessed despite of their separation and sizes. And indeed. the presence of *R. petersi* in Zaraninge. Askari. Kwamsisi. Madete. and Mbulizaga the forests which are found within Saadani National Park which gives the highest level of conservation and protection of the species *in situ* compared to the Gendagenda forest which is outside the park and managed by village cmmunity. Gendagenda forest is subjected to environmental degradation due to increasing of human activities (farming. fire and timber) in the forest that are threat on the survival of the *R. petersi* as observed in this.The distributions of *R.petersi* show a complex vegetation association in relation to their distributions. suggesting that a combination of a variety of vegetation variables contributes to microhabitat which support on their existence in these forests. The disjuncture pattern of forest patches in and around SANAPA could explained that, possibly the species could use some of small forest patches between the large ones probably for living and also as stepping stone to other forests which make the species to be present in all six forests studied.

The home range data were reduced due to the loss of two preyed *R. petersi* and the exclusion of the pregnant and one sub adult. Similarly. the presence of thicket vegetation in some parts in the study area hindered chances of seeing individual during tracking and hence failing in identification on which activity a particular individual was doing. All captured individuals by wire mesh traps. their traps were set in very distinct trail which can explain that. *R. petersi* uses distinct paths which are different from that of *Petrodromus tetradactylus* (FitzGibbon. 1995)this was also observed duringtraking.

For trapped individuals males had large weight than females. in the study of closely related species *R chrysopygus* no different in weight was observed between sexes (FitzGibbon. 1995). In that study. *R. chrysopygus* had body weight averaged to 536 g and a home range of about 4.1 ha against 453 g and a home range of about 2.64 ha in the current study. The difference in home range of these two species is linked to the large difference of body weight which has influence in home range of animal (May. 1976). The home range between sexes did not show any significant different in this study. this could be possibly due to the fact that *R. petersi* form a monogamy bond like *R. chryopygus* (Rathbun. 1979) and thus most of the time they move togethera and thus sharing same area. During the course of radio telemetry. *R. petersi* probably covered their entire home ranges in few days since we commonly used the same patterns during tracking. As more time spent on radio telemetry. animals were observed to use portion of their home range more intensely than other parts within its range.

The patterns of home ranges in this study probably could be due to equal demands for both sexes on the necessary requirements such as shelter. resource and mating (Burt. 1949), and indeed if the monogamy behavior is experienced (Rathbun. 1979).

Exceptional large home ranges were observed on a female RP 2 and a male RP 18 which was about twice large than the average home range recorded. This could possibly be due to the shifting of home range for looking resources or escaping of predators or forming a new bond with new partner. The overlap between males and females was observed only for two neighboring pair with high percentage recorded on females compared to males. Although we have limited interacted pairs, the boundary of home range areas observed suggest the animal exhibits a territorial behavior where a female occupy large part of male's home range areas. This was also evidently observed on male RP3 and RP4 where despite of being trapped in the same fish net separated with one meter they did not interact or overlap throughout the entire tracking period. This explains that males and females in most cases share their territories although each individual sleeps in its own nests.

Activity patterns of a species are considered to be an adaptation to seasonal and diurnal variation in environmental factors (Nielsen, 1983). According to Aschoff (1964), the daily activity pattern of animal results from complex compromise between optimal foraging time, social activities, and environmental constraints. The results from both radio telemetry and camera trap exhibited a definite diurnal pattern activity, a behavior which was also observed on *R. chrysopygus* (FitzGibbon, 1995). The activities patterns on radio telemetry indicate that *R. petersi* activities were more pronounced in mid-morning with a constant decrease in the middle of the day and increased just before sun set. The activity pattern observed on camera photograph (a passive method) during rainy season started just after 6.00 AM while during dry season activity starts between 4.00 and 5.00 AM. During dry season there is less cloudy compared to rainy season and that sun raise starts as early as 5.00 AM which possibly stimulate *R. petersi* to wake early and also to avoid hot times. High number of photographs observed during dry seasons compared to that in rainy season could probably be due to scarce of resources (invertebrates such as termites, beetles, earthworm) (Rathbun, 1979) which could increase the frequency of individual in search of food. Study by (Beisiegel and Mantovani, 2006) has shown the effect of rain on invertebrate's abundance. Similarly, the low number of photographs observed during rainy season could possibly due to limitation of movements of animal when it is raining such that the animal spend more time in their shelter, and also the increase of resources during rainy season could limit the frequency on movements.

An estimate of daily activity budget by radio telemetry *R. petersi* in Zaraninge forest was predominated with foraging as the main activities. During tracking some individuals were seen walking and or running in pair but separated latter where each spends much of its time alone including sleeping.

Our observed results from radio telemetry on nesting behavior of the black-and-rufous sengi nests sizes did not differ between sexes, this can be linked to their body sizes and weights which did not also differ significantly between sexes as seen also in FitzGibbon (1995). The shape of the nest hollow which was made like a boat shape makes an animal to fit and stay in a posture ready to flew. An architecture used by black-and-rufous sengi of using leaves are probably linked to regulate conditions such as excessive temperature and rain and also as a nest for youngs (Kolber and Janzen, 2002) as well as for cryptic protection. During tracking, individuals were observed to maintain a large number of nests which could probably be related to predation avoidance. Thus an animal is able to get into another nest any time it senses a danger, a phenomena which was observed in this study.

On nest placements, black-and-rufous sengi preferred more nests placements in shrubs; when compared between sexes, females preferred nest placements in shrubs while male's nests were more placed in leaf litter. Other variables though were statistically not significant in nest placements are suggested also to play a contribution on nest placements. Choosing nest placements is a prime prerequisite for all animals using nests as part of the main basic requirement for their survival (Wells *et al.*, 2006). The observed nest placement pattern is connected entirely to the home range of *R. petersi*, thus when the animal is feeding it can escape to any neighboring nests for protection and it may be also linked to vegetation structure requirements. As seen for female black-and-rufous sengi, placements of nests in shrubs take on account to the protection of the nests and the offspring which are born and cared until they have attain age to look for their own food and shelter. Shrubs are used presumably also to enhance cryptic environments for protection of nests instead of being placed at an open which minimize predation risks. Also during tracking some parts of shrubs' roots were seen dug by *R.petersi* an activity which could be part of searching invertebrates (Rathbun, 1979) also it might be utilizing some food materials from shrub roots. Males nest placements preferred more leaf litter areas but it uses both environments since it roams for food and mating. Like *R. chryopygus* (Rathbun, 1979) the species is considered to be monogamy, but it lacks paternal parental investments (Rathbun, 1979) thus les care is taken in nest placements and individual construct its own nests.

During tracking we did not observe two pair together in one 'nest although pairng were frequently observed. However, whether diurnal activity of *R. petersi* evolved is linked to the habit of constructing several nests remains debatable. Due to increasing of forest degradation especially ton protected habitats, this study recommends the conservation status based on ICUN to remain as vulnerable. Further studiesshould be conducted using passive methods (camera traps) to ascertain presence of *Rhynchocyon* species in other related habitats.

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CHAPTER FIVE

5.0 PAPER 4: Development of eight polymorphic microsatellite markers in the Black and Rufous sengi, *Rhynchocyon petersi.*

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ABSTRACT

The Black and Rufous sengi, *Ryhnchocyon petersi,* is endemic to a limited range in East Africa. We report the development of eight polymorphic microsatellites using next generation sequencing technology. Eighteen individuals from Zaraninge forest (Saadani National Park, Tanzania) were genotyped. The number of alleles per locus ranged from 2 to 6, while the observed and expected heterozygosities varied from 0.17 to 0.82 and from 0.25 to 0.81, respectively. No locus deviated from Hardy–Weinberg equilibrium. These microsatellite markers will be useful tools to study the effect of habitat fragmentation on the population genetic structure of *R. petersi.*

Keywords: *Rhynchocyon petersi*, vulnerable Afrotherian, Microsatellites, 454 sequencing

Introduction

Rhynchocyon petersi Bocage, 1880, the Black and Rufous sengi (elephant-shrew) is one of the four known giant sengi from the subfamily Rhynchocyoninae which belong to the supercohort Afrotheria grouping together aardvark, tenrecs, golden-moles, elephants, hyraxes and sea-cows. This species is endemic to East Africa, distributed in the Eastern Arc Mountains and coastal forests of Tanzania and Kenya. These habitats show a high level of biodiversity and endemism but are threatened by fragmentation and degradation due to agricultural expansion, timber for various uses and charcoal production (Rathbun and Butynski, 2008). *Rhynchocyon petersi* is categorized as vulnerable due to the loss of its habitats (IUCN, 2014). Little is known about this species because no detailed field studies have ever been conducted (Rathbun and Butynski, 2008). Our aim was to develop microsatellite loci for future genetic studies.

Methods

DNA was extracted using Nucleopsin kit (Machery Nagel) from a piece of ear. The library construction and microsatellite optimisation were outsourced to Genoscreen (Lille, France). The high-throughput method used by Genoscreen is based on coupling multiplex microsatellite enrichment and next-generation sequencing on a Roche 454 GS-FLX Titanium platform (Malausa et al. 2011). DNA from 11 individuals from 3 forest patches from Saadani National park (Zaraninge, UTM E 457957/N 9324703; Gendagenda, UTM E 460395/N 9383565; Kwamsisi, UTM E 455100/ N 9348677) were pooled for this step. In total 8310 sequenced reads allow the identification of 1833 sequences containing microsatellite motifs and validate 172 primer pairs framing a microsatellite motif. Seventy primer pairs were tested with 8 samples in PCR reactions of 10 µL final volume containing 6 pmol dNTP, 37.5 pmol $MgCl_2$, 10 pmol of each primer, 1 µL DNA (diluted 1/10) and 0.5 U of FastStart Taq DNA polymerase (Roche). The PCR conditions consisted in 10 min initial denaturation at 95°C, 40 cycles of 30 s at 95°C, 30 s at 55°C, 60 s at 72°C, and 10 min final extension at 72°C. 2 µL of PCR products were visualised on 2% agarose gels. Twenty-seven primer pairs were validated and we selected 24 pairs to study their polymorphism on 7 samples. PCR mix and conditions were similar to the previous step but with the forward primer labelled with a fluorescent dye (Table 1). 1 µL of the PCR amplicons were diluted and run on an ABI-3730xl DNA Analyzer using the GenScan-500LIZ (Applied Biosystems) as a size standard. Alleles were visualized and scored using GeneMapper 3.7 (Applied Biosystems). Nine microsatellite motifs showed polymorphism (sequences listed in Supplementary Material). However, for locus Rhpe20, the same 2 alleles were present in all individuals. The 9 polymorphic markers were pooled in 2 multiplex PCRs (Table 1) and tested for effectiveness on 15 additional samples of *R. petersi* from Zaraninge forest. The PCRs were performed in 10 µL reaction volume containing the QIAGEN Multiplex PCR Master Mix (1x), 0.2 µM of each primer and 1 µL of DNA. The PCR conditions consisted in 15 min initial denaturation at 95°C, 35 cycles of 30 s at 94°C, 90 s at 57°C, 90 s at 72°C, and 10 min final extension at 72°C. Microsatellite fragment analysis was performed as above. The number of alleles, and observed and expected heterozygosities were estimated by HW-QuickCheck (Kalinowski 2006). Tests for linkage disequilibrium were performed in GENEPOP 4.0 (Rousset 2008).

Table 5.1: Characteristics of 9 novel microsatellite markers developed in the Black and Rufoussengi. Results for 18 individuals from Zaraninge forest, Saadani National Park, Tanzania.

Locus	Dye	Size range (bp)	Multiplex	Primer sequences (5'–3')	Repeat motif	N_A	H_O	H_E	HWE P value
Rhpe02	6-FAM	103-106	1	F: GGAGCTTCACCACCCTATGA R: GGTTCTTGCTTTTAAGAATTTCACA	(AAC)6	3	0.61	0.56	0.42
Rhpe41	NED	141-162	1	F: TCCTTCCTTTACCTTGAGGC R: CAAGGAAGCAGGAGAGAAAAGA	(TCTA)10	6	0.82	0.81	0.61
Rhpe42	PET	118-130	1	F: TGGGTGTGGTTAAAACAGGG R: TGTTCCATCCAATTTCATCTG	(GATG)11	5	0.78	0.70	0.32
Rhpe64	NED	201-209	1	F: ACATAGAGGGATGGATAGACGAA R: TCCATCTGTCACTCATCATCTCTT	(TGG)5	2	0.22	0.20	0.83
Rhpe08	VIC	116-120	2	F: AAACGTGTTATGTTATTCATTTATTTG R: GGTGGGATGCATAGACCAA	(ATGT)6	2	0.33	0.41	0.38
Rhpe20*	NED	150-154	2	F: TGCAGTACCTAAAAGTAGCCCC R: TCAACTAAGCAGGCCTTCAA	(TTG)6	2	1	0.51	0.001
Rhpe33	PET	162-168	2	F: GACAGGGTGCATTTTAGGTCA R: GGGTAGGTTTCCCATCTGCT	(AAC)7	2	0.44	0.46	0.65
Rhpe43	NED	105-122	2	F: CGTTAGCTCACTTGATGGCA R: CCAGTGACTTGCCCAATTTAG	(CAA)13	5	0.82	0.75	0.34
Rhpe62	6-FAM	180-183	2	F: GGTGCACTTCAGGTCTGCTA R: GGTTTATGCTTTCCTTCCCA	(AAC)5	2	0.17	0.25	0.27

Results for 18 individuals from Zaraninge forest, Saadani National Park, Tanzania For each motif, the copy number listed is the one that was present among the next generation sequencing results. N_A observed number of alleles, H_O observed heterozygosity, H_E expected heterozygosity. *PS* primer sequence, *RM* repeat motif * For the locus Rhpe20, all individuals have the same genotype

Results

For the 18 samples from Zaraninge forest, allelic diversity ranged from 2 to 6 alleles per locus (Table 1). For the locus Rhpe20, all individuals show the same genotype Given this result, we would not recommend the use of this locus. The 8 remaining loci have an average allelic diversity of 3.4 alleles. Observed and expected heterozygosity averaged across all loci were both 0.52. Between pairs of loci, no significant linkage disequilibrium was detected after Bonferroni correction ($P < 0.002$). No evidence of deviation from Hardy–Weinberg equilibrium was detected. The new microsatellite markers should be useful for examining the impact of habitat fragmentation on the population structure and genetic diversity of this sengi species.

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CHAPTER SIX

6.0 PAPER 5: Genetic structure and diversity of the black and rufous sengi in Tanzanian coastal forests.

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Abstract

The black and rufous sengi *Rhynchocyon petersi* is restricted to the Eastern Arc Mountains and coastal forests of East Africa and considered vulnerable because of habitat fragmentation and degradation. Coastal forests are believed to have been isolated from each other for thousands of years due to climatic changes. Since *R. petersi* is described as strongly dependent on its forest habitat, we hypothesized that *R. petersi* from different forests would show genetic divergence. We investigated the genetic structure and diversity of this species in four coastal forests in Tanzania using eight microsatellites and cytochrome b sequences. In total, 45 individuals were captured after strenuous sampling efforts. For comparative purposes we also sequenced the cytochrome b of 57 individuals from a sympatric rodent forest species, *Beamys hindei*. The results indicate extant *R. petersi* have descended from a single population of high effective size (Ne) with no forest-distinctive signal. In contrast, *B. hindei* is more genetically structured: Although the most common haplotype is found in the three closest forests, each forest harbours private haplotypes. Moreover, *B. hindei* Ne appeared 10 times smaller than *R. petersi* in Zaraninge forest. While *B. hindei* results are consistent with the scenario of longterm isolation of coastal forests, the *R. petersi* are not. We suggest *R. petersi* may less depend on forest habitat than previously suspected, consistent with anecdotal reports of sengis nesting in intervening agricultural habitat. From a conservation viewpoint, this sengi species therefore appears robust to the current spatial and temporal scale of habitat fragmentation.

Keywords: *Rhynchocyon petersi*; vulnerable; conservation genetics; coastal forest *Beamys hindei*; genetic structure; genetic diversity; habitat fragmentation.

Introduction

The coastal forests of east Africa are believed to have had been separated from the Guineo-Congolian forests in West Africa by the up thrust of the central Tanganyika plateau about 35 million years ago (Dickinson, Burgess & Clarke, 1992). However, there were some periods of reconnection until complete disjunction about 3 million years ago (Dickinson et al.,1992). The long-term and slow gradual desiccation of the last 10 million years together with the most recent climatic fluctuations during the Holocene are likely responsible for the further reduction of forest cover inland of the Eastern African coast and the disjunction between sites (Burgess, Clarke &Rodgers, 1998). It is further suggested that recurrent anthropogenic fires dating back as far as about 50 000 B.C. separated the evergreen dry coastal forest patches from surrounding matrices, restricting them to fireproof sites in moister areas including hill tops, riverine and ground water areas (Dickinson et al., 1992). Currently most of the forests structure are small and highly fragmented varying in shape and structure with areas ranging from 1 to 50 km2 (Burgess et al., 1998). The forests are isolated from each other by less than one to several tens of kilometers by a vegetation matrix composed of a mixture of farmland, savannah woodland and thickets (Burgess, 2000). Globally, the East African coastal forests remain among 25 outstanding biological hotspots containing exceptional levels of endemism of major taxa (Myers et al., 2000). Among these endemic taxa are species of the genus *Rhynchocyon* (Rathbun, 1979).

The black and rufous sengi, often called elephant shrew *Rhynchocyon petersi* Bocage 1880, is one of the four known giant sengis from the sub-family Rhynchocyoninae which belong to the super-cohort Afrotheria. This species is endemic to East Africa with distribution limited to some coastal and Eastern Arc Mountain forests. Little is known about this species because detailed field studies are very scarce (Rathbun & Butynski, 2008). Most aspects of its natural history are assumed to be similar to the Golden-rumped sengi (Rathbun 1979), which are diurnal and live inmonogamous pairs with defined territories of about 1.5 ha. They build nests for shelters and each pair maintains 6 or 7 nests in a territory using several of them at one time. These nests are built with dry leaves in thick undergrowth or under a low bush tree (Hanna & Anderson, 1994). Their primary habitat is described as semi-deciduous forests and dense woodlands or coral rag scrub (on Zanzibar Island). Two important factors of this habitat are closed canopies to avoid aerial predation and thick leaf litter to build their nests (Corbet & Hanks, 1968; Hanna & Anderson, 1994; Coster & Ribble, 2005). Since *R. petersi* seems to be very dependent on this forest habitat, dispersal between populations of *R. petersi* from different coastal forests is likely to be severely restricted. Thus, long-term isolated coastal forests are expected to harbour divergent *R. petersi* genetic pools.

The International Union for Nature Conservation (IUCN) has categorized *R. petersi* on the IUCN Red List of Threatened Species as 'vulnerable' because of the fragmentation and degradation of their habitat due to anthropogenic activities (IUCN, 2015). This continued anthropogenic pressure includes forest clearing for subsistence agriculture, human settlements, extensive livestock grazing and overexploitation of natural resources for various use, for example, timber, firewood and charcoal production (Terborgh, 1992; Bloesch & Kl€otzli, 2002). Since habitat fragmentation has been shown to reduce genetic diversity, increase inbreeding and random genetic drift between populations (Soul_e, 1987; Frankham, 1995, 2005), it may result in irreversible consequences for the future of this geographically range limited species. Additionally, *R. petersi* has been suggested to have a much lower population density than the golden-rumped sengi (Hanna & Anderson, 1994;Coster & Ribble, 2005), which is listed as 'endangered' due to its even more restricted range (IUCN, 2015). As suggested by a recent study of the grey-faced sengi (Lawson et al., 2013), a better understanding of the genetic structure and diversity of *R. petersi* may allow clearer evaluation of the evolutionary history and conservation status of not just one, but all sengi species.

In this study, we use eight microsatellite markers and a mitochondrial gene to investigate the population genetics of *R. petersi* in four coastal forests in and around Saadani National Park in Tanzania. For comparative purposes, we also genotyped individuals from *Beamys hindei* captured in thes ame forests with the same mitochondrial gene. *Beamys hindei*, the lesser pouched rat, is a rodent species from the sub-family Cricetomyinae which is often found in sympatry with *R. petersi* (Clarke & Dickinson, 1995; Kiwia, 2009). As with the black and rufous sengi , the lesser pouched rat distribution is patchy within the coastal and Eastern Arc Mountains forests of East Africa (FitzGibbon, Leirs & Verheyen, 1995; Sabuni et al., 2015a), although its total geographic range is a little larger (IUCN, 2015). The lesser pouched rat is described as strongly dependent of forest habitat and sandy soils that facilitate burrow construction (FitzGibbon et al., 1995; IUCN, 2015; Sabuni et al., 2015a). We thus hypothesized that both species should show genetically structured populations in thefour forests.

Material and methods

Study sites

This study was carried out in coastal forests found in and surrounding Saadani National Park (SANAPA) (6°000S 38°450E) Tanzania. Data were gathered from four dry evergreen coastalforests: Zaraninge (~20 km2; 6°090S 38°380E), Kwamsisi (~10.5 km2; 5°510S 38°350E), Gendagenda (~14 km2; 5°330S 38°380E) and Askari (~1.2 km2; 5°590S 38°460E) (Fig. 1). The shortest distance between the edges of Zaraninge and Kwamsisi is ~16.6 km, and between Kwamsisi and Gendagenda ~34.6 km, while from Zaraninge to Askari ~18 km. Zaraninge and Askari forests are within

SANAPA, while Kwamsisi forest is managed by Kwamsisi village with only a small part of the water catchment under SANAPA management. Gendagenda is located north outside the Park and is managed by the Gendagenda village. A description of the vegetation of these forests is detailed in Sabuni et al. (2015a). The study area experiences bimodal rainfall with a high peak from March to May and a shorter rainy season from October to December (Bloesch & Kl€otzli, 2002). The four forests are separated by matrices of various types of mixed vegetation, wooded grassland, small patches of evergreen forests and thickets

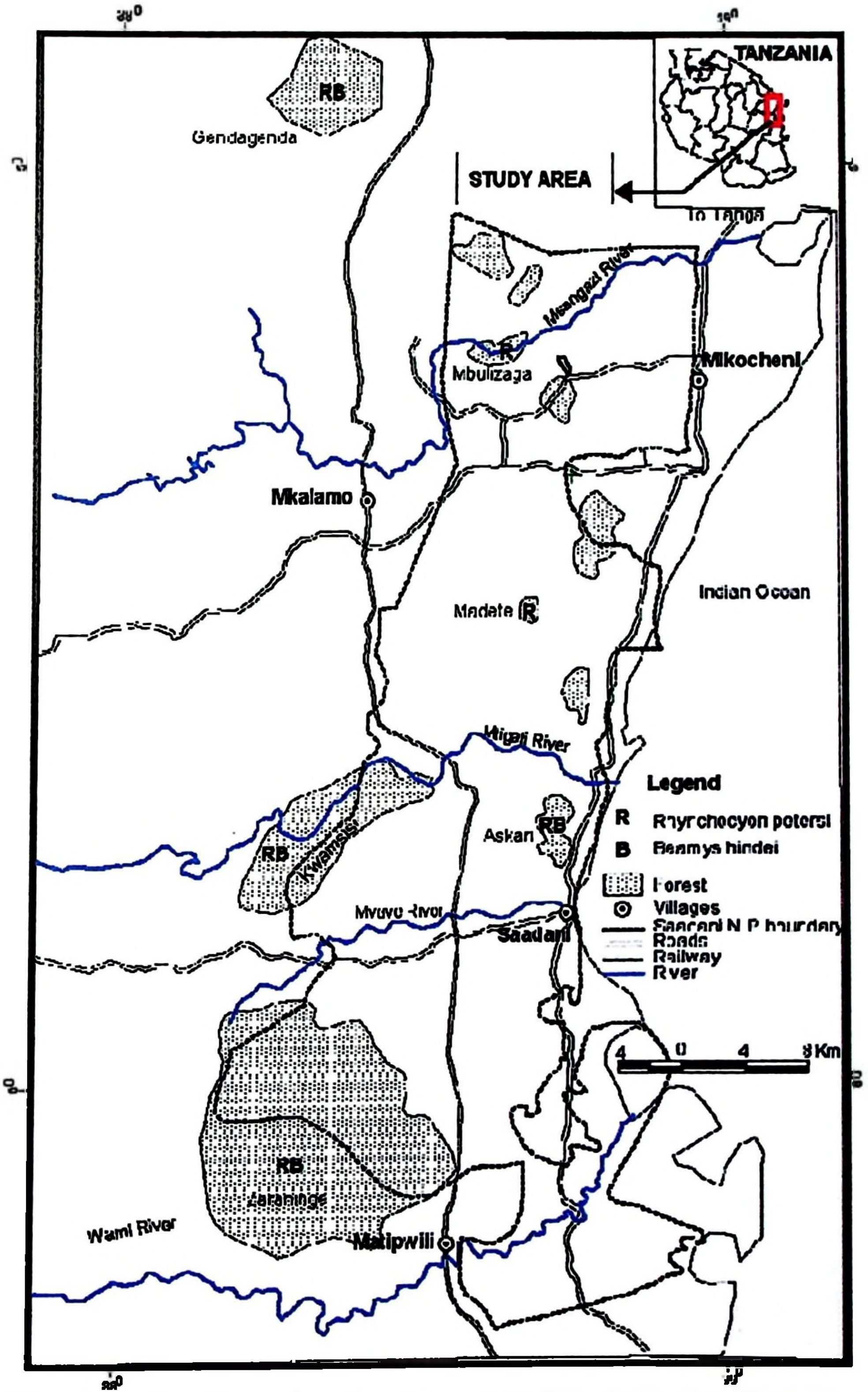

Figure 6.1: Location of the four forests used in this study (modified from Sabuni et al., 2015a). The letters R and B indicate the presence of *Rhynchocyon petersi* and *Beamys hindei* in the coastal forests

Sample collection

Trapping of *R. petersi* was conducted between October 2010 and May 2014. Trapping sengis is difficult as no bait able to attract sengis is known (Rathbun. 1979; Sabuni, Beddetti & Leirs. 2011). We used two trapping methods. wire-mesh and fish-net traps, in order to enhance the catch rate of live individuals: (1) twenty single-door non-collapsible wire-mesh traps (Tomahawk, model 102. 13 9 13 9 40.5 cm) and 25 medium collapsible single-door wire-mesh traps (Havahart Trap, 20 9 61 9 20 cm) were set in the trails and paths in the forests; (2) fifty nets laid following Rathbun (1979) in a narrow transect line of about 100 m. Both types of traps were left at each site at least for 3 weeks and inspected three times/day (morning. mid-day. and before sunset). Trapping effort was calculated as the number of traps 9 number of days. A small piece of ear was cut from trapped individuals and preserved in 96% ethanol. The ear was then disinfected and the animal released at the place where it was caught and monitored for a few minutes to ensure no adverse effects. Trapping of *B. hindei* was performed during the same period than *R. petersi* and a detailed description is available in Sabuni et al. (2015a). In total. 57 individuals were genotyped and distributed as follows (number genotyped/number captured): Zaraninge (32/158), Gendagenda (11/11), Kwamsisi (12/17) and Askari (2/2).

Genotyping

Genomic DNA was extracted using Nucleopsin kit (Macherey- Nagel. D€uren, Germany). Mitochondrial cytochrome b (cyt b) gene was amplified using MTCB-F and MTCB-R primers (Naidu et al., 2012) for R. petersi and L7 and H6 primers (Montgelard et al.. 2002) for B. hindei. PCR amplification was performed in 20 lL volume containing 0.2 lM of each primer, 0.2 mM of each dNTP, 2.5 mM MgCl2, 1X DreamTaq buffer, 1.25 unit of DreamTaq DNA Polymerase (Thermo Scientific Fermentas, Aalst, Belgium) and 1.5 lL of DNA template. The thermal cycling profile started with a denaturing step at 94°C (3 min), followed by 35 cycles at 94°C (45 s), 54°C (30 s) and 72°C (1 min) and ending with an extension step of 72°C (10 min). PCR products were purified and sequenced by VIB Genetic Service Facility (University of Antwerp, Belgium). Sequences were deposited in Genbank (AN: KU756138-KU756166).

Rhynchocyon petersi samples from Kwamsisi, Gendagenda and Askari were also genotyped at nine microsatellite loci as described in Sabuni et al. (2015b). Samples from Zaraninge were already genotyped (see Sabuni et al., 2015b). Alleles were visualized and scored using GeneMapper 3.7 (Applied Biosystems, Gent, Belgium). As previously found for all individualsfrom Zaraninge forest, all additional individuals showed the same genotype for the locus Rhpe20. This locus was thus excluded from the subsequent analyses. No microsatellite markers have been so far developed for *B. hindei* or closely related species impeding any comparison with *R. petersi* using these types of markers.

Mitochondrial DNA analysis of both species

Cyt b sequences were corrected and aligned in Geneious v.8. Haplotype diversity (h) and nucleotide diversity (Π) were calculated in DnaSP 5.10 (Librado & Rozas, 2009) and p-distances between haplotypes in Mega 6.06 (Tamura et al., 2013). A median-joining haplotype network was generated in Network 4.6 (Bandelt, Forster & RΕohl, 1999).

The demographic history was estimated using a Bayesian Markov Chain Monte Carlo (MCMC) coalescent approach implemented in BEAST 1.8.2 (Drummond et al.,2012). The Bayesian skyline plot (BSP) analysis uses MCMC sampling procedures to estimate a posterior distribution of effective population size through time from a sample of gene sequences, given the HKY model of nucleotide substitution (Drummond et al., 2005). The time dimension was calibrated by fixing the mean substitution rate to 0.05 per million years corresponding to an average over cyt b substitution rate in mammals (Nabholz, Glemin & Galtier, 2008). We used a Bayesian Skyline coalescent tree prior with five groups under a piecewise constant model. Analysis was run for 30 million MCMC generations sampled every 3000 generations and launched from a random starting tree. Tracer ver.1.6 (http://beast.bio.ed.ac.uk/ Tracer) was used to inspect chain convergence and performed the Bayesian skyline reconstruction using a stepwise skyline variant. BSP analysis was performed on three different datasets: all *R. petersi* individuals, *R. petersi* individuals from Zaraninge and *B. hindei* individuals from Zaraninge. For both species, we assumed an effective generation time of 1 year.

Population structure analysis of *R. petersi*

We estimated allelic richness corrected for sample size, and inbreeding coefficient (FIS) using FSTAT 2.9.3.2 (Goudet, 2001) for each microsatellite markers. Departures from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were tested in ARLEQUIN 3.0 (Excoffier & Lischer, 2010). For HWE exact tests, we set the Markov chain at 1 000 000 and thenumber of dememorization steps at 100 000 and applied Bonferroni correction to account for multiple testing. Analysis of molecular variation (AMOVA) was performed in ARLEQUIN3.0. These statistics were only calculated for the three forests where the number of sampled individuals was ≥12.

To infer the population structure of *R. petersi*, a Bayesian clustering approach was used on the microsatellite dataset as implemented in STRUCTURE version 2.3.4 (Pritchard, Stephens & Donnelly, 2000). The analysis was replicated 10 times for each value of K from 1 to 4 using 100 000 iterations burn-in followed by 1 000 000 iterations sampling the posterior.Graphic display of the STRUCTURE results was generated using CLUMPACK (Kopelman et al., 2015). NeEstimator 2.01 (Do et al., 2014) was used to determine the effective population size (Ne) of R. petersi taking all individuals as belonging to a single population. For the LD method, we assume that this sengi species has monogamous mating.

Results

Trapping of *R. petersi*

In total, we only trapped 45 R. petersi individuals despite extensive sampling efforts. In Zaraninge, we captured 18 sengis for a total of 4725 and 5250 trapping days of wire-meshtraps and fish nets respectively; in Kwamsisi and Gendagenda, we captured 13 and 12 sengis for a total of 4725 and 4520 trapping days of wire-mesh traps and fish nets respectively; finally we captured only 2 sengis in Askari for a total of 2835 and 3150 trapping days of wire-mesh traps and fish nets respectively.

Mitochondrial DNA analysis of both species

For *R. petersi*, we obtained 43 sequences of 1282 base pairs (bp) encompassing 16 bp of ND6, tRNA-Glu, full cyt b and part of the tRNA-Thr of the mitochondrial genome. Two samples with low-quality sequences were not included in the final dataset. These 43 sequences contained 31 polymorphic sites revealing 18 distinct haplotypes (Supporting Information Table S1; Fig. 2a) with a total haplotype diversity (h) = 0.907. The divergence among those haplotypes was low with a nucleotide diversity of 0.0031 and an average p-distance among different haplotypes of 0.4% (minimum distance between two haplotypes = 0.1% and maximum distance between twohaplotypes = 0.9%). For *B. hindei*, we obtained 57 sequences of 1121 bp covering the almost complete cyt b gene. Although the sampling size of *B. hindei* (57) was higher than the one of*R. petersi* (45), its cyt b sequence dataset was less polymorphic: *B. hindei* sequences contained 26 polymorphic sites revealing only 11 distinct haplotypes (Fig. 2b) with a total haplotype diversity (h) = 0.806. The divergence among B. hindei haplotypes was low with a nucleotide diversity of 0.0021 and an average p-distance among different haplotypes of 0.48% (minimum distance between two haplotypes = 0.1% and maximum distance between two haplotypes = 1.6%). Indeed, 10 haplotypes showed very low divergence with on average only 0.25% nucleotide difference per sites, while a single haplotype H11from Kwamsisi showed on average 1.51% nucleotide difference per sites with the other haplotypes (see Fig. 2b).

The haplotype networks of the two species showed different shapes: *B. hindei* network displays a star-like shape with the most common haplotype (H1) present in the three closest forests and at a central position, while all other haplotypes are private with very little divergent from H1 (Fig. 2). The exception is haplotype H11 (but see above). In contrast, the haplotypenetwork of *R. petersi* is scattered: The most common haplotypes are shared between 2 and 3 forest patches with H9, the most common, found in all forest patches (but not central to the network). There are only two instances where a given haplotype is found in at least two individuals and restricted to a single forest (H4 in three individuals and H10 in two individuals)(Fig. 2a). In summary, *B. hindei* populations appear genetically structured by forests, while *R. petersi* are not. The low divergence between haplotypes, which is not surprising atthat geographical scale, is consistent with a lower Ne for *B. hindei* compared to *R. petersi* resulting in lower maintenance of genetic diversity reflected by a lower number of haplotypes

relative to the sampling size.

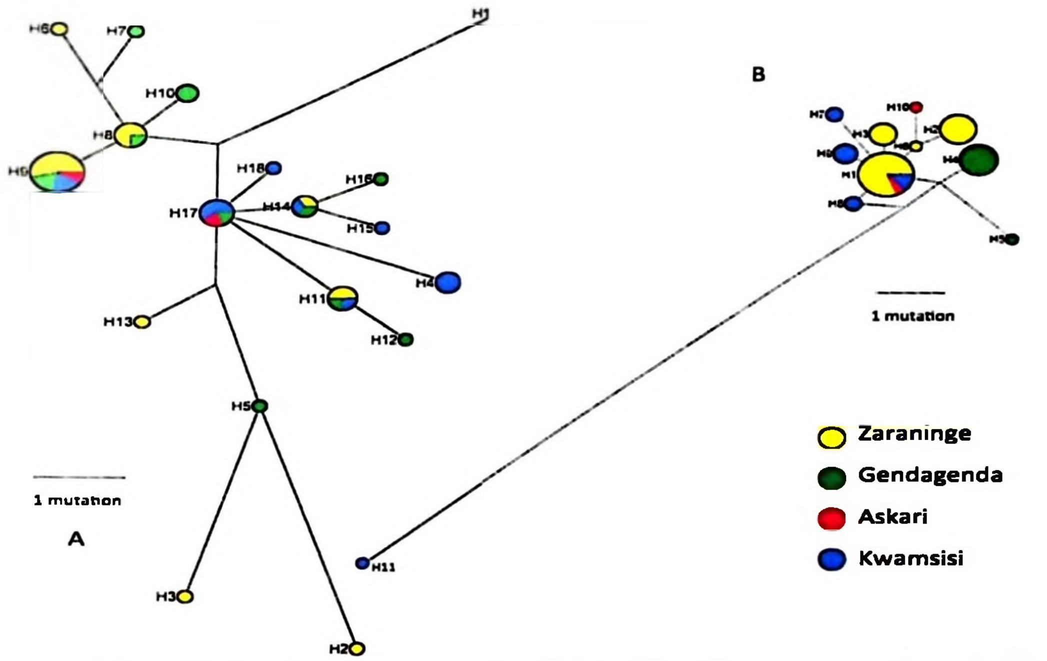

Figure 6.2: **Median-joining network of (a) *Rhynchocyon petersi* and (b) *Beamys hindei* cytochrome b haplotypes. The number of mutations between haplotypes is proportional to the length of branches, and circle sizes are proportional to the frequency of a given haplotype. The long branch of haplotype 11 in the *Beamys hindei* network is shortened and the numbe of mutational steps indicated in red. Colours reflect the forest origin of the haplotypes. For*R.petersi*, haplotype numbers correspond to Table S1 (Supporting Information)**

Demographic history of both species

Based on the previous results, we analysed the demographic history of *R. petersi* based on the complete dataset. For *B. hindei* in contrast, since we detected a pattern of genetic structure byforest, the demographic history of *B. hindei* was only investigated in the largest forest, Zaraninge, for which we have the largest sampling size and for which ecological data are available (Sabuni et al., 2015a). For comparative purposes, we also analysed the demographic history of the sengi in the same forest.

The analysis of demographic history based on our single mitochondrial marker suggested the black and rufous sengi Ne has remained relatively stable over the last ten of thousand years (Fig. 3) with relatively large Ne (~141 000 assuming an effective generation time of 1 year). However, these estimates came with wide confidence bounds [high posterior density (HPD) intervals], for example the current Ne estimate of the population lies between 8991 and 699 770 (95% HPD) (Table 1). In Zaraninge, *B. hindei* Ne was estimated at ~5550 individuals (HPD 14–50 087), the point estimate being 10 times lower than the sengi Ne in the same forest (Fig. 3,Table 1).

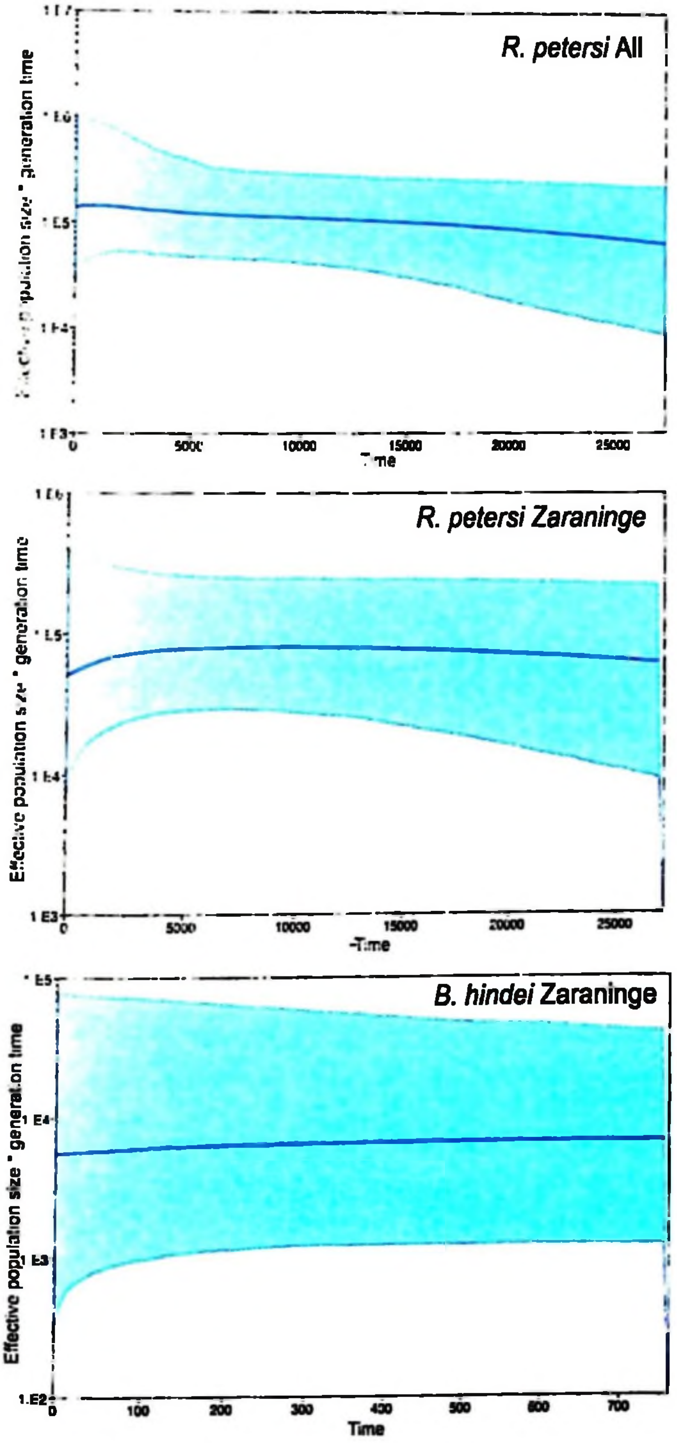

Figure 6.3: Bayesian skyline plots based on the mtDNA sequence data. The y-axis is the product of the effective population size and the generation time and the x-axis shows time. A mammalian average mutation rate of 5 9 10 8 was used and an effective generation time of 1 year is assumed.

Table 6.1: Current effective population size and 95% high posterior density (HPD) interval for *Rhynchocyon petersi* and Beamys hindei as estimated by Bayesian skyline plot reconstruction assuming for both species an effective generation time of 1 year

	R. petersi All	*R. petersi* Zaraninge	*B. hindei* Zaraninge
Number of individuals	43	16	32
Median *Ne*	140890	51795	5557
Geometric mean *Ne*	147420	51681	5366
95% HPD Interval	[8991-699770]	[1159-274490]	[14-50087]

Table 6.2: Microsatellite diversity of *Rhynchocyon petersi* in three coastal forests with allelic richness (A_R), inbreeding coefficient (F_{IS}), observed heterozygosity (H_O), expected heterozygosity (H_E) and *P* value of exact HW test (*P*)

Gendagenda (n=12)						Kwamsisi (n=13)					Zaraninge (n=18)				
Loci	A_R	F_{IS}	H_O	H_E	P*	A_R	F_{IS}	H_O	H_E	P*	A_R	F_{IS}	H_O	H_E	P*
Rhpe2	2	0.353	0.333	0.507	0.036	2.846	-0.301	0.692	0.538	0.081	2.995	-0.094	0.611	0.560	0.070
Rhpe41	4.917	-0.073	0.833	0.779	0.106	4	0.424	0.364	0.619	0.004	5.842	-0.014	0.824	0.813	0.102
Rhpe42	3	0.295	0.417	0.583	0.035	4.828	0.160	0.538	0.637	0.086	4.706	-0.110	0.778	0.703	0.088
Rhpe64	2	-0.100	0.250	0.228	0.125	1	•	•	•	•	1.983	-0.097	0.222	0.203	0.025
Rhpe08	2	-0.467	0.667	0.464	0.027	2	0.189	0.385	0.471	0.083	2	0.197	0.333	0.413	0.052
Rhpe33	2	-0.375	0.583	0.431	0.061	1.982	-0.043	0.154	0.148	0.143	2	0.029	0.444	0.457	0.057
Rhpe43	6	0.085	0.636	0.693	0.120	4.830	0.115	0.615	0.702	0.064	4.871	-0.103	0.824	0.749	0.094
Rhpe62	2	-0.100	0.250	0.228	0.125	2	0.593	0.154	0.369	0.011	1.995	0.329	0.167	0.246	0.031
Average	**2.980**	**-0.015**	**0.496**	**0.489**	**0.079**	**2.935**	**0.171**	**0.414**	**0.497**	**0.067**	**3.290**	**-0.015**	**0.525**	**0.518**	**0.065**

Table 6.3: Analysis of molecular variance (AMOVA) and hierarchical F statistics for *Rhynchocyonpetersi*

Source of variation	Sum of square	Variance components	Percentage variation	Fixation index
Among forests	6.642	0.048	2.440	0.0244 F_{ST}
Among individuals within forests	78.581	0.067	3.381	0.0346 F_{IS}
Within individuals	79.000	1.878	94.177	0.0582 F_{IT}

Population genetic analysis of *R. petersi*

Allelic richness (AR), inbreeding coefficient (FIS), observed heterozygosity (HO), expected heterozygosity (HE) and P value of exact Hardy–Weinberg test are presented in Table 2. Each of analysed loci was polymorphic with mean allelic richness across populations ranging from 2.98 to 3.29. The inbreeding coefficient FIS did not deviate significantly from zero, consistent with random mating within forests. No evidence of departure from HWE was found in any of the three forests and for any of the microsatellite loci, and neither for any of the microsatellite loci when combining all three localities together. AMOVA showed that the largest part of the variation in microsatellites was found within individuals (94.18%) with only 2.4% found among forests (Table 3). This result was confirmed by the Bayesian analysis of population structure: thebest estimated Ln Prob of data for STRUCTURE analyses for increasing numbers of genetic clusters (K = 1–4) were found for K = 1 (Supporting Information Figure S1) with no genetic structure detectable across forests (Fig. 4).

Genetic estimates of Ne based on different approaches varied considerably: The LD method provided a Ne of 66 (43–11595% CI), while the heterozygote excess and the co ancestry methods gave a point estimate of 'infinite'. These estimates should be regarded with caution: The LD method has been shown to be strongly biased when sample size is small (<100) and below the true Ne (England et al., 2006). The two last methods suggest there is no evidence of genetic drift in the sampled individuals though a larger sample size might give more accurate parameter estimation (Do et al., 2014).

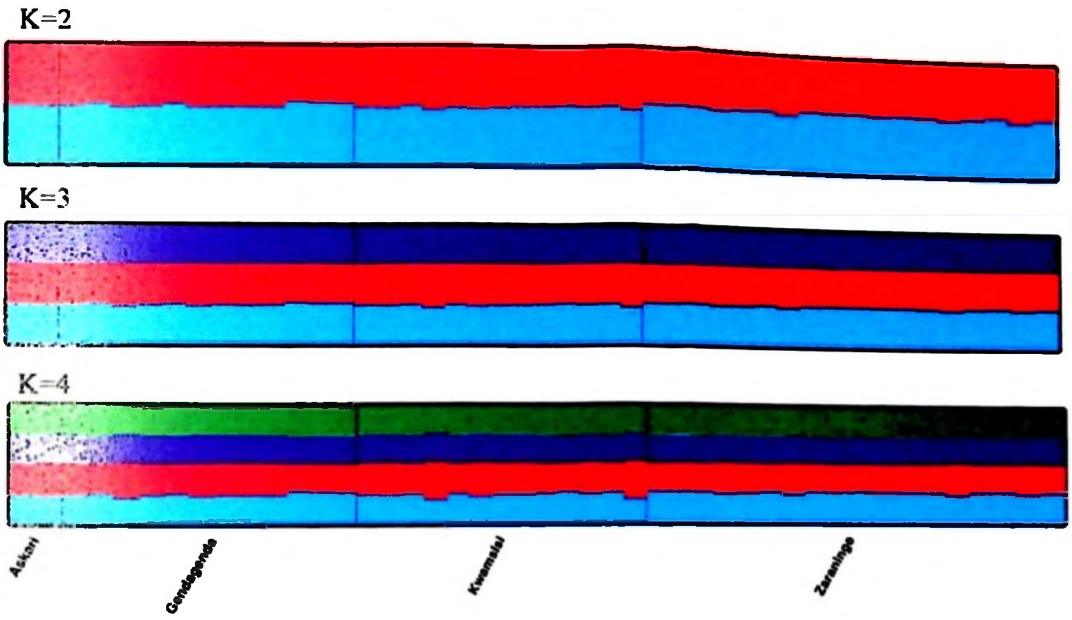

Figure 6.4: STRUCTURE summary plots of the estimated membership coefficient (y-axis) for each *Rhynchocyon petersi* individual for K = 2 (top plot) to K = 4 (bottom plot). Each individual is represented by a single vertical line broken into segments proportional to the membership coefficient for each of the population clusters. Individuals are arranged into forests from which they were sampled

Discussion

We investigated the population structure and diversity of *R. petersi* in four coastal forests of different size and at various distances from each other in and around SANAPA. Because this species is thought to be highly dependent on its forest habitat, we hypothesized that *R. petersi* from different forests would show genetic divergence since these forests are likely isolated from each other for thousand years. Contrary to our expectation, we found that these *R. petersi* cannot be distinguished from a sample of panmictic population with a likely very high effective population size. Two alternative scenarios could explain this result: (1) the isolation of the forests is more recent than previously thought – too recent to have left a signal detectable with our genetic markers; (2) the habitat between forests can support *R. petersi* and so dispersal and mating occur between them. The results of the sympatric murine species also described as forest specific, *B. hindei*, which showed genetic divergence according to forests, gives us some clues about the most plausible scenario.

The first scenario – fragmentation of the coastal forest is too recent to allow for genetic drift to be detected with our genetic markers – is plausible if the separation of the study forests is the result of the anthropogenic activities that have been increasing gradually around them for at least 50 years. Not only the high Ne detected in this study at the global scale, but also in Zaraninge forest would require many more generations than 50 (assuming a generation time of 1–2 years) to allow drift to shift allele frequencies (and their combinations) sufficiently to be able to distinguish samples from different fragments. However, it seems clear from the literature that the fragmentation of the coastal forest occurred a long time ago as a result of climatic changes spanning thousand years (Burgess et al., 1998) likely only accentuated more recently by the anthropogenic activities going on around the forests.

The alternative scenario, for example, the habitat between forests can support *R. petersi* but not *B. hindei*, appears the more likely explanation of the genetic pattern observed. Currentlythe four forests are separated by a matrix of different types of mixed vegetation with different levels of degradation. For example, a large part of SANAPA was formerly a ranch, while Zaraninge existed as a forest reserve before its inclusion in SANAPA in 2005 (Bloesch & Klötzli. 2002). Kwamsisi and Gendagenda existed as forest reserves with low-level management since around 1910 (Clarke & Stubblefield, 1995). In most cases, the forests are bordered by villages in which different activities are carried out such as agriculture and livestock keeping. We expected that this non-forested matrix would act as a barrier to gene flow between the forests. This barrier is visible for *B. hindei* with a more structured genetic pattern, while absent in *R. petersi.* The dependency on forest habitat may be thus stronger for the rodent than the sengi. Although no record of B. hindei individuals outside forest habitats is available inthe literature, *R. petersi* individuals have been observed to live and forage successfully in habitats disturbed or created by human activity: In Pugu Forest Reserve, nests of *R. petersi*were reported in mixed plantation of Cassia and Eucalyptus (Hanna & Anderson, 1994). A parallel study conducted on the home range of *R. petersi* in Zaraninge forest reports that thespecies utilizes habitats adjacent to the forest such as woodland and plantations which were previously part of the forest (C. A. Sabuni, unpubl. data). These 'secondary' habitats show muchlower densities of *R. petersi* than recorded in pristine forest nearby (Hanna & Anderson, 1994). If *R. petersi* is able to live and forage in secondary habitats, even at low density, then the matrix separating the different forest may not act as a complete barrier to gene flow and individuals could disperse between fragments separated by a few kilometres such as Zaraninge and Kwamsisi. It is more difficult to imagine that *R. petersi* can directionally disperse as far as 35 km between Kwamsisi and Gendagenda, but not so if some small forests located at the East of SANAPA act as stepping stones: Indeed, *R. petersi* have been also observed in Madete and Mbulizaga forests (C. A. Sabuni, unpubl. data). While our evidence supporting dispersal is indirect. direct evidence of dispersal connectivity would require enormous effort to trap sengi in low density areas.

Another result of our study is the high Ne estimated for both species, 10 times higher for *R. petersi* than for *B. hindei*. The recent study on the lesser pouched rat conducted in Zaraningeon a 2-ha grid reported that population abundance estimate fluctuates between 1 and 40 individuals/2 ha (Sabuni et al., 2015a). Extrapolation to the scale of the entire forest which is about 20 km2, the census population size could be ~2000 individuals. For *R. petersi*, the average home range for 18 individuals in Zaraninge has been estimated at 2.64 _ 0.31 ha (C. A. Sabuni. unpubl. data). If we assume that *R. petersi* occupies the forest evenly. the forest could supportabout 758 individuals (this is very conservative as a male and female from a pair of *R. petersi* have a partially overlapping home range). We should not expect relative census size size depends on trapping success, likely lower for high-visual acuity risk-averse animals such as sengis (the data used to calculate the range are based on radio telemetry). Second, Ne depends on the mating system and in particular on the variance in reproductive success. Strong inbreeding avoidance and low variance in reproductive success could allow *R. petersi* to maintain relatively high Ne for a given census size (e.g. Richmond et al., 2009). Third, the sengis in the four investigated forests may be part of a wider population that spans further than the sampled forests. In any case. our results suggest the black and rufous sengi in Tanzanian coastal forests is reasonably robust to the current spatiotemporal scale of habitat fragmentation.

In conclusion, *R. petersi* in the coastal forests in and around SANAPA is indistinguishable from a well-mixed population of high *Ne*. The IUCN categorization 'vulnerable' (rather than 'endangered') seems thus appropriate at least for this part of the species range. A future step would be to investigate if the 'population robustness' of *R. petersi* in the fragmented coastal forest habitat applies to other parts of its range, notably in the East Arc Mountain forests. In those latter, the more disjoint distribution of the species may be reflected in more divergent gene pools.

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CHAPTER SEVEN

7.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

Conservation of the species is not only depends on the number and or inventory but the knowledge on how the species concern interact with the environment. Studies to understand the ecology of small mammal have been limited to number of species (Leirs, 1996; Keesing, 1998; FitzGibbon, 1995) and in many active studies has been linked to species which are termed "pests" (Othiambo *et al.*, 2008). The current study had focused on trying to understand the two species of small mammal of which both leaves in coastal forests and they differ in trophic levels. The first two manuscripts investigated are linked to distribution population fluctuation and demographic pattern. It also looked on the behavior of *B. hindei* on the burrowing characteristic space use the diet. The distribution of *B. hindei* in the coastal forests was absent to some of the forests assessed which could be linked to probably absence of sential basic requirements for its survival.However, when this is linked to estimated abundance obtained where was low, and the climbing behavior observed and the burrowing behaviour it is concluded that the species is commonly foundin patch distribution as earlier documented by Kingdon (1974). This phenomenon is contributed due to the burrowing behavior and arboreal characteristics. The low recruitment and survival of the species behave differently from those of savannah species such (Keesing 1998; Leirs, 1996). Capture mark recapture a method which has been used to study population dynamic, gives also an opportunity to study home range of *B. hindei* where radio telemetry was not possible since the animal spends much more time in burrow and comes out only for food search and probably for mating. The number estimated from capture mark recapture per hectare was about the same obtained from home range as proxy for abundance estimates.

The third to six manuscripts dealt with the distribution and ecological status of the *Rhynchocyon petersi* in selected coastal forest.This is the first ecological study on *R. petersi* in Tanzanian coastal forests. Distribution of *R. petersi* was found in all forests assessed. Abundance of *R. petersi* was earlier linked to the nest index of that of related species *R. chrysopygus* (Coster and Ribble, 2005). In this study home range results from radio telemetry was used for estimation of abundance of *R.petersi*.Activity patterns were more pronounced in morning and evening this is probably the time when it is not hot. The study for genetic diversity and structure of *R.petersi* is the first. The development of 8 microsatellite markers was a first step which opened an avenue for the study of genetic diversity and structure. Results from this study indicate that genetic diversity was not detected from the populations on the three forest patches. The high genetic heterozygosity observed in the study populations impliesa relatively minor effect of inbreeding. From genetic perspective, there is evidence of dispersal of the species high enough to preclude extinction within these forests, demonstrating a panmictic population.

7.2 Recommendations

It interestingly that *R. petersi* occurred in all coastal forest assessed, the species being categorized as vulnerable (IUCN, 2014) calls attention for conservation and protection. Since individual is able to occupy an area of about 2.6 hectare, it imply that, those forest with small size it is likely this species are going to vanish if conservation mitigation to this species is not established. Though there is more effort put in conservation of charismatic species, yet there is little on cryptic elusive species including the current study species *Beamys hindei* and *Rhynchocyon petersi*. For example, *Beamys hindei* has been passing through different levels of conservation categories based on IUCN, now is categorized as "Least concerned," the fact that, there were some of the forest during the current study which did not record *B. hindei*, a survey in other forests using the method used in this study is recommended. On the *Rhynchocyon petersi* the species was found in all forest assessd. The study finds how useful the camera traps can be used to assess distribution and generated data used for association to environmental changes. Due to increasing use of forest resources especially in non protected habitats which results in the decreas of forest habitats it is recommended that, the conservation status of the *R. petersi* based on IUCNcategory should remain as vulnerable (VU). Also, since *B. hindei* is sympatry to *R. petersi* its status should be reviewed to become vulnerable. Studies for *B. hindei* should use at least 5 nights per site when trapping since the animal spend more time in the burrow and it may be missed if using few days. Camera traps should be encouraged to be used in similar studies to ascertain the distribution of *R. petersi* in other related habitats. The fact that there is now developed microsatellite markers for the genetic study of *Rhynchocyon* spp, we recommend further studies to be carried out in other forests to determine the genetic diversity and structure of *R. petersi* and other giant sengi in these coastal and other forests.Since the current study species depends on forests for their survival, conservation mitigation program should be in place to safe guard these species.

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APPENDICES

Appendix 1: A photo of Black and rufous sengi *Rhynchocyon petersi* captured from Reconyx HyperFire HC500 Semi-Covert IR Camera

Appendix 2: **A photo of lesser poched rat *Beamys hindei* on the tree after being released from Sherman trap**