engage local participants in biodiversity conservation and management. It is a chance to discuss their interests and concerns and discuss ways to contribute pragmatically. These exchanges are also an opportunity to show appreciation for their collaborations. Prioritize local team members in country to avoid unsustainable emission costs associated with transportation.

- Acknowledge local participants' contributions and include them as much as possible, in the decision-making process, e.g., field protocols, activities and conditions, and authorship. Fieldwork never goes as planned, and in challenging times, insights from local people can be particularly relevant for how to address crises. At a minimum, the field team should be active participants and should be given the opportunity to express their concerns and suggest alternative solutions, rather than serve as passive recipients taking directives. Respect their point of view and, if there are disagreements, respectfully discuss differences of opinion.

- Respect local Malagasy culture and superstitions. Scientific training does not give us the right to discredit local communities' beliefs and taboos. Even if taboos are rooted in misconceptions and fears, they are intrinsic to the fabric of Malagasy society. Our respect should supersede our incredulity. Incidentally, local knowledge rooted in superstition could be integrated into the research program or discussed in a broader context. Perhaps, myths and legends can help us understand some aspects of how biodiversity has been valued (or the opposite) in those regions.

- Please, stop thinking that money is always the solution in a developing country. Most foreign researchers in Madagascar come to the island with a preconceived idea that money can solve all the existing problems. In fact, the opposite may be true. On the one hand, offering money to speed up services, or to circumvent administrative requirements, perpetuates corruption in the local administrations, which most of us have witnessed and complained about. More importantly, it perpetuates inequality with local researchers who cannot afford to do the same. On the other hand, when you are in locations far from large towns, local villagers may prioritize access to balanced foods, medicine and health care, tools or items needed for their subsistence, while cash may be saved for bigger investments later. Finally, the sporadic giving of cash without context and without respecting local and socially acceptable terms can create confusion and false expectations, and thereby undermine the work of our colleagues.

- Follow and respect socially accepted ethical behaviours. We should not feel entitled to break or ignore local rules because they do not align with our own standards. Beliefs and traditional knowledge are important components of heritage and ignoring them is harmful and detrimental. We should also acknowledge that expeditions are impossible to carry out without local people, who are not only familiar with wildlife – which is critical to our data— but also help us navigate forests safely.

- Be legal. Follow local laws and regulations, whether it makes sense to you or not. This is particularly relevant for researchers attempting to enter protected areas and collect data without valid research permits and local authorizations. These behaviours are a form of neocolonialism. Plan ahead and contact local official and academic institutions for procedural details to avoid unnecessary delays. Incidentally, all relevant paperwork may be required by scientific Journals prior to manuscript submissions.

- Engage Malagasy students in your project, include them as members of your research team, provide mentorship, share the project rationale, objectives, and activities, and ask for feedback. Be prepared to adjust or change plans if students voice reservations or concerns. Establish a professional relationship that goes beyond collecting high-quality data and see students as potential long-term research collaborators and the emerging new generation of scientists and conservationists. In other words, mentor them. As much as possible, please work with the students to help them develop a side project integrated or not with the main project, a project that they can lead and/or publish. Such mentoring can tremendously increase their self-esteem and confidence in leading and disseminating their scientific results, and/or in developing new research projects and grant applications, and ultimately increase diversity in the current and future scientific workforce.

- Please, do not tokenize Malagasy students/collaborators. Take the time to seek people who are good matches for the proposed projects, ensuring their best interests are served, without assuming anybody could do the job just fine. Acknowledge their contributions with fairness and transparency, avoiding under- or overestimating their roles and responsibilities.

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New genetic evidence from the Ambatotsirongorongo / Petriky complex in southeast Madagascar calls for an immediate re-evaluation of conservation strategies focusing on the Bemanasy mouse lemur (*Microcebus manitatra*)

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Abstract

The diverse forest habitats of southeastern Madagascar support a complex arrangement of five putative species of mouse lemur (genus *Microcebus*). One of these species, the Critically Endangered Bemanasy mouse lemur (*Microcebus manitatra*), requires urgent evaluation. In this short article, we present findings from a series of expeditions to the Ambatotsirongorongo massif and the nearby littoral forest of Petriky. Genetic results confirm that the range of *M. manitatra* is extremely limited. Notably, we find that two *Microcebus* species (*M. manitatra* and the Anosy mouse lemur, *M. tanosi*) occupy the remaining forest fragments of the massif, with seemingly discreet distributions. We observed and captured *M. tanosi* in the remaining intact humid fragments, whereas we only found *M. manitatra* in degraded habitats on the lower eastern slopes. The forest fragment that comprised the original type locality of *M. manitatra* has been all but cleared in recent years. We further confirm the identity of the mouse lemur species present in nearby Petriky as *M. manitatra*, making this forest the foremost remaining habitat for the species. These findings have substantial implications for species conservation and management strategies at both sites.

Résumé

Les divers habitats forestiers du sud-est de Madagascar abritent un arrangement complexe de cinq espèces putatives de lémuriens souris ou microcèbes (genre *Microcebus*). L'une de ces espèces, le microcèbe de Bemanasy *Microcebus manitatra*, en danger critique d'extinction, nécessite une évaluation urgente. Dans ce court article, nous présentons les résultats d'une série d'expéditions dans le massif d'Ambatotsirongorongo et dans la forêt littorale voisine de Petriky. Sur la base des résultats génétiques, il est maintenant clair que l'étendue géographique de *M. manitatra* est plus restreinte que préalablement envisagée. Il est évident que deux espèces de microcèbes (*M. manitatra* et le microcèbe d'Anosy, *M. tanosi*) occupent les fragments forestiers restants du massif, avec des répartitions apparemment discrètes. Nous avons observé et capturé *M. tanosi* dans les fragments humides intacts restants, et *M. manitatra* dans les habitats dégradés des pentes inférieures orientales. Le fragment de forêt qui constituait la localité type d'origine de *M. manitatra* a été pratiquement défriché ces dernières années. Nous confirmons en outre l'identité de l'espèce de microcèbe présente à Petriky comme étant *M. manitatra*, faisant de cette forêt le principal habitat restant de l'espèce. Ces découvertes ont des implications substantielles pour l'espèce et les stratégies de gestion de la conservation sur les deux sites.

Introduction

The extreme southeast of Madagascar is characterized by marked environmental gradients (humidity, precipitation, and temperature) and consequently, notable habitat heterogeneity (Goodman *et al*., 2018). Beyond the large National Parks and reserves, many smaller relict forest habitats support important biodiversity. However, many of these forests are now disconnected and degraded as a consequence of long-term natural phenomena (Burney, 1992; Virah-Sawmy *et al*., 2010) and intensive anthropogenic practices (Harper *et al*., 2007; Vieilledent *et al*., 2018). The result is a highly fragmented landscape, with isolated islands of forest supporting unique assemblages of flora and fauna which reflect their positions along these gradients. These forests now serve as crucial refuges for species displaced by habitat loss.

The genus *Microcebus* is well represented in the southeast region, with five putative species occupying a range of habitats within 50 km2 (Rasoloarison *et al*., 2013; Hotaling *et al*., 2016). Although each species is largely constrained by habitat type (e.g., the Grey-brown mouse lemur *M. griseorufus* in dry spiny forest and *M. tanosi* in humid forest), two species appear to have particularly restricted ranges (*M. ganzhorni* and *M. manitatra*). Whilst Ganzhorn's mouse lemur (*M. ganzhorni*) is known only from the few remaining littoral forest fragments at Mandena, the precise distribution of the Bemanasy mouse lemur, *M. manitatra*, remains unclear. Despite being listed as one of the world's 25 most endangered primate species (Donati *et al*., 2019), and recognized as Critically Endangered by the IUCN (Blanco *et al*., 2018), a high degree of uncertainty surrounds our understanding of the geographic distribution and ecological needs of this species. Most significantly, its current conservation status is based on a series of distributional assumptions that require urgent validation.

Microcebus manitatra (Hotaling *et al*., 2016) has only officially been recognized from a single patch of forest on the Ambatotsirongorongo massif (Blanco *et al*., 2018), in the extreme southeast of Madagascar. The massif consists of a series of humid forest fragments that form the Nouvelle Aire Protégée Ambatotsirongorongo (decree 2015-792). Once a continuous habitat, these remaining forests now persist only on the south-facing, most inaccessible mountain slopes. Three main fragments remain; Lavasoa (54 ha; 300-800m a.s.l), Bemanasy (33 ha; 100-400m a.s.l) and Ambatotsirongorongo (< 5ha; 200-400m a.s.l). Despite recent protections, all fragments remain under severe human pressure (Donati *et al*., 2019), and logging is chronic at all elevations. In recent years, and notably during the Covid-19 pandemic, the forest fragment Ambatotsirongorongo has been greatly diminished and now exists as just a small cluster of trees (S. Mara, pers. comm., 2023). Crucially, this forest fragment represents the type locality for *M. manitatra*, based on the GPS data that accompanies the original species description (although the locality is commonly, but erroneously referred to as Bemanasy in the literature). Whilst it is widely assumed that *M. manitatra* occupies each of the three forest fragments (Blanco *et al*., 2018; Donati *et al*., 2019), previous work has suggested otherwise. Hapke *et al*. (2012) reported two *Microcebus* species from the massif, *M. cf. rufus* and *M. murinus*, each with discreet distributions. However, this report preceded two taxonomic revisions that bear directly on our understanding of *Microcebus* distributions in the region. First, the '*murinus*' species complex has been expanded to include *M. manitatra* and *M. ganzhorni*, and second, *M. tanosi* has been described as distinct from *M. rufus*. Following the description and naming of *M. manitatra* (Hotaling *et al*., 2016), all subsequent accounts omit the earlier observation of Hapke *et al*. (2012) and assume instead that all remaining forests support the new form, *M. manitatra*, exclusively.

Beyond the Ambatotsirongorongo massif, it is further hypothesized that the nearby (~5 km) littoral forest block

of Petriky (800 ha) also supports *M. manitatra*, based on mtDNA analyses (Hapke *et al*., 2012). Petriky represents a particularly important and highly threatened habitat type in itself, characterized as a sub-category of evergreen forest occurring on a sand substrate (Dumetz, 1999). Like other littoral forests, Petriky supports a rich biodiversity and is noted for its high floristic value (Consiglio *et al*., 2006; Temple *et al*., 2012). Petriky is the most southerly intact littoral forest patch in the region and is considered a transitional ecosystem, supporting a biota typical of both dry and humid littoral forest formations (Rabenantoandro *et al*., 2007). Notably, the composition of the lemur communities at both sites varies considerably, underscoring the specialized nature of both habitats. In this report, we aim to clarify the spatial distribution of the Critically Endangered *M. manitatra*.

Methods

Sample collection

As part of a broader sampling effort for mouse lemurs across the southeast, the Ambatotsirongorongo massif and the coastal forest of Petriky were sampled during three expeditions between October 2022 and June 2023. Animals were captured using a combination of Sherman trapping and direct hand capture. Animals were sedated using a standardized dose of ketamine (1 µl), administered into the quadriceps muscle. Lidocaine (5%) was applied topically to the ear pinna, and skin biopsies (ear clips) were collected and stored in 99% ethanol. All animals were released back to their initial capture sites after a period of post-operative monitoring, ensuring the effects of sedation had completely passed.

Genetic analysis

Whole genome re-sequence data was generated for two mouse lemurs sampled at Bemanasy forest (Am 2, Am10) and two mouse lemurs sampled at the Petriky (P1, P2) forest. The libraries for sequencing were built with illumina DNA prep workflow, with sequencing using an illumina Novaseq 6000 instrument at an approximate coverage of 10X. The read length was 150bp. We trimmed the raw reads to eliminate adaptors with Trimmomatic version 0.4 (Bolger *et al*., 2014). Clean reads were aligned to the *Microcebus murinus* reference genome version Mmur_3.0 (Genebank accession GCF_000165445.2) using BWA mem version 0.7.17 (Li and Durbin, 2009). The resulting BAM files were indexed and subsetted to the specific region of the cytochrome B gene (cytB), NC_028718.1:14222-15361. We then used SAMTOOLS version 1.7 (Li *et al*., 2009) to call SNPs and index the resulting vcf file. The consensus command of BCFTOOLS version 1.17 (Danecek *et al*., 2021) was used to extract the consensus sequence of each individual. The resulting fasta sequences were then concatenated together with all the cytB sequences used in Hotaling *et al.* (2016) to create a multi-fasta file used to build the phylogenetic tree. A maximum likelihood phylogenetic tree was created using the software iQtree version 1.6.12 (Nguyen *et al*., 2015) and visualized with Figtree version 1.4.4.

Results

We captured a total of 10 mouse lemurs on the Ambatotsirongorongo massif and 14 in the littoral forest of Petriky. We found two distinct morphological phenotypes on the Ambatotsirongorongo massif (Fig. 1) with the 'rufus' form genetically corresponding to *M. tanosi* (Fig. 2). The second phenotype corresponds both by morphology and location to *M. manitatra*, as described by Hotaling *et al.* (2016). In Petriky, we observed a single phenotype which also corresponds both morphologically and genetically to *M. manitatra*. Observations of other lemur species were recorded opportunistically at both sites during the expeditions (Tab. 1).

Discussion

The findings of this study have clear implications for

Tab. 1: Lemur inventories for the Ambatotsirongorongo massif and Petriky forests. Species lists derived from earlier works (Ganzhorn *et al*., 2007; Eppley *et al*., 2019), with contemporary observations included.

Locality	Species	Activity Pattern	Typical Affinity	Observed
Petriky	*Avahi meridionalis*	Nocturnal	Humid forest	Y
	Cheirogaleus cf. *thomasi*	Nocturnal	Humid forest	Y
	Microcebus manitatra	Nocturnal	Dry forest	Y
	Lemur catta	Diurnal	Dry forest	Y
	Propithecus verreauxi	Diurnal	Dry forest	X
Ambatotsirongorongo Massif	*Avahi meridionalis*	Nocturnal	Humid forest	Y
	Cheirogaleus lavasoensis	Nocturnal	Humid forest	Y
	Cheirogaleus thomasi	Nocturnal	Humid forest	X
	Daubentonia madagascariensis	Nocturnal	Humid forest	X
	Microcebus manitatra	Nocturnal	Dry forest	Y
	Microcebus tanosi	Nocturnal	Humid forest	Y
	Eulemur collaris	Cathemeral	Humid forest	X
	Hapalemur meridionalis	Cathemeral	Humid forest	Y
	Lemur catta	Diurnal	Dry forest	Y
	Propithecus verreauxi	Diurnal	Dry forest	X

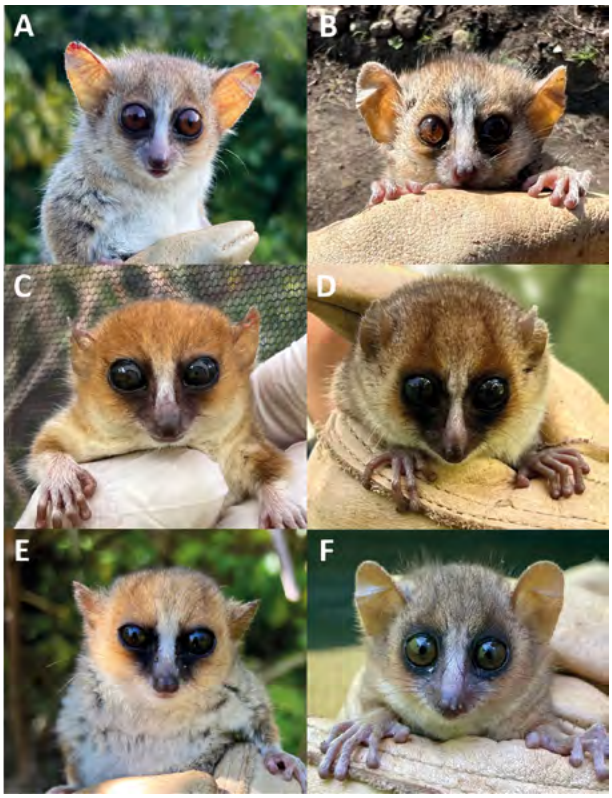

Fig. 1: Representative phenotypes from the study area. A+B) *Microcebus manitatra*; Ambatotsirongorongo / Sakoamaso forest. C+D) *Microcebus tanosi;* Bemanasy forest. E) *Microcebus tanosi*; Lavasoa forest. F) *Microcebus manitatra*; Petriky. All photographs by SHR.

the conservation of *Microcebus manitatra* and for the wider conservation management strategies at both the Ambatotsirongorongo massif and Petriky sites. First, we confirm the presence of two mouse lemur species in the Nouvelle Aire Protégée of Ambatotsirongorongo massif. Despite previous observations of *M. cf. rufus* reported on the massif (Hapke *et al*., 2012), recent population estimates, and distributional assessments are based on the premise that *M. manitatra* occupies the entire range of fragments (Blanco *et al*., 2020). However, we have determined that the remaining intact fragments at higher altitudes (Bemanasy and Lavasoa) support only *M. tanosi*, a humid forest species normally associated with the rainforests of Andohahela and Tsitongambarika. Whilst *M. manitatra* does evidently occur on the mountain (the area represents the type locality for the species), it appears to be distributed at lower altitudes and outside of intact standing forest, thus existing solely within the heavily degraded and secondary connective habitats on the eastern slopes (e.g., Ambatotsirongorongo and Sakoamaso). The forest fragment (Ambatotsirongorongo) from which *M. manitatra* was originally described (erroneously referred to as Bemanasy in Hotaling *et al.* (2016) and repeated in subsequent publications), has now been cleared. No individuals matching the *M. manitatra* phenotype were observed in either Bemanasy or Lavasoa, upholding the earlier observation of Hapke *et al.* (2012) that can now be interpreted as *M. manitatra* and *M. tanosi* having discrete distributions. Thus, the type locality of *M. manitatra* (Bemanasy mouse lemur), appears to have been incorrectly assigned. These findings suggest that the area of occupancy for *M. manitatra* on

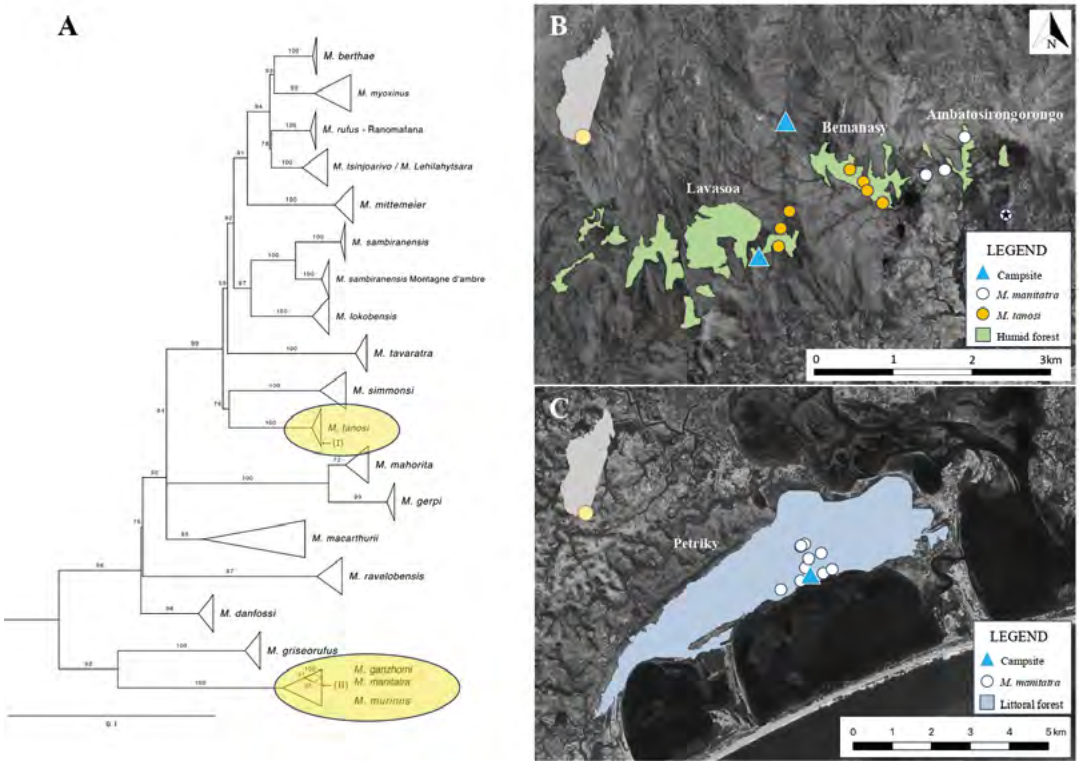

Fig. 2: A) Phylogenetic placement of sequenced individuals. Animals captured in Bemanasy (I) forest cluster with *M. tanosi* and individuals from Petriky (II) cluster with *M. manitatra*. *Cheirogaleus* samples (K133A, K152A and K180A) used as outgroups. B) Distribution of captured individuals on Ambatotsirongorongo massif (starred individual shows capture site of *M. manitatra* type specimen (Hotaling *et al.*, 2016). Forests in this Ambatotsirongorongo region are now heavily degraded. C) Distribution of individuals captured in Petriky.

the Ambatotsirongorongo massif is instead restricted to a series of highly degraded patches of connective and secondary forest, likely totalling ca. 10-15ha.

In the field, the two species (*M. tanosi* and *M. manitatra*) are clearly discernible by phenotype, with pelage coloration and ear size being clear diagnostic features (Fig. 1). Given the ease of visual identification, we can say that there does not appear to be any area where the two species are sympatric on the massif, although some interaction may occur towards the southern end of the Bemanasy forest fragment. We observed and captured several *M. tanosi* individuals in connective habitat outside of the Lavasoa forest fragment, indicating that the species readily utilizes degraded habitat beyond the sharp humid forest boundary. This distributional pattern may also be mirrored in the other Cheirogaleid species present on the massif; with the Lavasoa dwarf lemur *Cheirogaleus lavasoensis* occupying the intact rainforest blocks Lavasoa and Bemanasy, and Thomas' dwarf lemur *C. thomasi* present in degraded forest habitats (e.g., Vohisampa) on the lower eastern slopes (J. Rakotondranary, pers. obs., 2020).

Importantly, we further conclude that Petriky now represents the critical remaining habitat for *M. manitatra*. This roughly 800 ha littoral forest block is expected to undergo extensive mining in the coming years, reducing the remaining habitat to a 120 ha Conservation Zone (Temple *et al*., 2012), thus calling for urgent conservation evaluation of this locality. The proposed mining operation is anticipated to place extreme pressure on all plant and animal populations at the site. Whilst QIT Madagascar Minerals (QMM), along with Nature Based Solutions, are committed to partly restoring mined areas (c.225 ha) (Temple *et al*., 2012), their strategies, schedules and methodologies must be shared with wider stakeholders for transparency and constructive scrutiny. Confirmation that the *Microcebus* form present in Petriky corresponds to the Critically Endangered *M. manitatra* should invite local area managers at QMM to revisit their proposed biodiversity strategies, especially given that operations have not yet begun. Expansion of the existing Conservation Zone should be considered as an important strategy in Petriky, since *M. manitatra* population density appears to be highest in the protected areas (Malone *et al*., 2013). Of pressing concern, littoral habitats are poorly represented in the country's protected area network at present (Goodman *et al*., 2018), yet it is now clear that the three major littoral forest blocks in the southeast (Sainte Luce, Mandena and Petriky) each support a distinct form of *Microcebus* (*M. tanosi*, *M. ganzhorni* and *M. manitatra*, respectively).

Whilst the two sites, Ambatotsirongorongo and Petriky, are only separated by approximately 5 km, the intervening habitat is heavily modified, consisting mainly of rice paddies and pastures. Two small tomb forests (Amporoforo and Loharano) persist in between the two sites, although it is unclear whether these fragments still support any lemur species given their restricted size (9 ha and 4 ha, respectively). Under current strategies, once mining operations are completed, *M. manitatra* may only occupy a fragmented range of <150 ha. Although some slight phenotypic and genetic differences may exist between the populations on Ambatotsirongorongo massif and Petriky, these are likely explained by isolation and topographical effects and clearly do not extend to species delimitation.

We wholeheartedly echo the call for urgent conservation actions on the Ambatotsirongorongo massif (Eppley *et al*., 2019), given it clearly supports a diverse and unusual assemblage of lemur species, as well as numerous other threatened and range-restricted taxa (e.g., *Chrysalidocarpus prestonnianus*, the Madagascar fruit bat *Eidolon dupreanum*, the eastern falanouc *Eupleres goudoti*, and *Phelsuma antanosy*). However, we further urge that attention be immediately directed towards the littoral forests of Petriky. The IUCN Red List assessment for *M. manitatra* urgently requires updating in light of these new findings.

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Ethics and permissions

All field work was carried out with permission from the Malagasy authorities (Permits: No.290/22 and No.167/23/MEDD/SG/DGGE/DAPRNE/SCBE.Re). All animals were handled and treated according to IACUC protocol A163-22-09, registered to the corresponding author.

Data availability

The code and multi fasta file with the sequences used to build the phylogenetic tree are available at: https://github.com/CaroSegami/Ambatotsirongorongo_mouse_lemurs. New cytB sequences are available at NCBI under accession numbers OR754226 (Am10), OR754227 (Am2), OR754228 (P1) and OR754229 (P2).

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Phaner furcifer - The ghost lemur of northeastern Madagascar?

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Abstract

Fork-marked lemurs (*Phaner*) are a group of four medium-sized, gummivorous lemur species belonging to the Cheirogaleidae family, representing one of the least-studied primate genera in Madagascar. This study focuses on elucidating the presence and local knowledge of the Endangered *Phaner furcifer* in the Analanjirofo region of northeastern Madagascar. Field surveys included more than 300 km of nocturnal transect surveys across varying forest degradation stages, and 243 household interviews to gather local knowledge about this species. Results indicate the absence of *P. furcifer* in the study region, including areas with historical anecdotal reports. This species was not detected visually or acoustically during the extensive surveys, and only 1.6% of interviewees mentioned occasional sightings, but only in areas of previous anecdotal reports. This suggests either a recent decline of the population due to anthropogenic pressures and deforestation, or a genuinely disjunctive distribution of the species, potentially representing two distinct lineages. The study highlights the critical conservation status of *P. furcifer* and emphasizes the urgent need for conservation measures in the face of ongoing deforestation and climate change in Madagascar. Furthermore, it underscores the importance of taxonomic research to better understand the diversity and distribution of these enigmatic lemurs and the ecosystems they inhabit. Immediate action is imperative to safeguard the future of *P. furcifer* and other sympatric lemur species within this threatened region.

Introduction

Fork-marked lemurs (*Phaner*) from the Cheirogaleidae