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Research Article

# Diversity of Terrestrial Small Mammals in Mafia Island, Tanzania

**TM Sirilo[1]*, N Van Houtte[4], NE Mbije[1], CA Sabuni[2], A Laudisoit[3], S Gryseels[4] and H Leirs[4]**

[1]*Department of Wildlife Management, Sokoine University of Agriculture, Morogoro*

[2]*Institute of Pest Management, Sokoine University of Agriculture, Morogoro, Tanzania*

[3]*Eco Health Alliance, New York, USA*

[4]*Evolutionary Ecology Group, University of Antwerp, Belgium*

***Corresponding Author:** TM Sirilo, Department of Wildlife Management, Sokoine University of Agriculture, Morogoro.

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## Abstract

A study on the diversity of small mammals was conducted in different sites (Chole, Jibondo, and Juani) in Mafia Island from August to October 2021. The study involved two methods including Sherman traps which were used to capture small rodents and shrews and camera traps which were used for recording medium-sized mammals which are too big to be trapped in Sherman traps. Each trapped individual was immobilized with ethanol, identified, weighed, and sexed. From each captured animal, a small piece of the ear was cut under aseptic conditions and preserved in 1.5ml screw caped Eppendorf tubes containing 90% ethanol for further confirmatory species identification using molecular techniques. All captured rodents in Mafia Island were identified as *Rattus rattus* (*R.rattus*) using sequence analysis of the cytochrome b (cytb) gene. The identified *Rattus rattus* were observed to fall within the RrC lineage I. Only one shrew *Crocidura* sp. was captured which is yet to be characterized. The camera traps recorded Black and rufous sengi *Rhynchocyon petersi*, blue monkey *Cercopithecus mitis*, blue duiker *Cephalophus monticola*, the introduced Small Indian Genet Viverricula indica, and the red bush squirrel *Paraxerus palliatus*. Despite the limited time of data collection, results show a low diversity of small mammals on the islands of Mafia, with *R. rattus* as the dominant species in the islands. This finding recommends further studies in the rest of the forest patches in Mafia and exploring the diversity of flying mammals which was not included in this study.

**Keywords:** Diversity; Small mammals; *Rattus rattus; Mafia*

## Abbreviations

AMOVA: Analysis of Molecular Variance; BLAST: Basic Local Alignment Search Tool; CTR: Camera Trap Rate; Cytb: Cytochrome b; DNA: Deoxyrebosenucleic Acid; DNTPs : Deoxynucleoside Triphosphates; EVOECO: Evolutionary Ecology Group; H: Shannon Wiener Diversity Index; MEGA: Molecular Evolutionary Genetic Analysis; mtDNA: Mitochondria Deoxyribonucleic Acid; NC: Number of Captures; NCBI: National Center for Biotechnology Information, GenBank; NP: Number of Photos; NT: Number of Trap Night; PCR: Polymerase Chain Reaction; $P_i$ : Proportional of Individual Species; RrC: Rattus Rattus Complex; TN: Trap Night; TS: Trapping Success; VIB: Vlaams Institute for Biotechnology

## Introduction

Small mammals comprise many animal groups including flying (e.g., Chiroptera) and non-flying (e.g., rodents, mongoose, hedgehogs, shrews (Soricidae), elephant shrews or sengis (Macroscelidae), squirrels and hares [1]. This group is the largest among mammals in terms of abundance and species diversity, comprising about 47% of all known mammals [2].

Most of these animals are well adapted to nocturnal activity, efficient water conservation, control of body temperature, and rapid initiation of reproduction after drought. Often, small mammals have been disregarded when conservation strategies are initiated although they are a significant part of the fauna in the ecosystem [3]. Indeed, small mammals are keystone species that act in facilitating the carbon cycle and energy flow, soil fertility and serve as sensitive bio-indicators for environmental degradation [3,4].

The small mammals' communities' interactions with forest ecosystems are important in determining biodiversity ecosystem health [5]. They contribute to seed dissemination of vascular plants, decomposing organic matter and litter, regulating some invertebrates' populations and serve as food for predators such as birds of prey, snakes and medium to large size carnivorous mammals.

The global loss of coastal habitats particularly in the tropics has become an issue of increasing concern. Coastal habitats often provide resources on which the surrounding population depends for their survival and livelihood [6]. For centuries, island ecosystems have been the target for habitat loss, fragmentation, and degradation primarily for agricultural reasons, wood harvesting for fuel and residential cooking, and timber for construction [7]. Such habitat destruction not only directly damages the island flora but also reduces the faunal biodiversity such as small mammals as forested areas are diminished in which suitable habitats and food resources for fauna also diminish [7].

Mafia island forms part of a coastal tourism destination with unique biodiversity along the East African coast which is made up of coastal forests recognized as one of the major 25 biodiversity hotspots worldwide [8].

Even though during the recent past, numerous studies of small mammals have been conducted in various habitats in Tanzania, including both montane and coastal forests, as well as non-forested biotopes e.g. [9-15]. Yet there is still a paucity of information in the Mafia islands which consists also the remnants of coastal forests [16]. Nevertheless, the previous surveys have increased our knowledge of understanding the status, composition, and species diversity of small mammals on a large scale and some of these have yielded the discovery of new species [17]. Therefore, the current study of small mammals in Mafia Island aimed at first; providing a list of small mammal species and establishing the diversity in the existing habitats since conservation needs to understand what you have in these biotopes.

## Materials and Methods

### Study Area

The study was conducted in different habitats of Mafia Island including Jibondo, Chole, and Juani [16] between August and October 2021.

The islands, covering about 440km$^2$ of land,are in the Indian Ocean off the eastern coast of Tanzania, lying between latitudes 7$^0$ and 8$^0$ south, and longitude 39$^0$ and 40$^0$ east, approximately 20 km east of the mouth of the Rufiji River, and about 120 km south of Dar-es-Salaam City (Figure 1).


**Figure 1:** Map showing the study areas in Mafia and neighboring islands (Juani, Chole and Jibondo).

Mafia Island is characterized by its natural vegetation, which includes tidal mangrove thickets and scrubby coastal moorlands palm wooden grassland, and lowland rainforest. Also, baobab trees grow along with the native Albizia [18].

The forests within the islands are fragmented, small and are surrounded by marginalized communities that heavily depend on the islands for land and forest resources, such as timber and the production of charcoal observed in the Kua and Mlola forests [16].

The study was conducted in areas with natural vegetation which were Mlola and Kua-Juani forests; Utende and, Chole mangroves, and Juani shrubs.

### Trapping of small mammal

Trapping of the small mammals was conducted, using live folding aluminum-galvanized box traps (23 x 8 x 9 cm; H.B Sherman Traps, Inc. Tallahassee, Florida). Traps were positioned using two layouts; that transect layout (20m × 500m) with 150 traps, each trap was positioned at 10m while a distance from one transect line to another was 10m. Also, grid layout (70m × 70m) with 49 traps, each trap was positioned at 10 m following [11]. The transect layout was set at Mlola and Juani; while the grid layout was set at Utende, Chole and Jibondo. The two Sherman trap layouts at the different sites were chosen based on the nature of the topography and vegetation patches of the area.

A mixture of peanut butter and maize was used as the bait, and the traps were checked for captures every morning for five consecutive nights as described by [19]. Field identification of the captured small mammals followed [2]. From each captured animal, a small piece of the ear was cut under aseptic conditions and preserved in 1.5ml screw caped Eppendorf tubes containing 90% ethanol for further confirmatory species identification using molecular techniques. Thereafter, the animal was released in the same area where it was captured [9,14,19].

### Camera trapping

In each forest 10 camera traps (Reconyx Hyper fire HC 500 (Reconyx, Inc) semi-covert infrared emitters) were set spaced at about 100-200m apart. Around the specific camera locations, indicative signs were searched before setting the camera to maximize photographic capture rate, for example, distinct paths used by elephant shrews [19].

### Trapping success

The trapping success of captured animals was computed using the following formular.

$$TS = \frac{NC}{NT} \times 100\%$$

in which; TS = trapping success, NC = number of captures, NT = Number of trap night

### Camera trap rate

The Camera trap rate was computed using the following formular.

$$CTR = \frac{NP}{TN}$$

In which CTR = Camera Trap Rate, NP = Number of Photos, TN = Trap Night

### Species abundance

Small mammal's species abundance was determined as number of individual animals existing in the study site.

### Species richness

Small mammal's species richness was determined as number of species existing in the study site; this was obtained as sum of species of small mammals recorded in field.

### Species diversity

Diversity of small mammals, which included mammals captured by Sherman traps and in camera was computed using Shannon Wiener diversity index by using the following formular.

$$H' = -\sum_{i=1}^{s} Pi \ln(Pi)$$

in which; H = Shannon wiener diversity index

$P_i$ = Proportional of individual species

### Molecular Identification of Species

Species confirmatory was carried using a single mitochondrial marker (cytb gene). To avoid contamination, pre-amplification procedures and post-amplification analyses were performed in separate rooms in the Evolutionary Ecology Group (EVOECO) Laboratory at the University of Antwerp in Belgium. DNA was extracted from the tissue with NucleoSpin Tissue Kit (Macherey-Nagel) following manufacturer's instructions. Primer sets used to amplify the cytb genes are listed in table 1.

All amplifications were conducted in 15µL reactions containing about 1.5µL of extracted DNA, 0.3µL of dNTP, 0.8µM of each primer, and 0.2 units of Taq polymerase, 3µL of 10X buffer, 0.9mM of MgCl2 and 7.5µL of Nuclease free water.

Cycling conditions were as follows: one activation step at 94°C for 5min followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30s, elongation at 72°C for 90s, final exten-

| Designation | Gene name | Nucleotide sequence 5' T 3' | Annealing temperature | Fragment length |
|---|---|---|---|---|
| Cytb | Cytochromeb | | | |
| L14723 | | ACCAATGACATGAAAAATCATCGTT | 52°C | 1140bp |
| H15915 | | TCTCCATTTCTGGTTTACAAGAC | | |

**Table 1:** Primers and PCR cycling conditions used in this study.

sion at 72°C for 5min and lastly it ended with a final temperature of 22°C. Sequencing was done at Neuromics Support Facility (part of VIB, Vlaams institute for Biotechnology).

In this study, twenty-five (25) nucleotide sequences of rodents from five habitats were subjected to Basic Local Alignment Search Tool (BLAST) to determine species identity compared with other nucleotides of published rodent species available in GenBank database. Alignment of cytb gene sequences were performed using bioinformatics software platform Geneious Prime® 2022.2.1.

A BLAST search was performed for each sequence to locate nucleotide sequences of the related species available in National Center for Biotechnology Information (NCBI) GenBank.

### Molecular analysis

To determine the genetic relationship of rodent populations from different habitats, analysis of molecular variance (AMOVA) and population pairwise Fst were performed using Arlequin Version 3.0 [20]. Computation of the genetic distance for each population was calculated at $P < 0.05$ with the algorithm suggested by [20].

The Pairwise Fst test was performed using 10000 permutations to determine the genetic differentiation. The level of genetic differentiation was determined using [21], estimation of Wright's fixation index. Median-joining networks were constructed to determine the evolutionary relationships of mitochondria Deoxyribonucleic acid (mtDNA) rodent's haplotypes following the algorithms of [22], using Network 4.6.1.0 software (http://https://www.fluxus-engineering.com/sharenet.htm). The phylogenetic analysis was done using the Maximum Likelihood method, utilizing bootstrap test method with 100,000 replicates which are included in MEGA.

The evolutionary history of rodents found at Mafia Island was inferred by using the Maximum Likelihood method and Tamura 3-parameter model [23]. The percentage of a replicate of the optimal trees in which the associated taxa clustered are shown next to the branches. There were a total of 1124 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [24]. Sequences that were outside the nucleotide sequences from 1124 bp of the Cytb region were excluded from the molecular analysis.

## Results and Discussion

### Results

### Live trapping success

A total of 168 small mammals belonging to the family Muridae, *R.rattus* species, were captured in the five studied sites Mlola (n = 41), Kua-Juani (n = 37), Utende (n = 25), Chole (n = 20) and Jibondo (n = 45).

There was a significant variation on the trapping successes of the small mammals in Mafia Island in the selected fives sites (Figure 2). The variation in the trapping success ranged from 2.72%, in Chole, to 6.12% in Jibondo Island.

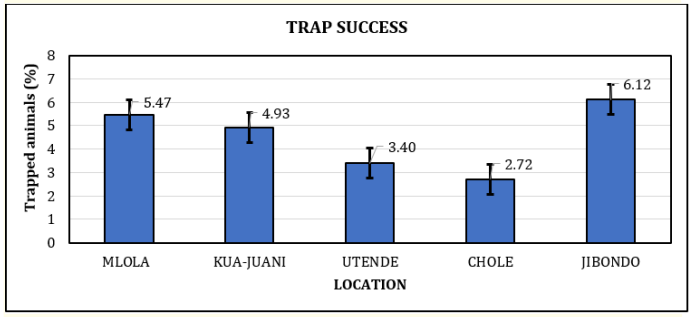


**Figure 2:** Trapping success in the five selected sites of Mafia Island.

### Camera trap rate

The camera trap rate results from the five studied sites in Mafia Island show a slight significant variation from one site to another (Figure 3).

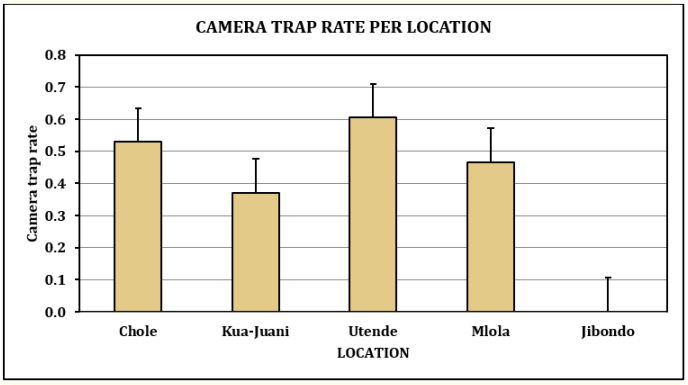


**Figure 3:** Camera trapping rate in five selected sites of Mafia Island.

## Species abundance

Species abundance of the five studied sites of Mafia Island was obtained showing that there was a clear distinction on the abundance of animals captured in the studied sites (Figure 4).

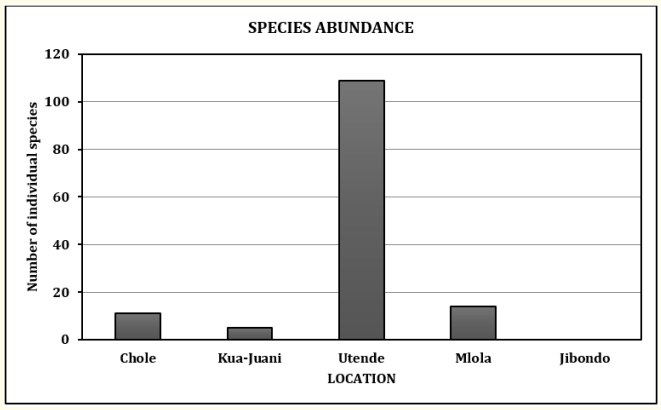


**Figure 4:** Species abundance in the five selected sites of Mafia Island.

Five species from five genera and five families were recorded from the camera traps (Table 2). From Sherman trapping, family Muridae was dominant with *R. rattus* strikingly accounting for all (n = 120) rodents captured in the five study sites.

## Species richness

The small mammals' species richness was obtained as shown in figure 5 and table 3.

| Common name | Scientific name | Family | Abundance |
|---|---|---|---|
| Blue monkey | *Cercopithecus mitis* | Cercopithecidae | 36 |
| Civet cat | *Civettictis civetta* | Viverridae | 11 |
| Red Bush Squirrel | *Paraxerus palliates* | Sciuridae | 27 |
| Blue duiker | *Philantomba monticola* | Bovidae | 17 |
| Sengis | *Rhynchocyon Petersi* | Macroscelidae | 48 |
| | Genera (5) | Family (5) | |

**Table 2:** Species abundance from Camera trap.

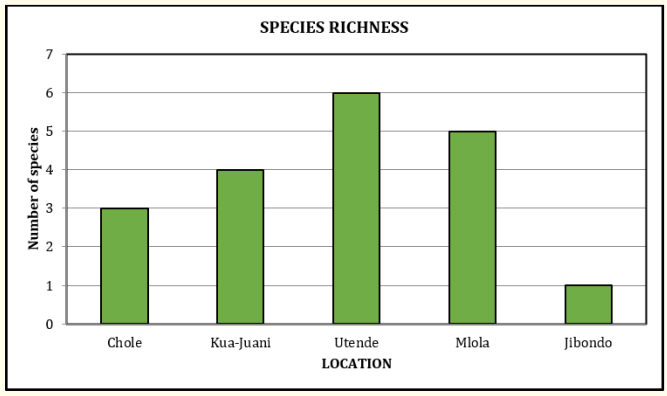


**Figure 5:** Species richness in five selected sites of Mafia Island.

| Common Name | Chole | Kua-Juani | Utende | Mlola | Jibondo |
|---|---|---|---|---|---|
| Rat | 1 | 1 | 1 | 1 | 1 |
| Blue monkey | 1 | 1 | 1 | 1 | 0 |
| Civet cat | 1 | 1 | 1 | 0 | 0 |
| Squirrel | 0 | 0 | 1 | 1 | 0 |
| Duiker | 0 | 1 | 1 | 1 | 0 |
| Sengis | 0 | 0 | 1 | 1 | 0 |
| Species richness | 3 | 4 | 6 | 5 | 1 |

**Table 3:** Species richness in Chole, Kua-Juani, Utende, Mlola and Jibondo islands.

## Species diversity

The species diversity in the selected five sites showed a slightly significant difference between Utende and the other four sights. There was, however, no clear significant difference on the species diversity between Mlola, Kua-juani, and Chole (Figure 6).

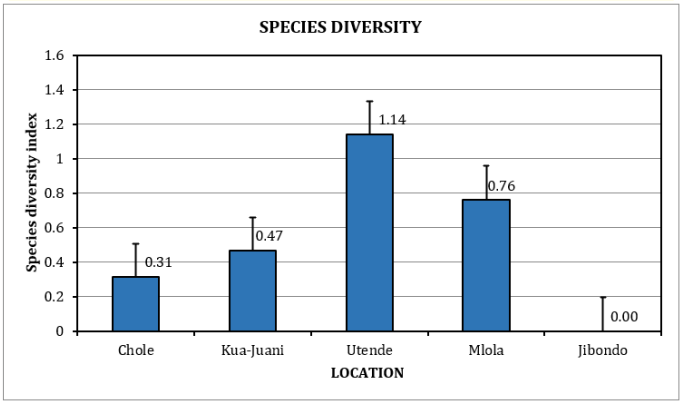


**Figure 6:** Species diversity of small mammals in Chole, Kua-Juani, Utende, Mlola and Jibondo sub-islands.

## Molecular identification of species

The obtained nucleotide sequences from Cytb mitochondrial gene, subjected to BLAST, were compared with previously published data from five different habitats in Mafia Island and were identified as *Rattus rattus* falling within the RrC lineage I [25] as shown by the Maximum Likelihood phylogenetic tree (Figure 7).

The analysis of molecular variance (AMOVA) based on haplotype frequencies revealed that 76.96% of the genetic variation occurred between populations, whereas only 23.04% of the genetic variation occurred within populations (Table 4). High genetic differentiation (Fst 0.76961) among the *R. rattus* populations was found. Strongly genetic structuring of the populations was revealed by Fst value ($P < 0.0001$), suggesting that there was greater genetic divergence between rodent populations found in different habitats at Mafia Island.

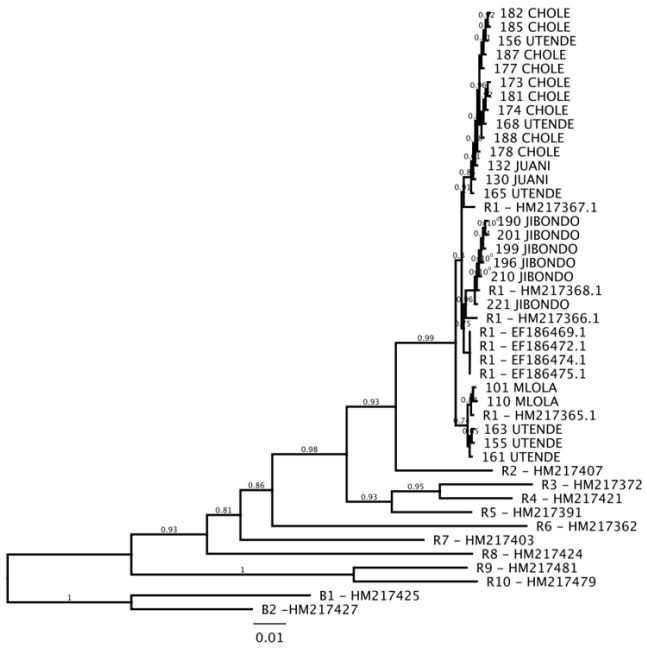


**Figure 7:** Maximum likelihood phylogenetic tree of the Rattus rattus Cytb sequences.

| Source of Variation | Degree of freedom | Sum of Squares | Variance Components | Percentage of Variations | $F_{ST}$ | P Value |
|---|---|---|---|---|---|---|
| Between Populations | **4** | 6.600 | 0.33405 Va | 76.96 | 0.76961 | 0.0001 |
| Within Population | 20 | 2.000 | 0.10000 Vb | 23.04 | | |
| Total | 24 | 8.600 | 213.98582 | | | |

**Table 4:** Molecular Variance (AMOVA) of of the *Rattus rattus.*

## Evolutionary relationship

In the evolutionary relationship, the median-joining network was constructed to understand the relationship of haplotypes of rodents from Mafia Island. The haplotype distribution revealed six distinct haplogroups of the *R. rattus* population found in the Mafia Islands (Figure 8). High haplotype diversity was observed between Jibondo, Chole, Mlola, and Juani populations.

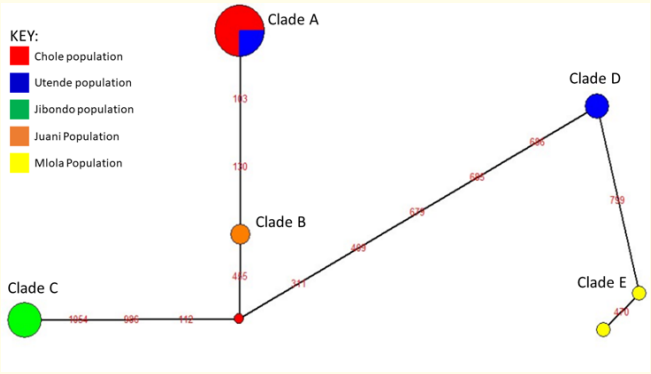


**Figure 8:** Median-joining network of six haplotypes observed in the R. rattus populations found in Mafia Island based on the polymorphic sites of the mitochondrial Cytochrome b gene.

## Discussion

In this study trapping of terrestrial small mammals was done using Sherman and camera traps. For the Sherman trap one species of rodent from the family Muridae, *rattus* from RrC Lineage I was captured. For camera trap, five terrestrial small mammals were captured, which included the Black and Rufous Sengi *Rhynchocyon petersi*, Blue Duiker *Philanthomba monticola*, the Small Indian Genet *Viverricula indica,* blue monkey *Cercopithecus mitis,* and the Red Bush squirrel *P. palliates*.

The biogeographic pattern of RrC Lineage I is present in Western India with broad distribution outside of mainland Asia in Europe, the Americas, Africa, and Madagascar, Australia, and various Pacific Islands [25].

*Rattus rattus* is extensively disseminated as a household as well as agricultural pest impacting cereals, vegetables, and palm plantations. It is also a dominant wild rodent in natural habitat [26-29].

Trapping of small mammals on Mafia and neighboring islands was low. This could be partly contributed to the capturing of non-target land hermit crabs (*Coenobita rugosus*) and the most common species of shell (*Terebralia palustris*) which are abundant in the study areas. These non-target species commonly entered the Sherman traps earlier, thus setting off the traps and restricting rodents from entering.

Mafia Island is a continental shelf island, which is physically connected to the mainland during low sea level periods. Due to this, these islands have a geological structure much like the mainland [30].

## Small mammals' species abundance, richness, and diversity

Species abundance, richness and diversity in Mafia Island is influenced by the type of habitat and human activities in an area. Small mammal abundance and diversity in various locations is attributed to the type of vegetation, food resource availability, cover, and human activity [1,4,12]. The same appears to be the case with Mafia and neighboring small islands.

The variation in species abundance in the five sites was likely due to the nature of the vegetation which limited some methods of capturing small mammals to be employed, hence the low trapping success of the traps. For instance, the transect layout was set at Mlola and Juani, with 150 traps for each site, since the areas had fewer physical trapping obstacles. The transect layout was therefore easy to apply. On the contrary, the grid layout at Utende, Chole and Jibondo, was more difficult because the areas were congested with vegetation and igneous rocks.

Indeed, species richness for terrestrial small mammals was recorded as low in Jibondo due to the failure of the camera traps to capture animals, due to high temperature during the day. This area was also predominantly rocky thus hindering good capturing by the camera. It is striking, however, that only *R. rattus* was captured, which is more commonly an indoor small mammal. It is possible that other small mammal species were missed in other habitats given the limited time of data collection.

## Evolutionary relationship and molecular identification

Haplotypes from this study on Mafia Island provide additional information on the colonization pathways of *R. rattus* in the West-

ern Indian Ocean region. It is sought that during the medieval maritime, trade routes by traders in the Indian Ocean caused further spread of RrC lineage I *R. rattus* from India into East Africa, the Indian Ocean Islands, and whole of the Mediterranean region [25,31,32].

Historically, in the eighteenth and nineteenth centuries, there was a strong interaction of the ocean-centered world trade, which took the East African coast, including the Mafia, as a trade center [32].

It is plausible, however, that the colonization by *R. rattus* in the islands most likely originated from East Africa, Madagascar, and the Arabian Peninsula and through independent shipping from all over the Western Indian Ocean [25,33]. The reason for this variation is also based on different colonization that occurred in the Island.

There was also an ambiguity in the morphological identification of rodents based on coloration, with *R. rattus* being confused with *Aethomys* spp, which has not been observed in the Western Indian Ocean. The rodent described to be *Aethomys* spp has a yellowish/grayish ventral coloration and a greyish dorsal part. This ambiguity was, however, cleared through DNA molecular identification of the species.

## Conclusion

The wide range of intra-specific morphological variation makes morphological criteria insufficient for accurate small mammal species identification because it has led to an over-description of species and confusing taxonomy, particularly because of an overabundance of synonyms.

Thus, using DNA molecular techniques for species identification, this study has succeeded in refining the taxonomy of one of the most difficult groups of mammals, *Rattus rattus* [34,35], as most species of rodents expected within the area were retrieved and turned to fall within the *Rattus* genus. Future studies should comprehensively include morphological, karyological, mitochondrial and nuclear markers data.

The study also recommends further studies in the rest of forest patches in Mafia and also explores the diversity of flying mammals, which was not included in this study. More studies based on DNA molecular identification of the small mammals should be done given the high accuracy of genetical approach in species identification.

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## Conflict of Interest

There is no any conflict of interest exists.

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