

# A New *Cheirogaleus* (Cheirogaleidae: *Cheirogaleus crossleyi* Group) Species from Southeastern Madagascar

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**Abstract:** A new species in the genus *Cheirogaleus* is described from Ranomafana and Andringitra national parks, Madagascar. Ranomafana National Park is a rainforest situated in a montane region, and Andringitra National Park is comprised of grassland, lowland and highland forests displaying great altitudinal variation. Both parks are known to harbor wide species diversity in flora and fauna. Genetic and morphometric analyses of the samples collected at these localities confirmed that this *Cheirogaleus* lineage represents a new species in the *C. crossleyi* group, and here we elevate it to species status as *Cheirogaleus grovesi*, for the British-Australian biological anthropologist, evolutionary biologist and taxonomist Colin Groves.

**Key Words:** *Cheirogaleus*, dwarf lemur, cryptic species, Madagascar

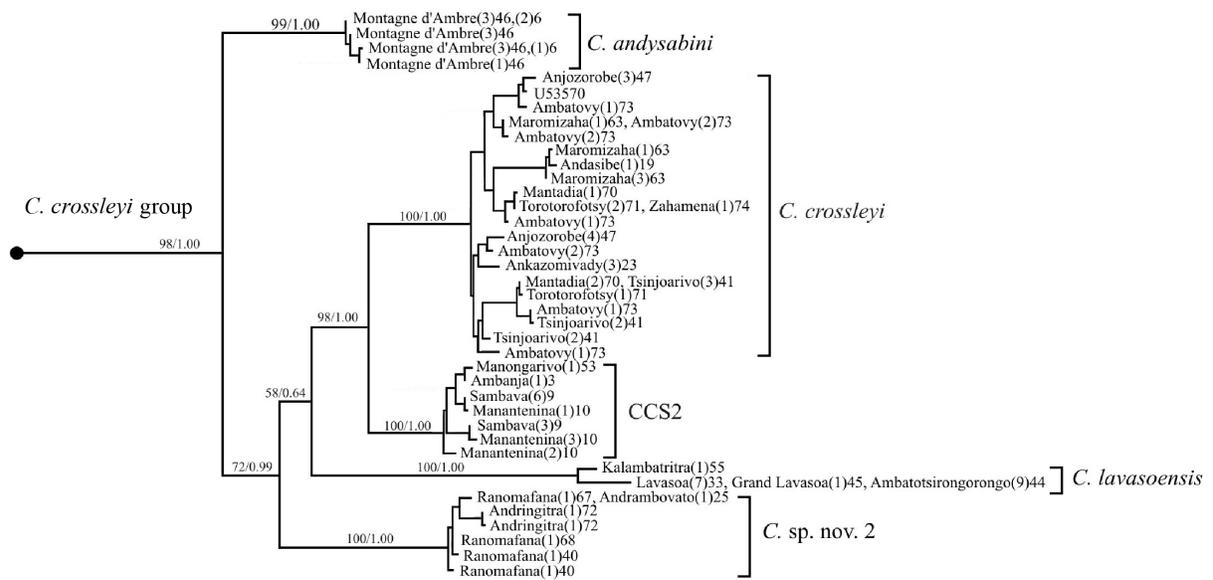
## Introduction

Dwarf lemurs, genus *Cheirogaleus*, are small, arboreal primates that are opportunistic omnivores subsisting largely on fruit. This genus has the ability to store fat in the tail to survive lean periods during the austral winter (Dausmann *et al.* 2005; Mittermeier *et al.* 2010). Previously unknown species diversity was identified in *Cheirogaleus* through extensive fieldwork coupled with molecular analyses (Lei *et al.* 2014 2015; Frasier *et al.* 2016). Seven new species (“confirmed candidate species” or “CCS”), and four possible new species (“unconfirmed candidate species” or “UCS”) were proposed by Lei *et al.* (2014). Elaborating on these publications, we describe here a new *Cheirogaleus* lineage endemic to southeastern Madagascar based on samples collected in and around the national parks of Ranomafana and Andringitra. These two parks are part of the Rainforests of Atsinanana UNESCO World Heritage Site (IUCN Technical Evaluation/UNESCO Designation; <<http://whc.unesco.org/en/list/1257>>).

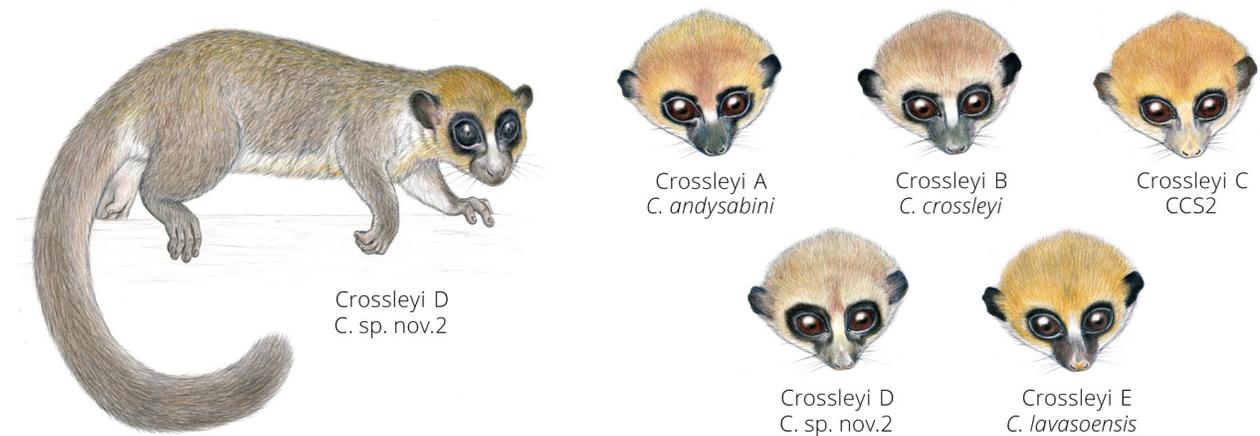
Ranomafana National Park, established in 1991, is a rainforest reserve of approximately 43,500 ha located in the Haute Matsiatra and Vatovavy-Fitovinany regions of Madagascar (Wright and Andriamihaja 2002; Gerber *et al.* 2010). The park is largely submontane rainforest and is crisscrossed by

at least 29 rivers of varying sizes. The largest of these rivers, the Namorona, runs alongside Route National 25 and bisects the park. Ranomafana is 400–1374 m above sea level, and receives about 2600 mm of rainfall annually (Razafimamondry 1988; Jenkins *et al.* 1999; Gerber *et al.* 2010). The park is home to rich species diversity, including 13 species of lemur—genera known to be present include *Avahi*, *Cheirogaleus*, *Daubentonia*, *Eulemur*, *Hapalemur*, *Lepilemur*, *Microcebus*, *Prolemur*, *Propithecus*, and *Varecia* (see Wright *et al.* 2012).

The Andringitra area was recognized by early naturalists for its unique geographic and biological characteristics and declared a strict nature reserve in 1927 (Rabetaliana and Schachenmann 1999; Rabetaliana *et al.* 1999). Andringitra National Park was established in 1999 and consists of approximately 31,160 ha of grassland, lowland rainforests, and higher elevation forests, as well as granite outcroppings of Precambrian rock at 500–2600 m above sea level (Rabetaliana *et al.* 1999). Andringitra is home to either 12 or 13 species of lemur—genera known to be present include *Avahi*, *Cheirogaleus*, *Daubentonia*, *Eulemur*, *Hapalemur*, *Lemur*, *Lepilemur*, *Microcebus*, and *Propithecus* (*Varecia* was formerly present but has not been recently reported and may be locally extinct) (Goodman and Rasolonandrasana, 2001; Mittermeier *et al.* 2010). Of these lemurs, the rufous mouse lemur



**Figure 1.** Subtree of the phylogenetic relationships between taxa within the *Cheirogaleus crossleyi* group based on *cytb* sequence data as presented in Lei *et al.* (2014; Fig. 2). Numbers on branches represent maximum likelihood values followed by posterior probability support. Tip labels include locality, followed by number of individuals carrying the haplotype in brackets, then the locality numbers.)



**Figure 2.** Illustration of *C. sp. nov. 2* and closely related species (Fig. 8 in Lei *et al.* 2014), Illustrations by Stephen D. Nash ©Conservation International. Photographs by Edward E. Louis, Jr. Top left panel represents *C. grovesi*. Top left panel represents a lateral view of *C. sp. nov. 2*, top right panel includes all lineages in the *Cheirogaleus crossleyi* group. Bottom photographs are of the holotype of *C. sp. nov. 2* (TRA8.81) at Andringitra National Park.

(*Microcebus rufus*) is found in the Andringitra area and north along a higher-elevation corridor to Ranomafana National Park, and it is possible that the newly identified *Cheirogaleus* occupies a similar range. The corridor between these areas is within the newly created protected area Corridor Forestier Fandriana-Vondrozo (CoFAV, 2015).

In 1999, a dwarf lemur from Ranomafana (RANO229) was immobilized to acquire morphometric data and genetic samples, and was determined to be *Cheirogaleus major* (E. E. Louis Jr. pers. comm.). Subsequently, samples from RANO229 were used as an outgroup in multiple genetic studies (Louis *et al.* 2006; Johnson *et al.* 2008). Groeneveld *et al.* (2009) were the first to incorporate this individual into a genetic study specific to *Cheirogaleus*. In this study, using mitochondrial sequence data, they demonstrated that RANO229 (GenBank accession: AY58448) was not *C. major*, but belonged to the *C. crossleyi* group in a subclade with an individual from Andrambovato/Oranjatsy (RMR146). Interestingly, RMR146 morphologically grouped with *C. major*, which the authors attributed to the possibility of hybridization (Groeneveld *et al.* 2009), lending credence to the initial field identification of RANO229. The Ranomafana-Andrambovato subclade inside the *C. crossleyi* group was recovered again in a study with additional Ranomafana samples (MB210, MB217) and morphometric data (Groeneveld *et al.* 2010); RANO229 was not included in this study. Thiele *et al.* (2013) proposed tentative species status for this lineage, identified as *C. sp.* Ranomafana Andrambovato in their analyses, which included MB210, MB217, and RMR146. Building on these works, Lei *et al.* (2014) expanded the genetic dataset for this proposed lineage with the inclusion of additional individuals from Ranomafana, which clustered with individuals from Andringitra National Park (Fig. 1). This Ranomafana-Andringitra clade was designated as *Cheirogaleus* sp. nov. 2 (Lei *et al.* 2014).

Here we describe a new species of dwarf lemur from the Haute Matsiatra region of Madagascar along the CoFAV based on specimens collected in and around the national parks of Ranomafana and Andringitra.

## Methods

### Sampling and morphology

See Lei *et al.* (2014) for a comprehensive list of localities and numbers of individuals that were represented in the most recent genus-wide study of *Cheirogaleus*. Here, we will only enumerate those belonging to the *C. crossleyi* group (Table 1). From the focal lineage, *C. sp.* nov. 2, two individuals from Ranomafana National Park and two individuals from Andringitra National Park were immobilized by the field team from the Madagascar Biodiversity Partnership (MBP; Table 2, Fig. 2). Morphometric measurements taken on sedated lemurs were recorded as in Louis *et al.* (2006) and Andrian-tompohavana *et al.* (2007). All lemurs were released at the point of capture. Interactions with the study subjects abided by Omaha's Henry Doorly Zoo and Aquarium's IACUC

(97-001, 12-101), and all collection and export permits were obtained from the appropriate authorities in Madagascar and the United States, respecting the Convention for International Trade in Endangered Species (CITES).

### Data generation and phylogenetic analyses

Methods used to identify this new species were presented in the "Methods" section of 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 1). 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 subunits 3, 4L, and 4 (ND3, ND4L, and ND4); as well as 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 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). Additionally, a Bayesian species delimitation analysis was performed using the bPTP webserver (<http://species.h-its.org>; Zhang *et al.* 2013) as in Lei *et al.* (2015). Use of species delimitation methodology without additional corroborative work has acknowledged limitations (Markolf *et al.* 2011). 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.

## Results

### Morphology

Morphological data were available for three individuals of *C. sp.* nov. 2: one adult male and one adult female from Andringitra, and one adult female from Ranomafana (Table 3). A juvenile from Ranomafana was excluded from the morphological data. The average weight of *C. sp.* nov. 2 was 0.453 kg.

### Phylogenetic Analyses

In the *cytb* sequence fragments, *C. sp.* nov. 2 differs from its closest genetic relatives in Lei *et al.* (2014) (*C. andysabini*, *C. lavasoensis* and *C. crossleyi*) in genetic distance by 6.3%±0.7%, 8.2%±0.7% and 6.5%±0.7%, respectively (Fig. 1). *Cheirogaleus* sp. nov. 2 is sympatric in the northern part of its range with *C. sibreei* and CCS5, a yet-to-be described species in the *C. major* group. The new species described

**Table 1.** Wild *Cheirogaleus* individuals sampled for this study. IDs correspond to Figure 2 and Figure 5 of Lei *et al.* (2014), except for the samples from Thiele *et al.* 2013 (denoted at the bottom of the table).

ID	Species Designation	Location	Latitude	Longitude
AMB5.22	<i>C. andysabini</i>	Montagne d'Ambre	-12.52731	49.17331
AMB5.23	<i>C. andysabini</i>	Montagne d'Ambre	-12.53017	49.17464
AMB5.27	<i>C. andysabini</i>	Montagne d'Ambre	-12.51722	49.17950
AMB5.28	<i>C. andysabini</i>	Montagne d'Ambre	-12.47881	49.21222
AMB5.29	<i>C. andysabini</i>	Montagne d'Ambre	-12.47922	49.21606
AMB5.30	<i>C. andysabini</i>	Montagne d'Ambre	-12.47917	49.21597
AMB5.31	<i>C. andysabini</i>	Montagne d'Ambre	-12.51083	49.19275
AMB5.32	<i>C. andysabini</i>	Montagne d'Ambre	-12.51242	49.18956
AMB5.34	<i>C. andysabini</i>	Montagne d'Ambre	-12.47822	49.21717
AMB5.35	<i>C. andysabini</i>	Montagne d'Ambre	-12.49519	49.20783
ANJZ1	<i>C. crossleyi</i>	Anjozorobe	-18.47750	47.93812
ANJZ2	<i>C. crossleyi</i>	Anjozorobe	-18.47750	47.93812
ANJZ3	<i>C. crossleyi</i>	Anjozorobe	-18.47750	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
RANO229	<i>C. sp. nov. 2</i>	Ranomafana (Talatakely)	-21.24833	47.42406
RANO2.95	<i>C. sp. nov. 2</i>	Ranomafana (Vatoharanana)	-21.29250	47.43842

KAL7.7	<i>C. lavasoensis</i>	Kalambatritra (Sahalava)	-23.53672	46.5335
GAR8	CCS2*	Manongarivo	-14.02369	48.27233
<b>Thiele et al. 2013 Samples</b>				
KC505933	<i>C. lavasoensis</i>	Petit Lavasoa	-25.0809	46.7622
KC505934	<i>C. lavasoensis</i>	Petit Lavasoa	-25.0809	46.7622
KC505935	<i>C. lavasoensis</i>	Petit Lavasoa	-25.0809	46.7622
KC505936	<i>C. lavasoensis</i>	Petit Lavasoa	-25.0809	46.7622
KC505937	<i>C. lavasoensis</i>	Petit Lavasoa	-25.0809	46.7622
KC505938	<i>C. lavasoensis</i>	Petit Lavasoa	-25.0809	46.7622
KC505939	<i>C. lavasoensis</i>	Petit Lavasoa	-25.0809	46.7622
KC505940	<i>C. lavasoensis</i>	Ambatotsirongorongo	-25.0780	46.7824
KC505941	<i>C. lavasoensis</i>	Ambatotsirongorongo	-25.0780	46.7824
KC505942	<i>C. lavasoensis</i>	Ambatotsirongorongo	-25.0780	46.7824
KC505943	<i>C. lavasoensis</i>	Ambatotsirongorongo	-25.0780	46.7824
KC505944	<i>C. lavasoensis</i>	Ambatotsirongorongo	-25.0780	46.7824
KC505945	<i>C. lavasoensis</i>	Ambatotsirongorongo	-25.0780	46.7824
KC505946	<i>C. lavasoensis</i>	Ambatotsirongorongo	-25.0780	46.7824
KC505947	<i>C. lavasoensis</i>	Ambatotsirongorongo	-25.0780	46.7824
KC505948	<i>C. lavasoensis</i>	Ambatotsirongorongo	-25.0780	46.7824
KC505949	<i>C. lavasoensis</i>	Grand Lavasoa	-25.0891	46.7447
<b>Groeneveld et al. 2009, 2010 Samples</b>				
EU825359	<i>C. sp. nov. 2</i>	Andrambovato (Oranjatsy)	-21.4959	47.4018
GQ243488	<i>C. sp. nov. 2</i>	Ranomafana (Talatakely)	-21.2639	47.4189
GQ243489	<i>C. sp. nov. 2</i>	Ranomafana (Talatakely)	-21.2639	47.4189
EU825360	<i>C. andysabini</i>	Montagne d'Ambre	-12.4748	49.2185
EU825361	<i>C. andysabini</i>	Montagne d'Ambre	-12.4748	49.2185
EU825362	<i>C. andysabini</i>	Montagne d'Ambre	-12.4748	49.2185
EU825348	CCS2*	Sambava	-14.3994	50.1739
EU825349	CCS2*	Sambava	-14.3994	50.1739
EU825350	CCS2*	Sambava	-14.3994	50.1739
EU825351	CCS2*	Sambava	-14.3994	50.1739
EU825352	CCS2*	Sambava	-14.3994	50.1739
EU825353	CCS2*	Sambava	-14.3994	50.1739
EU825354	CCS2*	Manantenina	-14.4910	49.8115
EU825355	CCS2*	Manantenina	-14.4910	49.8115
EU825356	CCS2*	Manantenina	-14.4910	49.8115
EU825357	CCS2*	Manantenina	-14.4910	49.8115
EU825358	CCS2*	Manantenina	-14.4910	49.8115
EU825363	CCS2*	Ambanja/Beandroana	-13.7030	48.5046
EU825364	CCS2*	Sambava	-14.3994	50.1739
EU825365	CCS2*	Sambava	-14.3994	50.1739
EU825366	CCS2*	Sambava	-14.3994	50.1739
EU825367	CCS2*	Manantenina	-14.4910	49.8115
GQ243481	<i>C. crossleyi</i>	Tsinjoarivo/Vatateza	-19.7208	47.8569
GQ243482	<i>C. crossleyi</i>	Tsinjoarivo/Vatateza	-19.7208	47.8569

GQ243483	<i>C. crossleyi</i>	Tsinjoarivo/Vatateza	-19.7208	47.8569
GQ243484	<i>C. crossleyi</i>	Tsinjoarivo/Vatateza	-19.7208	47.8569
GQ243485	<i>C. crossleyi</i>	Tsinjoarivo/Vatateza	-19.7208	47.8569
GQ243486	<i>C. crossleyi</i>	Tsinjoarivo/Vatateza	-19.7208	47.8569
GQ243487	<i>C. crossleyi</i>	Tsinjoarivo/Vatateza	-19.7208	47.8569
EU825368	<i>C. crossleyi</i>	Ankazomivady	-20.7800	47.1820
EU825369	<i>C. crossleyi</i>	Ankazomivady	-20.7800	47.1820
EU825370	<i>C. crossleyi</i>	Ankazomivady	-20.7800	47.1820
* CCS designations 2 and 6 are from Lei <i>et al.</i> 2014				

**Table 2.** List of dwarf lemurs, *Cheirogaleus* sp. nov. 2, from Ranomafana and Andringitra examined during this study using acronyms TRA and RANO to designate sites (see Table. 1). 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
TRA8.81	TTU-M 118807	TK 129245	Male	47317E2474	0.404	-22.22269	47.01889	11/16/2008
TRA8.82	TTU-M 118809	TK 129247	Female	4734461007	0.406	-22.22292	47.01950	11/18/2008
RANO2.95	TTU-M 118808	TK 129246	Female	N/A	0.550	-21.29250	47.43842	02/11/2002
RANO229			Female	N/A	0.268	-21.24833	47.42406	11/01/1999

Note: RANO229 is a juvenile.

**Table 3.** Ranomafana/Haute Masiatra dwarf lemurs, *Cheirogaleus grovesi*, examined in this study.

Class	No.	W (kg)	HC (cm)	BL (cm)	TL (cm)	ML (mm)	MW (mm)	F-Tb (cm)	F-LD (cm)	F-Hd (cm)	F-UR (cm)
Male	1	0.404±0.000	7.0±0.0	17.1±0.0	27.7±0.0	20.0±0.0	21.5±0.0	1.0±0.0	1.6±0.0	3.2±0.0	4.5±0.0
Female	2	0.478±0.102	6.0±0.2	21.6±4.0	29.7±1.0	19.0±0.0	20.0±0.0	1.2±0.0	1.5±0.0	3.9±0.2	5.1±0.1
Total	3	0.453±0.084	6.3±0.6	20.1±3.8	29.0±1.4	19.5±0.7	20.8±1.0	1.1±0.1	1.5±0.2	3.6±0.4	4.9±0.3

Note: W: weight; HC: head crown, HW: head width, BL: body length, TL: tail length, ML: muzzle length, MW: muzzle width, Forelimb F-Tb: thumb length, F-LD: longest digit length, F-Hd: hand length, F-UR: ulna/radius length.

**Table 3.** Continued.

Class	F-H (cm)	H-Tb (cm)	H-LD (cm)	H-Ft (cm)	H-T (cm)	H-F (cm)	UC (mm)	LC (mm)	TeL-R (mm)	TeW-R (mm)	TeL-L (mm)	TeW-L (mm)
Male	3.6±0.0	2.0±0.0	1.8±0.0	5.6±0.0	5.5±0.0	6.1±0.0	5.0±0.0	3.2±0.0	14.5±0.0	8.6±0.0	14.0±0.0	8.2±0.0
Female	4.1±0.8	1.8±0.4	1.6±0.1	5.9±0.2	5.6±0.7	5.1±0.8	4.0±0.0	3.0±0.7	NA	NA	NA	NA
Total	3.9±0.7	1.9±0.3	1.7±0.2	5.8±0.2	5.6±0.5	5.4±0.8	4.5±0.7	3.1±0.5	NA	NA	NA	NA

Note: F-H: humerus length, hindlimb H-Tb: thumb length, H-LD: longest digit length, H-Ft: foot length, H-T: tibia length, H-F: femur length, UC: upper canine length, LC: lower canine length, TeL-R: right testis length, TeW-R: right testis width, TeL-L: left testis length, TeW-L: left testis width.

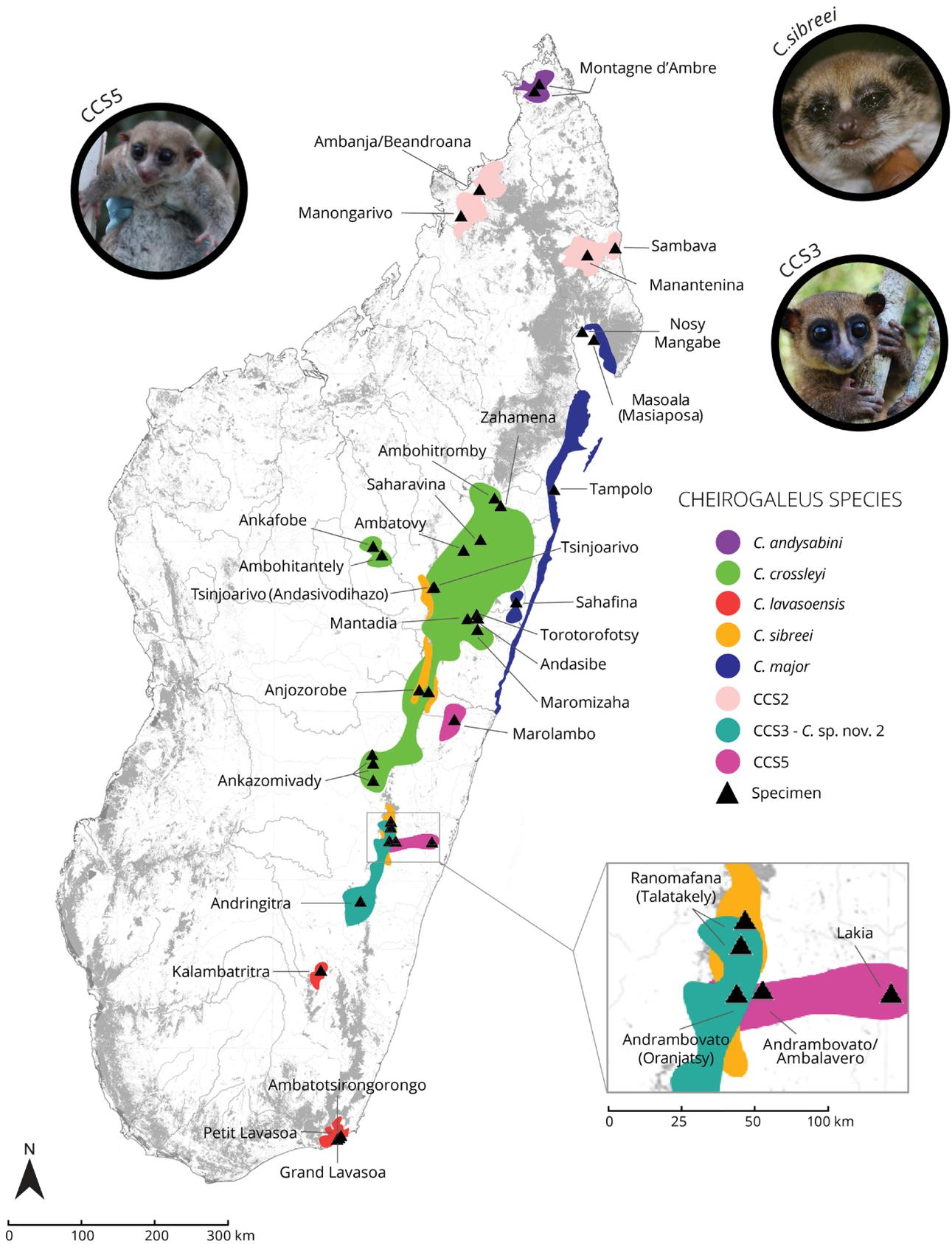
here differs from these two lineages by a genetic distance of 13.1%±0.9%, and 11.0%±0.8%, respectively (See Lei *et al.* 2014, Appendix II(g) for *cytb* genetic distance data). Additional analyses using D-loop, COII, and PAST mitochondrial gene regions resulted in *C. sp. nov. 2* segregating as a distinct lineage with a high degree of confidence (posterior probabilities equal to 1.00, with a single individual from Ranomafana scoring 89/0.99; Figs. 2–4 in Lei *et al.* 2014). These results were supported by analyses of three nuclear loci (CFTR-PAIRB, FIBA, vWF), with posterior probability in support of an independent lineage at 0.98 in a phylogenetic analysis including the CCS2 specimen from Kalambatritra, another possible, though as yet unconfirmed, new species in the *C. crossleyi* group known from multiple individuals on the northeastern (Sambava, Manantenina) and northwestern (Ambanja/Beandroana, Manongarivo) coasts (Figs. 5–6 in Lei *et al.* 2014). Use of the CCS2 specimens in the analysis significantly increased support, from 0.55 to 0.98, for the

independence of the *C. sp. nov. 2* lineage, likely because a more accurate picture of the genetic diversity within *C. crossleyi* was possible with the inclusion of a larger number of closely related species.

The population aggregate analysis (PAA) results are congruent with those presented in Lei *et al.* (2014). We obtained a Bayesian PTP support value of 0.96 indicating an excellent probability of the likelihood that *C. sp. nov. 2* is a species based on the given dataset.

## Discussion

Several new *Cheirogaleus* species were recently described via genetic analyses by Lei *et al.* (2014, 2015) and Frasier *et al.* (2016). Yet, there is still evidence of additional undescribed diversity in dwarf lemurs, in particular within the *C. crossleyi* group.



**Figure 3.** Map of Madagascar with the ranges of *C. sp. nov. 2* and other species in the *C. crossleyi* group: *C. andysabini*, *C. crossleyi*, *C. lavasoensis*, and CCS2. Map also includes lineages of other *Cheirogaleus* species groups that are partially sympatric, *C. sibreei* and CCS5. Additionally, *C. major* is included with the newly reported population at Sahafina. Photographs are provided to show the distinct phenotypic differences between sympatric lineages.

Lei *et al.* (2014) described the range of *C. crossleyi* as extending from Zahamena in the northeast south to Tsinjoarivo. Fecal samples collected since 2014 indicate a larger range for this species than previously suspected. *Cheirogaleus crossleyi* populations have now been identified as far north and west as the Ambohitantely National Park, as well as at the nearby community-run Ankafoabe Reserve about 110 km northwest of Antananarivo. Additional populations were identified some 80 km north and east of this location near Lac Aloatra and Ambatondrazaka at the sites of Ambohitromby and Saharavina. Historical sources support this larger range. The naturalist Alfred Grandidier collected the type specimen of *C. crossleyi*, now in the collection of the Harvard Museum of Comparative Zoology (MCZ 44952), near Lac Aloatra in the late 19th century. These remaining animals are isolated in surviving forests, and are likely not contiguous with populations located farther east and south. This population fragmentation and isolation is consistent with that found across the species' extended range (Andriaholinirina *et al.* 2014). *Cheirogaleus crossleyi* populations were also sampled at three sites around Ankazomivady, farther southwest than previously observed. Taken together, these additional samples indicate a greater range than previously known for *C. crossleyi*. Additional sites are being identified not just for *C. crossleyi*, but other *Cheirogaleus* lineages. A population of *C. major* was recently identified at Sahafina and confirmed with mitochondrial DNA (D-loop) sequenced from fecal samples. The presence of this population is being reported here for the first time (Fig. 3).

Here, we present a description of a new species, identified as *C. sp. nov. 2* (CCS3) within the *C. crossleyi* group by Lei *et al.* (2014), the range of which extends from Ranomafana to Andringitra national parks, and is supported by morphological and genetic data. Sufficient mitochondrial genetic divergence was observed to warrant elevation of this population as a new species (Table 2). Additional evidence used in elevating this population to species status was its geographic isolation from other genetically close populations of *Cheirogaleus*, *C. andysabini*, *C. lavasoensis* and *C. crossleyi*. Geographic isolation should not be the sole consideration for diagnosing a new species, but should be considered as one piece of evidence alongside other factors. In this case other factors include genetic divergence, size, pelage variation, and habitat elevation (Fig. 3; Table 2; Table 3).

Individuals of *C. sp. nov. 2* are considerably larger (0.18 kg on average) than *C. sibreei* individuals, a lineage with which they share the northern part of their range. It is unlikely that these two species are ecologically sympatric, as *C. sibreei* is found from 1128–1660 m above sea level while *C. sp. nov. 2* are found at 754–999 m. The distribution of *C. sp. nov. 2* also partly overlaps with the CCS5 lineage, a cryptic species within the *C. major* group identified at a lower altitudinal range (85–763 m), for which no morphological data are currently available (Lei *et al.* 2014). *Cheirogaleus sp. nov. 2* is notably larger than other lineages within the *C. crossleyi* species group, to which it belongs (Table 2; Table 3;

Lei *et al.* 2014, Table 4). *Cheirogaleus sp. nov. 2* individuals are as much as 0.11 kg heavier and approximately 5 cm longer than other species in the *C. crossleyi* group.

## Conservation Status

The conservation status of this species is unknown, although its presence in two national parks and a protected corridor indicates that it is possibly more secure than lemur species that are not resident in protected areas. Anthropogenic deforestation is a threat across Madagascar, and national parks are not immune to this threat. It is unknown if this species is hunted for bush meat, but this is certainly a possibility. Additional research is necessary to determine the conservation status of this species, so that protective measures can be taken to ensure the future of the lineage. The continuing identification of new primate species in Madagascar's remaining wild places highlights the need to protect this habitat from additional disturbance by human encroachment.

## Groves', Andringitra, or Haute Matsiatra Dwarf Lemur

### *Cheirogaleus grovesi*

Formerly *Cheirogaleus sp. nov. 2*, also CCS3/Crossleyi D (Lei *et al.* 2014), also *Cheirogaleus sp.* Ranomafana Andrambovato (Thiele *et al.* 2013).

**Holotype.** TRA8.81 (TTU-M 118807/K 129245); adult male; Permit number 239/08; 4 x 2.0 mm biopsies from ear pinna and 0.3 cc of whole blood; stored and curated at Museum of Texas Tech University (MTTU) Genetic Resources Collection, Natural Sciences Research Laboratory (NSRL); we placed a microchip subcutaneously between the scapulae and recorded as 47317E2474; collected by Edward E. Louis, Jr., Jean Richard Rakotonomenjanahary, and Jean Claude Randriamanana on 16 November 2008.

**Paratypes.** RANO2.95, RANO229 (juvenile); TRA8.82; RMR146, MB210, MB217

**Type locality.** Madagascar: Fianarantsoa Province, Haute Matsiatra Region, Andringitra National Park, S22.222694, E47.018889 at 763 m above sea level.

**Measurements of holotype.** Measurements recorded in field catalog: body length 17.1 cm; tail length 27.7 cm; head crown 7.0 cm; mass 404 g.

**Description.** The dorsum, limbs, and head are 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 continues onto the rufous-grey pelage of the ventrum.

**Diagnosis.** *Cheirogaleus grovesi* can be distinguished from *C. andysabini*, *C. crossleyi*, *C. lavasoensis* and CCS2 by 10, 9, 18 and 12 diagnostic characters in the *cytb* gene, respectively (Appendix II (k); Lei *et al.* 2014). *C. species nov. 2* has six diagnostic sites in the *cytb* sequence fragment such as G, G, C, G, C and C at the positions of 123, 432, 693, 918, 1078 and 1083, respectively, which differentiate *C. species nov. 2* from all other *Cheirogaleus* species. Despite being geographically close to CCS5 and *C. sibreei*, *C. grovesi* is distinct by six and 31 diagnostic characters from CCS5 and *C. sibreei*, respectively, while CCS5 is clustered in the *C. major* subgroup. An average weight of 0.410 kg was greater than all other members of the *C. crossleyi* species group, which has an average weight of less than 0.33 kg. *Cheirogaleus grovesi* is larger than *C. crossleyi*. Blackish-brown fur around eyes, rufous brown fur on dorsum, limbs, and head, venter is a mix of grey and rufous brown fur.

**Distribution.** *Cheirogaleus grovesi* is known from the national parks of Ranomafana and Andringitra, as well as surrounding areas, and likely occupies a fragmented range between the two parks across the Haute Matsiatra region of Madagascar. Observed at 754–999 m above sea level (Fig. 3).

**Etymology.** This species is named for the late British-Australian biological anthropologist, Professor Colin Groves (1942–2017), in recognition of his more than forty years of work in the fields of primatology, evolutionary biology, morphological analysis, mammalian taxonomy and associated disciplines. Professor Groves embodied the true spirit of collaboration. His fastidious research on historical collections incorporated the work of those that preceded him, which he combined with the efforts of his contemporaries, creating compositions that span hundreds of years of scientific exploration. At the time of his passing, Professor Groves was widely regarded as the greatest living primate taxonomist.

**Vernacular Names.** Groves', Andringitra, or Haute Matsiatra dwarf lemur.

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