



# LEMUR NEWS

The Newsletter of the Madagascar Section  
of the IUCN SSC Primate Specialist Group

Volume 23, 2021



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**Layout** Heike Klensang . Sophie Klevenow

**Front cover** Male grey-headed brown lemur (*Eulemur cinereiceps*) in Manombo Forest, south-eastern Madagascar. This species is classified as Critically Endangered on the IUCN Red List. © Inaki Relanzon / naturepl.com

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## Lemur News online

All 23 volumes are available online at [www.primate-sg.org](http://www.primate-sg.org), [www.aeecl.org](http://www.aeecl.org) and [www.dpz.eu](http://www.dpz.eu).  
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## Editorial

Another year has passed, yet again one with many challenges and restrictions owing to the continuing spread of different variants of the SARS-CoV-2 virus across the world and the associated hospitalisations, ICU admissions and deaths seen in virtually all countries. It would thus be easy enough to devote yet another *Lemur News* editorial to Covid challenges and mitigation measures. However, I will leave talking about Covid to others and rather devote this space entirely to lemurs and to the exciting activities of the Madagascar Section of the IUCN SSC Primate Specialist Group (PSG).

Since I wrote the editorial for the last volume of *Lemur News* (Vol. 22), Schüßler *et al.* (2020) described a new species of mouse lemur, *Microcebus jonahi*, thus bringing the number of species in the genus *Microcebus* to 25 (although the validity of one species, *M. mittermeieri*, is now disputed; Poelstra *et al.*, 2021) and the total number of lemur species to 109, in 113 taxa. Jonah's mouse lemur, *M. jonahi*, is a large-bodied, reddish-brown, and small-eared mouse lemur from Ambavala, about 20km west of Mananara-Nord, where it occurs sympatrically with Goodman's mouse lemur, *M. lehilahytsara*. It can be distinguished from the latter by its higher body mass, larger body size, and longer tail length. *M. jonahi* is named after my PSG Co-Vice-Chair for Madagascar and member of the editorial board of *Lemur News*, Prof. Jonah Ratsimbazafy, in recognition of his tireless work for lemur conservation. Jonah has also recently been honoured by the Malagasy Post Office, who have depicted him on a stamp (see news on page 3).

After a marathon of revising and updating all lemur Red List assessments over the last 24 months following the 2018 Red List assessment workshop in Antananarivo, we now have 112 recognised lemur taxa on the IUCN Red List, 109 of which were published between 2019 and 2021. The assessment update was led by PSG Red List Authority Coordinator, Kim Reuter, and involved more than 50 assessors. Almost all (95.5%) lemur taxa are now in one of the Red List's 'Threatened' categories, with 32% Critically Endangered, 40% Endangered, and 26% Vulnerable. For three taxa (*Cheirogaleus grovesi*, *Haplemur griseus gilberti*, *Microcebus boraha*), there were not enough data to assign them to any category, so they are considered Data Deficient. Two relatively widespread mouse lemur taxa (*Microcebus griseorufus*, *Microcebus murinus*) were assessed as of Least Concern. Further updates will be made to the Red List assessments as required, but we do not envisage any major revision or workshop in the coming years.

In 2021, Madagascar celebrated the 8th year of the now famous World Lemur Festival, created by GERP in 2014. The main event in Madagascar this year was held at Parc Botanique et Zoologique de Tsimbazaza, Antananarivo, on World Lemur Day, the 29th October. Alongside activities focusing on lemurs and the environment, there were also plenary sessions, round tables, oral presentations of research results, and presentations of scientific posters. The event at PBZT was sponsored by the Ministry of Environment and Sustainable Development. Other WLF events took place in Sahamalaza, CAZ, Ranomafana, Kianjavato, Montagne des Français and Sainte Luce national parks, in the SAVA and DI-

ANA regions and in the municipality of Ambohimahamasina. There were also many participating organisations in other parts of the world, from California to Tokyo.

The Lemur Conservation Network (LCN; [www.lemur-conservationnetwork.org](http://www.lemur-conservationnetwork.org)), directed by Lucía Rodríguez Valverde and Dr. Sehenó Corduant, has also gone from strength to strength over the last few years. The online platform aims to raise awareness of the precarious situation of lemurs, connect funders with conservation programmes and provide a forum to enhance communication and coordination between NGOs, researchers, corporates and the public. Since 2019, the LCN has increased its members to 65 organizations, zoos and conservation platforms that address the conservation of more than 100 lemur species. Its online engagement has significantly increased through its social media presence and website. The platform has put particular emphasis on increasing its Malagasy content and engagement, and now its biggest user audience comes from Madagascar.

And lastly, I am extremely pleased that the Council of the International Primatological Society has chosen Madagascar as the venue for its 2025 congress. A team of Malagasy and international colleagues, led by Jonah Ratsimbazafy and GERP, put together the successful bid. Well done everyone! I am looking forward to the best IPS Congress ever, and to showing the world the beauty of Madagascar and its lemurs.

The Margot Marsh Biodiversity Foundation, through re: wild's Primate Action Fund, kindly supported this volume of *Lemur News*.

Christoph Schwitzer

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Fig. 1: Jonah's mouse lemur, *Microcebus jonahi*. Photo: Dominik Schüßler



## News and Announcements

### Environmental Education working group Madagascar

Since early 2021, EE actors meet online once a month to share and discuss approaches, tools, and experiences. The objective of the working group is to connect Environmental Education (EE) practitioners working in Madagascar. More specifically, it aims to facilitate the development of collaborative actions, to share experiences and lessons learned, and potentially to develop new joint approaches and ideas in the field of environmental education.

The target groups are all interested practitioners working in Environmental Education (also known as Conservation Education and Education for Sustainable Development) in Madagascar. We aim to include all types of practitioners in the field: from governmental to non-governmental actors, including enterprises.

The initiative was started by a group of researchers and practitioners: Aina Brias-Guinart from University of Helsinki, Lena Reibelt from Madagascar Wildlife Conservation, Hanitra Rakotonirina and Matthias Markolf from the NGO Chances for Nature. The initiative has grown to 36 registered participants in July 2021.

Each session is led by a different organisation, who presents an education tool or project. After the presentation, the floor is open for discussion and exchange. A typical session lasts around an hour. The working group is open for interested environmental education actors to join, shape, and advance the initiative.

Do not hesitate to contact us for more information, or if you want to receive the invitations for our future meetings. We look forward to connecting with you!

Aina, Lena, Hanitra and Matthias

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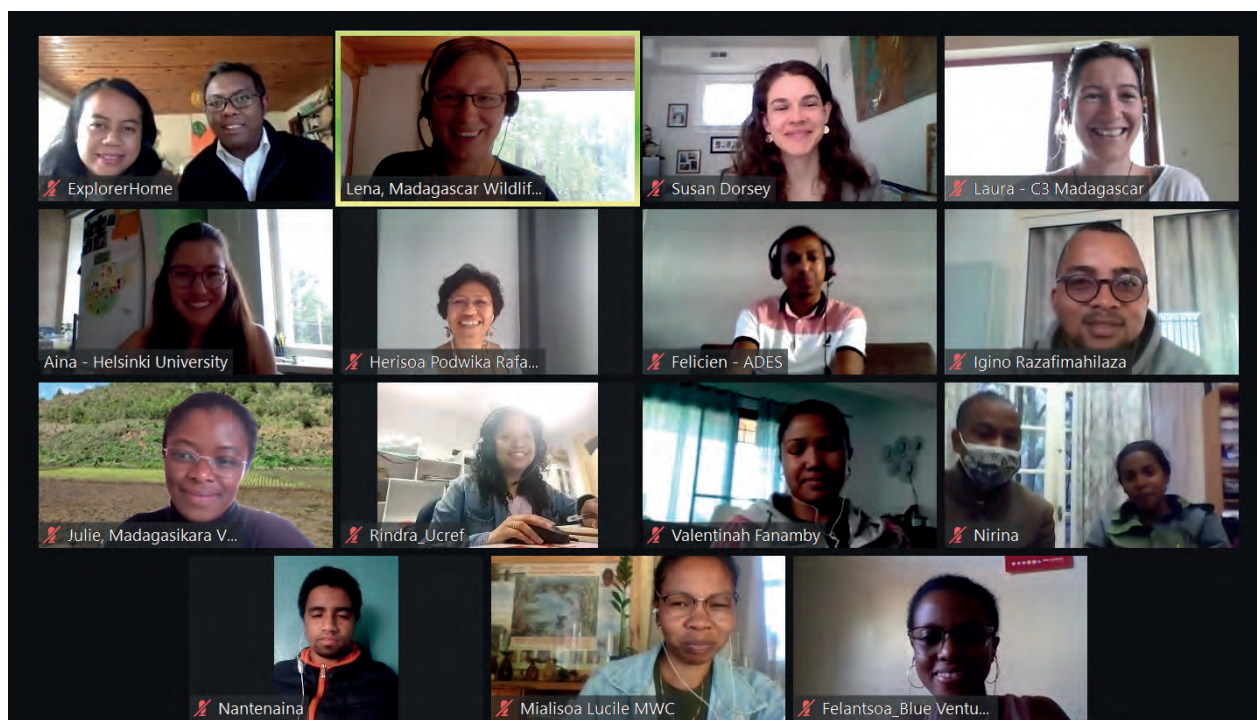
### Ebook available of revised version of 2018 Madagascar terrestrial protected area book

In 2018 Association Vahatra in Antananarivo published a three volume bilingual (French-English) book entitled The terrestrial protected areas of Madagascar: Their history, description, and biota. Working together with colleagues from Strand Life Sciences in Bangalore and financed by CEPF, the three volume set has been revised, converted to ebook format, and is now being distributed by The University of Chicago Press. The ebooks with a 2020 publication date have been separated into French and English sets and each volume needs to be purchased separately. See The University of Chicago Press website for further details at [https://press.uchicago.edu/ucp/books/publisher/pu3431914\\_3431915.html](https://press.uchicago.edu/ucp/books/publisher/pu3431914_3431915.html).

Another portion of this project with Strand was making available about 8000 pdf files that were used in writing the book and in the near future these will be posted on a cloud and accessible for free downloads to all that are interested. Revenues generated by sales of the ebook will be put to paying the annual fees of the cloud.

For those of you that are in Madagascar or have plans to travel to Madagascar in the near future and prefer the printed version of the book, a number of copies are still available at Association Vahatra for sale. We propose the notably reduced rate of 200,000 MGA (45 Euros or \$55 USD) for the three volume set.

Please transmit this message to your friends and colleagues that might be interested in obtaining the ebook or printed version.



Environmental Education working group Madagascar.

En 2018, l'Association Vahatra a publié un livre bilingue (En français et anglais) de trois volumes intitulé «Les aires terrestres protégées de Madagascar: leur histoire, description et biote». En collaboration avec des collègues de Strand Life Sciences à Bangalore et financés par CEPF, les trois volumes ont été révisés et convertis en format ebook, et qui sont maintenant distribués par l'Université de Chicago Press. Dans les ebooks publiés en 2020, les volumes sont séparés en ensemble français et anglais, et chaque volume doit être acheté séparément. Pour plus de détails, consultez le site Web de l'Université de Chicago Press à l'adresse [https://press.uchicago.edu/ucp/books/publisher/pu3431914\\_3431915.html](https://press.uchicago.edu/ucp/books/publisher/pu3431914_3431915.html).

Une autre partie de ce projet en collaboration avec Strand était de mettre à disposition environ 8000 fichiers pdf utilisés pour rédiger le livre et ceux-ci seront bientôt publiés sur un Cloud et disponibles en téléchargement gratuit pour tous ceux qui seront intéressés. Les revenus générés par la vente de l'ebook seront utilisés pour payer les frais annuels de l'hébergement du Cloud.

Pour ceux d'entre vous qui sont à Madagascar ou qui prévoient de voyager à Madagascar prochainement et qui préfèrent la version imprimée du livre, un certain nombre d'exemplaires sont encore disponibles en vente à l'Association Vahatra. Nous proposons le tarif particulièrement réduit de 200 000 MGA (45 Euros ou 55 USD) pour l'ensemble de trois volumes.

Veuillez transmettre ce message à vos amis et collègues qui pourraient être intéressés à obtenir le livre électronique ou la version imprimée.

Nous vous remercions à l'avance pour votre considération.

## Jonah Ratsimbazafy honoured with his own stamp

Madagascar, a blessed island rich in biodiversity, is home to lemurs – mysterious creatures whose beauty is matched only by their nobility and uniqueness. When we talk about lemurs, one name comes straight to mind: Jonah RATSIMBAZAFY. With unwavering determination, Jonah has devoted his life to the protection of these primates that can only be found in Madagascar.

As a committed social actor, Paositra Malagasy (the national post office of Madagascar) wished to pay tribute to Madagascar's endemic lemurs and to one of the country's best-known primatologists and conservationists. It was with this in mind that it was decided that five species of lemur, as well as Jonah himself, will be included in the next collection of Malagasy stamps.

An event that will be rooted in history, Jonah RATSIMBAZAFY will be the world's first primatologist to appear on

a stamp during his lifetime. These stamps carry a message of hope and symbolise the importance of our natural resources and the efforts undertaken by a large number of actors to safeguard our national heritage.

Indeed, the Ministry of Digital Development, Digital Transformation, Posts and Telecommunications is fully aware of the importance of the role of biodiversity in the development and well-being of future generations, and will continue to support all initiatives towards its preservation.

Rico Valiha Andrianirina  
GERP

## Short Communications

### Writing Fellowships for Malagasy Graduate Students and Early Career Conservationists

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#### Introduction

##### *Rationale for the program*

The onset of the COVID-19 global pandemic interrupted field research and conservation projects globally. In the months following the outbreak, the organisation Lemur Love ([www.lemurlove.org](http://www.lemurlove.org)), like many other organisations working in Madagascar, was unable to conduct field work. As such, we pivoted our within-Madagascar programming to address the anticipated impacts that COVID-19 would have on our organization's mission which is to protect lemurs, empower women, and further science. One aspect of our new programming included the launch of a 6-month writing fellowship for Malagasy early-career researchers (ECR, from now on, when referring to students, graduates, or researchers pursuing careers in academia and/or conservation). This built on previous work by Lemur Love to build the capacity of Malagasy researchers, including sponsored attendance at

scientific conferences (Reuter and LaFleur, 2019/20) and career development workshops (in collaboration with Ikala STEM). Here, our goal was to aid in the career progression of, and provide small stipends to, this next generation of ECRs while they worked to improve their scientific writing. We also wanted to provide an avenue through which talented ECRs could continue to develop professionally and prevent them from dropping out of the 'career pipeline' due to a lack of income as a result of the pandemic's impacts on the research/conservation



landscape (see below). The ability to write and publish in English are essential to ECRs who wish to participate in international academia (i.e. publishing research, applying for and reporting on grants, disseminating findings to the global public). Yet, these skills are not typically taught in Madagascar. Moreover, because university education in Madagascar (and generally in developing countries) is underfunded and often outdated, promising ECRs may never get the opportunity to disseminate their research. This is extremely problematic, as these ECRs are best situated to understand and protect the nature and culture of their country.

*The usual student cycle in Madagascar*

Malagasy ECRs are often reliant on foreign researchers (including foreign students) to conduct field research. This is because most Malagasy do not have the financial means to undertake field research on their own, and because foreigners are legally obliged to train and include Malagasy students as part of their research permits. While it is good that Malagasy students gain field experience alongside foreign researchers, the relationship often ends at the completion of the expedition or field season, which means the student is not included in data analysis or interpretation, and the publication process. Given the lack of preparation, guidance, and funding, Malagasy ECRs are significantly underrepresented as participants in academic arenas (e.g. conferences, publications), through no fault of their own. This trend is not limited to ECRs, as between 1960 and 2015 more than 90% of publications on Madagascar's biodiversity were led by researchers with foreign affiliations (Waeber et al., 2016). Promising Malagasy scholars often seek and attend graduate or postgraduate training overseas, in order to advance their skills and access academic opportunities. Though beneficial, this leads to a "brain drain" whereby Madagascar's most talented scientists take positions outside of Madagascar, sometimes permanently, and thus their skills may not be applied to the humanitarian and conservation challenges within their home country.

Our goal was to mentor promising Malagasy ECRs, through preparing their own first-authored scientific publication using data they had in hand. This allowed them to continue progressing in their careers, despite the COVID-19 pandemic disrupting a wide range of professional and income-generation opportunities. We believe that Malagasy ECRs who learn to publish their own research will significantly strengthen their skill set and may have access to academic and career opportunities they would not have otherwise.

**Methods**

We designed six-month writing fellowships, wherein Malagasy ECRs (n=7, from 18 applicants) were paired with a) a participating Lemur Love board member (n=3), and b) one or two external academic mentors from around the world (n=10). Fellows were selected that had existing data from previous field research which was pertinent to lemur conservation, no prior academic publications, and were able to communicate in English (we recognize that this would impede many Malagasy students, but not all mentors had the expertise to communicate in Malagasy or French). Fellow/mentor teams were asked to meet monthly, and mentors aimed to help the fellow turn their existing data into a scientific manuscript for submission to Lemur News and/or another appropriate journal. We anticipated that the fellows would develop or improve soft and technical skills as part of completing this fellowship. Fellows received a stipend (\$800USD per fellow, generously funded by Rewild's Lemur Conservation Action Fund sup-

ported by IUCN's Save Our Species (SOS) program). Mentors were not compensated and were not permitted to be listed as authors on the fellows' resulting manuscript. Fellowships started in November 2021 and concluded in May 2021.

**Results**

*Fellows*

We used Google Forms to have fellows assess their competency in several areas related to this fellowship, at the completion of the fellowship (Tab. 1).

Tab. 1: Fellows' (n=7) fellow self-assessed competency prior to and after Lemur Love Writing Fellowship. Scale 1-5, where 1= poor and 5= excellent.

Skill	Before (Average ± standard deviation)	After (Average ± standard deviation)
Email communication	2.1 ± 0.90	3.9 ± 0.38
Video conferencing	2.0 ± 0.90	3.9 ± 0.90
Responding to feedback	2.3 ± 1.33	4.3 ± 0.49
Academic writing	1.9 ± 0.82	4.0 ± 0.58
Writing in English	2.3 ± 0.38	3.7 ± 0.53
Speaking in English	2.8 ± 0.38	3.9 ± 0.38
Statistical analyses	3.4 ± 1.22	3.9 ± 0.95
Interpreting results	2.3 ± 0.90	4.2 ± 0.58
Situating results	2.4 ± 0.49	4.0 ± 0.69

Academic writing was reported by fellows to be the most improved skill. At the end of the six-month fellowship, only one fellow had a complete manuscript draft. However, within the month following the end of the fellowship, two more fellows completed manuscript drafts, and the remaining fellows expect to have drafts within 1-3 months post fellowship. Six out of seven fellows stated that in future they could write a manuscript without mentorship. All involved felt that this program was meaningful and should continue in future. One fellow stated anonymously that they "never thought they would be able to write an article in English", and another noted that this fellowship and their resulting article were like "a dream come true".

*Mentors*

In addition to meeting via video conference, we also used Google forms to request feedback from mentors. Of the respondents (n=4), all had a positive experience and would participate again. Mentors made several suggestions for how to improve the Lemur Love fellowship program and we have incorporated these into our future plans for mentoring (detailed below).

*Challenges*

Fellows and mentors noted several challenges through the duration of the fellowship. These included English competency in fellows, the duration (too short) and timing (coinciding with the North American academic calendar) of the fellowship, knowledge about academic writing and integrity, awareness of research ethics, and the fellows' ability to situate the significance of their research. We have used these 'lessons learned' to shape our proposed 3-year fellowship program which will support 30 of Madagascar's promising conservationists.

**Discussion**

*Plans for Lemur Love Writing Fellowship 2.0*

We have outlined a 3-year rotating fellowship program which we aim to find funding to support. Pandemic permit-



ting, we aim to start this program with our first workshops and retreat, in June 2022. This proposed program will address all the “challenges” encountered in the first Lemur Love Writing Fellowship.

All fellow/mentor teams will have one fellow, one Lemur Love mentor, and one to two external mentors. At least one mentor must be Malagasy. We hope to hire one full-time facilitator for this program. This person must be Malagasy and have experience with academia.

Fellows (n= 10 per year, and 30 in total) will be selected that have existing data sets related to Madagascar's biodiversity and need help to turn their data into a publishable manuscript. Additionally, fellows will not have submitted or published first-author publications prior to commencing the fellowship. Fellows will receive a stipend, and a letter of recommendation from their mentor team upon successful completion of their fellowship. Outgoing fellows are expected to attend the annual workshop (last day only), where they will present their research and greet incoming fellows, and the annual retreat. Lemur Love seeks funding to cover the fellows' stipends and all expenses related to the annual workshop and retreat.

Mentors (n=20 per year) will at least be Ph.D. candidates, have existing scientific publications, and be available for the duration of the fellowship. Malagasy mentors may be eligible for a stipend if funding is available. Non-Malagasy mentors will not be paid. All in-country expenses related to the workshop and retreat will be paid for mentors, but international travel will not be covered. Mentors will not normally be authors on their mentees' manuscripts.

## Summary

Madagascar possesses extraordinary biodiversity, however, much of the country's biodiversity is gravely imperiled. For instance, greater than 98% of all lemurs are at risk of extinction (IUCN, 2020). We hope that this program (Lemur Love Writing Fellowship 2.0) will enable and support Malagasy ECRs to embark on and establish careers in conservation science within their own country. We are committed to helping mentor and train Madagascar's most promising ECRs in conservation, as we believe they are best positioned to positively impact conservation of Madagascar's biodiversity, including lemurs.

## Acknowledgements

We would like to thank Rewild's Lemur Conservation Action Fund supported by IUCN's Save Our Species (SOS) program for funding this project. Additionally, are grateful to all the mentors who dedicated their time and expertise to support the Lemur Love Writing Fellows (Dr. Andrea Baden, Dr. Caitlin Eschmann, Dr. Caroline Amoroso, Dr. Meredith Gore, Dr. Katie Grogan, Dr. Dan Hending, Dr. Isabella Mandl, Mr. Malcom Ramsy, Dr. Melissa Seaboch, Mr. Dominik Schübler).

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## Similar gastrointestinal parasites infect two lemur species in Manombo forest, Farafangana

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## Abstract

Two Critically Endangered lemur species, *Varecia variegata editorum* and *Eulemur cinereiceps*, live in Manombo forest which suffers from many problems mainly due to human activity. A study was conducted in order to investigate gastrointestinal parasites in these two lemur species. A cross-sectional study was carried out between February and April 2019. We used fecal flotation and sedimentation methods to identify parasite species and the Mac Master counting technique to assess parasite abundance. We found that 95.83% of *Varecia variegata editorum* living in the Classified Forest, 28.57% living in the Special Reserve and 90.91% of *Eulemur cinereiceps* were parasitized by at least one species of gastrointestinal parasite. We identified 5 species of gastro-intestinal parasites, including *Callostoura* sp., *Lemuricola* sp., *Strongyloides* sp., *Ascaride*, and *Entamoeba* sp. Overall, the parasite diversity of the two lemur species was similar. *Callostoura* sp. infected both lemur species in both sites and had the highest mean abundance compared to the other parasite species. The *Varecia* in the Special Reserve was only infected with *Callostoura*, and lacked the diversity of parasites detected in the Classified Forest and in the *Eulemur cinereiceps*. These results raise questions about how human activity influences parasite diversity, and highlight the importance of future work on lemur health.

## Résumé

Deux espèces de lémuriens classifiées en danger critique d'extinction, *Varecia variegata editorum* et *Eulemur cinereiceps*, vivent dans la forêt de Manombo qui souffrent de nombreux problèmes principalement dus à l'activité humaine. Une étude a été menée afin d'étudier les parasites gastro-intestinaux chez ces deux espèces de lémuriens. Une étude transversale a été réalisée entre Février et Avril 2019. Nous avons utilisé les méthodes de flottation et de sédimentation fécales pour identifier les parasites, ainsi que la technique de coproscopie utilisant la lame Mac Master pour évaluer l'abondance parasitaire. Nous avons trouvé que 95,83% des *Varecia variegata editorum* vivant dans la Forêt Classée, 28,57% vivant dans la Réserve Spéciale et 90,91% des *Eulemur cinereiceps* étaient parasités par au moins une espèce de parasite gastro-intestinal. Nous avons identifié 5 espèces de parasites gastro-intestinaux, dont *Callostoura* sp., *Lemuricola* sp., *Strongyloides* sp., *Ascaride* et *Entamoeba* sp. Dans l'ensemble, la diversité parasitaire des deux espèces de lémuriens était similaire. *Callostoura* sp. a infecté les deux espèces de lémuriens dans les deux sites et avait l'abondance moyenne la plus élevée par rapport aux autres espèces de parasites. Les *Varecia* de la Réserve Spéciale n'ont

été infecté que par *Callistoura* et n'ont pas la diversité des parasites détectés dans la Forêt Classée et dans les *Eulemur cinereiceps*. Ces résultats soulèvent des questions sur l'influence de l'activité humaine sur la diversité des parasites et soulignent l'importance des travaux futurs sur la santé des lémuriens.

## Introduction

In the forest of Manombo, there are eight species of lemur, including *Varecia variegata editorum* and *Eulemur cinereiceps*, which are classified as Critically Endangered by the International Union for the Conservation of Nature (IUCN) (Ralainasolo et al., 2016, IUCN 2020). The biodiversity of this forest suffers from various forms of anthropogenic activities such as hunting, the exploitation of forest resources, vegetation fires, and slash-and-burn clearing for traditional agriculture (Johnson, 2002; Ratsimbazafy, 2002; Ralainasolo et al., 2016). Together, these activities degrade the natural habitats of wild animals, and can affect the long-term viability of lemurs (Ratsimbazafy, 2002; Ralainasolo et al., 2016). In addition to the deleterious effects of habitat loss and fragmentation on biodiversity, animals in degraded forests also can have suppressed immune systems, making them more prone to disease and parasitism (Gillespie and Chapman, 2006, 2008; Raharivololona and Ganzhorn, 2009).

The purpose of this study is to investigate gastrointestinal parasites in *Varecia variegata editorum* and *Eulemur cinereiceps* in the forest of Manombo.

## Methods

### Study site

The study was carried out in Manombo forest (Fig. 1) which is located in the south-eastern region of Madagascar, in the Farafangana district, former province of Fianarantsoa. The forest is located at 27km south of Farafangana along National Road 12. It extends from 22° 58' to 23° 07' E, and 47° 42' to 47° 47' S. The altitude ranges from 0 to 137m. The forest is divided into two parts. The Classified Forest of Manombo makes up an area of approximately 7,000ha and the Special Reserve with an area of 4,300ha (Ralainasolo et al., 2016). According to Ratsimbazafy (2002), the degree of deforestation is the same in the Classified Forest and the Special Reserve. All animals could be found in the two sites. Both forests were severely damaged after the Cyclone Grettelehit in Manombo in January 1997 (Ratsimbazafy, 2002).

### Study populations and period of study

Two Critically Endangered lemur species were studied: *Varecia variegata editorum* and *Eulemur cinereiceps*. We collected lemur feces from February 25, 2019 to March 25, 2019. Subsequent parasitological examinations were carried out at the National Veterinary Diagnostic Laboratory in Itaosy Antananarivo in April 2019.

### Sampling mode and sample size

With the help of guides, groups of lemurs were located daily. The groups were followed until fresh feces could be collected. For all the animals studied, fresh feces were collected within 2 minutes of defecation. We collected one fecal sample per individual. In total we collected 64 fecal samples: 24 from *Varecia variegata editorum* in the Classified Forest, 7 from *Varecia* in the Special Reserve, and 33 from *Eulemur cinereiceps* in the Special Reserve. We did not find any *Eulemur cinereiceps* within the Classified Forest.

### Sample collection

The collection of feces from the lemurs was done daily from 8AM to 5PM. Using a small spatula, feces were placed in 15 mL tubes containing 10% formalin solution, which was used to preserve the fecal samples until they could be analyzed. The tubes were sealed tightly with parafilm and shaken to allow maximum contact with the membranes of the parasites (either eggs or larvae) in the fecal matter with the formalin solution. Samples were stored at ambient temperature in the field until they could be transported to the lab for analysis. Feces were kept in the field for 3 weeks before being transferred to the laboratory for analysis.

### Laboratory analysis

Samples were stored and coproscopically analyzed in the National Veterinary Diagnostic Laboratory Itaosy Antananarivo. Qualitative analyses, including sedimentation, flotation, and a quantitative McMaster analysis were performed during this study. Each sample was subjected to two to three of these analyses. 1g of feces was weighed for each type of analysis.

### Data analysis

Data were processed and analyzed with R version 3.6.1 (R Core Team 2020) to describe the prevalence (fraction of the host population infected with a parasite), the abundance (number of parasite eggs or parasitic elements per gram of feces), and the parasite species richness (PSR), defined as the number of simultaneously present gastrointestinal parasite species in the feces of an individual host. We used Fisher's test to compare the prevalence between *Varecia* and *Eulemur* and the Mann-Whitney U-test for comparing Abundance and PSR between the two lemur species.

Results

## Results

During this study, we found that 23 of the 24 samples (95.83%) of *Varecia variegata editorum* living in the Classified Forest, 2 of the 7 samples (28.57%) of *Varecia* living in the Special Reserve and 30 of the 33 samples (90.91%) of *Eulemur cinereiceps* were parasitized by at least one species of gastrointestinal parasite.

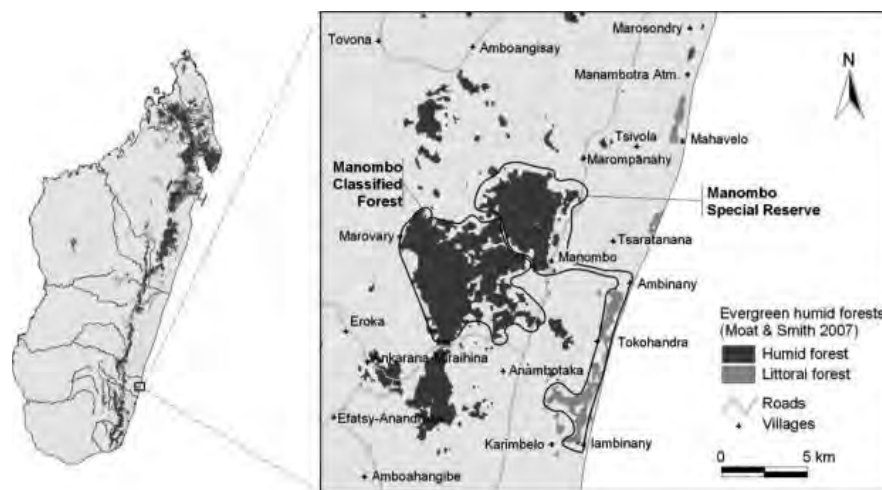


Fig. 1: Map of Manombo forest, Farafangana. (Source: Ralainasolo et al., 2008)



We identified five species of gastrointestinal parasites in the lemurs including *Lemuricola* sp., *Callistoura* sp., *Strongyloides* sp., *Ascaride* and *Entamoeba* sp. (Fig. 2). We also found mites of the genus *Chorioptes* and *Chirodiscoides* (Fig. 3), as well as arthropods and unidentified mite eggs in the feces of *Varecia variegata editorum* and *Eulemur cinereiceps*.

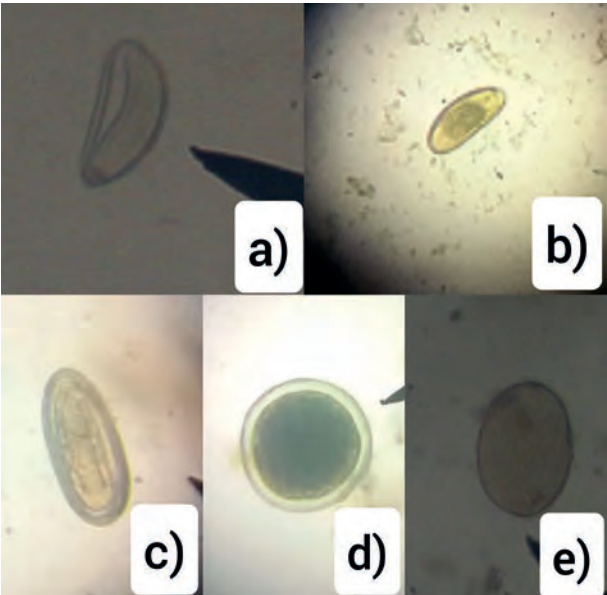


Fig. 2: Mites of the genus *Chorioptes* (a) and *Chirodiscoides* (b) found in the feces of *Varecia variegata editorum* and *Eulemur cinereiceps*. (Photo: Ratinarivo N.S.T)

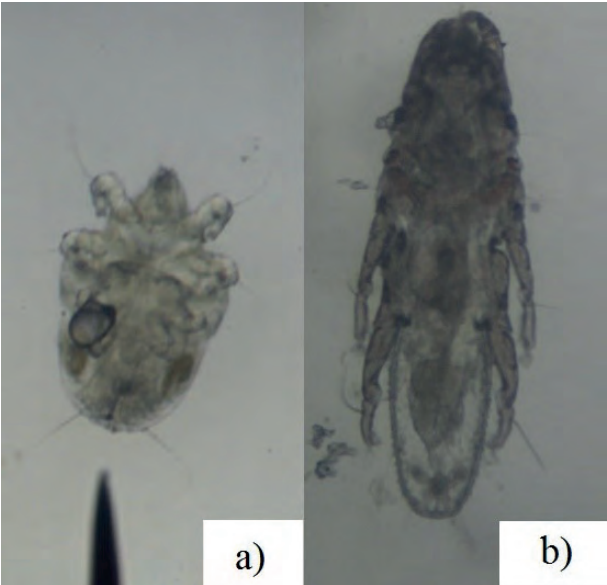


Fig. 3: Mites of the genus *Chorioptes* (a) and *Chirodiscoides* (b) found in the feces of *Varecia variegata editorum* and *Eulemur cinereiceps*. (Photo: Ratinarivo N.S.T)

Prevalence of each gastrointestinal parasite species in lemurs in the two sites

Less than half of the *Eulemur cinereiceps* individuals in the Manombo Special Reserve were infected by *Callistoura* sp. and *Entamoeba* sp., both at 48.48%. *Varecia* in the Classified Forest were more infested by *Callistoura* sp. (54.17%) than the *Varecia* living in the Special Reserve (28.57%) (Fig. 4). However, parasite species prevalence did not differ significantly between *Eulemur* and *Varecia* ( $p = 0.07$ ).

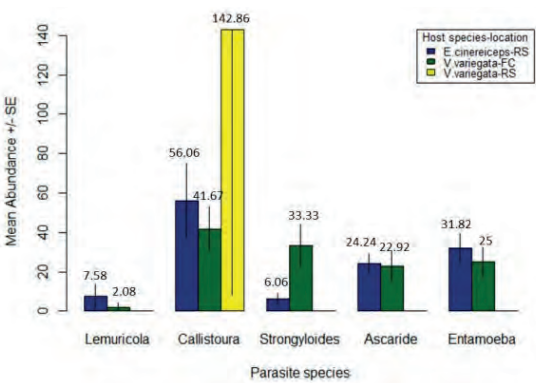


Fig. 4: Prevalence of each Parasite species in the two sites. SR refers to Special Reserve, CF refers to Classified Forest

Gastrointestinal parasite abundance in the two sites

The mean number of *Callistoura* eggs (i.e. abundance) of *Varecia* in the Special Reserve was the highest ( $142.86 \pm 134.7$ ) compared to the other parasite species (Fig. 5). The only parasite for which there was a significant difference in abundance between *Varecia* and *Eulemur* was *Strongyloides* ( $p = 0.04$ ).

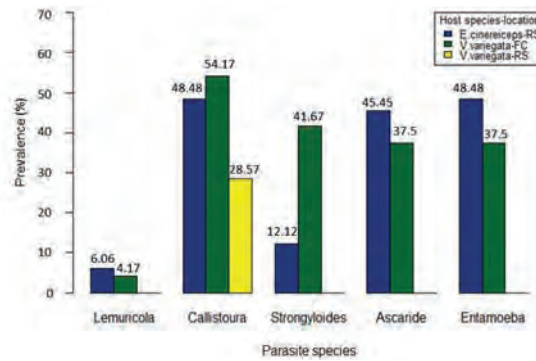


Fig. 5: Parasite abundance of lemurs in the two sites. SR refers to Special Reserve, CF refers to Classified Forest

The *Varecia* in the Special Reserve had a lower mean richness ( $0.29 \pm 0.18$ ) than the *Varecia* in the Classified Forest and the *Eulemur* (Fig. 6), but there is no significant difference between *Varecia* and *Eulemur* PSR ( $p = 0.45$ ).

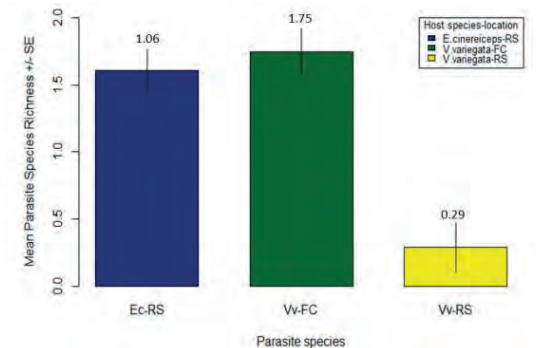


Fig. 6: Parasite Specific Richness in the two sites. SR refers to Special Reserve, CF refers to Classified Forest

Discussion

The major finding of this study is that there is a great deal of similarity overall between the parasite communities of *Varecia variegata editorum* and *Eulemur cinereiceps* in Monombo Forest. We also found discrepancy between parasite com-

munities of *Varecia variegata editorum* in two sites; however, more *Varecia* individuals were sampled in the Classified Forest than in the Special Reserve, which may have biased the results. Whereas five species of gastrointestinal parasites were identified from *Varecia* inhabiting the former, only one species was identified in *Varecia* sampled in the latter. The composition of the parasites of *Eulemur cinereiceps* in the Special Reserve closely resembled that of the *Varecia variegata* in the Classified Forest. One possible explanation for the observed pattern is that some combination of these five species of parasites comprises a typical gastrointestinal parasite community for lemurs in this forest. It is possible that, for some reason, human disturbance in the Special Reserve has disrupted the natural parasite community of *Varecia*, but not of *Eulemur cinereiceps*. However, other explanations related to sampling or random changes in parasite communities over time could also explain this pattern.

All gastrointestinal parasites species found in *Varecia variegata editorum* and *Eulemur cinereiceps* have a monoxenous life cycle (they infect their host directly without the need of an intermediate host) and are transmitted by the fecal-oral route. The lemurs become infected by incidentally ingesting eggs or larvae along with soil, fruit, or water that came in contact with feces (Radespiel et al, 2015; Rafalinirina, 2017). *Callistoura* sp. was the most prevalent parasite species in both lemur species and both sites. Also, the mean number of *Callistoura* eggs of *Varecia* in the Special Reserve was the highest ( $142.86 \pm 134.7$ ). This could be explained by the fact that *Callistoura* is considered a specific parasite of Malagasy lemurs (Chabaud et al., 1959, 1965; Irwin, 2009) and it confirms Rakotondrainibe's study about the high specificity of *Callistoura* in Lemuridae (Rakotondrainibe, 2008). However, *Callistoura* spp. are not responsible for any pathological signs (Rasambainarivo, 2008).

*Strongyloides* infect more *Varecia variegata editorum* (32.26%) than *Eulemur cinereiceps* (12.12%) and its abundance is also higher in *Varecia variegata editorum* (800) than *Eulemur cinereiceps* (200). This parasite is characterized by its direct development cycle. Although there are ecological differences among the two host species, it is not clear which of these differences would explain why this parasite is more prevalent in *Varecia* than *Eulemur*. The groups of *Varecia variegata editorum* in the Manombo forest could be in direct contact with *Strongyloides* sp. larvae (Radespiel et al., 2015) due to overlap in their home range and territory. The places where they sleep may have been contaminated by the feces of infected individuals. Strongyloidosis is a parasitic zoonosis whose natural hosts are non-human primates. In primates, this results in hemorrhagic diarrhea (Vandermeersh, 1990). They can be fatal for orangutans (*Pongo pygmaeus*), chimpanzees (*Pan troglodytes*), gibbons (*Hylobates lar*), patas monkeys (*Erythrocebus patas*), and woolly monkeys (*Lagothrix lagotricha*) (Elliott, 1994). Cutaneous, respiratory and digestive symptoms are encountered in humans (Vandermeersh, 1990). However, no clinical signs and no zoonoses have been reported concerning *Strongyloides* of lemurs.

The gastrointestinal parasite species identified during this study were all nematodes. The absence of trematode and cestode parasites could be explained by seasonal effects, as suggested by another study of *Eulemur* parasites (Clough et al., 2010). These two groups of parasites require intermediate hosts and specific environmental conditions such as heat and humidity for their development and reproduction (Andriatiavina, 2017).

According to a study carried out in the same Manombo forest in 2009, *Eulemur cinereiceps* presented eight species of helminths including: *Enterobius lemuri*, *Oesophagostomum*

sp., *Parababdonema* sp. and *Trichiuris* sp. in addition to the same four species reported here. *Entamoeba* sp. was not previously reported. The absence of certain species of parasites in this study could be an artifact of the duration and period of the study. Rakotoarivelo (2009) collected lemur feces in January-February (humid season) and September-October (dry season), while this study was restricted to February and March. In addition, our smaller sample size of *Eulemur cinereiceps* compared to that in 2009 could explain the smaller number of identified species of parasites in this study. In 2009, 78 samples of *Eulemur cinereiceps* (compared to 33 here) and 19 samples of *Varecia variegata editorum* (compared to 31 here) were collected.

We also found *Chorioptes* and *Chirodiscoides* mites in the feces of lemurs. In 2009, *Chorioptes* have already been identified in the two species of lemurs in the same Manombo forest (Rakotoarivelo, 2009). *Chorioptes* are mites causing scabies which are cutaneous and contagious. These mites live either in the epidermis, in the stratum corneum or on the surface of the skin.

In Mayotte, *Chirodiscoides* mites were also found in *Eulemur fulvus* (Negre, 2003). These pilicolous mites or Listrophoridae live permanently attached to mammalian hairs (Negre, 2003). Infestations by these parasites are most often asymptomatic (Negre, 2003). The mites are ingested during auto- or allogrooming with the lemurs' toothcombs, and hairs, ectoparasites, and eggs will pass through the digestive tract and will be eliminated via feces (Overdorff, 1993; Randriarimanana, 2012). Thus, finding these mites in feces suggests that they are present the lemurs' skin and hair.

This study shows that *Eulemur cinereiceps* and *Varecia variegata editorum* in Manombo forest are infected by at least five gastro-intestinal parasites. Parasites are essential components of ecosystems and act as regulators of host population dynamics and community structure (Kiene, 2021). In addition, the rate of gastrointestinal parasite infection is found to be one of the means of estimating the health of the population (Junge and Louis, 2005). Manombo is also home to other lemur species such as *Lepilemur jamesorum*, *Microcebus jollyae*, *Daubentonia madagascariensis*, *Haplemur meridionalis*, *Avahi ramanantsoavani* and *Cheirogaleus major* (Ralainasolo, 2016). Further work would be needed to describe the gastrointestinal parasites in these species.

## Conclusion

In conclusion, we found that parasite prevalence was relatively high in species living in a forest where the degradation index was high (Manombo, 2009) as is the case for *Varecia variegata editorum* and *Eulemur cinereiceps* in Manombo forest. This study describes the diversity of parasites in natural host populations, representing an important first step in understanding host-parasite relationships. Further study is needed to understand the health implications of these infections. New technological advances will offer opportunities to facilitate research and enhance conservation of lemurs. In addition, local populations of humans in the region have important contributions to make to wildlife and habitat conservation, which can be achieved through training, education, and involvement in lemur monitoring programs.

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## Preliminary data on lemurs of Kalanoro forest, in the District of Moramanga, Madagascar

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## Introduction

Protected Areas aim to preserve natural landscapes and especially the fauna and the flora in a geographically delimited area (Triplet, 2020). Planning the establishment of such areas helps conservationists keep wildlife populations healthy. The Malagasy government has been committed to increasing Protected Areas cover in Madagascar threefold since 2003. Lemurs are endemic to Madagascar. Unfortunately, lemurs are known to be among the highly threatened species, some of which are in critical condition, given the rate of destruction of their natural habitat (Mittermeier et al., 2010). The elaboration of the conservation plan for these primate species and their habitat in the Kalanoro forest is in process through the initiation of the "Ecovision Village" project. Kalanoro forest is part of the Ankeniheny-Zahamena Corridor (CAZ). The north part of the study area is covered by primary humid forest. And most of the southern parts are degraded forest, but it is in the process of natural regeneration. It is a strategic and key location that creates the link between Mantadia National Park, Analamazaotra Special Reserve and Vohimana Protected Area. This forest



may also contain key species of different vertebrate taxa and more sustainable natural resources, as it is surrounded by National Parks and Special Reserves (Brady and Griffiths, 1999; Andriamasimanana *et al.*, 2001; Andriambelo *et al.*, 2005; Dolch, 2008). It is thus worth considering it as a key area for biodiversity conservation.

In addition to the protection of these species, some of which are known to be seed dispersers and pollinators (Birkinshaw and Colquhoun, 1998; Ganzhorn *et al.*, 1999; Voigt *et al.*, 2004), this project also aims to reforest some forest fragments in the CAZ. Some lemurs play an important role in forest regeneration. A rapid assessment of these species is a start to achieve that goal and is likewise the first step of creating a Protected Area (Triplet, 2009). However, to date, no assessments have been carried out in this forest. That is the reason why we conducted the survey in this site.

Methods

Study site

The Kalanoro forest is located in central eastern Madagascar and is part of the CAZ (Fig. 1). The site is about 15km north from the Analamazaotra National Park (Périnet). A part of Kalanoro forest is connected to Mantadia National Park humid forest. Kalanoro is approximately 600ha in size, however only around 200 ha of primary and secondary forests remain at present. It is known to have been subject to selective logging, charcoal making, land clearing and slash-and-burn agriculture.

Observations

Direct observation following two transects was carried out from 16 to 21 February, 2021. The first transect, measuring 1400m (start: 18°53'44.5"S and 048°26'34.4"E; end: 18°53'26.1"S and 048°26'52.3"E), was established at the northwest part the Kalanoro forest, near the outer limit of the Mantadia National Park. The second one, of approximately 1600m in length (start: 18°53'35.8"S and 048°28'09.9"E; end: 18°53'15.1"S and 048°28'21.5"E) was located in the northeast of the study site. Observations took place from 6am to 9am for the diurnal species and from 7pm to 10pm for the nocturnal species. Each transect was visited once in the morning and once in the evening for three days by one observation team. The number of individuals seen per species during the survey, the age class for each individual and the traces of animal presence were noted. Species identification follows the description made by Mittermeier *et al.* (2010). Characterizations relate to the

size, the colour of the coat, the vocalisation, the local name as well as the behaviour of each encountered animal.

Results

Nine species of lemur, including four diurnal and five nocturnal, were observed from direct observation (Tab. 1). They all face the threat of extinction and are all stated in the IUCN Red List (UICN, 2020).

Tab. 1: List and conservation status of lemurs observed in the Kalanoro forest.

	Species name	Conservation status	Transects	
			TR 1	TR 2
Diurnal species	<i>Indri indri</i>	CR	-	+
	<i>Propithecus diadema</i>	CR	+	-
	<i>Eulemur rubriventer</i>	VU	-	+
	<i>Hapalemur griseus</i>	VU	+	+
Nocturnal species	<i>Avahi laniger</i>	VU	+	+
	<i>Lepilemur mustelinus</i>	VU	+	+
	<i>Cheirogaleus crossleyi</i>	VU	+	+
	<i>Cheirogaleus major</i>	VU	+	+
	<i>Microcebus lehilahytsara</i>	VU	+	+

TR 1: transect 1; TR 2: transect 2; CR: critically endangered; VU: vulnerable

*Indri indri*

Locally known as "Babakoto", this species is the largest of the living lemurs. A group of three adult individuals was found in transect 2. However, morning calls reveal that at least six groups are present in Kalanoro forest.

*Propithecus diadema*

Locally called "Simpona", *Propithecus diadema* is a diurnal species of the Indridae family. During the expedition, one group of three adults was recorded in transect 1 and another group of three adults outside the transects. Local guides claim to have observed up to eight individuals in a group at the site.

*Eulemur rubriventer*

The species *Eulemur rubriventer* or "Varika Mena" is apparently less abundant. Only one individual was observed in transect 2. However, traces of their presence were observed at transect 1. Those were fruit remnants of *Symphonia* sp. or "Kijy". The "Varika Mena" showed scared behaviour.

*Hapalemur griseus*

Locally named "Kotrika", the bamboo lemur (*Hapalemur griseus*) is a diurnal species living in groups. However, a solitary individual was encountered along transect 1 and another one along transect 2. These two adult individuals were the only ones observed during the survey.

*Avahi laniger*

*Avahi laniger* or "Fotsife" is a nocturnal species that lives in groups as observed in the Kalanoro forest. Its presence was checked in both transects. A group of three adults and a solitary individual were detected during the inventory.

*Lepilemur mustelinus*

*Lepilemur mustelinus* is locally named "Hataka". Seven solitary adults were counted in both transects. Two colour variations were noted among individuals of this species: one has a light grey coat and its tail is entirely light brown, while the other one is dark red and about 2/3 of its tail is black coloured towards the tip.

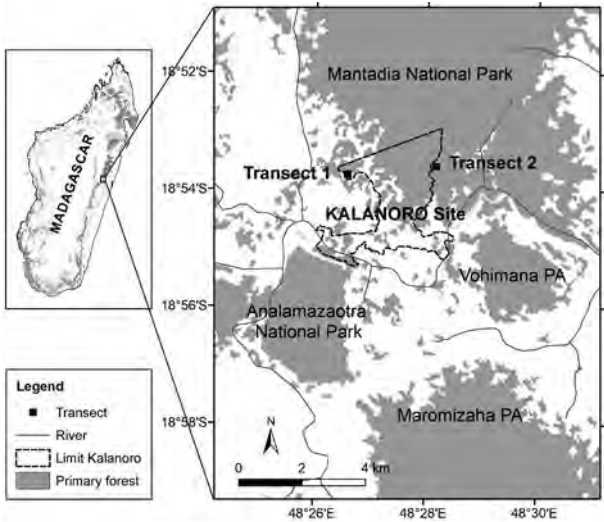


Fig. 1: Location of the Kalanoro forest study site.

*Cheirogaleus crossleyi* and *Cheirogaleus major*

There are two species of dwarf lemurs in the Kalanoro forest: *Cheirogaleus crossleyi* (n = 4) and *Cheirogaleus major* (n = 9). The local community call them “Tsidy” or “Matavirambo” and they were present in both transects. They still seem abundant in the forest.

*Microcebus lehilahytsara*

Locally called “Antsidy”, *Microcebus* cf. *lehilahytsara* is the most abundant species among lemurs in Kalanoro Forest, of which 32 individuals were counted. It is a nocturnal species and is one of the smallest of the lemurs.

**Discussion**

As Kalanoro forest is home to nine of the 14 species of lemurs existing between Zahamena National Park and Mantadia (Andriamasimanana et al., 2001), its conservation and restoration is a priority. All Kalanoro lemurs are listed as threatened by the IUCN and are at risk due to the destruction of their habitats. In addition, the absence of *Varecia variegata editorum* in this forest seems to be linked to the disturbance it suffered a few years ago. This species has become very rare even in Mantadia National Park and some groups are subject of translocation to the Analamazaotra Special Reserve for their preservation (Day et al., 2009). *Eulemur rubriventer* has become very sensitive to habitat disturbance and very difficult to observe in areas under pressure (Andriamasimanana et al., 2001). However, the morphological variation observed in the genus *Lepilemur* is worth special attention. *L. mustelinus* probably show an atypical coloration of the body (Mittermeier et al., 2010). This study could not determine whether this was an individual colour variation in this species or a distinctive character of two different species. Further study of this species is therefore necessary.

The initiative to conserve and restore the Kalanoro forest to connect three Protected Areas of CAZ is a key point for the long-term conservation of several species such as lemurs, amphibians and reptiles. Currently, it is in regeneration because the local people participate in its restoration and protection. The realization of this project offers the possibility of movement, migration and recolonization of these species in these areas (Schmid et al., 2005). Hence, it will facilitate allelic spotting within the population of these species. It is also one of the three main objectives of the Nagoya Protocol, signed in 2010 by several countries (CBD, 2011). This project confirms the importance of forest service that could be provided to the ecosystem (Pollini, 2009; Wendland et al., 2009). Future research should be focused on the study of species that can aid in the dispersal and germination of seeds, as well as in flower pollination in the Kalanoro Forest.

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## Techniques used for illegal lemur hunting in Ankarafantsika National Park, north-western Madagascar

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## Abstract

Lemurs are hunted illegally as bush meat inside protected areas in Madagascar. In 2016, we observed poachers hunting lemurs using blowpipes and snare traps in an area for scientific research in Ankarafantsika National Park, in northwestern Madagascar. To understand the techniques of lemur hunting, we describe hunting behavior, hunting equipment, and hunted prey. We encountered two poachers with a dog shooting brown lemurs (*Eulemur fulvus*) using a blowpipe. When the poachers fled, they dropped the blowpipe and their prey: one Milne-Edwards' sportive lemur (*Lepilemur edwardsi*) and three western avahis (*Avahi occidentalis*). All of the carcasses had had the digestive organs removed, probably to prevent decay and to give the offal to the dog as a reward. Blowpipe hunting is a threat to mid-sized nocturnal lemurs. After trained dogs detect the sleeping sites of nocturnal lemurs, hunters can easily shoot the sleeping or slow-moving lemurs. In the snare trap, called a *lalo*, a wood beam forms a horizontal bridge enabling access to decoy mangos. When the head of a lemur walking on the bridge is caught in a loop of plastic string, the lemur will hang. The *lalo* probably targets mid-sized frugivorous quadrupedal locomotors in a horizontal position, such as brown lemurs in Ankarafantsika. We found two brown lemur skulls under the trap. These hunting activities threaten lemurs in this region, and the eradication is imperative for Ankarafantsika National Park.

## Introduction

Lemurs are endemic to Madagascar, which is recognized as a biodiversity hotspot (Myers *et al.*, 2000). Unfortunately, over 90% of lemur species are threatened with extinction due to habitat destruction and hunting (Schwitzer *et al.*, 2013). Although hunting of all lemur species is prohibited by law in Madagascar (Durbin, 2007; Borgerson, 2015), lemurs are hunted illegally as bush meat, even inside nature reserves and national parks (Borgerson, 2015; García and Goodman, 2003; Golden *et al.*, 2014; Randrianandrianina *et al.*, 2010). Most of the studies of lemur hunting have focused on the species hunted. Based on the structure of snare traps using fruiting trees, Borgerson (2015) and Golden (2009) argued that frugivorous lemurs were vulnerable such as *Varecia* and *Eulemur*. However, only a few studies have reported on hunting activities and techniques (Anania *et al.*, 2019; Borgerson, 2015; Golden, 2009). Ankarafantsika National Park (ANP) protects the biggest fragment (ca. 132,400ha) of the dry forest ecosystem in western Madagascar (Du Puy and Moat, 2003). This park follows the concept of "Man and Biosphere" as defined by UNESCO (2005) and contains communities with over 2,000 residents who are basically agriculturalists of several ethnic groups (Aymoz, 2013). ANP consists of core areas with total protection, buffer zones with limited access, and zones for ecotourism and research where access by residents are prohibited (Madagascar National Parks, 2017). In 2016, we encountered poachers and snare traps for hunting lemurs

in the research zone. In this article, we describe the lemur hunting techniques and discuss the vulnerable targeted lemur species in each specific hunting technique.

## Methods

The study site was located at Ampijoroa Forestry Station (16°32'S, 46°82'E) in ANP, northwestern Madagascar (Fig. 1). Eight lemur species occur in ANP and some of them are listed as 'endangered' on the IUCN Red List (IUCN, 2020), three Cheirogaleidae [*Cheirogaleus medius* (VU), *Microcebus murinus* (LC), and *M. ravelobensis* (VU)], one Lepilemuridae [*Lepilemur edwardsi* (EN)], two Lemuridae [*Eulemur fulvus* (VU) and *E. mongoz* (CR)], and two Indriidae [*Avahi occidentalis* (VU) and *Propithecus coquereli* (CR)]. A rectangular trail system (500×600m<sup>2</sup>) called Jardin Botanique A (JBA) is placed in a dry primary deciduous forest for scientific research only. The area around JBA are used for both scientific research and ecotourism, and activities by local people are prohibited there. However, we encountered poachers hunting lemurs with a blowpipe within JBA on January 28, and we also found a snare trap with lemur prey north of JBA on May 21 in 2016 (Fig. 1). We observed the activities of blowpipe hunting and described the materials and structure of the snare trap *in situ*.

## Results and discussion

### Blowpipe Hunting

At around 16:00 on Jan 28, 2016, we encountered poachers and observed their hunting activities in the bush. The party of poachers consisted of two young Malagasy men and a dog. One man was shooting a brown lemur with a blowpipe and the other man carried the prey. The unleashed dog was barking at a group of brown lemurs. When the poachers noticed us, they discarded the blowpipe and prey and fled. We brought the tools and prey back to our campsite for measurement. The blowpipe was a 215cm-long steel pipe with an outside diameter of 15.9mm and inside diameter of 13.6mm, weighing 640g. It contained an iron dart with cotton from the fruit of the white silk cotton tree (*Ceiba pentandra*) at one end (Fig. 2A). This dart was made out of the same materials as the other five darts that we found in the forest. The six darts averaged 22.2±1.0cm in length and 3.9±0.7g in weight. The poached animals consisted of four individuals of two nocturnal lemur species: one Milne-Edwards' sportive lemur (*Lepilemur*

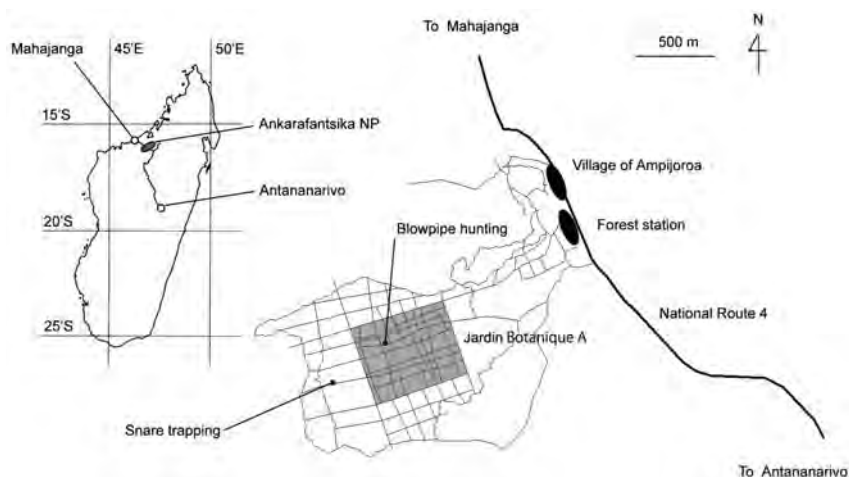


Fig. 1: Location of Ankarafantsika National Park (left) and the positions of blowpipe hunting and snare trapping in the trail system around Jardin Botanique A (right). Only scientific research is allowed within JBA (gray zone). Ecotourism and research are conducted in the trail system around JBA.



edwardsi) and three western avahis (*Avahi occidentalis*) (Tab. 1; Fig. 2B). All of the carcasses had been struck on the head and the femurs were all broken. In addition, some of the viscera had been removed through an abdominal incision, although the heart, lungs, liver, and kidneys remained; the abdominal space had been stuffed with tree leaves (Fig. 2C). In addition, ANP staff encountered another party of poachers composed of three men and two dogs at the northwestern corner of JBA during a patrol on Feb 16, 2016. Those poachers also escaped and left a blowpipe and a shoulder bag containing 17 darts. Two of the darts were smeared with animal blood. All of the tools were similar to those we found on Jan. 28. The blowpipe was 222cm long, 18.9mm in outside diameter, 13.4mm in inside diameter, and 730g in weight.

Tab. 1: List of lemurs taken by blowpipe hunting on January 28 in 2016.

ID	Species	Age	Sex	Head-body length (cm)	Tail length (cm)	Body-weight (g)*
1	<i>Lepilemur edwardsi</i>	Adult	Male	26.4	30.0	690.0
2	<i>Avahi occidentalis</i>	Adult	Male	25.3	32.0	570.0
3	<i>Avahi occidentalis</i>	Adult	Female	24.6	35.3	710.0
4	<i>Avahi occidentalis</i>	Infant	Female	16.2	20.4	210.0

\*Weight without abdominal organs

Blowpipe hunting was conducted in daytime and poachers were targeting day-active brown lemurs when we encountered them. However, all collected carcasses were nocturnal lemurs. During the daytime, sportive lemurs often sleep in tree holes, while avahis rest under the tree canopy. Although it is very difficult for humans to find sleeping lemurs in the dense vegetation, trained dogs with their keen olfactory sense are probably able to find them easily (see

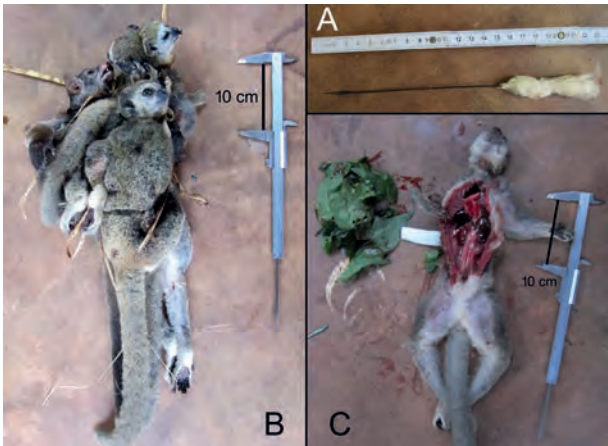


Fig. 2: The lemur carcasses and a blowpipe dart discarded by poachers on January 28, 2016. (A) a blowpipe dart, (B) carcasses consisted of one Milne-Edwards' sportive lemur (*Lepilemur edwardsi*) and three western avahis (*Avahi occidentalis*), and (C) abdominal incision in an adult female western avahi (*Avahi occidentalis*).

also Koster, 2009). The internal organs of the prey may have been removed to prevent decay and/or given to the dogs as a reward (Koster, 2009). After the dog detects a sleeping nocturnal lemur, the hunters can shoot the inactive lemurs. At JBA (ca. 30ha), there are an estimated 20 western avahis and 17 Milne-Edwards' sportive lemurs based on the population densities estimated by Ganzhorn *et al.* (1988). If poachers with dogs continuously hunt lemurs in JBA, a very important area for research in ANP, local extinction could easily happen within a few months.

Snare Trapping

On May 21, 2016, we found a snare trap north of JBA (Fig. 3A). The snare trap is called a *lalo* in the Ankarafantsika region. Fig. 3B illustrates the structure of the trap. A horizontal

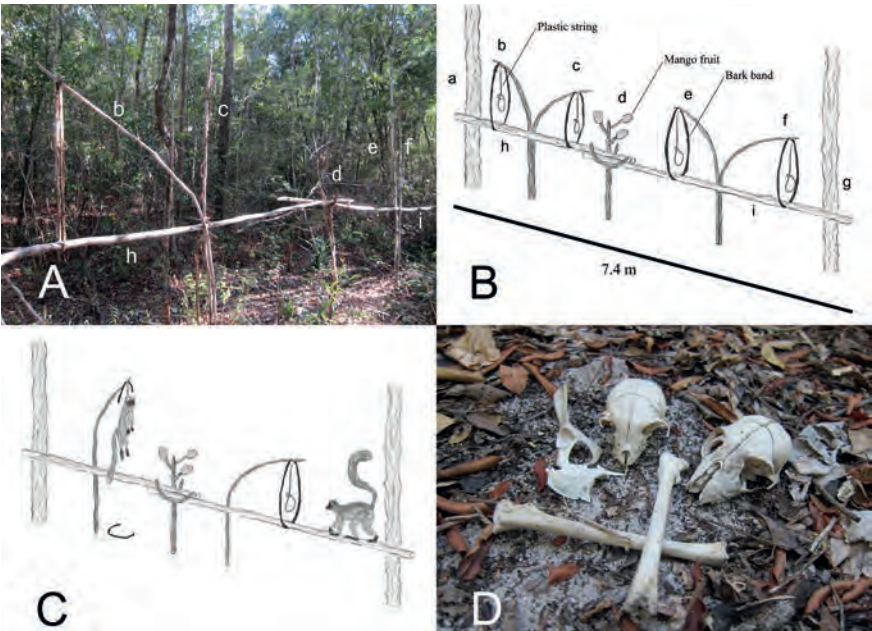


Fig. 3: Snare trap, *Lalo*. (A) Photo in the forest, (B) Structure, (C) Estimated scene of entrapping brown lemurs (*Eulemur fulvus*), and (D) skulls of brown lemurs under the snare trap. The components of the trap: (a, g) Pole using an alive tree, (b, c, e, f) hanging stick with a string, (d) Center pole with mango fruits on branches, (h, i) horizontal beam.

wooden beam bridged a 7.4m span between two live trees, 118cm above the ground. A branched pole was attached to the center of the bar, and mangos were attached to the branches (d in Fig. 3A, 3B). In addition, four snares were set on the bridge (b,c,e,f in Fig. 3A, 3B). Each snare consisted of a wooden stick with a plastic string forming a loop; the stick was held in a bent position by a fragile band of bark (b,c,e,f in Fig. 3B). Tab. 2 summarizes the dimensions of each part of this trap. If the head of a lemur walking on the bridge got caught in the loop, the bark band would break with the movement of the lemur, which would be hung (Fig. 3C). We found two brown lemur skulls (Fig. 3D) and several mango seeds under the trap.

The *lalo* snare probably targets brown lemurs in JBA based on three pieces of evidence. First, mangos will lure frugivores such as brown lemurs, but not folivores like sportive lemurs and avahis.

Second, the horizontal bridge is suitable for quadrupedal locomotors in a horizontal position, like brown lemurs, but not for vertical leapers such as sportive lemurs, avahis, and sifakas (*Propithecus*). Third, the snare parts (plastic loop and bark band) would be too big for small lemurs like dwarf (*Cheirogaleus*) and mouse (*Microcebus*) lemurs. The brown lemur skulls under the trap support these explanations. This snare trap is similar to a trap called *laly totoko* in Makira Forest (Golden, 2009) and *laly kodidy* around Masoala National Park. (Borgerson, 2015) in northeastern Madagascar. These two traps involve bridges between two fruiting trees using a wood beam with several snares without mangos (Borgerson, 2015; Golden, 2009). Similar to the targeted lemurs in Ankarafantsika, the *laly kodidy* in Masoala also mainly catches frugivorous quadrupedal locomotors, such as white-headed lemurs (*Eulemur albifrons*) (Borgerson, 2015). As the populations of white-headed lemurs were largely degraded by *laly kodidy* (Borgerson, 2015), *Eulemur* is likely vulnerable to this trapping method because of its frugivorous habits. *Eulemur* is the most important and largest seed disperser in Ankarafantsika (Sato, 2012). This lemur hunting method is unsustainable (Golden, 2009); unsustainable hunting will lead to the collapse of forest regeneration systems, given that Ganzhorn *et al.* (1999) found low densities of saplings of large-seeded plants in degraded forest with no *Eulemur*.

Tab. 2: Length and diameter of the aspects of the snare trap. The ID of each part match the letters in Fig. 3.

ID	Description	Length	Diameter
a	Pole using an alive tree	ca. 18m in height	18.0 cm
b	Hanging stick with a string	201 cm	15.5 mm
c	Hanging stick with a string	221 cm	14.3 mm
d	Center pole with mongo fruits on branches	174 cm	25.9 mm
e	Hanging stick with a string	222 cm	17.6 mm
f	Hanging stick with a string	233 cm	16.1 mm
g	Pole using an alive tree	ca. 10m in height	7.5 cm
h	Horizontal beam	340 cm	3.0 cm
i	Horizontal beam	500 cm	3.5 cm

In March 2019, we revisited JBA and confirmed the presence of brown lemurs, avahis, and sportive lemurs. However, we encountered two poachers with blow pipes there, and one of them was finally identified by the managers of ANP. The poacher was a local resident living at a town in the periphery of the park. In the periphery of ANP, the populations are growing rapidly and ANP are not able to manage their illegal activities including wildlife hunting in the park (Aymoz *et al.*, 2013). In the situation of an increase in demand for bush meat in urban areas near protected forests (Randrianandrianina *et al.*, 2010), the explanation and education of conservation policies are necessary not only within the park but also in the periphery and neighboring urban areas of ANP.

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**Microcebus griseorufus using artificial refuge to face the changing environment in the Bezà Mahafaly Special Reserve in southwestern Madagascar**

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Introduction

Mouse lemurs (*Microcebus* spp.) are present throughout Madagascar wherever there remains an appropriate natural habitat, including primary and secondary forests and even in disturbed and degraded habitats (Knoop *et al.*, 2018; Ramsay



et al., 2019). These nocturnal lemurs are often among the most abundant mammals in the areas where they are found (Mittermeier et al., 2014).

Twenty-four species of mouse lemur are now recognized in Madagascar (Schüßler et al., 2020). The xerophytic forests of southwestern Madagascar, one of the driest and most seasonal environments in all of Madagascar (Ratsirarson et al., 2001; Mittermeier et al., 2014), constitute also habitats for mouse lemurs. In an extensive taxonomic revision of populations from 12 localities in western and southern Madagascar, seven species of *Microcebus* were recognized, including *Microcebus griseorufus*. Species were distinguished by morphometrics, and by differences in coat color and dental and other morphological characteristics (Richard et al., 2016). The geographical distribution of *Microcebus griseorufus* in Madagascar is very restricted and extends only from the southwestern part to the south of the island. Based on molecular studies (Heckman et al., 2006; Richard et al., 2016), *Microcebus griseorufus* has been reported as the only species of *Microcebus* present in the Bezà Mahafaly Special Reserve in southwestern Madagascar. In the Bezà Mahafaly forest Reserve, these mouse lemurs are located in gallery forests and sleep mostly in tangles of vegetation (Rasoazanabary, 2004). *Microcebus griseorufus* is omnivorous, and feeds on vegetative parts of plants such as fruits, flowers, buds, gums, as well as some insects' larvae and adults (Randrianarimalalaso, 2008).

In this short communication, we report the accidental observation of *Microcebus griseorufus* using artificial habitat such as house attic as their refuge. In June 2021, three individuals of *Microcebus griseorufus* were observed, during the winter period (average temperature 2010-2020 and in June 2021 respectively:  $T_{\min} = 17^{\circ}\text{C}$  and  $12^{\circ}\text{C}$ ;  $T_{\text{mean}} = 25.7^{\circ}\text{C}$  and  $22^{\circ}\text{C}$ ;  $T_{\max} = 34.3^{\circ}\text{C}$  and  $32^{\circ}\text{C}$ ), using the attic, a space contained between the ceiling (inside) and the roof (outside) of buildings located in the Bezà Mahafaly Special Reserve camp, as a refuge. This camp is located next to a gallery forest dominated by tamarin trees (Ratsirarson et al. 2001; Rasamimanana et al., 2012; Ranaivonasy et al., 2016). These constructions have been there for more than 20 years, but this is the first time that these lemurs have been observed using this attic for refuge. A male individual was observed in the attic of a building uncovered during its repair (Fig. 1). There were tufts of leaves and stems found in this attic. Two other individuals (one male and one female) were also observed emerging from the attic of the Museum building in the Bezà Mahafaly camp followed by a snake which tried to chase them away. We also saw tufts of leaves and stems in this Museum attic after checking it.

These observations of lemurs using attic spaces for refuge may show habitat adaptation of *Microcebus griseorufus* fol-

lowing the changing of its environment. Mandl et al. (2018) have observed *Lepilemur sahamalaza*, choosing sleeping sites that are more confined like cavities in dead or living trees especially in colder periods. Morland (1993a, b), Balko (1998) and Vasey (2005) have also shown behavioral change of *Varecia variegata* and *Varecia rubra*, especially during winter, where they have been observed coping with food shortages by reducing activity and increasing energy conservation. Changing climate parameters and availability of food resources may be possible factors influencing behavioral changes in *M. griseorufus* at the Bezà Mahafaly Reserve.

The objective of this work is therefore to identify whether the change in climate parameters and the availability of food resources in the forests are the possible explanations for behavioral plasticity in sleeping site choice for these Mouse lemurs at the Bezà Mahafaly Special Reserve. We hypothesize the following:

1. Over the last five to ten years in the southern Madagascar, there is a drought caused by the increase in average annual temperature and the decrease in annual rainfall below the normal pattern. A drought has been defined as a period of time when an area or region experiences below-normal precipitation (Panagoulia, 1998).
2. The drought impacted the availability of food for lemurs over the last five to ten years. We believe that food shortage, due to drought, decreased the vital energy of *M. griseorufus*, which prevented it from coping during cold winter periods. Thermoregulatory and energy-conserving behavior often occurs in areas with a prolonged dry season (Sato et al., 2014). We thus hypothesized that these lemurs used this artificial refuge as a thermoregulatory strategy.

### Methodology

The observation we made in *M. griseorufus* was just accidental and we did not intend to study the refuge behavior of these lemurs. However, having seen these *Microcebus* at Bezà Mahafaly using attics as refuge for the first time, we tried to determine the reasons why this species uses these artificial environments for their refuge.

Regular and systematic records of daily temperature and rainfall have been carried out for more than 20 years in the Bezà Mahafaly Special Reserve. To find out whether this southwestern part of Madagascar, in particular the Special Reserve of Bezà Mahafaly and its surroundings, was marked by drought during the last five years, which might have had some impact on the daily activities and behavior of this mouse lemur, we focused our observations on: (i) the general pattern of the annual and seasonal temperature (aver-



Fig. 1: (a) *Microcebus griseorufus* on the ground falling from the attic of the wooden house, (b) *Microcebus griseorufus* falling from the attic held by the building constructor.



age, maximum, minimum) over the last ten years from 2010 to June 2021; (ii) the general pattern of the total annual precipitation from 2010 to June 2021, as well as the number of annual rainy days. As the diet of *M. griseorufus* are composed of vegetative parts of plants such as fruits and flowers, we collected regular data on plant phenology since 2005 twice a month every year in two permanent transects of the Bezà Mahafaly Reserve (Rasamimanana *et al.*, 2012). We used the phenology data from individual trees which had available leaves, flowers and fruits, indicating the availability of food resources for lemurs.

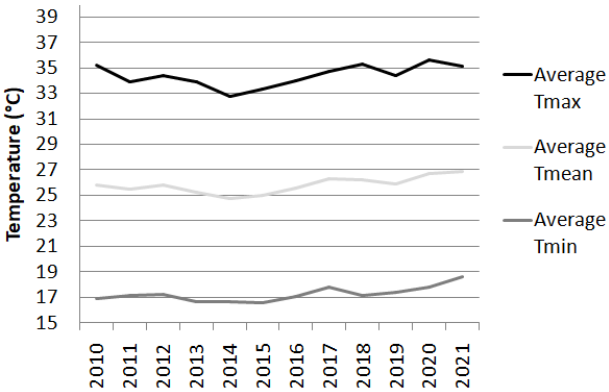


Fig. 2: General pattern of the average annual temperature (average maximum T<sub>max</sub>, mean T<sub>mean</sub> and minimum T<sub>min</sub> temperatures) in the Bezà Mahafaly Special Reserve from 2010 to June 2021.

Results and discussion

Overall, we observed an increase in annual temperature (average, maximum and minimum) from 2017 at Bezà Mahafaly. The last five years were warmer than the years prior to 2017. We have observed a difference in annual temperature (average maximum, mean and minimum temperatures) between 2010 and June 2021 (Fig. 2). The maximum temperature exceeds 35°C after 2017. The average annual temperature is normally between 25°C and 26°C but continued to increase from 2017 reaching almost 27°C in 2021. The minimum temperature which is around 17°C has climbed to reach 18 to 19°C in 2020-2021, an increase of almost 1 to 2°C in ten years.

The total annual precipitation and the number of rainy days (Fig. 3) were very low in 2010 in the area of Bezà Mahafaly, which is similar to report from the rest of southern Madagascar (Van Eeckhout & Hervieu, 2010). The total annual precipitation and the number of rainy days continued to decrease from 2014 and 2017 (in 2017, 700mm of rain fell over 44 days,

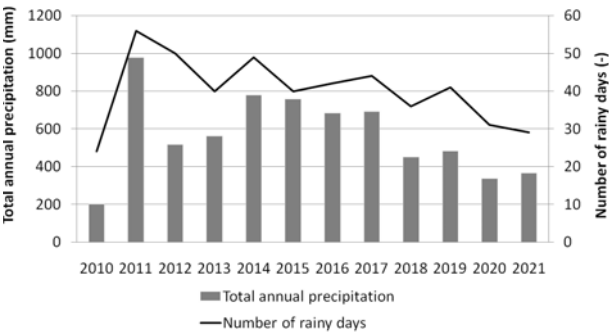


Fig. 3: Total annual precipitation and number of rainy days in the Bezà Mahafaly Special Reserve from 2010 to June 2021.

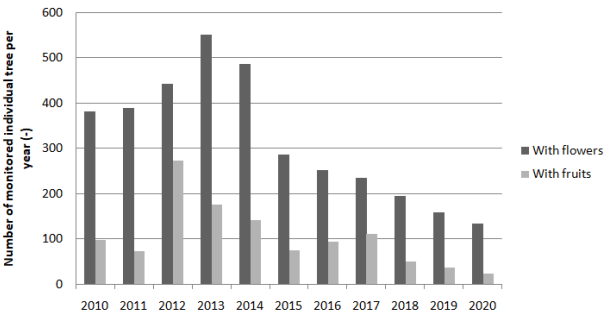


Fig. 4: Number of monitored individual tree with flowers and fruits in the Bezà Mahafaly Special Reserve from 2010 to 2020.

in 2020 less than 330 mm of rain fell in less than 30 days). The increase in temperature and the decrease in rain confirms the drought in southern Madagascar over the past five years (2017-2021) having significant impacts on agriculture and the threat of famine, but also on the availability of food for lemurs like *Microcebus griseorufus* (Fig. 4). Indeed, since 2013, we also observed the decline of the number of individual trees having flowers and fruits in the two permanent monitoring transects at the Bezà Mahafaly Reserve (Fig. 3, Fig. 4). There is a clear positive correlation between the precipitation pattern and the availability of food for lemurs.

The increase in temperature and the decrease in rainfall over the past five years suggest that the southwestern area is facing a drought, which can lead to starvation not only for humans but also for wildlife. Our results showed that the decreased precipitation correlated with reduced availability of flowering and fruiting of individual trees in the forest, and thus that increasing annual temperature and decreasing rainfall may have an impact on the availability of vital food resources for lemurs, especially for *Microcebus griseorufus* in the Bezà Mahafaly Reserve. The lack of food may lead mouse lemurs to have reduced energy with which to cope with the changing environment, driving them to use artificial refuge during colder winter periods (T<sub>min</sub> in June 2017 was about 13.6°C, and continued to decrease every year, reaching approximately 12°C in June 2021, unpublished data). This may be the reason that these lemurs took refuge in an artificial habitat such as the attic of buildings in the camp of the Bezà Mahafaly Reserve, which is warmer than in their natural habitat. We hypothesize that these lemurs have limited available energy to nest in the tangles of vegetation with the cold winter temperatures and prefer to use a man-made artificial structure nearby instead. Even if tree holes or entanglement of vegetation in living trees are effective in keeping the heat (Schmid, 1998), the colder temperature during winter may no longer bearable for these lemurs to stay outside in the surrounding forest. They may prefer to use this artificial (attic) environment which might be more comfortable, warmer and easier to use. With the challenges of environmental changes (deforestation, climate change), these mouse lemurs might have to move and use other more comfortable areas to survive or to take refuge as observed as well by Mandl *et al.* (2018) in *Lepilemur sahamalaza*.

The impacts of climate change could explain the use of this artificial environment by *Microcebus griseorufus*, but detailed studies remain to be systematically explored, including the number of natural refuge sites available in the surrounding gallery forest. Close observation of the behavior of these nocturnal mouse lemurs need to be monitored to better understand their adaptation to the continued environmental changes.

Global warming (disruption of the rainy seasons, disruption of the crop calendar, etc.) threatens the animal emblem of Madagascar, the lemurs. According to Andriantsoarana *et al.*, 2021, southern Madagascar is now in its fourth consecutive year of drought which has wiped out harvests and led to food insecurity for local populations. It has shown also from our study the negative impact of drought on lemurs' food availability. With the persistent drought, the lemurs' habitats may no longer be viable for them. These lemurs may have to move to other habitats to survive. They will have to migrate, to leave the patches of degraded forests to seek refuge elsewhere (Tétaud, 2018). Wright (2006) hypothesized that lemur traits evolved to cope with the unpredictable and climatically difficult island of Madagascar, including their adaptations to save energy or maximize the use of scarce resources. However, although lemurs are resilient, this resilience has its limits. The effects of rapid climate change on the ecology and long-term survival of lemurs are significant. Faced with these changes, lemurs may seek refugia in human-made structures to adapt to environmental change, taking risks in doing it. In the long-term, solutions must be found so that these animals can live in their natural habitats. Our study showed that mouse lemurs could adopt artificial nest boxes for their sleeping sites (see Baden, 2019), in their natural habitat. In addition, restoration of their forest habitats to maintain a thick and viable forest cover is very important to allow these lemurs to adapt to the cold winter temperatures. Other disturbing factors, which may also be the origin of these behavioral changes, must be studied carefully in order to ensure an effective conservation strategy for these nocturnal lemur species in the southwestern region of Madagascar.

### Acknowledgments

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## Intensive hunting of *Varecia variegata* in Andriantantely, section of the new Protected Area “Corridor Ankeniheny Zahamena”

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Biodiversity Conservation Madagascar (BCM) is a registered Non-Governmental Malagasy Organisation dedicated to conservation. The organization was established in 2002, and serves as the Madagascar conservation arm of Bioculture. BCM's main goal is to conserve vulnerable forests with great biodiversity value, particularly rich in lemurs.

The forest of Andriantantely has been identified as a major priority for conservation in Madagascar (Schmid and Alonso, 2002). Andriantantely is in a section of the new Protected Area named “Corridor Ankeniheny Zahamena” managed by Conservation International Madagascar (CI). In February 2021, a joint mission comprising teams from BCM and CI visited Andriantantely to assess the effectiveness and current level of ecosystem management by the local communities in Andriantantely.

During our survey of Andriantantely forest, three lemur trap lines were found. These traps were for the capture of black-and-white ruffed lemur (*Varecia variegata*) and other lemur species (e.g. *Eulemur fulvus*, *E. rubriventer*) with similar behaviour. The hunters had chopped trees over an area of up to 1,600m<sup>2</sup> (straight-line area of 50 x 10m, 100 x 10m, and 160 x 10m for the three trap lines respectively; Fig. 1).



Fig. 1: Linear gaps created in continuous forest to install lemur traps. Photo: Radosoa Andrianaiavarivelo

A total of 12 functional traps with one dead individual captured (*V. variegata*) in the first trap were found (Fig. 2). However, the presence of lemur hairs on eight traps testifies the effectiveness of the traps and its reuse on several occasions and therefore, we assumed, at least 8 individuals were caught in these traps prior to our arrival.

The traps were fixed at a distance of about 25m apart, on a tree trunk mounted in a horizontal position forming a bridge connecting the two forest boundaries on either side of the trap line. The consequence is not limited to the threat of survival of the lemurs, particularly of *V. var-*

*iegata*, but also to the whole Andriantantely ecosystem, since 16,000 to 24,000 trees (DBH > 12 cm) are cleared for making the trap lines. Urgent *in-situ* conservation measures should be put in place to curb such ecosystem degradation and lemur poaching in Andriantantely, otherwise at the current threat level, variegated black-and-white ruffed lemurs will disappear within a decade. Based on discussions with the local community and our investigation (February 2021) in this forest it seems that *Eulemur* sp. no longer exist in the inspected forest areas, although they once occurred there. A plausible reason for this is that these animals have been the subject of intensive hunting with the same hunting method and have disappeared over time. Indris (*Indri indri*) and diademed sifakas (*Propithecus diadema*) both still occur at Andriantantely.

The Malagasy NGO Biodiversity Conservation Madagascar which runs the nearby Sahafina Reserve and the Beanka Reserve on the west coast, is currently in discussion with CI and the Malagasy Government to employ a minimum of 10 forest guards and establish a community-based project at Andriantantely to counter the hunting threat and ongoing slash and burn agriculture (tavy) within this protected area. Based on BCM's experience at Sahafina, the best way to ensure the survival of this forest and its fauna is to establish a locally based team of well-paid forest guards with a vested interest in the protection of this forest. Protected Area status alone is not sufficient to ensure the long-term survival of this biodiversity rich forest.

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Fig. 2: Snared *Varecia variegata* in the forest of Andriantantely in 2021. Photo: Radosoa Andrianaiavarivelo



## Articles

### Range Extension of the Hairy-eared Dwarf Lemur, *Allocebus trichotis*, in north-central Madagascar

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#### Introduction

One of the rarest and least-studied primates is the Hairy-eared Dwarf Lemur, *Allocebus trichotis* (Günther, 1875), a solitary, nocturnal lemur, comparable in size to mouse lemurs of the genus *Microcebus*. Originally identified as a *Cheirogaleus* (Günther, 1875), it was later reclassified to the monospecific genus *Allocebus* by Petter-Rousseaux and Petter (1956). This species was believed to be extinct until 1989 when it was rediscovered (Meier and Albignac, 1991). *Allocebus trichotis* is currently Endangered (Louis et al., 2020), though there is little published data on its ecology, including a comprehensive understanding of distribution and population size.

Since its rediscovery in 1989 along the Mananara River (Meier and Albignac, 1991), the geographical distribution of *A. trichotis* has undergone significant revision. Though remaining within the moist evergreen forests of eastern Madagascar, *A. trichotis* has been observed in several protected areas (Fig. 1; Tab. 1), including Analamazaotra Special Reserve (Garbutt, 2001), Marojeje National Park (Goodman and Raselimanana, 2002), Anjanaharibe-Sud Special Reserve (Schütz and Goodman, 1998; Schmid and Smolker, 1998), Masoala National Park (Sterling and Rakotoarison, 1998), and Marotrandrano Special Reserve (Mittermeier et al., 2008). Although it remains a rare animal (Coppeto and Harcourt, 2005), it appears to be more widely distributed than originally thought. Having an accurate understanding of the geographic range of *A. trichotis* is an important component to advancing conservation strategies (Schwitzer et al., 2013).

During a field study at Bemanevika Protected Harmonious Landscape, we discovered multiple individuals of *A. tricho-*



Fig. 1: Geographic distribution of *Allocebus trichotis* in Madagascar, location of Bemanevika Protected Area, and locations of observed and captured individuals therein.

*tis*, capturing one of them. This paper presents details on the presence of this species in the north-central forests of Madagascar.

#### Methodology

##### Study site

The Bemanevika Protected Harmonious Landscape (14° 10' - 14° 35' S, 48° 25' - 48° 50' E; Fig. 1) is located 40km northwest of Bealanana in the Sofia Region. The area consists of rugged terrain, hills, and mountain ranges interspersed with valleys, covering a total area of 35,605ha, of which slightly over 20,000ha are forested (Peregrine Fund, 2014; Goodman et al., 2018). The protected area falls within an altitudinal range of between 700 and 1,800m (Rabearivony et al., 2010).

##### Capture and Data Recording

All research was authorized by Madagascar's Ministry of the Environment, Ecology, and Forests and complies with protocols approved by the IACUC of Omaha's Henry Doorly Zoo and Aquarium (97-001, 12-101). The aim of our research is to provide an update about the number of all nocturnal lemur species in the site and to do a systematic revision. The field expedition was carried out from Dec 5-7, 2017. A team of 7 individuals conducted line transects surveys along established trails (Hilário et al., 2012) over the three days. The capture of a single individual of *A. trichotis* was by hand and after handling the animal was released at the capture site. We administered Telazol (Tiletamine and Zolazepan, Fort Dodge Animal Health, Iowa USA; 10mg/kg of the animal's live weight) as the anesthetic sedative to properly handle the animal. We conducted a full medical examination of the animal, recording sex, temperature in Celsius, pulse and respiratory rates, weight in grams, and morphometric measurements in millimeters. Additionally, blood and tissue samples were taken for

Tab. 1: Locations where presence of *Allocebus trichotis* has been confirmed. Localities are presented from north to south. N/A indicates that the information was not available in the citation.

Location	Latitude	Longitude	Altitude	Reference
Bemanevika Protected Harmonious Landscape	S 14° 21' 35.5"	E 48° 35' 46.6"	1600m	Goodman <i>et al.</i> , 2018
Marojejy National Park	S 14° 25.6'	E 49° 36.5'	1175m	Goodman and Raselimanana, 2002
Anjanaharibe-Sud Special Reserve	S 14° 44.7'	E 49° 27.7'	1260m	Schütz and Goodman, 1998
Makira Natural Park	N/A	N/A	N/A	Goodman <i>et al.</i> , 2018
Masoala National Park	N/A	N/A	N/A	Sterling and Rakotoarison, 1998
Marotandrano Special Reserve	S 16° 26' 20"	E 49° 38'	N/A	Pers. Comm. J. Ralison in Mittermeier <i>et al.</i> , 2010
Mananara	S 16° 28'	E 49° 38' 30"	N/A	Meier and Albignac, 1991
Mananara	S 16° 26' 20"	E 49° 38'	N/A	Meier and Albignac, 1991
Andranomahitsy	S 16° 12'	E 49° 37'	N/A	Pers. Comm. A. Peyri��ras in Meier and Albignac, 1991
Ambavala	S 16° 12'	E 49° 37'	N/A	Meier and Albignac, 1991
Antsahanadraitry Forest	S 16° 39' 31.91"	E 49° 40' 56.38"	N/A	Miller <i>et al.</i> , 2015
Ambodiriana Forest	S 16° 40' 19.51"	E 49° 42' 0.63"	N/A	Miller <i>et al.</i> , 2015
Zahamena National Park	N/A	N/A	N/A	Rakotoarison, 1998
Ankeniheny-Zahamena Natural Resource Reserve	N/A	N/A	N/A	Goodman <i>et al.</i> , 2018
Mantadia National Park	N/A	N/A	N/A	Goodman <i>et al.</i> , 2018
Ampasipotry-Anivonimaro/Ambalafary Forest	S 19° 02' 38"	E 48° 20' 55"	995m	Lagade�� and Goodman, 2010
Ambatovy-Analamay	N/A	N/A	N/A	Ralison, 2010
Torotorofotsy Protected Area	N/A	N/A	N/A	Goodman <i>et al.</i> , 2018
Analamazaotra Special Reserve	N/A	N/A	N/A	Garbutt 2001
Maromizaha Natural Resource Reserve	N/A	N/A	N/A	Pers. Comm. J. Zaonarivelo in Mittermeier <i>et al.</i> , 2010
Vohimana Forest	N/A	N/A	N/A	Pers. Comm. N. Garbutt in Mittermeier <i>et al.</i> , 2010
Torotorofotsy Forest	N/A	N/A	N/A	Rakotondratsimba <i>et al.</i> , 2013
Vohidrazana Forest	N/A	N/A	N/A	Rakotoarison <i>et al.</i> , 1997

later analysis. Finally, we placed a transponder containing an alphanumeric code specific to the individual, subcutaneously between the scapula's for future identification of the lemur.

#### Measurements

We followed morphometric measurement guidelines described by Louis *et al.* (2006), recording all measurements in millimeters. We recorded the head crown (total length from tip of the nose [soft tissue of the nose not included] to the occipital crown), the crown body length (total length of body from the occipital crown of the head to the base of tail), the tail length (total length from base of tail to the end of the last caudal vertebra), the muzzle length (total length from the tip of nose [soft tissue of the nose is not included] to the medial corner of the eye), the ear length (total length from tip of the ear to the base), and the ear width (total width across widest portion of the ear pinna). We collected two 2mm in diameter biopsy punches from the ear pinna, which were stored in tubes containing a mixture of 0.5 ml saturated NaCl buffer solution, 20% Dimethyl Sulfoxide (DMSO), and 250 EDTA 16mM pH7.5 (Longmire *et al.*, 1992). We collected a blood sample from the femoral vein (1cc of whole blood per kilogram of weight of the animal), immediately storing the sample stored at ambient temperature in a solution of 0.5ml sodium salt buffer solution 0.1M EDTA, 0.1M TRIS base, 2% SDS (Longmire *et al.*, 1992). After taking a blood sample, we administered approximately 2cc of Ringer's Lactate (Abbott Laboratories, Chicago, Illinois, 60064, USA) subcutaneously to rehydrate the animal.

#### Results and discussion

During our survey of the Bemanevika Forest, we identified several genera of nocturnal lemurs, including *Microcebus*,

*Lepilemur*, *Avahi*, *Daubentonia* (verified by the presence of traces), and *Allocebus*. This capture of *A. trichotis* confirms previous observations by J. Mittermeier and R. Lilyarison in the forest west of Lac Matsaboribe on September 13, 2016 (S 14° 21.052' E 048° 35.865' Alt: ca. 1600m) and R. Mittermeier, J. Mittermeier and R. Lilyarison on September 26, 2016 at the edge of the Marataolana Marsh in Bemanevika (S 14° 19.822' E 048° 34.949' Alt: 1600m and S 14° 19.897' E 048° 35.046' Alt: 1600m), verifying its presence in north central Madagascar. We observed three individuals, capturing one (S 14° 21' 35.5" E 048° 35' 46.6" Alt: 1615m; Fig. 2). Measurements of this individual were comparable to ones taken by this survey team on *A. trichotis* in the eastern forest of Ambatovy, Madagascar in August 2008 at 18° 50' 55"S; 48° 17' 55"E coordinate point.

Until recently, *A. trichotis* had only been recorded in moist evergreen forests of the east, as far south as For  t de Vohidrazana (Rakotoarison *et al.*, 1997), and north to Anjana-



Fig. 2: Photos of *Allocebus trichotis* in Bemanevika Protected Area. Photos: John C. Mittermeier, left, and Nicolas Bezan-dry, right.



haribe-Sud Special Reserve (Schütz and Goodman, 1998; Schmid and Smolker, 1998), and east to Masoala National Park (Sterling and Rakotoarison, 1998). Previous elevation ranged from approximately 900–1300m (Tab. 1). Documenting this species in the Bemanavika Protected Area extends its distribution further north, as well as increasing its altitudinal range to over 1600 meters.

It is likely that the distribution of *A. trichotis* extends through the Marojejy-Anjanaharibe Sud-Tsaratanana corridor (COMATSA) to Bemanavika, which would expand its current range further north. Future research should focus on identification of other forests within this species range, such as Mahimborondro, and examination of behavioral idiosyncrasies of *A. trichotis* across its range.

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## Communicative Variation and Multimodality in Ring-Tailed Lemurs (*Lemur catta*)

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## Abstract

The study of multimodal communication in primatology has increased only recently. At present, we are not aware of any on-going investigations of multimodal communication in ring-tailed lemurs (*Lemur catta*), despite the body of research on this species. This study investigated how different sensory modes of *L. catta* inter-individual multimodal communication are socially coordinated and integrated by examining frequencies of occurrence within four potential biological and social factors: age, troop affiliation, sex, and dominance rank. Research was conducted over four months (May to August 2019) at the Duke Lemur Center, Durham, NC, on 14 individuals from three separate troops of semi-free-ranging *L. catta*. Results demonstrate communicative variation in unimodal signals, but not multimodal signals, which correlate to sex and rank in this species. Dominant females appear to utilise visual signal components more frequently than males, while males rely more on auditory means of communicating, consistent with troop spatial organization. This research provides a comparative baseline for future investigations into primate multimodal communication.



## Introduction

Primates, as social animals, often utilize a number of different subtle and explicit signals to communicate with conspecifics (Partan and Marler, 1999). All communicative signals engage at least one sensory channel in the receiver of that message: auditory, visual, tactile, olfactory, and in lemurs – like many other mammals – taste and the vomeronasal organ (VNO), although these latter two modalities were not assessed in this study (see Colquhoun, 2011; Smith *et al.*, 2015 for more on VNO). Still, it is erroneous to assume every signal makes use of only one sensory mode of communication (Liebal and Oña, 2018). Lemurs, like other primates including humans, create complex multimodal signals to communicate with one another (Fig. 1). While multimodal communication is by no means a novel concept, its incorporation into primatology has only recently begun to appear in the literature (see Singletary and Platt, 2020; Fröhlich and van Schaik, 2018). At present, there are no investigations of multimodal communication in the ring-tailed lemur (*Lemur catta*), despite the large body of research on this species in particular. In contrast to being the most common primate species in captivity (LaFleur *et al.*, 2017), the potentially rapidly dwindling wild populations of *L. catta* (see Murphy *et al.*, 2017) are highly threatened by anthropogenic changes to their native landscape, such as habitat loss, agricultural intensification, and mining enterprises (Gould and Sauther, 2016; Estrada *et al.*, 2018; LaFleur and Gould, 2020). Multimodal research provides a more accurate representation of the complexities of animal communication, including that of humans, and offers a novel approach to the study of social complexity in primates (Peckre *et al.*, 2019).

This investigation explores how multimodal communication is utilized in a semi-free-ranging, population of *L. catta* by examining multimodal signal composition and occurrence. This study takes a multimodal approach to data collection

which will expand our understanding of the evolution of communication on an ultimate level (see Fröhlich and van Schaik, 2018).

## Methods

Observational data on all social behaviours were collected over four consecutive months from May through August 2019, in Durham, North Carolina, at the Duke Lemur Center (DLC) for a total of 85 research days. Only social actions, defined here as either those occurring in proximity of or directly involving another individual, as best as could be determined during the observation period, were counted toward scoring for this project. For example, individual grooming (i.e. autogrooming) was not recorded, but grooming of another individual (i.e. allogrooming) was recorded since it represents a form of tactile communication (see Hager, 2020 for additional detail). Continuous focal-animal sampling (Altmann, 1974) was used to collect frequency of occurrence data on the three larger troops of outdoor free-ranging *L. catta* at the DLC. From these three troops (troop 1  $n=4$ , troop 2  $n=4$ , troop 3  $n=6$ ), four males and ten females were observed, totalling 14 individuals and ranging in age from three to 28 years old. Research days were divided into “morning” (9AM – 12PM) and “afternoon” (1PM – 4PM) sampling periods of three hours each, for a total of six hours of observations per day and 36 hours per individual. This allowed for alternation between focal individuals every day to collect data from both “morning” and “afternoon” contexts for each individual, and to control for behaviour and activity levels that may vary between these two time periods (see Hager, 2020 for additional detail). Tallied field data were recorded and combined with individual life history information provided by the DLC, including rank, sex, age, and familial relation relative to the other individuals within the same enclosure and to the captive population sampled ( $n=14$ ). To facilitate comparison between individuals,

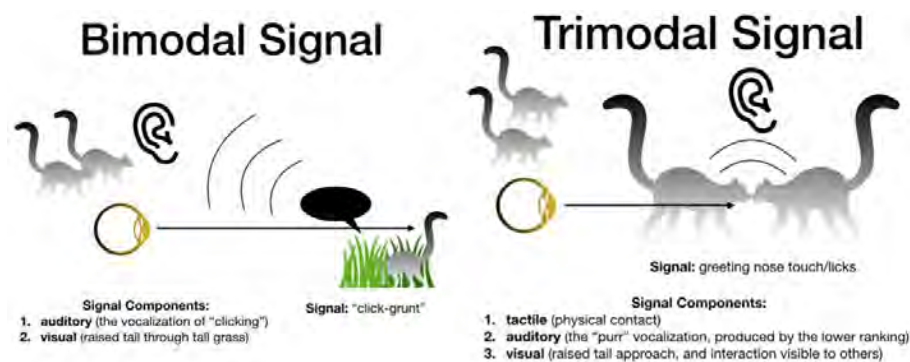


Fig 1: Example of multimodal signals in *Lemur catta*. Note that the trimodal signal example may also include olfactory, taste, and vomeronasal organ (VNO)/accessory olfactory system (AOS) involvement, but these likely constitute more “background” components in the signal relative to the three listed above (see Colquhoun, 2011; Smith *et al.*, 2015 for more on VNO).

and analysis to determine whether individual *L. catta* show a preference for different communicative mode components (auditory, visual, tactile, olfactory), including combinations thereof, and whether factors like individual age, troop affiliation, sex and dominance rank correlate with communicative mode frequencies. The results demonstrate the extent to which inter-individual variation in multimodal communication is present and how that variation is expressed across different demographic and biological factors. This research establishes a comparative baseline for future investigations into the multimodal communication of lemurs in the wild,

the proportion of each communicative mode used by an individual was calculated relative to that individual's total mode use (i.e., the occurrence of all modes). The data were then collated into different groupings to assess the potential impacts that troop affiliation, age, rank, and sex had on mode-use proportions. Further analysis was conducted in RStudio® (version 1.2.1335) to investigate the statistical significance of results ( $p<0.05$ ) using MANOVAs (multivariate analysis of variance, visualized in boxplots using the package *ggplot2*), two-way ANOVAs (analysis of variance), and one-factor ANOVAs (where each mode proportion was a “factor”) where applicable. After the initial analysis of all 14 individuals, multimodal data analysis was focused on a subset of six individuals to optimize comparisons: the three dominant females from each troop as well as the lowest ranking males. These two groups specifically exhibited the most interesting comparisons to pursue further analysis. As the first study of multimodal communication in *L. catta*, including the description of signal components in both unimodal and multimodal signals, this analysis represents a novel approach to this type of investigation.

## Results

*Potential Factors: troop affiliation, age, dominance rank, sex*

Initial analysis examining troop affiliation and age returned no statistically significant differences in the proportions of modes used between the three troops ( $n=14$ ). However, the range of mode proportions within each troop did vary (e.g.: auditory modes ranged in troop 1 from 23 to 46%, in troop 2 from 38 to 42%, and in troop 3 from 25 to 47%).

There were significant differences among dominance ranks for auditory (3 and 10 degrees of freedom,  $\text{Pr}( > F ) = 0.04892$ ,  $n=14$ ) and visual components (3 and 10 degrees of freedom,  $\text{Pr}( > F ) = 0.01983$ ,  $n=14$ ) only. Auditory signals were lowest and visual signals highest in the highest-ranking individuals, while lower ranking individuals did not follow a clear trend for these signals. Further analysis of dominance rank within each individual troop demonstrated statistically significant results for troop 1 (6 degrees of freedom,  $\text{Pr}( > F ) = 0.0085$ ,  $n=4$ ) and troop 3 (9 degrees of freedom,  $\text{Pr}( > F ) = 0.0026$ ,  $n=6$ ), but not for troop 2.

There were statistically significant differences between females and males for all four modalities ( $\text{Pr}( > F ) = 0.03411$ ,  $n=14$ ; Fig. 2). The differences were strongest for olfactory ( $\text{Pr}( > F ) = 0.04015$ ) and visual ( $\text{Pr}( > F ) = 0.01155$ ) modes, and non-significant for auditory and tactile modes. When sex and rank were examined together to separate the dominant female from those lower in rank, the MANOVA returned a statistically significant result ( $\text{Pr}( > F ) = 0.048$ ). *Post hoc* one-factor ANOVA analysis revealed statistically significant differences for auditory ( $\text{Pr}( > F ) = 0.047$ ) and visual ( $\text{Pr}( > F ) = 0.032$ ) mode component proportions, with marginal significance for olfactory ( $\text{Pr}( > F ) = 0.058$ ).

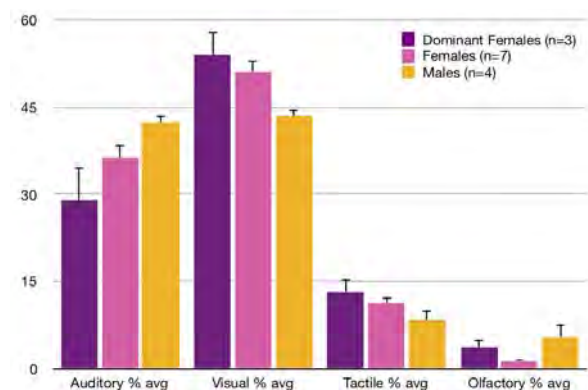


Fig. 2: Average mode component use by sex and rank ( $n=14$ ). Each bar represents the mean of the proportional use of a communicative sensory mode. Error bars represent the standard error for these grouped data, calculated by dividing the standard deviation by the square root of ( $n$ ).

### Multimodal Analysis: signal type and composition

In five of the six focal individuals for this analysis, the frequency of unimodal versus multimodal signalling approximated 50:50, although males demonstrated greater intrasexual variation (Fig. 3). The male from troop 3 used a relatively higher proportion of unimodal signals (approximately 61% of his total recorded signals) than all other individuals examined (ranging from 48 to 51%; Fig. 3). Further analysis demonstrated no statistically significant differences in proportions of unimodal and multimodal signal use between the dominant females and males ( $\text{Pr}( > F ) = 0.6989$ ). For all individuals ( $n=6$ ), bimodal signals were the most common multimodal signal type

observed. Trimodal and tetramodal signals were relatively infrequent.

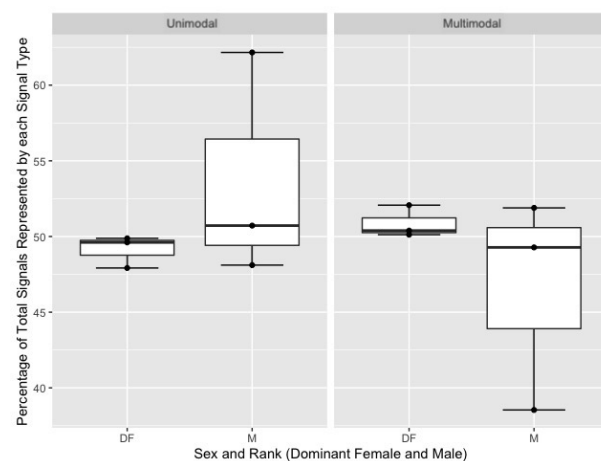


Fig. 3: Signal type (unimodal or multimodal) use by sex and rank. Where “DF” is dominant females ( $n=3$ ) and “M” is males ( $n=3$ ). The line between either hinge represents the median, the upper hinge the upper quartile and the lower hinge the lower quartile. Whiskers depict the highest and lowest value. Data points have been displayed to show the distribution of values within each box.

Variation in the composition of unimodal signals per individual was non-significant ( $\text{Pr}( > F ) = 0.5812$ ), but did show a fairly consistent high occurrence of visual components, with tactile and olfactory modes representing relatively small proportions of the total signals for most individuals. The male from troop 3 showed a higher relative frequency of olfactory components over tactile, and the male from troop 2 showed the highest frequency of auditory components in this sample. The *post hoc* one-factor ANOVA revealed that the proportions of visual components in unimodal signals were significantly different between the dominant females and males ( $\text{Pr}( > F ) = 0.02856$ ), while auditory, tactile, and olfactory components showed no statistically significant differences.

The composition of multimodal signals revealed fairly consistent values across all four modes ( $\text{Pr}( > F ) = 0.8475$ ) with only some, non-significant variation between individuals. Visual components represented the majority component in multimodal signals with no significant differences between males and females ( $\text{Pr}( > F ) = 0.202$ ). Auditory and tactile components were the next most common signal components for both males and females, with the male from troop 3 as the only exception. While there was slight variation between males and females in both tactile and olfactory component proportions, differences again were not statistically significant ( $\text{Pr}( > F ) = 0.4488$  and  $0.4662$  respectively; see Hager, 2020 for additional detail).

## Discussion

From this analysis, *L. catta* appear to use multimodal signals for approximately half of their total means of communication and the majority of those are bimodal: consisting of two sensory modes. Generally, there is some support for the frequency of occurrence of the sensory mode an individual uses to communicate varying according to their rank and sex. The composition of multimodal signals is relatively consistent between individuals in contrast to that for unimodal signals. For unimodal signals, dominant females displayed visual-based signals more frequently than

males, while males used more auditory-based signals. This finding is consistent with the typical spatial organization of this species, where female individuals are more likely to be close to troop-mates than males who often occupy the peripheries of a troop (Oda, 1996; Nakamichi and Koyama, 1997; Jolly, 2012; Gabriel *et al.* 2014; Bolt and Tennenhouse, 2017). Tactile signals did not appear to correlate with any of the factors examined, producing proportions that were roughly even across all 14 individuals examined. Olfactory components, on the other hand, did appear to vary significantly when compared between males and females, and marginally between dominant females, subordinate females, and males.

The findings from this research, despite the relatively small number of individuals studied, may suggest one of two things: 1) *L. catta* unimodal signals are more open to individual variation, whereas their multimodal signals might be more constrained to following a specific “repertoire”; or 2) these results may be indicative of the challenge of conducting research on multimodal signals using the current methods available. In this study, observations were limited to human perception, which misses the more complicated multimodal signals involving relatively subtle components like chemical signals. Nevertheless, this work represents a stepping-stone to continuing studies of multimodal communication by focusing this analysis on a single species and, it seems, is the first to compare unimodal to multimodal signals in this fashion. Future research should be conducted on larger populations in the wild to capture more natural stimulants, the possibly of year-round variation, as well as an overall larger sample size to strengthen confidence in the present results. From an evolutionary standpoint, the flexibility of an organism in the ways in which it communicates, and its ability to utilize multiple modalities to do so, may be indicative of greater social complexity, behavioural plasticity, and an ability to adaptively respond to current and growing anthropogenic pressures (Singletary and Tecot, 2020; Peckre *et al.*, 2019; Papworth *et al.* 2013). Researching this species to better understand their communication and behavioural ecology can contribute to current knowledge of the evolution of primate behaviour broadly, in addition to improving conservation action to prevent the extinction of this endangered species in the wild (see LaFleur and Gould, 2020).

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## Étude des préférences d'habitat et des comportements de lémuriens nocturnes de l'Aire Protégée Mangabe – Ranomena – Sahasarotra

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## Abstract

Five nocturnal lemur species, including *Avahi laniger*, *Daubentonia madagascariensis*, *Cheirogaleus major*, *Lepilemur mustelinus*, and *Microcebus lehilahytsara*, which are all clas-



sified as threatened, are present within Mangabe Reserve. This study aims to understand their habitat use (ecology) and their behaviors. The habitat used (substrate size, forest cover, microhabitats, tree size according to the CBH and height of the animal above the ground) by lemurs was studied within a 100m<sup>2</sup> plot where the animals were found along the transect (1000m). The animals' behavior were recorded during 30 minutes to an hour of animal survey. 58.6 % of *A. laniger*, *M. lehilahytsara*, *C. major* and *L. mustelinus* were frequently observed and living inside the forest. Their habitats vary according to the species: *M. lehilahytsara* frequents small supports, using a substrate between 0.5 to 5 meters above the ground (level 1); *L. mustelinus* prefer large and medium supports, occurring on level 2 substrate; *C. major* can be seen on large supports, approximating level 4 substrate and *A. laniger* use large supports on level 2 substrates. These species of nocturnal lemurs generally use all parts of the vegetative systems of the tree; the animal is found either inside or at the periphery of the tuft such that: *A. laniger* and *L. mustelinus* select the trunks at rest and during movement; *C. major* and *M. lehilahytsara* frequently localized on branches during food intake. Four types of behavior and reactions towards humans were marked during the study; their activities are: resting, moving, grooming and feeding. The duration of the common "rest" activity varies from 20 - 29 minutes for these four species. These lemurs have been placed in higher threat categories due to increased human pressure (Tavy) in the Protected Area. These assemblages are closely related to undisturbed forests, even those adapted to open areas, and the restoration of their forest habitats will be recommended for their survival.

**Keywords:** Nocturnal lemurs, behaviour, habitat, preference, Mangabe Protected Area

## Résumé

La réserve de Mangabe abrite cinq espèces de lémuriens nocturnes dont *Avahi laniger*, *Daubentonia madagascariensis*, *Cheirogaleus major*, *Lepilemur mustelinus*, and *Microcebus lehilahytsara* qui sont toutes classées menacées. Cette étude vise à comprendre leurs utilisations de l'habitat (écologie) et leurs comportements. Les habitats utilisées (taille substrat, couvert forestier, microhabitats, taille de l'arbre en fonction de la CBH et hauteur de l'animal au-dessus du sol) par ces lémuriens ont été évalués dans une parcelle de 100m<sup>2</sup> où les animaux ont été trouvées le long du transect (1000m). L'étude de comportements s'effectue pendant 30 minutes à une heure et leurs activités ont été enregistrées. 58,6% des *A. laniger*, *M. lehilahytsara*, *C. major* et *L. mustelinus* ont été fréquemment observées et vivants à l'intérieur de la forêt. Leurs habitats varient selon les espèces: *M. lehilahytsara* fréquentent les petits supports, utilisant un substrat entre 0,5 à 5 mètre par rapport au sol (niveau 1); *L. mustelinus* préfèrent les larges et moyens supports, se trouvant sur le substrat de niveau 2; *C. major* s'observent sur des larges supports, rapprochant les substrats de niveau 4 et *A. laniger* utilisent les larges supports sur des substrats de niveau 2. Ces espèces des lémuriens nocturnes utilisent en général toutes les parties des appareils végétatifs de l'arbre; l'animal se trouve soit à l'intérieure soit à la périphérie de la touffe tels que: *A. laniger* et *L. mustelinus* sélectionnent les troncs au repos et au cours de ses déplacements; *C. major* et *M. lehilahytsara* localisées fréquemment sur les branches au cours de la prise de sa nourriture. Quatre types des comportements et réactions envers l'homme ont été marquées pendant l'étude; leurs

activités sont: repos, En mouvement, toilettage et Alimentation. La durée de l'activité commune «repos» varie de 20-29 minutes pour ces quatre genres. Ces lémuriens ont été placés dans des catégories de menaces plus élevées en raison de l'intensification des pressions humaines (Tavy) dans l'Aire Protégée. Ces faunes sont étroitement liés à des forêts non perturbées, même celles adaptées aux zones ouvertes, et la restauration de leur habitats forestiers sera recommandée pour leur survie.

**Mots-clés:** lémuriens nocturnes, comportement, habitat, préférence, Aire Protégée Mangabe

## Introduction

Selon IUCN, près d'un tiers (31%) de toutes les espèces de lémuriens de Madagascar sont aujourd'hui En danger critique, à seulement un pas de l'extinction, et 98% d'entre elles sont menacées. La présente mise à jour montre que 33 espèces de lémuriens sont En danger critique d'extinction, 103 parmi les 107 espèces encore en vie étant menacées d'extinction, principalement en raison de la déforestation et de la chasse à Madagascar (IUCN, 2020). D'après cette mise à jour, toutes les cinq espèces de lémuriens nocturnes vivants dans la Reserve de Mangabe sont toutes classées menacées. Dans l'objectif de conserver sa biodiversité ainsi son écosystème spécifique que Madagasikara Voakajy (MV) a initié la création de l'Aire Protégée (AP) Mangabe avec l'accord du gouvernement de Madagascar par le décret n°2015/725 du 21 Avril 2015. Ce site a indiqué la présence des neuf espèces (Keane et al., 2012; Andriantsimanarilafy et al., in press) dont: deux diurnes (*Propithecus diadema* et *Indri indri*), deux cathémérales (*Eulemur fulvus* et *Haplemur griseus*) et cinq nocturnes (*Avahi laniger*, *Daubentonia madagascariensis*, *Cheirogaleus major*, *Microcebus lehilahytsara* et *Lepilemur mustelinus*) qui font parties des cibles de conservation du dite AP. Vue la dominance des espèces nocturnes qui sont souvent ignorés; l'objectif de l'étude a de bien comprendre les espèces avec leurs exigences pour orienter leur conservation.

## Méthodes

### Site d'étude

L'AP Mangabe (Fig. 1) est localisée entre la latitude Sud 19°00 et 19°28 ainsi que la longitude Est 48°05 et 48°25 dans District de Moramanga, Région Alaotra-Mangoro et rattachée aux deux communes rurales (Ambohibary et Mangarivotra). Elle s'étend sur une superficie d'environ 27 346 ha où abrite deux espèces de lémuriens En Danger critique d'Extinction: *Indri indri* (Gmelin, 1788) et *Propithecus diadema* (Bennett, 1832). L'étude s'est fait en deux périodes : du 19 Janvier au 22 Février 2018 dans la partie nord à Mangabe (site 1), à Andranomavo (site 2) et du 29 janvier au 10 Mars 2019 dans la partie sud à Lakambato (site 3), à Ambodirotra (site 4) et à Avolo (site 5).

### Etude d'habitat

L'étude d'habitat s'est fait le long de 30 transects (1000m) de suivi dans des quadrats de 100m<sup>2</sup>. Le choix de l'emplacement des quadrats ainsi que l'espèce étudiée dépendait du nombre d'individu et de la composition des espèces observées sur le transect. Les différents paramètres collectés sont listés dans le Tab. I.

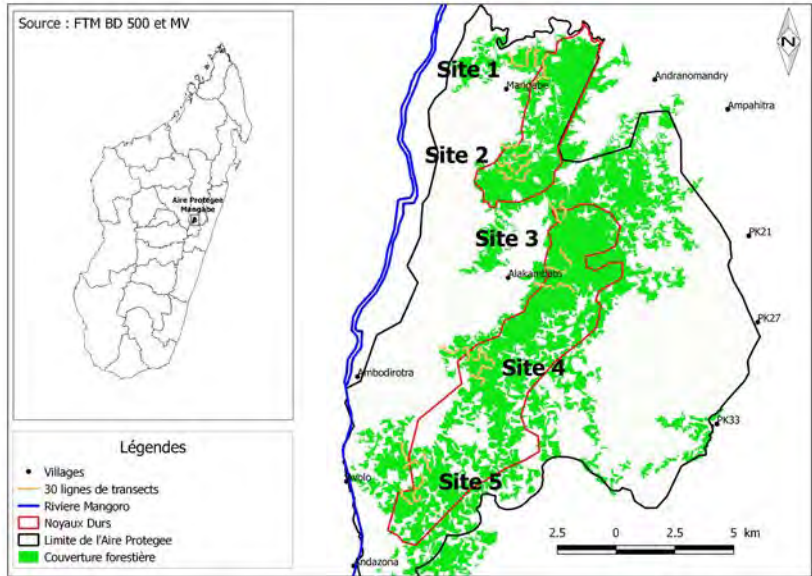


Fig. 1: Carte du site d'étude (Aire Protégée Mangabe – Ranomena – Sahasaroetra).

Tab. 1: Paramètres habitats collectées pour les lémuriens nocturnes observés.

Caractéristiques	Paramètres collectées
Espèces	Nombres, âge, taille de l'arbre, type et taille du substrat, hauteur de l'animal, position de l'animal sur l'arbre, comportement, réaction de fuite envers l'homme
Microhabitats	Espèces de plantes, Circonférence Bois Hauteur poitrine (CBH), hauteur des arbres et couverture canopée dans une aire de 100m <sup>2</sup>
Taille de l'arbre suivant le CBH	Petit < 30 cm
	Moyen ] 31 – 60] cm
	Large >60 cm
Type des substrats	Ce sont les parties des appareils végétatifs utilisées par les lémuriens nocturnes pendant la période d'observation à chaque suivi. Celles – ci peuvent être: des branches, des troncs et des tiges d'arbres.
Position de l'animal par rapport à la touffe d'arbre	L'animal observé se trouve dans la touffe d'arbre et se classe de la manière suivante: - sur des branches intérieures et branches périphériques; - sur des troncs secondaires et troncs principales; - à l'intérieure et aux périphériques
Hauteur de l'Animal par rapport au sol	Niveau 1 ] 0 – 5] m
	Niveau 2 ] 6 – 10] m
	Niveau 3 ] 11 – 15] m
	Niveau 4 >15m

Tab. 2: Descriptif des comportements observés.

Activités	Description / Définition
Alimentation	Pour obtenir de la nourriture (liquides, solides et insectes consommés) en saisissant à la patte ou en mangeant directement
Repos	Rester inactif (repos, couché horizontalement avec les quatre membres, position assise droite, position bouclée); ne participe à aucune autre activité qui peut être identifié
En mouvement	Locomotion de toute description (se déplacer; se déplacer lentement à quatre pattes; se déplacer verticalement, en haut des arbres, des branches; sauter entre les branches)
Toilettage	Frottez la fourrure de façon répétée à l'aide du peigne dentaire et/ou de la langue (soit mutuel, soit individuel sur n'importe quelle partie de leur corps)

L'analyse des données est effectué avec le logiciel «SPSS Statistics 17.0» en utilisant le test Chi – deux ( $\chi^2$ ).

**Suivi éthologique des lémuriens nocturnes**  
 Une méthode d'échantillonnage par observation directe des comportements d'un lémurien nocturne a été réalisée pendant 30 minutes à une heure (Altmann, 1974). Leur activité est notée au première période de l'observation de l'animal. Une sorte d'éthogramme (Dockery et Reiss, 1996) a été établi pour la collecte des données (Tab. 2).

**Résultats**

Au total, nous avons étudié 624 individus dont 385 *M. lehilahytsara*, 126 *A. laniger*, 90 *C. major* et 23 *L. mustelinus*. Cette étude révèle que *M. lehilahytsara* est plus actif tôt dans la nuit de 19h48 à 21h45 et *A. laniger*, *L. mustelinus*, *C. major* sont plus actifs tard dans la nuit à partir de 20h00.

**Habitats utilisés par les différentes espèces de lémuriens nocturnes**

4,7-58,6% des quatre lémuriens nocturnes étudiés fréquentent et vivent à l'intérieure de la forêt contre 1,4-63,4% se localisent dans la bordure de la forêt. Cette préférence se repartisse desquels: *L. mustelinus* (1,4% Bordure forêt (BF); 4,7% Intérieure forêt (IF)); *C. major* (14,1% BF; 13,0% IF) et *A. laniger* (21,1% BF; 23,7% IF) enfin *M. lehilahytsara* (63,4% BF, 58,6% IF). La plupart des *L. mustelinus* et *A. laniger* sont enregistrés à l'intérieure de la forêt. Par contre, *C. major* et *M. lehilahytsara* utilisent et s'observent à la bordure de la forêt. Une différence hautement significative est détectée sur le choix de la bordure et l'intérieure de la forêt ( $\chi^2=629,944$ ;  $df=12$ ;  $p<0,001$ ). Ces lémuriens n'exploitent pas la même façon les bordures et les intérieures de la forêt restante.

La taille de support (substrat) varie suivant l'espèce dont : 79,0% *M. lehilahytsara* fréquentent les petits supports (31,1% Large (L); 52,8% Moyenne (M); 79,0% Petite (P)); 36,7% *A. laniger* utilisent les larges substrats (36,7% L; 29,7% M; 9,6% P); 10% *L. mustelinus* préfèrent des larges supports (10,0% L; 4,1% M; 1,1% P); finalement 22,2% *C. major* s'observent sur des larges supports (22,2% L; 13,3% M; 10,3% P). Une différence hautement significative est observée sur la fréquentation selon la taille de supports ( $\chi^2=101,646$ ;  $df=12$ ;  $p<0,001$ ). Ces lémuriens ne choisissent pas la même façon les tailles de supports disponibles.

Selon la disponibilité de types des substrats, leurs fréquentations s'expliquent: 6,8% *L. mustelinus* rampent sur des troncs d'arbres (1,0% Branches (B); 6,8% Troncs d'arbre (TA); 0,0%Tiges (T)); 22,4% *C. major* s'observent sur des branches d'arbres (22,4% B; 6,4% TA; 6,7% T); 90% *M. lehilahytsara* fréquentent les tiges (64% B; 56,6% TA; 90% T) enfin 30,2% *A. laniger* utilisent des troncs d'arbres (12,6% B; 30,2% TA; 3,3% T). Une différence hautement significative est enregistrée sur la préférence aux types des substrats ( $\chi^2=170,800$ ;  $df=20$ ;  $p<0,001$ ). Ces lémuriens ne profitent pas la même façon les types de substrats disponibles.

**Répartition verticale des différentes espèces de lémuriens nocturnes**

Pendant l'étude, la hauteur d'espèces de lémuriens sur les arbres par rapport au sol est enregistrée. Les besoins de chaque espèce se distinguent les unes des autres: 85,8% *M. lehilahytsara* fréquentent le niveau 1 (85,8% Niveau 1 (N1);

45,1% Niveau 2 (N2); 23,0% Niveau 3 (N3); 15,4% Niveau 4 (N4)); 6,4% *L. mustelinus* s'observent sur le niveau 2 (2,6% N1; 6,4% N2; 0,0% N3; 0,0% N4) et 33,9% *A. laniger* se trouvent sur le niveau 2 (8,6% N1; 33,9% N2; 25,7% N3; 15,4% N4) tandis que 69,2% *C. major* utilisent le niveau 4 (3,0% N1; 14,6% N2; 51,4% N3; 69,4% N4). Une différence hautement significative est observée sur l'utilisation de la hauteur des substrats au cours de leurs activités ( $\chi^2=550,871$ ;  $df=16$ ;  $p<0,001$ ). Ces quatre espèces n'exploitent pas la même façon les hauteurs de substrats disponibles.

#### Position des différentes espèces de lémuriens nocturnes par rapport à la touffe d'arbre

La localisation des lémuriens par rapport aux touffes d'arbres est très variée: 29,8% *A. laniger* sont détectées sur des troncs (16,7% Branches (B); 15,8 Intérieurs (I); 12,0% Périphériques (P); 29,8% Troncs (T)); 31,0% *C. major* sont observées sur des branches (31,0% B; 13,8% I; 25,9% P; 6,7% T); 68,8% *M. lehilahytsara* sont décelées à l'intérieure des touffes (50,0% B; 68,8% I; 61,1% P; 56% T) enfin 7,6% *L. mustelinus* sont repérées sur des troncs (2,4% B; 1,6% I; 0,9% P; 7,6% T). Une différence hautement significative est observée sur la position de l'animal par rapport à la touffe ( $\chi^2=270,927$ ;  $df=16$ ;  $p<0,001$ ). Ces espèces n'exploitent pas la même façon les touffes d'arbres libres.

#### Comportements des différentes espèces de lémuriens nocturnes

Cette étude permet d'enregistrer quatre types de comportements pour les lémuriens nocturnes vivants dans la réserve de Mangabe (Fig. 2). Ces comportements se différencient d'une espèce à l'autre. Trois types d'activités ont été constatés chez *A. laniger* dont le repos (R) occupait 38,5% de leurs activités nocturnes; 5,2% En mouvement (EM) et 3,9% Alimentation (A). Trois sortes de mouvements ont été rédigé pour *C. major* (13,6% R; 14,3% EM; 17,5% A). Quatre comportements ont été noté avec *M. lehilahytsara* (41,6% R; 79,2% EM, 50,0% Toiletage (T); 77,7% A). Quatre attitudes ont été marqué pour *L. mustelinus* (6,3% R; 1,3% EM; 50,0% T; 1,0% A). Une différence hautement significative est enregistrée pendant les activités de comportements ( $\chi^2=353,838$ ;  $df=16$ ;  $p<0,001$ ). Ces espèces ne présentent pas de compétitions sur l'utilisation des habitats et sur la prise des nourritures.

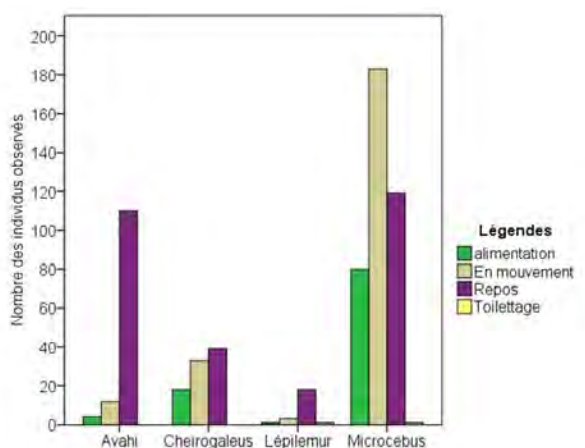


Fig. 2: Différents types d'activités par rapport aux nombres des espèces de lémuriens nocturnes observées.

#### Discussion

Cette étude signale que 58,6% de *M. lehilahytsara*, *A. laniger*, *L. mustelinus*, *C. major* fréquentent et vivent à l'intérieure de la forêt (Lehman, 2006). Ces lémuriens auront besoin des forêts pour leur survie (nourritures et habitats).

*M. lehilahytsara* est l'un des plus petits lémuriens étudié, avec une longueur tête-corps d'environ 9 cm et un poids de 45 à 48 g (Kappeler et al., 2005); cette charge permet à lui d'utiliser les substrats moyens et les branches d'arbres de petite dimension. Le choix des supports affecte autant au mode de locomotion et à la morphologie des membres des lémuriens; on observe une proportionnalité entre membres et supports utilisés par ses animaux; les petits lémuriens observés utilisent les branches d'arbres de petites tailles (Grassi, 2002).

*A. laniger* est localisé sur la plupart des troncs d'arbres mais en même temps utilise des branches d'arbres lors de la prise de sa nourriture (Ganzhorn et al., 1985). Ainsi, il se trouve en fréquence à l'intérieure des touffes et sur de troncs principales; cette sélection des microhabitats est en relation avec sa mode de locomotion qui est marquée par son déplacement d'un arbre à l'autre en sautant verticalement de tronc en tronc lors de sa locomotion en utilisant des larges substrats (Ganzhorn, 1989; Thalmann, 2003). En général, *L. mustelinus* est observé sur des troncs d'arbres à titre de substrat; est localisé sur le tronc principal par rapport à la touffe et trouve sur des larges et moyens supports lors des activités «En mouvement» ou déplacement (Rasoamanarivo, 2011).

*C. major* et *M. lehilahytsara* sont enregistrées sur les branches d'arbres à l'intérieure et au périphériques de touffes. Ces deux espèces semblent similaires sur l'utilisation de tous types de supports selon la petite taille de l'animal qui ne sélectionnent plus leurs habitats mais traversent tous les branches d'arbres supportant leurs poids dans l'endroit où ils étaient observés. Notons que ceux deux genres fréquentent dans les sites à fortes densité de petits arbres (Andrianasolo et al, 2006).

Trois lémuriens nocturnes parmi les cinq étudiés sont folivores à l'exception *Daubentonia madagascariensis* et *M. lehilahytsara*. Ce dernier était omnivore, observé solitaire en avalant des fruits et attrapant des insectes lors de cette étude (observation personnel Pierre Razafindraibe). Leur alimentation est plus diversifiée et évolue également en fonction de la saison (Radespiel, 2006). *A. laniger* et *L. mustelinus* sont des espèces folivores mangeant des feuilles d'arbres. Ce sont des aliments pauvres en énergie; leur digestion nécessite beaucoup de temps pour assurer ce mécanisme. La plupart des études antérieures montre que ces deux genres passent la plupart de temps à l'activité «Repos» (Hladik, 1978; Powzyk, 1997).

La durée de comportement était transcrite; l'activité commune «repos» a été une durée variable de: 29 min chez l'*A. laniger*; 25 min pour *M. lehilahytsara*; finalement 20 min avec *C. major* et celle de *L. mustelinus* (Rasoamanarivo, 2011). Ceux-ci montrent que ces animaux perdent une grande partie de son temps au repos (Harcourt, 1987). Cette étude prouve que *M. lehilahytsara* consacre son temps en mouvement (20 min) et en toiletage (29 min).

Chaque espèce nocturne répond différemment à la présence de l'homme. Les activités «En mouvement et en Repos» qui étaient communes, remarquables et occupaient 6–53,2% de leur réactions envers l'homme ( $\chi^2=218,256$ ;  $df=16$ ;  $p<0,001$ ). Dans les endroits à forte fréquentations humaines, quatre types d'activités (Repos, En Mouvement, Toiletage et Alimentation) ont été enregistrées. Ces animaux étaient habitués par la présence des villageois riveraines vivantes et utilisant des voies de communications à



l'intérieure de cette forêt. Ces changements de comportements risquent de diminuer leurs nombres, inciter les braconnières au chasse et à manger leurs viandes (Jenkins *et al.*, 2011; Rakotondratsimba *et al.*, 2013).

## Conclusions

Cette étude aidera déjà à comprendre la situation actuelle, les besoins et l'utilisation des habitats disponibles par ces lémuriens nocturnes vivants dans l'AP Mangabe. Des études approfondies une à une de ces espèces de lémuriens nocturnes seront primordiales pour la gestion et le maintien de leurs habitats. Vu les différentes menaces (tavy, défrichement, chasses) enregistrés, des mesures de conservation pérenne seront prises dans l'immédiat pour maintenir et conserver l'état actuels de leurs habitats afin d'éviter l'extinction de ses espèces menacées restantes.

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## Census of the red-bellied lemur (*Eulemur rubriventer*) in the Manirisoa-Samivar forest fragments east of Ranomafana National Park, Madagascar

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## Abstract

Habitat fragmentation and degradation are serious threats to biodiversity. Knowledge on rare species' demography in disturbed habitat is relevant for conservation plans. In Madagascar, habitat alteration is known to affect both lemur density and distribution. We conducted a 40-day daylight census of an endangered lemur species, the red-bellied lemur (*Eulemur rubriventer*), in a fragmented and degraded forest in the southern part of its geographic range. With this preliminary study, we report that this species occurs in small fragments and populates a mosaic area east of the Ranomafana National Park, in southeastern Madagascar. Using a total count method, we estimated a minimum population of 30 individuals, a density of 1.05 individuals/km<sup>2</sup>, and a mean group size of 3.3 individuals. Slash-and-burn agriculture, logging, and the presence of free-ranging dogs appear as the major threats to lemur survival and likely contributed to the disappearance of three species (*Eulemur rufifrons*, *Propithecus edwardsi*, *Varecia variegata*). In the future, management strategies based on field data will be crucial to the survival of the lemur population in the Ranomafana area, which is likely home to the largest population of red-bellied lemurs.

## Résumé

La fragmentation et la dégradation de l'habitat font partie des menaces les plus graves pour la biodiversité. Les connaissances sur la démographie des espèces rares dans les habitats perturbés sont de plus en plus pertinentes pour l'élaboration de plans de conservation efficaces. À Madagascar, l'altération de l'habitat affecte à la fois la densité et la répartition des lémuriens. Nous avons mené un recensement d'une espèce de lémurien en voie de disparition, le lémur à ventre roux (*Eulemur rubriventer*), dans la partie sud de son aire de répartition, dans une zone très dégradée et fragmentée. Cette étude préliminaire confirme la présence de cette espèce dans de très petits fragments forestiers localisés dans zone très anthropisée à l'est du parc national de Ranomafana. Nous avons estimé une population minimale de 30 individus, une densité de 1.05 individus/km<sup>2</sup> et une taille moyenne de groupe de 3.3 individus. La dégradation et la perte d'habitat et la présence de chiens en liberté semblent être les principales menaces pour la survie des lémuriens et ont probablement contribué à la disparition de trois espèces (*Eulemur rufifrons*, *Propithecus edwardsi*, *Varecia variegata*). À l'avenir, des stratégies de gestion basées sur des données de terrain seront cruciales pour la survie de la population de lémuriens dans la région de Ranomafana qui abrite probablement la plus grande population de lémurs à ventre roux.

## Introduction

Habitat fragmentation and degradation are among the greatest global threats to biodiversity. The evaluation of their effects on species is receiving attention from scientists (Radespiel and Bruford, 2014; Alroy, 2017), particularly in biodiversity hotspots such as Madagascar (Kling et al., 2020). While the direct effects of fragmentation on species is challenging to measure (Fahrig, 2003; Irwin, 2008), studying species' persistence and abundance in anthropogenically-changed habitat may provide useful data about which conservation measures can be put in place *in situ*. This is especially true for endangered and scarcely-known species which need urgent actions for their preservation.

In Madagascar, habitat alteration affects several aspects of lemur physiology, behavioral ecology and demography, including density and distribution (Johnson and Overdorff, 1999; Irwin, 2008; Irwin et al., 2010). Some species of true lemurs (genus *Eulemur*), for instance, suffer from physiological stress and a higher parasite load when living in a degraded habitat (Schwitzer et al., 2010; Balestri et al., 2014) and their distribution is negatively affected by habitat fragmentation (Eppley et al., 2020). The red-bellied lemur (*Eulemur rubriventer*) is a cathemeral lemur living in pairs and groups (Tecot et al., 2016) in northern and eastern Madagascar and whose range is mostly restricted to primary rainforests (Irwin et al., 2020). This species is sensitive to habitat quality degradation (Andriambololoniaina, 2009; Andriamasimanana et al., 2001), prefers closed-canopy habitats (Rafidimanana et al., 2017), and is not edge-intolerant, being also distributed at the edge of forests (Lehman et al., 2006). Despite being frugivorous, the abundance of this species in disturbed forests is greater than in undisturbed forests (Johnson et al., 2003), but the probability of presence increases inside protected areas (Eppley et al., 2020). Physiological response to habitat disturbance appears strongly attenuated (Tecot, 2013), but reproductive success is clearly affected by it (Tecot and Overdorff, 2005). There is no updated data about the global population of this species, which is listed on Appendix I of CITES and is considered "Vulnerable" by the IUCN Red List (Irwin et al., 2020). *Eulemur rubriventer* is thinly distributed

and considerably more rare than other sympatric *Eulemur* species (Irwin et al., 2020). The southern distribution of this species was assessed 20 years ago (Irwin et al., 2005) and needs to be updated. *Eulemur rubriventer* was mostly studied in pristine habitats such as Ranomafana National Park and its demography is poorly known in degraded and fragmented habitat. A deeper knowledge of these lemurs' occurrence in disturbed habitats is crucial because they are seed dispersers (Razafindratsima et al., 2014), and therefore, they potentially play a major role in reforestation (Manjaribe et al., 2013; Chapman and Dunham, 2018).

The main goal of this study is to provide preliminary data about the presence, abundance, density, and group size of red-bellied lemurs in a degraded and fragmented area in the southern part of this species' geographic range, in the nearby of the Ranomafana National Park.

Because of the landscape of the study area, composed of dispersed small forest patches in a preponderant matrix, we expect density over the whole area to be smaller than in Ranomafana. As the level of habitat disturbance does not affect *E. rubriventer* group size (Herrera et al., 2011), we expect group size in our site to be comparable to Ranomafana. We additionally formulate hypotheses about the disappearance of those species whose presence we found no evidence. We finally provide conservation and ethnobiological notes from the area.

## Methods

### Study area

The study forest (21°12'S, 47°38'E) is located in southeastern Madagascar, Region of Vatovavy, District of Ifanadiana, Commune rurale of Tsaratanana. The area is under administration of the Sahofika and the Ambodigoavy *fokontany* administrative unit. The forest is 530 km southeast of the capital city Antananarivo and the Sahofika village is nearly 40 km from the entrance to Ranomafana National Park.

Tanala people inhabit the area and manage it through two recently-created community-based organizations, the Samivar and the Manirisoa VOIs (Vondron'Olona Ifotony), established respectively in 2018 and 2020 (after our study). French Association Helpsimus and its partners, Malagasy NGO IMPACT Madagascar and Ranomafana National Park, have long established conservation and development projects in the area with campsites in Volotara and Sahofika villages. The majority of the villagers rely on agriculture and small-scale poultry farming. A smaller part of the community is also involved in trade, artisanal rum distilling, pig (*Sus domesticus*) and zebu (*Bos taurus indicus*) breeding.

In the region, climate is seasonal with both rainfall and temperatures being higher during the months from December to March, corresponding to the warm, wet season (King et al., 2011).

The area is crossed longitudinally by the Faravory river and is fragmented as a result of human activities. Forest fragments consisted of exploited and under-regeneration secondary rainforest. The matrix landscape was composed of patches of bamboo forests (*Valiha diffusa*), herbaceous and shrubby fallow lands, *Eucalyptus* and pine plantations, and agricultural lands. Cultivation included rice paddies and agricultural crops such as coffee (*Coffea* sp.), cassava (*Manihot esculenta*), and sugar cane (*Saccharum officinarum*). The area delimited by the two VOIs covers overall 2858 ha (Manirisoa: 615 ha; Samivar: 2243 ha). Most forest fragments are severely degraded and have a low canopy. Several areas are subject to active human pressures. The forest hosts populations of red-bellied lemurs, greater bamboo lemurs (*Prolemur simus*), Ranomafana bamboo lemurs (*Hapaplemur*

*griseus ranomafanensis*), Peyrieras' woolly lemurs (*Avahi peyrierasi*), mouse lemurs (*Microcebus* spp.), and dwarf lemurs (*Cheirogaleus* spp.) (Helpsimus, unpubl. data).

#### Lemur census

Prior to start our census, we organized meetings with village chiefs, elders from the main villages, and villagers living nearby the fragments to collect local knowledge concerning lemur presence and distribution. We asked permission to local authorities to enter the forests where there were Tanala cemeteries or *vatoahy* (ancestors' stones).

We surveyed 28 forest fragments in the Manirisoa and Samivar VOIs (Fig. 1) during 40 days from 8 July to 26 August 2019 for a total of ~121h of survey efforts. We only visited secondary forest patches, whose areas ranged 0.07–5.54 ha. Fragments reached maximum altitude of 659 m (Vohizahana fragment). To identify fragments, we considered the presence of natural barriers like rivers and the interposition of non-forested areas like rice plantations. We considered the Réserve Scolaire and Sahalava patches as one fragment, as the presence of a short matrix and the absence of natural barriers between the two areas suggested the movement of lemurs from one to the other to be likely.

The team was composed of a researcher (AA) and two or more local guides. In some cases, local trackers joined the team. We adopted the total count method (Ross and Reeve, 2011; Plumtre *et al.*, 2013), which has been used for primates (Cabral *et al.*, 2018). We considered this method reliable and suitable for these reasons: 1) patches to be surveyed were so small that almost the whole area could be covered during the survey, 2) forest was degraded and not very dense, so animals could be easily detected, and 3) group size of this species is relatively small. Moreover, total count enabled us to collect more reliable data on group

size, as contact time with the group was not restricted as with distance sampling methods. We made the following assumptions: 1) we were able to count all individuals of the red-bellied lemur community within the census areas, 2) we could cover the whole area, and 3) the study species does not live in the matrix and no forest patch was left unvisited. Each fragment was visited at least twice, except one fragment (Amparihimilalo) which has been visited once for logistical and time constraints. The number of repeat surveys for each fragment ranged from one to ten (Tab. 1). For each encountered group, we tried to repeat counts by revisiting the fragment. The number of repeat counts obtained per group ranged from one to five. The maximum count was taken as the group size. As red-bellied lemurs are territorial and travel over a defined home range (Overdorff, 1993), we identified a distinct group based on its location and group size/composition. Considering the average home range of the species (12–15 ha; Irwin *et al.*, 2020) and the small size of fragments (all < 6 ha), we also assumed that each fragment could not be used by more than a group, excluding the possibility of neighboring groups in the same patch. Census sessions varied between 7 a.m. and 5 p.m. Reports by local guides about lemur movements between fragments and a check on group composition/size helped us to minimise the likelihood of double counting.

At every sighting, we collected date, time, age class (infant/juvenile/adult) of individuals, group composition and size. Collecting data on sex was possible as this species has a clear sexual dimorphism. Once a group was detected, we observed it as long as necessary to ensure that all individuals were counted. Group's location was recorded using a Garmin GPSMap 64st. We took note of the sightings made fortuitously by local guides and trackers during the off-census time and in the absence of the researcher between late June and late August 2019. Red-bellied lemur density (individuals/km<sup>2</sup>) was calculated by dividing the total number of counted individuals by the area comprising the two VOIs (including the non-surveyed matrix).

To double-check group presence, we compared our survey data with presence/absence data collected from camera-traps set by Helpsimus in 2019 to monitor the overall lemur population. Helpsimus installed the Coolife 21MP cameras in the low to middle canopy in three fragments: one camera in Analafady-Vatonandroka, three cameras in Ankolona, and one camera in Manasaka (active only in August 2019). We analyzed videos with the VLC player.

During our census, we additionally collected data from the observations of other lemur species and we took note of traces (faeces and consumed plant material) left by all lemur species.

#### Conservation and ethnobiological notes

We took note of plausible factors of disturbance such as village dogs and logging activities. AA and a local dialect-speaking translator had informal conversations in a private setting with six local men about taboos (*fady*) against lemur hunting and forest logging.

## Results

#### Lemur census

We directly observed the red-bellied lemur (locally known as *kirioka*) in nine forest fragments (Table 1) and we assume observations to correspond to nine distinct groups. We found two groups in very small fragments (< 1 ha; S8 and S11 in Fig. 1). In three fragments (Avohimanombo, Mandrizavona, Analafady-Vatonandroka), local guides and trackers reported the sighting of overall three groups (occurred between

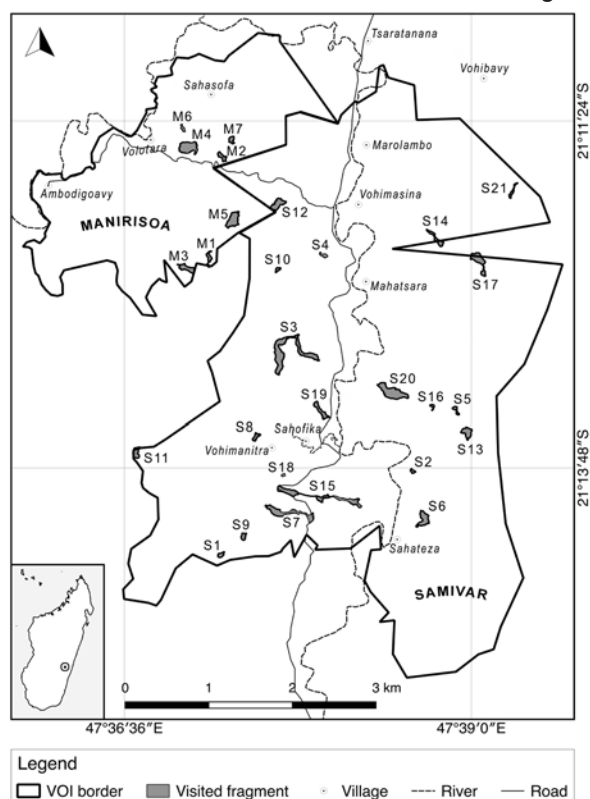


Fig. 1. Location of study fragments. The complete list of fragment names and associated codes is in Tab. 1. IMPACT Madagascar provided the VOI layers.



June and August 2019) but we did not observe them. In all those fragments, we found supposed faeces and/or fruit bites of *E. rubriventer*. In the most frequently visited fragment Analafady-Vatonandroka, where we only found fruit marks made allegedly by red-bellied lemurs, the camera trap did not detect their presence. Camera traps confirmed the presence of the observed groups in Ankolona and Manasaka in August 2019.

Individuals were detected between 07:47am and 03:27pm, and they have been observed at an altitude ranging from 457m to 627m. Total population estimated from direct observations was 30 individuals. Considering guides and trackers' reports, the number of individuals was 41. Group size varied between three and four individuals (mean:  $3.3 \pm 0.5$ ). Prior to our census, a male from a three-individual group had apparently been chased by an adult male and was travelling alone across the same fragment as the pair. We considered all of them as one group. Based on direct observations, the estimated density of red-bellied lemurs across the whole observation area is 1.05 ind/km<sup>2</sup>, with 0 ind/km<sup>2</sup> in Manirisoa and 1.34 ind/km<sup>2</sup> in Samivar.

We directly observed Peyrieras' woolly lemurs in two fragments and several groups of the greater bamboo lemur. We did not observe *Hapalemur* species although guides stated they saw *H. griseus* in two fragments and the Volotara village. However, we found foraging traces left on bamboo leaves allegedly by *Hapalemur* individuals in four fragments. Despite

the report by a villager, who claimed the presence of the red-fronted lemur (*Eulemur rufifrons*) in the Tsingovy fragment, in the whole surveyed area we found no evidence of this species' existence, and the same is true for Milne-Edwards' sifakas (*Propithecus edwardsi*) and black-and-white ruffed lemurs (*Varecia variegata*).

### Conservation and ethnobiological notes

Slash-and-burn agriculture (*tavy*) represents one of the major threats in the area (Peters, 1999) as it entails forest clearing and leads to habitat loss and fragmentation. Locals practice logging also to get firewood and for housing construction. We found evidence of recent logging activity and we heard axe blows in some fragments. In two forest fragments (Manasaka and Ambodivoasary), we observed the presence of free-ranging dogs.

We found no evidence of the presence of the fossa (*Cryptoprocta ferox*), the largest extant carnivore in Madagascar (Gerber et al., 2012). Local guides claimed they have not observed it in recent years, and camera traps set in two fragments since 2018 never detected its presence (Helpsimus, *unpubl. data*).

According to a local man, nearly 50 years ago, three men died after cutting trees in the Analafady fragment, and since then, logging has been taboo there. Another respondent explained that as it is forbidden to practice *tavy* in places where Tanala cemeteries are present, forest fragments

Tab. 1: Presence and abundance of the red-bellied lemur in the study fragments as emerging from direct observations, finding of faeces, sightings by guides and trackers, and camera trap data. All data were collected between June and August 2019. +: identification/recording; -: never recorded.

Code	VOI	Fragment	Forested area (ha)	N surveys	<i>Eulemur rubriventer</i>			
					Max observed ind., this study	Observed faeces, this study	Additional individuals seen by guides and trackers	Camera traps detections
M1	Manirisoa	Amboatavo	0.48	2	0	+		
M2	Manirisoa	Ambolanga	0.57	4	0			
M3	Manirisoa	Amparihimilalo	1.05	1	0			
M4	Manirisoa	Analafady-Vatonandroka	2.43	10	0		2	-
M5	Manirisoa	Ankaranila	1.96	4	0			
M6	Manirisoa	Marosay	0.16	3	0			
M7	Manirisoa	Sahamaintso	0.38	4	0			
S1	Samivar	Ambakamaniry	0.33	2	0			
S2	Samivar	Ambatolampitsara	0.19	2	0			
S3	Samivar	Ambodialanana	5.54	2	3	+		
S4	Samivar	Ambodivoasary	0.18	2	0			
S5	Samivar	Ambohibe	0.29	2	0			
S6	Samivar	Ambohimirary	1.52	2	4	+		
S7	Samivar	Ankolona (Ambatoafo)	2.41	8	3			+
S8	Samivar	Asitongandeona	0.39	4	3	+		
S9	Samivar	Avohimanombo	0.38	6	0	+	4	
S10	Samivar	Bevoapaka	0.20	2	0			
S11	Samivar	Madiolambo	0.77	2	3	+		
S12	Samivar	Manasaka	1.38	7	4	+		+
S13	Samivar	Mandrivazona (Sahataky)	1.11	2	0	+	5	
S14	Samivar	Marohady	0.82	2	0			
S15	Samivar	Réserve scolaire-Sahalava	3.14	6	3			
S16	Samivar	Sahavotelo	0.15	2	0			
S17	Samivar	Tsingovy	1.81	2	4			
S18	Samivar	Vohimanitra	0.07	2	0			
S19	Samivar	Vohimarirana	1.05	3	0			
S20	Samivar	Vohitrakondro	3.53	2	3			
S21	Samivar	Vohizahana	0.34	1	0			
		TOTAL	32.65	91	30			

with tombs have been better preserved than those without them. Our experience suggests that these fragments are effectively not cleared, but they appear degraded as the others. One informant stated that in the area, those youngsters that do not respect laws preferably hunt *Hapalemur*. During our conversations, there was no agreement among respondents on whether lemurs were taboo for the ancestors or not. One person told us that the Milne-Edwards' sifaka was fady for all Tanala people. Two informants revealed that some villagers consider fady for pregnant women to consume lemurs and that doing so would result in the birth of "lemur-like" disabled children. According to a local belief reported by an informant, if you are so lucky to observe a lemur's parturition and leaves used by the female fall down on the ground, it is a good practice to keep and identify the leaves: a drink of those leaves facilitates women's parturition as well.

## Discussion

Our preliminary study confirmed the presence of the red-bellied lemurs in the area. As expected, the density of red-bellied lemurs in our study sites was significantly smaller than in Ranomafana and in almost all forests cited in the literature (Tab. 2). The resulting low density in the area including the two VOIs may be due to the highly fragmented structure of the area, in which the matrix is preponderant. Moreover, we were not able to conduct nocturnal surveys and this fact may have limited our chances to detect groups as *E. rubriventer* appears to be more frequently detected during nocturnal than diurnal surveys (Holmes et al., 2015).

Group size is comparable with that of other forests (Tab. 2), such as the Fandriana-Marolambo forest corridor (Lehman and Ratsimbazafy, 2000), the Ambatovy-Analamay forest (Ralison, 2010), and, as predicted, Ranomafana National Park (Razafindratsima et al., 2013), which is overall much less disturbed than our study sites.

The black-and-white ruffed lemur, the Milne-Edwards' sifaka, and the red-fronted lemur, which are found in Ranomafana National Park (Herrera et al., 2011), are absent in the surveyed area. The absence of *V. variegata* is not surprising. This species is a highly specialized frugivore (Herrera et al., 2011), particularly sensitive to fruit availability and habitat degradation (Balko and Underwood, 2005). Among the lemur species of Ranomafana, it is considered the most susceptible to disturbance and one of the first to become locally extinct face to habitat loss (White et al., 2005). Still in the 2000s, *V. variegata* was hunted in south-east Madagascar (Lehman et al., 2006). Despite the existence of a taboo in the Ranomafana area (Jones et al., 2008), *P. edwardsi* could also be a favorite prey item by locals (Lehman et al., 2006). Because of the feeding strategy, *P. edwardsi* is particularly exposed to the risk of hunting or predation by the fossa (Overdorff et al., 2002) or free-ranging dogs. Moreover, the low net reproductive growth rate (Pochron et al., 2004) makes this species vulnerable to anthropogenic disturbances (Lehman et al., 2006). All listed factors combined with forest alteration could explain its local extinction.

The reasons why *E. rufifrons* was locally eradicated as opposed to *E. rubriventer* deserve further consideration. *Eulemur rufifrons* and *E. rubriventer* are cathemeral, frugivorous,

Tab. 2. A literary review on the density and group size of the red-bellied lemur across Madagascan forests. \*: Mean density and SD have been calculated using data from the paper.

Site	Mean density (ind/km <sup>2</sup> )	Mean density ST. DEV	Density range (ind/km <sup>2</sup> )	Mean group size (ind)	Group size range (ind)	Reference
Ambato	3.48					Rakotosamimanana et al., 2004
Ambatovy-Analamay forest	30*	44*	16 - 130	3.4 ± 1.3	2 - 5	Ralison, 2010
Analamay-Mantadia Forest Corridor	11					Ralison et al., 2015
Andasibe-Mantadia National Park						
Analamazaotra Special Reserve	7.04					Rakotosamimanana et al., 2004
Mantadia National Park	5.69					Rakotosamimanana et al., 2004
Ankerana	15					Ralison et al., 2015
Betsakafandrika Region				3.7 ± 1.1	3 - 5	Lehman and Wright, 2000
Fandriana-Marolambo forest corridor	35			3.7 ± 1.5	2 - 5	Lehman and Ratsimbazafy, 2000
Maromizaha Protected Area						
Maromizaha P.A.	3					Ralison et al., 2015
E-Maromizaha	6.28					Rakotosamimanana et al., 2004
W-Maromizaha	0.52					Rakotosamimanana et al., 2004
Ranomafana National Park						
Ranomafana N.P.			15 - 30			Overdorff, 1991
Ranomafana N.P.	30					Glander et al., 1992
Ranomafana N.P.				3	2 - 4	Overdorff, 1993
Ranomafana N.P.				3	3 - 4	Overdorff, 1996
Ranomafana N.P.	5.25	0.64				Irwin et al., 2005
Ranomafana N.P.	7.08				1 - 3	Karpanty, 2006
Vatoharanana site	13.96					Herrera et al., 2011
Talatakely site	8.17					Herrera et al., 2011
Ranomafana N.P.	5.46	0.7	15 - 25			Wright et al., 2012
Ranomafana N.P.				3.44 ± 0.55		Razafindratsima et al., 2014
Samivar + Manirisoa	1.05			3.3 ± 0.5	3 - 4	This study
Torotorofotsy (N/E site)	1.84	0.9				Rakotondratsimba et al., 2013
Tsinjoarivo	6.65	2.39				Rakotomalala et al., 2017
Vohibola III	26.7	10.2				Lehman et al., 2006
Vohimana Reserve					2 - 4	Anania, unpubl. data

congeneric lemur species, which compete with each other due to the dietary overlap (Overdorff *et al.*, 1999; Erhart and Overdorff, 2008; Wright *et al.*, 2012; Holmes, 2017). The abundance and density of *E. rufifrons* are affected by habitat disturbance more significantly than *E. rubriventer* (Herrera *et al.*, 2011). Red-fronted lemurs are also slightly larger in body size (Razafindratsima *et al.*, 2014), move in larger groups (Overdorff, 1996; Kappeler and Fichtel, 2016) and are more active (Overdorff, 1996) than red-bellied lemurs. They are less used to rest in the upper story and canopy trees than red-bellied lemurs (Overdorff, 1996). While foraging, they are far less cryptic than their congener (Overdorff *et al.*, 2002). They forage on the lower forest levels more frequently than *E. rubriventer* and, like sifakas and alike red-bellied lemurs, they feed on the ground (Overdorff *et al.*, 2002). All these traits could make red-fronted lemurs more easily detectable and put them at a higher risk of predation and hunting. Based on our few conversations, we had no evidence of a taboo forbidding the consumption of true lemurs in our study area. In the Ranomafana area, *E. rufifrons* was uncommonly considered taboo and was the target of traps (Jones *et al.*, 2008) and in the Kianjavato area, which is some 50 km from our study sites, it is one of the most commonly hunted lemurs (Rafidimanana *et al.*, 2017). Ultimately, niche competition, differential sensitivity to habitat disturbance, and unequal hunting and predatory pressures may be the driving factors for the differential survival among these congeners.

In our study sites, the major threats to lemur survival appeared to be forest clearing and degradation. The resulting fragmentation prevents lemurs from dispersing to larger forest areas and may eventually result in crowding, exacerbating the state of shortage of resources (Tecot, 2008). While taboos forbidding lemur consumption and logging in some fragments exist, spread and adherence level need further systematic investigation. Despite the disappearance of the lemurs' largest natural terrestrial predator (the fossa), lemurs can experience predatory pressure from dogs, which are known to attack lemurs (Anania *et al.*, 2018; Brockman *et al.*, 2008). Dogs' presence has both direct (harassment, predation) and indirect (disease transmission, competition) effects on lemurs (Farris *et al.*, 2019; Zohdy *et al.*, 2019), influencing their survival and distribution, as reported for the red-bellied lemur in Ranomafana (Farris *et al.*, 2019).

## Conclusion

Despite being a preliminary analysis, this work describes a population of *E. rubriventer* never studied before and provides updated information on the distribution of this vulnerable species on the southern part of its geographic range. We found a relatively low-density population living in a fragmented and degraded landscape. Different anthropogenic factors threaten the survival of this population, including forest clearing, selective logging, and the presence of free-ranging dogs. These factors, coupled with hunting, are likely responsible for the disappearance of three lemur species (*E. rufifrons*, *P. edwardsi*, *V. variegata*). Further work on the actively-visited forest fragments (i.e. composition, level of disturbance) and the behavioral ecology of *E. rubriventer* (i.e. diet, home range) may reveal essential information about this species' responses to habitat alteration and provide more explanations as to how this species has survived whilst others have disappeared from the same area. This population of red-bellied lemur lives in a very fragile and threatened habitat. The recent creation of the two VOIs constituted a first step for the protection of this area. In the future, the strategies of management implemented by these

community-based organizations should be based on periodic lemur censuses. Integrated action of species monitoring, education, and community-driven sustainable management of the fragments will be crucial to the survival of the lemur population in this area which, including Ranomafana, is likely home to the largest population of red-bellied lemurs in Madagascar.

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## Lemur inventory of the spiny and transition forests of the Anosy region in southern Madagascar

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### Introduction

The latest IUCN Red List assessment of the conservation status of lemurs in 2018 indicated continuing declines of populations for most lemur species and assignments of species into higher categories of threat. Among other issues were the status of *Lemur catta* and *Propithecus verreauxi* and of *Lepilemur leucopus* in the south of Madagascar. While *L. catta* and *P. verreauxi* have a large area of distribution, the actual presence of *Lemur catta* at many sites within this area of distribution is uncertain and thus the area of occupancy might be substantially smaller than suggested by maps showing the area of distribution (Gould and Sauther, 2016; LaFleur et al., 2016; Murphy et al., 2017).

The case of *L. leucopus* is somewhat different. The long history of studies on what was assumed to be the widespread *L. leucopus* (starting with Charles-Dominique and Hladik, 1971) leaves the impression that *L. leucopus* would be one of the better-known lemur species. Yet, these studies were completed exclusively in Berenty, a site that is now considered to be outside the range of *L. leucopus* (Eppey et al., 2020a; Louis et al., 2020). The animals studied in Berenty are actually *L. petteri* that has been described to occur between the Mandrare river to the east and the Onilahy river in the west. This leaves us with only fragmentary information on *L. leucopus* (Feistner and Schmid, 1999; Rakotoarisoa et al., 2008; Lei et al., 2017). As a consequence of the taxonomic revision, *Lepilemur leucopus* has turned out to be one of the neglected lemur species restricted to a small area of spiny and dry forest below 300m above sea level between the Mandrare river to the west and the humid rainforest of Andohahela towards the east (Fig. 1). The area falls in part into Parcel 2 of Andohahela National Park. North and south of Parcel 2, most of the dry and spiny forests have been cleared, limiting the species to an area of no more than 2300 km<sup>2</sup> and resulting in its categorization as [Endangered; B1ab(i,iii,v)] (Lei et al., 2017; Eppey et al., 2020a, based on the IUCN Red Listing Workshop Antananarivo, 2018).

Given the high anthropogenic pressure on the remaining forests and given that the species is known only from Parcel 2 of Andohahela NP and a few surrounding forests, more information on its actual distribution are needed, as well as a better understanding of the causes of anthropogenic pressure in the region. Thus, the objective of the study was to determine the occurrence of *Lepilemur leucopus*, *Lemur catta* and *Propithecus verreauxi* between the humid forest of Andohahela and the Mandrare river, updating previous inventories (Fenn et al., 1999; Ralison, 2008).

### Methods

Between October 2020 and June 2021, we inventoried 15 sites for the occurrence of lemur species (Tab. 1; Fig. 1), supplemented by accidental observations during other long-term studies in Mangatsiaka, Parcel 2 of Andohahela National Park.

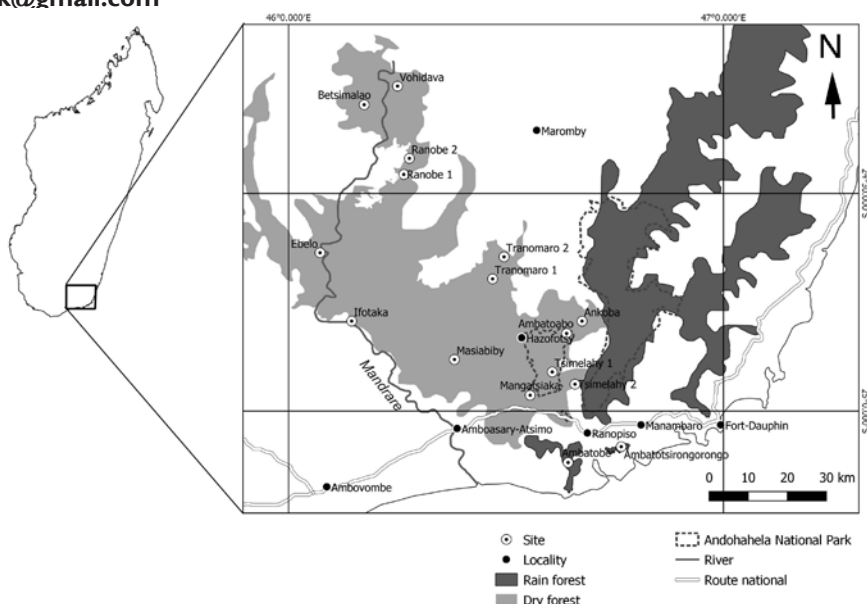


Fig. 1: Map of sites inventoried; circles mark towns; stars mark survey sites; the Mandrare river is in italics (modified from Google Earth).

Tab. 1: Sites inventoried for *Lepilemur* spp., *Propithecus verreauxi* and *Lemur catta* in 2020 (if not specified otherwise).

N°	Date	Site	Latitude	Longitude	Transect length (m)	Transect walks Day/ Night
1	19-Oct-21	Ranobe (Site 1)	S24° 27' 22.95"	E046° 15' 54.08"	1500	1/1
2	20-Oct-21	Ranobe (Site 2)	S24° 25' 10.06"	E046° 16' 40.10"	1000	1/0
3	22-Oct-21	Tranomaro (Site 1)	S24° 41' 46.70"	E046° 28' 09.29"	3000	1/0
4	25-Oct-21	Tranomaro (Site 2)	S24° 38' 44.52"	E046° 29' 43.76"	1000	1/0
5	27-Oct-21	Ebelo	S24° 38' 10.30"	E046° 04' 20.18"	1000	1/0
6	28-Oct-21	Ifotaka	S24° 47' 36.34"	E046° 08' 42.70"	1000	1/0
7	30-Oct-21	Masiabiby	S24° 52' 53.29"	E046° 22' 53.41"	1000	1/0
8	31-Oct-21	Tsimelamy (Site 1)	S24° 54' 36.59"	E046° 36' 23.40"	1000	1/0
9	16-Nov-21	Tsimelamy (Site 2)	S24° 56' 17.67"	E046° 39' 31.35"	1000	1/0
10	03-Nov-21	Ambatoabo	S24° 49' 17.79"	E046° 38' 22.09"	2000	1/1
11	03-Nov-21	Ankoba	S24° 47' 36.64"	E046° 40' 30.00"	1500	1/1
12	06-Nov-21	Ambatobe	S25° 07' 06.26"	E046° 38' 34.33"	1000	1/0
13	06-Nov-21 May-July 2021	Ambatotsirongorongo	S25° 04' 55.12"	E046° 45' 54.14"	400 Intensive monitoring <sup>1</sup>	0/1 Camera traps for 3 months
14	Feb.-June 2021	Vohidava (east of the Mandrare river) <sup>2</sup>	S24° 25'	E046° 30'	Intensive monitoring <sup>2</sup>	31/16
15	Feb.-June 2021	Betsimalao (west of the Mandrare river) <sup>2</sup>	S24° 33'	E046° 17'	Intensive monitoring <sup>2</sup>	62/30
16	Since 2009	Mangatsiaka / Andohahela Parcel 2.3	S24° 58'	E046° 33'	chance observations <sup>3</sup>	Day + Night; several months

<sup>1</sup>Rakotondrany et al. (unpublished a); <sup>2</sup>details presented in Jaonasy et al. (in press); <sup>3</sup>Rakotondrany et al. (unpublished b)

Surveys in Vohidava-Betsimalao were standardized as described in Jaonasy et al. (2021). In brief, 10 transects, of 1150m to 2000m length were established, in different parts of the site using existing trails. These trails were walked repeatedly during the day and at night with individual animals being recorded (in case of *P. verreauxi* and *Lepilemur* spp.) or groups of animals in case of *L. catta*. At the other sites, inventories consisted of transects walked along pre-existing trails during the day and at night, specific searches for animals with the help of local assistants, and interviews of the local human population. Systematic night walks could not be completed at all sites for security reasons. Where possible, night surveys were only to confirm the presence of *Lepilemur* if people had indicated its presence, but the spe-

cies had not been found during the day. Each transect was walked only once with 4 people searching for lemurs. The numbers of animals seen during the day cannot be used for density calculations that would be comparable with other density estimates. But the numbers of animals seen during the day provide an indication of relative densities (Tab. 2). All survey sites were located in dry or spiny forest, except for Tsimelamy (Site 1) that represents a gallery forest, and Ambatotsirongorongo with remnants of transitional forests (Eppley et al., 2020b).

Tab. 2: Survey results; sites correspond to the sites listed in Tab. 1. For *Lepilemur leucopus* (Ll) the number indicates the number of animals seen per 1 km transect. Color variations of *L. leucopus* are indicated in brackets: bl = black; br = brown, w = white. For *Propithecus verreauxi* (Pv) and *Lemur catta* (Lc), the numbers indicate the number of groups/km transect.

N°	Site and forest type	Disturbance	Transects			Interviews		
			Ll	Pv	Lc	Ll	Pv	Lc
1	Ranobe (Site 1) (Dry/Spiny forest)	dis-turbed	0.7 (bl)	0.7	0	+	+	+
2	Ranobe (Site 2) (Dry/Spiny forest)	dis-turbed	3 (bl)	2	1	+	+	+
3	Tranomaro (Site 1) (Dry/Spiny forest)	dis-turbed	1.3 (br, w)	1.6	0	+	+	+
4	Tranomaro (Site 2) (Dry/Spiny forest)	dis-turbed	4 (br)	0	0	+	?	?
5	Ebelo (Dry/Spiny forest)	intact	2 (br)	3	0	+	+	+
6	Ifotaka (Dry/Spiny forest)	intact	5 (br)	1	3	+	+	+
7	Masiabiby (Dry/Spiny forest)	dis-turbed	4 (bl, br)	2	0	+	+	+
8	Tsimelamy (Site 1) (Gallery forest)	intact	2 (bl, br)	0	0	+	+	+
9	Tsimelamy (Site 2) Gallery forest	dis-turbed	1 (w)	0	0	+	?	?
10	Ambatoabo (Dry/Spiny forest)	dis-turbed	0	0	0	+	+	?
11	Ankoba (Dry/Spiny forest)	dis-turbed	0	0	0	+	?	?
12	Ambatobe (Transitional forest)	dis-turbed	0	0	0	-	+	?
13	Ambatotsirongorongo (Transitional forest)	dis-turbed	0	0	1	-	+	+
14	Vohidava I (Dry/Spiny forest)	intact	abundant	abundant <sup>2</sup>	abundant <sup>2</sup>	+	+	+
15	Betsimalao I (Dry/Spiny forest)	intact	abundant	abundant <sup>2</sup>	abundant <sup>2</sup>	+	+	+
16	Mangatsiaka (Dry/Spiny forest)	intact	abundant	present	rare	+	+	+

<sup>1</sup>For details see Jaonasy et al. (in press); <sup>2</sup>high densities also indicated by Ramanorintsoa (2017); "?" indicates that people were not sure whether the species still exists at the site; "-" indicates absence



## Results and discussion

*Lepilemur leucopus* were reported at all sites north of the Route Nationale running east-west from Tolagnaro – Manambaro-Ranopiso-Amboasay. *Lepilemur* sp. had not been reported from Ambatotsirongorongo in previous surveys either and does not seem to occur there (Eppeley *et al.*, 2020b). Thus, the form now classified as *Lepilemur leucopus* occurs in all dry forests between the humid forests of Andohahela in the west and the Mandrare River in the east. Within this region, the *Lepilemur* differed markedly in coloration between sites, ranging from black markings to reddish/brown and white/grey variants, making species identification impossible without genetic analyses. The northernmost forests (Vohidava-Betsimalao) encompasses the headwater of the Mandrare River and might include individuals from *L. leucopus* (supposed to be east of the Mandrare river) and *L. petteri* (supposed to be west of the Mandrare river), if the Mandrare river would separate the two forms. A more extensive survey of Vohidava-Betsimalao showed high variation in pelage colors, though the animals shown in the photos from Vohidava-Betsimalao (Jaonasy *et al.*, 2021) resemble more the animals found in the National Park Andohahela Parcel 2 (i.e., *L. leucopus*) than they resemble *Lepilemur petteri* at Tsimanampetsotse. If so, the range of *Lepilemur leucopus* extends west, beyond the Mandrare river at least in the headwaters.

*Propithecus verreauxi* also occurred over the whole survey region north of the Route Nationale. In 2006, the species was still present and easy to see at Tsimelahy but it was not seen in the present survey, though it still is reported by local people to occur there. The species is absent south of the Route Nationale, except for a remnant population at Ambatotsirongorongo. There, only one *P. verreauxi* was reported during several days of extensive surveys. The species reaches high densities in the protected area of Vohidava-Betsimalao (Ramanorintsoa, 2017; Jaonasy *et al.*, 2021).

*Lemur catta* has actually been seen only at Ifotaka and far north at Ranobe and Vohidava-Betsimalao, though people still indicated its presence at most other sites. Given its patchy distribution in the spiny forest (e.g., Kasola *et al.*, 2020), the species might still be present and simply might not have been covered by the transects. Yet, *Lemur catta* is recorded reliably from Ambatotsirongorongo in all surveys (Eppeley *et al.*, 2020) and reaches high densities in the protected area of Vohidava-Betsimalao (Ramanorintsoa, 2017; Jaonasy *et al.*, 2021). The lack of physical encounters at the other sites indicates low population densities.

### Conservation issues

The conservation situation of the region remains precarious. In 2021 the ongoing drought resulted in excessive famine (Randrianady *et al.*, 2021). Under the present conditions, intensification of agriculture on the basis of annual crops does not seem to be a sustainable and viable option, though additional forest might be cleared when rain will fall. Some sort of agroforestry with perennial plant species might be better able to buffer agricultural production against environmental variability (Estrada *et al.*, 2012). For the time being, people have to rely on forest resources, possibly intensifying hunting pressure and charcoal production. Mining of mica, malachite and semi-precious stones represents other options to earn some money. In the north-east (Tranomaro) people mine mica, selling it for 100-300 Ariary/kg (100 Ar = 0.025 US\$). The mineral is transported by trucks and new dirt roads dissect the remaining forests, including the National Park of Andohahela. During our stay in the area, at least 10 trucks with mica passed our camp per day. In 2021,

there was substantial migration out of the region towards the towns in search for work (J.-B. Ramanamanjato, TBSE pers. comm.). It remains to be seen whether town will be able to generate new sources of income and whether or not people will move permanently. Reinforcing the present agricultural system in the spiny forest region is not a sustainable option.

On the positive side, *Lepilemur* spp. were reported at most sites and are abundant at several sites. In addition, *Propithecus verreauxi* and *Lemur catta* have been found or been reported from several sites within the region, basically occurring over the entire region. It remains to be seen whether hunting pressure on these species has diminished during the last few years or whether previous records had assumed lower occurrences of these species than is really the case. At some sites, such as at Ebelo, “sacred forests” protected by the community remain strongholds for biodiversity conservation (e.g., Bodin *et al.*, 2006; Tengo *et al.*, 2007; Ferguson *et al.*, 2013, 2014), though the safeguarding effect of these forests vary (e.g., Nopper *et al.*, 2017).

Issues to be followed up concern the identification of *Lepilemur* and some conservation problems. Our personal experience with *Lepilemur petteri* and *L. leucopus* is based on observations of *Lepilemur* in Parcel 2 of Andohahela (*L. leucopus*), Berenty and Tsimanampetsotse (*L. petteri*). Based on these experiences we would classify all animals seen during this survey as *L. leucopus*. If so, the species is wide-spread with several subpopulations and occurs in three protected areas (Andohahela Parcel 2, Ifotaka and Vohidava-Betsimalao). But genetics might tell a different story. While *lepilemurs* are hunted at Ambatoabo and Ankoba, the species is «fady» at the other sites.

The National Park of Andohahela was the only site in southern Madagascar, where the dry forests of the west were still connected to the humid forests of the east (Rakotondranary *et al.*, 2011). In 2008, there were still two continuous corridors between the dry and the humid forest. The northern corridor had been very narrow and may now already be severed, though we did not visit this site in 2020 and could not interpret the present biological state from Google Earth images. The southern forest corridor between Tsimelahy and Ebosika is about to disappear as the woody plants are converted to charcoal (Fig. 2). Both sites would be good areas for reforestation initiatives.



Fig. 2: Charcoal production between Tsimelahy and Ebosika in 2020, severing the last link between dry and wet forests in the southern half of Madagascar. Photo: Jacques Rakotondranary; November 2020)

The forests south of the Route Nationale have not been surveyed systematically and have largely been destroyed since we worked there in 2008 (Gligor *et al.*, 2009). There are a few remnants left west of Ambatsirongorongo which we could not visit. Given that the littoral forests of the southeast represent rather unique systems and that they extend into the dry region of the south, remnants south of the Route Nationale 13 might provide (or could have provided) interesting information on the biogeographic history of the region.

## Conclusion

Locally, there seems to be little that can be done to stop mining for mica as long as there are no income alternatives and the central government neither implements the existing laws nor upholds basic human rights standards (Cardiff and Andriamanalina, 2007).

Apart from these general concerns, the most encouraging results of the study were

1. The still wide distribution of all three lemur species considered;
2. The very good condition of some forests along the Mandrare river.

Gallery forests have been identified as some of the most threatened forest systems in Madagascar (e.g., Richard and Ratsirarson, 2013). In view of climate change, they have become more important than ever to serve as areas of retreat for species that can no longer survive in the dry forests due to increasing aridity, such as seems to be happening to *Lemur catta* in Tsimanampetsotsa National Park (Kasola *et al.*, 2020).

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## Natural habitat evolution of lemur species in the Mahavavy-Kinkony Wetland Complex using ecosystem land-cover accounting

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Ecosystem accounting is an essential tool to assess the spatial and temporal changes of ecosystem services and ecosystem conditions for national and regional planning decisions (UN, 2014). Ecosystem accounts integrate environmental data and economic information into a common framework. In Madagascar, a number of natural capital and ecosystem valuation and accounting assessments have been developed at the national (Conservation International, 2015; WAVES, 2016) and regional levels (Holmes *et al.*, 2008; Portela *et al.*, 2012; GTE/CECN, 2017; Rakotoniaina *et al.*, 2018) based on methodologies such as composite indices and the system of environmental-economic accounts. The importance of ecosystem accounting, which provides reliable information on which the conservation of Madagascar's unique ecosys-

tems and biodiversity depends, is increasingly recognized (Reuter *et al.*, 2019). Applying ecosystem accounting to protected areas management is currently among the most cited policy priorities in developing countries (UNCEEA, 2021). This study produced land-cover accounts that are part of the ecosystem accounts to monitor land-use evolution in the Mahavavy-Kinkony Wetland Complex Protected Area (MKWC).

The MKWC Protected Area (45°27' to 46°10'E, 15°57' to 16°15'S) is located in northwestern Madagascar, Boeny Region (Fig. 1) and covers an area of approximately 302,400ha (Asity Madagascar, 2014). The Protected Area is listed as IUCN category V (Protected Landscape/Seascape). The MKWC Protected Area comprises human settlements, cropland, tourism infrastructure, and various natural ecosystems (dry forest, mangrove, grassy and tree savannas, wetlands). The MKWC is rich in faunal biodiversity, including lemurs that are present in the forested areas (Biodev, 2014): *Eulemur mongoz* (Critically Endangered), *Propithecus coronatus* (Critically Endangered), *Propithecus deckenii* (Critically Endangered), *Cheirogaleus medius* (Vulnerable), *Eulemur rufus* (Vulnerable), *Haplemur occidentalis* (Vulnerable), *Microcebus myoxinus* (Vulnerable), *Microcebus murinus* (Least Concern).

We followed the Convention on Biological Diversity (CBD) methodology called Ecosystem Natural Capital Accounts (ENCA) to produce the land-cover accounts (Weber, 2014). Four Landsat 8 OLI images (scene 160-071) of the MKWC in 2013 and 2018 were downloaded free of charge from the United States Geological Survey earth explorer ([earthexplorer.usgs.gov/](http://earthexplorer.usgs.gov/)). Land-cover classification was performed under supervised classification

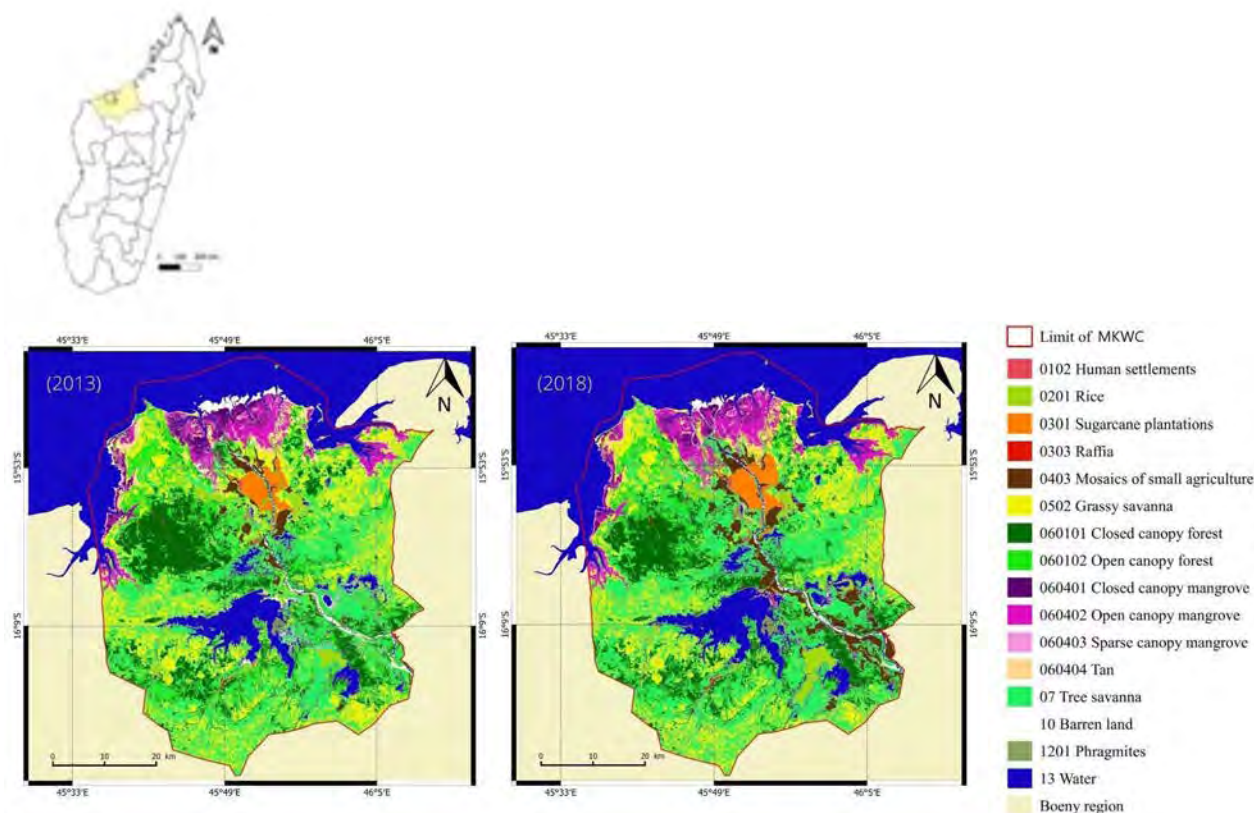


Fig. 1: Land-cover ecosystem units maps of the Mahavavy-Kinkony Wetland Complex (MKWC) for 2013 and 2018. Overall classification accuracies of 2013 and 2018 maps are 89.9 and 90.8% respectively. Sixteen ecosystems units were identified in MKWC. We distinguished two sub-classes of dry forest (closed and open canopy) and three subclasses of mangrove (closed, open and sparse canopy) from map analysis and field observations. Two types of savannas exist in MKWC: grassy and *Bismarckia nobilis* tree savannas.



using the RandomForest algorithm (Breiman, 2001). Land-cover ecosystem units (LCEU) of the MKWC were defined based on 15 aggregated ecosystem units proposed by ENCA (Weber, 2014). Validation activities of the LCEU maps included field observations and accuracy assessments, as described by Olofsson *et al.* (2013). Changes including land-cover formation/expansion and consumption/decrease between the accounting years are generally allocated to anthropogenic activities (e.g. artificial development, agricultural expansion) or in some cases to changes due to natural causes such as climatic anomalies or hazards (Weber, 2014).

Land-cover accounts for the MKWC (Tab. 1) indicated that dry forests (open and closed canopy) had the largest area in 2013 covering a quarter (26%) of the Protected Area. A previous study using supervised classification of 2005 Landsat images found 37% dry forest cover in the MKWC (Andriamasimanana *et al.*, 2013), suggesting forest cover loss between those years. Between 2013 and 2018, all forest land types decreased in area except open canopy mangrove. The expansion of this type of mangrove came as a result of internal conversion of the closed canopy mangrove. Overall, area losses of 15% and 0.9% were recorded in dry forests and mangroves respectively in five years. The major factors behind dry forests cover losses were degradation of forest land to savannas and agriculture expansion, while conversion to tan, cropland and urban areas were the main threats to mangroves. In this period, the increase in agricultural land by 81% of its initial area due to high migration to the Protected Area (Asity Madagascar, 2014) occurred mostly in savannas and dry forests. Savannas expansion were mainly due to deforestation of dry forests and *Bismarckia nobilis* tree savanna had the largest area occupying 24% of the MKWC in 2018.

Tab. 1: Aggregated land-cover stock and flow accounts for the Mahavavy-Kinkony Wetland Complex (2013 and 2018) in ha. Land-cover formation and consumption on these two dates are grouped in land-cover flow classes: artificial development, agriculture extension, internal conversions within land-cover classes, management and alteration of forested land, restoration and development of habitats and changes due to natural causes.

Land-cover ecosystem units (LCEU)	Opening stock (2013)	Total formation	Total consumption	Closing stock (2018)
Closed canopy forest	50,031	7,899	11,427	46,503
Open canopy forest	42,800	14,692	25,576	31,916
Closed canopy mangrove	6,816	304	2,190	4,930
Open canopy mangrove	14,482	5,519	3,028	16,973
Sparse canopy mangrove	3,047	955	1,783	2,219
Grassy savanna	63,937	20151	16,257	67,831
Tree savanna	82,737	28,926	26,732	84,931
Cropland	14,801	12,367	246	26,922
Other	72,111	8,089	11,663	68,537
Total	350,762			350,762

Conversion of dry forests – the primary habitat for lemurs in the MKWC (Andriamasimanana *et al.*, 2013) – to savannas and agricultural land is of great regional concern for *Propithecus coronatus* and *Propithecus deckenii* as the Protected

Area is one of their largest ranges in western Madagascar (Andriamasimanana and Cameron, 2014). The dry forests within the MKWC will disappear in approximately 25-30 years if the current rate of deforestation continues, which could lead to population declines or even local extinction of these species primarily threatened by habitat loss (Razafindramanana *et al.*, 2020; King and Rakotonirina, 2020). The land-cover evolution of the MKWC therefore negatively impacts biodiversity habitat. Conservation measures for dry forests such as their classification at site level as priorities for biodiversity conservation (Andriamasimanana *et al.*, 2013) should be strengthened. This study contributes to understanding land-cover trends to potentially inform future MKWC management plans.

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## Présence de *Cheirogaleus medius* dans la Nouvelle Aire Protégée d'Antrema

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**Keywords:** Antrema, *Cheirogaleus medius*, census, nocturnal lemurs

### Abstract

In order to monitor the lemur population trends at the New Protected Area (NAP) Antrema, located in the northwestern of Madagascar, an inventory of lemur species present in the site was performed. Our study was carried out in the NAP of Antrema, from February 1<sup>st</sup> to March 14<sup>th</sup>, 2020. Diurnal and nocturnal surveys were carried out in eight different forest fragments in Antrema, Kapahazo, Ampampamena, Antsoherimasiba, Ambato, Antsahelika, Ambanjabe, Ankoririka and Bako. In addition, villager surveys (n=39) were carried out in order to bring more information on lemurs and their habitat. In total, six species of lemurs have been recorded, including *Cheirogaleus medius*, a species newly recorded for this area. The majority of people surveyed (n=38), did not know about the existence of *C. medius* in the NAP. However, one person interviewed in Ambanjabe reported that he had seen an individual similar to *C. medius*. Thus, NAP Antrema hosts six species of lemurs: *Eulemur mongoz*, *Eulemur rufus*, *Propithecus coronatus*, *Cheirogaleus medius*, *Microcebus murinus* and *Lepilemur aeeclis*, the latter three being nocturnal species. The occurrence of *Cheirogaleus medius* in the NAP Antrema is reported here, for the first time.

### Résumé

Dans l'objectif de suivre les populations de lémuriens de la Nouvelle Aire Protégée (NAP) Antrema dans le nord-ouest de Madagascar, un inventaire des lémuriens présents et un recensement de l'espèce *Eulemur mongoz* ont été réalisés. Cette étude a été menée dans la NAP d'Antrema,

du 1<sup>er</sup> Février au 14 Mars 2020. Des prospections, diurne et nocturne, ont été menées dans huit différents fragments forestiers à Antrema, Kapahazo, Ampampamena, Antsoherimasiba, Ambato, Antsahelika, Ambanjabe, Ankoririka et Bako. D'autre part, des enquêtes auprès des villageois (n=39) ont été réalisées afin d'obtenir des compléments d'information sur les lémuriens et leur habitat. Au total, six espèces de lémuriens ont été recensées dont *Cheirogaleus medius* une espèce recensée pour la première fois dans cette zone. La majorité des personnes enquêtées (n=38), ne connaissaient pas l'existence de *C. medius* dans la NAP. Toutefois, une personne à Ambanjabe a signalé qu'elle a déjà observé un individu semblable à *C. medius*. Ainsi, la NAP Antrema héberge six espèces de lémuriens: *Eulemur mongoz*, *Eulemur rufus*, *Propithecus coronatus*, *Cheirogaleus medius*, *Microcebus murinus* et *Lepilemur aeeclis*. Ces trois dernières étant des espèces nocturnes. Notre étude rapporte pour la première fois la présence de *Cheirogaleus medius* dans la NAP Antrema.

**Mots-clés:** Antrema, *Cheirogaleus medius*, recensement, lémuriens nocturnes

### Introduction

Actuellement, plus de 110 espèces de lémuriens sont connues, toutes endémiques de Madagascar (Schwitzer et al., 2013). Récemment, la découverte d'une nouvelle espèce, *Microcebus jonahi* (GERP, 2020) a donné l'espoir dans l'exploration des endroits moins étudiés en termes de biodiversité lémurienne. Cela encourage aussi le renforcement des mesures de conservation face aux diverses pressions et menaces qui pèsent sur ces animaux. Parmi les espèces nocturnes, dans la famille des Cheirogaleidae, le genre *Cheirogaleus* est un petit lémurien nocturne qui entre en période de torpeur saisonnière s'apparentant à l'hibernation (Mittermeier et al., 2014). Le genre *Cheirogaleus* est très diversifié. Selon la liste rouge de l'IUCN en 2021, il existe neuf espèces, *C. major* (VU), *C. medius* (VU), *C. crossleyi* (VU), *C. grovesi* (DD), *C. sibreei* (CR), *C. lavasoensis* (EN), *C. thomasi* (EN), *C. shethi* (EN), *C. andysabini* (EN). L'habitat de *C. medius* est réparti dans tout l'ouest et le sud de Madagascar depuis Tolagnaro (Fort-Dauphin) au sud-est, en passant par la brousse à épineux du sud, dans la partie sud-ouest de l'île, puis en remontant vers le nord par les forêts sèches de l'ouest jusqu'à la région du Sambirano (Mittermeier et al., 2014). La répartition de cette espèce est limitée aux forêts sèches de l'ouest de Madagascar incluant la Nouvelle Aire Protégée (NAP) d'Antrema. Une seule espèce de la famille de Cheirogaleidae, *Microcebus murinus*, a été observée durant les différentes investigations antérieures réalisées à Antrema (Ramanamiasa et al., 2014; Ravelomandrato, 2017; Rokshane, 2018). Une mise à jour des données sur la densité des lémuriens a été effectuée entre 2019 et 2020. Cela s'est concentré surtout sur les espèces *Propithecus coronatus* et *Eulemur mongoz*. Pour ce dernier, les informations obtenues lors du suivi écologique sont des données de références. Cela a permis aussi de recenser les espèces de lémuriens sympatriques, en particulier les autres espèces nocturnes. La présente étude a pour objectif de faire une mise à jour de l'inventaire des espèces nocturnes présentes dans la NAP Antrema et le recensement de *E. mongoz*.

### Matériels et méthodes

Cette étude a été menée du 1<sup>er</sup> février au 14 mars 2020 dans la Nouvelle Aire Protégée (NAP) d'Antrema, au nord-ouest de Madagascar. Elle se situe dans le District de Mitsinjo, commune rurale de Katsepy, Fokontany Antrema. Elle se

trouve à 12 km de Katsepy et est limitée au nord-ouest par le Canal de Mozambique, au sud par la route qui mène vers Mitsinjo, à l'est, par la route qui mène vers le phare de Katsepy. Ce site s'étend sur une superficie de 20.620ha dont 1.000ha pour le Parc marin. Sa géolocalisation se trouve entre 15°42 à 15°50 de latitude Sud et 46° à 46°15 de longitude Est (Gauthier *et al.*, 1999). En ce qui concerne la formation végétale, la NAP Antrema fait partie de la zone éco-floristique occidentale de basse altitude (0 à 800m) (Faramalala et Rajeriarison, 1999) et la végétation climacique correspond à des forêts denses sèches semi-caducifoliées, de série à *Dalbergia*, *Commiphora* et *Hildegardia* (Koechlin *et al.*, 1974).

Huit zones différentes (Antrema, Kapahazo, Ampampamena, Antsoherimasiba, Ambato, Antsahelika, Ambanjabe, Ankori-rika et Bako) ont été l'objet de cette étude. Pour recenser les lémuriens présents dans le site, la méthode de ligne de transect (Randrianambinina *et al.*, 2010; Rakotondravony et Rabenandrasana, 2011) a été utilisée dans les différents fragments forestiers susceptibles d'abriter des lémuriens (Fig. 1). Trois séries d'observation ont été effectuées à savoir le matin (6h00-10h), l'après-midi (14h00-16h00) et la nuit (18h30-22h), sur les 33 pistes existantes à l'intérieur de chaque fragment forestier, et sur 2 transects utilisés lors des études antérieures (cas d'Ankori-rika et d'Antrema). La longueur de ces pistes varie entre 0,500Km à 2,500Km et entre 300 à 500m pour les transects. Chaque piste ou transect a été visité par deux observateurs, durant les trois séries d'observation, pour le suivi des espèces diurnes et nocturnes. Parfois une ou deux séries d'observation seulement ont pu être réalisées à cause des difficultés d'accessibilité dans les fragments forestiers. La vitesse d'observation est de 1km.h<sup>-1</sup>. Au total, 12 jours d'observation nocturnes ont été effectués pour cette étude. Les espèces nocturnes sont facilement repérées par le reflet du «*tapetum lucidum*» au contact de la lumière émise par une lampe avec une forte intensité (Wright, 1999). Ainsi, à chaque rencontre d'un individu de l'espèce le nom, les coordonnées géographiques, l'heure de rencontre, le nom de l'arbre support et l'activité de l'animal sont notés. Pour les espèces nocturnes, une observation à l'aide de lampe de forte intensité type maglight suivie d'une prise de vue à l'aide d'un appareil photo (Canon Rebel EOS T6i, focal 200mm) ont été effectués pour mieux identifier l'animal rencontré. Le comportement des animaux peut

aider dans leur identification, ainsi une espèce nocturne est facilement reconnaissable lorsqu'on l'observe à la lumière d'une lampe de forte intensité. Le mode de déplacement quadrupède et lent permet de distinguer *Cheirogaleus* des autres espèces nocturnes telles que *Microcebus* ou *Mirza*. Une des particularités de *Cheirogaleus* est que l'anus de l'animal se situe aussi au début de la queue, mais on ne peut observer cette particularité que si l'on est près de l'animal ou si on le tient en main (Mittermeier *et al.*, 2014). De plus, la meilleure chance de l'observer est au cours de sa saison d'activité. *Cheirogaleus* sort généralement de sa torpeur juste avant le début de la saison des pluies qui débute généralement en novembre (Schülke et Ostner, 2007). Toutefois, pour maximiser les données obtenues, 39 personnes ont été enquêtées pour connaître la présence éventuelle des lémuriens ainsi que les caractéristiques de leur habitat dans la NAP d'Antrema. Ces personnes, dont six femmes et 33 hommes, sont issues de neuf villages différents. Ils sont âgés de 24 à 76 ans et ayant des activités variées. La plupart sont des agriculteurs (n=11) et des agents qui travaillent au Parc (n=8). Les autres personnes enquêtées occupent diverses autres fonctions (n=20). Des séries de questions ont été posées lors de l'enquête. Elles se répartissent comme suit: Q1: À propos des personnes interrogées: Lieu /âge/ sexe / occupation / ville d'origine. Q2: Questions sur les connaissances générales sur les lémuriens: Combien de lémurien avez-vous connaissance dans la NAP Antrema? Connaissez-vous le sifaka, Gidro mena, Raipaka, tsitsidika?. Des questions sur les endroits ou types d'arbres où ils ont rencontré l'espèce, ont été également posées. Le livre «Lémuriens de Madagascar» (Mittermeier *et al.*, 2014) a été utilisé pour montrer des illustrations et faciliter ainsi la reconnaissance des espèces par les personnes enquêtées.

## Résultats et discussion

Au total, six espèces ont été détectées à savoir l'espèce diurne *Propithecus coronatus*, les espèces cathémérales *Eulemur rufus* et *Eulemur mongoz*. Parmi les espèces nocturnes, la présence de *Microcebus murinus*, et de *Lepilemur aeeclis*, a été confirmée celle de *Cheirogaleus medius* a été constatée pour la première fois. En effet, deux individus de *Cheirogaleus* ont été observés dans deux endroits différents à Kapahazo. Un individu a été observé le 9 février 2020 vers 19h50 dans forêt de Matsaborilava (S15.76574; E046.11079) sur un *Grewia madagascariensis* à une hauteur de 15m en quête de nourriture et un autre a été observé à la même date vers 22h00 dans la localité de Kaokabo (S15.76987; E046.11497) sur une espèce d'arbre *Grewia* sp à une hauteur de 10m. La quadrupédie et le déplacement lent de ces individus ont été observés lors de notre suivi. De plus, la période de notre descente sur le terrain a coïncidé avec la saison d'activité de *Cheirogaleus*. En observant de près la Fig. 2, l'orifice anal situé à la base de la queue peut être remarqué, entouré de poils un peu plus clairs que le reste de la queue.

En se basant sur la distribution du genre *Cheirogaleus* (Mittermeier *et al.*, 2014), on peut penser qu'on est probablement en présence de *C. medius*. En effet, selon Mittermeier et ses collaborateurs (2014) la forêt sèche de la NAP Antrema fait partie de l'aire de répartition de *Cheirogaleus medius*. On signale pour la première fois la présence de *C. medius* dans la NAP Antrema, étant donné que seules cinq espèces de lémurien y étaient connues et présentes (Ramanamisa *et al.*, 2014). Seuls *Microcebus murinus* et *Lepilemur aeeclis* y étaient signalés comme espèces nocturnes (Ravelomandrato, 2017). Dans la partie nord-ouest, cette espèce s'observe dans plusieurs endroits tels que dans le district de Mitsinjo ou dans la forêt de Mariarano



Fig. 1: Délimitation des zones d'étude (google Earth, 2020, modifiée par l'auteur).





Fig. 2: *Cheirogaleus medius* dans la forêt de Matsaborilava – Kapahazo. (cliché de Randrianarison Heriniaina)

(Petter et al., 1977; Ibouroy et al., 2013; Gardner, 2016) et dans le Parc National Ankarafantsika (Mittermeier et al., 2014). Ce résultat est conforté par les informations recueillies auprès des villageois. Sur les 39 personnes enquêtées, seules 3 ont signalé l'existence de six espèces de lémuriens *Eulemur mongoz*, *Eulemur rufus*, *Lepilemur aeeclis*, *Microcebus murinus*, *Avahi* sp., *Propithecus coronatus* hormis *Cheirogaleus medius*. Deux personnes parmi ces 3 ne sont pas originaires d'Antrema. La majorité d'entre eux ( $n=36$ ) ont avoué n'avoir vu que 2 à 5 espèces de lémuriens dont *Propithecus coronatus* et *Eulemur rufus*. La présence de *Avahi* n'a pas été observée lors des études antérieures, ni par la présente étude. Ces résultats semblent montrer que *Cheirogaleus* est très rarement observé par les villageois dans la NAP Antrema. Toutefois, un des villageois enquêtés à Ambanjabe a révélé qu'il a remarqué un individu semblable à *Cheirogaleus medius* vers 2014, en exploitant les Ravinala (Hatrandra). Il a nommé cette espèce «Gara maso». D'autre part, *Cheirogaleus medius* a été observé dans le village d'Antrema, malheureusement aucune photo de l'individu n'a été prise (Gauthier, communication personnelle). Vu la conservation des rituels et l'attachement au respect des coutumes ancestrales dans cette zone et selon les enquêtes effectuées, la consommation des lémuriens est tabou pour les natifs d'Antrema (Harpet et al., 2008). Ainsi, la faible densité des espèces pourrait être davantage liée à la destruction de leur habitat.

Notre étude semble montrer que *Cheirogaleus medius* peut être trouvé dans la NAP d'Antrema dans la zone de Kapahazo, alors que les résultats antérieurs n'ont jamais signalé la présence de cette espèce. Si les dires de la personne enquêtée sont vérifiés, on pourrait potentiellement trouver cette espèce dans la zone d'Ambanjabe. Le village d'Antrema serait également un site d'observation de l'espèce. Étant donné que la capture des lémuriens reste un tabou pour la population d'Antrema, seules les analyses génétiques basées sur des collectes d'échantillons fécaux seraient l'unique possibilité de confirmer l'espèce rencontrée. Dans ces condi-

tions ni les mensurations, ni les prélèvements d'échantillons de tissus ne sont réalisables. C'est pour cette raison qu'une étude plus poussée serait adéquate pour mieux confirmer l'identification de cette espèce et sa répartition au sein de la NAP d'Antrema.

En conclusion, notre étude rapporte pour la première fois la présence de *Cheirogaleus* dans la NAP Antrema. Nos observations semblent montrer qu'il s'agit bien du genre *Cheirogaleus*. Cependant, des études plus poussées seraient nécessaires pour confirmer s'il s'agit bien de *Cheirogaleus medius*.

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## The potential distribution of the giant mouse lemurs (*Mirza coquereli*, *Mirza zaza*) with implications for their conservation

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### Abstract

The giant mouse lemurs (*Mirza* spp.) of Madagascar are among the understudied lemur species with persistent knowledge gaps concerning their behavior, ecology, biogeography and distribution.

We therefore aim to investigate the potential distribution of *M. zaza* and *M. coquereli*, to assess their bioclimatic niche divergence and to deduce implications for their conservation. We derived occurrence records from the literature and used MaxEnt-based species distribution models to determine the distribution of suitable habitats for both species across Madagascar.

The niches of both species are significantly different from each other and *M. zaza* is predicted to have a very limited geographic distribution, whereas *M. coquereli* occurs and could occur across vast stretches along the west coast of Madagascar. Habitats for both species are highly fragmented with <16.5% of their potential distributions being still covered with forests.

Our findings highlight the need to invest in further studies concerning these two species, to understand their ecological requirements, their adaptability towards land use changes and the human dimension of their protection, to initiate tailored measures for their conservation. Particularly for *M. zaza* with its narrow and highly fragmented distribution.

**Keywords:** Habitat fragmentation, endangered species, species distribution modeling

### Résumé

Les lémuriens souris géants (*Mirza* spp.) de Madagascar font partie des espèces de lémuriens peu étudiées, avec des lacunes persistantes dans les connaissances concernant leur comportement, leur écologie, leur biogéographie et leur distribution.

Nous souhaitons donc à utiliser des modèles de distribution des espèces pour étudier la distribution potentielle de *M. zaza* et *M. coquereli*, d'évaluer leur divergence de niche bioclimatique et d'en déduire des implications pour leur conservation. Nous avons dérivé des enregistrements d'occurrence de la littérature et utilisé des modèles de distribution d'espèces basés sur MaxEnt pour déterminer la distribution des habitats appropriés pour les deux espèces à travers Madagascar. Les niches des deux espèces sont significativement différentes l'une de l'autre et on prévoit que *M. zaza* a une distribution géographique très limitée, alors que *M. coquereli* est présent et pourrait être présent sur de vastes étendues le long de la côte ouest de Madagascar. Les habitats des deux espèces sont très fragmentés avec <16,5% de leurs distributions potentielles encore couvertes de forêts.

Nos résultats soulignent la nécessité d'investir dans des études supplémentaires concernant ces deux espèces, afin de comprendre leurs exigences écologiques, leur adaptabilité aux changements d'utilisation des terres et la dimension humaine de leur protection, pour initier des mesures adaptées à leur conservation. En particulier pour *M. zaza* avec sa distribution étroite et très fragmentée.

**Mots-clés:** Fragmentation de l'habitat, espèces menacées, modélisation de la distribution des espèces

### Introduction

The genus of giant mouse lemurs (*Mirza*) constitutes of two medium sized nocturnal lemur species found in Western Madagascar (Mittermeier *et al.*, 2010). The first species, *Mirza coquereli*, was described in 1867 by Grandidier, while the second one, *Mirza zaza*, was only acknowledged scientifically in 2005 (Kappeler *et al.*, 2005). Distinctiveness between these two species has so far been hypothesized based on molecular evidence (Kappeler *et al.*, 2005; Herrera and Dávalos, 2016), behavioral differences (Markolf and Kappeler, 2019) and morphological discrepancies (Kappeler *et al.*, 2005; Rode-Margono *et al.*, 2016). However, the exact ranges of these two species are still not yet fully resolved. *M. coquereli* is known to occur along the lower western coast of Madagascar with the northernmost accounts from the region of the Tsingy de Bemaraha National Park (NP; Dammhahn *et al.*, 2013), a core zone in the Menabe region (Kappeler *et al.*, 2005; Dolch *et al.*, 2011) and the southernmost occurrences reported from the Fiherenana river just north of Toliara (Gardner *et al.*, 2009; Fig. 1). In contrast to that, *M. zaza* is known from the Sambirano region from the northwestern coast of Madagascar, including the Sahamalaza and Ampasindava peninsulas and the region around Ambanja (Kappeler *et al.*, 2005; Markolf *et al.*, 2008a; Webber *et al.*, 2020; Fig. 1).

The distribution of the two *Mirza* species is disjunct, with real absence records from the Boeny and Betsiboka regions (Olivieri *et al.*, 2005; Markolf *et al.*, 2008a). However, there has been one account on the presence of *Mirza* spp. in the Tsingy de Namoroka NP (Kappeler *et al.*, 2005) with yet unclear species status. Independent of its actual affiliation, this location would represent a potentially isolated relict population for both *Mirza* species (Markolf *et al.*, 2008a). Here we aim to (1) delimit the potential distributions of *Mirza* spp. in Madagascar, and to (2) identify areas of conservation concern for this genus.

### Methods

We compiled presence data for *Mirza* spp. from our own observations in the Sahamalaza peninsula (June 2017, NRR),



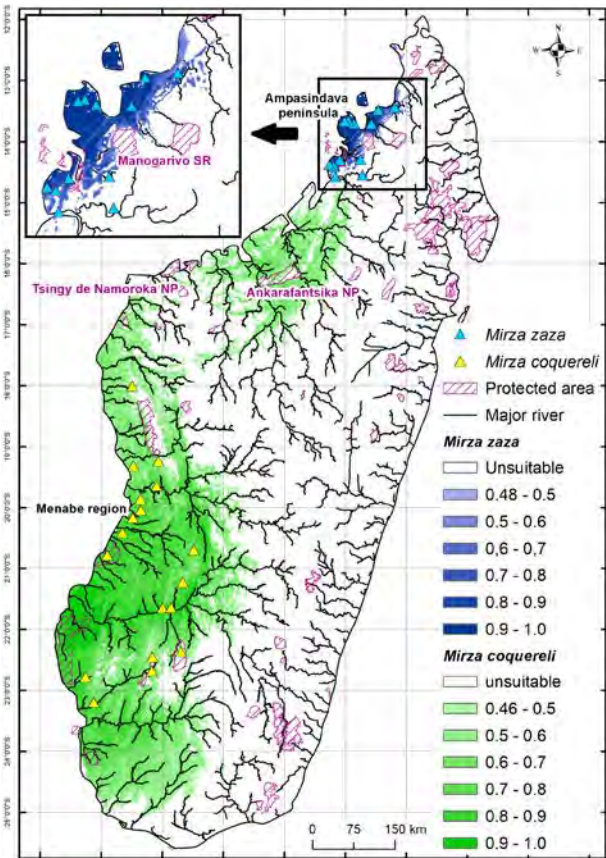


Fig. 1: Spatially filtered occurrence records and potential distribution of *Mirza zaza* and *Mirza coquereli*, based on the bioclimatic niche suitability truncated by the 10th percentile of the training presences.

the literature, LemursPortal ([www.lemursportal.org](http://www.lemursportal.org)) and GBIF ([www.gbif.org](http://www.gbif.org)). A total of 30 validated and spatially filtered (Boria *et al.*, 2014) occurrence records could be used for species distribution modeling (*M. zaza*:  $n = 12$ , *M. coquereli*:  $n = 18$ ) applying the MaxEnt algorithm as implemented in R (Phillips *et al.*, 2017; R Core Team, 2019). Models were constructed using the ENMtools R package (Warren *et al.*, 2021) and a set of 12 bioclimatic variables from the CHELSA database (Karger *et al.*, 2017), which are frequently used in Madagascar (e.g., Blair *et al.*, 2013; Kamilar *et al.*, 2016). These 12 variables (temperature related: Bio03-06, Bio08-09; precipitation-related: Bio12-17, Karger *et al.*, 2017) were transformed using a principal component analysis to exclude collinearity (RStoolbox package, Leutner *et al.*, 2019). The first 3 principal components were selected for modeling, which captured together 94.34% of the variation.

The best model was selected from a set of candidate models with varying model parameters (randomization multiplier from 1-6 and feature classes L, Q, P, H and all combinations) using a jackknife cross-validation approach (in ENMeval R package; Pearson *et al.*, 2007; Peterson *et al.*, 2011; Kass *et al.*, 2021) and the AICc as selection criterion (Warren and Seifert, 2011; Muscarella *et al.*, 2014; Kass *et al.*, 2021). We assessed model performance using the AUC (area under the Receiver Operating Curve) and the seemingly more robust CBI (continuous Boyce index; Hirzel *et al.*, 2006; Warren *et al.*, 2019) based on the jackknife cross-validation in ENMeval (Kass *et al.*, 2021). Niche breadth (B2 according to Levins, 1968) and niche overlap (I, D and rank correlations according to Schoener, 1968 and Warren *et al.*, 2008) in geographic

and environmental space were calculated using ENMtools R package. Geographic space is thereby defined as concerning purely spatial representations of suitable habitats whereas environmental space refers to the macro-environmental suitability range based on the input variables used. For significance testing, we used the identity test as proposed by Warren *et al.* (2008) with 99 replicates. Binary distribution maps were produced using the suitability value at the tenth percentile of training presences as a threshold (Escalante *et al.*, 2013).

Lastly, the binary distribution maps were clipped to the latest forest cover maps for Madagascar (i.e., 2017, 30x30 m resolution; Vieilledent *et al.*, 2018) to evaluate the amount of forest in the potential range of *Mirza* spp. and to illustrate patterns of habitat fragmentation. The protected area network was derived from the WDPA database ([www.protectedplanet.net](http://www.protectedplanet.net)). Occurrence records and the suitability maps are deposited here: [doi.org/10.25625/ICIQ70](https://doi.org/10.25625/ICIQ70).

Results

The selected species distribution models were of acceptable quality with AUC-values of 0.989 and 0.829 and CBI-values of 0.595 and 0.736 for *M. zaza* and *M. coquereli*, respectively. For *M. zaza*, the predicted distribution was limited to the lowland areas of the Sambirano region excluding the slopes and mountains of the Manogarivo Special Reserve (Fig. 1). *M. coquereli* instead occurs and is predicted to occur in the dry deciduous forests all along the west coast of Madagascar. This is also reflected in the geographic and environmental niche breadth estimates being 0.120 and 0.080 for *M. zaza* and notably wider for *M. coquereli* with 0.546 and 0.266, respectively. Both species inhabit significantly different niches in geographic and environmental space with very little overlap (Tab. 1; Fig. 1;  $p=0.010$ ).

Tab. 1: Niche breadth (Levins B2) and niche overlap in geographic and environmental space. Niche overlap for all metrics significantly different ( $P = 0.010$ ).

	Niche breadth		Niche overlap		
	M. zaza	M. coquereli	D	I	Rank correlation
Environmental space	0.080	0.266	0.088	0.247	0.342
Geographic space	0.120	0.546	0.304	0.565	0.099

The distribution of the suitable bioclimatic niche of *M. zaza* is predicted with a size of 6,256km<sup>2</sup> of which only 16.5% (1,031 km<sup>2</sup>) were still forested in 2017. The largest remaining forest block in the potential range of *M. zaza* is found on the Ampasindava peninsula with a few more fragments on the Sahamalaza peninsula and around the city of Ambanja (Fig. 2). *M. coquereli* instead has a potential distribution of 204,905 km<sup>2</sup> of which 13.0% (26,678 km<sup>2</sup>) were still forested in 2017. However and in accordance to the range of its sister species, the range of *M. coquereli* is highly fragmented with the largest forest fragments being located along the Tsingy de Bemaraha NP, Menabe-Antimena Protected Area, Kirindy-Mitea NP and Zombitse-Vohibasia NP.

Discussion

*Potential distributions*

The predicted distributions based on the bioclimatic niches of the two species were significantly different from each other, both in their location (in geographic and environmental space) and their niche breadth. Furthermore,



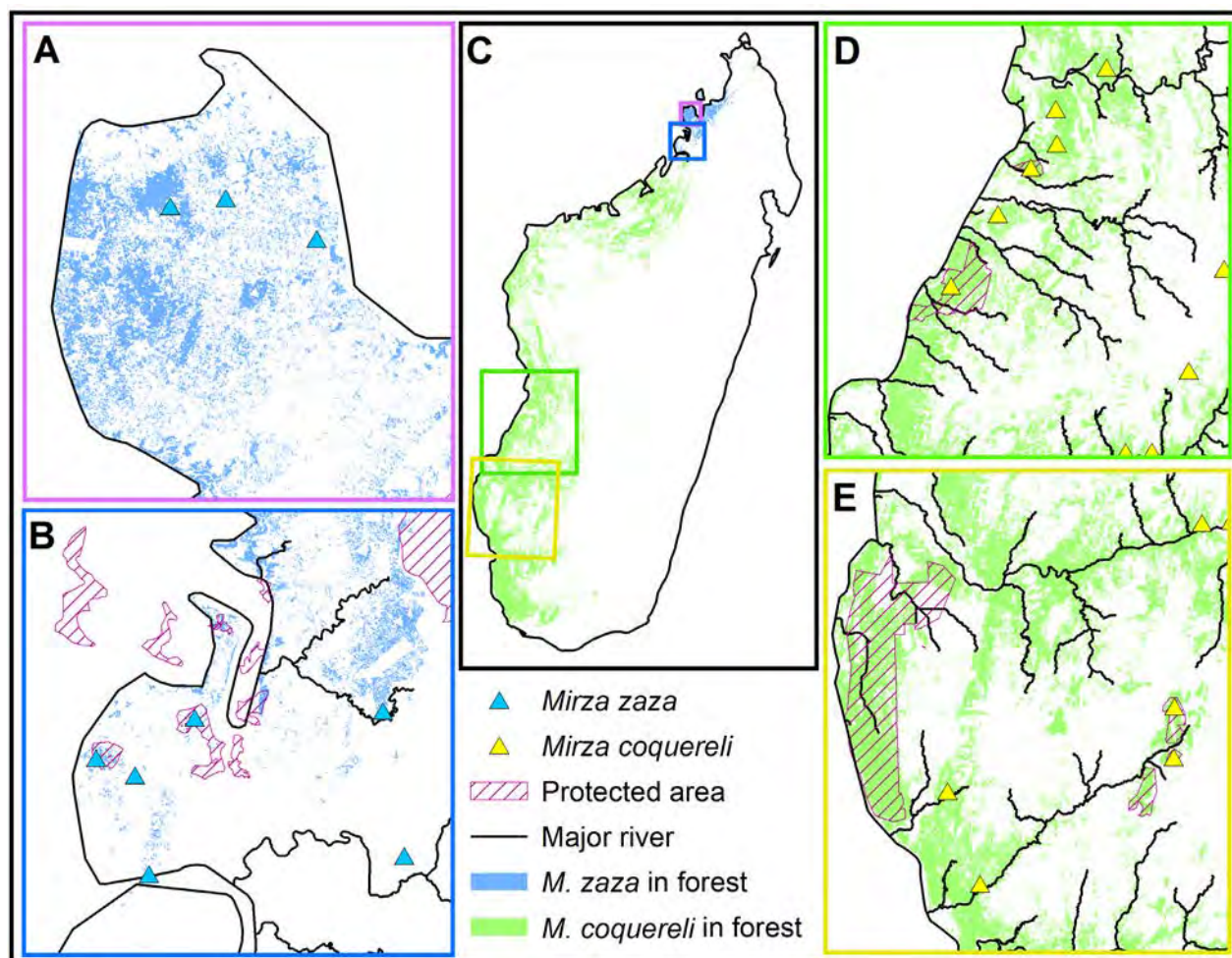


Fig. 2: Forested areas in the potential distribution of *Mirza zaza* and *M. coquereli*. A: The Ampasindava peninsula, B: The Sahamalaza peninsula with Sahamalaza Iles-Radama NP, C: Overview map with color-coded inlays, D: Western Madagascar with Menabe-Antimena Protected Area (north) and Kirindy-Mitea NP (south), E: Mikea NP (west) and Zombitse-Vohibasia NP (east).

both species occur in distinctive climatic regions: *M. zaza* is predicted to find suitable climates in a limited region characterized by lowland tropical monsoon forests (category Am in Köppen-Geiger climate classification, Beck *et al.*, 2018) of the Sambirano region. The area is characterized by a humid to sub-humid climate, high precipitation, warm temperature throughout the year and a transitional vegetation from rainforest towards dry deciduous forest at the coast (Koechlin, 1972). Our prediction is in line with actual presence and absence records for more southern locations (Markolf *et al.*, 2008a) and the slopes and mountains of the Manogarivo Special Reserve (Goodman and Schütz, 2000). Our results are congruent with Markolf *et al.* (2008a), supporting the hypothesis of *Mirza zaza*'s distribution being restricted by the Mahavavy Nord and Maevarano rivers adding an altitudinal range limit of <400 m a.s.l. to accurately describe its range.

We predicted a much wider bioclimatic niche for *M. coquereli*, including the tropical savannahs with dry winters and arid steppes with hot summers (categories Aw and BSh, respectively), found all along the west coast of Madagascar. This region is characterized by high atmospheric aridity during the six to eight months long dry season, a rainfall gradient decreasing towards the south and dry deciduous forest a main vegetation type (Koechlin, 1972). There are no records about this species from the southernmost areas of this prediction (south of the large Onila-

hy river), and true absences of it north of the Betsiboka river (Olivieri *et al.*, 2005), although suitable habitat could be found ahead of these rivers. Given the case that no new occurrences are provided by more extensive expeditions, these two rivers may be the ultimate barriers for *M. coquereli*. The region in question for unidentified *Mirza* spp., the Tsingy de Namoroka NP, falls right into the region of suitable climates for *M. coquereli* and is about 180km away from the northernmost occurrence records for this species (i.e., Beanaka forest, Dammhahn *et al.*, 2013). It falls outside the predicted suitability of *M. zaza* and we therefore conclude from our evidence, that the potential population at Namoroka is most likely *M. coquereli*.

#### Implications for conservation

For both species, it has been estimated that less than 16.5% of their potential distribution was still forested in 2017. With recent reports on ongoing deforestation, especially during the COVID-19 crisis, this figure may be considered even smaller. Irrespective of the actual amount of forested habitats within their bioclimatic niches, all forests are highly fragmented and core areas can only be found in the yet established protected areas. Studies on how these species can cope with land use change (i.e., deforestation, agroforestry) are still very limited, but first assessments may indicate that *Mirza* spp. can tolerate selective logging of forests (Ganzhorn, 1995), can adapt to agroforestry plantations (Web-

ber *et al.*, 2020) and may be able to effectively re-colonize accessible forests after a population bottleneck (Markolf *et al.*, 2008b). They can be sometimes found in high population densities, particularly in transitional forests and outside the protected areas (Hending, 2021). However, it is unknown, whether *Mirza* spp. actually occurs in all of the above described forests and the actual inhabited areas may be even smaller than the figures found in our analysis.

The limited scientific body concerning these two species, together with our study highlighting the low amount of forested but highly fragmented areas throughout their potential ranges, emphasizes the critical need to address further questions: What habitats are core areas/population sources for *Mirza* spp.? Are there disturbance thresholds predicting their occurrences? Could agroforestry corridors be used to re-connect forest fragments? What role do human perceptions of this species play in terms of human-*Mirza*-coexistence? To answer these questions, further studies are needed throughout the range of *Mirza* spp., particularly in the highly fragmented landscapes of the Sahamalaza peninsula or the agroforestry key areas around Ambanja for *M. zaza* and the larger protected areas, the riverine forest corridors and the northern range extent of *M. coquereli*.

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## Lemur inventories at the Vohidava-Betsimilaho New Protected Area

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### Introduction

A portion of the remote Vohidava and Betsimilaho Massifs in the upper Mandrare River valley was designated as a New Protected Area (NPA) in 2015, comprising 18,169 ha of spiny thicket vegetation. The process to designate the site was led by Missouri Botanical Garden (MBG), and this NGO now supports a site-based team that implements a conservation program here in collaboration with the local community. MBG invested in this site primarily because of its diverse flora that includes a number of threatened and locally endemic species (Goodman *et al.*, 2018). The fauna of this area was poorly known. However, recently, lemur surveys have been initiated within this NPA to provide a basic inventory of this site and also to provide a baseline from which the status of the reserve can be tracked. This article presents preliminary data from these surveys.

### Methods

#### Study site

The study was carried out in the new protected area of Vohidava-Betsimilaho (V-B), located between 46°10' - 46°20' eastern longitude and 24°11' - 24°26' southern latitude (Fig. 1). The area is part of the District of Amboasary and surrounded by the communities of Mahaly, Marotsiraka, Tsivory and Ranobe. Annual precipitation is around 990 mm but subject to high annual fluctuations. Subsequent droughts and famines exacerbate social unrest.

The spiny thicket vegetation of the V-B NPA is almost pris-

tine. In part, this is due to the area's very low human population density, attributable to the location's sub-arid and unpredictable climate and its history scarred by insecurity related to cattle theft. The high quality of the spiny thicket is also related to the local population's appreciation of the vegetation as a site where they can hide themselves and their possessions including, most importantly, their cattle, at times of bandit attack. Parts of the V-B forest are also traditional burial sites and within such areas cutting of trees is strictly forbidden. The local population is dominated by Antandroy who are well known for their many traditional behavioural restrictions, or fady. Some of these contribute to conservation, including restrictions on killing and eating lemurs and tortoise. Distant from large towns, and accessible only by very poorly maintained tracks, V-B and its fauna and flora remain poorly known. Botanical inventories to-date have recorded 277 native plant species, of which 226 are endemic to Madagascar. Four species are known only from this site, while 8 species are known from this site and fewer than 5 other locations. In addition to V-B's importance for biodiversity conservation, it is likely that this extensive thicket helps to maintain water flow in the Mandrare River throughout the year. This river is the water source for most of the Androy Region's population.

While currently the biodiversity of V-B is almost free from human disturbance, potential future threats including large scale charcoal production and artisanal mining of mica, malachite, and a variety of semi-precious stones are all too evident further south. An additional potential threat is a resurgence of shifting cultivation in the forest which may occur if farmers become less fearful of bandits and willing to farm further from home.

Lemur inventory, frequency of encounter and lemur density To undertake an inventory of the different lemur species present in the V-B NPA and to estimate their respective encounter frequencies and densities, we established 10 transects, of 1150 to 2000 m length, across the site (Fig. 1 and Tab. 1). The locations for the transects were determined by existing trails and the need to represent different parts of the reserve. Between 2 March and 28 March 2021 and 17 April and 20 May 2021, three people slowly (speed approximately 1 h. 40 mins. per km during the day, 2 h. 30 mins. per km at night) and quietly walked along the transect and counted and identified to species level any lemurs that were detected. The transects were not always surveyed in the same direction. Encounter frequency was expressed as the number of animals or groups encountered per kilometre of transect. To estimate the density of each lemur species, for each encounter we estimated the perpendicular distance of the animal (if there was a sole individual) or centre of the group from the observer (following methods as detailed in Buckland *et al.*, 2001). During the day, perpendicular distance was calculated using a range finder (Suaoki Golf Range Finder 656 IP54) to measure distance and a compass to estimate the angle from the surveyor between the transect and the sighting. At night the perpendicular distance was estimated by eye. These data were used to determine the maximum reliable sighting distance and thus to estimate the effective transect width for each species. These distance estimates were classified in intervals and following methods as described by Müller *et al.* (2000, p. 252) the distance interval at which the number of detections dropped to two-thirds or less of the preceding interval (the "fall-off" distance) was defined as the distance from the transect within which animals of the particular species are reliably detected. This distance was doubled to give the transect width. For *Lemur catta* (Hira or Ring-tailed Lemur) it was not possible



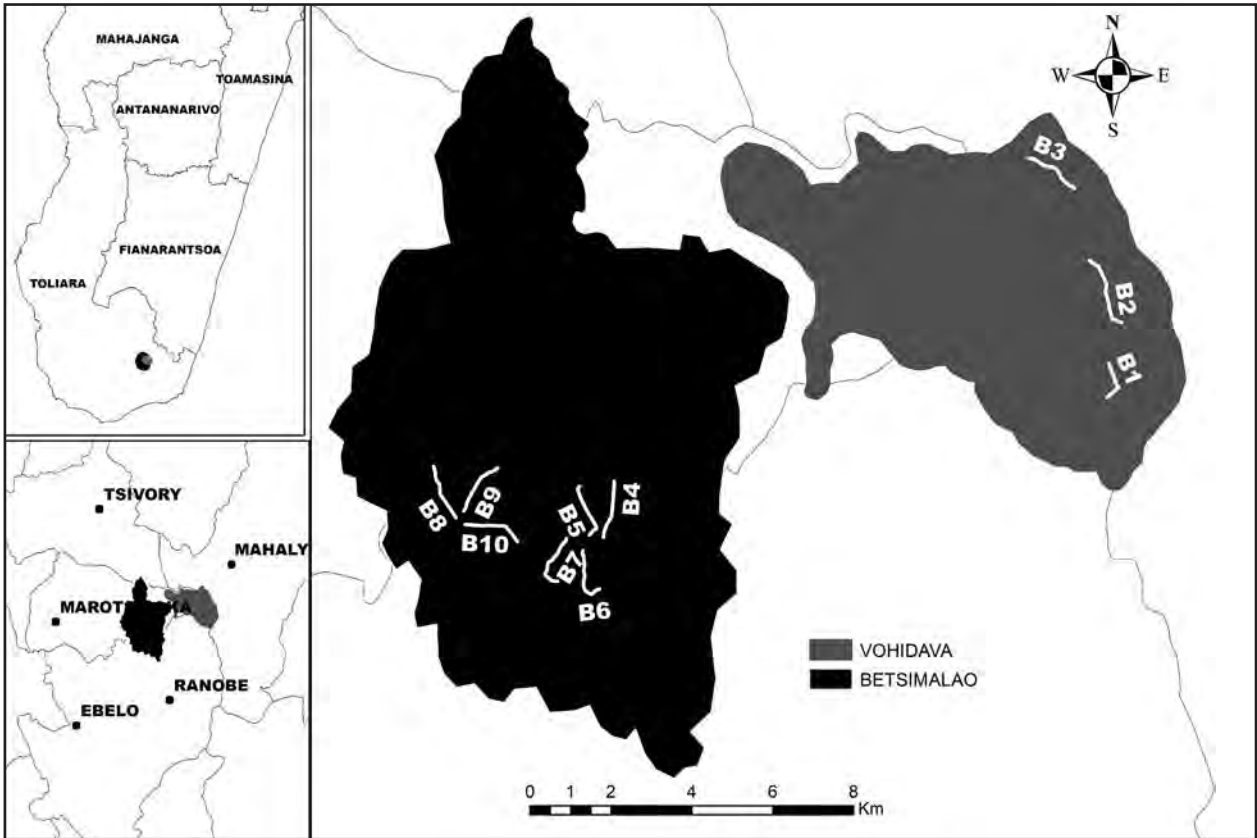


Fig. 1: Sites inventoried in Vohidava-Betsimalaho; circles mark towns; lines mark transects.

to count the number of individuals, as animals were fearful and fled before they could be counted. For this species, we noted the number of groups encountered rather than the number of individuals and estimated density in terms of the number of groups. Monitoring was conducted both during the day (between 07:00-10:30 and 15:00-18:00) and at night (18:30-22:30). Any diurnal species encountered during the night were not counted and vice versa.

Tab. 1: Characteristics of transects for lemur surveys in Vohidava-Betsimalaho; coordinates were taken at mid-point of the transects.

Area	Tran-sect	Length of transect (m)	Latitude	Longi-tude
Vohidava (N = 3)	B1	1150	-24.274145	46.304957
	B2	2000	-24.254082	46.302494
	B3	1500	-24.226627	46.290341
	Total	4650		
Betsimalaho (SE) (N = 4)	B4	1500	-24.318253	46.175566
	B5	1500	-24.314319	46.167337
	B6	1500	-24.303148	46.176378
	B7	1500	-24.302882	46.182456
	Total	6000		
Betsimalaho (SW) (N = 3)	B8	1500	-24.299291	46.14056
	B9	1500	-24.297419	46.14931
	B10	1500	-24.306401	46.153793
	Total	4500		
Total (N = 10)		15510		

Results and Discussion

In total, we completed 137 km of transect walks during the day and 59 km of transect walks at night. Four different lemur species were encountered: *Lemur catta*, *Propithecus*

*verreauxi* (Sifaka or Verreaux's Sifaka), a species of *Microcebus* (Mouse Lemur) and a species of *Lepilemur*. According to the published biogeographic distribution, the mouse lemur at V-B should be *M. griseorufus* (Songiky or Grey-brown Mouse Lemur) (Ganzhorn *et al.*, 2020). This identification is also supported by the observed individuals' phenotypes which are similar to the phenotype of *M. griseorufus* present in the dry parts of Andohahela and in Tsimanampesotse (Ganzhorn, pers. comm.). The biogeographic situation with *Lepilemur* is more complex in this region. Further south, the Mandrare river is considered to mark the limit between *Lepilemur leucopus* (Hataka or White-footed Sportive Lemur) to the east and *L. petteri* to the west (Eppley *et al.*, 2020; Louis *et al.*, 2020). However, in the landscape of Vohidava-Betsimalaho, the Mandrare does not represent a true barrier for the dispersal of animals, as the phenotype of observed individuals on both sides of the river resembles the *Lepilemur* of Andohahela Parcel 2 (i.e. *L. leucopus*) rather than those of Tsimanampesotse or Berenty (i.e. *L. petteri*). Thus, provisionally, we name V-B's sportive lemur (Fig. 3) as *Lepilemur leucopus* until fine-grained phylogeographic genetic analyses can resolve the biogeographic distribution of *Lepilemur* species in southeastern and southern Madagascar.

All four lemur species inhabit both the Vohidava and the Betsimalaho Massifs (Tab. 2). *Lepilemur leucopus* was encountered most frequently with a mean of 4.7 animals seen per km of transect walk (Tab. 2). *P. verreauxi* was the next most frequently encountered species with a mean of 3.1 individuals seen per km of transect walk. Group size for this species ranged from 2 to 9 with a mean of 4.4 individuals. However, it should be noted that since we could not identify the different groups, it is likely that the same groups were counted repeatedly and the larger groups more often, thus inflating our calculated mean group size.



Fig. 2: *Microcebus griseorufus* from Vohidava-Betsimilaho. Photo: Maël Jaonasy).

On average, 3.0 individuals of *M. griseorufus* were seen per km transect walk and, finally, 0.2 groups of *L. catta* were encountered per km of transect walk. Encounter rates were notably higher for *L. leucopus* and *P. verreauxi* in the south-eastern part of Betsimilaho. This may be due to its relatively remote location and associated low human presence or to the high abundance of *Alluaudia ascendens* (Didiereaceae) here. This plant is much frequented by these two lemur species perhaps because its spines, high level of branching and the obtuse angle between the branches and the main trunk makes it an excellent refuge from both terrestrial and aerial predators (Razafindraibe, 2011, Ganzhorn, pers. comm.). The leaves and flowers of Didiereaceae have also been reported as a key food for *L. leucopus* (Charles-Dominique and Hladik, 1971).

Tab. 2: Lemur encounter rates per kilometer transect walk (March to May 2021).

Transect	Number of transect walks day/night		Total length of transect walks (m)		Lemur catta (groups/km)	Propithecus verreauxi (inds./km)	Lepilemur leucopus (inds./km)	Microcebus griseorufus (inds./km)
	Day	Night	Day	Night				
B1	9	7	9900	4975	0.4	3.2	1.4	3.8
B2	13	5	23910	7120	0.0	0.9	2.9	3.2
B3	9	4	12850	6000	0.2	1.2	1.0	1.7
B4	9	3	13000	4500	0.2	2.5	7.3	4.9
B5	8	4	12000	4100	0.2	8.0	8.3	5.6
B6	7	5	10500	6200	0.1	5.3	6.6	1.5
B7	8	3	11710	4500	0.1	4.7	6.4	2.7
B8	9	5	13500	6800	0.1	1.4	5.6	1.5
B9	10	6	15000	8450	0.2	2.5	4.4	1.8
B10	10	4	15000	6000	0.3	1.7	3.5	3.7
Mean encounter rate per km					0.2	3.1	4.7	3.0

Tab. 3: Lemur densities in the Vohidava-Betsimilaho NPA (May 2021).

	Lemur catta (groups/km²)	Propithecus verreauxi (inds./km²)	Lepilemur leucopus (inds./km²)	Microcebus griseorufus (inds./km²)
Transect width	15 m x 2	15 m x 2	20 m x 2	15 m x 2
Density per km²	4.6	79.1	112	90

The population densities of *L. catta*, *P. verreauxi*, *L. leucopus* and *M. griseorufus* within the protected area were estimated respectively as 4.6 groups per km², 79.1 individuals per km², 112.0 individuals per km², and 90.0 individuals per km². It is remarkable that *L. catta* was recorded in all but one of the ten transects established at V-B, as

the species is typically reported to have a patchy distribution in spiny forest (Feistner and Schmid, 1999; Fenn et al., 1999; Ralison, 2008; LaFleur et al., 2016; Murphy et al., 2017; Ramanorintsoa, 2017; Kasola et al., 2020; but see also Murphy et al. (2017) for a critique of some of these studies). All other lemur species were also encountered more frequently here than at other sites of the region (see references above), except for the well-protected reserves of Berenty and Beza-Mahafaly (e.g., Richard et al., 1991; Sussman, 1991, Jolly et al., 1982, 2002, Axel and Maurer, 2011). Ongoing surveys, during different seasons, will help to confirm this result.

This study therefore suggests that Vohidava-Betsimilaho may be a stronghold for lemur conservation in southern Madagascar. This situation is probably due to the apparent rarity of lemur hunting in this zone that in turn is related to low human population density and persisting respect for traditional rules forbidding the consumption of lemurs (Behevitra; Manager V-B NPA, pers. comm.). It is remarkable that these “fady” remain intact, as this area is part of a zone impacted by recurrent droughts leading to famine (e.g., Gould et al., 1999), most recently, at the time of this study. Presumably the persistence of this belief system if associated with the relative isolation of communities in the northern parts of the Mandrare valley. However, in contradiction to the observed rarity of lemur hunting is the fearful behaviour of *L. catta* that suggests that, at least historically, some hunting may have occurred.

Missouri Botanical Garden, the formal managers of this protected area, are in the rare and fortunate position within Madagascar of facilitating the conservation of a natural ecosystem that is little degraded (with the exception of the presumed historic loss of megafauna) and currently little threatened. However, this situation could change rapidly given the diverse threats that are all too evident further south, and thus the challenge for these managers will be to prepare for the anticipated challenges. One way to do this would be to maximise now the engagement of local communities with all aspects of site management by building capacity and creating employment at all levels. Thus, for example, rather than hiring one technician from outside the community to patrol the site using a drone, it may be better to provide part-time employment for a score of local people to do the same job.

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Fig. 3: *Lepilemur leucopus* from Betsimilaho (left) and Vohidava (right). Photos: Maël Jaonasy

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## Genetic confirmation of the Anjamangirana sportive lemur in the Anjajavy Forest

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## Abstract

Most of Madagascar's lemurs are nocturnal, and most nocturnal lemurs are cryptic, making congeners difficult to differentiate due to their morphological similarity. Sportive lemurs (genus *Lepilemur*) are a great example and have been the subject of ongoing taxonomic debate for decades. Twenty-six sportive lemur species are currently recognized, based on early cytogenetic and more recent genetic stud-



ies. As a consequence of taxonomic rearrangements, species distributions have changed significantly over the years. During fieldwork at Anjajavy, a dry deciduous forest along the coast of the Inter River System (IRS) III, we opportunistically collected a tissue sample from a female *Lepilemur*. Although census work previously identified *L. grewcockorum* in Anjajavy and other locations in the IRS III, the only genetic confirmation for this species comes from the inland forests of Anjamangirana and Ambongabe. We sequenced a marker gene (Cytochrome B) and compared results to a gene database assembled from GenBank. Our results genetically confirm the individual from Anjajavy as *L. grewcockorum*. Additional genetic analyses, coupled with known census sites, might render this species more widely distributed than originally thought. We encourage further survey, genetic, and behavioral work within the remaining forest patches of the IRS III to clarify the true range, population estimates, and ecological characteristics of *L. grewcockorum*. This study demonstrates the value of using genetics to identify species that are morphologically similar and to determine the boundaries of their geographic ranges.

## Introduction

Madagascar is home to a rich array of lemur species, nearly all of which are threatened with extinction (IUCN, 2021). Whereas the diurnal lemurs are typically listed as flagship species for conservation efforts, somewhat ironically, the majority of lemur diversity is in the cryptic and nocturnal lineages (Mittermeier et al., 2010). In recent years, the nocturnal lemur lineages have undergone significant taxonomic revision (the aye-aye, *Daubentonia madagascariensis*, is a notable exception), as genetic approaches allow us to ‘see’ the differences between morphologically similar species (e.g., Andriantompohavana et al., 2007; Frasier et al., 2016; Schübler et al., 2020). The sportive lemurs are a classic example of an understudied nocturnal lineage that has been the subject of much taxonomic debate (Lei et al., 2017). Sportive lemurs are elusive and challenging to research. They are widely distributed throughout Madagascar, but “are relatively uniform in appearance, morphology, behavior, and ecology” (Thalmann and Ganzhorn, 2003, p. 1336), rendering species assignments challenging.

Sportive lemurs were first classified within the *Lepilemur* genus by Geoffroy Saint-Hilaire (1851) (Dunkel et al., 2012) which was placed within the family Lepilemuridae by Gray (1870) (Mittermeier et al., 2010). The name ‘sportive lemur’ was given by Forbes (1894) regarding the agility of this species, as they are excellent clingers and leapers (Dunkel et al., 2012). Hill (1953) classified the genus instead within the Lemuridae family based on morphological and karyological evidence, but Petter et al. (1977) favored maintaining them separately in the Lepilemuridae family (Thalmann and Ganzhorn, 2003). Tattersall and Schwarz (1985) placed the genus as sister to the extinct *Megaladapis* genus, within the Megaladapidae family, based on dental characteristics (Thalmann and Ganzhorn, 2003). By 2005, however, accruing genetics studies re-established *Lepilemur* and *Megaladapis* as independent lineages (Yoder et al., 1999; Karanth et al., 2005). Recent genomic data supports these early genetic findings and established Lepilemuridae and Cheirogaleidae as sister lineages (Marciniak et al., 2021).

While gaining clarity into the higher-level relationships between sportive lemurs and other lemurs, recent years have also seen a rapid increase in the number of species within the genus. Historically, only two species were included in the *Lepilemur* genus: *L. mustelinus* in the east and *L. ruficaudatus* in the west and south (Thalmann and Ganzhorn, 2003). Petter

et al. (1977) elevated 5 additional subspecies to species status, based on karyological evidence, though Tattersall (1982) favored synonymizing them all as subspecies within *L. mustelinus* (Thalmann and Ganzhorn, 2003). By 2000, genetic studies and karyological evidence led the field to largely recognize 7 full species (Thalmann and Ganzhorn, 2003). Since the early 2000s, accruing molecular, morphometric, and karyological studies support at least 26 species distributed around Madagascar (Andriaholinirina et al., 2006; Craul et al., 2007; Lei et al., 2017; Louis et al., 2006; Rabarivola et al., 2006; Rumpler et al., 2008). Many of these species were first described, and remain known today, only from single type localities and few samples or individuals. As more species within this genus continue to be described, questions remain regarding each species’ geographic distributions and ecological characteristics. Here, we add to our growing knowledge about the *Lepilemur* genus by sequencing a marker gene (cytochrome B) from an individual sportive lemur that was opportunistically sampled in the Anjajavy forest. Anjajavy, a dry deciduous forest in northwest Madagascar, sits along the coast between the Sofia and Maevarano rivers in the Inter River System (IRS) III. Based on the new lemur assessments released by the IUCN Red List of Threatened Species (2020), and the potential for rivers to establish lemur biogeographical patterns (Wilmet et al., 2014), we predict the sportive lemur from Anjajavy to be *L. grewcockorum*.

*Lepilemur grewcockorum*, also known as the Anjamangirana sportive lemur, was first identified by Louis et al. (2006) as *L. grewcocki* in the Classified Forest of Anjamangirana (15°09'14.9"S, 47°43'41.0"E) in the former range of *L. edwardsi*, based on mitochondrial DNA. Near the same locality, Craul et al. (2007) described specimens as *L. manasamody*, from Ambongabe (15°19'38.3"S, 46°40'44.4"E) and Anjamangirana I (15°09'24.6"S, 47°44'06.2"E). Zinner et al. (2007) indicated that *L. manasamody* is probably a junior synonym of *L. grewcocki*, as sampling sites were separated by less than two kilometers, with no obvious geographic barrier. The synonymizing of *L. grewcockorum* and *L. manasamody* was confirmed by a molecular genetic analysis by Lei et al. (2017). During this period of taxonomic ambiguity for the Ambongabe samples, Hoffmann (2009) noted that *L. grewcocki* was an incorrect original spelling and the species name was amended to *L. grewcockorum*.

The Anjamangirana sportive lemur is found in northwestern Madagascar (Louis et al., 2020). The known distribution is limited to the inland sites of Ambongabe and Anjamangirana, as confirmed by genetic analysis (see Fig. 1). Both sites are situated in the IRS III which is delimited by the Sofia River in the south and Maevarano river in the north (Olivieri et al., 2005; Craul et al., 2007). During census surveys, Randrianambinina et al. (2010) reported *L. grewcockorum* at three additional sites, including Anjajavy (S15°01'39.6" E47°16'38.4"), Ambarijeby (S14°53'20.9" E47°43'17.8") and Bekofafa (S14°53'20.9" E47°43'17.8"), though none have been confirmed genetically. According to these surveys, the encounter rates of *L. grewcockorum* are rare (Randrianambinina, 2010). The species is currently listed as Critically Endangered, due to its tiny extent of occurrence (EOO) covering only 143 km<sup>2</sup> (IUCN, 2020), which does not include the census sites that lack genetic confirmation.

## Methods

### Sample collection

The subject was a female sportive lemur opportunistically sampled from the Anjajavy forest. The individual was caught on July 15<sup>th</sup>, 2018, by hand from a tree hole, while searching



Fig. 1: Map of Madagascar showing the IUCN ranges in the northwest of *L. grewockorum* in the IRS III and the neighboring *L. otto* in the IRS II, *L. edwardsi* in the IRS I, and *L. sahamalaza* in the IRS IV against the Maevarano, Sofia, Mahajamba rivers. Sampling locale at Anjajavy is depicted as a star.

for a radio-collared dwarf lemur that was hibernating in the adjacent tree. While in hand, the individual was placed in a cloth bag and brought back to the campsite to be given a physical exam by project veterinarians. At camp, the sportive lemur was briefly anesthetized with Ketamine (10 mg/kg body mass) for morphometric data collection, and a small tissue biopsy was obtained from the left ear for genetic analysis. The sample was immediately submerged in 90% ethanol and stored at room temperature until extraction and subsequent analysis. The individual was given water after recovery and released at her initial capture location at sunset the same day. Although this individual was a by-catch, and not the target of our research project, we followed approved research practices for nocturnal species, following the guidelines established by the International Primatological Society in "International Guidelines for the Acquisition, Care and Breeding of Nonhuman Primates". In addition to sampling, this was an opportunity to conduct a comprehensive biomedical exam by two early-career wildlife veterinarians (ER & HAR) overseen by an expert lemur veterinarian (RS).

#### DNA extraction and amplification

DNA was extracted from the tissue sample *in situ* at Anjajavy within 2 weeks of capture using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). DNA concentration was quantified on a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA).

We used primers CYT-LEP-L (5'- AATGATATGAAAAAC-CATCGTTGTA -3') and CYT-LEP-H (5'- GGCTTCAAGGCCGGGGTAA -3') following Andriaholinirina et al. (2006) in the U.S. to amplify the mitochondrial cytochrome B (*cytb*) gene. The 25  $\mu$ L PCR reaction included 12.5  $\mu$ L Qiagen HotStartTaq Master Mix, 2.0  $\mu$ L Ambion Ultrapure non-acetylated Bovine Serum Albumin (20 mg/mL), 1.0  $\mu$ L each of 10  $\mu$ M forward and reverse primers and 4.0  $\mu$ L of template DNA. Following an activation step at 95°C for 15 min, PCR cycling conditions (40 cycles) were: 94°C for

60 sec, 50°C for 60 sec, 72°C for 90 sec. The final extension was at 72°C for 10 min. PCR product was visualized via agarose gel electrophoresis, enzymatically purified and sequenced at the Duke DNA Analysis Facility on an Applied Biosystems 3730 Genetic Analyzer using both the PCR primers and internal sequencing primers CYT-LEP-L400 5'- TGAGGACAAATATCATTCTGAGG - 3' and CYT-LEP-H545 5'- TGGAGTGCGAAGAATCGGGT- 3' following Andriaholinirina et al. (2006). The chromatogram was visually inspected using FinchTV v 1.5.0 (Geospiza).

#### Data analysis

We downloaded available ( $n=146$ ) sportive lemur complete cytochrome B sequences in GenBank, representing all 26 currently-recognized sportive lemur species (IUCN, 2021). We removed duplicate sequences, resulting in a final dataset of 124 sequences. The newly generated data for the sportive lemur was collated to the datamined sequences and aligned using MUSCLE v3.8.31 (Edgar, 2004). The alignment was visually inspected using AliView (Larsson, 2014). The best scoring maximum likelihood tree was estimated using RAxML (Stamatakis, 2006) using the rapid bootstrap analysis algorithm (Stamatakis et al., 2008) with 1000 bootstrap replicates and a general time-reversible (GTR) nucleotide substitution model with a gamma distribution for rate heterogeneity. A GTR model was chosen because it has been found to perform at least as well as other models in phylogenetic reconstruction under a variety of conditions (Arenas, 2015). An eastern woolly lemur (*Avahi laniger*; DQ451106.1) was used as an outgroup. For the construction of the final tree, we removed a handful of samples with unclear provenance in GenBank and identical sequences from conspecifics.

#### Results

The Anjajavy sample is placed as sister to the *L. grewockorum* sequence (Fig. 2), collected from Anjiamangirana (Lei et al., 2017). Bootstrap support for this placement was high (98). We provide morphometrics from the focal subject in Tab. I, along with published values and descriptions for individuals from other sites.

#### Discussion

Our results support the assignment of the sportive lemur from Anjajavy as *L. grewockorum*. This represents a confirmed range expansion for the species, which is currently listed in the IUCN Red List for Threatened Species in only a tiny fragment far inland of our locality. Importantly, census data placed *L. grewockorum* as variably distributed at intermediate locations between Anjajavy and Anjiamangirana (Randrianambinina, 2010), suggesting that this species is present throughout the IRS III. It is becoming clearer that sportive lemur species, like mouse lemurs, are allopatric in the northwest and confined to specific IRS (Olivieri et al., 2007; Roos et al., 2021; Wilmet et al., 2014). We encourage the IUCN to update the range maps for this species to include Anjajavy and the census sites of Ambarijebby and Bekofafa. We also encourage further survey, genetic, and behavioral work within the remaining forest patches of the IRS III to clarify the true range, population estimates, and ecological characteristics of *L. grewockorum*.

The case of *L. grewockorum* highlights the importance of using genetics to confirm the boundaries of species' ranges. Within those boundaries, morphological characteristics can be used as general descriptors to guide census, behavioral, and survey work. But morphological and visual features, like coat color, can be subjective and variable across popula-

Tab. 1: Morphometrics from published sources and this study: BM: body mass in kg, BL: body length in cm, TL: tail length in cm, HW: head width in cm. NA: data not available

Species	BM (Mean + SD)	BL (Mean + SD)	TL (Mean + SD)	HW (Mean + SD)	Notes
<i>L. grewcockorum</i> (n=3)*	0.78 (0.20)	24.8 (2.1)	28.5 (1.8)	NA	Predominantly gray color pattern. Area around the mandible and dorsal surface of the snout is whitish-pink in coloration. A dark stripe is present on the dorsal midline surface of the head... Unlike <i>L. edwardsi</i> , which has a consistently white-tipped tail, the tail of <i>L. grewcockorum</i> is entirely gray.
<i>L. manasamody</i> (now <i>L. grewcockorum</i> , n=8)**	0.939 (96.97)	NA	28.1 (15.24)	37.61 (2.29)	Dorsal pelage is predominantly grey-brown. The ventral pelage is generally grey to creamy. Face and forehead are essentially grey. From the middle of the upper skull, a dark diffuse line runs down the spine. Tail is grey-brown to deep brown, sometimes with a white tail.
*Louis et al., 2006; **Craul et al., 2007					
Anjajavy (This study)	1.09	29	30	NA	Predominantly gray color pattern with a white-grey ventral side and a pronounced white tail tip.

tions and individuals (see Tab. 1). Species described from a small number of lemurs within single populations might miss some morphological variations. This is the case with the white tail-tip, which was thought to be descriptive of *L. edwardsi* and absent in *L. grewcockorum* (Louis et al., 2006) but also turns out to be variably present among *L. grewcockorum* individuals (Craul et al., 2007; this study). The case of *L. grewcockorum* at Anjajavy, coupled with the recent confirmation of sympatric *M. danfossi* (Blanco et al. 2020), also highlights the potential for research-informed conservation at Anjajavy. Anjajavy boasts a new protected area under Category V (Harmonious Landscape) that comprises >10,000ha of mangrove, tsingy, dry deciduous forest,

and recovering agricultural land. Although the site is perhaps best known for its high-end ecotourism in the smaller private reserve, a growing research program across the entire protected area aims to characterize and monitor the endangered species endemic to this heterogenous landscape.

Acknowledgements

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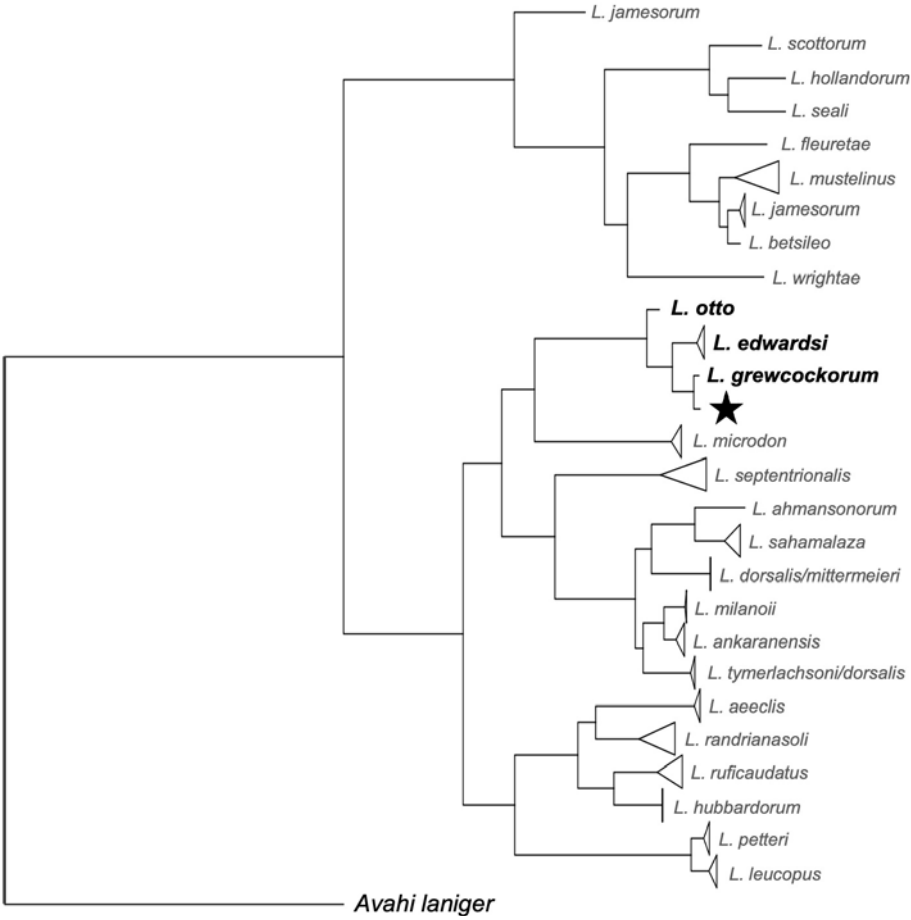


Fig. 2: Maximum likelihood phylogenetic tree of cytochrome B sequences with bootstrap support. The star denotes the sample from Anjajavy. The length of the branch connecting Avahi to the sportive lemur clade is minimized for ease of visualization.



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## Description of the gastrointestinal parasites of *Propithecus diadema* (Primates: Lemuridae) in the New Protected Area of Maromizaha, Eastern Madagascar

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### Abstract

The aim of this work is to identify and describe the gastrointestinal (GI) parasites of the lemur *Propithecus diadema* from the New Protected Area of Maromizaha – Andasibe, East Madagascar. 218 fecal samples were analyzed from adult females and males from two different groups. These *Propithecus diadema* host six morphotypes of GI parasites including: 1) four Nematode, of which two Oxyuridae (*Lemuricola* sp. and unidentified sp.), one Trichostrongylidae (*Pararhabdonema* sp.), and one other Nematode unidentified sp.; 2) one Cestode (*Hymenolepis* sp.); and 3) one Protozoan of the Coccidia order. This study expands upon the known GI parasites of diademated sifaka.

### Introduction

Parasites affect host survival and reproduction and thus are an important selective force shaping host physiology, ecology, and behavior (Coltman et al., 1999; Nunn and Altizer, 2006; Wood and Johnson, 2015, cited in Springer and Kappeler, 2016). Specifically, intestinal helminths and protozoa can lead to decreased energy absorption, pathological damage, and decreased reproductive success in their hosts (Hudson et al., 1992, 1998; Delahay et al., 1995; Hillegass et al., 2010, cited in Springer and Kappeler, 2016). Thus, the study of parasites is proving to be necessary to aid in the conservation of animal species (Altizer et al., 2007)

*Propithecus diadema* is Critically Endangered (Irwin, 2020). Few parasitological studies have been carried out on this species, and one species of Strongylidae, *Pararhabdonema longistriata*, has been reported to infect these sifaka inhabiting the Tsinjoarivo Protected Area in central eastern Madagascar (Irwin, pers. comm). The present study will describe the gastrointestinal parasites present in *Propithecus diadema* of Maromizaha which will complete the data on the natural history of lemur parasites. This is a first step for the knowledge of the parasites of this species in the protected area of Maromizaha which will lead us to other more extensive studies in the future that will contribute to the improved conservation of this endemic species of Madagascar. We examined the GI parasites present in diademed sifaka (*Propithecus diadema*) at the New Protected Area of Maromizaha. We predict that Maromizaha will have higher GI parasite diversity in diademed sifaka, when compared to Tsinjoarivo, because this location is hotter (average annual maximum temperatures: Maromizaha 19.4°C (Ranoarisoa, 2017), Tsinjoarivo 16.7°C (Holiarimino, 2013)) and has lower annual rainfall (average Maromizaha = 1850 mm/year (GERP,2015), Tsinjoarivo two principal sites were enregistred: Mahatsinjo= 2083 mm/year and Vatateza 2632 mm/year (Irwin *et al.*, 2019)). Parasite richness positively correlates to ambient temperature (Benavides *et al.*, 2012) and humidity (Nunn and Alitzer, 2006). Results from this study can help us understand variation in the parasite diversity in this species of sifaka.

Methods

Study Site

The Forest of Maromizaha, is located in the East of Madagascar (geographical coordinates 18°56'49"S - 48°27'53"E), in the Alaotra-Mangoro Region, District of Moramanga and within the Rural Communes of Andasibe and Beforona. It covers an area of 1880ha (GERP, 2015). It is located 140km

east of Antananarivo and 225km from Toamasina. The Maromizaha forest is located in the southeastern part of the Andasibe area and runs along the RN2 for 6.5km opposite the Analamazaotra Special Reserve. Straddling the Rural Municipalities of Andasibe and Ambatovola. The western part of the Maromizaha forest borders the southern part of the RN2 from the quarry of Amboasary (PK: 128 on RN-2) to the village of Anevoka (PK: 131). This protected area covers an area of approximately 1,880ha (GERP, 2015). The region studied is located on the steep eastern side of the island, overlooking the Betsimisaraka cliff. It is formed by a series of high hills separated by narrow valleys. The relief is very uneven, the slopes are strong and in general higher than 40% and the altitude varies between 700 and 1000m except on the highest point located at 1213m.

Vegetation

The forest of Maromizaha has a high rate of endemism of plants of the order of 77%. It is, because the vegetation is characterized by a typical species of the family of LILIACEAE: *Dracaena* known as "Dragon Trees", also called "Rainforest of Dragon Trees". This forest is well stratified and heterogeneous and the presence of several plant forms has been noted (trees and shrubs, lianas, bushes, epiphytes including orchids with a hundred species, herbaceous). The undergrowth is particularly dense with numerous lianas (GERP, 2008).

Climate

The Alaotra Mangoro Region has a humid, temperate, high altitude climate, with long, hot, overcast summers from December to March; however, winters in July and August are short and very cold, cool, and clear overall. The climate is rainy throughout the year. Over the course of the year, the temperature generally ranges from 11 to 27°C and is rarely

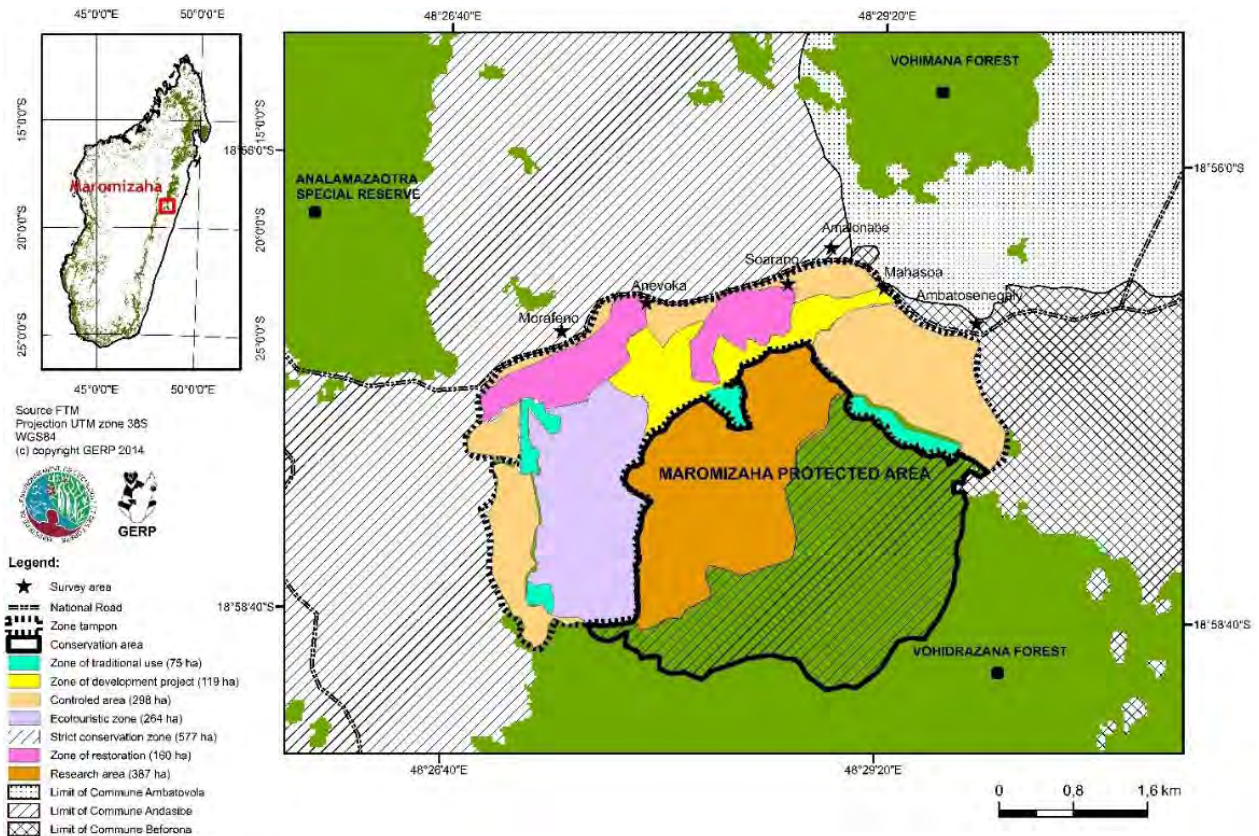


Fig. 1: Location of study site in Madagascar, the New Protect Area Maromizaha (GERP, 2015).



below 9°C or above 29°C. The study area is a frequent passage of tropical cyclones (GERP, 2015).

Temperature and precipitation

The most abundant precipitation occurs between December and March with an average of 288mm; the least rainy months are from August (99mm) to October (62.5mm). It is a humid tropical climate with an average annual rainfall of 1850mm and an average temperature of 20.4°C. In a year, it rains for 207 days of which 81 days are from December to March and 126 days from April to November. December to March are the hottest months with an average temperature of 21°C; and the freshest months are between June and September with an average temperature of 15.4°C. The mean minimum and maximum temperatures are equal to 14°.9°C in July and 21.2°C in February (GERP, 2015).

Data collection

The New Protected Area (NPA) of Maromizaha harbors nine groups of *Propithecus diadema*. We followed two groups (group 1, group 2) of habituated *Propithecus diadema* for a total of 480 hours, over two data collection periods of 20 days during 2019. The first data collection period was April to May and the second data collection period was July to August. Each group had one adult male and one adult female. Each animal was followed for five days per data collection period. We used continuous focal animal sampling (Altmann, 1974) during the animal survey, and collected all fecal samples after animal defecation.

Fecal sample analysis

We collected 218 fecal samples from the four focal individuals of diademed sifaka. Samples immediately collected and preserved in the tube containing 4% formalin after this defecation. 300mg of the faecal sample were analysed. Samples were analyzed through the modified protocol of the McMaster flotation egg counting technique (Sloss *et al.*, 1994) by using a potassium iodide reagent (Meyer-Lucht and Sommer, 2005).

Samples were triturated and mixed with 4.5ml of potassium iodide solution and filtered through a fine mesh sieve to obtain the preparation to be examined. Once we have filled the two chambers of the Mc Master slide were filled with this preparation, the slide was rested for 10 minutes on the microscope stage while the eggs rise to the surface. We observed and counted the eggs and larvae using 10x magnification for counting, and 40x for identification. Each type of parasite observed was measured, described, photographed and counted separately. This coproscopy was undertaken in the laboratory of the Mention Anthropobiologie et Développement Durable at the University of Antananarivo.

The identification of the parasites was based on egg morphology and made from several documents, books, studies made by specialists and previously published research (Leger *et al.*, 1977; Irwin and Raharison 2009; Raharivololona, 2009; Huffman and Chapman, 2009; Rambeloson, 2014).

Results

Parasite specificity

A total of 218 samples obtained from 4 individuals (2 males and 2 females from two groups) were analyzed for intestinal parasites. We detected eight morphotypes: 1) six Nematode whose, two Oxyuridae (*Lemuricola* sp. and unidentified sp.), two Trichostrongylidae (*Pararhabdonema* sp. and unidentified sp.), one Strongylidae (unidentified sp.), and one Nema-

tode (unidentified sp.); 2) one Cestode (*Hymenolepis* sp.); and 3) one Protozoan of the Coccidia order (Tab. 1).

Tab. 1: List of parasites observed in *Propithecus diadema* of Maromizaha

Class	Family/ Order	Genus
Nematode	Family: Oxyuridae	<i>Lemuricola</i> sp.
	Family: Oxyuridae	Unidentified species
	Family: Trichostrongylidae	<i>Pararhabdonema</i> sp.
	Unidentified Nematode	Unidentified species
Cestode	Family: Hymenolepididae	<i>Hymenolepis</i> sp.
Protozoan	Order: Coccidia	Unidentified species

Parasite descriptions

Parasite 1: *Lemuricola*

Kingdom: Animalia  
Phylum: Nemathelminthes  
Class: Nematoda  
Order: Oxyurida  
Family: Oxyuridae  
Subfamily: Enterobiinae  
Genus: *Lemuricola*

Description: The egg is generally ellipsoid of light brown or colorless color. The two poles are reduced and equal. The shell is double, smooth and fine. The egg contains a morula or cluster of cell or blastomeres. Size: 40-45µm x 80-105µm (Fig. 2).

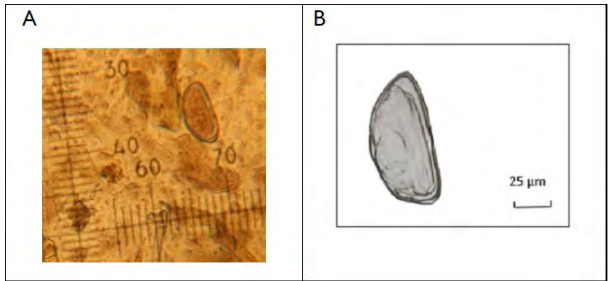


Fig. 2: Pictures (A) and schemas (B) of *Lemuricola* (source: N. Raveloson).

Parasite 2: Unidentified Oxyurids

Kingdom: Animalia  
Phylum: Nemathelminthes  
Class: Nematoda  
Order: Oxyurida  
Family: Oxyuridae  
Genus: Unidentified  
Species: Unidentified

Description: The eggs are elongated, asymmetrical with one flat side and another convex side. The shell is simple, smooth and rather thick. Size: 30-40 x 80-115µm (Fig. 3).

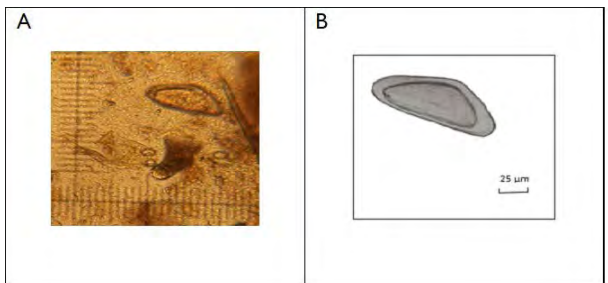


Fig. 3: Pictures (A) and schemas (B) of unidentified oxyurids (source: N. Raveloson).



**Parasite 3: *Pararhabdonema* sp.**

Kingdom: Animalia

Phylum: Nematelminthes

Class: Nematodes

Order: Strongylida

Family: Trichostrongylidae

Genus: *Pararhabdonema* sp.

Description: The egg has a somewhat ovoid shape with two symmetrical poles. It is surrounded by a thin wall and contains a polysegmented embryo, it is a morula more than 16 blastomeres. Size: 75-80µm x 40-45µm. During the fecal analysis, different states of development are found in this egg, like the number of morula and a clearly visible embryo (Fig. 4).

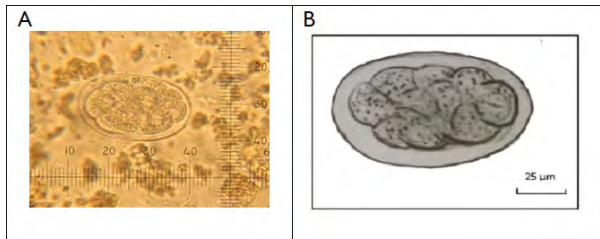


Fig. 4: Pictures (A) and schemas (B) of *Pararhabdonema* sp. (source: N. Raveloson).

**Parasite 4: Unidentified Nematode**

Kingdom: Animalia

Phylum: Nematelminthes

Class: Nematoda

Family: Unidentified

Genus: Unidentified

Species: Unidentified

Description: The egg has an asymmetrical shape with a thick double membrane shell. The morula occupies the whole content of the egg. The egg is of brown color. Size: 50 x 30µm (Fig. 5).

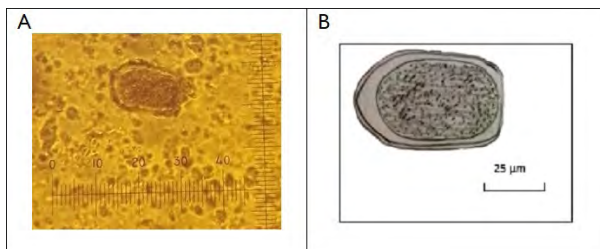


Fig. 5: Pictures (A) and schemas (B) of Unidentified Nematode (source: N. Raveloson).

**Parasite 5: *Hymenolepis* sp.**

Kingdom: Animalia

Phylum: Platyhelminthes

Class: Cestoda

Order: Cyclophyllidae

Family: Hymenolepididae

Genus: *Hymenolepis*

Species: Unidentified

Description: It is a cestode egg with brown color, rounded shape approximately 75µm long. Double membrane shell and without polar filaments, the inner shell is slightly thickened. This egg has a hexacanth embryo and the six hooks move two by two inside (Fig. 6).

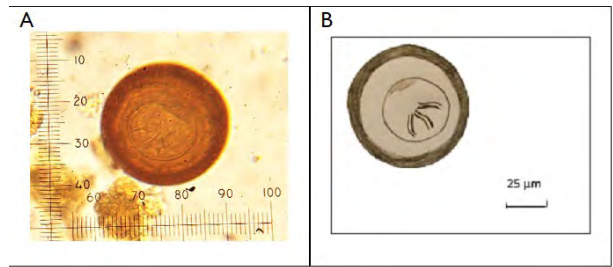


Fig. 6: Pictures (A) and schemas (B) of *Hymenolepis* sp. (source: N. Raveloson)

**Parasite 6: *Coccidia***

Kingdom: Animalia

Class: Protozoa

Order: Coccidia

Family: not identified

Genus: not identified

Species: not identified

Description: the oocyst is round and of brown color with a simple, thick and rough shell (Fig. 7). The nucleus occupies almost the entire content of the cyst and has a vacuole at its apical side. Size: 30 – 40µm.

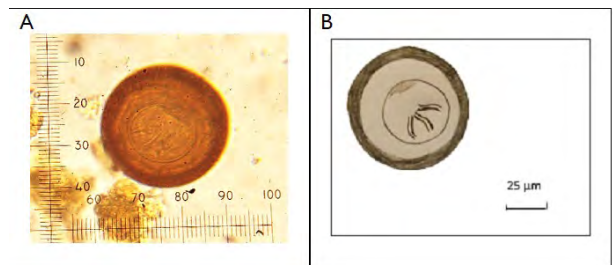


Fig. 7: Pictures (A) and schemas (B) of *Coccidia* (source: N. Raveloson).

**Parasite prevalence**

For this study of intestinal parasites in *Propithecus diadema*, 218 tubes containing feces from 4 individuals were analyzed, 54 from the adult male of group 01 and 61 from the male of group 02; 46 from an adult female of group 01 and 57 from the adult female of group 02. This difference in numbers is due to the difference in the number of defecations in these target individuals during the follow-up. We collected these 218 samples for 40 days with 10 days for each individual. Of the four individuals tested, a cestode was found only once in the male from group 01.

**Discussion**

Six parasite egg morphotypes were encountered in the 218 fecal samples from four *Propithecus diadema* individuals inhabiting the forest of the New Protected Area of Maromizaha. The fecal material of this lemur species in Maromizaha contained more parasite egg morphotypes than that in Tsinjoarivo (Irwin, pers. com.). In this forest, only one parasite species, *Pararhabdonema longistriata*, has been reported to infect *Propithecus diadema* (Irwin, pers.com). This difference could be due to several factors, as many authors have already reported on the interaction of intrinsic and extrinsic factors on the parasite load of an animal species. Benavides *et al* (2012) observed that parasite richness was positively correlated to day range and temperature in wild social primate population. Maromizaha appears to be warmer than Tsinjoarivo, average daily high Maromizaha is 19.4°C (Ranoarisoa, 2017), which could have resulted in greater diversity

Tab. 2: Number of positive samples and parasite prevalence.

	Number of tests	Lemu-ricola	oxyu-ridea	Pararhab-donema sp	Nema-tode ID	Hymi-nolepis	Coc-cidia
Male 01	54	1	3	42	4	1	52
Male 02	61	3	11	48	4	0	56
Female 01	46	0	1	30	0	0	42
Female 02	57	1	2	45	1	0	54
Total	218	5	16	165	9	1	204
Number of individuals tested		4	4	4	4	4	4
Number of individuals tested		3	4	4	3	1	4
Prevalence (%)		75	100	100	75	25	100

in parasite species infecting *P. diadema* in Maromizaha forest than in Tsinjoarivo forest. Other factors, as several authors have reported, could also cause differences in the parasite species richness that animals harbor, such as habitat size and quality. Individual *Microcebus murinus* living in a small fragment in the Mandena littoral forest, Southeastern Madagascar, harbor more parasite species than that from a large fragment (when both fragments are good quality) (Raharivololona and Ganzhorn, 2009). Maromizaha is smaller (1880ha) (GERP, 2015) than Tsinjoarivo (26,471ha) (Randriantsizafy, 2004), which may thus have impacted on species richness. The *Pararhabdonema* that we have here may be the same one found in *Propithecus diadema* from Tsinjoarivo, but with the coproscopy method, it is difficult to determine with certainty exactly the genus and species. It is necessary to take into account also the threats. According to our studies during the fieldwork and the report of GERP, the habitat of these group of *P. diadema* does not present any threat of human origin; this might explain the frequency and length of time spent on the ground, which could be an area of high contamination. Maromizaha is a research and tourism site where there is permanent presence of humans such as guides, researchers, rangers and tourists. These human activities in the wildlife area could have impacts on their parasitic infestations. According to Ragazzo et al. (2018), the distance to human settlements explains the variation in *Entamoeba histolytica* infection observed in lemurs in the Ranomafana National Park in Southeastern Madagascar.

Conclusion

Six egg morphotypes of gastrointestinal parasites were identified from fecal samples of *Propithecus diadema* inhabiting the forest of the New Protected Area of Maromizaha. This lemur population appears to harbour more parasite diversity, when compared to the only other existing study documenting parasitism in diademed sifaka. The climatic and seasonal factor plays an important role in the parasite infestation. More data are needed to fully understand the parasites of the diademed sifaka, including the effect of season.

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## Captive populations of lemurs in European zoos: mismatch between current species representation and ex-situ conservation needs

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### Abstract

Captive breeding programmes in zoological institutions can be important tools for conservation. Lemurs are popular zoo animals and are present in hundreds of zoos outside of Madagascar. But are captive lemur populations integrated into ex-situ conservation efforts? Are lemur species in zoos chosen because of their conservation value, popular appeal, or some other considerations? Here, we address these questions, focusing on zoological institutions of the European Association of Zoos and Aquaria (EAZA) network. We assess whether lemur species presence in EAZA zoos is linked to taxonomy, International Union for the Conservation of Nature (IUCN) threat category and/or biological traits (body mass and diet). We find that a total of 22 of 109 lemur species are currently kept in EAZA zoos (July 2021). Our results show that some species (e.g. *Lemur catta*, *Varecia variegata*) and genera (e.g. *Eulemur*) are over-represented in zoos, whereas some species-rich genera are poorly represented (*Microcebus*) or not represented at all (*Lepilemur*). Body mass and diet are strong indicators of presence in captivity, with larger or frugivorous species overrepresented, and small or folivorous species underrepresented. A total of 15 species are currently bred under collaborative European ex-situ programmes. There is no link between severity of IUCN status and species presence in zoos, and endangered or critically endangered species are not more likely to be found in captivity. These results suggest that species in EAZA zoos have predominantly been chosen due to their appeal to the public, ease of husbandry or other practical and administrative constraints, rather than based on potential benefits for conservation. Addressing the imbalance between the EAZA's current collection of captive lemur species and the lemur species of conservation priority would lead to better representation of the threatened biodiversity of lemurs under active ex-situ population management, potentially acting as a failsafe against extinction.

### Introduction

Lemurs are a diverse but highly endangered group of primates endemic to the island of Madagascar. Of the 109 ex-

tant species of lemur, 103 (94%) are considered threatened by the International Union for Conservation of Nature (IUCN), that is, they are currently classified as vulnerable, endangered or critically endangered (IUCN, 2021). Given the large number of species at risk and the increasing level of threats (habitat destruction, hunting), lemur conservation efforts have become multifaceted and employ a variety of strategies (Schwitzer *et al.*, 2013a). These strategies must focus on assuring viability of wild populations in their natural habitats, but, given the rise of anthropogenic threats in Madagascar, it has also become increasingly important to maintain conservation-focused captive breeding ex-situ programmes (Schwitzer *et al.*, 2013b). Ex-situ conservation through captive assurance colonies can have multiple advantages: complementing and supporting local conservation programmes in Madagascar, maintaining genetic diversity, aiding population recovery and reintroductions, as well as raising awareness through educational and visibility activities (Kleiman, 1989; Zimmermann, 2010; Schwitzer *et al.*, 2013b). Several lemur ex-situ conservation programmes are currently running in Madagascar, with strong links to in-situ management initiatives (King *et al.*, 2013; Schwitzer *et al.*, 2013b). Beyond Madagascar, captive breeding with conservation purposes has also been set up in various countries all over the world. Under the “One Plan” approach, populations of a lemur species within and outside of Madagascar, in the wild and in captivity, should all be managed as a meta-population, increasing the chances of success in an unpredictable future (Byers *et al.*, 2013; Schwitzer *et al.*, 2013b). In addition to ex-situ captive-breeding programmes, lemurs are also currently kept in zoos worldwide due to their attractiveness and ability to attract visitors (Carr, 2016). Due to their cuteness, exoticism and popularity, lemurs have gone global – they are found in zoological institutions on six continents. While many populations in zoological collections have a link to conservation (often indirect, through raising awareness), lemurs are not always held in captivity with the aim of protecting them and many lemur populations are not part of formal ex-situ conservation programmes. Lemurs are often kept for purely commercial or entertainment purposes (e.g. roadside zoos or tourist attractions) both in Madagascar and abroad (Reuter *et al.*, 2019). Previous research in mammals has shown that the selection of mammalian families represented in zoos is strongly linked to body size and the degree of human-perceived attractiveness (Frynta *et al.*, 2013). Mammals that are perceived as less attractive to zoo visitors tend to be underrepresented in zoos, even if they are of high conservation priority (Frynta *et al.*, 2013). Therefore, we can expect the representation of lemurs in zoos to also not be tightly correlated with conservation needs, but to be driven by other considerations. For example, some lemur species, such as the ring-tailed lemur (*Lemur catta*), are zoo “stars”, able to attract visitors due to their recognizable morphological features and behaviour, and are frequently portrayed in popular culture, nature documentaries and cartoons (Sauter *et al.*, 2015; Clarke *et al.*, 2019). Furthermore, unlike ring-tailed lemurs, which are omnivorous and have a flexible behaviour and ecology, not all lemur species are easily and viably kept in zoos, as husbandry constraints can influence welfare, survivorship and ability to breed under captive conditions (Caravaggi *et al.*, 2018; Bailes *et al.*, 2020).

In this study, we focus on the species of lemurs that are currently being held in zoological institutions that are members of the European Association of Zoos and Aquaria (EAZA). The EAZA currently has over 400 member institutions in 48 countries, most of them in Europe, but also includes a



few institutions on other continents. The current collection of lemur species in EAZA institutions has been partly shaped by historical and regulatory contingencies. EAZA institutions often do not have a choice as to the species of lemurs they can include in their collections, as there are several bodies at play which help decide which species will be housed. Information on the origin of lemur populations in EAZA institutions is patchy, with most founders coming from the wild in Madagascar or others from institutions elsewhere (Zootierliste, 2021). Records show that several species of lemur have been imported from Madagascar to European zoological collections over the years (Zootier-liste, 2021), often with mixed results, with some species doing well and others not surviving in captivity. For example, eight indris (*Indri indri*) imported from Madagascar to the Jardin des Plantes in Paris in 1939 died within a month of arrival due to stress and malnutrition (Crandall, 1964; Zootierliste, 2021). The first European zoos were mostly interested in collecting rare or “exotic” species to show to European audiences, and were not focused on conservation. As attitudes towards conservation changed, zoos felt the need to combine efforts, and the first European-based captive breeding programs with conservation goals in mind were set up in 1985 (Nogge, 2007). This eventually led to the creation of the current EAZA-run European Ex-situ Programs (EEP's), which aim to maintain long-term viable healthy captive populations of various threatened species (Nogge, 2007). EAZA's EEP's are typically managed by a zoo which holds the species and acts as a coordinator. The EEP programme manages population size, genetic diversity and demography of the species, coordinates exchange of individuals between partner institutions, and facilitates fundamental research. EEPs involve inter-zoo collaboration on husbandry, studbooks (registry of the captive individuals of a species) and exchange of individuals to preserve genetic diversity. Shortly after the first EEPs were established, a review of lemur captive breeding was published, entitled “The role of zoos and captive breeding in lemur conservation” (Durrell, 1989). In that review, the author referred to a total of 22 extant species of lemur, 17 of which were being held in ex-situ programs at the time. There have been substantial changes since the publication of the review by Durrell – for example, since 1989 dozens of new species have been discovered and described (Mittermeier *et al.*, 2008, 2014). Therefore, a review of the status of captive breeding of lemurs is overdue and it may allow us to gain insight into current gaps in lemur species representation.

In this study, we list and characterize the lemur species that are currently kept in captivity in EAZA member institutions. We assess whether species currently held in captivity were chosen mostly for conservation reasons, popular appeal, or biological constraints. We aim to answer the following questions: i) what is the species composition of lemur populations in European zoos and how are these integrated into ex-situ conservation programmes? ii) which characteristics have influenced the choice of lemur species that are currently represented in ex-situ collections? To answer ii) we focus on taxonomy, IUCN threat category, body mass and diet of the lemur species. If species have been chosen based on their conservation priority, we would expect species with more severe IUCN threat statuses (e.g. endangered, critically endangered) to be well represented in zoos. If species have been chosen for their popular appeal, we may expect larger-bodied species to be overrepresented, as visitors are known to show greater interest in large animals (Moss and Esson, 2010). Finally, we may expect species with generalist or less specialized diets to be favoured in living collections, given that replicating natural diet as accurately

as possible is essential for species survival in captivity, with some species with specialized diets being particularly challenging or costly to feed (Sha, 2014).

## Methods

All data used in this study are provided in Tab. S1 (available at: <https://data.mendeley.com/datasets/6wxpfrmjz25/1>). From the IUCN website (IUCN 2021), we obtained the list of extant lemur species currently recognized by that organisation. For each species we gathered their IUCN Red List status (as of July 2021). For completeness, we added one recently described lemur species that is not currently on the IUCN list, *Microcebus jonahi* (Schübler *et al.*, 2020), which we classified as ‘not evaluated’. We obtained mean body mass data for each species from a published dataset of body masses of wild lemurs (Taylor and Schwitzer, 2012). We classified species into the following categories: <0.2kg; 0.2kg to 1kg; 1kg to 2kg; >2kg. For 13 recently described species, body mass data were not available in Taylor and Schwitzer (2012), so for those species we gathered data from other sources or inferred the mean body mass category based on the modal body mass category for the genus. All these cases and respective references are indicated in Tab. S1. Data on diet were obtained from the IUCN website (IUCN, 2021). Lemur diets can be difficult to categorize, as diets can be diverse, highly seasonal and are often insufficiently studied or unknown (Godfrey *et al.*, 2004; Beeby and Baden, 2021). We chose to classify species into broad categories based on their most common dietary categories: “bamboo”, “frugivorous”, “folivorous”, “gummivorous”, “insectivorous”, “omnivorous”. These diets are not rigid and are “fluid”, but using this classification scheme we aimed to highlight general patterns in diet. For several species, diet data were not available on the IUCN website, and for these we assumed their diet to be the same as for other congeners (based on the genera for which data on diet were available on the IUCN website, diet under the broad categories we use is highly conserved within genera).

We obtained data on the lemurs that are currently held in zoological institutions that are members of EAZA (Tab. S1). Our focus on EAZA collections is due to the fact that there is relatively up-to-date recordkeeping and a good overview of the data for zoos that are part of this association. The Species360 Zoological Information Management System (ZIMS), a database of wild animals under care, was used to extract data on: identity of lemur species currently held in captivity, number of species and number of zoos that keep each lemur species (ZIMS, 2021). In addition, we used the database Zootierliste, which compiles information on current and former holdings in EAZA member institutions, to obtain information on lemur species that were held in the past but are not currently held (Zootierliste, 2021). When compiling data from these databases, no data were excluded, hybrids were included under one of the parent species and subspecific taxa were lumped together. The number and identity of species that are currently held in EAZA institutions reported by ZIMS and Zootierliste were the same. The number of institutions currently holding lemurs varies between both databases, so for this metric we favoured using ZIMS, as it is a more formally managed database. We obtained information on current EAZA ex-situ programmes (EEP's) from the EAZA website (EAZA, 2021).

We assessed whether the fact that a species is currently held in captivity within an EAZA institution is related to the species' taxonomic classification (genus), IUCN Red List status, body mass and diet. These explanatory variables were plotted against the proportion of all species for

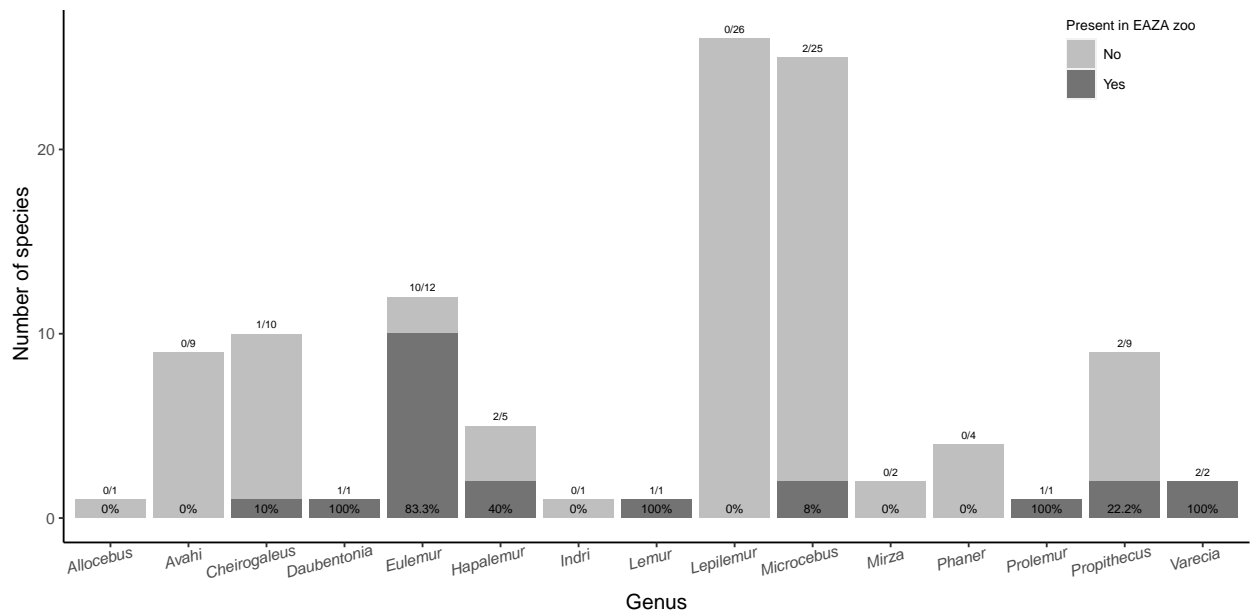


Fig. 1: Number and percentage of species of lemurs held in EAZA institutions as of July 2021. Numbers above the bars represent number of species in captivity/ total number of species in the genus. Percentage numbers shown on bars represent percentage of species present in EAZA zoos for each genus (July 2021).

each variable category that are currently held in captivity. We statistically tested for an effect of IUCN status, body mass and diet on the proportion of species under captivity using a test of equal proportions, where we compared the proportion of species of each category that are present in captivity, testing the null hypothesis that the proportions in several categories are the same. We used the function ‘prop.test’ in R, which is part of R’s basic “stats” package. As sample sizes are low, we did not test for interactions between variables, and treated each variable separately. However, we acknowledge that variables can be correlated, and that the interaction of different variables (e.g. diet and body mass) may influence the representation of species in captivity.

Tab. 1: Lemur species that were previously held in European zoological institutions, but which are no longer held, according to ZIMS and Zootierliste (2021).

Species	First record	Last record	IUCN status 2021
<i>Allocebus trichotis</i>	1991	2002	EN
<i>Cheirogaleus crossleyi</i>	1952	1961	VU
<i>Cheirogaleus major</i>	1906	2019	VU
<i>Eulemur sanfordi</i>	Unknown	2003	EN
<i>Hapalemur griseus griseus</i>	1893	2011	VU
<i>Indri indri</i>	1939	1939	CR
<i>Lepilemur ruficaudatus</i>	1986	1993	CR
<i>Microcebus myoxinus</i>	1890	Unknown	VU
<i>Microcebus rufus</i>	1970	2005	VU
<i>Mirza coquereli</i>	1885	1917	EN
<i>Mirza zaza</i>	1986	2009	VU
<i>Phaner furcifer</i>	1908	1996	EN
<i>Propithecus diadema</i>	1908	Unknown	CR
<i>Propithecus verreauxi</i>	1900	1912	CR

Results

As of July 2021, 22 different species of lemur are represented in zoological institutes that are members of EAZA, representing 20.2% of all extant lemur species (total 109). A total

of 236 EAZA zoos currently hold at least one lemur species. According to ZIMS and Zootierliste (ZIMS 2021; Zootierliste 2021), at least 14 lemur species previously held in European collections are currently absent. These species are shown in Tab. 1. None of these were part of the priority list by Schwitzer *et al.* (2013b), but several of them are currently highly threatened.

Taxonomic coverage

The percentage of lemur species per genus currently held in captivity is unequal (Fig. 1). Of the largest genera in terms of numbers of species, the most widely represented in zoos is *Eulemur*, with 10 out of 12 species currently in captivity. Genus *Hapalemur* has less than half of its species in EAZA zoos (2 out of 5). Four species-poor genera have all their species in zoos: *Daubentonia*, (n=1 species); *Lemur*, (n=1 species); *Prolimur*, (n=1 species); and *Varecia*, (n=2 species). By contrast, the most species-rich genera are poorly represented: no species of *Lepilemur* (out of 26 species) and fewer than 10% of *Microcebus* species (out of 25 species) are represented in EAZA zoos. In fact, 6 out 15 genera of lemurs are not present at all in EAZA zoos.

At the species level there is also great unevenness (Fig. 2). If we use the number of institutions keeping a species as a proxy for number of individuals, just three species (*Lemur catta*, *Varecia variegata* and *Varecia rubra*) make up over 60% of the captive lemur ‘population’, while the other 19 species combined make up around 40%. *Lemur catta* is by far the most commonly kept species in EAZA zoos, held in 212 institutions. The majority of species are kept in fewer than 20 zoos (Fig. 2).

Body mass and diet

The presence of a particular lemur species in zoos is strongly linked to body mass and diet. Large body sized species are overrepresented in zoos and small body sized species are underrepresented (Fig. 3, test of equal proportions:  $\chi^2= 30.61$ ,  $df = 3$ ,  $p<0.001$ ). Species with frugivorous and omnivorous diets are more likely to be currently kept in captivity (Fig. 3, test of equal proportions:  $\chi^2= 22.855$ ,  $df = 5$ ,  $p<0.001$ ). The three

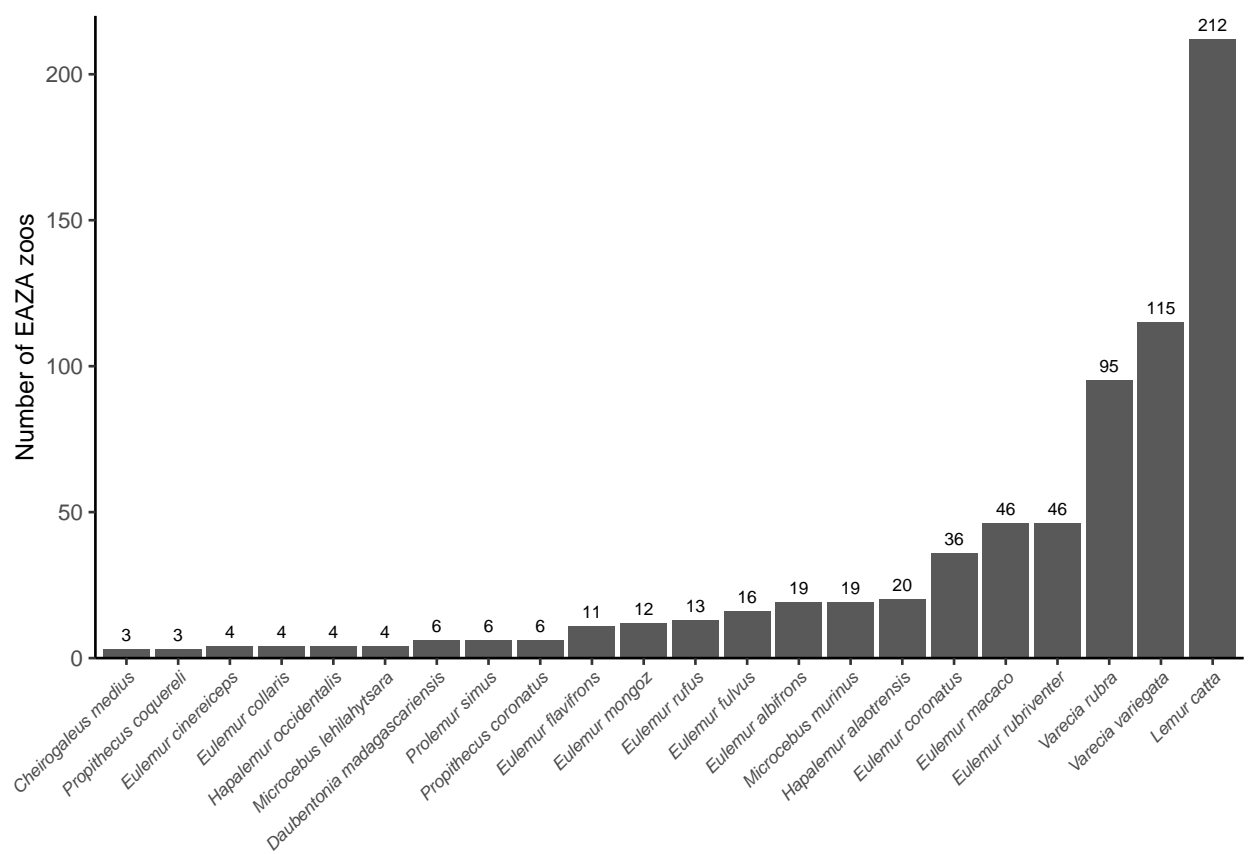


Fig. 2: Number of EAZA institutions that hold at least one lemur species, as of July 2021. Numbers on top of bars represent number of zoos that hold the given species.

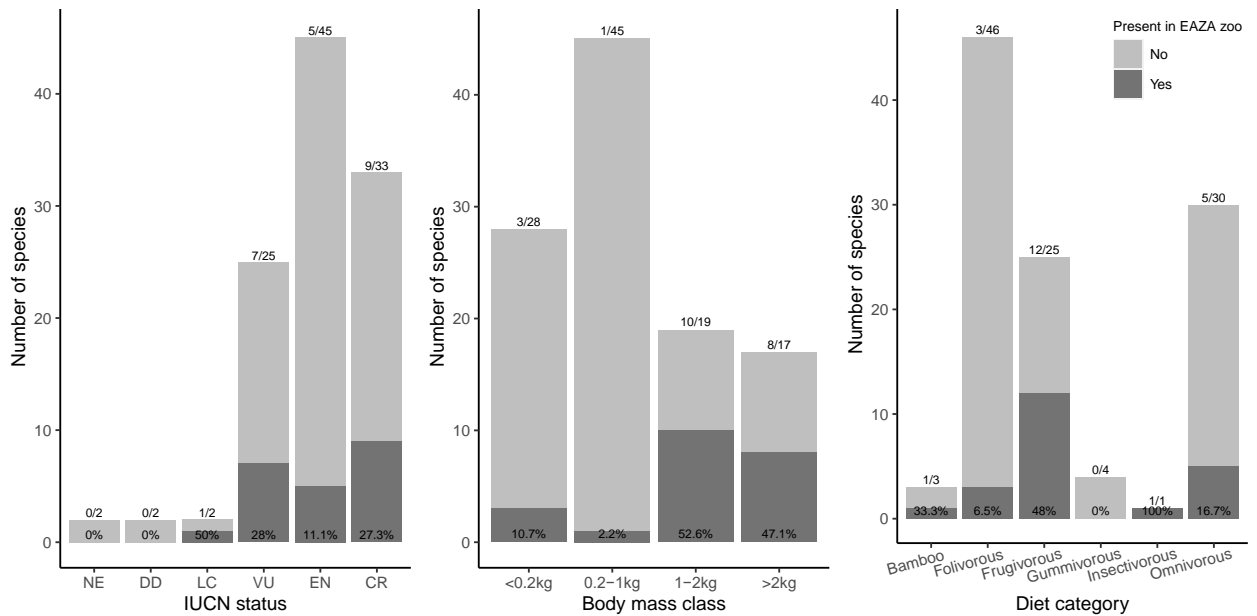


Fig. 3: Representation of lemur species in EAZA zoos by IUCN status (a), body mass class (b) and diet (c). Number and percentage of species for each class held in EAZA zoos, as of July 2021. Numbers above the bars represent number of species in captivity/ total number of species in the category. Percentage numbers shown on bars represent percentage of species present in EAZA zoos for each category (July 2021).

most common diet types across all lemur species are folivory, frugivory and omnivory, all with more than 20 species each. However, species that are mostly frugivorous are clearly overrepresented, with 12 out of 25 species in zoos, whereas species that are mostly folivorous are underrepresented, with only 3 out 46 species in zoos.

**Conservation status and EEPs**  
Of the 22 species currently held in EAZA zoos, 21 are classified as threatened with extinction by the IUCN (threat categories ‘vulnerable’, ‘endangered’ or ‘critically endangered’), and one as ‘least concern’ (*Microcebus murinus*). The fact that the majority of species in captivity are threatened



is not surprising, given that only two out of all lemur species are currently classified as non-threatened (“least concern”). Importantly, for the threatened lemur species in captivity, there is no link between severity of threat status and the existence of an ex-situ program. The level of threat according to IUCN status is not a good predictor of the presence of an ex-situ population (Fig. 3, test of equal proportions:  $\chi^2 = 6.392$ ,  $df = 5$ ,  $p > 0.05$ ). In other words, more threatened species are not more likely to be currently found in captivity than expected by chance. Finally, out of the 22 species currently held in captivity, 15 receive active coordination in captive breeding in the form of an EEP (July 2021).

## Discussion

A total of 22 lemur species, about one fifth of all extant species, are currently held in at least one EAZA member zoo. Many species of lemurs have only been discovered in the last 20 years, are extremely rare or difficult to find in the wild (Mittermeier *et al.*, 2014). Thus, the number (22) and percentage (20.1%) of species currently held in zoos can be considered respectable. Lemurs may have an “advantage” over many other taxa when it comes to zoo representation, as prosimians (which include strepsirrhines) were ranked as the second most attractive group of mammals to zoo visitors (Whitworth, 2012), which likely makes it economically beneficial for zoos to add species of lemur to their collections. With one in five species held in zoos, lemurs are well represented compared to threatened terrestrial vertebrates in general, for which the value is one in seven (Conde *et al.*, 2011).

Our analysis of the current situation of lemur ex-situ population composition in Europe suggests that there is bias in the species that are currently represented. We found that representation of lemur species in EAZA zoos is uneven with regards to taxonomy (genus), body mass and diet, with some categories being more widely represented than others. By contrast, we find that IUCN threat status does not play a role in which species are currently represented in zoos. While there may be species not present in EAZA zoos that are currently held in captivity in non-member institutions (e.g., on other continents), we do not expect that number to be high. For example, all the 12 species of lemur that are currently held in captivity (July 2021) in the most diverse collection of lemurs outside of Madagascar - the Duke Lemur Center in the USA - are all also currently held in EAZA zoos (Duke Lemur Center, 2021). Our results and discussion in terms of species representation are therefore likely demonstrative of the global status of lemur captive colonies outside of Madagascar. However, it should be noted that our results regarding lemur species representation are to some extent dependent on active bookkeeping and regular updates on ZIMS.

### Biological traits that influence current representation in zoos

Two key predictors for the presence of a lemur species in zoos were found to be body mass and diet. Species with large body mass are clearly overrepresented in zoos. A total of 18 out of the 22 captive species (82%) weigh more than 1 kg, despite large body mass species making up only 33% of the total species of lemurs. Small body size categories (below 1 kg) are rarely represented in zoos, despite representing a majority of lemur species. The fact that large species are favoured in zoos is well known also in other types of animals (Moss and Esson, 2010; Frynta *et al.*, 2013). Large animals are appealing to visitors and easier to spot in enclosures, and this may be behind the decision to favour these types of lemurs in European zoos. Indeed, the level of

attraction and interest of zoo visitors was previously found to be positively correlated with body size (Moss and Esson, 2010). Perhaps for these reasons, zoo animal species tend to be larger than their close relatives not held in zoos (Martin *et al.*, 2014).

In terms of diet, frugivorous lemur species are found in zoos at higher numbers than expected by chance, while folivorous and gummivorous are underrepresented. While diet is unlikely to influence the level of attractiveness for visitors, it affects the chances of sustaining an ex-situ population. Species with narrow dietary requirements (e.g. feeding on leaves of specific plant species) are more difficult to keep in a captive environment. In the field of animal husbandry, folivorous diets are considered to be one of the most difficult to replicate (Sha, 2014). Leaves of endemic plants to which species are specialized may contain compounds that are difficult to provide in a captive setting. For example, indri (*Indri indri*) are particularly difficult to keep in captivity (LaFleur *et al.*, 2020) which may be partly due to the fact that this species has a largely folivorous diet (Quinn and Wilson, 2002).

We also found that certain genera are overrepresented in zoos. *Eulemur* and *Varecia*, both genera with large body-sized and mostly frugivorous species, are well represented in zoos. Species-rich genera with poor representation in zoos are either exclusively folivorous (*Avahi*, *Lepilemur*, *Propithecus*), or exclusively composed of species with small body mass (*Microcebus*). There are likely other factors at play that we did not consider here that may have also influenced the choice of species brought in captivity. For example, a good candidate is activity pattern (diurnal/nocturnal), as nocturnal species may be harder to maintain in zoos, require special conditions for visitors to be able to see them, and species with low diurnal activity may be less attractive to visitors (Moss and Esson, 2010). Indeed, several lemur genera with poor or no representation in zoos are exclusively nocturnal (*Lepilemur*, *Microcebus*, *Phaner*). Nevertheless, nocturnal lemurs are not completely absent from zoos. The aye-aye (*Daubentonia madagascariensis*), and the fat-tailed dwarf-lemur (*Cheirogaleus medius*), are examples of nocturnal lemur species that are currently held in EAZA facilities, the aye-aye even being part of an EEP. *Eulemur* species, many of which are in zoos or are subject of an EEP, can be both diurnal or nocturnal. Other traits that may be interesting to examine in the future are mating system, arboreality, or behavioural traits related to stress, all of which can affect the ability of species to survive and/or breed in captivity. Finally, it is likely that the interaction between traits rather than a specific trait per se may be the determining factor for the selection of lemurs for captive breeding.

### Ex-situ populations and conservation

A total of 87 species of lemurs are currently absent from EAZA zoos, including 40 endangered and 24 critically endangered species that are of high conservation priority (IUCN 2021). A key result of our study is that the current representation of lemur species in European zoos is not linked to the severity of their IUCN status. For example, critically endangered or endangered species are not more likely to be found in zoos than species classified as vulnerable. If the choice of species were mostly conservation driven, it would seem good practice to give higher priority to species that are more endangered, but that does not seem to be the case. Research on birds and mammals has previously showed that current species representation in zoos is not related to conservation needs (Frynta *et al.*, 2013; Martin *et al.*, 2014). In the case of lemurs, there may be several reasons for this: threatened lemur

species may be intrinsically more difficult to breed in captivity (e.g., diet, habitat or climate specialists), captive programmes are costly and funding is limited, or highly threatened species may by chance be less attractive to visitors (e.g., small body size, nocturnal). Another possible reason could be linked to the finding of Frynta *et al.* (2013) that species-rich mammalian clades tend to be poorly represented in terms of proportion of species, as a few individuals are perceived as sufficient to represent the group to most visitors.

Another noteworthy result is the fact that only 15 species are currently managed under EEPs, which means that several species currently held in captivity are not actively managed as part of European-wide breeding programmes. Species currently in captivity but not formally part of an EEP include one taxon classified as critically endangered (*Eulemur cinereiceps*) and one classified as endangered (*Eulemur collaris*). In 2013, Schwitzer and colleagues (Schwitzer *et al.*, 2013b) proposed a list of priority lemur species for ex-situ conservation. However, many of those priority species are still not yet held in captivity in EAZA institutions, including critically endangered *Cheirogaleus sibreei*, *Lepilemur sahamalensis*, *Microcebus berthae* and *Propithecus candidus*. Of course, expanding species breadth for ex-situ conservation is not a simple endeavour, as it may require extensive preparation to ensure animal welfare. Therefore, embarking on improved husbandry methods to make it possible to incorporate priority species into EEPs should be an important next step. However, even if good captive conditions can be established, adding new species to the global zoo collection is challenging, particularly if new founding populations need to be established from the wild, as permits and public opinion make it difficult to capture and export wild individuals.

For captive breeding outside of Madagascar to be meaningful, it should have a measurable positive effect on in-situ conservation in the country, with captive colonies acting as a reservoir of individuals and genetic diversity stock for the future of the species, and not just be used for human entertainment or commercial reasons. Arguably the most direct way to do this is to eventually release animals into the wild. Releases and translocations of lemurs into wild settings are rare and have had mixed results (Donati *et al.*, 2007; Day *et al.*, 2009; Schwitzer *et al.*, 2013b). An attempt was made to release 13 captive-born black and white ruffed lemurs (*Varecia variegata*, CR) into their native wild range (Britt *et al.*, 2004). Five of them survived in the wild for more than a year and three of them had offspring. The project was found to be a relative success, showing how captive breeding can reinforce wild lemur populations (Britt *et al.*, 2004). Another advantage of captive breeding is that it provides a 'failsafe' population in case the animal goes extinct in the wild. The benefit of ex-situ populations also lies with the education opportunities they offer. If the public is to care for conservation of lemurs, it first needs to learn about them. A zoological institution is a place where that can happen, potentially forming a bond and giving visitors motivation to care for the natural environment (Scott, 2012).

We hope our analysis offers insight into the representation of biological diversity of this threatened group of primates under captive breeding programmes, highlighting points for improvement when considering which species to keep in zoos. Biases in the selection of species in zoos have previously been shown in mammals (e.g. Frynta *et al.*, 2013), so we would not expect the situation for lemurs to be different. However, we may have expected to see a shift in lemur species held in captivity for conservation programmes since the publication of the strategic prioritization plan for lemur ex-situ conservation (Schwitzer *et al.*, 2013b). In order for zoos to truly represent

the diversity of Madagascar's unique primates, more attention needs to be paid to species selection and new collaborative breeding programs should be established. This is particularly the case for genera that are currently not represented (*Allocebus*, *Avahi*, *Indri*, *Lepilemur*, *Mirza* and *Phaner*) in EAZA collections. Furthermore, the fact the ring-tailed lemur (*L. catta*) is being kept in over 200 different institutions could be seen as excessive, given that so many lemur species in urgent need of protection are not represented at all.

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## Survey of nocturnal lemurs of Mangabe-Ranomena-Sahasarotra Reserve, Moramanga District, Alaotra-Mangoro Region

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### Abstract

We conducted the first focused survey of nocturnal lemurs in the Mangabe reserve in order to assess their status within the reserve and provide recommendations for their conservation. We combined distance sampling and camera trapping to determine species occurrences and estimate their relative abundance within the reserve. The fieldwork was done in January to February 2018 in the northern and February to March 2019 in the southern part of the reserve. We surveyed 30 transects of one kilometer and each transect was visited three times. We installed eight camera traps; three in October 2017 and a further five were added in May 2018. Five species, *Avahi laniger*, *Cheirogaleus major*, *Daubentonia madagascariensis*, *Microcebus lehilahytsara* and *Lepilemur mustelinus*, were encountered and abundance differed between sites. *D. madagascariensis* and *L. mustelinus* are rare and should be prioritized for conservation actions in the future. The other lemur species including *M. lehilahytsara*, *C. major* and *A. laniger* can be used as key attractions for ecotourism within the reserve given their higher density.

**Keywords:** Nocturnal, Lemurs, Conservation, Density, Mangabe

### Résumé

Nous avons mené une première recherche focalisée sur les lémuriens nocturnes dans la réserve de Mangabe depuis sa création afin d'évaluer leur statuts dans cette réserve et de donner des recommandations pour leur conservation. Nous avons utilisé la méthode d'itinéraire échantillon et la piège photographique pour étudier leur distribution et abondance. Les travaux sur terrain ont été faits entre Janvier et Février 2018 dans la partie nord ainsi que Février et Mars 2019 pour la partie sud de la réserve. Trente transects de 1 km ont été utilisés dont chaque transect a été visité trois fois. Nous avons installé huit pièges photographiques dont trois sont installés depuis Octobre 2017 alors que cinq ont été placés en Mai 2018. Cinq espèces ont été recensées et leur abondance varie pour chaque site. *D. madagascariensis* et *L. mustelinus* sont rare et doit être priorisé dans les activités de conservation dans la future alors que les autres espèces comme *M. lehilahytsara*, *C. major* et *A. laniger* peuvent être utilisées parmi les attraits touristiques de la réserve vu qu'elles sont abondantes et facile à observer.

**Mots-clés:** Nocturne, Lémuriens, Conservation, Densité, Mangabe

### Introduction

All of Madagascar's five lemur families are endemic to the country and represent more than 20% of the world's primate species and 30% of family-level diversity (Schwitzer *et al.*,



2014). Lemurs across Madagascar face extinction risks driven by human disturbance of forest habitats and they are considered to be the most threatened mammal group on earth (Schwitzer *et al.*, 2014). Nocturnal lemurs in particular are highly threatened due to habitat loss and illegal bushmeat hunting (Fa and Brown, 2009; Schwitzer *et al.*, 2014). This applies also to the nocturnal lemurs within the Alaotra-Mangoro region (Jenkins *et al.*, 2011). Protected areas are essential for lemur conservation as they are in their natural habitat (Mittermeier *et al.*, 2010). The process to create Mangabe-Ranomena-Sahasarotra reserve (hereafter Mangabe) started in 2008 in order to save Madagascar's endemic and threatened species, especially the golden mantella frog *Mantella aurantiaca* and large diurnal lemurs. Based on their distribution range depicted in the book "Lemurs of Madagascar" (Mittermeier *et al.*, 2010), Mangabe reserve may be home to nine lemur species including two diurnal (*Indri indri* and *Propithecus diadema*), two cathemeral (*Eulemur fulvus* and *Haplorhina griseus*) and five nocturnal species (*Avahi laniger*, *Cheirogaleus major*, *Daubentonia madagascariensis*, *Lepilemur mustelinus*, and *Microcebus lehilahytsara*). Since then, in-depth study of the distribution of diurnal lemurs has been conducted (Keane *et al.*, 2012) but no such work has been undertaken on the nocturnal species. This research was conducted to assess the status of nocturnal lemurs within Mangabe reserve and investigate how best to protect its nocturnal lemurs.

Materials and methods

Study site

Mangabe Reserve (latitude S19.045, longitude E48.151) is situated within the Moramanga District, Alaotra-Mangoro Region, and eastern Madagascar (Fig. 1). It is included in the eastern mid-altitude bioclimatic zone with vegetation dominated by evergreen humid forest characterized by high and closed canopy (Du Puy and Moat, 1996). Slash and burn agriculture, selective logging, illegal gold mining and hunting are the main threats that occur within the reserve and can affect all of its biodiversity including nocturnal lemurs (Madagasikara Voakajy, 2015, unpubl).

Direct observations

We used distance sampling with line transects (Buckland *et al.*, 2001; modified according to Thomas *et al.*, 2010) to detect species presence. Surveys were undertaken from five camp sites located around the two strict protected zones of interest in the north, Mangabe and Andranomavo; and in the south, Lakambato, Andasivilona and Avolo (Fig. 1). Transects were placed from the edge to the interior of the forest and spaced at least 200m apart (Meyler *et al.*, 2012). Each transect was visited three times between 7:00-10:00PM by four people composed of two researchers and two local guides. The interval between two surveys of the same transect was at least 72 hours to minimize disturbance. Accurate perpendicular distance of each animal from the transect line at its first detection was measured using a tape measure. Fieldwork was done in January-February 2018 in the northern part of the Reserve and in January-March 2019 in the south. Density

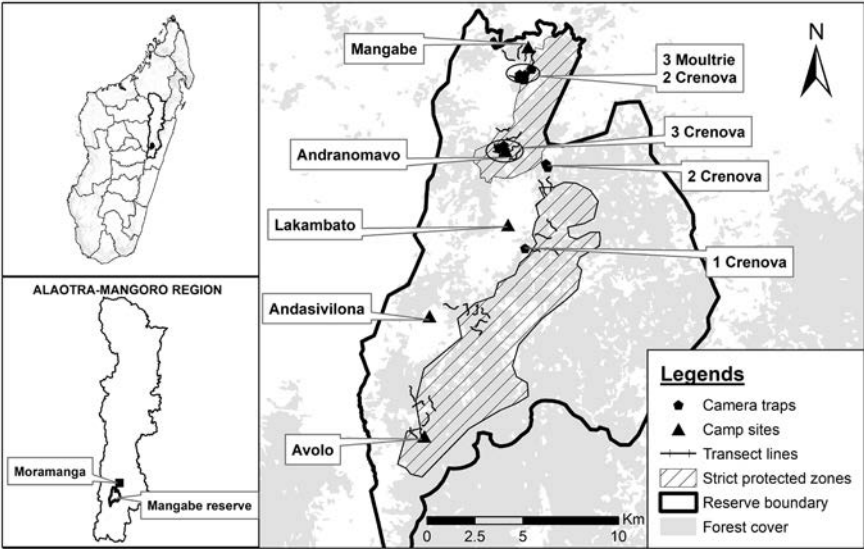


Fig. 1: Location of the Mangabe Reserve within the Moramanga District with the location of camp sites/ transect lines and camera traps (Moultrie and Crenova).

was estimated using Distance software version 7.0 following combinations of key functions and adjustments suggested by Thomas *et al.* (2010).

Camera traps

Camera traps were used to complement data from the transect surveys. Initially, camera traps were used to track for the Aye-aye (*Daubentonia madagascariensis*) as it could not be observed by eye in the wild. In October 2017, three camera traps (Moultrie) were installed at three localities in the northern core conservation area. In May 2018, we set up eight additional camera traps (Crenova) of which five were in the north and three in the south (Fig. 1). Camera traps were set to collect photos at three second intervals. Data from the camera traps were collected every three months and photos were scanned manually using excel database. Data analysis was based on the number of records of each species during the covered period of data analysis.

Results

Species richness and distribution

Five nocturnal lemur species were encountered during the field surveys in Mangabe reserve: the mouse lemur (*Microcebus lehilahytsara*), the woolly lemur (*Avahi laniger*), the sportive lemur (*Lepilemur mustelinus*), the dwarf lemur (*Cheirogaleus major*) and the Aye-aye (*Daubentonia madagascariensis*) (Tab. 1). They are all threatened on the IUCN Red List of threatened species including one Endangered and four Vulnerable.

Tab. 1: Species distribution and richness per camp site.

Species	IUCN Status	Man-gabe	Andra-nomavo	Lakam-bato	Andasi-vilona	Avo-lo
<i>M. lehilahytsara</i>	VU	obs	obs	obs	obs	obs
<i>C. major</i>	VU	obs	obs	obs	obs	obs
<i>L. mustelinus</i>	VU	obs	obs	obs	obs	obs
<i>A. laniger</i>	VU	obs	obs	obs	obs	obs
<i>D. madagascariensis</i>	EN	CMT			obs	obs
Observed species		5	4	4	5	5

obs: direct observation, CMT: observed by camera trap, VU: Vulnerable, EN: En Danger, IUCN: International Union for Conservation of Nature

Density

In total we walked 30 transects (12 in the north and 18 in the south) of one kilometre three times each. Density of *Daubentonia madagascariensis* was not estimated because there were only two direct observations from Andasivilona and Avolo; and three photos captured by three different camera traps in the Mangabe site. *Microcebus lehilahytsara* was the most frequently observed followed by *Avahi laniger*, *Cheirogaleus major* and *Lepilemur mustelinus* (Tab. 2). Density differs between species and sites. The Mangabe site had the highest density for all species except *A. laniger* which is most abundant in Lakambato. *M. lehilahytsara*, *C. major*, and *L. mustelinus* are highest from the north strict protected zone than the south in contrary to *A. laniger* which the density is quite similar from both.

Tab. 2: Density (ind/km<sup>2</sup>) of each species from each study site and from all of the reserve.

	Mangabe	Andranomavo	Lakambato	Andasivilona	Avolo	Mangabe reserve
<i>M. lehilahytsara</i>	397	354	186	195	325	268
<i>C. major</i>	123	55	34	51	46	66
<i>L. mustelinus</i>	22	13	18	2	5	11
<i>A. laniger</i>	97	60	119	86	16	72

Camera trapping

Since October 2017 when the first camera traps were installed until May 2019, the total effort was 1,738 nights of camera trapping. We captured a total of 348 lemur shots of which 16 were *Indri indri*, 63 *Propithecus diadema*, 191 were *Eulemur fulvus*, 63 of *Avahi laniger*, eight *Microcebus lehilahytsara*, four *Cheirogaleus major* and three *Daubentonia madagascariensis*. We did not capture any photos of *Lepilemur mustelinus* with the camera traps.

Discussion

This survey confirmed the presence of five nocturnal lemurs in Mangabe Reserve. All species can be encountered in the northern and southern part of the Reserve, but their encounter rates vary between the species and the sites. Overall, *Microcebus lehilahytsara* is the most frequently observed but it was rarely captured on the camera traps. This is probably due to the fact that *Microcebus* has a high density in the degraded environment favoured by the opening of the forest and the abundance of small trees (Ralison et al., 2015) while the camera traps which targeted *Daubentonia madagascariensis* were set in less disturbed areas as Farris et al., (2011) found evidence of higher aye-aye abundance and activity levels in non-degraded forest. Sightings of Aye-aye were rare, both from direct observation and the camera traps. The species has huge individual home ranges and long interbirth intervals which may translate to low population densities (Perry et al., 2012). With this very low number, the Aye-aye is highly threatened and requires more attention for conservation actions such as increasing effort to localise the species and its requirement within the reserve and deploy strategy to increase its population. The second rarest species is *Lepilemur mustelinus*. This can be explained by the site history which was exploited for wood production before and big trees were cut. Rasoloharijaona et al., (2008) suggested that the survival of this species will be strongly dependent on the availability of mature rain forests with suitable hollow trees. Densities of nocturnal lemurs identified in the Mangabe reserve during the present study are most similar to other

sites such as from Andasibe (Ganzhorn, 1998) which has been protected for more than 50 years and Maromizaha (Ralison et al., 2015) a new protected area (Tab. 3).

Tab. 3: Densities (ind. Km<sup>-2</sup>) of nocturnal species from Mangabe and other sites.

Species	Mangabe	Andasibe	Maromizaha
<i>M. lehilahytsara</i>	268	110	206
<i>A. laniger</i>	72	72	63
<i>C. major</i>	66	68	67
<i>L. mustelinus</i>	12	13	39

Mangabe forest is home to four Critically Endangered species such as the Golden mantella frog (*Mantella aurantiaca*), Pronk's day gecko (*Phelsuma pronki*), Indri (*Indri indri*) and Diademend sifaka (*Propithecus diadema*) which are all forest dependant. Ecotourism is one of the activities expected to generate income in Mangabe Reserve and sustain the conservation of biodiversity. Given their high densities, circuits can be organized to facilitate observation of *M. lehilahytsara*, *C. major* and *A. laniger* with consideration of the needs of others rare species such as *L. mustelinus* and *D. madagascariensis*. This enables important income generation whilst protecting the rarest species in the area.

Conclusion

Mangabe reserve is home to five nocturnal lemur species which are all listed as threatened on the IUCN Red List. Mangabe's nocturnal lemurs are threatened by hunting and slash-and-burn agriculture. Efforts to conserve the forest of Mangabe and its current target species, *Mantella aurantiaca*, *Indri indri* and *Propithecus diadema* will also contribute to the conservation of nocturnal lemurs. We recommend further studies to investigate the distribution and ecology of the rarest species: *Daubentonia madagascariensis* and *Lepilemur mustelinus*.

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## Responses of *Varecia rubra* to a frequently disturbed habitat by cyclones in Masoala National Park, Madagascar

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**Keywords:** *Varecia rubra*, Cyclone, demography, habitat, disturbances, adaptation, Masoala

### Résumé

Le mois d'Avril 2000, la forêt de Masoala a été dévastée par un cyclone très intense de catégorie 4, dénommé Hudah. Des études ont été menées sur les impacts de cette catastrophe naturelle sur la population du lémurien diurne

*Varecia rubra* qui ont été menées un an (2001) et quatre ans (2004) après son passage dans un site nommé Antsahamanara, situé sur la côte Est de la presqu'île de Masoala, région la plus affectée par ce cyclone. La présente étude est menée en 2018 dans le même site, pendant la même saison et appliquant les mêmes méthodes de collecte de données que ceux utilisés pendant les études précédentes. Elle consiste à déterminer les variations sur les paramètres démographiques et de l'habitat, le budget de temps et la disponibilité de la nourriture dix-huit ans après le passage du cyclone Hudah. De cette étude, des changements au niveau de ces paramètres ont été rapportés suggérant une stratégie d'adaptation de *Varecia rubra*, à la dégradation de son habitat malgré sa vulnérabilité.

### Introduction

*Varecia rubra* is a critically endangered lemur species, endemic to the Masoala peninsula, located in the northeastern coast of Madagascar. Emblematic of Masoala, in the National Park, *Varecia rubra* plays an important ecological role as it is among the principal seed dispersal agents for habitat sustainability and restoration (Martinez *et al.*, 2014).

In Madagascar, 16% of primate taxa are vulnerable to cyclones (Zhang *et al.*, 2019), amongst them the lemur *Varecia rubra*. This species is sensitive to habitat degradation as a decrease in its populations has been recorded in severely disturbed habitat by a cyclone (Ratsisetraïna, 2013). In addition to intensifying cyclones that hit the region every year, the park has suffered degradation following extensive illegal logging of precious woods. Between 2008 and 2011, the rate of forest change across Masoala National park was 1.27%, higher than the most recent annual deforestation for all of Madagascar (Allnutt *et al.*, 2013).

In 2000, Masoala peninsula was hit by a strong cyclone called Hudah with wind speeds of more than 250km/h. It was the strongest tropical cyclone ever recorded in the region before. According to the climate projection for Madagascar, by the end of the 21st century, cyclones will increase in intensity, although their annual frequency will not change. In addition, they will mostly come from the Indian Ocean and likely to land in the northeast of the island accompanied by stronger winds. This is to say that Masoala peninsula remains at risk from tropical cyclones in the future that are intensifying with climate change (Rabefitia *et al.*, 2008). In 2001, the impact of the cyclone Hudah on the population of *Varecia rubra* in two sites: Antsahamanara that was described as moderately affected by the cyclone and Sahafary as severely damaged (Birkinshaw *et al.*, 2001; Ratsisetraïna, 2001) was studied. Compared to its state before the cyclone, a population decrease in a severely damaged habitat was recorded (Ratsisetraïna, 2001). Then in 2004, research on the recovery of the population following the cyclone disturbance in the two sites was conducted. Between the two study sites, compared to the population state before the cyclone (year 2000) population recovery was slow and low in the severely disturbed forest (Ratsisetraïna, 2013). The current project was set up to fill the information gap of fourteen years (2004 to 2018) on the species demography.

The goal of this study is to provide up-to-date information on the state of the population of *Varecia rubra* in a particular site within Masoala national park. The main objectives are to: 1) provide information on the state of the species' population eighteen years (2000 to 2018) after the habitat disturbance by the cyclone Hudah and 2) describe the resilience or ability of the species to respond to habitat disturbances in a site frequently disturbed by cyclones. Re-



sults from this study will serve as a reference in formulating long-term conservation strategies for *Varecia rubra*.

Methods

Site description

The project is held in Masoala National Park, situated in the Masoala Peninsula in the northeastern coast of Madagascar. The geographic coordinates are: 15° 30' 48" S, 50° 07' 20" E. The previous studies (2000, 2001, 2004) were held in the two study sites (Antsahamanara and Sahafary) (Fig. 1). As the forest within Sahafary has completely disappeared for cultivation owing to population growth, the current project was only conducted in the site of Antsahamanara. This study site, located in the northeastern portion of the peninsula, has been monitored annually since 1996 (Merenlender *et al.*, 1998). The sampling surface of Antsahamanara site is 3.72km<sup>2</sup>.

Sahafary and Antsahamanara location

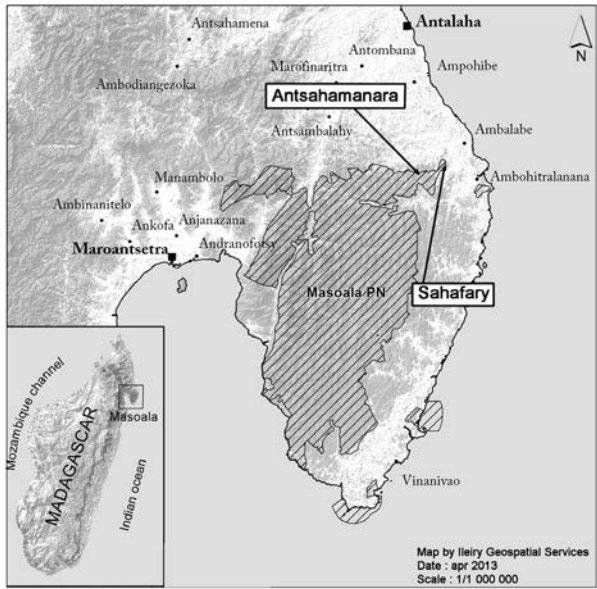


Fig. 1: Study site location.

From 2000 to 2017, the northeastern coast of Madagascar, where the Masoala National park is situated, were the landfall area of nine significant tropical cyclones (Tab. 1). Among them, 56% have been classified as category 4 (with wind speeds between 210 and 249km/h) or category 5 (with wind speeds of more than 249km/h) and are both classified as severe tropical cyclone (Probst *et al.*, 2017). Fig. 2 depicts the trajectories of the nine tropical cyclones around the Masoala Peninsula, northeastern coast of the country.

Tab. 1: Significant tropical cyclones hitting the Masoala peninsula, northeast of Madagascar. (Source: European Union, 2017)

Name	Year	Equivalent SSHS Landfall
HUDAH	2000	Category 4
HARY	2002	Category 5
GAFILO	2004	Category 4
INDLALA	2007	Category 3
JAYA	2007	Category 1
IVAN	2008	Category 4
JADE	2009	Category 1
BINGIZA	2011	Category 2
ENAWO	2017	Category 4

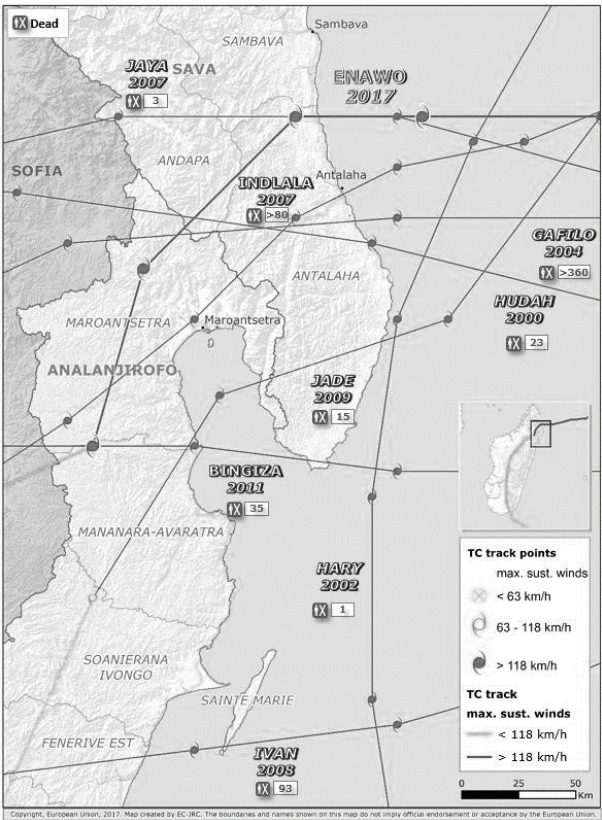


Fig. 2: Trajectories of tropical cyclones in the study area over 2000-2017. (Source: European Union, 2017)

Demographic analyses

Fieldwork was undertaken over two periods: July to August 2017 (cold rainy season) and January to February 2018 (hot rainy season). The work has begun with recovering all census transects previously used for population monitoring in 2000, 2001 and 2004. Geographic coordinate points were collected at every 100m from the beginning until the end of each transect. Although three of these transects (A, B, C, Fig. 3) are connected to each other (and therefore not completely independent). Our primary aim was to collect data that were comparable to the earlier years, therefore we repeated the same methodology. Each transect was visited eight times (over eight consecutive days) during each period as we did for previous studies. We recorded the total number of individuals including adults and infants encountered in each group. Censuses were begun early in the morning from 6h.30, whereas the end time of the transect depended on the length of the respective transect and the time we spent counting and observing the animal activities (mean time of observation: 3h.42mn±0.04, maximum duration: 5h.20mn; minimum duration: 01h.55mn). We moved slowly along the transect with frequent stops to better locate noises and vocalizations of the animals (Merenlender *et al.*, 1998). We carried out lemur censuses on four transects of 5920 m in total (Fig. 1).

For each survey, the following information were noted:

- Date
- Transect name
- Time of the beginning and the end of the census.

Every time a lemur group was encountered, the following data were recorded:

- The time at which the first individual or group was seen or heard
- The total number of individuals observed
- The location on the transect at which the first animal was seen
- The number of individuals per age: adult (over one year), young (aged less than one year), infant (less than three months). Since age could not be reliably estimated from visual observations, animals were grouped according to size. Large individuals were considered as adults, those of a medium size are considered as juveniles and small ones that were still carried by another individual as infants.
- The relative distance of the animal to the observer
- The angle from the observer to the animal spotted
- The perpendicular angle between the transect and the animal
- The animal activities during the observation (moving, feeding, resting, mating, grooming, etc.)
- The geographical coordinates of the location where the animal is first seen.

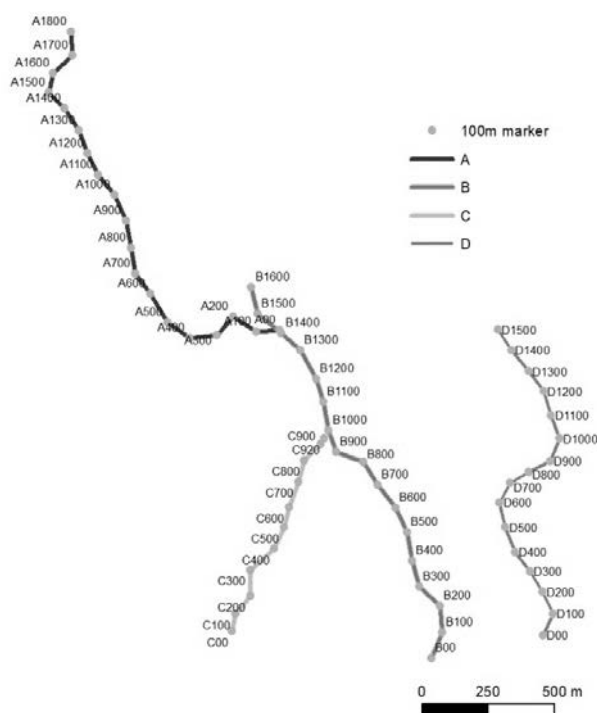


Fig. 3: Lemur census transects.

#### Habitat analysis

The methods used for the vegetation study are taken from that described by Gounot (1969) and White and Edwards (2001). Temporary vegetation plots of 50 x 4m size were established every 200m along the lemur census transects. Additionally, permanent plots of 100 x 20m were set up in each transect in a manner to represent different altitudes: higher (508m) in Plot A, high (357m) in Plot D, middle (215m) in Plot B and low (99m) in Plot C. In addition to phenology data, we collected plant species samples encountered within the permanent plots for identification.

All trees having diameter at breast height (DBH) greater than or equal to 5 cm were recorded within the temporary plots as we performed during our previous studies in 2001 and 2004, and trees from DBH 10cm and above (Birkinshaw *et al.*, 2001) for the newly established permanent plots. For each tree, we noted: the leaves (whether 75% of them were

young, mature or older), flowers (absence or presence), and fruits (absence or presence).

The habitat survey was conducted during two seasons; dry (August-September 2017) and wet (January-February 2018) to get phenology data such as the abundance of leaves, flowers and fruits. The temporary plots were surveyed in the dry season and the permanent plots in the wet season.

In total, twenty-nine temporary vegetation plots of 50m x 4m in 2017 and 4 permanent plots of 100m x 20m were established at transect A: 800; C: 200; B: 800; D: 1400 (*cf.* Fig. 3) in 2018. In addition, we collected plant sample of those observed to be consumed by the red ruffed lemurs for identification.

#### Data analysis

##### Group identification

The identification of the groups was done according the following two criteria: 1) Groups which were met more than three times at the same place or the surrounding area on the ground during the eight days of observations were considered belonging to the same group. 2) Groups identified in trees were verified and justified during data processing as follows: we established vital domains of each group in a circular model of a radius of 500m to obtain sampled surface. The aim was to establish a circular radius of the home range by reporting on the geographical points of the census transects the location point of each group observed. If two or more groups have a significant overlap area with on average (mean) more than 60%, they were considered as belonging to the same group.

##### Group size

Comparison of demographic data collected from different periods: 2000 (before cyclone Hudah), 2001 (after cyclone Hudah), 2004 (four years after cyclone), 2018 (current study). We used Excel software to obtain summary and descriptive statistics of the data.

##### Density

The density was calculated as the relationship between the number of individuals of all social groups assigned (abundance) and "surfaces" area sampled in the study site (3.72 km<sup>2</sup>). Abundance or population size is the total number of individuals determined for each group. The sampling surface of each group is calculated from the overall length of the census transect and their respective maximum radius of vital domain of 500m. (Merenlender *et al.*, 1998). We compared data obtained during the four periods of study (2000, 2001, 2004, 2018) as they were collected in the same season (hot rainy season).

##### Activity budget

As we recorded the activities of each animal or group encountered during each census, we counted how often they were sleeping, feeding, vocalizing, moving and resting. Percentages of each activity were calculated, and we compared the data obtained during the four periods of study (2000, 2004, 2017, 2018) as data on activities from 2001 were missing.

##### Food availability

We calculated the percentage of trees with flowers and fruits out of the total number of trees recorded within permanent plots as they were surveyed during the flowering and fruiting periods. Trees having DBH more than 40cm in all temporary plots referring to previous study on vegetation by Rigamonti (1993).

Results

Group identification

Fig. 4 shows the identification of the number of groups according to their maximum home range (500m of diameter). In 2018, two groups occasionally shared their home range to form only one group and split into sub-groups as suggested for a species exhibiting a fission-fusion social system. The same year, we observed red ruffed lemur feeding in a group of ten individuals, a group size that has never been documented in our study site before.

Group numbers, population size and group size

The number of groups increased to five one year after the cyclone and to six four years after the cyclone Hudah. Yet, group numbers decreased years after the cyclone and remain the lowest compared to the previous study periods with 3 groups in total. There was a fluctuation of the population size over the periods of study (Tab. 2). The total number of individuals recorded in 2018 was higher (13 individuals) than those in 2000 (before the cyclone Hudah) with 10 individuals, which slightly reached the size of recovered population (14 individuals) four years after cyclone. Compared to the period after the cyclone in 2001, *Varecia rubra* lived in small groups ( $1.60 \pm 0.54$ ) and tended to live in a larger group with  $4.33 \pm 0.58$  individuals years after (in 2018).

Tab. 2: Group numbers, total individuals and group size over the four years of study.

Periods	Group numbers	Total individuals	Group size
Before cyclone Hudah (2000)	4	10	$2.50 \pm 1.29$
One year after cyclone Hudah (2001)	5	8	$1.60 \pm 0.54$
Four years after cyclone Hudah (2004)	6	14	$2.30 \pm 0.81$
2018	3	13	$4.33 \pm 0.58$

Density

Population density changed following habitat disturbances (Tab. 3). Population was less dense ( $2.68 \text{ individuals/km}^2$ ) before the cyclone and density decreased to  $2.15 \text{ individuals/km}^2$  just after cyclone Hudah has hit the site. Population density then increased to  $3.49 \text{ individuals/km}^2$  in 2018 despite successive cyclone disturbances after 2004.

Tab. 3: Changes in population density.

Periods	Density (individuals/Km <sup>2</sup> )
Before cyclone Hudah (2000)	2.68
One year after cyclone Hudah (2001)	2.15
Four years after cyclone Hudah (2004)	3.76
2018	3.49

Activity budget

Tab. 4 shows the number of times the animals or groups were observed carrying out each type of activity over the three study periods. The same groups or individuals are often reobserved during the transect revisit. In some cases, none of the groups nor individuals were recorded during the daily visit of transect.

Tab. 4: Number of times individuals seen or heard carrying out each activity during lemur census.

Activity	2000	2004	2017	2018
Vocalizing	38	10	4	7
Moving	6	5	1	3
Feeding	0	5	1	6
Resting	8	7	2	0
Sleeping	1	0	0	0
Total (sighting+hearing)	53	27	8	16

In 2000, we did not see red ruffed lemur feeding, however we saw them resting several times and sleeping. Conversely, in 2018 we did not see them resting nor sleeping indicating that they might be most active in habitats disturbed by the cyclones. Besides, they spent large amount of their time vocalizing or moving. The species has been rarely spotted during the cold rainy season (in 2017) compared to the other season. To sum up, their activities appear to have changed after Hudah and other cyclones, tending to allocate most of their time to feeding compared to other periods. As indicated in Fig. 5, the species spent 37% of the time budget feeding, 19% moving and 44% vocalizing.

Food availability

Classes of Diameter at Breast Height (DBH)

Red-ruffed lemur is a canopy dwelling species; they sleep,

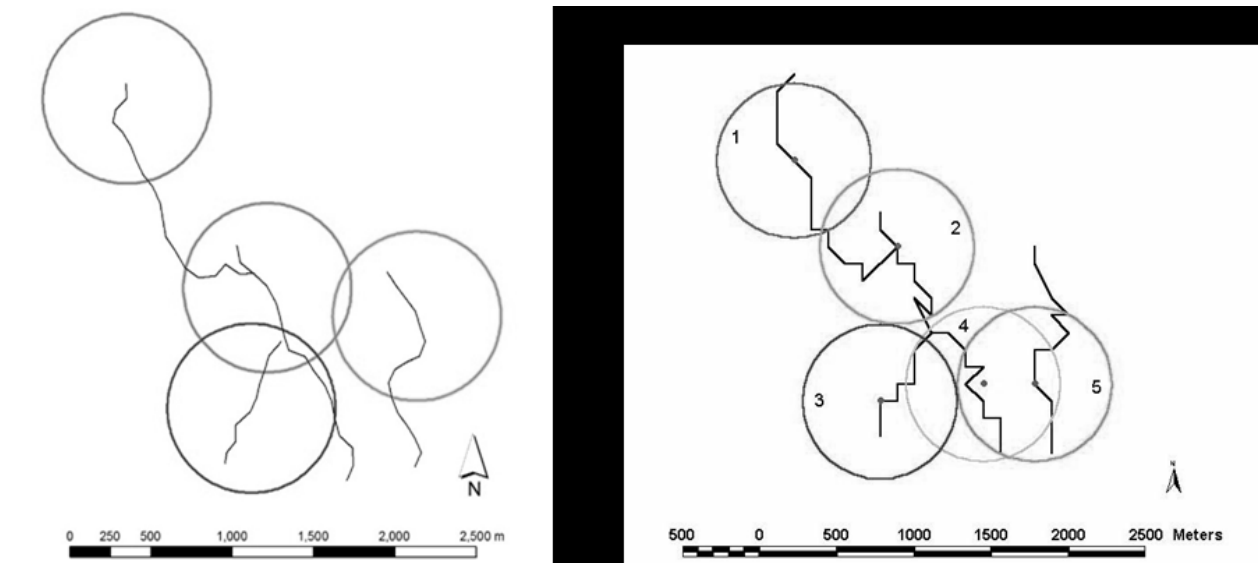


Fig. 4: Identification of group using 500m home ranges: 2018 in the left and 2001 in the right.



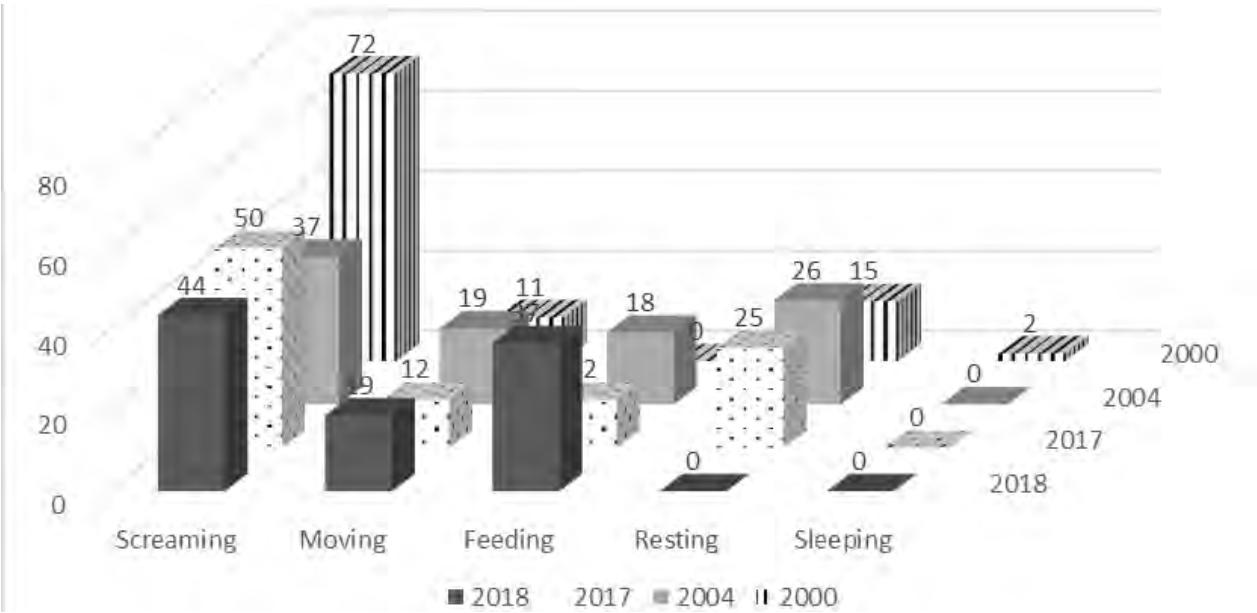


Fig. 5: Percentage of the activity budget of red ruffed lemur in different period (2018, 2017, 2004, 2000).

feed and rest in large trees. Our latest study on vegetation found that out of more than 1286 trees recorded within the twenty-nine 50mx4m temporary plots, 2.64% (34 trees) have DBH between 40.2cm and 95.1cm, demonstrating that the forest is dominated by small size trees (Tab. 5).

Tab. 5: Percentage of trees having breast height diameter (DBH) more than 40cm.

	Number	Total	DBH (cm)	Percentage (%)
Transect A	11	506	49.5 – 95.1	
Transect B	4	339	41.4 – 85.5	
Transect C	8	139	40.2 – 87.2	
Transect D	11	302	41.5 – 69.3	
Total	34	1286		2.64

Flowering and fruiting trees

For this study, 391 species of plants spread over 36 families were recorded within the four permanent vegetation plots of 0.08Ha in total. Only one species (*Grewia* sp, MALVACEAE) or 0.26% contained flowers and 5 species (1.28%) were fruiting during the period of January to February 2018. The five tree species fruiting were: *Dillenia triquetra* (DILLENIACEAE), *Ravenala madagascariensis* (STRELITZIACEAE), *Allophylus masoalensis* (SAPINDACEAE), *Xylopia buxifolia* (ANNONACEAE), *Colubrina faralaotra* (RUBIACEAE). Apart from *Ravenala madagascariensis*, none of these species were observed to be consumed by the red ruffed lemur during the census. During our study, only four species *Canarium madagascariensis* (BURSERACEAE), *Mimusops masoalensis* (SAPOTACEAE), *Ravenala madagascariensis* (STRELITZIACEES) and *Uapaca littoralis* (EUPHORBIACEAE) were seen to be consumed by *Varecia rubra*. Only *Canarium madagascariensis* was not recorded within the plots but was observed to be consumed by the lemurs outside the vegetation plots. The other three species are common in the four permanent plots.

Discussion

The population in 2018 tended to reach its level before the cyclone Hudah disturbance in 2000, suggesting that ruffed lemurs are able to adapt to habitats frequently dis-

turbed by cyclones. Despite its vulnerability to habitat degradation (Ratsisetraina, 2013), the species might have been becoming more resilient and adapted more effectively to the degraded habitat. Alternatively, the population has just recovered after cyclone disturbances as observed in 2004. However, we do not have any information on the status of the population and the habitat between 2004 and 2018. On one hand, individual recruitment following immigration from other locations might explain the rise of the population density in the study area. Many sites would lose their forest since 2004 like the case of Sahafary that would accentuate the species migrations. In this case, frequent immigration into the study area would suggest that the study site might be more prosperous than others after the extensive illegal logging of precious woods, land conversion to agricultural purposes, and the frequent tropical cyclone disturbances. On the other hand, the increase of population and group size might be explained by a high rate of birth that might have occurred in the site. Increase of birth rate might be the result of recruitment of more females than males within the site and/or the groups. Additionally, during this study we encountered the largest group that had ever been recorded before in this area. This phenomenon might indicate that the species have experienced recent ecological disorders (Ratsimbazafy, 2002) from successive cyclones that significantly impacted the forest structure (Birkinshaw, 2001) and food availability. Accordingly, fission-fusion dynamics can be an adaptation behavior pattern adopted by the groups in the face of the habitat disturbance (Holmes et al., 2016). Generally, most lemur species tend to merge their groups to reduce feeding competition in the face of fruit scarcity (Baden, 2015) and when food is scarce (Balko et al., 2005) as observed in our study site with only 1.28% of trees fruiting. Nonetheless, presence of trees that can provide sufficient food appears to be key to the establishment of the groups (Balko et al., 2005). Before cyclone Hudah, we noticed the presence of ruffed lemurs by hearing them vocalizing several times about 200m away from the census transect. In this study, we discovered them more often when they are feeding, but less often when they were resting compared to other periods. Other findings, hence, support the notion that ruffed lemurs travel less

in resource-scarce periods (Vasey, 2005). Thus, activity budget might be responsive to current food distribution and availability and the vegetation structure that might change following successive cyclones. Other research stated that this species uses and exploits intensively only small portions of their total home range each month (for a period of two or three month) and shift partially to new zones (Vasey, 2005). In 2018, we observed them feeding in a small patch of the transect C400, B1000, B600 and D800.

According to a previous study on the diet by Rigamonti (1993), red-ruffed lemurs fed on fruits, leaves and flowers of 42 different tree species from 28 species with preference to seven food species including *Ficus lutea* and *Ficus reflexa*, *Ocotea* sp., *Garcinia* spp. Yet, a year-long fruit utilization analysis reported a high degree of preference for several species of trees (Balko, 2005). However, flowers and fruits were rare during our data collection periods (August to September 2017 and January to February 2018). Our findings showed that the number of food tree species of red-ruffed lemur decreased with only three species compared to previous research demonstrating that food is scarce even during the period it is supposed to be abundant (Rigamonti, 1993). This situation would be the consequences of the recent successive powerful cyclonic disturbances accompanied by human activities causing changes in the forest structure and forest degradations. According to Balko (2005), intense disturbances, caused by heavy logging or severe cyclones have long-lasting impacts on fruit production. Additionally, regional changes in temperature and rainfall patterns due to climate change might affect the tree phenology, or the frequency of intense cyclones may affect the distribution of some species (Metcalf et al., 2008).

Red ruffed lemur is a canopy dwelling species, they sleep, feed and rest in large trees with an average DBH of 59.8cm and ranging between 41 and 80cm (Rigamonti, 1993). Few trees (2.64%) having these sizes were recorded, suggesting that the forest is currently dominated by rather small size trees. Moreover, the study after cyclone Hudah on the vegetation reported that large trees with DBH from 40cm and more were the most affected by the strong winds (Ratsisetraina, 2001).

## Conclusion

The study was conducted in a site that was moderately affected by cyclone Hudah and was not subjected to extensive illegal rosewood logging. Results indicate that the population size of red-ruffed lemurs is stable compared to that observed before the cyclone Hudah has hit the region. In other words, despite the frequency and the intensity of cyclones hitting the site since 2000, the species could recover and adopt strategies to adapt and to survive in a habitat frequently disturbed by strong winds. Red-ruffed lemurs are highly frugivorous in terms of diet, strategies may include migration to a more prosperous habitats, a tendency to live in a larger group or group fusion and finally, activity shifts. This finding could serve as a reference to further studies on the species for instance: population modelling and projection, long-term population and habitat monitoring to enable formulate adequate strategies for sustainable conservation of the species and the whole biodiversity of Masoala in a changing climate with intensifying tropical cyclones.

## Acknowledgements

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## Theses completed

Hager, H. 2020. Do Actions Speak Louder than Words? Communicative Frequencies and ultimodality in Ring-Tailed Lemurs (*Lemur catta*). Master's Thesis, Anthropology, The University of Western Ontario, London, Canada. [Electronic Thesis and Dissertation Repository. 7355: [ir.lib.uwo.ca/etd/7355](http://ir.lib.uwo.ca/etd/7355)].

The study of multimodal communication in primatology has increased only recently. At present, there are no ongoing investigations of multimodal communication in ring-tailed lemurs (*Lemur catta*), despite the body of research on this species. I investigated how different modes of *L. catta* inter-individual multimodal communication are socially coordinated and integrated by examining frequencies of occurrence within four potential biological and social factors: age, troop affiliation, sex, and dominance rank. Research was conducted over four months at the Duke Lemur Center, Durham, NC, on 14 individuals from three separate troops of captive, free-ranging *L. catta*. Results demonstrate communicative variation in unimodal, but not multimodal, signals correlating to sex and rank in this species. Dominant females appear to utilise visual signal components more frequently than males, while males rely more on auditory means of communicating, consistent with troop spatial organization. This research provides a baseline for future investigations into primate multimodal communication.

Tiafinjaka, O. 2020. Inventaire des lémuriens par l'utilisation des pièges photographiques dans les forêts fragmentées de Kianjavato, Sud-Est de Madagascar. Masters thesis. Sciences de la vie et de l'environnement. Université de Mahajanga.

Cette étude a été effectuée dans la forêt classée de Kianjavato au Sud-Est de Madagascar, pendant quatre mois (Mai-Août 2019). Les objectifs consistent à confirmer la présence des espèces de lémuriens et à déterminer les caractéristiques de la végétation et l'influence des pressions anthropiques sur leur distribution. Pour atteindre ces objectifs, plusieurs méthodes ont été utilisées notamment: l'installation de 60 caméras dans trente stations. Les caméras arboricoles ont été installées à des hauteurs de 6 à 14 m et les caméras terrestres à 0.5 m dans les cinq fragments; et un plot botanique circulaire a été utilisé pour étudier les caractéristiques des habitats à chaque station. Les résultats ont montré que la caméra arboricole est plus efficace pour l'étude des lémuriens par rapport à la caméra terrestre. Les neuf espèces de lémuriens présentes ont été détectées par les caméras et tous les fragments hébergent les lémuriens mais le nombre d'espèces varient entre un et sept selon le fragment. *Eulemur rufifrons* est la seule espèce observée dans tous les fragments et détectée dans les deux types de caméras. Nous n'avons pas pu examiner la probabilité d'occupation de toutes les espèces mais trois espèces seulement, qui sont *Eulemur rufifrons*, *Cheirogaleus major* et *Microcebus jollyae*. Le résultat a montré qu'*Eulemur rufifrons* occupe 47% de la surface du site d'étude. En général, la présence de toutes les espèces n'ont pas de relation avec la structure de végétation. En revanche, la destruction de l'habitat, causée par les hommes ont une influence sur la présence des lémuriens. Les sentiers et les coupes illicites sont très nombreux, et les fragments avec un indice de pression élevée sont ceux avec une richesse taxonomique élevée par rapport aux autres qui ont des indices de pression faible.



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## Journal article

Ranaivoarisoa, J.F.; Ramanamahefa, R.; Louis, Jr., E.E.; Brenneman, R.A. 2006. Range extension of Perrier's sifaka, *Propithecus perrieri*, in the Andrafiomena Classified Forest. *Lemur News* 11: 17-21.

## Book chapter

Ganzhorn, J.U. 1994. Les lémuriens. Pp. 70-72. In: S.M. Goodman; O. Langrand (eds.). Inventaire biologique; Forêt de Zombitse. Recherches pour le Développement, Série Sciences Biologiques, n° Spécial. Centre d'Information et de Documentation Scientifique et Technique, Antananarivo, Madagascar.

## Book

Mittermeier, R.A.; Konstant, W.R.; Hawkins, A.F.; Louis, E.E.; Langrand, O.; Ratsimbazafy, H.J.; Rasoloarison, M.R.; Ganzhorn, J.U.; Rajaobelina, S.; Tattersall, I.; Meyers, D.M. 2006. Lemurs of Madagascar. Second edition. Conservation International, Washington, DC, USA.

## Thesis

Freed, B.Z. 1996. Co-occurrence among crowned lemurs (*Lemur coronatus*) and Sanford's lemur (*Lemur fulvus sanfordi*) of Madagascar. Ph.D. thesis, Washington University, St. Louis, USA.

## Website

IUCN. 2008. IUCN Red List of Threatened Species. <[www.iucnredlist.org](http://www.iucnredlist.org)>. Downloaded on 21 April 2009.

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