

## A New Species in the Genus *Cheirogaleus* (Cheirogaleidae)

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**Abstract:** The genus *Cheirogaleus*, the dwarf lemurs (Infraorder Lemuriformes), has been identified as harboring cryptic species diversity. More comprehensive fieldwork combined with improvements in genetic research has revealed a larger radiation of species than was initially described in a number of lemur genera, including *Avahi*, *Lepilemur*, *Microcebus*, and *Mirza*. Available genetic and morphological evidence suggests that *Cheirogaleus* is among the genera where diversity was previously underestimated, and additional fieldwork may reveal even more species. A population of *Cheirogaleus* from northern Madagascar in and around Montagne d'Ambre National Park, surveyed during an expedition in 2005, was recently identified and proposed as a new species. Additional specimens were obtained during fieldwork in February of 2015. Subsequent genetic and morphological analyses of the data collected have determined that this population is an independent lineage, and herein we describe this new species, which we name *Cheirogaleus andysabini* after New York philanthropist Andy Sabin.

**Key Words:** *Cheirogaleus*, cryptic species, dwarf lemurs, Madagascar, new species

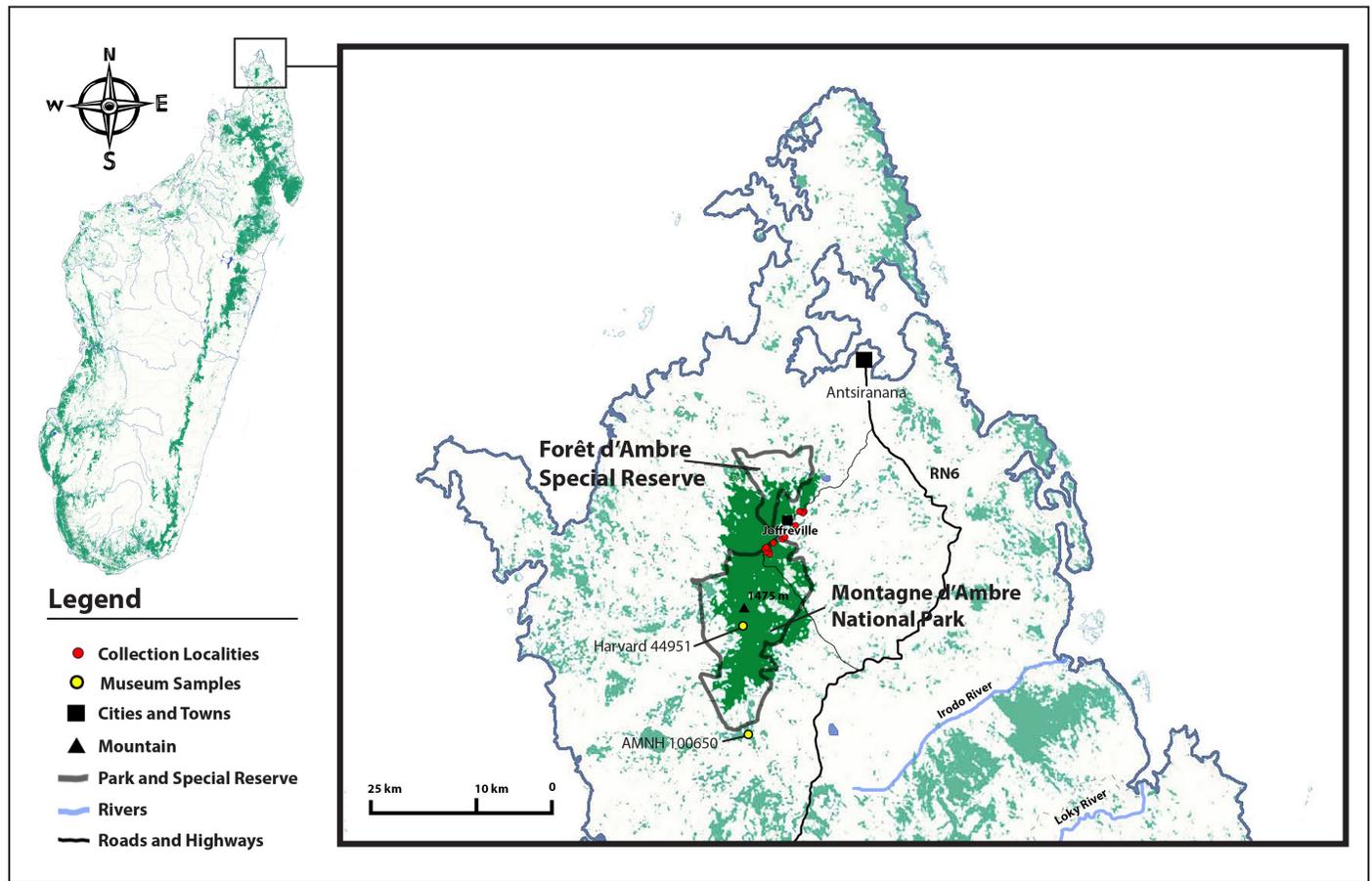
### Introduction

The dwarf lemurs (genus *Cheirogaleus*) are a radiation of small, arboreal primates endemic to the island of Madagascar (Mittermeier *et al.* 2010). The taxonomy and species status in *Cheirogaleus* has been contentious; a situation exacerbated by the limited number of specimens available for study in collections (Schwarz 1931; Groves 2000; Lei *et al.* 2014). Groves (2000) recognized seven *Cheirogaleus* species (*C. major*, *C. medius*, *C. crossleyi*, *C. adipicaudatus*, *C. sibreei*, *C. ravus*, and *C. minusculus*); a number that has been challenged as overly conservative in recent fieldwork and genetic analyses, suggesting a larger radiation in this genus (Hapke *et al.* 2005; Groeneveld *et al.* 2009, 2010; Thiele *et al.* 2013; Lei *et al.* 2014).

Concerns about overenthusiastic species descriptions in the lemuriform radiation raised by Tattersall (2007, 2013) have been addressed previously in Lei *et al.* (2014). Application of the Phylogenetic Species Concept and access to data from molecular technology (Eldredge and Cracraft 1980; Wheeler and Platnick 2000) has nearly tripled the number of recognized lemur species since the early 1980s (36 to >103; Tattersall 1982; Mittermeier *et al.* 2010). *Cheirogaleus* has not experienced the type of genus-level expansion seen in

*Lepilemur* (Louis *et al.* 2006) or *Microcebus* (Mittermeier *et al.* 2010); genera with the greatest increases in recognized species diversity. Nevertheless, *Cheirogaleus* appears to be harboring greater diversity than previously suspected (Thiele *et al.* 2013; Lei *et al.* 2014).

A new species of *Cheirogaleus* was identified via extensive fieldwork in and around Montagne d'Ambre National Park (Fig. 1) in November of 2005 (Lei *et al.* 2014), with additional fieldwork in February of 2015. The massif of Montagne d'Ambre is of volcanic origin (Segalen 1956), and this rain forest ecotype may have been separated from the surrounding dry deciduous vegetation for millions of years (Raxworthy and Nussbaum 1994), with the last volcanic eruptions occurring as recently as 2 mya (DuPuy and Moat 1996). It has a distinctive microclimate that is in part the result of greater than average precipitation in comparison to the surrounding areas. Raxworthy and Nussbaum (1994) noted the presence of species there that were altitudinal specialists, with those above 900 m living in moist rainforest, and those below 900 m living in a transitional area with a dry deciduous forest. Montagne d'Ambre's combination of geology and climate has resulted in a unique community of microendemic fauna and flora; plants (Mathieu 2003; Callmander *et*



**Figure 1.** Map of Montagne d'Ambre and the surrounding region in northern Madagascar. Red circles in and around Montagne d'Ambre represent collection localities of *Cheirogaleus* sp. nov. 1 individuals listed in Tables 1 and 2. Yellow circles represent two museum samples, Harvard Museum of Comparative Zoology (MCZH) 44951 received in January 1929 as part of the Grandidier collection and American Museum of Natural History (AMNH) 100650 collected November 7, 1930 by Austin L. Rand, that are holotype candidates (The MCZH 44951 is missing in inventory, 1994.).

al. 2009), amphibians (D'Cruze et al. 2010; Rakotoarison et al. 2015), reptiles (Raxworthy and Nussbaum 1994; D'Cruze et al. 2008; Glaw et al. 2009; Ratsavina et al. 2011), birds (Wilmé 1996), and mammals (Louis et al. 2008; Goodman et al. 2015).

The first herpetological survey in the area of Montagne d'Ambre was carried out in 1893, and subsequent expeditions have cataloged the regional endemism of the park and surrounding area (D'Cruze et al. 2008). The national park and surrounding region continues to be an exciting source of new species, including a chameleon in the genus *Furcifer* (Glaw et al. 2009). The area is also home to a number of lemurs, including two species in the genus *Eulemur* (*E. coronatus* and *E. sanfordi*), the Montagne d'Ambre fork-marked lemur (*Phaner electromontis*), the aye-aye (*Daubentonia madagascariensis*), and the recently described Montagne d'Ambre mouse lemur (*Microcebus arnholdi* Louis et al., 2008). Malagasy endemic carnivores are also found in the park, including the rare falanouc (*Eupleres goudotii*) and the ring-tailed mongoose (*Galidia elegans*) (Mittermeier et al. 2010).

The area around Montagne d'Ambre National Park and the Forêt d'Ambre Special Reserve were initially protected in the 1920's as a forest station called 'Les Roussettes'. In

1958, Montagne d'Ambre became the first national park created in Madagascar, and the Forêt d'Ambre S.R. was created the same year (Raxworthy and Nussbaum 1994). Subsequent deforestation has reduced the size of both the national park and the special reserve, in particular impacting the lowland rainforests (those below 900 m). More than twenty years ago, Raxworthy and Nussbaum (1994) observed a noticeable difference in forest cover based on a comparison of aerial photographs from the 1950s and the early 1990s. Ongoing deforestation has since been noted by Glaw and Vences (2007) and D'Cruze et al. (2008), among others.

Montagne d'Ambre National Park, like much of the remaining forested areas of Madagascar, is under the greatest threat from a combination of slash-and-burn agriculture (*tavy*) and charcoal extraction (Nicoll and Langrand 1989). The growth of the nearby port city of Antsiranana (formerly Diego Suarez) has accelerated deforestation by greatly increasing the demand for charcoal, a primary source of fuel for cooking. The town of Joffreville, named for the French General Joseph Joffre of First World War fame and located just east of the national park, is a center of charcoal extraction. As of 2001, 98% of the population of Joffreville was employed primarily in agriculture, with 90% farming and 8%

**Table 1.** Free-ranging *Cheirogaleus* samples used in this study. IDs correspond to Figure 2 and Figure 5 (Lei *et al.* 2014), with the exception of the samples from Thiele *et al.* 2013 (denoted at the bottom of the table, as well as on the map).

ID	Species designation	Location	Latitude	Longitude
AMB5.22	<i>C. sp. nov. 1</i>	Montagne d'Ambre	-12.52731	49.17331
AMB5.23	<i>C. sp. nov. 1</i>	Montagne d'Ambre	-12.53017	49.17464
AMB5.27	<i>C. sp. nov. 1</i>	Montagne d'Ambre	-12.51722	49.1795
AMB5.28	<i>C. sp. nov. 1</i>	Montagne d'Ambre	-12.47881	49.21222
AMB5.29	<i>C. sp. nov. 1</i>	Montagne d'Ambre	-12.47922	49.21606
AMB5.30	<i>C. sp. nov. 1</i>	Montagne d'Ambre	-12.47917	49.21597
AMB5.31	<i>C. sp. nov. 1</i>	Montagne d'Ambre	-12.51083	49.19275
AMB5.32	<i>C. sp. nov. 1</i>	Montagne d'Ambre	-12.51242	49.18956
AMB5.34	<i>C. sp. nov. 1</i>	Montagne d'Ambre	-12.47822	49.21717
AMB5.35	<i>C. sp. nov. 1</i>	Montagne d'Ambre	-12.49519	49.20783
ANJZ1	<i>C. crossleyi</i>	Anjozorobe	-18.4775	47.93812
ANJZ2	<i>C. crossleyi</i>	Anjozorobe	-18.4775	47.93812
ANJZ3	<i>C. crossleyi</i>	Anjozorobe	-18.4775	47.93812
JOZO4.7	<i>C. crossleyi</i>	Anjozorobe	-18.46789	47.94131
JOZO4.8	<i>C. crossleyi</i>	Anjozorobe	-18.46789	47.94131
JOZO4.9	<i>C. crossleyi</i>	Anjozorobe	-18.46789	47.94131
JOZO4.10	<i>C. crossleyi</i>	Anjozorobe	-18.46789	47.94131
MIZA16	<i>C. crossleyi</i>	Maromizaha	-18.97375	48.46461
MIZA19	<i>C. crossleyi</i>	Maromizaha	-18.97067	48.46431
MIZA6.1	<i>C. crossleyi</i>	Maromizaha	-18.95694	48.49236
MIZA6.2	<i>C. crossleyi</i>	Maromizaha	-18.95694	48.49236
MIZA7.1	<i>C. crossleyi</i>	Maromizaha	-18.95694	48.49236
TAD4.10	<i>C. crossleyi</i>	Mantadia	-18.80942	48.42731
TAD4.11	<i>C. crossleyi</i>	Mantadia	-18.80942	48.42731
TAD4.12	<i>C. crossleyi</i>	Mantadia	-18.80942	48.42731
TOR6.2	<i>C. crossleyi</i>	Torotorofotsy	-18.83658	48.34719
TORO8.11	<i>C. crossleyi</i>	Torotorofotsy	-18.77044	48.42814
TORO8.16	<i>C. crossleyi</i>	Torotorofotsy	-18.76856	48.42475
TVY7.12	<i>C. crossleyi</i>	Ambatovy	-18.85086	48.29256
TVY7.196B	<i>C. crossleyi</i>	Ambatovy	-18.86433	48.31136
TVY7.197	<i>C. crossleyi</i>	Ambatovy	-18.86658	48.30972
TVY7.199	<i>C. crossleyi</i>	Ambatovy	-18.87294	48.305
TVY7.20	<i>C. crossleyi</i>	Ambatovy	-18.84797	48.29433
TVY7.200	<i>C. crossleyi</i>	Ambatovy	-18.86883	48.30975
TVY7.206	<i>C. crossleyi</i>	Ambatovy	-18.87289	48.30453
TVY7.207	<i>C. crossleyi</i>	Ambatovy	-18.87178	48.30297
TVY7.22	<i>C. crossleyi</i>	Ambatovy	-18.85017	48.292
TVY7.33	<i>C. crossleyi</i>	Ambatovy	-18.85086	48.29256
ZAH240	<i>C. crossleyi</i>	Zahamena	-17.48917	48.74722
TRA8.81	<i>C. sp. nov. 2</i>	Andringitra (Ambarongy)	-22.22269	47.01889
TRA8.82	<i>C. sp. nov. 2</i>	Andringitra (Ambarongy)	-22.22292	47.0195

Table continued on next page

Table 1, continued

ID	Species designation	Location	Latitude	Longitude
RANO229	<i>C. sp. nov. 2</i>	Ranomafana (Talakely)	-21.24833	47.42406
RANO2.95	<i>C. sp. nov. 2</i>	Ranomafana (Vatoharanana)	-21.2925	47.43842
KAL7.7	<i>C. lavasoensis</i>	Kalambatritra (Sahalava)	-23.53672	46.5335
POLO5.2	<i>C. major</i>	Tampolo	-17.28989	49.40753
ZOM6.2	<i>C. medius</i>	Zombitse	-22.88631	44.69375
GAR8	CCS2*	Manongarivo	-14.02369	48.27233
ANK5.18	CCS6*	Ankarana	-12.96631	49.13808
ANK5.19	CCS6*	Ankarana	-12.96631	49.13808
ANK5.20	CCS6*	Ankarana	-12.96631	49.13808
ANK5.21	CCS6*	Ankarana	-12.96631	49.13808
FIA5.19	CCS6*	Andrafiomena (Anjakely)	-12.91539	49.31956
FIA5.22	CCS6*	Andrafiomena (Anjakely)	-12.91539	49.31956
MATY5.31	CCS6*	Analamera (Ampasimaty)	-12.76556	49.48358
MATY5.40	CCS6*	Analamera (Ampasimaty)	-12.76703	49.48358
MATY5.42	CCS6*	Analamera (Ampasimaty)	-12.77136	49.48303
JOZO4.17	<i>C. sibreei</i>	Anjozorobe	-18.46789	47.94131
<b>Thiele <i>et al.</i> 2013 Samples (Fig. 5)</b>				
AH-04-131	<i>C. lavasoensis</i>	Ambatotsirongorongo	-25.0780	46.7824
AHMG-06-201	<i>C. lavasoensis</i>	Grand Lavasoa	-25.0891	46.7447
AH-X-00-181	<i>C. lavasoensis</i>	Petit Lavasoa	-25.0809	46.7622

\* CCS designations 2 and 6 are from Lei *et al.* (2014).

engaged in pastoral activities (predominantly cattle grazing) (Cornell University/USAID/FOFIFA/INSTAT Census 2001). Timber extraction, quarrying, and land clearance for cattle grazing are also threats to the endemic wildlife. These threats have been exacerbated by rapid population growth, low levels of education, and extreme poverty; factors that have led the human population to devastate the ecosystem to obtain needed resources (Gezon and Freed 1999; Marcus and Kull 1999; D’Cruze *et al.* 2008).

Genetic samples, measurements and photographs of several sedated *Cheirogaleus* individuals were taken prior to their release (Table 2). The samples taken from these animals were subsequently analyzed in the context of a larger phylogenetic study of the genus *Cheirogaleus* by Lei *et al.* (2014), and determined to be sufficiently distinct to warrant elevation as a new species in the *C. crossleyi* species group. The Montagne d’Ambre individuals were designated *Cheirogaleus* sp. nov. 1 by Lei *et al.* (2014), as were six other “confirmed candidate species” (CCS) that likely warrant elevation to full species status. Here we describe a new species of dwarf lemur endemic to the area around Montagne d’Ambre.

## Methods

Methods used to identify this new species were presented in Lei *et al.* (2014). Briefly, extracted genomic DNA taken

from safely immobilized animals was subjected to a series of wet bench and computational analyses (Table 2). The mitochondrial regions analyzed were: Cytochrome b (cytb) (Irwin *et al.* 1991); Cytochrome oxidase subunit II (COII) (Adkins and Honeycutt 1994); the displacement loop or control region (D-loop) (Baker *et al.* 1993; Wyner *et al.* 1999); a fragment of the Cytochrome oxidase subunit III gene (COIII); NADH-dehydrogenase sub units 3, 4L, and 4 (ND3, ND4L, and ND4); and the tRNA<sup>Gly</sup>, tRNA<sup>Arg</sup>, tRNA<sup>His</sup>, tRNA<sup>Ser</sup>, and partial tRNA<sup>Leu</sup> genes (PAST) (Pastorini *et al.* 2000). Three nuclear loci were also sequenced: alpha fibrinogen intron 4 (FIBA), von Willebrand Factor intron 11 (vWF), and Cystic Fibrosis Transmembrane conductance (CFTR-PAIRB) (Heckman *et al.* 2007; Horvath *et al.* 2008). All genetic data were then analyzed using Maximum Likelihood (ML) and Bayesian phylogenetic analyses, and subjected to a battery of tests to examine the strength of the results (Lei *et al.* 2014). Phylogenetic trees were constructed based on these analyses and used to evaluate genetic divergence between lineages (Lei *et al.* 2014, Figs. 2–6, Appendix 1a–1d).

A Bayesian species delimitation analysis was performed using the bPTP webserver with 100,000 Markov Chain Monte Carlo generations, which is sufficient for datasets of less than 50 taxa (<http://species.h-its.org>; Zhang *et al.* 2013). The bPTP server uses a Bayesian Poisson tree processes model to add Bayesian support values to proposed species lineages on a

user-supplied tree using the number of mutations in a lineage. The mitochondrial combined guide tree included representative populations from the total sample set of Lei *et al.* (2014), with all individuals sampled at Montagne d'Ambre (*Cheirogaleus* sp. nov. 1) and all other individuals in the *Cheirogaleus crossleyi* group (*C. crossleyi*, CCS2, *C. sp. nov. 2*). Representatives from the other *Cheirogaleus* species groups (*C. lavasoensis*, *C. major*, *C. medius*, *C. sibreei*), as well as *Microcebus berthae* as an outgroup species, were also included in the bPTP analyses (Fig. 2).

Additionally, D-loop sequences were generated from fecal samples collected in February 2015 from Montagne d'Ambre (locations marked on Fig. 1), which were identical to those from blood and tissue samples. Because of the identical nature of these samples, they were not used in the analyses. The pelage of *Cheirogaleus* sp. nov. 1 was also compared to closely related species (Figs. 3 and 4).

## Results

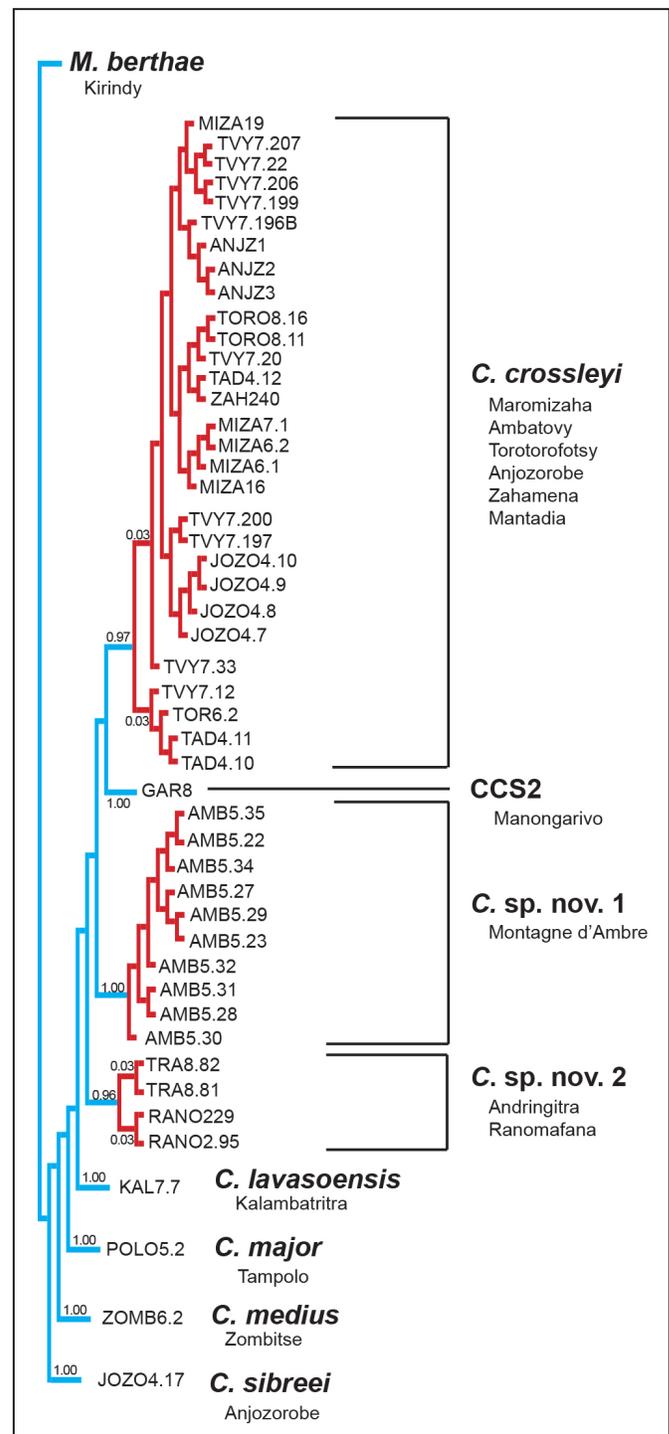
In the *cytb* sequence fragment analyses, *Cheirogaleus* sp. nov. 1, differs from its closest genetic relatives in Lei *et al.* (2014) (CCS2, CCS3, *C. lavasoensis* and *C. crossleyi*) in genetic distance by  $5.6\% \pm 0.7\%$ ,  $6.3\% \pm 0.7\%$ ,  $8.1\% \pm 0.8\%$  and  $6.2\% \pm 0.6\%$ , respectively. Despite being relatively geographically close to CCS6, with the Irodo River as a barrier, *C. sp. nov. 1* is distinct from CCS6. The latter is clustered in the "Medius" subgroup clade with a genetic distance of  $11.7\% \pm 0.9\%$  between *C. sp. nov. 1* and CCS6. Additional analyses using the mitochondrial loci listed in the Methods section above resulted in *C. sp. nov. 1* segregating as an independent lineage with a high degree of confidence, but the results with nuclear loci were less robust among lineages even when large geographic distances (up to 900 km) were a factor. This may be the result of a number of factors, including incomplete lineage sorting, ancient introgression, or the small size of the nuclear sample analyzed (Lei *et al.* 2014, Fig. 5, Appendix Ib–Ic). The mitochondrial and nuclear phylogenetic trees generated in this study are available in Lei *et al.* (2014, Figs. 2–6, Appendix Ia–Id).

The population aggregate analysis (PAA) results were congruent with those presented in Lei *et al.* (2014). We obtained a Bayesian PTP support value of 1.00 indicating an excellent probability of the likelihood that this lineage is an independent species based on the given mitochondrial dataset (Fig. 2).

## Discussion

Sufficient mitochondrial genetic divergence was observed, in conjunction with differences in pelage, to warrant elevation of this population as a new species. Additional evidence used in elevating this population to species status was its geographic isolation from other populations of *Cheirogaleus*. *Cheirogaleus* sp. nov. 1 is found northwest of the Irodo River, and is isolated from other *Cheirogaleus* species by this boundary (Fig. 5). A similar geographical situation exists with the

Montagne d'Ambre mouse lemur (*Microcebus arholdi*) and the Tavaratra mouse lemur (*Microcebus tavaratra*), with the Irodo River acting as a barrier between the two lineages; pelage differences are visible between these two *Microcebus* species as well (Mittermeier *et al.* 2010). Geographic



**Figure 2.** Results of the Bayesian species delimitation analyses of the combined mitochondrial dataset using the bPTP webserver (<http://species.h-its.org>). A total of 100,000 Markov Chain Monte Carlo generations were run with a rooted tree including the outgroup species *Microcebus berthae*. The Montagne d'Ambre population, site designation AMB (*Cheirogaleus* sp. nov. 1), had robust support (1.00) as an independent lineage within the *C. crossleyi* group.

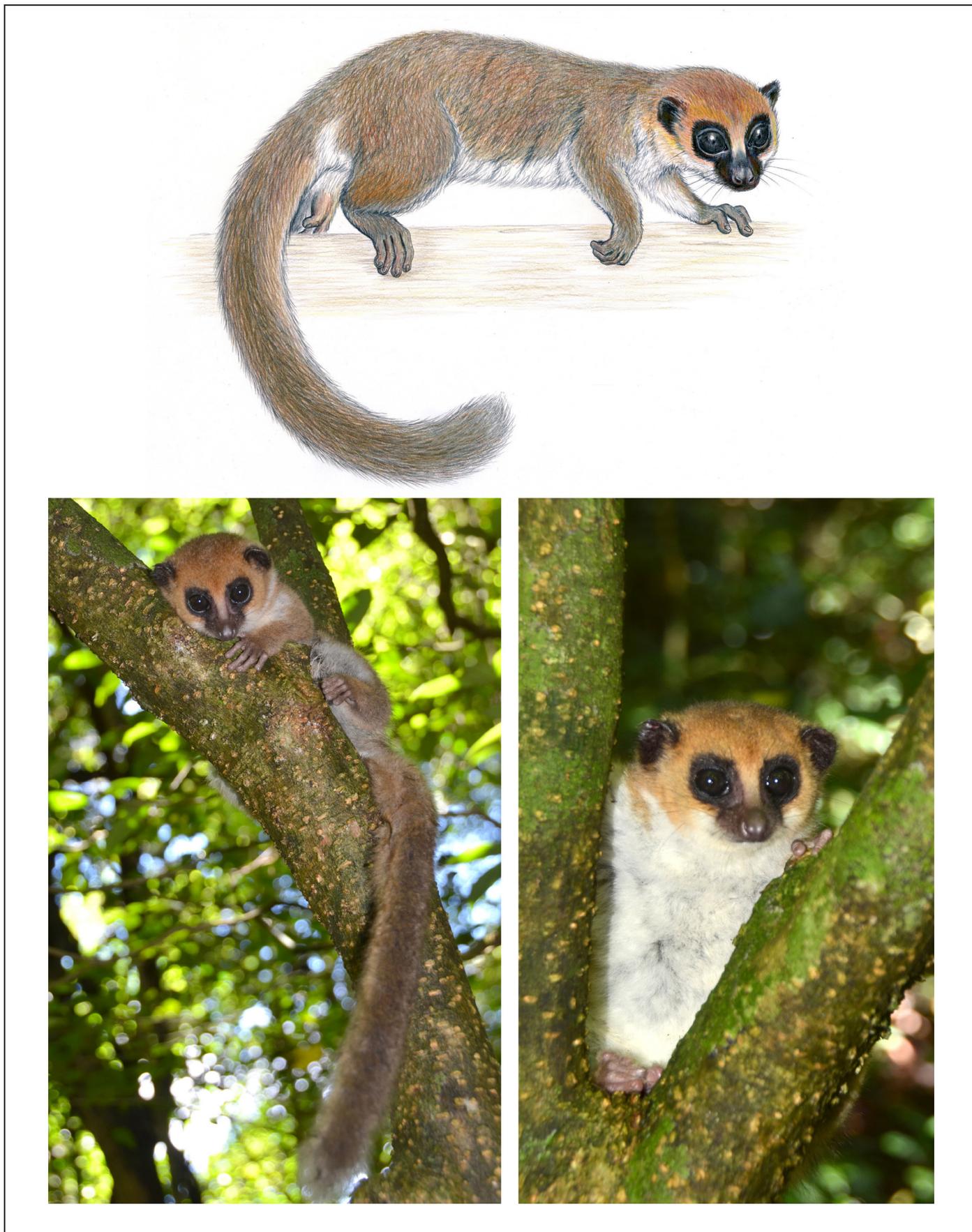
**Table 2.** List of dwarf lemurs, *Cheirogaleus* sp. nov. 1, from Montagne d’Ambre examined during this study using site designation acronyms for specimens, AMB and DAMB, collected by Omaha’s Henry Doorly Zoo and Aquarium (OHDZA) and Madagascar Biodiversity Partnership (MBP) field teams. Catalog and tissue accession numbers from the Museum of Texas Tech University (TTU-M).

ID No.	Catalog No.	Tissue No.	Sex	Microchip ID	Weight (kg)	GPS		Sampling Date
AMB5.22			Male	466A1C1004	0.271	-12.52731	49.17331	11/21/2005
AMB5.23			Male	463D653640	0.256	-12.53017	49.17464	11/21/2005
AMB5.27	TTU-M 118801	TK 129239	Female	4669753C7D	0.310	-12.51722	49.17950	11/22/2005
AMB5.28			Female	466A1D6C48	0.391	-12.47881	49.21222	11/22/2005
AMB5.29	TTU-M 118802	TK 129240	Female	463B507D20	0.336	-12.47922	49.21606	11/22/2005
AMB5.30	TTU-M 118803	TK 129241	Male	467314576F	0.296	-12.47917	49.21597	11/22/2005
AMB5.31			Female	466C4A392F	0.311	-12.51083	49.19275	11/22/2005
AMB5.32*			Male	465E502450	0.110	-12.51242	49.18956	11/23/2005
AMB5.34			Male	462C017435	0.291	-12.47822	49.21717	11/22/2005
AMB5.35			Female	466C5E121D	0.321	-12.49519	49.20783	11/23/2005
DAMB15.4			Male	476F11225A	0.240	-12.52716	49.17191	02/28/2015
DAMB15.5			Female	47741F1E78	0.200	-12.52461	49.17236	02/28/2015
DAMB15.6			Male	4767432A7C	0.164	-12.52425	49.17302	02/28/2015

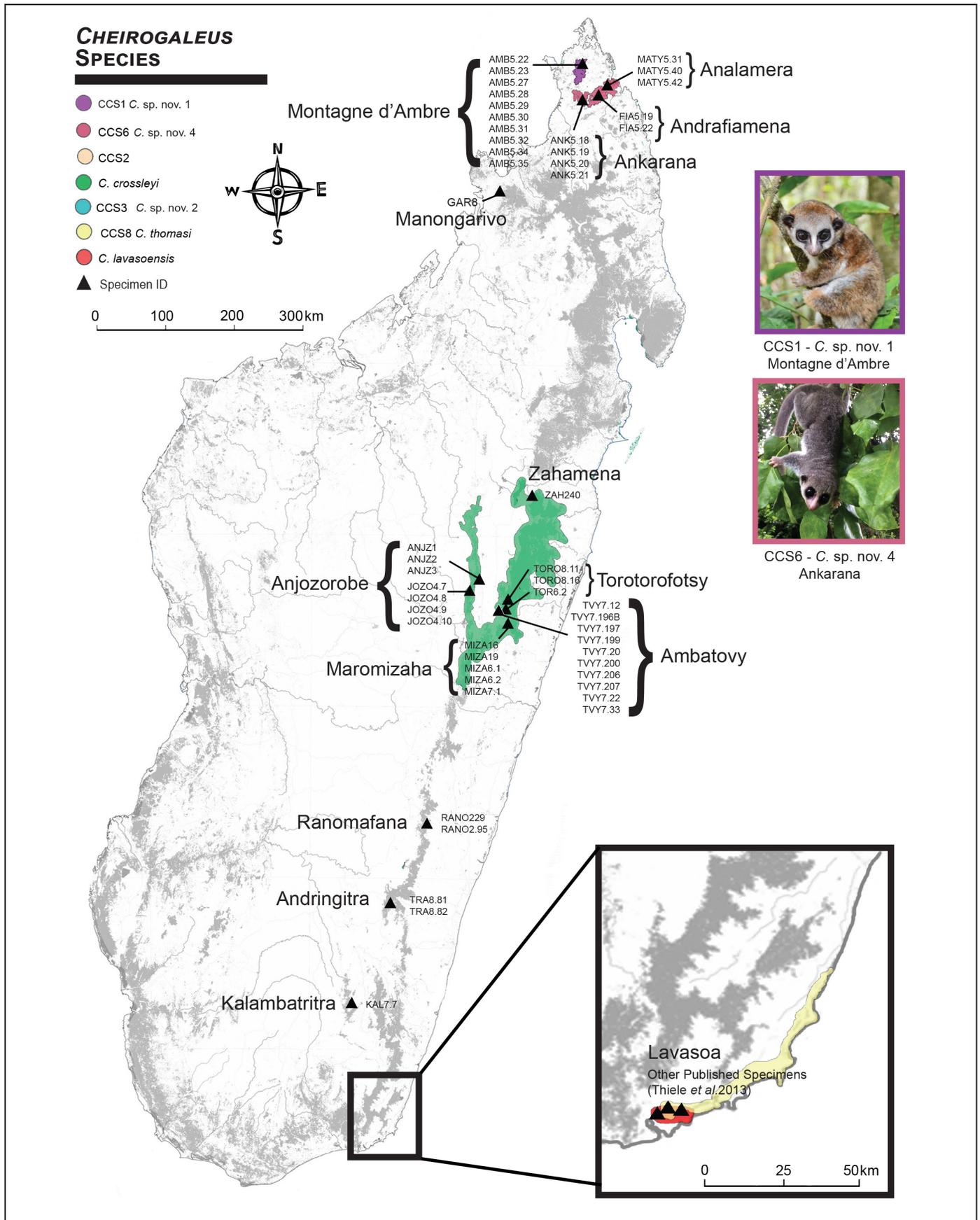
\*Juvenile



**Figure 3.** Illustrations of *Cheirogaleus* lineages based on archived photographs (Museum of Texas Tech University; Fig. 8 in Lei et al. 2014). Left panel represents all *Cheirogaleus* lineages, except *C. sibreei*. Right panel represents the *Cheirogaleus crossleyi* group, including *Cheirogaleus* sp. nov. 1, here named *Cheirogaleus andysabini*.



**Figure 4.** Illustration of *Cheirogaleus andysabini* and photographs of Omaha's Henry Doorly Zoo and Aquarium (OHDZA) catalogue acronym for specimen DAMB15.4 taken at Montagne d'Ambre. Illustrations by Stephen D. Nash ©Conservation International. Photographs by Edward E. Louis, Jr.



**Figure 5.** Map of Madagascar with the ranges of *Cheirogaleus sp. nov. 1* (*Cheirogaleus andysabini*) and closely related *Cheirogaleus* species highlighted to show the geographic distance between lineages. ID numbers on the map correspond to ID numbers of animals listed in Table 1. Photographs of *Cheirogaleus sp. nov. 1* and CCS6 (Lei et al. 2014), the Ankarana/Andrafiamena/Analamera/Bekaraoka lineage is provided to show a clear difference in pelage and the distance between the ranges of the two lineages. The Irodo River acts as a northern barrier to CCS6.

isolation alone is not evidence for the elevation of a new species. However, when considered as one factor alongside mitochondrial genetic divergence and pelage variation it provides additional justification for the description of a new species. *Cheirogaleus* sp. nov. 1 has a deeper reddish-brown dorsal coat, and a white rather than grey ventral coat that distinguish its pelage from that of the closely related *C. crossleyi* (Figs. 3, 4; See also Lei *et al.* 2014, Fig. 8). The average size of *C. sp. nov. 1* is generally smaller than *C. crossleyi* individuals, although there is overlap in body size among some individuals (Lei *et al.* 2014, Table 4).

The Bayesian species delimitation analysis provided posterior delimitation probabilities in support of our elevation of the Montagne d’Ambre *Cheirogaleus* group as an independent species. We acknowledge the limitations of any species delimitation methodology when used independent of other corroborating methods (Carstens *et al.* 2013). In the case of this *Cheirogaleus* group the bPTP species delimitation is presented as additional evidence of our assertion that this group constitutes a new species.

### Conservation Status

Insufficient information is available about the conservation status of this species, but the rate of anthropogenic ecological destruction in this region of Madagascar (and Madagascar overall) is severe. The population of this new species is found in and just outside the boundaries of Montagne d’Ambre National Park. A region of unprotected forest remains between the northern boundary of the national park and the Forêt d’Ambre Special Reserve (Mittermeier *et al.* 2010). The conservation status of this species cannot be determined at present, but the proximity to the park boundary and the settlement of Joffreville brings this species into close contact with humans and the possibility of forest clearance and hunting. The large port city of Antsiranana (formerly Diego Suarez) is *c.* 30 km away, and the demand for cooking charcoal from the sizeable population there has led to increased stress on the forest ecosystem at Montagne d’Ambre. Furthermore, the clearing of forest for the production of khat (*Catha edulis*) in the past decade has increased dramatically (E.E. Louis, Jr. pers. obs.). Therefore, with combinations of threats such as hunting, deforestation, and subsistence farming, even lemur populations in parks and reserves are not necessarily protected (Dufils 2003; Schwitzer *et al.* 2014). Given the microendemism found in Montagne d’Ambre, it is imperative to safeguard this habitat for this newly described *Cheirogaleus* species and other rare wildlife confined to this unique and isolated rainforest.

### *Cheirogaleus andysabini* sp. nov.

Formerly *Cheirogaleus* sp. nov. 1, also CCS1 (Lei *et al.* 2014).

Holotype: AMB5.27 (AMB is the Henry Doorly Zoo designation for Montagne d’Ambre); adult female; Permit number 181; 4 × 2.0 mm biopsies from ear pinna and 0.3 cc

of whole blood; stored and curated at the Museum of Texas Tech University (MTTU, catalog number: TTU-M 118801/K 129239) Genetic Resources Collection, Natural Sciences Research Laboratory (NSRL); We placed a microchip subcutaneously between the scapulae and recorded as 4669753C7D; Collected by Jean Freddy Ranaivoarisoa, Ravaka Ramana-mahefa, Nirina Jean de Dieu Andriamadison, Joseph Désiré Rabekinjaja, Gérard Nalanirina, François Randrianasolo on 22 November 2005.

Paratypes: AMB5.28-5.32, 5.34-5.35, DAMB 15.4-15.6

Other specimens: Possible (unconfirmed), Harvard Museum of Comparative Zoology (MCZH) 44951, skull and skin from Montagne d’Ambre accessioned in January 1929 as part of the Grandidier Collection by Robert Barbour received from Louis Lavauden (missing in inventory since 1994). Additional specimen in the collection of the American Museum of Natural History (AMNH) M-100650, skull and skin taken in November of 1930 by Austin Rand at a site near Montagne d’Ambre “15 miles southwest of Tsarakimby.”

Type locality: Madagascar: Antsiranana Province, Diana Region, District Antsiranana II, Montagne d’Ambre National Park, S12.52720, E49.17950 at 1073 m above sea level.

Measurements of holotype: Measurements (in cm and g) recorded in a field catalog: Body length 18.2 cm; Tail length 27.1 cm; Head crown 5.8 cm; Mass 310 g.

Description: The dorsum, limbs, and head are a rufous brown. The areas around the orbits are brownish-black, with a white patch proximal to the fleshy part of the nose in the inter-ocular space. The pelage on the ventral surface of the mandible is white, which is continuous onto the white pelage of the ventrum. Size is generally smaller than *C. crossleyi*, braincase higher, with very poorly expressed temporal lines (Fig. 4).

Diagnosis: *Cheirogaleus andysabini* can be distinguished from *C. crossleyi*, CCS2, CCS3 and *C. lavasoensis* by 10, 7, 10 and 16 apomorphic characters in the cytochrome b gene, respectively (Appendix II(k); Lei *et al.* 2014). *Cheirogaleus andysabini* has four diagnostic sites in the cytb sequence fragment such as G, A, G and G at the positions of 297, 303, 306 and 1071, respectively, which differentiate *C. andysabini* from all other *Cheirogaleus* species. Despite being geographically close to CCS6, with the Irodo River as a barrier, *C. andysabini* is distinct by nine diagnostic characters from CCS6, which is clustered in the “Medius” subgroup clade. An average weight of 0.282 ± 0.61 kg, dark fur around eyes, rufous brown fur on dorsum, limbs, and head, venter is white. Table 3 contains measurements of captured individuals.

Distribution: *Cheirogaleus andysabini* is known from the Montagne d’Ambre National Park and areas nearby around the town of Joffreville, northwest of the Irodo River in northern Madagascar (Fig. 1). Observed at 541–1073 m above sea level.

Etymology: This new species is named after Andy Sabin, a well-known New York philanthropist committed to species conservation, especially turtles, amphibians and primates. In particular, he has supported many projects in Madagascar,

**Table 3.** Morphological data for adult *Cheirogaleus* species nov. 1, from Montagne d’Ambre

Class	No.	W (kg)	HC (cm)	HW (mm)	BL (cm)	TL (cm)	EL (mm)	EW (mm)	ML (mm)	MW (mm)	IW (mm)
Male	6	0.253±0.048	6.4±0.8	28.4±1.5	16.5±1.9	26.0±2.1	21.0±2.4	14.6±1.0	16.7±0.7	18.1±0.9	12.2±0.4
Female	6	0.312±0.062	6.0±0.5	27.6±3.2	17.1±1.5	26.4±2.3	22.2±1.6	15.0±2.4	15.0±0.2	18.5±1.0	12.0±0.8
Total	12	0.282±0.061	6.2±0.7	27.9±2.4	17.6±1.6	26.2±2.1	21.8±2.0	14.8±1.7	15.7±1.0	18.3±0.9	12.1±0.6

Note: W: weight; HC: head crown, HW: head width, BL: body length, TL: tail length, EL: ear length, EW: ear width, ML: muzzle length, MW: muzzle width, IW: intra-orbital width.

**Table 3.** Continued.

Class	F-Tb (cm)	F-LD (cm)	F-Hd (cm)	F-UR (cm)	F-H (cm)	H-Tb (cm)	H-LD (cm)	H-Ft (cm)	H-T (cm)	H-F (cm)	UC (mm)	LC (mm)
Male	1.1±0.0	1.4±0.3	3.4±0.2	4.1±0.4	3.5±0.7	1.6±0.2	1.5±0.2	5.0±0.1	5.2±0.5	5.7±0.5	3.2±0.3	3.1±0.4
Female	1.0±0.1	1.3±0.1	3.4±0.2	4.3±0.2	3.3±0.7	1.6±0.1	1.5±0.2	5.0±0.3	5.2±0.5	5.7±0.5	2.8±0.4	3.1±0.1
Total	1.0±0.1	1.4±0.2	3.4±0.2	4.2±0.3	3.4±0.7	1.6±0.2	1.5±0.2	5.0±0.2	5.2±0.5	5.7±0.5	3.0±0.4	3.1±0.3

Note: F-Tb: thumb (forelimb), F-LD: longest digit (Forelimb), F-Hd: hand, F-UR: ulna/radius, F-H: humerus, H-Tb: thumb (hindlimb), H-LD: longest digit (hindlimb), H-Ft: foot, H-T: tibia, H-F: femur, UC: upper canine, LC: lower canine.

including research on lemurs, tortoises and frogs. His long-term interest, his enthusiasm, and his generosity have helped to encourage many researchers and conservationists, young and old alike.

Vernacular names: Montagne d’Ambre or Andy Sabin’s dwarf lemur.

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### Literature Cited

- Adkins, R. M. and R. L. Honeycutt. 1994. Evolution of the primate cytochrome c oxidase subunit II gene. *J. Mol. Evol.* 38: 215–231.
- Baker, C. S., A. Perry, J. L. Bannister, M. T. Weinrich, R. B. Abernethy, J. Calambokidis, R. H. Lien, J. U. Lamberesen, O. Ramirez, P. Vasquez, J. Clapham, A. Alling, S. J. O’Brien and S. R. Palumbi. 1993. Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. *Proc. Natl. Acad. Sci. U.S.A.* 90: 8239–8243.
- Callmander, M. W., L. Gautier and S. M. Trigui. 2009. *Pandanus nusbaumeri* Callm. & L. Gaut. (Pandanaceae), a new species from northern Madagascar. *Candollea* 64: 213–218.
- Carstens, B. C., T. A. Pelletier, N. M. Reid and J. D. Salter. 2013. How to fail at species delimitation. *Mol. Ecol.* 22: 4369–4383.
- Cornell University/USAID/FOFIFA/INSTAT Ilo Commune Census. 2001. Census of 1395 communes. Website: <<http://www.ilo.cornell.edu/ilo/data.html>>.
- D’Cruze, N., J. Köhler, M. Franzen and F. Glaw. 2008. A conservation assessment of the amphibians and reptiles of the Forêt d’Ambre Special Reserve, north Madagascar. *Madag. Conserv. Dev.* 3: 44–54.
- D’Cruze, N., J. Köhler, M. Vences, and F. Glaw. 2010. A new fat fossorial frog (Microhylidae: Cophylinae: *Rhombophryne*) from the rainforest of the Forêt d’Ambre Special Reserve, northern Madagascar. *Herpetologica* 66: 182–191.
- Dufils, J. M. 2003. Remaining forest cover. In: *The Natural History of Madagascar*, S. M. Goodman and J. P. Benstead (eds.), pp.142–146. University of Chicago Press, Chicago, IL.
- Dupuy, D. and J. Moat. 1996. A refined classification of the primary vegetation of Madagascar based on the underlying geology: using GIS to map its distribution and to assess its conservation status. In: *Biogéographie de Madagascar*, W. R. Lourenço (ed.), pp.205–218. Editions de l’ORSTOM, Paris.
- Eldredge, N. and J. Cracraft. 1980. Phylogenetic patterns and the evolutionary process: Method and theory in comparative biology. Columbia University Press, New York.
- Gezon, L.L. and B.Z. Freed. 1999. Agroforestry and conservation in northern Madagascar: hopes and hindrances. *Afr. Stud. Quart.* 3: 29–37.

- Glaw, F. and M. Vences. 2007. *A Field Guide to the Amphibians and Reptiles of Madagascar*. 3rd edition. Köln (Cologne), Germany.
- Glaw, F., J. Köhler and M. Vences. 2009. A distinctive new species of chameleon of the genus *Furcifer* (Squamata: Chamaeleonidae) from the Montagne d'Ambre rainforest of northern Madagascar. *Zootaxa* 2269: 32–42.
- Goodman, S. M., B. Ramasindrazana, K. M. Naughton and B. Appleton. 2015. Description of a new species of the *Miniopterus aelleni* group (Chiroptera: Miniopteridae) from upland areas of central and northern Madagascar. *Zootaxa* 3936: 538–558.
- Groeneveld, L. F., D. W. Weisrock, R. M. Rasoloarison, A. D. Yoder and P. M. Kappeler. 2009. Species delimitation in lemurs: multiple genetic loci reveal low levels of species diversity in the genus *Cheirogaleus*. *BMC Evol. Biol.* 9: 30.
- Groeneveld, L. F., M. B. Blanco, J. L. Raharison, V. Rahalinarivo, R. M. Rasoloarison, P. M. Kappeler, L. R. Godfrey and M. T. Irwin. 2010. MtDNA and nDNA corroborate existence of sympatric dwarf lemur species at Tsinjoarivo, eastern Madagascar. *Mol. Phylogenet. Evol.* 55: 833–845.
- Groves, C. P. 2000. The genus *Cheirogaleus*: unrecognized biodiversity in dwarf lemurs. *Int. J. Primatol.* 21: 943–962.
- Hapke, A., J. Fietz, S. D. Nash, D. Rakotondravony, B. Rakotosamimanana, J. B. Ramanamanjato, G. Randria and H. Zischler. 2005. Biogeography of dwarf lemurs: genetic evidence for unexpected patterns in southeastern Madagascar. *Int. J. Primatol.* 26: 873–901.
- Heckman, K. L., C. L. Mariani, R. Rasoloarison and A. D. Yoder. 2007. Multiple nuclear loci reveal patterns of incomplete lineage sorting and complex species history within western mouse lemurs (*Microcebus*). *Mol. Phylogenet. Evol.* 43: 353–367.
- Horvath, J. E., D. W. Weisrock, S. L. Embry, I. Fiorentino, J. P. Balhoff, P. Kappeler, G. A. Wray, H. F. Willard and A. D. Yoder. 2008. Development and application of a phylogenomic toolkit: resolving the evolutionary history of Madagascar's lemurs. *Genome Res.* 18: 489–499.
- Irwin, D. M., T. D. Kocher and A. C. Wilson. 1991. Evolution of the cytochrome b gene of mammals. *J. Mol. Evol.* 32: 128–144.
- Lei, R., C. L. Frasier, A. T. McLain, J. M. Taylor, C. A. Bailey, S. E. Engberg, A. L. Ginter, C. P. Groves, R. A. Mittermeier and E. E. Louis Jr. 2014. Revision of Madagascar's dwarf lemurs (Cheirogaleidae: *Cheirogaleus*): designation of species, candidate species status and geographic boundaries based on molecular and morphological data. *Primate Conserv.* (28): 9–35.
- Louis Jr., E. E., S. E. Engberg, R. Lei, H. Geng, J. A. Sommer, R. Randriamampionona, J. C. Randriamanana, J. R. Zaonarivelo, R. Andriantompohavana, G. Randria, Prosper, B. Ramaromilanto, G. Rakotoarisoa, A. Rooney and R. A. Brenneman. 2006. Molecular and morphological analyses of the sportive lemurs (Family Megaladapidae: Genus *Lepilemur*) reveals 11 previously unrecognized species. *Spec. Publ. Mus., Texas Tech Univ.* 49: 1–47.
- Louis Jr., E. E., S. E. Engberg, S. M. McGuire, M. J. McCormick, R. Randriamampionona, J. F. Ranaivoarisoa, C. A. Bailey, R. A. Mittermeier and R. Lei. 2008. Revision of the mouse lemurs, *Microcebus* (Primates, Lemuriformes), of northern and northwestern Madagascar with descriptions of two new species at Montagne d'Ambre National Park and Antafondro Classified Forest. *Primate Conserv.* (23): 19–38.
- Mathieu, G. 2003. New endemic *Peperomia* species (Piperaceae) from Madagascar. *Syst. Geogr. Pl.* 73: 71–81.
- Marcus, R. R. and C. Kull. 1999. Setting the Stage: The Politics of Madagascar's Environmental Efforts. *Afr. Stud. Quart.* 3(2): 1–8.
- Mittermeier, R. A., E. E. Louis Jr., M. Richardson, C. Schwitzer, O. Langrand, A. B. Rylands, F. Hawkins, S. Rajaobelina, J. Ratzimasafy, R. Rasoloarison, C. Roos, P. Kappeler and J. Mackinnon. 2010. *Lemurs of Madagascar*. 3rd edition. Conservation International, Arlington, VA.
- Nicoll, M. E. and O. Langrand. 1989. *Revue de la conservation et des aires protégées*. WWF, Gland, Switzerland. 374pp.
- Pastorini, J., M. R. J. Forstner and R. D. Martin. 2000. Relationships among brown lemurs (*Eulemur fulvus*) based on mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* 16: 418–429.
- Rakotoarison, A., A. Crottini, J. Müller, M.O. Rödel, F. Glaw and M. Vences. 2015. Revision and phylogeny of narrow-mouthed treefrogs (*Cophyla*) from northern Madagascar: integration of molecular, osteological, and bioacoustic data reveals three new species. *Zootaxa* 3937: 61–89.
- Ratsoavina, F. M., E. E. Louis Jr., A. Crottini, R. D. Randrianiaina, F. Glaw and M. Vences. 2011. A new leaf tailed gecko species from northern Madagascar with a preliminary assessment of molecular and morphological variability in the *Uroplatus ebenauui* group. *Zootaxa* 3022: 39–57.
- Raxworthy C. J. and R. A. Nussbaum. 1994. A rainforest survey of amphibians, reptiles and small mammals at Montagne d'Ambre, Madagascar. *Biol. Conserv.* 69: 65–73.
- Schwarz, E. 1931. A revision of the genera and species of Madagascar Lemuridae. *Proc. Zool. Soc. Lond.* (1931): 399–426.
- Schwitzer, C., R. A. Mittermeier, S. E. Johnson, G. Donati, M. Irwin, H. Peacock, J. Ratzimasafy, J. Razafindramana, E. E. Louis Jr., L. Chikhi, I. C. Colquhoun, J. Tinsman, R. Dolch, M. LaFleur, S. D. Nash, E. Patel, B. Randrianambinina, T. Rasolofoharivelo and P. C. Wright. 2014. Averting lemur extinctions amid Madagascar's political crisis. *Science* 343: 842–843.
- Segalen P. 1956. La gibbsite dans les sols derives de roches volcaniques basiques à Madagascar. *Bulletin du groupe français des argiles* 8: 11–15.

- Tattersall, I. 1982. *The Primates of Madagascar*. Columbia University Press, New York.
- Tattersall, I. 2007. Madagascar's lemurs: cryptic diversity or taxonomic inflation? *Evol. Anthropol.* 16: 12–23.
- Tattersall, I. 2013. Species-level diversity among Malagasy lemurs. In: *Leaping Ahead: Advances in Prosimian Biology*, J. M. Masters, M. Gamba and F. Genin (eds.), pp.11–20. Springer, New York.
- Thiele, D., E. Razafimahatratra and A. Hapke. 2013. Discrepant partitioning of genetic diversity in mouse lemurs and dwarf lemurs—biological reality or taxonomic bias? *Mol. Phylogenet. Evol.* 69: 593–609.
- Wheeler, Q. D. and N. I. Platnick. 2000. The phylogenetic species concept (*sensu* Wheeler and Platnick). In: *Species Concepts and Phylogenetic Theory: A Debate*, Q. D. Wheeler and R. Meier (eds.), pp.55–69. Columbia University Press, New York.
- Wilmé, L. 1996. Composition and character of bird communities in Madagascar. In: *Biogéographie de Madagascar*, W. R. Lourenço (ed.), pp.349–362. Editions de l'ORSTOM, Paris.
- Wyner, Y. M., G. Amato and R. Desalle. 1999. Captive breeding, reintroduction, and the conservation genetics of black and white ruffed lemurs, *Varecia variegata variegata*. *Mol. Ecol.* 8: S107–115.
- Zhang, J., P. Kapli, P. Pavlidis and A. Stamatakis. 2013. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* 29(22): 2869–2876.

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