Journal of Zoology

ZSL LET'S WORK FOR WILDLIFE

Journal of Zoology. Print ISSN 0952-8369

Genetic structure and diversity of the black and rufous sengi in Tanzanian coastal forests

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

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Editor: Jean-Nicolas Volff

Received 11 April 2016; revised 2 July 2016; accepted 19 July 2016

doi:10.1111/jzo.12384

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 long-term 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.

Introduction

The coastal forests of east Africa are believed to have had been separated from the Guineo-Congolian forests in West Africa by the upthrust 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 are small and highly fragmented varying in shape and structure with areas ranging from 1 to 50 km^2 (Burgess *et al.*, 1998). The forests are isolated from each other by less than one to several tens of kilometres 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 in monogamous 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 & Klötzli, 2002). Since habitat fragmentation has been shown to reduce genetic diversity, increase inbreeding and random genetic drift between populations (Soulé, 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 the same 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 the four forests.

Materials and methods

Study sites

This study was carried out in coastal forests found in and surrounding Saadani National Park (SANAPA) (6°00′S 38°45′E) Tanzania. Data were gathered from four dry evergreen coastal forests: Zaraninge (~20 km^2; 6°09′S 38°38′E), Kwamsisi (~10.5 km^2; 5°51′S 38°35′E), Gendagenda (~14 km^2; 5°33′S 38°38′E) and Askari (~1.2 km^2; 5°59′S 38°46′E) (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 & Klötzli, 2002). The four forests are separated by matrices of various types of mixed vegetation, wooded grassland, small patches of evergreen forests and thickets.

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 × 13 × 40.5 cm) and 25 medium collapsible single-door wire-mesh traps (Havahart Trap, 20 × 61 × 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 × 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, Düren, 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

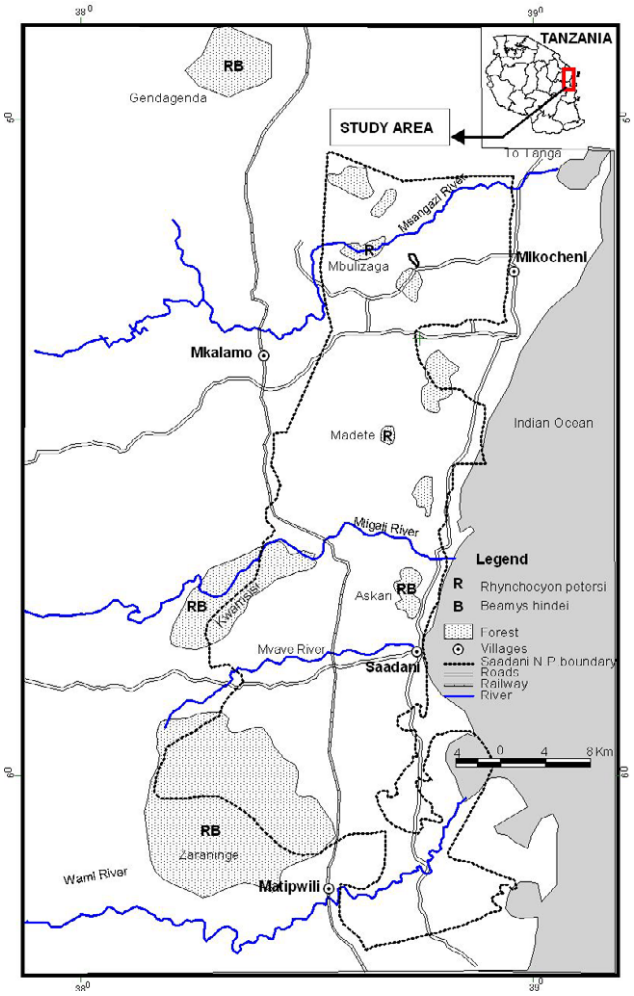

Figure 1 Location of the four forests used in this study (modified from Sabuni *et al.,* 2015a). The letters R and B indicate the presence of *Rhynchocyon petersi* and *Beamys hindei* in the coastal forests.

et al., 2002) for *B. hindei*. PCR amplification was performed in 20 μL volume containing 0.2 μM 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 μL 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 individuals from 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 & Röhl, 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 (F_{IS}) 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 the number of dememorization steps at 100 000 and applied Bonferroni correction to account for multiple testing. Analysis of molecular variation (AMOVA) was performed in ARLEQUIN 3.0. 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-mesh traps 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 two haplotypes = 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 H11 from Kwamsisi showed on average 1.51% nucleotide difference per sites with the other haplotypes (see Fig. 2b).

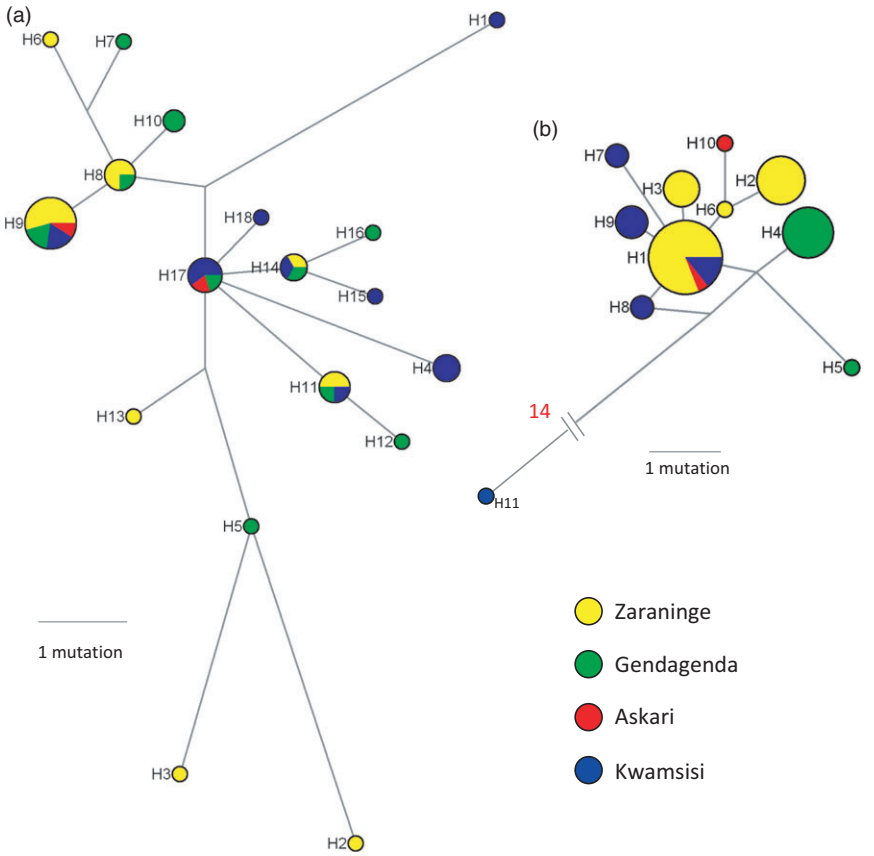

Figure 2 Median-joining network of (a) *Rhynchocyon petersi* and (b) *Beamys hindei* cytochrome b haplotypes. The number of mutations between haplotypes is proportional to the length of branches, and circle sizes are proportional to the frequency of a given haplotype. The long branch of haplotype 11 in the *Beamys hindei* network is shortened and the number 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).

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 haplotype network 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 at that 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.

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 by forest, 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).

Population genetic analysis of *R. petersi*

Allelic richness (A_R), inbreeding coefficient (F_{IS}), observed heterozygosity (H_O), expected heterozygosity (H_E) 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 F_{IS} 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: the best 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).

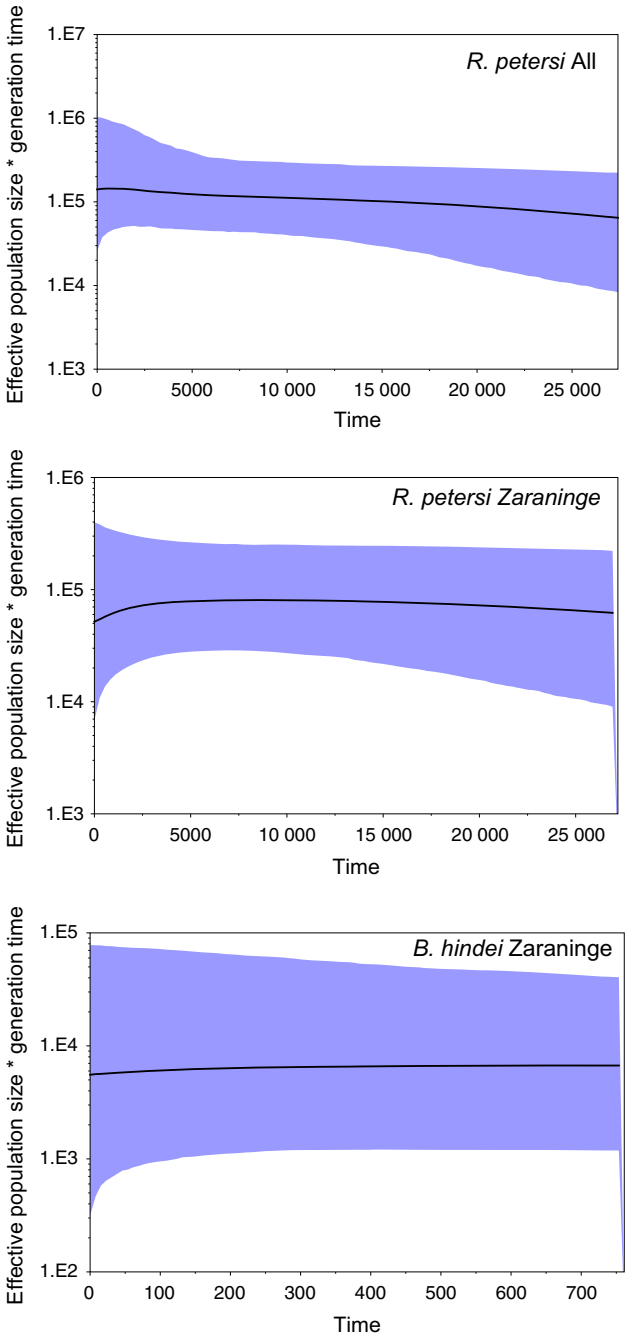

Figure 3 Bayesian skyline plots based on the mtDNA sequence data. The *y*-axis is the product of the effective population size and the generation time and the *x*-axis shows time. A mammalian average mutation rate of 5×10^{-8} was used and an effective generation time of 1 year is assumed.

Table 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

	Rhynchocyon petersi All	*Rhynchocyon petersi* Zaraninge	*Beamys hindei* Zaraninge
Number of individuals	43	16	32
Median *Ne*	140 890	51 795	5557
Geometric mean *Ne*	147 420	51 681	5366
95% HPD interval	[8991–699 770]	[1159–274 490]	[14–50 087]

Genetic estimates of *Ne* based on different approaches varied considerably: The LD method provided a *Ne* of 66 (43–115 95% CI), while the heterozygote excess and the coancestry 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).

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, i.e. the habitat between forests can support *R. petersi* but not *B. hindei*, appears the more likely explanation of the genetic pattern observed. Currently the 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 in the 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

Table 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	0.288	2.846	−0.301	0.692	0.538	0.566	2.995	−0.094	0.611	0.560	1.000
Rhpe41	4.917	−0.073	0.833	0.779	0.845	4	0.424	0.364	0.619	0.030	5.842	−0.014	0.824	0.813	0.693
Rhpe42	3	0.295	0.417	0.583	0.276	4.828	0.160	0.538	0.637	0.599	4.706	−0.110	0.778	0.703	0.759
Rhpe64	2	−0.100	0.250	0.228	1.000	1	–	–	–	–	1.983	−0.097	0.222	0.203	1.000
Rhpe08	2	−0.467	0.667	0.464	0.215	2	0.189	0.385	0.471	0.577	2	0.197	0.333	0.413	0.558
Rhpe33	2	−0.375	0.583	0.431	0.488	1.982	−0.043	0.154	0.148	1.000	2	0.029	0.444	0.457	1.000
Rhpe43	6	0.085	0.636	0.693	0.956	4.830	0.127	0.615	0.702	0.448	4.871	−0.103	0.824	0.749	0.280
Rhpe62	2	−0.100	0.250	0.228	1.000	2	0.593	0.154	0.369	0.075	1.995	0.329	0.167	0.246	0.271
Average	2.980	−0.015	0.496	0.489	0.634	2.935	0.173	0.414	0.497	0.067	3.290	−0.015	0.525	0.518	0.695

Table 3 Analysis of molecular variance (AMOVA) and hierarchical *F* statistics for *Rhynchocyon petersi*

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}

& Anderson, 1994). A parallel study conducted on the home range of *R. petersi* in Zaraninge forest reports that the species 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 much lower 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 Zaraninge on 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 km^2, 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 support about 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 and effective size to be related for these species. First, census 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 radiotelemetry). 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

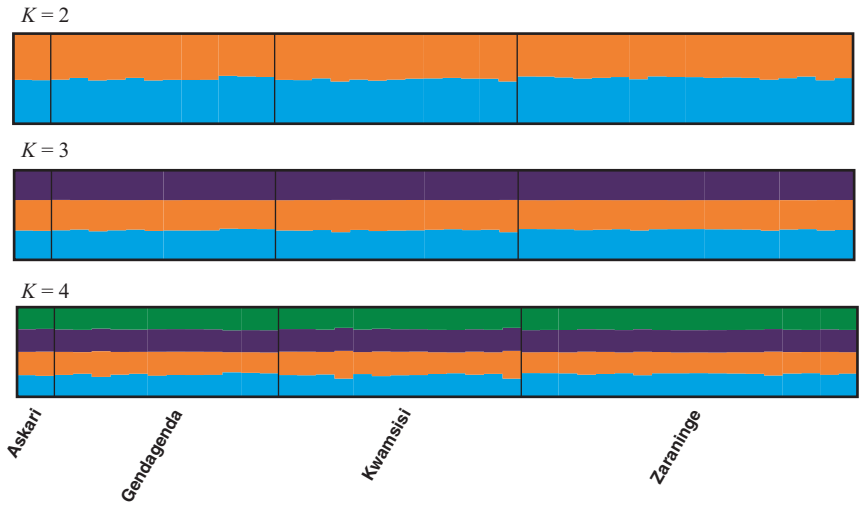

Figure 4 STRUCTURE summary plots of the estimated membership coefficient (*y*-axis) for each *Rhynchocyon petersi* individual for $K = 2$ (top plot) to $K = 4$ (bottom plot). Each individual is represented by a single vertical line broken into segments proportional to the membership coefficient for each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

East Arc Mountain forests. In those latter, the more disjoint distribution of the species may be reflected in more divergent gene pools.

Acknowledgements

The authors wish to thank all persons involved in the realization of this study. Special thank goes to the field assistants Omari Kibwana, Dege Hussein, Victor Morris and Samweli Shaba for their commitment during the whole period of data collection. The Tanzania Wildlife Research Institute and Tanzania National Parks gave permission to conduct the study. The staffs of Saadani National Park are to be thanked for their support especially Mr Hassani, Chief Park Warden and Miss Halima Penga, Warden for Ecology. Funding was obtained through the VLIR – Own Initiative Program. Dr. Galen Rathbun provided valuable comments on methodology for field collections of the study species and Dr. Stuart J. E. Baird helpful comments and English proofreading of the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Estimated Ln Prob of the data of *Rhynchocyon petersi* for STUCTURE runs from $K = 1$ to $K = 4$.

Table S1. Date of sampling, localities, sample identification, mitochondrial DNA haplotypes (mt hap) and microsatellite alleles.