Genetic characterization, ecology, and population dynamics of the house mouse (*Mus musculus*) in Ankazobe District, Madagascar

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Abstract

On Madagascar, little is known regarding the natural history of the widespread introduced species, *Mus musculus* (family Muridae). The aim of this study was to identify the subspecies occurring on the island, and aspects of its ecology and population dynamics, in order to implement control measures for zoonotic surveillance. We implemented a longitudinal study at six localities of the Ankazobe District of the Central Highlands between 2018 and 2020. Six small mammal trapping sessions in houses were conducted. A total of 265 individuals of *M. musculus* were captured during the two-year project. A portion of the cytochrome *b* gene for 12 individuals was amplified and sequenced in order to identify the form occurring in this area of the island. The associated molecular analysis showed that individuals from Ankazobe formed a single clade and suggest a monophyletic group. No significant seasonal fluctuation was found in terms of abundance during the six trapping sessions. Morphometric analyses of adults revealed significant variation between trapping sessions, but no significant difference was observed between localities or sex. These findings suggest that *M. musculus* abundance is quite stable and control should be undertaken regularly in this district to avoid plague emergence associated with this species.

Keywords: fluctuation, *Mus musculus* cf. *gentilulus*, control, population characteristics, Central Highlands

Résumé détaillé

A Madagascar, la peste demeure une maladie zoonotique d'importance majeure, représentant une menace persistante pour la santé publique, en particulier dans les zones rurales où les interactions entre l'homme, les réservoirs animaux et les vecteurs sont étroites. Trois espèces introduites de petits mammifères à Madagascar sont connues comme étant réservoirs de *Yersinia pestis* : *Rattus rattus* et *R. norvegicus* (famille des Muridae) et *Suncus murinus* (famille des Soricidae). A ces espèces pourrait s'ajouter *Mus musculus* (famille des Muridae), une espèce largement distribuée dans l'île mais encore peu étudiée dans le contexte de la peste.

Introduit à Madagascar depuis plusieurs siècles, *M. musculus* est actuellement présent dans les habitats anthropisés, les marais, les zones humides et les habitats montagnards éricoïdes. Cependant, sa dynamique de population et sa bio-écologie restent mal connues. Ainsi, l'objectif de cette étude est : (i) d'identifier la sous-espèce de *M. musculus* présente dans un foyer de peste actif, (ii) de caractériser sa bio-écologie à travers des données morphométriques, et (iii) d'analyser la dynamique temporelle de sa population pour mieux orienter les stratégies de lutte.

Une étude longitudinale a été conduite entre 2018 et 2020 dans six localités rurales du District d'Ankazobe, sur les Hautes Terres centrales, une zone connue pour la récurrence des cas de peste humaine. Lors des six sessions de piégeage, des petits mammifères terrestres ont été capturés à l'intérieur des habitations à l'aide de pièges grillagés BTS et de pièges Sherman. Au total, 265 individus de *M. musculus* ont été capturés. Pour l'identification taxonomique, l'ADN mitochondrial de 12 individus représentatifs des six localités a été extrait, et le

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gène du cytochrome *b* a été amplifié et séquencé. Les analyses phylogénétiques de séquences obtenues ont révélé que tous les individus analysés appartiennent à un même clade, suggérant que ces individus forment un groupe monophylétique.

Sur le plan écologique, l'analyse de la dynamique de population à l'aide d'un modèle linéaire mixte généralisé (GLMM) n'a montré aucune variation saisonnière significative du taux de capture, que ce soit selon le sexe, l'âge ou la période de capture. Cela suggère une stabilité relative de l'abondance de cette espèce tout au long de l'année dans ce district. Par ailleurs, les données morphométriques recueillies sur les individus adultes ont été analysées par Analyse en Composantes Principales (ACP). Cette approche a mis en évidence une variabilité significative entre les sessions de capture, probablement due à des facteurs environnementaux et à la disponibilité des ressources alimentaires. Aucune différence significative n'a toutefois été détectée entre les localités ou entre les sexes.

Ces résultats indiquent que les individus d'Ankazobe sont du même groupe, cf. *gentilulus,* que ceux séquencés de Tsimbazaza, Antananarivo. *Mus musculus* est une espèce commensale bien établie dans les habitats humains d'Ankazobe, dont la population reste stable au fil des saisons. Cette stabilité, associée à sa proximité avec l'homme, pourrait favoriser une interaction avec les vecteurs de la peste. Par conséquent, il est recommandé d'inclure cette espèce dans les programmes de surveillance épidémiologique et de mettre en place des mesures de contrôle régulières pour limiter son abondance. Une telle approche permettrait de réduire les risques écologiques et sanitaires liés à cette espèce potentiellement impliquée dans le cycle de transmission de la peste à Madagascar.

Mots clés : fluctuation, *Mus musculus* cf. *gentilulus*, contrôle, caractéristiques des populations, Hautes Terres centrales

Introduction

The house mouse, *Mus musculus* Linnaeus, 1758, within the family Muridae is recognized worldwide as a major invasive terrestrial small mammal species (Goodman, 1995; Marshall, 1977; Soarimalala & Goodman, 2011; Soarimalala *et al*., 2019; Ramasindrazana *et al*., 2022a, Norbury *et al*., 2023). *Mus musculus* is known for its adaptability and ability to colonize and breed in a wide variety of habitats ranging from commensal (Pocock *et al*., 2004) to non-commensal habitats (Fitzgerald *et al*., 1996; Cuthbert & Hilton, 2004). In particular, households form ideal habitats where *M. musculus* finds stable environmental conditions and food supplies, which favor its reproduction throughout the year (Pocock *et al*., 2004). Because of its habitat preferences, *M. musculus* may impact human societies in different ways, through food contamination (Pocock *et al*., 2001), damage to agricultural crops (Brown *et al*., 2007), and anthropogenic structure destruction associated with their gnawing behavior (Sidorov & Putin, 2010). *Mus musculus* is also reported to be a host of different infectious zoonotic such as bacteria (e.g.: *Leptospira interrogans*, *Orientia tsutsugamushi*), viruses (e.g., Astroviruses, lymphocytic choriomeningitis virus), and parasites (*Trypanosoma cruzi*) that can cause public health problems (Meerburg *et al*., 2009; Ramasindrazana *et al*., 2022a).

A previous phylogenetic study of *M. musculus* revealed the presence of different subspecies worldwide (Suzuki & Aplin, 2012) which included a southern Asian subspecies (*M. m. castaneus*: CAS), a northern Eurasian subspecies (*M. m. musculus*: MUS), a western European subspecies (*M. m. domesticus*: DOM), and a subspecies found in Afghanistan and Pakistan (*M. m. bactrianus*). Subsequent analysis of mtDNA control region sequences found one other highly divergent lineage in populations from Yemen (Prager *et al*., 1998), as well as in other areas, involving prehistoric to recent introductions, including on Madagascar (Duplantier *et al*., 2002).

The presence of the house mouse in Madagascar was first documented in the late 19th century. In 1899, Grandidier described a new species, *Mus auratus*, from specimens collected in Morondava on the western coast, distinguishing it from previously described species based on its geographic isolation. Subsequent molecular analyses by Duplantier *et al*. (2002) using mitochondrial markers (DLoop) demonstrated that Malagasy mice belong to a lineage closely related to populations from the Arabian Peninsula, specifically Yemen, indicating that their introduction to Madagascar likely occurred via historical maritime trade and represents a relatively recent and singular colonization event. Indeed, Malagasy mtDNA lineage of *M. musculus* consists of one monophyletic group that belongs to the *gentilulus* group (Duplantier *et al*., 2002; Sakuma *et al*., 2016). Recent whole genome analyses based on few samples revealed that house mice occurring on Madagascar showed strong genetic affinity to *M. m. castaneus* with a tiny contribution of *M. m. domesticus* (Fujiwara *et al*., 2022). *Mus musculus* colonized a wide range of habitats (Rakotomalala & Goodman, 2010; Ramasindrazana *et al*., 2022a) and is the second most common rodent found in human settlements throughout the island

Regarding the role of this species on Madagascar as a reservoir of zoonotic agents, several studies revealed the presence of such pathogens in *Mus* (Brook *et al*., 2017; Raharinosy *et al*., 2018; Parany *et al*., 2025). It is important to mention that the District of Ankazobe in the Central Highlands lies in a plague-endemic area and is considered an active focus zone (Ramasindrazana *et al*., 2022b). For this reason, it is a critical region to survey rodent-associated zoonotic risks, particularly considering that this species is a major component of small mammal assemblages in non-forested portions of the area. To date, little is known regarding population characteristics of *M. musculus* in Madagascar in general or specifically the Central Highlands. The aim of this study was to identify *M. musculus* group in this district, characterize its ecology and population dynamics in order to reduce house mouse populations in human settlements and limit the risk of disease transmission.

Materials and methods

Study area

This study was conducted in the Ankazobe District, a rural area of the Central Highlands of Madagascar, a plague focus zone where *M. musculus* lives in a synanthropic manner (Rahelinirina *et al*., 2021). Six villages were investigated: Ambohitromby (18°25’36.31’’S, 47°9’7.14’’E), Ambolotarakely (18°1’24.02’’S, 47°23’33.08’’E), Antakavana (18°1’16.76’’S, 47°23’34.16’’E), Kiangara (17°54’29.12’’S, 47°1’16.38’’E), Talata-Angavo (18°12’22’’S, 47°5’59.16’’E), and Ankazobe I (18°12’23.49’’S, 47°5’53.63’’E) (Figure 1). Except Ankazobe I, which lies in the district capitol and the most urbanized among communes in Ankazobe District, the other five sites typically consist of 20-150 households grouped on hill tops. Houses, usually with two levels, are made of mud or bricks, generally with thatched roofs. The lower level of these houses is generally used for crop storage or living space for

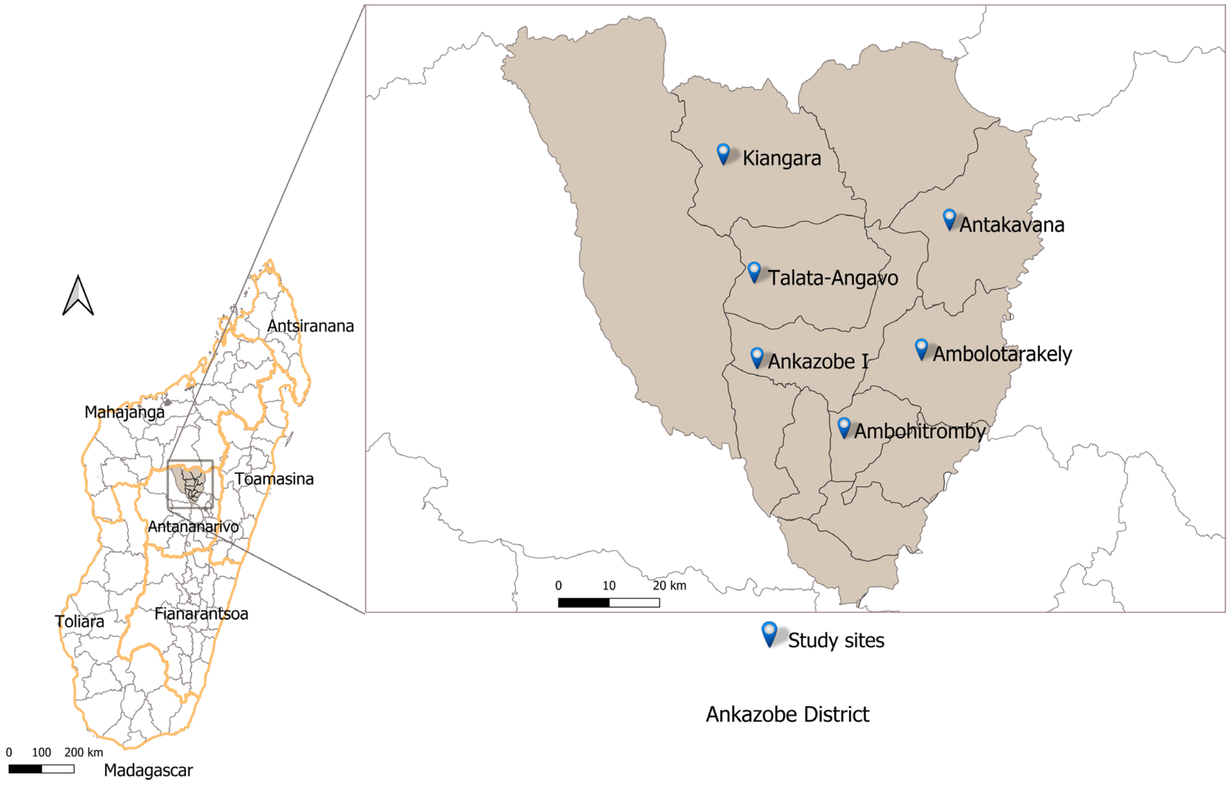

Figure 1. Map of the study sites in the Ankazobe District. Left panel: map of Madagascar with the six ex-provinces and showing the location of the Ankazobe District. Right panel: zoomed-in view of the Ankazobe District with each of the six villages where small mammals were captured.

domestic animals like zebu, pig or poultry and the second level used for human occupation.

Small mammal capture

A detailed description of field protocols of the small mammals sampling in the Ankazobe District was provided in Parany *et al*. (2025). Briefly, small mammal trapping was carried out in the six villages from December 2018 to June 2020 over a total of six trapping sessions (TS), which were planned to cover the four seasonal periods, namely the rainy (TS1 and TS5) and dry (TS3) seasons, as well as the transitional periods from rainy-to-dry (TS2 and TS6) and dry-to-rainy (TS4). In the context of this study, *M. musculus* was captured only inside houses and these data are employed herein associated with *Mus* population analyses (Parany *et al*., 2025). Thirty-six traps were installed inside houses within 18 households in the six villages. Inside each house, one BTS (Besançon Technical Service, Besançon, France, BTS; 30 cm long x 10 cm wide x 10 cm high) and one Sherman (H.B. Sherman Trap, Inc., Tallahassee, FL; 23 cm long x 7.5 cm wide x 9 cm high) traps were installed for three successive nights. Traps were baited with dried onions and dry fish and checked and rebaited twice a day (morning and afternoon).

All small mammals captured were euthanized by cervical dislocation and identified at the species level based on morphological characters. External measurements included: total length: TL; head-body length: HB; tail length: TV; ear length: EAR; and hind foot length (without the claws): HF – all in mm, and weight (in g). All external measurements were taken by a single field worker, ensuring consistency and avoiding inter-collector variation in measurement techniques. The spleen was collected from each individual and stored in ethanol 95° for molecular analyses.

Age and reproductive status

An analysis of age and reproductive variation of *M. musculus* sampled at the six localities was conducted. Individuals were classified as juveniles or adults based on reproductive status. External and internal reproductive characteristics of both male and female individuals were recorded. In males, animals with scrotal testes and developed epididymis were considered reproductively active and classified as adult. In females, the external condition of the vagina (perforated or non-perforated) was noted. All females that were pregnant, lactating, or with placental scars were considered reproductively active and adult. The balance of the individuals was classified as juveniles.

DNA extraction, sequencing, and phylogenetic analyses of *Mus musculus*

A total of 12 individuals of *M. musculus* from the six sites were included in our phylogenetic analysis. Total DNA was extracted from the spleen sample using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). A 1,140 bp fragment mitochondrial region encoding for cytochrome *b* was then amplified by Polymerase Chain Reaction (PCR) using primers L14723 (5’ ACC AAT GAC ATG AAA AAT CAT CGT T3’) and H15915 (5’TCT CCA TTT CTG GTT TAC AAG AC3’) (Ducroz *et al*., 2001; Shivambu *et al*., 2023). The amplification conditions included an initial denaturation phase at 94°C for 3 min, followed by 35 cycles each of denaturation at 92°C for 30 s, hybridization at 55°C for 45 s, elongation at 72°C for 1 min, and a final elongation phase at 72°C for 10 min. PCR products were sent to Genoscreen (Lille, France) for Sanger sequencing. All new sequences were deposited in GenBank under accession numbers PV054343 to PV054354.

Sequences were obtained and aligned using the multiple alignment algorithm implemented in CLUSTALW, and they were subsequently aligned with sequences uploaded from GenBank (Table 1) of *M. m. castaneus* (n =11), *M. m. domesticus* (n = 10), *M. m. musculus* (n =5), as well as of *M. m. gentilulus* (n = 5) from Tsimbazaza, a locality from the Central Highlands and in urban Antananarivo. The best fit substitution model was selected based on the lowest Akaike Information Criterion (AIC) calculated using jModelTest2 (Darriba *et al*., 2012; Guindon & Gascuel, 2003). A phylogenetic tree was inferred by Maximum Likelihood methods with HKY + G model and 1,000 bootstrap replicates as a robustness proxy using MEGA X (Kumar *et al*., 2018).

Population data analysis

The capture rate of *M. musculus* per locality was estimated from the overall trap success, which is defined as the total number of individuals captured divided by the number of trap-night, expressed as a percentage.

We used the Generalized Linear Mixed Model (GLMM) to assess whether the capture rate of *M. musculus* varied according to sex, age, and trapping session based on a Poisson distribution model

Table 1. Details of cytochrome *b* sequences from *Mus musculus* used in this study.

Subspecies	Localities	Accession Number	Reference
Mus musculus castaneus	India	AB819908	Suzuki *et al*. (2013)
Mus musculus castaneus	India	AB820897	Suzuki *et al*. (2013)
Mus musculus castaneus	Philippines	AB649500	Suzuki *et al*. (2013)
Mus musculus castaneus	China	AB819914	Suzuki *et al*. (2013)
Mus musculus castaneus	Russia	AB820911	Suzuki *et al*. (2013)
Mus musculus castaneus	India	AB819909	Suzuki *et al*. (2013)
Mus musculus castaneus	Japan	AB820914	Suzuki *et al*. (2013)
Mus musculus castaneus	India	AB819910	Suzuki *et al*. (2013)
Mus musculus castaneus	India	AB649499	Suzuki *et al*. (2013)
Mus musculus castaneus	India	AB649490	Suzuki *et al*. (2013)
Mus musculus castaneus	India	AB649488	Suzuki *et al*. 2013
Mus musculus domesticus	South Africa	MZ353139	Shivambu *et al*. (2023)
Mus musculus domesticus	South Africa	MZ353141	Shivambu *et al*. (2023)
Mus musculus domesticus	South Africa	MZ353138	Shivambu *et al*. (2023)
Mus musculus domesticus	South Africa	MZ353140	Shivambu *et al*. (2023)
Mus musculus domesticus	South Africa	MZ353137	Shivambu *et al*. (2023)
Mus musculus domesticus	Australia	AB649474	Suzuki *et al*. (2013)
Mus musculus domesticus	France	AB649458	Suzuki *et al*. (2013)
Mus musculus domesticus	Germany	AB649456	Suzuki *et al*. (2013)
Mus musculus domesticus	Greece	AB649461	Suzuki *et al*. (2013)
Mus musculus domesticus	Cyprus	AB649469	Suzuki *et al*. (2013)
Mus musculus musculus	China	AB649539	Suzuki *et al*. (2013)
Mus musculus musculus	China	AB649540	Suzuki *et al*. (2013)
Mus musculus musculus	Japan	AB649571	Suzuki *et al*. (2013)
Mus musculus musculus	Korea	AB649554	Suzuki *et al*. (2013)
Mus musculus musculus	Japan	AB649572	Suzuki *et al*. (2013)
Mus musculus gentilulus	Madagascar	LC644158	Fujiwara *et al*. (2022)
Mus musculus gentilulus	Madagascar	LC644159	Fujiwara *et al*. (2022)
Mus musculus gentilulus	Madagascar	LC644160	Fujiwara *et al*. (2022)
Mus musculus gentilulus	Madagascar	LC644161	Fujiwara *et al*. (2022)
Mus musculus gentilulus	Madagascar	LC644162	Fujiwara *et al*. (2022)
Mus musculus Ankazobe	Madagascar	PV054343	This study
Mus musculus Ankazobe	Madagascar	PV054344	This study
Mus musculus Ankazobe	Madagascar	PV054345	This study
Mus musculus Ankazobe	Madagascar	PV054346	This study
Mus musculus Ankazobe	Madagascar	PV054347	This study
Mus musculus Ankazobe	Madagascar	PV054348	This study
Mus musculus Ankazobe	Madagascar	PV054349	This study
Mus musculus Ankazobe	Madagascar	PV054350	This study
Mus musculus Ankazobe	Madagascar	PV054351	This study
Mus musculus Ankazobe	Madagascar	PV054352	This study
Mus musculus Ankazobe	Madagascar	PV054353	This study
Mus musculus Ankazobe	Madagascar	PV054354	This study
Mus spretus	Mediterranean	NC025952	Chang & Hwang (2016)

(Breslow & Clayton, 1993) using the *lme4* package. The model included age, sex, and trapping session as fixed effects, while locality was included as a random effect to account for potential variability among sampling sites. In addition, we fitted a null model including only the random effect of locality to test its influence.

Comparisons of external measurements (TL, HB, TV, HF, EAR, and WT) between males and females according to their age (adult or juvenile) were performed using a non-parametric Wilcoxon test. A Chi-square test was used to analyze the variation of individual male and female active reproduction during the six trapping sessions. The effect of trapping session on the proportion of pregnant females was tested using a non-parametric Kruskal-Wallis test (Dytham, 2011).

Principal Component Analysis (PCA) was performed to visualize and investigate multivariate patterns of morphological variation in *M. musculus* adults. ANOVAs were then performed on individual coordinates along Component 1 and 2 in order to test statistically potential morphological differences between trapping sessions, localities or sexes.

All analyses were conducted under R software version 4.3.2 (R Core Team, 2023) stating $P < 0.05$ as statistical significance threshold.

Results

Species abundance

A total of 690 individuals of small mammals were captured inside houses during 3,888 trapping-nights in the six localities in the Ankazobe District. *Rattus rattus* was the most abundant (n = 410), followed by *Mus musculus* (n = 265), *Suncus murinus* (n = 13), and *R. norvegicus* (n = 2). *Mus musculus* represented 38.4% of the total number of animals captured. All *M. musculus* individuals were captured with Sherman traps leading to a trap success of 13.6%.

Phylogenetic characterization *Mus musculus* from Ankazobe based on cytochrome *b* gene

The phylogenetic analysis based on the mitochondrial cytochrome *b* gene indicates that the 12 individuals of *M. musculus* from the six localities captured in the context of this study form a monophyletic clade together with sequences obtained from *M. musculus* from Tsimbazaza (Antananarivo) (Figure 2). This clade corresponds to a group identified as *M. m.* cf. *gentilulus.*

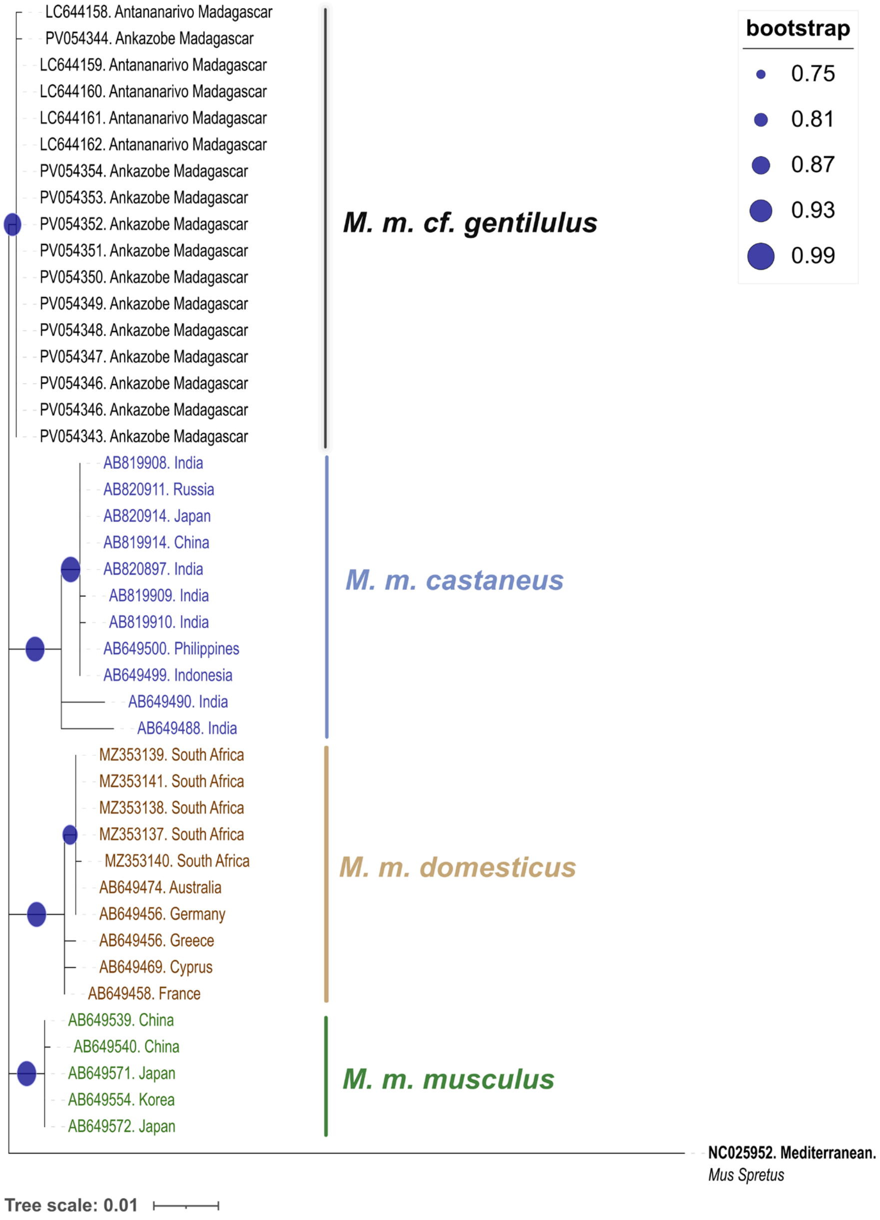

Figure 2. Phylogenetic tree of *Mus musculus* using cytochrome *b* (1140 bp) gene constructed by the Maximum Likelihood method (HKY + G model) with 1000 bootstrap replicates. Bootstrap values are placed in major nodes.

Variation in *Mus musculus* trap capture rate

Our GLMM model performed better than the null model and showed a significant difference in *Mus musculus* captured based on age and sex parameters. We found that juvenile individuals were less abundant than adults and that females were more abundant than males across all age classes (Table 2, Figure 3). However, no significant difference was observed with respect to capture rate and trapping session. Regarding reproductive status, no significant

Table 2. Parameter estimates from models for age, sex, and trapping session (TS) variables on the capture rate of *Mus musculus* based on a GLMM analysis.

Fixed effects	Estimate	SE	z-value	Pr(>|z|)	Significance
Intercept	1.567	0.166	9.44	2.00E-16	***
Age_juvenile	-1.092	0.258	-4.23	1.91E-05	***
Sex_male	-0.466	0.127	-3.66	0.000	***
Trapping_sessionTS2	-0.200	0.247	-0.81	0.417	
Trapping_sessionTS3	0.053	0.217	0.24	0.807	
Trapping_sessionTS4	-0.178	0.227	-0.78	0.433	
Trapping_sessionTS5	0.185	0.203	0.91	0.363	
Trapping_sessionTS6	0.027	0.216	0.12	0.902	

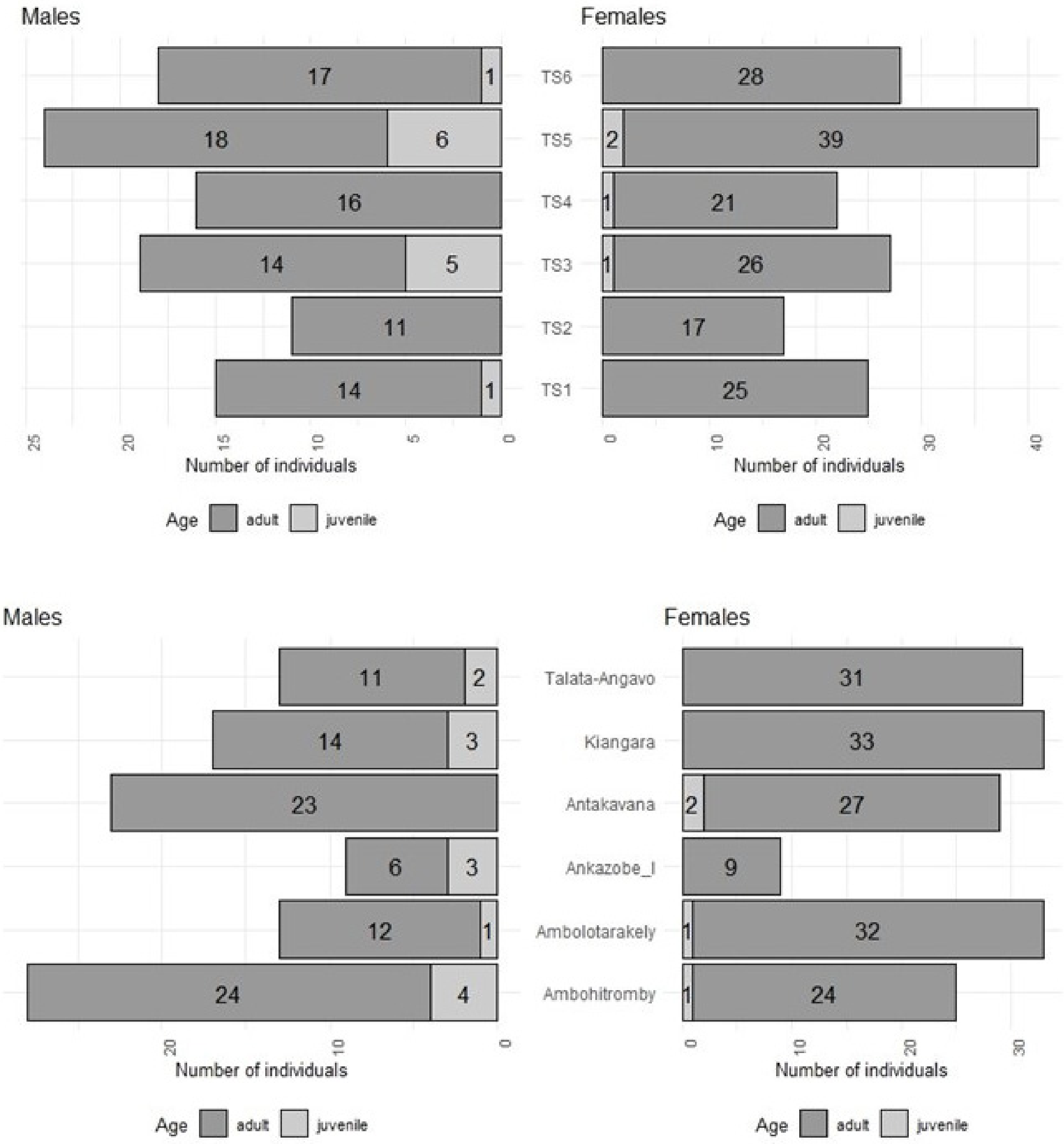

Figure 3. Number of individuals of *Mus musculus* from the Ankazobe District captured per trapping session (TS) (A) and locality (B) by age and sex. Bar plot illustrates the total number of individuals captured by age group (Juveniles are colored in light grey and adults in dark grey).

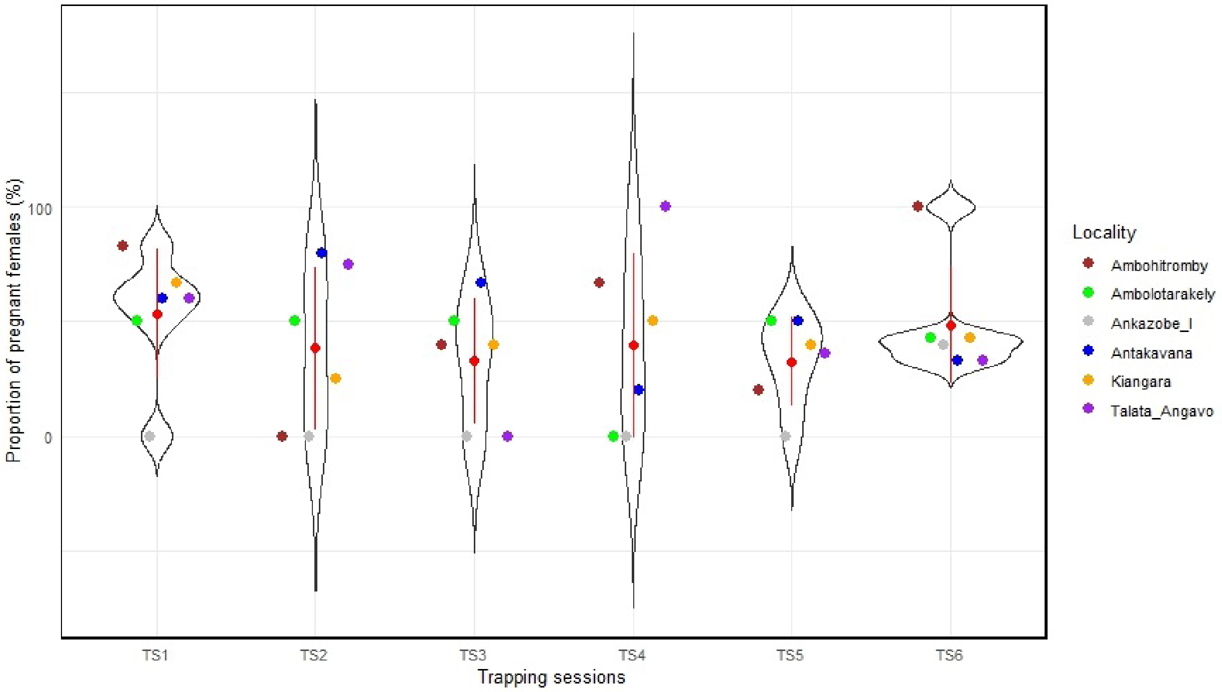

Figure 4. Proportion of pregnant *Mus musculus* females during each trapping session (TS1 to TS6) at the six study sites in the Ankazobe District. Colored points correspond to the mean proportion of pregnant females at each locality and violin shapes illustrate the distribution of proportions across trapping sessions. Red dot represents the mean proportion of pregnant female for each TS.

difference was observed in the proportion of males (χ^2 = 3.282, df = 5, *P* = 0.667) and reproductively active females (χ^2 = 3.707, df = 5, *P* = 0.592) across the six trapping sessions. In addition, no significant variation was observed on the proportion of pregnant females across the six trapping sessions (Kruskal-Wallis, χ^2 = 5, df = 5, *P* = 0.431), suggesting the presence of at least a portion of the population at each site reproducing throughout the year (Figure 4).

Morphological analysis

External measurements of 265 individuals were analyzed, including 156 adult females, 90 adult males, four juvenile females, and 13 juvenile males (Table 3) (data for two individuals were not available and excluded in the morphological analysis). Among adults, significant difference between male and female was observed for TL (*P* = 0.007), WT (*P* < 0.001) and HF (*P* < 0.001), whereas HB, TV, and EAR did not differ significantly between sexes. For juveniles, no

Table 3. Variability in five external measurements of *Mus musculus* captured at six villages during six trapping sessions: TL: total length, HB: head to body, TV: tail length, EAR: ear, HF: hind-foot without claws and WT: Weight. Measurements are presented as mean ± standard deviation, minimum and maximum measurements of individuals, n: number of individuals. P-value was obtained with non-parametric Wilcoxon test.

	Adult			**Juvenile**		
	Female	**Male**		**Female**	**Male**	
	N = 156	N = 90	p-value	N = 4	N = 13	p-value
TL	158.9 ± 10.40 (120-185)	155.1 ± 10.35 (120-178)	**0.007**	139.2 ± 22.08 (109-160)	141.7 ± 20.64 (92-173)	1
HB	79.2 ± 5.68 (63-98)	75.9 ± 5.31 (63-88)	0.058	70.2 ± 10.31 (55-77)	68.9 ± 9.70 (46-82)	0,376
TV	79.9 ± 7.08 (32-91)	79.4 ± 6.09 (56-92)	0.695	69.8 ± 12.89 (54-83)	73.1 ± 11.76 (46-93)	0,814
EAR	12.9 ± 1.36 (7-19)	12.9 ± 1.39 (10-18)	0.228	12 ± 1.41 (10-13)	12.1 ± 1.29 (10-15)	0,571
HF	14.8 ± 1.64 (12-18)	15.2 ± 1.77 (11-19)	**< 0.001**	13.2 ± 1.50 (12-15)	14.2 ± 1.52 (11-17)	0,821
WT	13.2 ± 2.94 (6.5-25)	11.8 ± 2.20 (6.5-16)	**< 0.001**	8.9 ± 3.33 (4-11.5)	9.2 ± 3.11 (4-15)	1

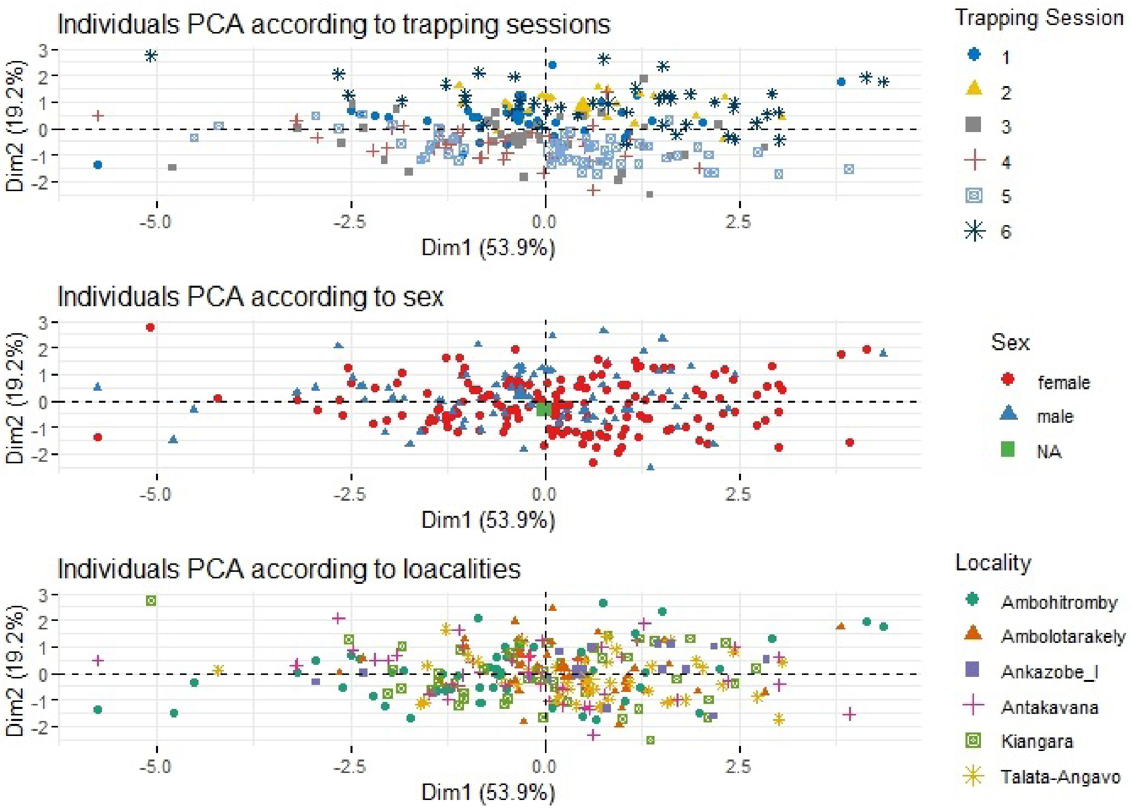

Figure 5. PCA plot of the first two components of adult mice collected at six study sites in the Ankazobe District. Each dot represents an individual. Individuals were labelled according to their (A) trapping session (TS), (B) sex, and (C) trapping site.

significant morphological differences were observed between males and females for any of the measured variables (Table 2). Multivariate analysis of external morphometrics based on a principal component (PC) analysis for adult individuals showed that the first two components explained 73.1% of the total variance (Figure 5).

When ANOVAs was performed on PC coordinates, statistically significant differences along PC1 were observed according to trapping session (TS) ($P < 0.001$) with mean TL being higher during TS2 and TS6 corresponding to March-May. However, no significant difference was observed for locality ($P > 0.05$) and sex ($P > 0.05$) along PC1 coordinates. No significant difference was observed along PC2 coordinates according to TS ($P = 0.05$) and locality ($P > 0.05$), while statistically significant difference was observed according to sex ($P = 0.001$).

Discussion

Mus musculus represented more than 38% of small mammals captured inside houses during six trapping sessions at six localities in the Ankazobe District of the Malagasy Central Highlands. Several studies conducted in rural area of Madagascar showed that *M. musculus* can coexist with other introduced small mammals, including *Rattus rattus*, *R. norvegicus*, and *Suncus murinus* within households (Rahelinirina *et al*., 2022; Parany *et al*., 2025). *Mus musculus* was captured inside houses only with Sherman traps and never with BTS traps. This suggests that Sherman traps are more suitable for the capture of *M. musculus*. The capture rates reported by Rahelinirina *et al*. (2021) further support this interpretation: of 589 trap nights with Sherman traps, 162 *M. musculus* were captured, whereas only 9 were captured in 1159 trap nights with BTS traps.

MtDNA analysis suggest that animals from the Ankazobe District belong to the *M. m.* cf. *gentilulus* lineage, which is considered as a separate subspecies distinct from the three other major subspecies (Duplantier *et al*., 2002). Our mtDNA-based study is also consistent with a previous study, with the Ankazobe individuals forming a single monophyletic clade with other Malagasy individuals that were previously referred as to *M. m.* cf. *gentilulus* (Fujiwara *et al*., 2022). This suggests a common ancestor and a single colonization event for this district. Considering that *Mus* likely arrived in Madagascar via maritime trade, it is plausible that they subsequently dispersed broadly on the island via human commerce. Given the extensive historical maritime exchanges from the

Arabian Peninsula along the African coast towards the Comoros islands and Madagascar (Allibert, 1988), an Arabian origin of the Malagasy population of *Mus* makes good sense and the phylogenetic relationships of other populations on the island needs to be verified to assess the possibility of this multiple colonization events from different source populations.

On the basis of our Principal Component Analysis followed by ANOVA, there is no morphological difference in *M. musculus* between the six localities and between male and females in the Ankazobe District. However, the period of trapping was found to have a significant effect on external measurements (as expressed by PC1 coordinates). Seasonal differences in food availability would explain the presence of large individuals during TS2 and TS6 (March to May), specifically for total length, as this period corresponds to that of rice harvesting in the fields and storage inside houses (Soarimalala *et al*., 2019), which in turn would explain the increase in body size for certain individuals during the rice harvest period.

We found no significant variation in the abundance of *M. musculus* across trapping sessions, regardless of the locality and that a certain number of individuals were collected, suggesting that their relative abundance remains stable throughout the year in rural households. This stability is likely due to the consistent environmental conditions and abundant food resources provided by human dwellings. Similarly, the proportion of pregnant females, a clear indicator of reproductive activity probably associated with food availability, did not vary significantly between sessions, indicating year-round reproduction in *M. musculus* populations in the Ankazobe District. These findings are consistent with previous studies reporting continuous reproduction of *M. musculus* in commensal environments in Córdoba, Argentina (Gomez *et al*., 2009). Consequently, the number of juveniles captured remains relatively low, likely due to a rapid growth to adults. In addition, like in other rodents (Ramasindrazana *et al*., 2022a), house mice can reach sexual maturity in less than two months and produce litters as large as 10-12 pups at intervals of one to two months (Yuan *et al*., 2023). Adults may be more prone to capture following more active search for resources and mates and/or more aggressive behavior (Drickamer *et al*., 1999; Panti-May *et al*., 2012). The abundance of adults compared to juveniles has also been reported in previous studies (Gomez *et al*., 2009; Sidorov & Putin, 2010; Panti-May *et al*., 2012; Khanam *et al*., 2017). Altogether, both relative abundance and reproduction of house mice in the Ankazobe District appeared stable across seasons, thus suggesting that in this area they often live in human habitations and that regular control measures could effectively reduce their population.

The sex ratio is female-biased. This may indicate that females are more actively involved in foraging for food and searching for breeding sites, which likely increases their chances of being trapped. Moreover, *M. musculus* lives in small units or demes with one dominant male and several females and subordinate males. Migration between demes is rare and likely occurs through female movement, which may increase their probability of being captured (Reimer & Petras, 1967).

A survey of small mammals over the course of two years in the Ankazobe District provides a baseline for studying the ecology of *M. musculus* living inside human habitations; additional analyses of the dataset will also be conducted. Indeed, tissue samples of *M. musculus* collected in different areas the island should be sequenced using a range of different markers (including nuclear) and analyzed to understand the colonization history of the species. In addition, it is important to mention that among the 265 individuals of *Mus* captured during the survey, we found two individuals that were seropositive for plague (Parany *et al*., 2025), indicating previous exposure to *Y. pestis*. These results highlight the potential status of *M. musculus* as a candidate plague reservoir in the Central Highlands. Thus, prospective and retrospective analyses should be conducted on house mice in Madagascar to understand their role in plague epidemiology and on zoonotic maintenance and transmission.

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