

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/325855577>

Re-discovering the forgotten Phaner population of the small and isolated Analafiana forest (Vohémar, SAVA)

Article · May 2018

CITATIONS

0

READS

39

18 authors, including:



Jordi Salmons

Paul Sabatier University - Toulouse III

51 PUBLICATIONS 494 CITATIONS

SEE PROFILE



Emmanuel Rasolondraibe

University of Mahajanga

19 PUBLICATIONS 166 CITATIONS

SEE PROFILE



Ando Nantenaina Rakotonanahary

University of Mahajanga, Madagascar, Mahajanga

9 PUBLICATIONS 40 CITATIONS

SEE PROFILE



Tantely Ralantoharijaona

University of Mahajanga

8 PUBLICATIONS 26 CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



Climate change impacts on lizards [View project](#)



Inferring demographic history from genetic and genomic data [View project](#)

Re-discovering the forgotten *Phaner* population of the small and isolated Analafiana forest (Vohémar, SAVA).

Jordi Salmona^{1,2,3}, Emmanuel Rasolondraibe⁴, Fabien Jan¹, Ando Nantenaina Rakotonanahary⁴, Tantely Ralantoharijaona⁴, Barbara Le Pors¹, Dhurham Said Ali Oussen⁴, Isa Aleixo-Pais^{1,8,9}, Adam Joseph Doncheff Marques¹, Gabriele Maria Sgarlata¹, Helena Teixeira^{1,5}, Vivien Gabillaud¹, Alexandra Miller^{1,6}, Mohamed Thani Ibouroi^{4,10,11}, John Rigobert Zaonarivelo⁹, Nicole Volasoa Andriaholinirina⁴, Lounès Chikhi^{1,2,3}

¹Instituto Gulbenkian de Ciência, Rua da Quinta Grande, 6, P-2780-156 Oeiras, Portugal

²CNRS, Université Paul Sabatier, IRD ; UMR5174 EDB (Laboratoire Évolution & Diversité Biologique), 118 route de Narbonne, F-31062 Toulouse, France

³Université Toulouse 3 Paul Sabatier, CNRS, UMR5174 EDB, F-31062 Toulouse, France

⁴Faculté des Sciences, de Technologie et de l'Environnement, Université de Mahajanga, BP 652, (401) Mahajanga, Madagascar

⁵Institute of Zoology, University of Veterinary Medicine Hanover, Hanover, Germany

⁶School of Human Sciences, University of Western Australia, Crawley, WA 6009

⁷EPHE, PSL Research University, CNRS, UM, SupAgro, IRD, INRA, UMR 5175 CEFE, F-34293 Montpellier, France

⁸Organisms and Environment Division, Cardiff School of Biosciences, Cardiff University, Cardiff CF10 3AX, United Kingdom

⁹Centre for Research in Anthropology (CRIA-FCSH/NOVA), 1069-061, Lisbon, Portugal

¹⁰Institut des Sciences et Industrie du Vivant et de l'environnement, AgroParisTech, 19 Avenue de Maine 75015 Paris, France

¹¹Département des Sciences de la Nature et de l'Environnement, Université d'Antsiranana, 201 Antsiranana, Madagascar

*Corresponding authors: jordi.salmona@gmail.com, chikhi@igc.gulbenkian.pt

Key words: *Cheirogaleidae*, *Phaner*, fork-marked lemurs, dry forest, isolated population, mitochondrial phylogeny, morphological data

Abstract

Despite the fact that lemurs are the most threatened group of mammals and live in one of the world's richest biodiversity hotspots, there are still lemur lineages and regions of Madagascar that suffer from a lack of naturalistic data. This is particularly striking for the genus *Phaner* as well as for many unprotected forests of Northeastern Madagascar. We detected the presence of *Phaner* in the isolated lowland dry forest of Analafiana, located south of the Manambato River and close to the city of Vohémar. Little is known about the presence of *Phaner* in this region. The only record of *Phaner* in the area around Analafiana comes from museum specimens (Groves and Tattersall, 1991) geographically assigned to Vohémar and identified as belonging to a “*doubtful subspecies*”. Here we provide morphological and genetic (mitochondrial DNA) data for a single juvenile individual captured in Analafiana. We compare our sequence data to the only four *Phaner* sequences publicly available: two *P. electromontis* individuals sampled in Montagne d'Ambre and Ankarana, and two other from *P. pallescens* and *P. parienti* species. We find that the new mtDNA sequence is divergent from the two *P. electromontis* sequences, but closer to these than other *Phaner* sequences. We also note that the Analafiana *Phaner* individual is morphologically more similar to *P. electromontis* than to the other species. Altogether, this data suggests this unique and isolated population of *Phaner* survives in the unprotected Analafiana forest in northeastern Madagascar. It is urgent to carry out more genetic, behavioral, acoustic, and demographic research in this region and on this population, whose exact taxonomic status should be urgently clarified. Our results strongly suggest that Analafiana's *Phaner* population likely holds the memory of a unique evolutionary history.

Introduction

In the last twenty years our understanding of Malagasy primates has profoundly changed (Mittermeier *et al.*, 2010; Schwitzer *et al.*, 2013). Genera such as *Microcebus* and *Lepilemur* have seen the number of recognized species increase tremendously (Hotaling *et al.*, 2016; Lei *et al.*, 2016; Louis and Lei, 2016), and regions rarely visited are now more thoroughly studied and sampled. These two changes are not independent and the identification of new species has been to some extent driven by the genetic analyses of samples obtained over large geographical areas. However, there are still regions of Madagascar that have been sparsely sampled and visited. This is true, for instance, for forests located in northeast Madagascar, south of the Manambato River (Fig. 1). These regions are mostly covered by humid forests but there are also a few isolated dry lowland forests, such as Analafiana, located ~20 km from Vohémar, and ~20 km south of the Manambato River.

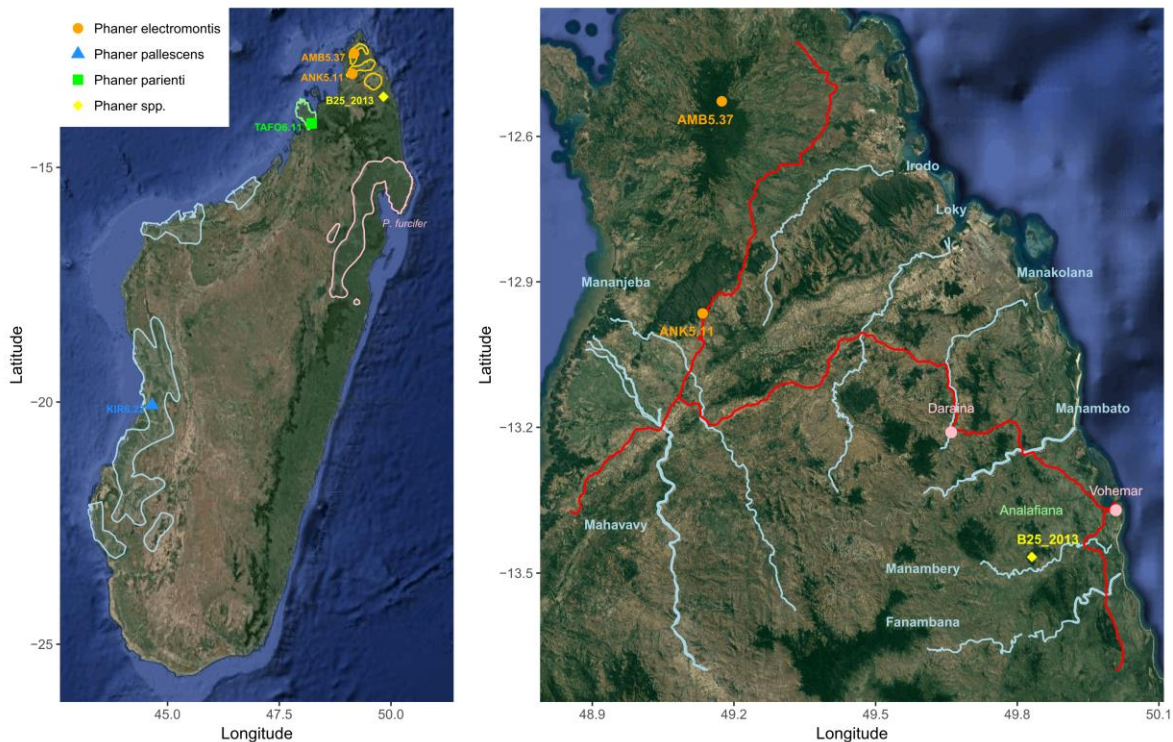


Fig. 1: Map of *Phaner* samples used in this study. The left panel represents the 5 samples used in the phylogeny reconstruction (see also Tab. 2). The samples B25_2013 correspond to the only juvenile *Phaner* individual captured in Analafiana in the present study. The right panel shows a closer view of Northern Madagascar allowing a better representation of the grassland/forest landscape and of the river system in the region. Rivers are represented in light blue, and roads in red. The figure was built using the R package *RgoogleMaps* (R CoreTeam, 2014). Distribution ranges of the left panel plot were downloaded from the IUCN red list pages (IUCN, 2012d; 2012a; 2012c; 2012b).

The genus *Phaner*, commonly called fork-marked lemurs, belongs to the Cheirogaleidae family, which includes also the genus *Microcebus* (mouse lemurs), one of the most speciose lemur genera. Four subspecies of *Phaner* were described by Groves and Tattersall (1991), based on a limited number of morphological differences identified among *Phaner* museum specimens. Groves (2001) elevated all subspecies to the species rank, and this has been the consensus until now (Mittermeier *et al.*, 2010). Their distribution is discontinuous, and most southern and eastern regions of Madagascar do not host any *Phaner* species (Fig. 1; Mittermeier *et al.*, 2010). Three of the four species are distributed in the north of Madagascar: *P. parienti* in the north-west, *P. electromontis* in the north-northeast, and *P. furcifer*, in the east.

Mittermeier *et al.* (2010) noted that based on field observations (R.A. Mittermeier in 1995) the southernmost *P. electromontis* populations located between the Loky and the Manambato rivers could belong to a distinct species. This region is also known as Daraina, and is interestingly the only region where the golden-crowned sifaka (*Propithecus tattersalli*) can be found, hence supporting the idea that

other taxonomic groups could have been isolated therein. Groves and Tattersall (1991) also identified a *Phaner* museum sample from Vohémar (Iharana), located south of the Daraina region, and thus further south than the possibly distinct *P. electromontis* population or putative species. However, the lack of samples and data from these regions have not allowed researchers to go beyond suggestions.

To summarize, it is unclear whether some of the gaps in the distribution (Fig. 1) reflect real discontinuities, or lack of knowledge from regions that are still poorly studied. Analyzing the few samples available from these regions for which little is known but where deforestation is taking place, is an increasingly important objective. In this study we present new genetic and morphological data obtained from one juvenile *Phaner* individual captured in Analafiana. Our report benefits from morphometric measurements and mitochondrial DNA material of a caught individual with precise locality and with the construction of a phylogeny placing it within the *Phaner* evolutionary tree.

Material and Methods

Study site and sample collection

Between the 25th and the 31st of August 2013 we visited the Analafiana dry deciduous lowland forest, located between the Manambato and Manambery rivers, ~20km west of the town of Vohémar, Eastern Madagascar (Fig. 1; <https://google/maps/analafiana>). The Analafiana forest is the southernmost dry forest of northern Madagascar. The closest dry deciduous forests are located north of the Manambato River. It is mainly surrounded by grasslands, open landscape, and montane humid forests, the closest being the Salafaina forest on the West. Few naturalistic reports mentioning the Analafiana forest exist, most of which focuses on plants and reptiles (e.g. Raxworthy *et al.*, 2008; Leong Pock Tsy *et al.*, 2013). Due to this isolation, the Analafiana forest is expected to host a unique fauna and flora diversity.

We visited the Analafiana forest to survey lemur populations. We used line transect visual and acoustic distance sampling, trapping, and other survey methods that are described in our previous works in Northern Madagascar (Meyler *et al.*, 2012; Salmona *et al.*, 2013; Sgarlata *et al.*, 2017). We captured one individual of the genus *Phaner* by hand. We handled it after tranquilizing with an injection of Ketamine Hydrochloride (~3 mg/kg), collected ear biopsies and measured 18 morphological traits listed in Tab. 1. Field handling and sampling procedures followed Code of Best Practices for Field Primatology of the International Primatological Society, adhered to the legal requirements of France, Madagascar, and Portugal, and were approved by the Malagasy Ministry of Water and Forests.

Tab. 1: Morphometric measurements of the Alanafiana *Phaner* individual. This table shows the measures obtained for the juvenile individual sampled in Alanafiana together with published data for the other *Phaner* species.

Morphological traits (in mm)	<i>Phaner</i> spp. This study	<i>Phaner</i> spp. Vohemar	<i>P. furcifer</i>	<i>P. pallescens</i>	<i>P. parienti</i>	<i>P. electromontis</i>
Head length	50.3	59.5-54.6 ^b	-	53.1 ^b	55 ^b	56 ^b
Body length	124.0		-	-	-	-
Head-Body length	174.3		230 - 290 ^a	237 ^c -263 ^d	~238 ^d	272 ^d
Tail length	262.0		290 - 370 ^a	319 ^c -321 ^d	~401 ^d	344 ^d
Head width	26.4		-	-	-	-
Ear length	24.8		-	-	-	-
Ear width	15.9		-	-	-	-
Snout length	18.3		-	-	-	-
Intra orbital breadth	11.0		-	-	-	-
Inter orbital breadth	28.0	15.8-13.5 ^b	34.2 ^b	32.2 ^b	32.6 ^b	35 ^b
Tight length	48.3		-	-	-	-
Tibia length	60.5		-	-	-	-

Foot length	38.0		-	-	-	-
Radius length	35.0		-	-	-	-
Humerus length	34.2		-	-	-	-
3rd toe length	15.5		-	-	-	-
4th toe length	16.3		-	-	-	-
Tail circumference	26.0		-	-	-	-
Weight (g)	140.0		-	327 ^c -340 ^d	~360 ^d	387 ^d

References: a: Tattersall, (1982); b: Groves and Tattersall (1991); c: Schülke (2003); d: Mittermeier *et al.* (2010).

Sample conservation and laboratory procedures

We stored the biopsies in Queens Lysis Buffer (Seutin *et al.*, 1991) at ambient temperature during the fieldwork period and subsequently at -20°C. Genomic DNA was extracted from biopsies using a DNeasy Blood & Tissue Kit (QIAGEN #69506). Adaptations to the protocol included the addition of 20 µL of 1 M Dithiothreitol (Sigma-Aldrich #646563) during ligation and before eluting DNA in 80 µL of Buffer AE (QIAGEN).

We amplified and sequenced two mitochondrial (mtDNA) loci: cytochrome b (*cyt b*; 1150 bp), (Irwin *et al.*, 1991); and the cytochrome c oxidase subunit II (*cox2*; 684 bp) (Adkins and Honeycutt, 1994). Amplification of *cyt b* was carried out in a 10 µl reaction with 2.5 µL of 5X GoTaqFlexi Buffer, 20mM of MgCl₂, 3 mM of each dNTP, 8 µM of each primer (L14724; H15915) and 1U of GoTaqFlexi DNA polymerase (Promega #M7801). Amplification of *cox2* was carried out in a 10 µL reaction comprised of 3.6 µL of 2X MyTaq HS Mix (BioLine #BIO-25045), and 2 µM of each primer (L17553 and H8320). In both reactions, 70 ng of DNA template was used. Standard PCRs were run with an initial denaturing temperature of 95 °C for 2 min, the *cyt b* reaction followed with 35 cycles of 1 min at 94 °C, 45 sec at 52.5 °C, and 90 sec at 72 °C (*cox2* followed with 28 cycles of 30 sec at 95 °C, 1 min at 45 °C, and 75 sec at 72 °C), and a final extension of 10 min at 72 °C (Guschanski *et al.*, 2006; Horvath *et al.*, 2008). PCR products were sequenced in an ABI 3130 XL Genetic Analyzer (Applied Biosystems #4359571) and edited using Geneious v10.1.3 (Kearse *et al.* 2012).

Sequence alignment and phylogeny

We retrieved available mitochondrial genomes of the *Phaner* genus and of a *Cheirogaleus major* individual, KM112323.1 (Tab. 2; Louis and Lei, 2016), from NCBI. We aligned *cyt b* and *cox2* and the above-mentioned mitochondrial genomes using MAFFT v7.031b (Kato and Standley, 2013), visually checked the alignment in GENEIOUS v7 (Kearse *et al.*, 2012) and subsequently truncate the alignment to keep the two aligned regions (*cyt b* and *cox2*). The final alignment consisted of two concatenated loci of 1346 bp. We used jModelTest v2.1.5 7 (Darriba *et al.*, 2012) to select the best-fitting model of nucleotide substitution for the concatenated loci (best model: GTR; Generalized Time-Reversed). We built a phylogeny using RAXML (Stamatakis, 2014), with 500 rapid bootstrap and 20 ML replicates, the GTR nucleotide sequence evolution model with CAT approximation of rate heterogeneity, partitions of each locus and codon positions and using the *Cheirogaleus major* sequence as outgroup (Tab. 2). The tree was graphically represented using the *ape* R package (Paradis *et al.*, 2004; R CoreTeam, 2014).

Tab. 2: Sequences used in the phylogeny reconstruction.

Voucher	Species	Locality	Latitude	Longitude	Reference	Accession
AMB5.37	<i>Phaner electromontis</i>	Montagne d'Ambre	-12.5274	49.1742	Louis and Lei (2016)	KM112338.1
ANK5.11	<i>Phaner electromontis</i>	Ankarana	-12.9651	49.1336	Louis and Lei (2016)	KM112339.1
KIR6.22	<i>Phaner pallescens</i>	Kirindy	-20.0660	44.6549	Louis and Lei (2016)	KM112340.1
TAFO6.11	<i>Phaner parienti</i>	Antafondro	-14.0466	48.2248	Louis and Lei (2016)	KM112341.1
B25_2013	<i>Phaner spp.</i>	Anafiana	-13.4669	49.8311	<i>This study</i>	MG813795 - MG813796

MAS6.10	<i>Cheirogaleus major</i>	Masoala (Masiaposa)	-15.6719	49.9662	Louis and Lei (2016)	KM112323.1
---------	---------------------------	------------------------	----------	---------	-------------------------	------------

Accession: NCBI accession number.

Results and Discussion

The captured individual was identified as a juvenile female (specimen B25_2013; Tab. 1 and Fig. 2). With its tail longer than its head-body length (Tab. 1), a darkening pattern limited to the last third of the tail, and the presence of clear fork mark and dorsal stripe (Fig. 2), the individual seems to best fit the description of *P. electromontis* than that of the other *Phaner* species, according to the determination key and species diagnosis proposed by Groves and Tattersall (1991). Unfortunately, out of the 18 measures that we obtained (Tab. 1), only two (head length and inter orbital breadth) were also used by Groves and Tattersall (1991) making quantitative comparisons tentative at best. However, we note that the head width and inter-orbital breadth of our individual are smaller than the values reported by these authors for *P. electromontis* (Tab. 1) and for the “doubtful subspecies” population of Vohémar. These differences can be attributed to the individual being a juvenile.

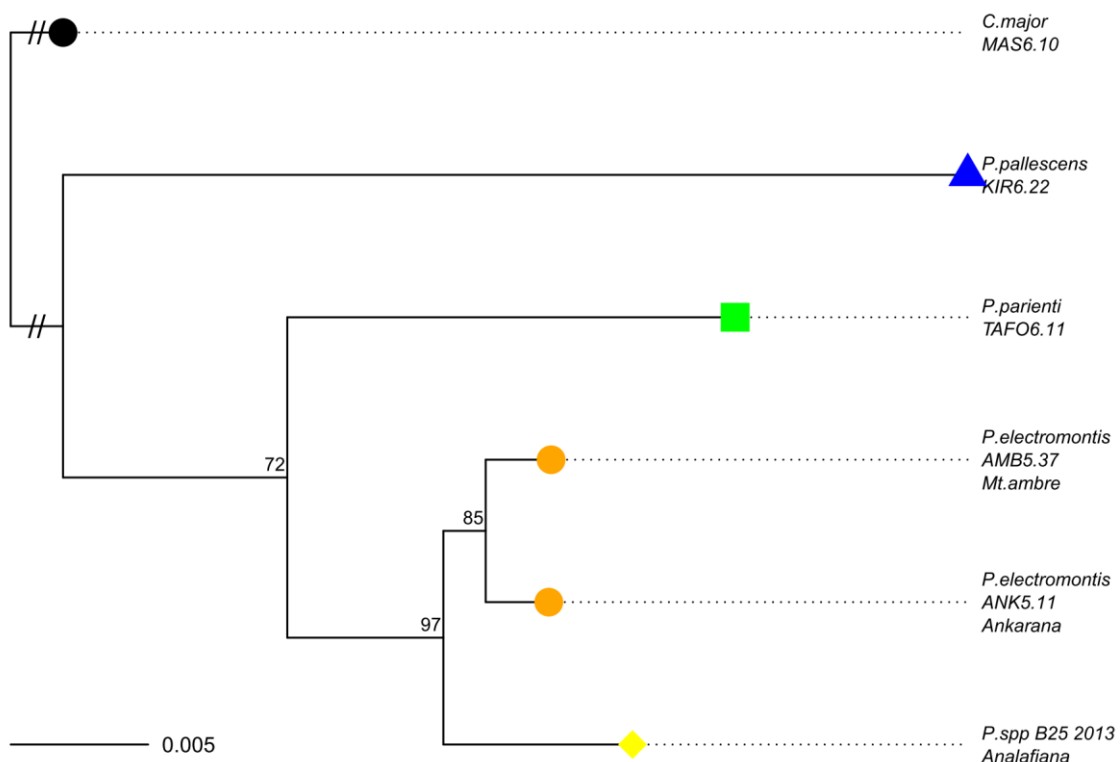


Fig. 2: Photographic illustrations of the coat color patterns of the juvenile *Phaner* specimen B25_2013. a) Dorsal view of the specimen; b) zoomed view of the specimen’s tail coloration pattern and c) zoomed profile view of the specimen’s head.

The phylogenetic tree represented in Fig. 3 was based on two DNA fragments with a total length of 1346 bp. Other *Phaner* species sequences published by Louis and Lei (2016) were much longer, however, the topology obtained here was consistent and our bootstrap values are generally high. The first point to notice is that the two *P. electromontis* sequences of Louis and Lei (2016) group together. Moreover the Analafiana *Phaner* sequence forms a sister lineage to *P. electromontis* (Fig. 3). The Analafiana *Phaner* sequence is divergent from the two *P. electromontis* sequences, but closer to these than other *Phaner* sequences. This suggests that if our individual belongs to a new species, it is genetically closer to *P. electromontis* than *P. parienti* in the north-west.

Altogether, we identified for the first time a population of *Phaner* that is isolated, apparently genetically different from known populations of *Phaner*, and is likely under serious threat. Mittermeier *et al.* (2010) noted that *Phaner* are very vocal and it is easy to acoustically detect their presence. Surveys by our

group over several field seasons provided very little support for *Phaner*'s presence in the surrounding humid forests (JS and LC, unpublished data), whereas their presence was obvious in Analafiana.

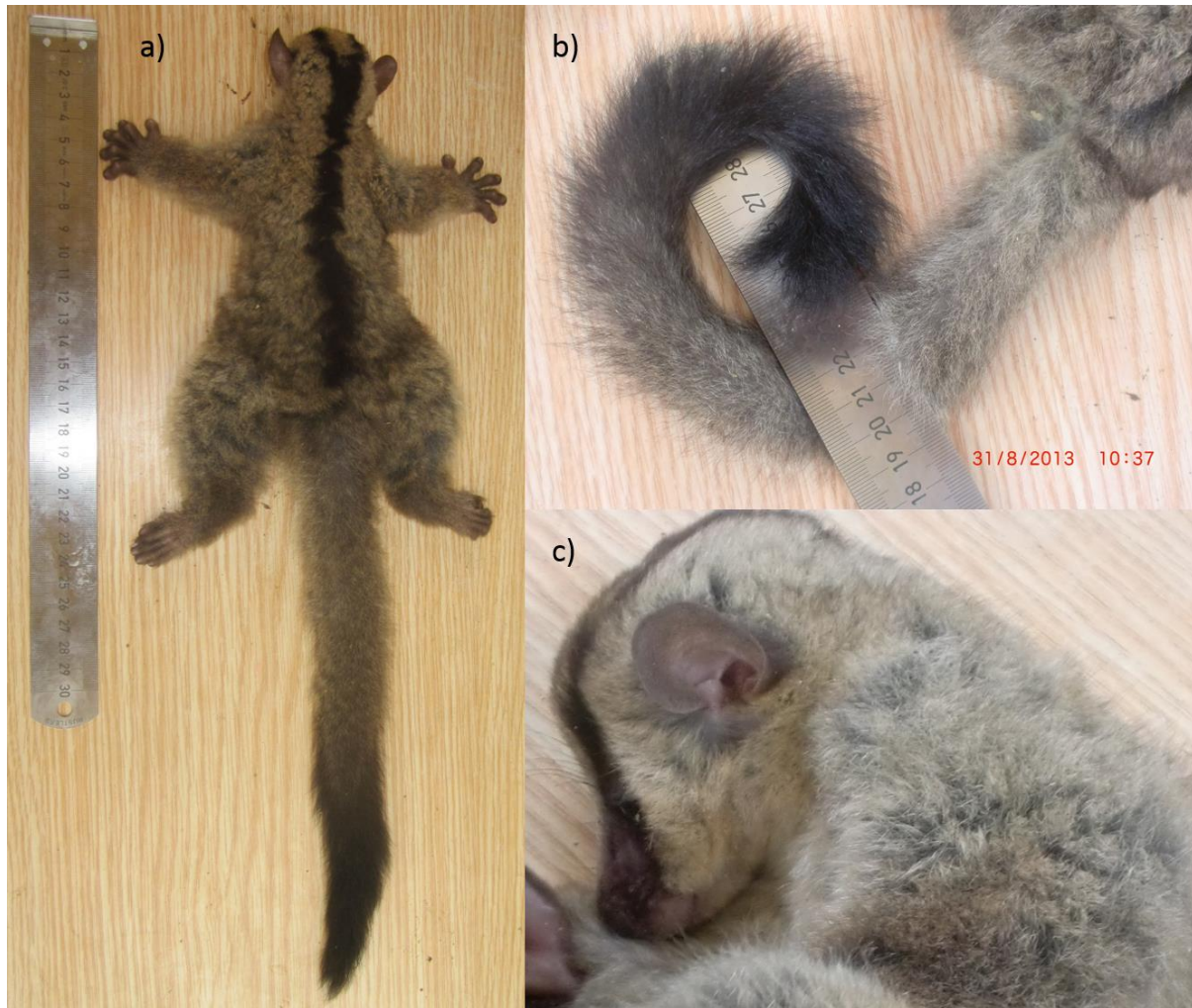


Fig. 3: Phylogeny of the *Phaner* genus obtained from *cyt b* and *cox2* mitochondrial loci. Hashed edges of the root (*C. major*) have been shortened for graphical purposes and its branch lengths are therefore not to scale. Tree tip symbols and colors correspond to those of figure 1. Bootstraps values are indicated as percentage derived from 500 replications.

Our data do not allow us to determine whether a new species has been discovered, and more work would be needed across all species of *Phaner* to increase our understanding of within and between populations, and species diversity. The population between the Loky and Manambato River was identified as a possibly distinct species; it has not yet been systematically investigated (Mittermeier *et al.*, 2010). Additional research is needed to determine the exact taxonomic status of these new lineages. To be comprehensive and integrative, this research should include 1) extensive morphological, genetic and behavioral characterization of the *Phaner* species and populations (museum specimens should be revisited), 2) characterizing species distribution with presence-absence surveys (acoustic surveys at dusk are an efficient tool for *Phaner*; Charles-Dominique and Petter, 1980; Salmons, 2014) and 3) acoustic characterization of the populations (shown to be potentially informative criteria for nocturnal cryptic species differentiation; (Braune *et al.*, 2008). As noted above, *Phaner* are very vocal animals. Do we wish to hear the sound of silence (Simon and Garfunkel, 1964).

Acknowledgments

We thank CAFF/CORE, the ‘Direction Générale de l’Environnement et des Forêts’, the ‘Direction Régionale de l’Environnement et des Forêts région SAVA’. The field work was possible thanks to the continuous support of the ‘Département de Biologie Animale et Ecologie’, University of Mahajanga, the University of Antsirana and to a large extent, thanks to the participation of the Malagasy master students the field assistants and volunteers, and many great local guides and cooks which we warmly thanks for their help in the field and for sharing their incomparable expertise of the forest, misaotra anareo jiaby. Finally we would like to thanks the IGC Sequencing Service for their collaboration. Financial support for this study was also provided by the ‘Fundação para a Ciência e a Tecnologia’ (grant number SFRH/BD/64875/2009 to J.S., PD/BD/114343/2016 to G.M.S and PTDC/BIA-BIC/4476/2012, PTDC/BIA-BEC/100176/2008 to L.C.), the Optimus!Alive-IGC to H.T (2013), the GDRI Madagascar, the ‘Laboratoire d’Excellence’ (LABEX) entitled TULIP (ANR-10-LABX-41; ANR-11-IDEX-0002-02), ‘Rufford Small Grant Foundation’ (grant number 10941-1 to J.S. and 12973-1 to I.M.T), the Instituto Gulbenkian de Ciência, the LIA BEEG-B (Laboratoire International Associé - Bioinformatics, Ecology, Evolution, Genomics and Behaviour) (CNRS), and the 5582015-2016 BiodivERsA COFUND call for research proposals, with the national funders ANR (ANR-16559EBI3-0014), FCT (Biodiversa/0003/2015), and PT-DLR (01LC1617A).

References

- Adkins, R.M.; Honeycutt, R.L. 1994. Evolution of the primate cytochrome c oxidase subunit II gene. *Journal of Molecular Evolution* 38: 215-231.
- Braune, P.; Schmidt, S.; Zimmermann, E. 2008. Acoustic divergence in the communication of cryptic species of nocturnal primates (*Microcebus* spp.). *BMC biology* 6: 19.
- Charles-Dominique, P.; Petter, J.J. 1980. Ecology and social life of *Phaner furcifer*. *Nocturnal Malagasy primates: Ecology, physiology and behavior* 75-96.
- Darriba, D.; Taboada, G.L.; Doallo, R.; Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature methods* 9: 772-772.
- Groves, C.P. 2001. *Primate taxonomy*. Washington DC: Smithsonian Institution Press.
- Groves, C.P., Tattersall, I. 1991. Geographical variation in the fork-marked lemur, *Phaner furcifer* (Primates, Cheirogaleidae). *Folia Primatologica* 56: 39-49.
- Guschanski, K.; Olivieri, G.; Funk, S.M.; Radespiel, U. 2006. MtDNA reveals strong genetic differentiation among geographically isolated populations of the golden brown mouse lemur, *Microcebus ravelobensis*. *Conservation Genetics* 8: 809-821.
- Hall, T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95-98.
- Horvath, J.E.; Weisrock, D.W.; Embry, S.L.; Fiorentino, I.; Balhoff, J.P.; Kappeler, P., Wray, G.A.; Willard, H.F.; Yoder, A.D. 2008. Development and application of a phylogenomic toolkit: resolving the evolutionary history of Madagascar’s lemurs. *Genome Research* 18: 489-499.
- Hotaling, S.; Foley, M.E.; Lawrence, N.M.; Bocanegra, J.; Blanco, M.B.; Rasoloarison, R., Kappeler, P.M.; Barrett, M.A.; Yoder, A.D.; Weisrock, D.W. 2016. Species discovery and validation in a cryptic radiation of endangered primates: coalescent-based species delimitation in Madagascar’s mouse lemurs. *Molecular Ecology* 25: 2029-2045.
- Irwin, D.M.; Kocher, T.D.; Wilson, A.C. 1991. Evolution of the cytochrome b gene of mammals. *Journal of Molecular Evolution* 32: 128-144.
- IUCN. 2012a. Andriaholinirina, N.; Baden, A.; Blanco, M.; Chikhi, L.; Cooke, A.; Davies, N.; Dolch, R.; Donati, G.; Ganzhorn, J.; Golden, C.; Groeneveld, L.F.; Hapke, A.; Irwin, M.; Johnson, S.; Kappeler, P.; King, T.; Lewis, R.; Louis, E.E.; Markolf, M.; Mass, V.; Mittermeier, R.A.; Nichols, R.; Patel, E.; Rabarivola, C.J.; Raharivololona, B.; Rajaobelina, S.; Rakotoarisoa, G.; Rakotomanga, B.; Rakotonanahary, J.; Rakotondrainibe, H.; Rakotondratsimba, G.; Rakotondratsimba, M.; Rakotonirina, L.; Ralainasolo, F.B.; Ralison, J.; Ramahaleo, T.; Ranaivoarisoa, J.F.; Randrianahaleo, S.I.; Randrianambinina, B.; Randrianarimanana, L.; Randrianasolo, H.; Randriatahina, G.; Rasamimanana, H.; Rasolofoharivelo, T.; Rasoloharijaona, S.; Ratelolahy, F.; Ratsimbazafy, J.; Ratsimbazafy, N.; Razafindraibe, H.; Razafindramanana, J.; Rowe, N.; Salmons, J.; Schwitzer, C.; Seiler, M.; Volampeno, S.; Wright, P.; Youssouf, J.; Zaonarivelo, J.; Zaramody, A. *Phaner electromontis*: The IUCN Red

- List of Threatened Species. International Union for Conservation of Nature. <https://doi.org/10.2305/IUCN.UK.2014-1.RLTS.T16875A16114199.en>
- IUCN. 2012b. Andriaholinirina, N.; Baden, A.; Blanco, M.; Chikhi, L.; Cooke, A.; Davies, N.; Dolch, R.; Donati, G.; Ganzhorn, J.; Golden, C.; Groeneveld, L.F.; Hapke, A.; Irwin, M.; Johnson, S.; Kappeler, P.; King, T.; Lewis, R.; Louis, E.E.; Markolf, M.; Mass, V.; Mittermeier, R.A.; Nichols, R.; Patel, E.; Rabarivola, C.J.; Raharivololona, B.; Rajaobelina, S.; Rakotoarisoa, G.; Rakotomanga, B.; Rakotonanahary, J.; Rakotondrainibe, H.; Rakotondratsimba, G.; Rakotondratsimba, M.; Rakotonirina, L.; Ralainasolo, F.B.; Ralison, J.; Ramahaleo, T.; Ranaivoarisoa, J.F.; Randrianahaleo, S.I.; Randrianambinina, B.; Randrianarimanana, L.; Randrianasolo, H.; Randriatahina, G.; Rasamimananana, H.; Rasolofoharivelo, T.; Rasoloharijaona, S.; Ratelolahy, F.; Ratsimbazafy, J.; Ratsimbazafy, N.; Razafindraibe, H.; Razafindramanana, J.; Rowe, N.; Salmona, J.; Schwitzer, C.; Seiler, M.; Volampeno, S.; Wright, P.; Youssof, J.; Zaonarivelo, J.; Zaramody, A. *Phaner furcifer*: The IUCN Red List of Threatened Species. International Union for Conservation of Nature. <https://doi.org/10.2305/IUCN.UK.2014-1.RLTS.T16872A16114295.en>
- IUCN. 2012c. Andriaholinirina, N.; Baden, A.; Blanco, M.; Chikhi, L.; Cooke, A.; Davies, N.; Dolch, R.; Donati, G.; Ganzhorn, J.; Golden, C.; Groeneveld, L.F.; Hapke, A.; Irwin, M.; Johnson, S.; Kappeler, P.; King, T.; Lewis, R.; Louis, E.E.; Markolf, M.; Mass, V.; Mittermeier, R.A.; Nichols, R.; Patel, E.; Rabarivola, C.J.; Raharivololona, B.; Rajaobelina, S.; Rakotoarisoa, G.; Rakotomanga, B.; Rakotonanahary, J.; Rakotondrainibe, H.; Rakotondratsimba, G.; Rakotondratsimba, M.; Rakotonirina, L.; Ralainasolo, F.B.; Ralison, J.; Ramahaleo, T.; Ranaivoarisoa, J.F.; Randrianahaleo, S.I.; Randrianambinina, B.; Randrianarimanana, L.; Randrianasolo, H.; Randriatahina, G.; Rasamimananana, H.; Rasolofoharivelo, T.; Rasoloharijaona, S.; Ratelolahy, F.; Ratsimbazafy, J.; Ratsimbazafy, N.; Razafindraibe, H.; Razafindramanana, J.; Rowe, N.; Salmona, J.; Schwitzer, C.; Seiler, M.; Volampeno, S.; Wright, P.; Youssof, J.; Zaonarivelo, J.; Zaramody, A. *Phaner pallescens*: The IUCN Red List of Threatened Species. International Union for Conservation of Nature. <https://doi.org/10.2305/IUCN.UK.2014-1.RLTS.T16874A16114392.en>
- IUCN. 2012d. Andriaholinirina, N.; Baden, A.; Blanco, M.; Chikhi, L.; Cooke, A.; Davies, N.; Dolch, R.; Donati, G.; Ganzhorn, J.; Golden, C.; Groeneveld, L.F.; Hapke, A.; Irwin, M.; Johnson, S.; Kappeler, P.; King, T.; Lewis, R.; Louis, E.E.; Markolf, M.; Mass, V.; Mittermeier, R.A.; Nichols, R.; Patel, E.; Rabarivola, C.J.; Raharivololona, B.; Rajaobelina, S.; Rakotoarisoa, G.; Rakotomanga, B.; Rakotonanahary, J.; Rakotondrainibe, H.; Rakotondratsimba, G.; Rakotondratsimba, M.; Rakotonirina, L.; Ralainasolo, F.B.; Ralison, J.; Ramahaleo, T.; Ranaivoarisoa, J.F.; Randrianahaleo, S.I.; Randrianambinina, B.; Randrianarimanana, L.; Randrianasolo, H.; Randriatahina, G.; Rasamimananana, H.; Rasolofoharivelo, T.; Rasoloharijaona, S.; Ratelolahy, F.; Ratsimbazafy, J.; Ratsimbazafy, N.; Razafindraibe, H.; Razafindramanana, J., Rowe, N., Salmona, J., Schwitzer, C., Seiler, M., Volampeno, S., Wright, P.; Youssof, J.; Zaonarivelo, J.; Zaramody, A. *Phaner parienti*: The IUCN Red List of Threatened Species. International Union for Conservation of Nature. <https://doi.org/10.2305/IUCN.UK.2014-1.RLTS.T16873A16114500.en>
- Katoh, K.; Standley, D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular biology and evolution* 30: 772-780.
- Kearse, M.; Moir, R.; Wilson, A.; Stones-Havas, S.; Cheung, M.; Sturrock, S.; Buxton, S.; Cooper, A.; Markowitz, S.; Duran, C.; Thierer, T. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.
- Laird, P.W.; Zijderveld, A.; Linders, K.; Rudnicki, M.A.; Jaenisch, R.; Berns, A. 1991. Simplified mammalian DNA isolation procedure. *Nucleic Acids Research* 19: 4293.
- Lei, R.; Frasier, C.L.; Hawkins, M.T.; Engberg, S.E.; Bailey, C.A.; Johnson, S.E.; McLain, A.T.; Groves, C.P.; Perry, G.H.; Nash, S.D.; Mittermeier, R.A. 2016. Phylogenomic reconstruction of sportive lemurs (genus *Lepilemur*) recovered from mitogenomes with inferences for Madagascar biogeography. *Journal of Heredity* 108: 107-119.

- Leong Pock Tsy, J.-M.; Lumaret, R.; Flaven-Noguier, E.; Sauve, M.; Dubois, M.-P.; Danthu, P. 2013. Nuclear microsatellite variation in Malagasy baobabs (*Adansonia*, Bombacoideae, Malvaceae) reveals past hybridization and introgression. *Annals of botany* 112: 1759-1773.
- Louis, E.E.J.; Lei, R. 2016. Mitogenomics of the family Cheirogaleidae and relationships to taxonomy and biogeography in Madagascar. *The Dwarf and Mouse Lemurs of Madagascar: Biology, Behavior and Conservation Biogeography of the Cheirogaleidae* 73: 54.
- Martin, R.D. 1995. Prosimians: From Obscurity to Extinction? In Alterman, Em L.; Doyle, G. A.; Izard, M.K. (eds.). *Creatures of the Dark City*: Springer US. pp. 535–563.
- Meyler, S.V.; Salmona, J.; Ibouroi, M.T.; Besolo, A.; Rasolondraibe, E.; Radespiel, U.; Rabarivola, C.; Chikhi, L. 2012. Density estimates of two endangered nocturnal lemur species from Northern Madagascar: new results and a comparison of commonly used methods. *American Journal of Primatology* 74: 414-422.
- Mittermeier, R.A.; Louis, E.E.; Richardson, M.; Schwitzer, C.; Langrand, O.; Rylands, A.B.; Hawkins, F.; Rajaobelina, S.; Ratsimbazafy, J.; Rasoloarison, R.; Roos, C.; Kappeler, P.M.; Mackinnon, J. 2010. *Lemurs of Madagascar*. Third edition. Conservation International, Tropical field guide series.
- Myers, N.; Mittermeier, R.A.; Mittermeier, C.G.; da Fonseca, G.A.B.; Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403: 853-858.
- Olivieri, G.; Zimmermann, E.; Randrianambinina, B.; Rasoloharijaona, S.; Rakotondravony, D.; Guschanski, K.; Radespiel, U. 2007. The ever-increasing diversity in mouse lemurs: Three new species in north and northwestern Madagascar. *Molecular Phylogenetics and Evolution* 43: 309-327.
- Paradis, E.; Claude, J., Strimmer, K. 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20: 289-290.
- R CoreTeam. 2014. R: A language and environment for statistical computing. <http://www.R-project.org>
- Radespiel, U.; Olivieri, G.; Rasolofoson, D.W.; Rakotondratsimba, G.; Rakotonirainy, O.; Rasoloharijaona, S.; Randrianarison, R.M. 2008. Exceptional diversity of mouse lemurs (*Microcebus* spp.) in the Makira region with the description of one new species. *American Journal of Primatology* 70: 1033-1046.
- Raxworthy, C. J.; Pearson, R. G.; Zimkus, B.M.; Reddy, S.; Deo, A. J.; Nussbaum, R.A.; Ingram, C.M. 2008. Continental speciation in the tropics: contrasting biogeographic patterns of divergence in the *Uroplatus* leaf-tailed gecko radiation of Madagascar. *Journal of Zoology* 275: 423-440.
- Rumpler, Y.; Dutrillaux, B. 1979. Chromosomal evolution in Malagasy lemurs. IV. Chromosome banding studies in the genera *Phaner*, *Varecia*, *Lemur*, *Microcebus*, and *Cheirogaleus*. *Cytogenetics and cell genetics* 24: 224-232.
- Salmona, J.; Jan, F.; Rasolondraibe, E.; Zaranaina, R.; Saïd Oussen, D.; Mohamed-Thani, I.; ... Chikhi, L. 2013. Survey of the critically endangered Perrier's sifaka (*Propithecus perrieri*) across most of its distribution range. *Lemur News* 17: 9-12.
- Salmona, J. 2014. Conservation, distribution and population size update of endangered nocturnal lemur species from Northern Madagascar, Rufford Small Grant final report (Rufford Small Grant Final Report No. 1) p. 21.
- Salmona, J.; Rakotonanahary, A.; Thani, I.M.; Zaranaina, R.; Ralantoharijaona, T.; Jan, F.; Rasolondraibe, E.; Barnavon, M.; Beck, A.; Wholhauser, S; Ranirison, P. 2014. Estimation des densités et tailles de population du Microcèbe Roux du Nord de (*Microcebus tavaratra*) dans la région Loky-Manambato (Daraina). *Lemur News* 18: 73-75.
- Schneider, N.; Chikhi, L.; Currat, M.; Radespiel, U. 2010. Signals of recent spatial expansions in the grey mouse lemur (*Microcebus murinus*). *BMC Evolutionary Biology* 10: 105.
- Schülke, O. 2003. *Phaner furcifer*, fork-marked lemur, vakihandry, tanta. *The Natural History of Madagascar*. Chicago: University of Chicago Press. pp. 1318–1320.
- Schwitzer, C.; Mittermeier, R.A.; Davies, N.; Johnson, S.; Ratsimbazafy, J.; Razafindramanana, J.; Louis, E.E.; Rajaobelina, S. 2013. *Lemurs of Madagascar: a strategy for their conservation 2013-2016*. IUCN SSC Primate Specialist Group, Bristol Conservation and Science Foundation, Conservation International, Bristol, UK.
- Seutin, G.; White, B.N.; Boag, P.T. 1991. Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology* 69: 82-90.

- Sgarlata, G.M.; Salmons, J.; Aleixo-Pais, I.; Rakotonanahary, A.; Sousa, A.P.; Kun-Rodrigues, C.; Chikhi, L. 2017. Genetic differentiation and demographic history of the northern rufous mouse lemur (*Microcebus tavaratra*) across a fragmented landscape in Northern Madagascar. *Submitted*.
- Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.
- Tattersall, I. 1982. *The primates of Madagascar*. New York: Columbia University Press.
- Wilmé, L.; Goodman, S.M.; Ganzhorn, J.U. 2006. Biogeographic evolution of Madagascar's microendemic biota. *Science* 312: 1063-1065.