ISSN 0898-6207

PRIMATE CONSERVATION

The Journal of the IUCN SSC Primate Specialist Group

Number 30 2016

Primate Conservation is produced and circulated courtesy of the Margot Marsh Biodiversity Foundation, Conservation International, and the Department of Anatomical Sciences of the Stony Brook University, New York, USA.

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Anatomical Sciences

Primate Conservation

The journal of the IUCN SSC Primate Specialist Group

Conservation International
2011 Crystal Drive, Suite 500, Arlington, VA 22202, USA

ISSN 0898-6207 Abbreviation: *Primate Conserv.*

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Front cover: Sheth's dwarf lemur, *Cheirogaleus shethi* sp. nov., from Andrafiamena (Anjakely), Antsiranana Province, Madagascar, described here by Cynthia L. Frasier and colleagues (see pages 59–72). Photograph by Richard Randriamampionona.
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This issue of *Primate Conservation* was kindly sponsored by the Margot Marsh Biodiversity Foundation, Virginia, USA, and the Department of Anatomical Sciences of Stony Brook University, Stony Brook, NY, USA.

Contents

Neotropical Region

Madagascar

Africa

Primate Conservation 2016 (30): 1–6

Legal International Trade in Live Neotropical Primates Originating from South America

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Abstract: Neotropical primates have fascinated Europeans since they first arrived in the Americas. Trade, shipping the monkeys back to Europe, began in the 16th century, and continues today, sending them to countries worldwide for zoos, privately owned pets, and for research. In this study, we characterize the legal trade of Neotropical primates from South America, with particular emphasis on Brazil, as evidenced in the CITES Trade Database website. Taking into account wild animals between 1977 and 2013, there were nearly 1,300 transactions, over half of which (60%) were reported to be commercial. Imports by zoos (10%) and for scientific purposes (6.5%) involved almost 90,000 primates. Sixty-three countries imported Neotropical primates and, with the exception of Ecuador, all South American countries exported live animals. The individual contribution of different countries to the trade fluctuated over the years. Only eight species showed numbers superior to 1% of the total number of exported primates. Considering captive animals, there were nearly 300 transactions, involving 4,827 individuals, with a smaller number of species and exporters. The amount of traded primates is relevant and deserves monitoring. Nevertheless, we noticed a trend towards stabilization of the numbers of species and total numbers of exported individuals over the last decade.

Key Words: Neotropical primates, CITES, legal trade, South America

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Introduction

Neotropical primates have fascinated Europeans since they first arrived in the Americas over 500 years ago. Monkeys were shipped backed to Europe mainly to serve as exotic pets for the nobles (Fragaszy *et al*. 2004; Urbani 2007). There are records of primates being taken from northeastern Brazil to the European continent dating back to 1511 (Urbani 1999). This trade continues; countries worldwide import these animals for zoos, privately owned exotic pets, and for scientific research (Mack and Mittermeier 1984).

The 1973 Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) was drafted as a result of a resolution adopted in 1963 at a meeting of members of IUCN. The text was agreed at a meeting of representatives of 80 countries in Washington, DC, on 3 March 1973. It entered into force on 1 July 1975, its aim being to regulate and promote the sustainability of wildlife trade, ensuring that the international trade in specimens of wild animals does not compromise the survival of natural populations to the point of their endangerment. When the government of a State decides that it will be bound by the provisions of CITES, it can 'join' the Convention by making a formal declaration in writing to the Depositary Government (Switzerland). Once the document has been received, the Convention enters into force in 90 days. Each country that implements CITES is called a Party, and has to designate a Management Authority and Scientific Authority to carry out the treaty. Collectively, the member countries to CITES are referred to as the Conference of the Parties.

Currently there are 182 parties. The process of making a declaration to be bound by CITES is called 'ratification,' 'acceptance,' 'approval' or 'accession.' Ratification, acceptance and approval are legally equivalent actions but are only applicable in relation to the States that signed the Convention when it was open for signature (between 3 March 1973 and 31 December 1974). Acceptance and approval are the actions taken by certain States when, at national level, constitutional law does not require a treaty to be 'ratified'. All States that had signed the Convention have now ratified, accepted or approved it. 'Accession' is used in relation to the States that did not sign the Convention (CITES 2015, for South America

Table 1. Countries which entered the process of making a declaration to be bound by the provisions of CITES, type and period of the process (CITES 2015).

Country	Type	Date of joining	Entry into force
Ecuador	Ratification	11/02/1975	01/07/1975
Chile	Ratification	14/02/1975	01/07/1975
Uruguay	Ratification	02/04/1975	01/07/1975
Peru	Ratification	27/06/1975	25/09/1975
Brazil	Ratification	06/08/1975	04/11/1975
Paraguay	Ratification	15/11/1976	13/02/1977
Guyana	Accession	27/05/1977	25/08/1977
Venezuela (Bolivarian Republic of)	Ratification	24/10/1977	22/01/1978
French Guiana	Approval	11/05/1978	09/08/1978
Bolivia (Plurinational State of)	Ratification	06/07/1979	04/10/1979
Suriname	Accession	17/11/1980	15/02/1981
Argentina	Ratification	08/01/1981	08/04/1981
Colombia	Ratification	31/08/1981	29/11/1981

see Table 1). CITES defines criteria and standards for transactions to be followed by the signatory countries that assumed responsibility for authorizing and registering the international trade in specimens, parts and derivatives, obtained from nature or not, by their national Management Authority.

Once CITES was instituted, the international trade in primates declined significantly (Fragaszy *et al*. 2004), and by 1981 all South American countries had become signatories (CITES 2015). Approximately 5,600 species of animals are, in principle, protected against legal overexploitation between countries by means of CITES. They are listed in three appendices, each defining different levels of protection from over-exploitation according to the degree of threat to which the trade subjects them (CITES 2015). All non-human primates are included in Appendices I or II of the Convention; their trade requires, therefore, the approval of CITES authorities from all the countries involved—origin and destination. Twenty-one of the 174 Neotropical primate species currently recognized by CITES are listed in Appendix I, the most restrictive Appendix, in which trade in specimens is permitted only in exceptional circumstances. The remaining species are included in Appendix II that includes species in which trade must be controlled in order to avoid utilization incompatible with their survival. Exceptional cases are listed in Appendix III (CITES 2015). Here we characterize the trade in Neotropical primates originating from South America between 1977 and 2013.

Methods

We obtained the report on legally traded Neotropical primates from the *CITES Trade Database* website (<http://trade.cites.org/>) (CITES 2015), following the *Guide to Using the CITES Trade Database* (UNEP and WCMC 2013). Data were available for the years 1977 to 2013.

We analyzed the annual numbers of live specimens traded by species and by country of origin and destination by consolidating the information reported by the traders—importers and/or exporters (gross import/export quantities, which included re-exports). We included animals originating from the wild (W), seized or confiscated (I), of unknown origin (U) and from captive origin (codes C, F and R – see UNEP and WCMC 2013). When available, we used the numbers provided by the importing country; when lacking, we used the number declared by the exporter. When one of the parties informed more than one value for the same species and year, we considered the highest. We excluded data missing part of the transaction information, as well as records for which the taxonomic identity was above the level of genus.

Results

We recorded the trade of 89,358 primates, W, I or U (Table 2) belonging to at least 43 species (only *Ateles geoffroyi frontatus* was cited as a subspecies) originating from South America. Sixty-three countries imported Neotropical primates and, with the exception of Ecuador, all South American countries, including Chile that has no native species, exported live monkeys at a given point during the examined time frame. For those 36 years (1977−2013), the average of number of individuals per taxa traded between two countries was 70. The largest single transaction for a single taxon in a given year was 2,466 individuals of *Saimiri sciureus* exported by Guyana to the United States in 1986. However, we can see a gradual reduction in volume and number of species involved over the years (Figs. 1 and 2).

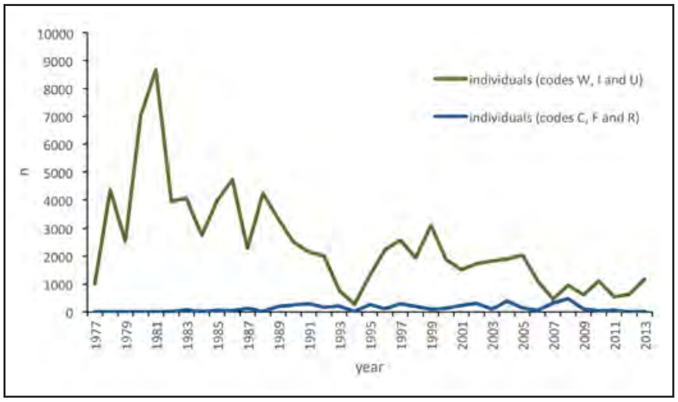

Figure 1. Number of live Neotropical primates exported from South America between 1977 and 2013.

Table 2. Number of live Neotropical primates exported by genus and country from South America between 1977 and 2013, only animals originating from the wild, apprehended or of unknown origin (codes W, I and U) (AR = Argentina, BO = Bolivia, BR = Brazil, CL = Chile, CO = Colombia, GF = French Guiana, GY = Guyana, PE = Peru, PY = Paraguay, SR = Suriname, UY = Uruguay, VE = Venezuela).

Genus	AR	BO	BR	CL	CO	GF	GY	PE	PY	SR	UY	VE	Total
Aotus		1368			109			1,817	174				**3,468**
Ateles		36			1		47	1		14		22	**121**
Cacajao		4	4										**8**
Alouatta	69	90	6		1		171	1	36	2		12	**388**
Callicebus		181	2					18					**201**
Callimico		27			1			5					**33**
Callithrix[1]	3	691	523		14			307	385	32	2		**1,957**
Cebus[2]	95	654	54	38	2		7,058	38	259	558	2	8	**8,766**
Chiropotes		6					126			4			**136**
Lagothrix		80	12		38			6					**136**
Leontopithecus	2		33					1					**36**
Pithecia							300			2			**302**
Saguinus[3]		5353	24	1	8		3,522	3,153		1,062		2	**13,125**
Saimiri	12	14,454	6	2	84	33	37,862	2750		5,476		2	**60,681**
Total	**181**	**22,944**	**664**	**41**	**258**	**33**	**49,086**	**8,097**	**854**	**7,150**	**4**	**46**	**89,358**

[1] Includes *Callibella, Cebuella, Mico*. [2] Includes *Sapajus*. [3] Includes *Leontocebus*.

Of almost 1,281 recorded transactions (defined as the movement of one taxon between two countries in a given year), over half (60%) were categorized as commercial, followed by imports by zoos (10%) and for scientific purposes (6.5%).

The individual contribution of different countries to this trade fluctuated over the years (Fig. 3). Bolivia was the main primate-exporting country from 1978 to 1983, peaking in 1981, with the export of a little more than 7,000 monkeys, very largely *Saimiri* and *Saguinus*. Bolivia almost ceased its legal exports in 1987. Guyana was the principal exporter from 1984, gradually increasing its numbers from 1977 to 1988, peaking in 1986 (4,024 individuals) and in 1988 (3,747), but dropping to 1,659 by 1991. Guyana's exports dropped in 2007, averaging 602 from 2007 to 2013. Squirrel monkeys, *Saimiri*, comprised the majority of its exports (2,562) from 2007 to 2013. Significant numbers of monkeys (6,971) were also exported from Suriname between 1995 and 2013. Between 1977 and 2004, Peru exported between 60 and 920 primates per year, peaking in 1987. From 2005 to 2013 numbers were minimal.

Historically, Guyana accounts for 54.9% of all exported primates and only Bolivia, Peru and Suriname, following this order, exhibit percentages higher than 1%. The principal importers were the United States and Japan, accounting for almost 70% of all traded primates; the United States alone for nearly half of all imports (49.7%). During the 36-year period we studied, Brazil legally exported 664 primates (0.7%).

Only eight species (of five genera) showed export numbers superior to 1% of the total number of exported primates (Fig. 4). The most frequently recorded taxon was *Saimiri sciureus*, including Bolivian squirrel monkeys that currently are classified as *S. boliviensis*, corresponding to 59,982, or 67.1% of all individuals, followed by *Cebus* (= *Sapajus*) *apella* (6,547: 7.3%), *Saguinus labiatus* (5,227: 5.8%), *Saguinus midas* (4,608: 5.2%), *Aotus trivirgatus* (2,694: 3%) (undoubtedly

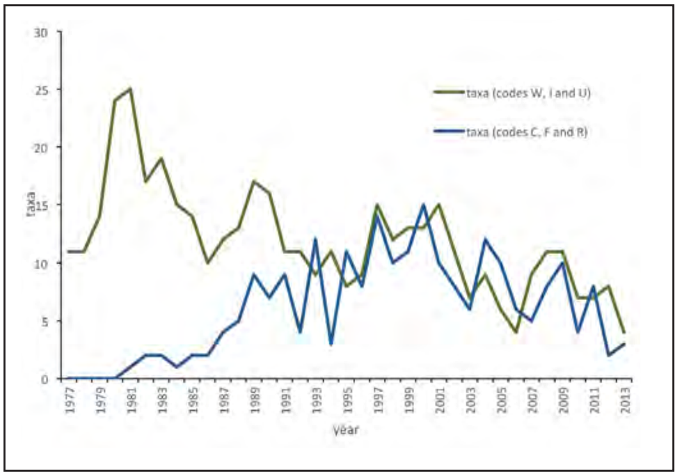

Figure 2. Number of Neotropical primate species (taxa) exported by South American countries between 1977 and 2013.

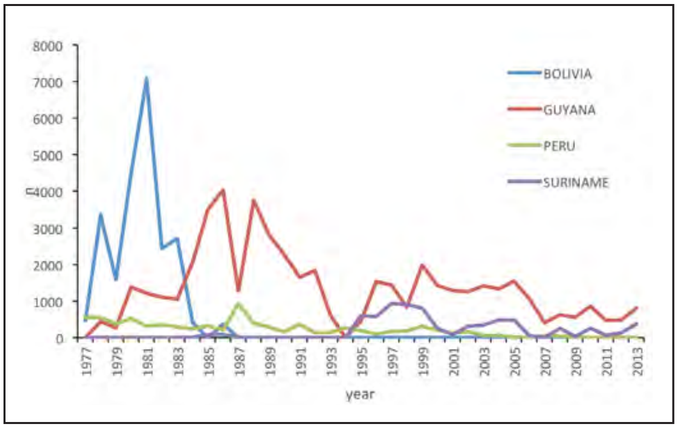

Figure 3. Number of live Neotropical primates exported by the four most important South American countries (>1% in total number) between 1977 and 2013, only animals originating from the wild, apprehended or of unknown origin (codes W, I and U).

a mix of the currently recognized species), *Saguinus mystax* (2,111; 2.4%), *Cebus olivaceus* (1,363: 1.5%), and *Callithrix jacchus* (1,221: 1.4%). In most recent years, there was a notable peak in trade in *Callithrix* (*Cebuella*) *pygmaea* originating from Peru, *Cebus* (= *Sapajus*) *apella*, *Cebus olivaceus*, *Chiropotes chiropotes* (= *C. satanas*), and *Saguinus midas* from Guyana, and *Saimiri sciureus* from Guyana as well as Suriname.

In South America's export trend, *Saimiri* is the main genus exported (60,681; 67.9%), with *Saguinus* being a rather distant second (13,125; 14.7%). Brazil is slightly distinct from the overall pattern, *Callithrix jacchus* accounted for more than two-thirds of all transactions (450: 67.8%), mostly in 1979−1980. It was followed by *Cebus* (= *Sapajus*) *apella* (54: 8.1%) and *Callithrix geoffroyi* (38; 5.7%).

The trade of captive animals (codes C, F and R) was considerably lower than the trade of W, I and U animals. It involved less specimens (4,827), taxa and countries (Table 3, Fig. 1), although the number of species exported annually from the 90's is very similar between both types of trend (Fig. 2). *Saimiri* remains as one of the main genera involved, but *Callithrix* is the principal export.

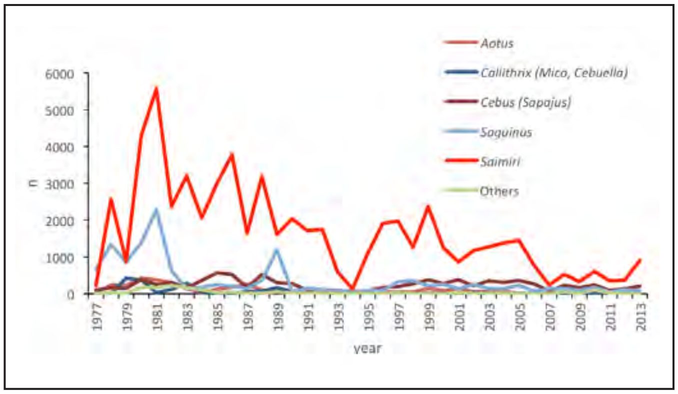

Figure 4. Number of live Neotropical primates by genus exported from South America between 1977 and 2013, only animals originating from the wild, apprehended or of unknown origin (codes W, I and U).

Although Brazil's participation in the trade of animals from the wild was minimal, it is the main source of animals that come from captivity considering the period analyzed, with a small, but constant volume over the years— mostly *Callithrix* (85%).

Discussion

Records from the CITES Trade Database have known limitations (Mack and Mittermeier 1984; UNEP and WCMC 2013). We here highlight two that surfaced during our analyses, but which we believe did not compromise the patterns we report: a trade may be registered twice in two consecutive years, either by the exporter, or the importer; and a group of animals may be identified as a given taxon by the exporter and as another by the importer.

The numbers of traded primates is relevant and deserves monitoring. Nevertheless, we noticed a trend towards stabilization of the species richness and total number of exported individuals over the last decade. Exceptionally high figures were registered for 1964 and 1980, when over half a million specimens, approximately 60% of them *Saimiri*, were taken from South America (notably Peru and Colombia) into the United States (Mack and Mittermeier 1984). CITES was in the early stages of implementation in 1977, and many countries had yet to become signatories.

Until 1973, Peru and Colombia were the principal suppliers to the United States, the main Neotropical primate importer. After these countries banned the commercial export of primates, Bolivia and Guyana became the major exporters to the United States (Mack and Mittermeier 1984), with high numbers of primates exported during the 1980s by these

Table 3. Number of live Neotropical primates exported by genus and country from South America between 1977 and 2013, only animals originating from captivity (codes C, F and R) (AR = Argentina, BO = Bolivia, BR = Brazil, CL = Chile, CO = Colombia, GF = French Guiana, GY = Guyana, PE = Peru, SR = Suriname, VE = Venezuela).

Genus/Country	AR	BO	BR	CL	CO	GF	GY	PE	SR	VE	Total
Alouatta	32		3							26	61
Aotus								918			918
Ateles							4	9		10	23
Callimico									3		3
*Callithrix*¹	25		1472		2			231			1730
*Cebus*²	86	1	113	64			120	18		14	416
Lagothrix			3					6			9
Leontopithecus			95		1		14				110
Pithecia								2			2
*Saguinus*³			51		4		50	63	9		177
Saimiri	13					539	416	324	86		1378
Total	**156**	**1**	**1737**	**64**	**7**	**539**	**604**	**1571**	**98**	**50**	**4827**

¹Includes *Callibella, Cebuella, Mico*. ²Includes *Sapajus*. ³Includes *Leontocebus*.

countries between 1977 and 2013. In the late 1980s, Guyana replaced Bolivia as the principal New World primate exporter, because the latter stopped exporting the Bolivian squirrel monkey (Abee 2011). This explains the peak in *Saimiri* trade between Guyana and the United States observed in 1986. In 2013, Guyana was the fifth most important provider of primates to the United States (IPPL 2015). The lack of trade originating from Ecuador is a consequence of the countries' stricter legislation.

Saimiri was also the taxon most traded prior to CITES and the Brazilian, Peruvian and Colombian legislation restricting primate exports (Mack and Mittermeier 1984). Although most exports are listed as commerce, scientific testing is likely to be the true driver behind the majority of the primate trade. That is certainly the case for the squirrel monkey (*Saimiri*), which is the most extensively used Neotropical taxon in scientific and biomedical research (Abee 2011), as well as the main target for the pet trade (Mack and Eudey 1984).

The United States is not only the largest importer of South American primates, as shown in our analyses, but also of primates worldwide (IPPL 2015). In 2013, the country imported 18,934 primates, 97.7% of which were reported bred in captivity and 98.5% from Asian countries (IPPL 2015), primarily China (Hsu 2011). The United States is also the country with the highest use of Neotropical primates in experimental (biomedical) research, where squirrel monkeys (*Saimiri*), owl monkeys (*Aotus*), marmosets (*Callithrix jacchus*), and tamarins (*Saguinus mystax*) are the main taxa (Abee 2011).

CITES tends to adopt a conservative taxonomy, as nomenclatural changes can disrupt and confuse the provision of permits and other processes, which incurs significant delays in its implementation. Primate nomenclature follows Wilson and Reeder (2005), with several additions concerning individual species or genera. Once every three years CITES evaluates the utility of updating its nomenclature at its Conference of the Parties. Despite this, given the increase in taxonomic studies of primates, resulting in the continual updating and changes in taxonomic lists (see Groves 2005; Rylands and Mittermeier 2008, 2014; Rylands *et al.* 2012), we here highlight the need for systematic updates in the CITES checklist. Considering their prominence in the primate trade, it is urgently necessary to incorporate revisions in the taxonomies of the squirrel monkeys *Saimiri* (see Lynch Alfaro *et al*. 2015) and capuchin monkeys, the latter now divided into two distinct genera (Lynch Alfaro *et al.* 2012a, 2012b)—*Cebus* for the gracile, untufted capuchins and *Sapajus* for the robust, tufted capuchins (Lynch Alfaro *et al.* 2012b; Boubli *et al*. 2012). Trade records for *Saimiri sciureus* and *Cebus apella* undoubtedly comprise now other recognized species—notably, for the former, the distinct *Saimiri boliviensis* from Bolivia—and some of them may be threatened. Having a more discerning taxonomy will better allow for the tracking of records of species exported from countries where they are not native, although they could be re-exportations, as is the case for the small numbers exported from Uruguay and Chile, which have no wild primate populations.

Since the last global assessment of the primate conservation status by IUCN, only one taxon categorized as threatened was involved in trade, in this case, *Ateles paniscus*, considered Vulnerable (VU).

The limitations of the report made available by the CITES Trade Database are already acknowledged in the user guide provided by UNEP and WCMC (2013). We highlight here, in addition to the outdated taxonomy, that a trade could be registered twice in two consecutive years, either by the exporter, or the importer, and that a group of animals could be identified as a given taxon by the exporter and as another by the importer.

Finally, another important issue is the fact that Guyana and Suriname, the two main exporters of primates in South America, do not have a list of threatened species, although the commonly exported taxa are not currently listed as threatened on the IUCN Red List of Threatened Species (IUCN 2015).

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Received for publication: 22 March 2016
Revised: 14 June 2016

Primate Conservation 2016 (30): 7–13

Decline in Population Density and Group Size of Mona Monkeys in Grenada

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Abstract: Mona monkeys (*Cercopithecus mona*) were introduced from West-Central Africa to the Caribbean island of Grenada about 250 years ago. Little is known, however, about the recent trends in size and conservation status of this non-native primate population. We estimated the population density and abundance of mona monkeys in and around the forested areas located in central Grenada (including the Grand Etang National Park). We used repeat line transect distance sampling, a standard method for census surveys of forest-dwelling primates. The estimated group density, individual density, average mixed-sex and all-male group sizes, and total population size throughout the rain forest habitat on the island were 0.7 group/km², 6.0 individuals/km², 10.3 ± 3.0 individuals/mixed-sex group, 2.1 ± 0.3 individuals/all-male group, and 289 individuals, respectively. The comparison of these values with those obtained from a previous study conducted 20 years ago, in the same area and with the same method, showed a marked population decline. This decrease may be explained by the ecological devastation of Hurricane Ivan in 2004, combined with persistent over-hunting. We discuss the theoretical implications of this study for evolutionary processes and biodiversity patterns.

Key Words: distance sampling, population density, hurricane, hunting pressure, bushmeat, pet trade, introduced primates, *Cercopithecus mona*

Introduction

Accurate and updated information on the status and trends of primate populations is a prerequisite for successful conservation programs (Plumptre and Cox 2006). Evidently, most conservation efforts consist in preserving the diversity of species and subspecies in their native habitats (Dobson and Lyles 1989). However, knowledge of non-native primate populations, i.e., those introduced outside the species' native range as a direct or indirect consequence of human action (Heinsohn 2003), can also have major implications for conservation issues and further our understanding of evolutionary patterns and processes (Schlaepfer *et al.* 2011).

Research on differences in the ecology, demography, life history, behavior, genetics, and health status between native and non-native primate populations provides valuable information with regard to habitat disturbance (González-Martínez 2004), phenotypic plasticity (Corlett 2004), genetic diversity (Aarnink *et al.* 2014), phylogenetic inertia (Sol *et al.* 2008), biogeographic history (Denham 1987), hybridization/speciation (Oliveira and Grelle 2012), pathogen transmission (Hamilton *et al.* 2014), and conservation strategies (Hernandez-Pacheco *et al.* 2016). Therefore, even though a major tenet of conservation management holds that non-native animals are ecologically harmful, recent research suggests some introduced primate species can have beneficial impacts, and at least, a heuristic value.

This is particularly true for relatively small, inbred primate populations, historically introduced to island ecosystems. Examples include: green monkey species, *Chlorocebus sabaeus*, introduced to the West Indian islands of Anguilla, Barbados, Nevis, Saint Kitts, and Saint Martin (Denham 1987); long-tailed macaques, *Macaca fascicularis*, introduced to the island of Mauritius (Sussman and Tattersall 1986), and across (east of) Wallace's Line into Papua and the Lesser Sundas (Heinsohn 2001); East Javan langurs, *Trachypithecus auratus*, into Lombok (Heinsohn 2001); and rhesus macaques, *Macaca mulatta*, and patas monkeys, *Erythrocebus patas*, introduced to Puerto Rico (González-Martínez 2004). After a founder event, such as the arrival of a few members from an original population onto an island, the founding population experiences a bottleneck (i.e., a loss in genetic diversity due to a reduction in population size and geographic isolation for several generations) and is more vulnerable to genetic drift (Nei *et al.* 1975). This is why island ecosystems are excellent models for research on evolutionary processes and biodiversity patterns (Vitousek *et al.* 1995).

Mona monkeys (*Cercopithecus mona*) were introduced to the Caribbean island of Grenada from West-Central Africa during the slave trade. Although we lack direct historical information about their exact date of introduction, the most likely scenario based on trade patterns covers a range of dates between the late 1600s and 1807, most probably in the mid-1700s (Glenn 1996; Glenn and Bensen 2013). A recent genetic study, using a mitochondrial DNA analysis, showed that the total founding population of mona monkeys introduced to Grenada was extremely small, and originated from the island of São Tomé, Gulf of Guinea, rather than directly from mainland Africa (Horsburgh *et al.* 2002). This complex history of introduction makes the Grenadian mona monkeys good candidates for the study of ecological flexibility in fragmented habitats and speciation processes (Glenn and Bensen 2013).

Even though mona monkeys are still the only nonhuman primates on Grenada island today, the insular population trend is unknown. Two first surveys were conducted in 1987 and 1989 (Lippold 1989), but the information was not systematically collected, as it was based on opportunistic sightings in different locations, and possibly not reliable, as some population estimates were drawn from interviews with hunters (cf. Glenn 1996). The only reliable evaluation of the mona monkey population density in Grenada comes from a five-month survey conducted in 1994 and 1995 that used the repeat line-transect distance sampling technique; this study found that the population density estimate averaged 42.1 ± 35.7 individuals/km², and the extrapolated island-wide population estimate was 2,021 individuals based on the cover of available forested areas (Glenn 1998). However, assessing population trends requires at least two data points (ideally more) obtained several years apart and using the same methodology (e.g., Leca *et al.* 2013).

An updated demographic assessment is necessary for three reasons. First, unlike most other non-native insular primates that are considered invasive (Sussman and Tattersall 1986; Denham 1987; González-Martínez 2004), local biological and socio-economic effects of the mona monkeys in Grenada have not been quantified and are largely unknown. Even though the monkeys occasionally raid crops, particularly in the agricultural areas along the forest edge (Glenn 1996), no cost-benefit analysis is available. In light of the aforementioned valuable scientific information derived from small, inbred, insular populations, the conservation status of the mona monkeys of Grenada should be monitored regularly.

Second, although the mona monkeys of Grenada have few food competitors and no animal predators, they face the major anthropogenic threat of persistent and considerable hunting. For decades, the monkeys have been extensively hunted for bushmeat, served at local restaurants and food fairs (Lippold 1989; Glenn 1998). Recently, adult monkeys have also been hunted for the purpose of capturing live youngsters to sell them as pets (Michael Sanderson, pers. comm.). However, there is a disagreement on whether such intense hunting rates are sustainable (Bensen and Glenn 1997) or represent a risk for the future of this non-native primate in Grenada (Lippold 1989). Third, this monkey population recently faced a natural disaster that might have led to another bottleneck effect: the devastation of most of the forested habitat on the island by Hurricane Ivan in 2004 (Glenn and Bensen 2008). A post-Ivan demographic evaluation of their population is needed, in order to assess whether a management program should be put into practice for the survival of these introduced monkeys (Levy 2005).

Figure 1. Adult male mona monkey, *Cercopithecus mona*, in the Grand Etang National Park (photo by N. Gunst).

This study aimed to evaluate possible demographic changes in the population of mona monkeys (Fig. 1) in and around the forested areas located in central Grenada (including the Grand Etang National Park). The demographic parameters estimated include group and individual densities, group size, and total population size. We discussed our results from the perspective of the threats faced by the mona monkeys in Grenada, and suggested management plans for the protection of this primate population.

Methods

Study species

The mona monkey is a forest-dwelling arboreal guenon, originally ranging in West-Central Africa (i.e., Benin, Cameroon, Ghana, Nigeria, and Togo), inhabiting a variety of forested habitats, and exhibiting an omnivorous diet (Matsuda Goodwin 2007; Glenn *et al.* 2013). It forms one-male multi-female groups, multimale-multifemale groups, and all-male groups (Glenn, 1996; Matsuda Goodwin 2007). Solitary males also occur (Glenn 1996, 1997; Matsuda Goodwin 2007).

Data collection

This study was conducted over two months in March and April 2014, and in two types of habitats in central Grenada:

(1) forested areas, including the Grand Etang National Park and Forest Reserve, covering approximately 1,540 ha of montane tropical rain forest between 340 and 710 m above sea level, and (2) cultivated areas along the forest edge, between 220 and 410 m above sea level (Table 1, Fig. 2; cf. Glenn 1996 for further information about habitat types in central Grenada).

On a daily basis (except on rainy days) between 6:30 am and 4:30 pm, NG and JBL used the repeat line-transect distance sampling technique, with records of the perpendicular distances from the transect line to the estimated center of the group of the study subjects (Buckland *et al.* 1993). Successive walks on the same transect were separated by at least 48 hours. Observers walked on the transects at a constant speed of 1.5 km/h, looking ahead and sideways to detect the monkeys, and occasionally using binoculars to determine group sizes. We used a pen and paper and a Garmin GPSmap 60CSx to record, for each encounter, the following data: (1) time, (2) GPS coordinates of the detection point on the transect, (3) the perpendicular distance, estimated by eye, from the transect line to the position on the ground directly under the center of the group of individuals, and (4) the number of individuals detected during each encounter (referred to as group size). We used the term "group" to refer to a cluster/aggregation of mona monkeys at a given moment in time, and that were located within a maximum of 300 m of each other (cf. Matsuda Goodwin 2007). During our transect walks, we also recorded the presence and location of anthropogenic activities or artefacts (e.g., possible hunting activity as inferred by fresh dog footprints and recently discarded empty shell cases). We sampled a total of nine transects throughout the study area (covering 10.3 km²; Table 1, Fig. 2) and the distance surveyed was 173.3 km.

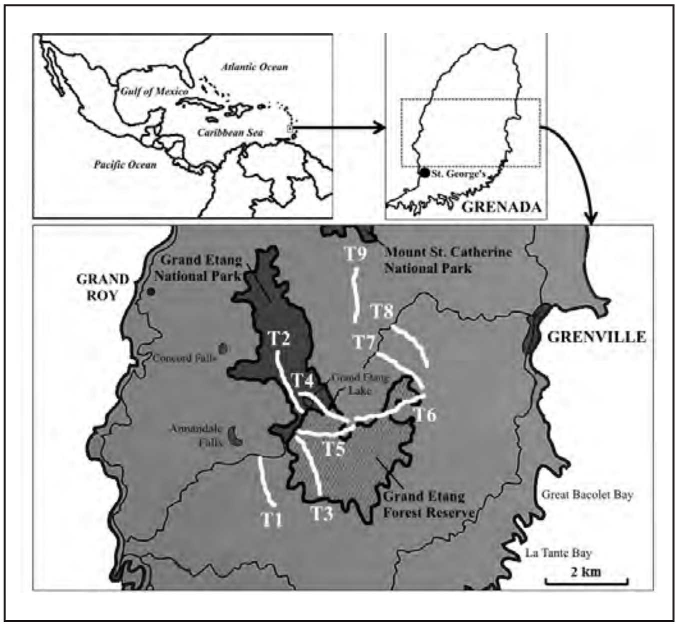

Figure 2. The study site in Grenada showing the location of the nine survey transects.

We recorded perpendicular distance data by categorizing them into 13 distance intervals, namely 0−5 m, 5−10 m, 10−15 m, 15−20 m, 20−25 m, 25−30 m, 30−40 m, 40−50 m, 50−60 m, 60−70 m, 70−80 m, 80−90 m, and 90−100 m. To ensure that perpendicular distances would be estimated accurately, observers were trained on evaluating distances by eye prior to the onset of the study (cf. Leca *et al.* 2013). Data collection started only after they reached 95% accuracy. We recorded a total of 46 encounters during the transect walks. An encounter was defined as the visual detection from the transect of at least one individual monkey. This research adhered to the legal requirements of the Republic of Grenada.

Data analysis

The perpendicular distances that we measured were used to estimate a detection function (i.e. the probability that a monkey is detected, as a decreasing function of its distance from the line), which in turn, allows for the calculation of the density of monkeys (or groups of monkeys) in the study area (Buckland *et al.* 1993). In order to provide estimates of density and abundance of mona monkeys in the study area,

Table 1. The nine transects surveyed, with the two main habitats encountered, number of times (N) walked, transect length (km), and total distance sampled (km). [a] Lower montane forest (between 340 and 400 m elevation); [b] Upper montane forest (between 500 and 710 m elevation); [c] between 220 and 410 m elevation.

Transect	Transect name	Main habitat	N walked	Length km	Total walked km
T1	Black Forest	Montane forest[a]	11	1.0	11.0
T2	Mount QuaQua	Montane forest[b]	17	1.5	25.5
T3	Après-Toute	Montane forest[b]	18	1.0	18.0
T4	Shoreline	Montane forest[b]	19	1.5	28.5
T5	Cross Trail	Montane forest[b]	20	1.3	26.0
T6	Seven Falls (down)	Montane forest[b]	19	1.1	20.9
T7	Seven Falls (up)	Cultivated area[c]	18	1.0	18.0
T8	Gangadee	Cultivated area[c]	11	1.0	11.0
T9	Spring Garden	Cultivated area[c]	12	1.2	14.4
Total	-		145	-	173.3

we used the computer software program Distance 6.0 (Buckland *et al.* 1993). Our sample of encounters reached the size required by this program, i.e. at least 40 encounters for fitting the detection function (cf. Buckland *et al.* 1993).

We examined the distribution of distances and found that no data were collected beyond 50 m. Therefore, no extra adjustment terms—sometimes required to fit a long tail to the detection function (Leca *et al.* 2013)—were necessary, and we did not truncate our distance data prior to analysis (Buckland *et al.* 1993). To estimate the mean group size, we averaged the number of individuals detected (i.e., seen not heard) during our 46 encounters. The encounter rate was defined as the number of groups detected per unit length of transect, i.e. per kilometer walked. After testing different detection function models, we selected the one that best fit our data set, based on well-established criteria (cf. Buckland *et al.* 1993): the half-normal key with cosine adjustments.

Results

Estimates of group and individual densities, group size, and estimated total population size

We plotted the detection function, superimposed on the histogram showing the detection probability as a decreasing function of the distance from the transect line to the monkeys detected (Fig. 3). The result of our chi-square goodness-of-fit test ($\chi^2 = 1.94$, df = 5, p = 0.858) showed that the model selected fit our data well (cf. Buckland *et al.* 1993).

The detection function allowed for the calculation of the main estimated values for our study of the population density and abundance of mona monkeys in and around the forested areas located in central Grenada. In the 10.3 km² of study area, we found, on average, 0.7 ± 0.1 group/km² and 6.0 ± 1.5 individuals/km². The estimated number of individuals in the study area was 62 ± 13. Based on the assumption that the population density of mona monkeys was constant across all rain forested areas in Grenada (covering approximately 48 km²; cf. Glenn 1998), the projected island-wide total population estimate was 289 individuals. Of the 46 encounters during the transect walks, we recorded 30 mixed-sex groups (mean group size: 10.3 ± 3.0 individuals/group, range: 5−15), nine all-male groups (2.1 ± 0.3 individuals/group, range: 2−3), and 7 solitary males.

Spatial distribution of mona monkeys in and around the forested areas of central Grenada

Table 2 shows the group abundance and size in the different transects sampled. This preliminary assessment of the spatial distribution of mona monkeys in the study area showed statistically significant local differences in the mean numbers of encounters per km walked (Chi-square goodness-of-fit test: $\chi^2 = 78.67$, df = 8, p < 0.001) and individuals detected per km walked ($\chi^2 = 658.02$, df = 8, p < 0.001). With regards to the mean number of encounters/km, the lowest values were

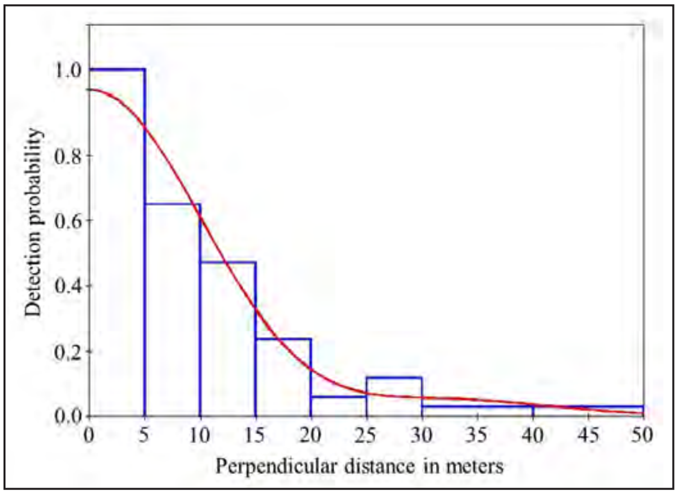

Figure 3. Histogram showing the detection probability as a function of the perpendicular distance from the transect line (interval distances), as generated by the analytical program Distance 6.0. The curve represents the detection function obtained with the detection function model that best fit the data.

Table 2. Cumulative and mean numbers of encounters and individuals detected in the different transects sampled ([a] encounters include groups and solitary individuals).

Transect	Total number of encounters[a] (cumulated over visits)	Mean number of encounters[a] per km	Total number of individuals detected (cumulated over visits)	Mean number of individuals detected per km	Mean ± SD (min-max) number of individuals detected per encounter[a]
T1	3	0.27	33	3.00	11.0 ± 1.7 (10-13)
T2	5	0.20	9	0.35	1.8 ± 0.4 (1-2)
T3	5	0.28	37	2.06	7.4 ± 3.6 (2-11)
T4	10	0.35	68	2.39	6.8 ± 5.5 (1-15)
T5	5	0.19	30	1.15	6.0 ± 4.8 (1-11)
T6	8	0.38	67	3.21	8.4 ± 2.7 (3-11)
T7	2	0.11	2	0.11	1.0 ± 0.0 (1-1)
T8	3	0.27	37	3.36	12.3 ± 0.6 (12-13)
T9	5	0.35	53	3.68	10.6 ± 6.2 (2-15)
Total	46	0.27	336	1.94	7.3 ± 4.8 (1-15)

found on transects T7, T5, and T2, and the highest values on T4, T9, and T6 (Table 2). With regards to the mean number of individuals detected/km, the lowest values were found on transects T7, T2, and T5, and the highest values on T6, T8, and T9 (Table 2).

We also found significant local differences in the estimated mean group sizes across transects ($\chi^2 = 16.55$, df = 8, p = 0.035). The smallest group sizes were recorded on T7 and T2, and the highest group sizes were recorded on T9, T1, and T8 (Table 2).

Effect of potential anthropogenic disturbance on the encounter rate

During our transect walks in the forested areas, we recorded 20 spots or artefacts of potential anthropogenic disturbance (e.g., indirect evidence of hunting activity as inferred by fresh dog footprints and recently discarded empty shell cases; four spots/artefacts on T3, five on T4, five on T5 and six on T6). The occurrence of such signs of anthropogenic disturbance significantly decreased the encounter rate on the same day (Mann-Whitney U test: $z = -3.32$, $p = 0.001$). We never encountered mona monkeys on a trail where we found indirect evidence of recent hunting activity.

It is also noteworthy that the temporary presence of the military in the Grand Etang National Park, including soldiers setting up camp around the T2 and T4 transects for about 10 days during the study period, resulted in a lower encounter rate in this area during this particular sub-period (mean number of encounters/km during the military presence: 0.15, versus 0.31 before the military presence, and 0.38 after the military presence). However, the difference in the encounter rate across these three sub-periods was not statistically significant (Kruskal-Wallis test: $\chi^2 = 4.3$, df = 2, p = 0.114).

Discussion

With regards to the demography of the mona monkeys in central Grenada, all the estimated values provided by the current study were markedly lower than those found 20 years before by Glenn (1998): (1) the estimated group density was 0.7 group/km² in 2014 versus 2.3 groups/km² in 1995, that is a 70% decrease; (2) the estimated individual density was 6.0 individuals/km² in 2014 versus 42.1 individuals/km² in 1995, that is a 86% decrease; and (3) the projected island-wide total population estimate was 289 individuals in 2014 versus 2,021 individuals in 1995, that is a 86% decrease. Moreover, the estimated mixed-sex group size was 10.3 individuals/group in 2014 versus 18.0 individuals/group in 1995 (Glenn 1997), that is a 43% decrease. Overall, the direct comparison of our current data with findings obtained 20 years before in the same area and with the same sampling method leads to the conclusion that there has been a marked decrease in the group and individual densities, group size, and overall abundance of the mona monkey population between these two points in time.

The decrease in group size of non-habituated mona monkeys over the past 20 years, also confirmed by long-term forestry staff (Kester Charles, pers. comm.) is particularly alarming. The current average size of mixed-sex groups is also lower than that estimated at Lama Forest, Benin (13.3 individuals/group: Matsuda Goodwin 2007). In guenons (including mona monkeys), there may be a minimum group size—defined as the minimum number of individuals in a group necessary for continued functioning of the group—below which the entire population may be at risk of extinction (Young and Isbell 1994).

Despite the claim by the forestry staff that the recent decrease in primate encounters and group size was not seasonal (Kester Charles, pers. comm.), we cannot definitely rule out any seasonal effect. Indeed, our survey was conducted during the dry season, with relatively low fruit availability in the montane forest areas (Glenn 1996). It could be argued that several groups of monkeys had moved from the Grand Etang National Park to lower elevations to find more abundant food patches. However, our results on the spatial distribution of mona monkeys did not show a clear pattern of higher densities in cultivated areas than in the montane forest (Table 2).

On September 4, 2004, Hurricane Ivan devastated most of the island of Grenada, including the forested areas of the Grand Etang National Park and Forest Reserve that were completely flattened (Levy 2005; Glenn and Bensen 2008). Even though no demographic study has been conducted in the immediate aftermath of Hurricane Ivan, it is likely that the majority of the mona monkey population was wiped out during the storm (Glenn cited in Levy 2005). In the weeks following this natural disaster, there were many reports of hungry mona monkeys being driven into the streets of mountain villages and sometimes accidentally run over by cars or shot to prevent them from stealing food (Levy 2005). The combination of massive die-offs following Hurricane Ivan and the forced movement of the surviving monkeys into unfamiliar and dangerous areas may have put severe restrictions on the viability of the mona monkey population in Grenada.

Previous studies have shown that local habitat devastation associated with powerful tropical cyclones generally lead to population reduction, decrease in group size, social disorganization, and behavioral changes in ranging and foraging activities in nonhuman primates (Dittus 1985; Pavelka *et al.* 2003). Even though these dramatic effects are generally temporary (Pavelka *et al.* 2003), any concomitant hunting pressure could put primate populations at increased risk of extinction (Young and Isbell 1994).

Even though a two-year hunting moratorium was decided by the Wild Game and Conservation Association of Grenada in the aftermath of Hurricane Ivan, its implementation and effect were impossible to assess due to the lack of systematic wildlife monitoring on the island (Michael Sanderson, pers. comm.). According to the Forestry Department, the mona monkey hunting season is typically open from October through December and only outside the Grand Etang National Park. Former and current hunters, however, harvest mona monkeys throughout the year, and there are no guards to monitor the protected areas in Grenada (Glenn 1997). Although we did not record any direct evidence of hunting, we recorded a series of indirect indications for a persistent hunting pressure, including in the protected areas of the Grand Etang National Park and Forest Reserve: (1) fresh dog footprints and recently discarded empty shell cases in the protected forested areas, (2) accounts from several interviewed hunters, who often team up and mainly use shotguns to kill monkeys of all age and sex classes, and occasionally use dogs to retrieve dead individuals, and (3) the recent account of the killing by a hunter of a free-ranging but resident male mona monkey around Annandale Falls (Row Murrell, pers. comm.). Moreover, our results showed that hunting artefacts were associated with a decrease in the mona monkey encounter rate. In line with the considerable hunting pressure on the mona monkeys in the mid-1990s (Glenn 1997), two of the hunters we interviewed confirmed that the harvest rate was substantial and were concerned about the viability of the monkey population in Grenada in the near future. However, with few hunters interviewed, much caution is required when discussing a trend in hunting over time.

In Grenada, there is no subsistence hunting of mona monkeys, nor any organized monkey meat market. However, monkey meat is considered a delicacy by some Grenadian people and often consumed in abundance during special celebrations in a few local restaurants and at regular "wild meat fairs" in Black Bay Beach, West Grenada (Kester Charles, pers. comm.). Inextricably linked to the bushmeat trade is the sale of orphaned mona monkeys as pets, which has recently become a major concern in Grenada (Michael Sanderson, pers. comm.). Many wealthy residents and foreign students acquire young monkey pets (sold for up to US$1,100 and advertised via flyers in touristic areas; N. Gunst, pers. obs.), which is legally allowed on the island. As part of the exotic pet trade, mona monkeys are also exported from Grenada upon the agreement of the Forestry Department (Anthony Jeremiah, pers. comm.). Such a frequent demand for young monkey pets had led several local hunters to specialize in killing adult members of a group in order to catch a live infant (Michael Sanderson, personal communication).

Overall, the current population decrease is probably the consequence of the ecological devastation of Hurricane Ivan in 2004, combined with persistent over-hunting. To better monitor the population dynamics of the mona monkeys in Grenada, we urge for the replication of the same study design in the same area, at least every five years. Although this primate species is able to cope with a certain degree of habitat disturbance and appears to be resilient to hunting (Linder and Oates 2011), it is not clear whether this primate population can sustain such a dramatic decline. Introduced free-ranging animals can be grouped into three categories, depending on whether their local biological and socio-economic effects are deemed mainly negative, positive, or neutral/unknown (Schlaepfer *et al.* 2011). Until a quantitative cost-benefit analysis is conducted on the mona monkeys of Grenada, they should be grouped into the third category before contemplating any population management plan. Considering the wealth of scientific information derived from small inbred insular populations, "it may not be a lost cause to conserve very small populations of forest primate species in habitat fragments" (Glenn and Bensen 2013; p. 413).

Acknowledgments

The field work of this study was funded by the American Institute of Bisexuality, and we thank John Sylla for his support. Additional funds were provided by the Natural Sciences and Engineering Research Council of Canada, the Leakey Foundation, and the Office of the Dean of Arts at the University of Lethbridge. In Grenada, we thank the Department of Forestry for their collaboration: Anthony Jeremiah, Kester Charles, and Raldon Thomas. We thank the staff at the Grand Etang National Park: Alfred Mark, Patricia Pierre, Ashley Abraham, Pat. Paterson, Leslie Debelotte, Angela Welch, and Benjamin Hannibal. We thank Telfor Bedeau, Bruno and Iris Delvallée, Anne-Sophie Duval, Pierre-Luc Gagnon (CIRADD, Canada), Ernest Henry John, Terence McPhail, Row Murrell, Lambert Rechea, Michael Sanderson (Wild Game and Conservation Association), and Tom Sanderson. We are grateful to the Honourable Minister of Tourism, Alexandra Otway-Noel. We thank Anthony Rylands and two anonymous reviewers for their insightful comments on a previous version of the manuscript.

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Received for publication: 10 June 2015
Revised: 27 February 2016

Primate Conservation 2016 (30): 15–20

First Record of *Lagothrix flavicauda* on the Eastern Side of the Río Huallaga: An Expansion of its Known Geographic Distribution

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Abstract: In this report we present the first record of *Lagothrix flavicauda* in montane forests on the eastern side of the Río Huallaga. The record of five groups occurred during primate censuses between the villages of Monopampa and Libertad, along the horseshoe that connects the basins of the ríos Huallaga and Pozuzo. With this record, the known distribution of *L. flavicauda* is extended to the southeast, up to the boundary with the Región Pasco. Five other primates, among them *Lagothrix lagothricha tschudii*, occur in the forests there, at elevations above 1900 m above sea level. As in other parts of the Región Huánuco, deforestation is the main threat for the primates and the wildlife in general.

Key words: Primates, Andean montane forest, *Lagothrix flavicauda*, new record, distribution

Introduction

The Peruvian yellow-tailed woolly monkey (*Lagothrix flavicauda*), one of the world's 25 most endangered primates (Mittermeier *et al.* 2012), is the only species that has been studied in Peru's montane forests. The studies are as yet insufficient, however, and not least regarding its geographic distribution, occurrence and causes of seasonal migrations, sympatric primate species and competition for food resources, and comparative population densities in its range, all aspects that are important for the conservation of this primate and others that inhabit the montane forests.

A number of studies of *L. flavicauda* have been carried out in the Región Amazonas, all in relatively restricted areas, and oriented towards behavior and food resources (Leo Luna 1980, 1982, 1987; Cornejo 2008), threats (Leo Luna 1984), activity budgets and behavior patterns (Shanee and Shanee 2011), population densities (Leo Luna 1982, 1987; Shanee and Shanee 2011; Cornejo 2007; Aquino *et al*. 2015), and its geographical distribution (Shanee *et al*. 2008; Shanee 2011). Aquino (2006) was the first to report the occurrence of *Lagothrix flavicauda* in the Región Huánuco, on the right bank of the Río Crisnejas, boundary with the Región San Martin. One group was seen and no exploration or censuses were carried out to the south to delimit their geographic distribution. Aquino and Ramos (2010) believed that the distribution of *L. flavicauda* southwards could reach the microbasin of the Río Chinchao and would include montane forests of the ríos Monzón, Patuy Rondos and Carpish. Shanee (2011) considered its range in Huánuco to cover the montane forests from the boundary with the province of Tocache (Región San Martín) to the Río Magdalena, whilst Aquino *et al*. (2015) considered the montane forests on the left bank of the Río Alto Huallaga as the probable limit to its distribution to the south. Prior to this study, *L. flavicauda* was observed only in montane forests of the microbasin of the Río Chontayacu (Aquino *et al*. 2015), and recently in forest near the village of Alto Azul in the microbasin of the Río Santa Martha (Aquino *et al*. 2016). Based on this finding and interviews, Aquino *et al*. (2016) considered the montane forests of the left bank of the Río Alto Huallaga to be the limit of its distribution to the south. There were still doubts, however, about the limit of its distribution southeast of Huánuco, right bank of the Río Huallaga. During interviews carried out in September 2010 (R. Aquino, pers. obs.) in the village of Muña, on the right bank of the Río Huallaga, we were informed of the existence of a monkey known as "coto negro" living in the highest elevations of montane forests between Monopampa and Río Pozuzo, the phenotypic characteristics of which, as described by those interviewed, corresponded to *L. flavicauda*.

This motivated us to conduct this study to (1) confirm the existence of this species between the ríos Alto Huallaga and

Pozuzo, (2) to determine the current status of its populations and the limit of its distribution to the southeast, and (3) record the primates with which it shares its habitat. It is appropriate to mention that we have no knowledge of any biodiversity studies conducted for this part of the Región Huánuco. It is certainly rich in both plants and animals, many of them possibly endemic and new to science. We conducted transect censuses from 5 to 29 April 2016 between Monopampa and Libertad, and brief exploratory incursions and interviews from 6 to 21 May 2016 in forests near the villages of César Vallejo and Fujimori, located in the microbasin of the Río Tulumayo and the villages of Herminio Valdizán, Ugartechi, Río Azul and Cedropampa, all located near to the boundary with the Región Ucayali. In this report we present the results.

Methods

Study area

The study area comprises the montane forests between the basins of the ríos Alto Huallaga and Pozuzo (Fig. 1). The area is suffering deforestation for the cultivation of coffee, corn and other crops, as well as for cattle ranching, but there are still patches of relatively extensive primary forest with primates and larger mammals such as spectacled bears (*Tremarctos ornatus*) and dwarf brocket deer (*Mazama chunyi*). To search for *L. flavicauda* and other primates, we used mules to travel along the horseshoe that connects the basins of the ríos Huallaga and Pozuzo to the sectors defined for censuses. The horseshoe is about 35 km in length, and settled with seven small villages, including Monopampa, Oso Mayo and Libertad. These settlements served as references for the three survey sites in the section between Monopampa and Libertad: Pantano, Oso Mayo, and Palizada (Fig. 1). Patches of primary forest, moderately disturbed by timber extraction for house construction and livestock pens, predominated at Oso Mayo and Palizada. In Pantano, in the Monopampa sector, the vegetation was dominated by remnant forest patches heavily disturbed by selective logging for commercial purposes, and particularly for sale as firewood. We also interviewed people in the villages of César Vallejo, Fujimori, Herminio Valdizán, Ugartechi, Río Azúl and Cedropampa (all located north of the Huallaga-Pozuzo horseshoe) to inquire about the presence of *L. flavicauda*, and made short exploratory trips into forests nearby, to define the limits of its distribution northward.

Distance censused at survey sites

The purpose of the censuses was to find *L. flavicauda* and incidentally record the presence of other primates that share its habitat in this part of Amazonia. For censuses we used trails of loggers and settlers, and occasionally trails along the horseshoe between Monopampa and Libertad. For each survey site we walked between five and seven trails, with lengths that ranged from 1 to 2.5 km in length. Each trail was walked up to three times, from 07:00 to 16:00 h. Two teams of two observers simultaneously walked along the trails at an average speed of 0.4 km/hour. Every time a group of *L. flavicauda* or other primate was sighted we recorded group size (when possible), perpendicular distance from the transect to the first individual seen, height (in the vertical stratum of the forest), activity at the time of detection, and presence of juveniles and infants. In all, we walked 213 km, corresponding to 532 hours of censusing (Table 2).

Results

Groups registered

We saw five groups during the surveys (Table 3). Contact with the first group occurred on 11 April at 15:35 h. The group of 12 was seen in Oso Mayo, more than 2 km from our camp at 2,071 m above sea level. It was detected by the noise caused during travel. On seeing us, individuals shook the branches and emitted alarm calls. After a few minutes the group quietened down, and began to forage, allowing us to count the group members for approximately 45 minutes. When contacted, all the groups were approachable, and sometimes even challenged us, indicating that they had not been exposed to hunting (Fig. 2). Good counts of adults, subadults, and juveniles were achieved for only two of the groups—the one of 12 in Oso Mayo, and a second of 15 in Palizada. Each group had three dependent infants indicating that April–May was the breeding season. Persistent rain and thick fog limited our vision to about 30 m. Field guides assured us that groups could exceed 20 individuals, without counting infants. All the groups observed between Oso Mayo and Palizada, in the Chorropampa sector, were at elevations above 1,930 m above sea level (Table 3). We found no groups at Pantano.

Geographic distribution

In Huánuco, *L. flavicauda* inhabits the montane forests on both sides of the Río Huallaga; on the western side, its range extends from the Río Crisnejas, at the boundary with the Región San Martin, southwards to the left bank of the Río Alto Huallaga, and on the eastern side, from near the Río Alto Huallaga to the Río Pozuzo and from the Río Tulumayo in the north, to the boundary with the Región Pasco in the south (Fig. 3). It may also be inhabiting the montane forests of the regiones Ucayali and Pasco near the boundary with the Región Huánuco; something important to investigate. On the western side of the Río Huallaga, however, *L. flavicauda* is locally extinct over a large part of its range, the main cause being the destruction of its habitat for agriculture and cattle-ranching. On the eastern side of the Huallaga, this primate is still present almost throughout its range there.

Sympatric primates

In addition to *L. flavicauda*, we registered the Peruvian woolly monkey (*Lagothrix lagothricha tschudii*), the black spider monkey (*Ateles chamek*), red howler (*Alouatta seniculus*), the large-headed capuchin (*Sapajus macrocephalus*), the Marañón white-fronted capuchin (*Cebus yuracus*), and the night monkey (*Aotus* sp.). All but *A. chamek* were found above 1,900 m; sharing as such the habitat with *L. flavicauda*,

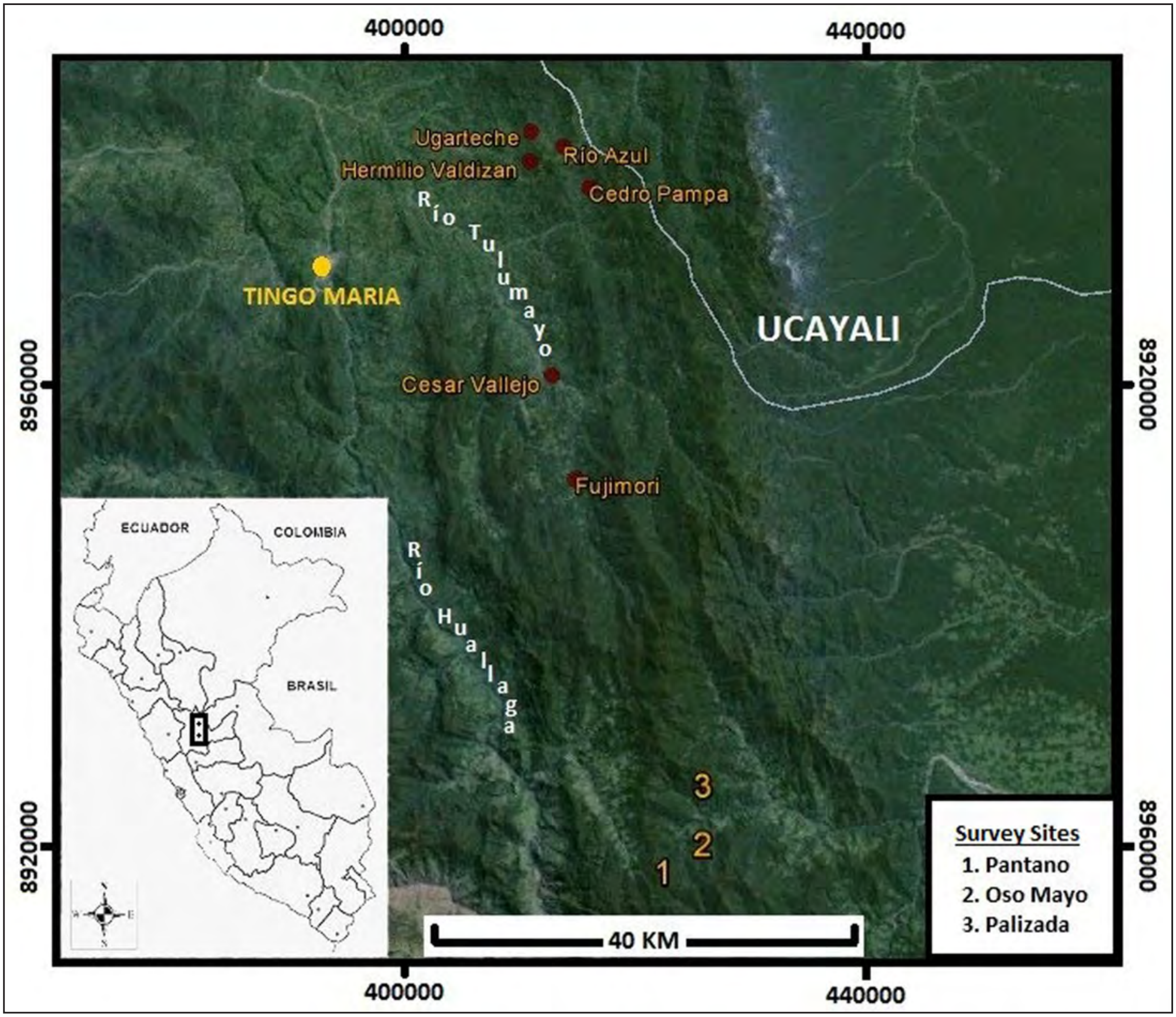

Figure 1. The survey sites and villages where interviews and brief explorations were conducted.

Table 1. Surveys sites for *L. flavicauda* between the basins of the ríos Alto Huallaga and Pozuzo.

Sectors	Survey sites*	Coordinates (UTM)	Altitude (m.a.s.l.)	Human disturbance level
Monopampa	1. Pantano	421584 / 8917132	2196	Very high
Valle de Chorropampa	2. Oso Mayo	425542/ 8921294	2079	Moderate
	3. Palizada	425527 / 8924368	1705	Moderate

*Numbers correspond to the survey sites in Figure 1.

while *A. chamek* was observed only below 1,500 m above sea level. With the exception of *C. yuracus*, all other species were recorded just once, which would indicate a low abundance in forests where they share the habitat with *L. flavicauda*. It would be appropriate, however, to conduct surveys in this area in the dry season, to investigate any seasonal differences in abundance.

Activities that constitute threats

The main threat in the region is deforestation for agriculture (Fig. 4) and cattle-ranching, affecting forests along the horseshoe between Monopampa and Río Pozuzo, up to 1 km either side of the horseshoe. The collection of firewood and material for house construction also results in deforestation (Fig. 5) that for now is concentrated in the Monopampa sector, including the Pantano survey site where the road ends. In this sector, patches of residual forest are severely degraded by intense deforestation for the purposes outlined above, the firewood and timber being transported to the towns of Chaglla and Panao to be commercialized in markets and to supply bakeries. Hunting is very sporadic and evidently does not seriously affect *L. flavicauda*. The species hunted are *S. macrocephalus* and *C. yuracus*, and sometimes *Tremarctos ornatus*, that enter the cornfields to feed on immature corn known as "choclo." They are repelled with firearms, and sometimes killed.

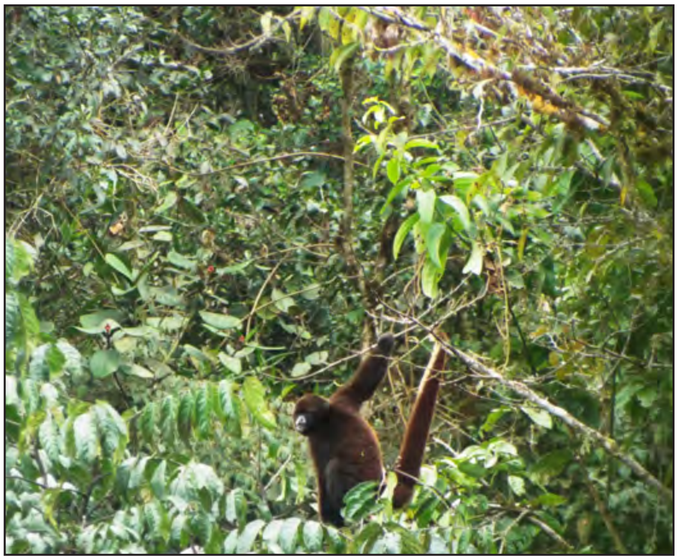

Figure 2. Adult male of *Lagothrix flavicauda* observed in the Oso Mayo survey site at 2071 m above sea level.

Table 2. Length walked (km) and hours of censusing in each survey site in the study area.

Sectors	Survey sites	Distance walked (km)	Effort (hours censused)
Monopampa	Pantano	40	100
Valle de Chorropampa	Oso Mayo	74	185
	Palizada	99	247
Total		213	532

Discussion

This expansion of the distributional limits of *L. flavicauda* in southern Huánuco takes it range beyond that proposed by Shanee (2011), who indicated the Río Magdalena as the probable limit southward, and also the montane forests of the left bank of the Río Alto Huallaga, as was proposed by Aquino *et al*. (2016).

With the exception of *L. flavicauda*, the species composition of these forests was similar to those reported by Aquino *et al.* (2015) for Miraflores and Santa Ana, both located on the right bank of the Río Alto Huallaga, but was much higher than those in the other census sites, including premontane and montane forests of the regiones San Martin, Huánuco and Amazonas registered by Shanee *et al*. (2013). The absence of *L. flavicauda* between Miraflores and Monopampa (Aquino *et al*. 2015) and the scarcity of *A. chamek* between Monopampa and Libertad (this study) could be related, among other factors, with competition for habitat and food resources. Indeed, there are marked differences in the number of groups observed between the two study areas with similar characteristics in terms of sampling effort; thus, between Miraflores and Santa Ana we reported eight groups of *A. chamek* and none of *L. flavicauda* (Aquino *et al.* 2015), whereas in this study we observed five groups of *L. flavicauda* and only one of *A. chamek*. Another characteristic to note is that all groups of *L. flavicauda* in the study area were observed above 1,900 m, coinciding with the information of the people living in this part of Huánuco, who told us that this primate, known there as "coto negro," lives in the highest forested elevations in the mountains, while from 1,900 m down they are occupied by *A. chamek* and *L. lagothricha tschudii*, potential competitors of *L. flavicauda*, and that are absent on the left bank of the Río Huallaga, which would explain the presence of *L. flavicauda* even to 1,500 m, as indicated by the records in the microbasin of the Río Chontayacu, where all groups except one were observed between 1,572 m and 1,823 m.

The constant rain and dense fogs during the winter season (January to April) made finding groups of *L. flavicauda* and other primates difficult, due to the noise caused by wind and raindrops on the leaves of trees, and the poor visibility. For now, though, we can indicate that the populations of *L. flavicauda* and other primates that inhabit this part of Huánuco are relatively secure because they are not exposed to hunting and because the patches of primary forest are still relatively

Table 3. Groups of *L. flavicauda* observed between the basins of the ríos Alto Huallaga and Pozuzo.

Groups	Survey sites	Altitude m.a.s.l.	Activity	Group size	Observations
1	Oso Mayo	2071	Locomoción	12	+3 infants
2	Oso Mayo	2270	Locomoción	>8	
3	Oso Mayo	1930	Alimentación	>5	
4	Palizada	2161	Locomoción	>10	
5	Palizada	2010	Alimentación	15	+3 infants

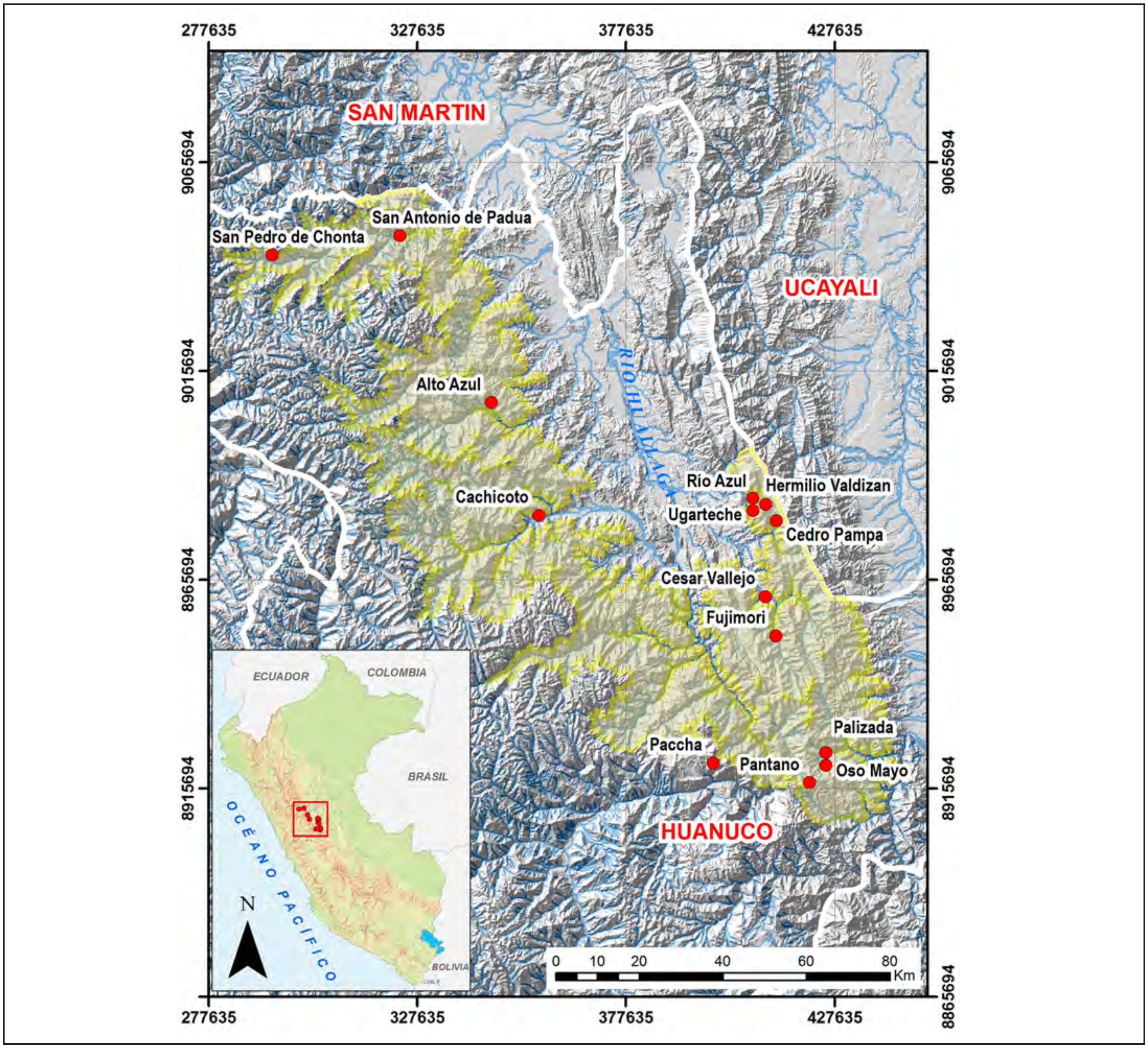

Figure 3. Geographic distribution of *Lagothrix flavicauda* in the Región Huánuco.

Figure 4. Forest replaced by coffee plantations in the Oso Mayo survey site, Sector Chorropampa.

Figure 5. Felling trees for commercial firewood, Sector Monopampa.

large, especially to the north where the largest population of *L. flavicauda* and other species would be found. The situation for these primates could, however, change quickly because of plans to continue construction of the road to connect the basins of the ríos Alto Huallaga and Pozuzo. This would facilitate access by an increased number of settlers, and intensify deforestation for agriculture, livestock and commercial logging. One way to mitigate these threats would be to propose alternatives to the settlers such as the creation of community conservation areas, which could sustain ecotourism, and guarantee the existence of a high diversity of wildlife, including *L. flavicauda* and the other five primates, *T. ornatus*, *Mazama chunyi* and *Puma concolor* among the mammals, *Rupicola peruviana*, *Penelope montagni*, *Andigena hypoglauca* among the birds, and many other species of mammals, birds, amphibians, reptiles and molluscs, many of them probably endemic and new to science.

Acknowledgment

We are grateful to The Mohamed bin Zayed Species Conservation Fund for the financial support that allowed us to continue the inventory and primate assessment northward and southeast of the Región Huánuco. We also thank the Universidad Nacional Mayor de San Marcos for the material support and field equipment. We are grateful to our guides of the village of Monopampa for their dedication in searching for primates in heavy rain and thick fog. Also, our thanks to Pedro Pérez for putting together the map, and a special thanks to Anthony Rylands for improving and polishing the English text.

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Submitted for publication: 1 June 2016
Revised: 19 September 2016

Primate Conservation 2016 (30): 21–29

Conservation Status and Threats to Atelids in the Northeastern Peruvian Amazon

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Abstract: Of the seven species of atelids occurring in Peru, three are present in the northeast of the Amazon; one of them, *Ateles belzebuth*, is listed as Endangered on the IUCN Red List of Threatened Species. These primates are preferred game, but detailed information on group size, population density and conservation status is scarce. We conducted transect censuses in forests with different levels of human disturbance (low, moderate and high), and also surveyed forests along the northern and southern banks of the Río Marañón. We recorded 232 groups of three species along 2,126 km of transect censuses. Group size and population densities of *Lagothrix lagothricha poeppigii* and *Alouatta seniculus* were lower in forest where human disturbance was ranked as high, compared to forests of low and moderate human disturbance. *Ateles belzebuth* had been extirpated in the area of high human disturbance. Besides hunting, increasing deforestation is a major threat to the survival of atelids in the northeast of the Peruvian Amazon.

Key Words: Atelids, northeast Amazon, conservation status, threats

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Introduction

Primates play an important role in forest regeneration since they are key pollinators and seed dispersers besides contributing to insect control. They are also predators and prey, and decreases in population numbers can lead to ecological impacts such as altering the floristic composition. They have economic importance, because the natives and mestizos who live along the riverbanks depend on primates and other wildlife for food and even some additional income from the sale of pets, the most in demand being infants of spider monkeys (*Ateles*) and woolly monkeys (*Lagothrix*), and small species such as pygmy marmosets *Cebuella pygmaea*, tamarins (*Saguinus*) and squirrel monkeys (*Saimiri*).

Six ateline taxa occur in the Peruvian Amazon (Aquino and Encarnación 1994; Groves 2001; Aquino *et al*. 2015). They are highly sensitive to habitat disturbance and fragmentation (Cowlishaw and Dunbar 2000). Even small-scale disturbance such as seismic exploration preceding actual oil drilling may affect their populations (Kolowski and Alonso 2012). They are also the monkeys most hunted for subsistence and commercial markets (Bodmer *et al*. 1997, 1999; Aquino *et al.* 2000a; Peres 2000), and overhunting has resulted in *Ateles belzebuth* being classified as Endangered on the IUCN Red List of Threatened Species (IUCN 2015). It has already been extirpated in some microbasins and sub-basins (Aquino *et al*. 2013a).

Several inventories and assessments of the status primates have been conducted in the Peruvian Amazon, most of them along the larger river basins (Neville *et al*. 1976; Freese *et al*. 1982; Castro *et al*. 1990; Encarnación and Castro 1990; Aquino *et al*. 2000a, 2009, 2013a; Aquino 2005), but virtually no surveys to determine the status of primate populations have been carried out to the north of the ríos Amazonas and Marañón. The exceptions are the microbasins of the ríos Curaray (Heymann 2000; Heymann *et al.* 2002; Colowski and Alonso 2012; Aquino *et al*. 2013a), Pucacuro (Aquino *et al*. 2000b), and Alto Itaya (Aquino *et al*. 2009). The scarcity of information on primates, particularly atelids in the northeastern Peruvian Amazon, motivated us to conduct this study. Our objectives were oriented to determining the current status of these primates and identifying threats to the survival of their populations. In this report, we present our findings for a series of expeditions between 2004 and 2013 in different areas north of the Peruvian Amazon.

Methods

Expeditions

Transect censuses were carried out along the following rivers: the Río Baratillo, tributary of the Río Pucacuro from May to June 2007; the ríos Curaray and Aguas Verdes, both tributaries of the Río Napo, from November to December 2012; and the ríos Huanganayacu, Alto Itaya and Alto Nanay from February to June 2013. These sites are located north of Río Marañón and west of the ríos Amazonas and Napo. Detailed information on the geographical location, semi-quantitative estimates of the level of human disturbance at the sampling sites, and the distances traveled (km) are shown in Figure 1 and Table 1. According to the human disturbance level (hunting, deforestation, logging, harvesting of forest products, and exploration of hydrocarbons), forests of these six sampling sites were grouped into the following three categories.

Low human disturbance. Forests of the Río Curaray. There the main activity is logging, with camps and trails being found near the river bank. The trails (7–8 m wide and 200–300 m long) are used to transport the timber from the interior of the forest to the river edge. The area is also hunted, but it is occasional and only subsistence, and limited to riparian forest, and *Ateles* and *Lagothrix* are still tolerant of human presence.

Moderate human disturbance. Forests of the ríos Aguas Verdes and Baratillo. They are inaccessible during the dry season (June–December), so logging and hunting are restricted to the wet season (January–May). Hunting, logging, and harvesting of forest products are practiced only by the native communities.

High human disturbance: Forests of the ríos Huanganayacu, Alto Nanay and Alto Itaya. High forest disturbance is manifested by the presence of many trails and camps in use by hunters, loggers and harvesters of palm leaves and wild fruits. Empty cartridges are frequently found on the trails, and skulls and other bones, particularly of primates and rodents, litter the camps. Primates and other wildlife flee in the presence of humans. This level of disturbance is typical of a large part of the forests of the northeast of the Peruvian Amazon

A number of shorter expeditions were conducted in January and August 2006 along the ríos Pastaza, Morona and their tributaries such as Chuinda and Chapuli; from November to December 2009 on the Río Huallaga and its tributaries such as the ríos Paranapura and Aipena; and from October to November 2012 on the Río Marañón (for more details, see Figure 2 and Table 2). These expeditions allowed us to gather information on the conservation status of these primates, understanding that the indigenous communities of various ethnic groups settled in these rivers traditionally have a preference for hunting primates for subsistence, most particularly spider monkeys and howler monkeys.

Transect censuses

Four transects were set up at each sampling site. They ranged in length from 4.0 to 5.0 km. The censuses were carried out from 06:30 h to 12:00 h (outgoing) and from 14:00 h to

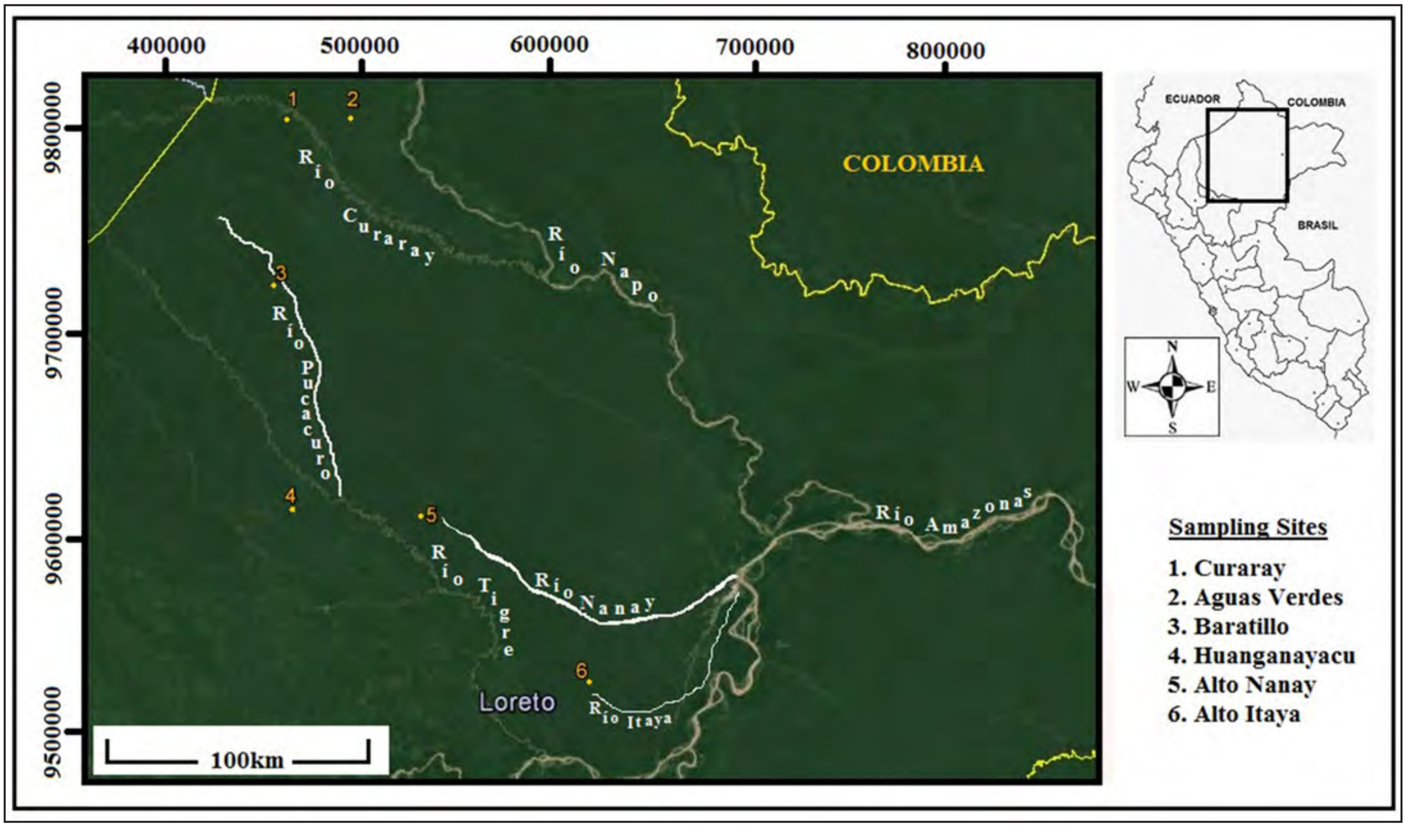

Figure 1. Location of the six sampling sites in the northeast of the Peruvian Amazon.

17:30 h (return). Two teams, each consisting of two observers (a researcher and a field assistant) walked different transects at an average speed of 1 km/hour. Each transect was walked three or four times. When primates were encountered, the following was recorded: the species; perpendicular distance to the transect of the first individual detected; number of individuals detected; height; activity; and the presence of dependent infants. Complete counts of group size were obtained whenever possible. A total of 2,126 km were walked; of them, 1211 km was in forest with high levels of human disturbance, 430 km in forest with low levels of disturbance, and 485 km in moderately disturbed forests (Table 1).

Data analysis

Excepting *L. l. poeppigii*, the low number of atelid groups observed in forests of the three levels of human disturbance was not sufficient to estimate population density by applying the software Distance (Laake *et al*. 1994). We used instead the formula D = N/2dL (Burnham *et al*. 1980; Peres 1999), where N is the number of groups found along the transects, L is the total length travelled (km), and d the mean perpendicular distance to the transect of the first animal sighted (different for each species). The number of individuals per square kilometer was then calculated by multiplying D by the mean group size. This method is widely used in assessments of wildlife

Table 1. Survey areas and sites for atelids in the northeast Peruvian Amazon.

Area	Site	Code	Coordinates (UTM)	Level of human disturbance	Total length of transect walked (km)
Ríos Curaray − Napo	Curaray	1	475731/9821084	Low	430
	Aguas Verdes	2	505540/9820535	Moderate	162
Ríos Tigre − Pucacuro	Baratillo	3	469723/9735776	Moderate	323
Ríos Tigre − Corrientes	Huanganayacu	4	473383/9627228	High	406
Ríos Tigre − Nanay	Alto Nanay	5	542669/9617188	High	403
	Alto Itaya	6	615679/9540649	High	402

Table 2. Additional sampling sites for atelids in the northeast of the Peruvian Amazon.

Area	Site	Code	UTMS Coordenates	Level of human disturbance	Type of evidence	Species[1]
Río Pastaza May−June, 2006	Andoas Viejo	1	342710/ 9678351	High	I	n.p.
	Huagramona	2	334891/9660952	High	I	n.p.
	Alianza Cristiana	3	334715/9617444	High	I	n.p.
	Santa María	4	326127/ 9582939	High	I	n.p.
	Puerto Alegre	5	330876/9578724	High	I	n.p.
	Puerto Pijuayal	6	336061/9570386	High	I	Llp, As
	Nuevo Ucayali	7	315775/9557531	Moderate	I	Ab, Llp, As
	Puerto Requena	8	304514/9533469	Moderate	O	Ab(2),As(1)
	Puerto Egipto	9	298431/9540596	Moderate	I	Ab ,Llp, As
	Nueva Yarina	10	284599/9531730	Moderate	I	Ab, Llp, As
Río Morona May−June, 2006	San Juan	11	206506/9662848	Moderate	I	Ab, Llp, As
	Kuzuime Chico	12	204642/9655460	Moderate	I	Ab, Llp, As
	Santa Cruz	13	210304/9646300	High	I	Llp, As
	Nueva Alegría	14	212402/9627396	Moderate	O	Ab(1), Llp(1)
	Caballito	15	2440277/9576092	High	I	Llp, As
	Unanchay	16	257772/9564232	High	O	Llp(2)
	Fernando Rosas	17	251742/9559806	High	I	n.p.
	San Salvador	18	254522/9562976	High	I	n.p.
	Fortaleza	19	240232/9532104	High	I	Llp
	Puerto Alegría	20	253964/9520796	High	O	As(1)
Río Marañón July, 2012	Borja	21	216955/9509472	High	O	As(2)
	Santa Rosa	22	211300/9489580	High	I	n.p.
Río Huallaga October−November, 2009	San Antonio	23	364985/9423516	Moderate	I	Llp, As
	Progreso	24	394986/9376145	High	I	n.p.
	Balsapuerto	25	327379(9355102	High	I	n.p.
	Canoa Puerto	26	323957/9350338	High	I	n.p.

[1] Numbers of groups observed are indicated in parentheses
n.p.= no presence; O = observation; I = interview
Ab =*Ateles belzebuth*; Llp = *Lagothrix lagothricha poeppigii*; As = *Alouatta seniculus*

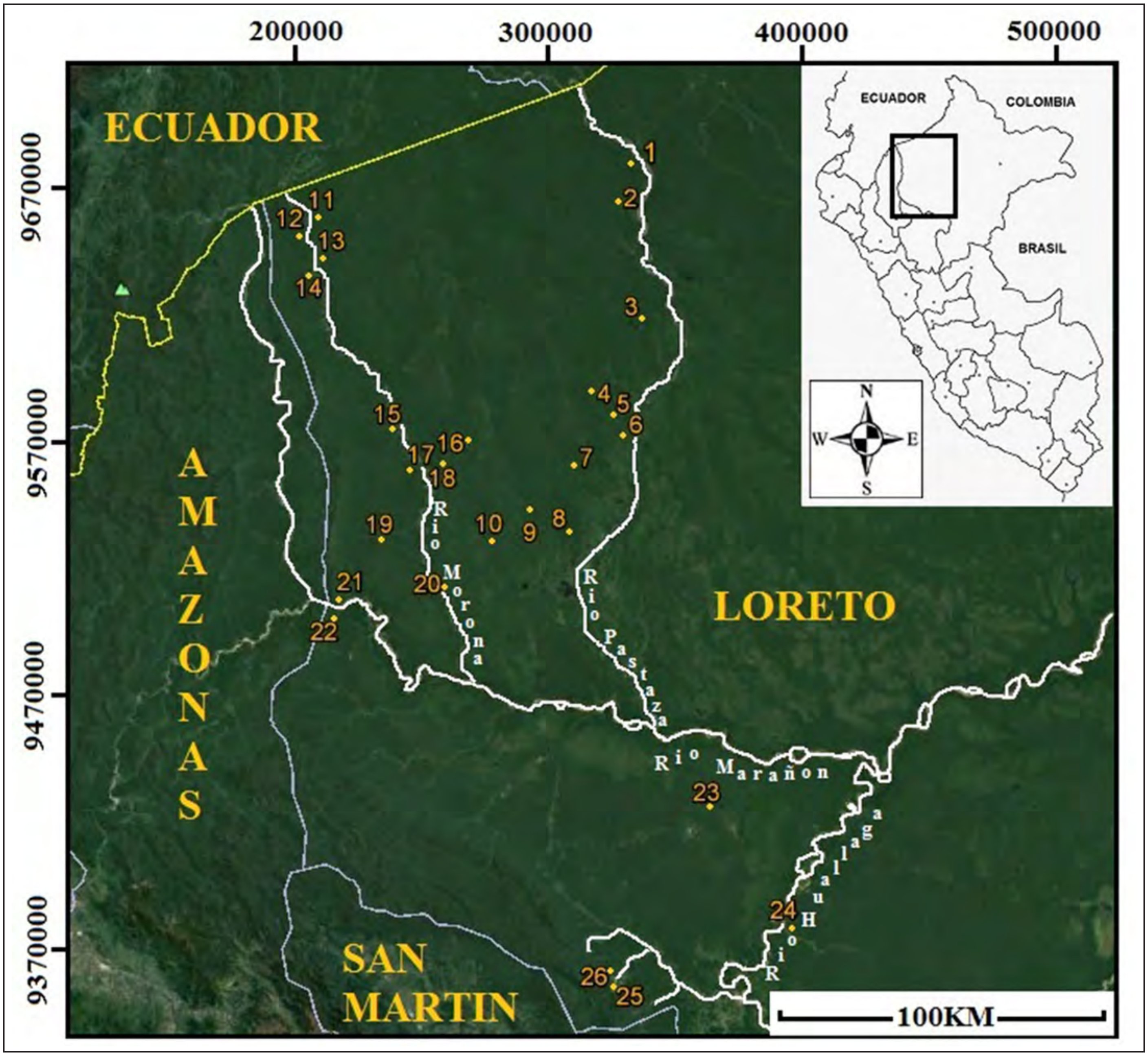

Figure 2. Location of the 26 additional sampling sites on the northern and southern banks of the Río Marañón.

with few records. We also calculated the relative abundance by the number of sightings per 10 km of transect walked. In order to examine the effect of human disturbance on group size, we compared the mean group size between forests of different levels of human disturbance with the Kruskal-Wallis test in BioStat 5.0.

Results

Species and groups recorded

In all 232 groups of the three atelid species—*Alouatta seniculus*, *Ateles belzebuth*, and *Lagothrix lagothricha*—were sighted during the 2,126 km walked along the transect surveys (Table 3). Ninety-eight, 41% of all sightings, were observed in forests with low human disturbance; just a little less than double the encounters in the forests with high human disturbance (54 groups or 24% of the groups sighted). *Lagothrix l. poeppigii* groups were seen 143 times, making it the most commonly recorded species in the study area. *Alouatta seniculus* was the least common, with only 43 groups, equivalent to 18.5% of the total. In forests with high human disturbance, *Ateles belzebuth* was not seen at all, while *A. seniculus* was seen only rarely; eight groups in total.

Group size and range

Variation in the range of group sizes and the mean group sizes for these three atelids are shown in Table 4, besides the results of the full group counts that we achieved. In general, larger groups were recorded in forests with low human disturbance. Comparing species, *L. l. poeppigii* was seen in larger groups that, depending on the level of human disturbance, varied from 9 to 23 individuals (mean size 16 individuals) in forests with low disturbance and between 8 and 13 (mean size 11 individuals) in forests with high human disturbance. The difference is statistically significant (0.0039, $p<0.05$). Group sizes in the forests with low and moderate levels of disturbance were not significantly different (0.13, $p>0.05$). *Alouatta seniculus* lives in smaller groups that varied from 6 to 11 (mean size 7 individuals) in forest with low human disturbance, and from 2 to 4 (mean size of 2.6 individuals) in forests with high human disturbance. This difference is also significant (0.0002, $p<0.05$), as was the difference in observed group sizes between forests with moderate and high disturbance (0.014, $p<0.05$).

Sighting rates and population density

Sighting rates for *L. l. poeppigii* and *A. seniculus* were low in the forest with high human disturbance, and up to four times higher in forests with low disturbance (Table 5). Similarly, the population densities for both species were low in forest with high disturbance, and higher in low and moderate human disturbance forests; true in particular for *L. l. poeppigii* with an estimated density of 49.6 individuals/km². *Ateles belzebuth* was not seen in forests with high disturbance, indicating local extinctions.

Table 3. Number (N) and proportion (%) of atelid groups observed in forests with different levels of human disturbance.

Disturbance level	Species			
	A. belzebuth	***L. l. poeppigii***	***A. seniculus***	**Total**
Low disturbance				
N	31	49	18	98
%	67%	32%	42%	41%
Moderate disturbance				
N	15	48	17	80
%	33%	35%	39%	35%
High disturbance				
N	-	46	8	54
%	-	33%	19%	24%
Total N	**46**	**143**	**43**	**232**

Table 4. Group sizes of atelids in forests with different levels of human disturbance.

Species	Range and mean group size according to the level of human disturbance			Group size range in other areas
	Low	**Moderate**	**High**	
Ateles belzebuth				
Range	6–18	2–11		2–11[a]
Mean size ± SD	12.2±5.5	4.2±2.8		
Total groups	31	15		
Groups with complete counts	5	11		
Lagothrix lagothricha poeppigii				
Range	9–23	12–18	8–13	5–24[b]
Mean size ± SD	16±5.6	16±3.4	11±2	
Total groups	49	48	46	
Groups with complete counts	6	5	10	
Alouatta seniculus				
Range	6–11	3–7	2–4	2–13[c]
Mean size ± SD	7±2.4	5.2±2.1	2.6±1	
Total groups	18	17	8	
Groups with complete counts	8	6	6	

Sources: [a]Río Samiria: Aquino and Bodmer (2006); [b]Río Itaya: Aquino *et al.* (2009); [c]Pacaya Samiria: Soini (1986).

Table 5. Sighting rates and population density estimates for atelids in forests with different levels of human disturbance.

Species	Encounter rate and population density according at each level of human disturbance			Other areas	
	Low	Moderate	High	Arabela	Samiria
Ateles belzebuth					
No of sightings/10 km of transect walked	0.7	0.3			
Groups/km^2	1.9	0.8			
Ind./km^2	23	3.6		13.3[a]	5.1[b]
Lagothrix lagothricha poeppigii					
No. of sightings/10 km of transect walked	1.1	1	0.4		
Groups/km^2	3.1	2.7	1.3		
Ind./km^2	49.6	44	14.3	30.6[a]	14.9[c]
Alouatta seniculus					
No. of sightings/10 km of transect walked	0.4	0.3	0.1		
Groups/km^2	1.4	1.6	0.3		
Ind./km^2	9.8	8.3	0.7	n.a.	16.1[c]

Source: [a]Kolowski and Alonso (2012), [b]Aquino and Bodmer (2006), [c]Aquino *et al*. (2001). n.a.= not available.

Conservation status and threats

In the Peruvian Amazon, atelid populations and other medium-sized primates are subject to constant threats from human activities, not only in lowland rainforest but also in submontane forest that is included in the ranges of some of the species. Where we carried out transect censuses, and in the other areas that we explored, we identified the following threats to the survival of these primates: hunting, deforestation for pasture and agriculture, logging, fruit harvesting, and the exploration of hydrocarbons. The levels of disturbance of each of these activities at the sites we visited varied according to the distance from the nearest towns.

Hunting. With the exception of the forests at the headwaters of the ríos Curaray and Nashiño (tributaries of the Río Napo), Tangarana, Baratillo, Corrientes and Macusari (tributaries of the Río Tigre), Chapuli, Chuinda and Sicuanga between the Ríos Pastaza and Morona, hunting is the main threat to the survival of atelids and other long-lived mammals which are favored as game. The absence of populations of *A. belzebuth* over a large part of the study area, and the very reduced numbers of *A. seniculus* can be ascribed to hunting. After *A. belzebuth*, *A. seniculus* is evidenlty most vulnerable to hunting because it has a habit of hiding in trees with abundant vines when alarmed, which allows the hunter to climb up the tree to kill the entire group. Hunting is related to logging; the logging teams spend extended periods in the forest living on game (Fig. 3).

Deforestation. This is the most pervasive and common threat in the area we surveyed. Deforestation is for agriculture, cattle-ranching and logging. Its impact is habitat loss and degradation (Fig. 4). Logging was more intense in the forests ranked as highly disturbed, where we had encounters with logging teams of more than 15 people during the censuses,

Figure 3. Adult male red howler *Alouatta seniculus* hunted for subsistence by the Brigade loggers in the Río Huanganayacu basin, April, 2013.

Figure 4. Logging road for towing commercially valuable timber in the Río Alto Itaya basin, March, 2013.

Figure 5. Seismic line used by hunters to access the forest in the Alto Nanay basin. June, 2013.

hindering our work because of the noise of chainsaws and falling trees. In the forests with moderate and low human disturbance, logging was modest and temporary, and we had no difficulties during the censuses.

Hydrocarbon exploration. This is the third major threat to atelid populations and those of other primates. The trails opened for Environmental Impact Assessments (EIA), and for seismic exploration, in particular in forests already with high human disturbance, contribute to increase hunting because they are used by hunters (Fig. 5). Air traffic, noise generation, presence of people, the construction of heliports, the installation and stocking of encampments, the opening of seismic lines, detonation of explosives, and numerous other activities related to Environmental Impact Assessments and seismic exploration undoubtedly generates temporary displacement causing intraspecific conflicts over home ranges, as well as changes in the behavior of the atelids, undoubtedly involving stress, as shown by the scared behavior of the groups of *A. belzebuth* and *L. l. poeppigii* contacted during the censuses, even in the forests with low human disturbance. This exploratory activity does not apparently cause major impacts, while a positive outcome that results in the mining of gas or petroleum is obviously disastrous for the local wildlife.

Harvest of wild fruits. Harvesting fruits is particularly serious for the frugivorous atelids. It is more common in forests with easy access and close to the villages. Fruits of *Couma macrocarpa*, *Rhigospira* sp. and *Parahancornia peruviana*, all of the family Apocynaceae, and the palms, *Mauritia flexuosa* and *Oenocarpus bataua* (family Arecaceae) are among the main food resources for atelids, as well as other primates, and these are also favored by the local people. They are in high demand in the markets of Iquitos, Nauta, San Lorenzo and others. Although generally quite abundant, people, against all good sense, cut them down to harvest their fruits and in the case of *Couma* to harvest the latex.

The absence of atelids, in particular of *A. belzebuth*, in much of the northeastern Peruvian Amazon is a consequence of human activities, principally hunting and deforestation. Currently the major populations of these primates remain in intact forests or those of low human disturbance that are either remote (headwaters of rivers) or difficult to reach (narrow and shallow rivers, and palm swamps known as *aguajales* in Peru). In the northeast, this conforms to a narrow strip along the border with Ecuador, with the headwaters of the ríos Nashiño and Curaray (tributaries of the Río Napo), Tangarana, Baratillo, Corrientes and Macusari (tributaries of the Río Tigre), Chapuli, Chuinda, Manchari, Huitoyacu, Siquanga, Anaza and Situche, between the ríos Pastaza and Morona, Cordillera Campanquiz between the ríos Morona and Santiago and the Río Aipena (tributary of the Río Huallaga). In the areas of the additional surveys, records of *A. belzebuth* near the indigenous communities of Puerto Requena and Nueva Alegría settled between the ríos Pastaza and Morona show that forests in the rivers mentioned above are still relatively well preserved and abundant in wildlife.

Discussion

The high percentage of records of *A. belzebuth* and *L. l. poeppigii* in the forests with low or moderate human disturbance indicates that these forests are still relatively well preserved; disturbed only by the occasional hunting and small scale, temporary logging of valuable timbers. They compare with the highly disturbed forests suffering from massive logging and overhunting. The absence of *A. belzebuth* is due to overhunting—among all Neotropical primates spider monkeys are the most sensitive and vulnerable to hunting. The second most vulnerable species in highly disturbed forest is *A. seniculus* due to its inclination to hide rather than flee when approached by humans, as already mentioned.

The mean sizes of the *L. l. poeppigii* groups in the low and moderately disturbed forests were similar to those estimated for the remote Sierras de Contamana where there is no hunting (Aquino *et al*. 2005), and likewise Alto Itaya, which when surveyed was regarded as only moderately disturbed (Aquino *et al*. 2009). All means were higher than the group sizes estimated in the highly disturbed forests reported here and of Pucacuro (Aquino *et al.* 2000b).

Regarding *A. belzebuth*, the mean group size estimated for the forests with low disturbance was higher than that for moderately disturbed forests in this study, as well in forests along the Río Pucacuro (Aquino *et al*. 2000b) and Río Samiria (Aquino and Bodmer, 2006), that are subjected to high hunting pressure. It was similar to the mean group sizes recorded for the forests of Curaray-Nashiño and Curaray-Arabela also considered to be little disturbed (Aquino *et al*. 2013a).

In the case of *A. seniculus*, the mean group size estimated for the forests with low human disturbance was higher than those for the moderate and highly disturbed forests; it was also higher than those reported for the ríos Urubamba and Tambo (Aquino *et al*. 2013b), but was much lower than the mean group sizes recorded in Pacaya-Samiria (Soini 1986) and *aguajales* in the Río Samiria basin (Aquino, 2005). These differences undoubtedly result at least in part from the different vegetation types surveyed. The censuses in the little disturbed forests were mostly conducted on terra firme and not in flooded forests such as *aguajales*, where howler monkeys densities tend to be higher because of the abundance of fruits of *Ficus* spp. (Moraceae) and *Mauritia flexuosa* preferred by howler monkeys in the flooded forests (Aquino 2005; Haugaasen and Peres 2005).

The population density of *A. belzebuth* estimated for the forests with low human disturbance was similar to estimates obtained for forests between the ríos Curaray and Nashiño and Curaray-Arabela (Aquino *et al.* 2013a), but higher than that reported for the Río Pucacuro (Aquino *et al.* 2000b), and Río Samiria (Aquino and Bodmer 2006), both subject to high hunting pressure, and Arabela-Tangarana (Colowski and Alonso 2012). In the case of Arabela-Tangarana it is possible that this results from the relatively short distance surveyed (less than 165 km). The lack of sighting in highly disturbed forest indicates local extinction by overhunting.

Estimated densities of *L. l. poeppigii* for forests with low human disturbance were higher than those of high human disturbance in this study and in the Río Pucacuro basin (Aquino *et. al*. 2000b) and Río Samiria (Aquino 2005) considered to be areas of high hunting pressure. They are also higher than the estimates for *L. l. tschudii* in hunted parts of the Manú National Park (Endo *et al.* 2012) and between the ríos Urubamba and Tambo (Aquino *et al*. 2013b); areas characterized by low hunting pressure, but marked by low fruit productivity that corresponds to a montane forest. Density estimates for this species in moderately disturbed forest in this study were similar to those reported for *L. l. lagothricha* in the Río Algodón basin (Aquino *et al*. 2007) where there is virtually no hunting due to its remoteness.

The population density of *A. seniculus* in little disturbed forest was higher than the density estimates for the Río Pucacuro basin (Aquino *et al.* 2000) and hunted sites in the Manú National Park (Endo *et al.* 2012), lower than that estimated for the Río Samiria (Aquino and Bodmer, 2006), and similar to the results for the ríos Urubamba and Tambo (Aquino *et al*. 2013b). The relatively low population density obtained in little disturbed forest is largely a result of the censuses being mainly on terra firme, where red howlers are naturally less abundant than in palm swamps and flooded terraces (Aquino 2005; Haugaasen and Peres 2005).

In the northeast Peruvian Amazon, atelid populations are threatened variably by human activities such as hunting (both subsistence and commercial), deforestation, logging and hydrocarbon exploration. The main threat is hunting, with obvious effects on population density and group size, particularly for *A. belzebuth* with low group (0.8 groups/km^2) and individual (3.6 indiv./km^2) densities even in moderately disturbed forest and its total absence in highly disturbed forest. The effect of hunting and the accompanying forest degradation on population densities is in line with findings for other atelids throughout Amazonia (Peres 1990, 2000; de Souza-Mazurrek *et al*. 2000; Haugaasen and Peres 2005; Nuñes-Iturri and Howe 2007). Atelid population density is also influenced, however, by habitat quality (Symington, 1988), and is evidently the case for the moderately disturbed forest, although the effect of hunting was obvious. There, the spider monkeys flee from people, the opposite occurs in little disturbed forests, where spider monkeys even approach people, dropping and throwing branches at them.

Acknowledgments

Our gratitude to The Mohamed bin Zayed Species Conservation Fund for the financial support that allowed us to assess the status of the atelids in northeastern Peru. We are also grateful to the Faculty of Forestry Science of the Universidad Nacional de la Amazonía Peruana and Instituto de Investigaciones de la Amazonía Peruana for allowing us to participate in the wildlife evaluation on the rivers Pastaza, Morona, Huallaga and tributaries. Our thanks to Conservation International for its support through the Primate Action Fund for expeditions to the ríos Marañón and Santiago. Our thanks to Idea Wild for the donation of essential field equipment, and to the Universidad Nacional Mayor de San Marcos for material support and field equipment. Our thanks to our local field assistants and the local authorities of the villages Santa Elena and 28 de Julio, Río Tigre. Special thanks to Anthony B. Rylands for improving and polishing the English text.

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Received for publication: 6 July 2016
Revised: 9 September 2016

Primate Conservation 2016 (30): 31–37

Distribution and Current Status of the Peruvian Yellow-tailed Woolly Monkey (*Lagothrix flavicauda*) in Montane Forests of the Región Huánuco, Peru

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Abstract: In this report we provide information on the geographical distribution and current status of the Peruvian yellow-tailed woolly monkey (*Lagothrix flavicauda*) and of other primates that inhabit the montane forests of the Región Huánuco. For this purpose we conducted transect censuses and interviews during the months of July, September and October 2015 and January 2016 in the montane forests of the ríos Chontayacu, Santa Martha, Monzón and Derrepente, and also in the upper basin of Río Huallaga. The results indicate that the southern distributional limit of *L. flavicauda* is the Región Huánuco in the montane forests on the left bank of the Río Alto Huallaga. During the survey, we also recorded 28 groups of five species of other primates, the most frequently observed of which was *Leontocebus leucogenys*. In all locations surveyed except for the montane forests between the ríos Chontayacu and Magdalena, populations of *L. flavicauda* and other primates are in the process of local extinction, principally due to intense deforestation for agriculture, cattle ranching and timber extraction, but also due to hunting.

Key Words: *Lagothrix flavicauda*, Andean montane forest, distribution, Neotropical primates, current status

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Introduction

The Peruvian yellow-tailed woolly monkey (*Lagothrix flavicauda*), more commonly known in Huánuco as *coto negro*, is one of the 25 most endangered primate species in the world (Mittermeier *et al*. 2012). This Peruvian endemic inhabits the montane forests of Amazonas, La Libertad, San Martín and Huánuco, but the limits to its geographic range in the south, and the primates with which it shares its habitat there, are still poorly understood, particularly in the Region Huánuco.

A number of studies have clarified the behavior and feeding (Leo Luna 1980, 1982, 1984, 1987; Cornejo 2008), activity budget and behavior patterns (Shanee and Shanee 2011). and geographical distribution (Aquino aqnd Encarnaciòn 1994; Shanee *et al*. 2008; Shanee 2011) of *L. flavicauda* in the Peruvian montane forests where it lives. Aquino and Ramos (2010) argued that the southern limit to the occurrence of *L. flavicauda* could reach the microbasin of the Río Chinchao, and included the montane forests surrounding the Río Monzón with the Patuy Rondos and Carpish forests close to Tingo Maria. In this regard, Shanee (2011) indicated that the limits might extend through the montane forests from the border of the province of Tocache (Región San Martin), south as far as the Río Magdalena. Aquino *et al*. (2015a), however, suggested that the probable limit of its range in the south would be the pre-montane and montane forests on the left bank of the Río Alto Huallaga.

In November of 2011, R. Aquino came across a juvenile female of *L. flavicauda* in the locality of Santa Rosa del Oso; its mother had been shot by a hunter on the right bank of the Río Chontayacu. This showed that primates in this part of Huánuco are subject to subsistence hunting or are hunted simply to obtain infants for sale as pets in local markets. Aquino *et al*. (2015a) observed 13 groups of *L. flavicauda* in montane forests near the community of Cocalito on the left bank of the Río Chontayacu, confirming that it occurs on both sides of the Río Chontayacu. The limit to its distribution in the south remained undefined, however.

The only references to other primate species inhabiting the montane forests of Huánuco are found in Shanee *et al*. (2013) and Aquino *et al*. (2015b). They reported the presence of four and six species respectively. The minimal information concerning abundance, group size and habitat conditions for *L. flavicauda* in many of the sites where it had been recorded inspired a plan of action with following objectives: (1) to determine its extent of occurrence in southern Huánuco;

(2) to determine the status of the species based on group size and abundance, and (3) to identify the locations with the largest populations. This information would prove important in the proposal of community managed conservation areas for the species, implementing, besides, ecotourism opportunities with local participation. To achieve these objectives, transect censuses were conducted during July, September and October of 2015, and in January of 2016 in forests close to the communities of the microbasins of the ríos Chontayacu, Santa Martha, Monzón and Derrepente, and in the upper basin of the Río Huallaga. The results obtained are presented in this report.

Methods

Study area

The montane forests of the Región Huánuco are in the central Peruvian Amazon. In this region, the forests have been subjected to intense deforestation for agriculture, cattle ranching, floriculture and timber extraction, leaving only "patches" of highly disturbed, degraded, remnant forests and moderately disturbed, more extensive, primary forest. Subsistence hunting and culling to control crop-raiding are also threats. Our exploration and censuses were conducted in these primary forest and remnant forest fragments in four microbasins and the upper basin of the Río Huallaga (Fig.1). The geographical coordinates, height and disturbance level of the survey sites are shown in Table 1. The sectors were as follows.

Microbasin of the Río Chontayacu. Deforestation has destroyed forests from the community of Santa Rosa del Oso upstream as far as the community of San Pedro de Chonta. Primary forest still exists further downstream, however, but it is suffering commercial timber extraction and subsistence hunting. Even though deforestation for agriculture is still moderate, there has been a notable increase since the end of 2012 with the opening of the Uchiza – Huacrachuco road. In this sector, the survey sites were placed near to the communities of San Antonio de Padua and Santa Rosa del Oso. No surveys were carried out in San Pedro de Chonta, only interviews.

Microbasin of the Río Santa Martha. The primary forest which exists at the headwaters of this river is being cut down for agriculture and the illegal cultivation of coca (*Eritroxilum coca*). Timber extraction has been facilitated by the road. The

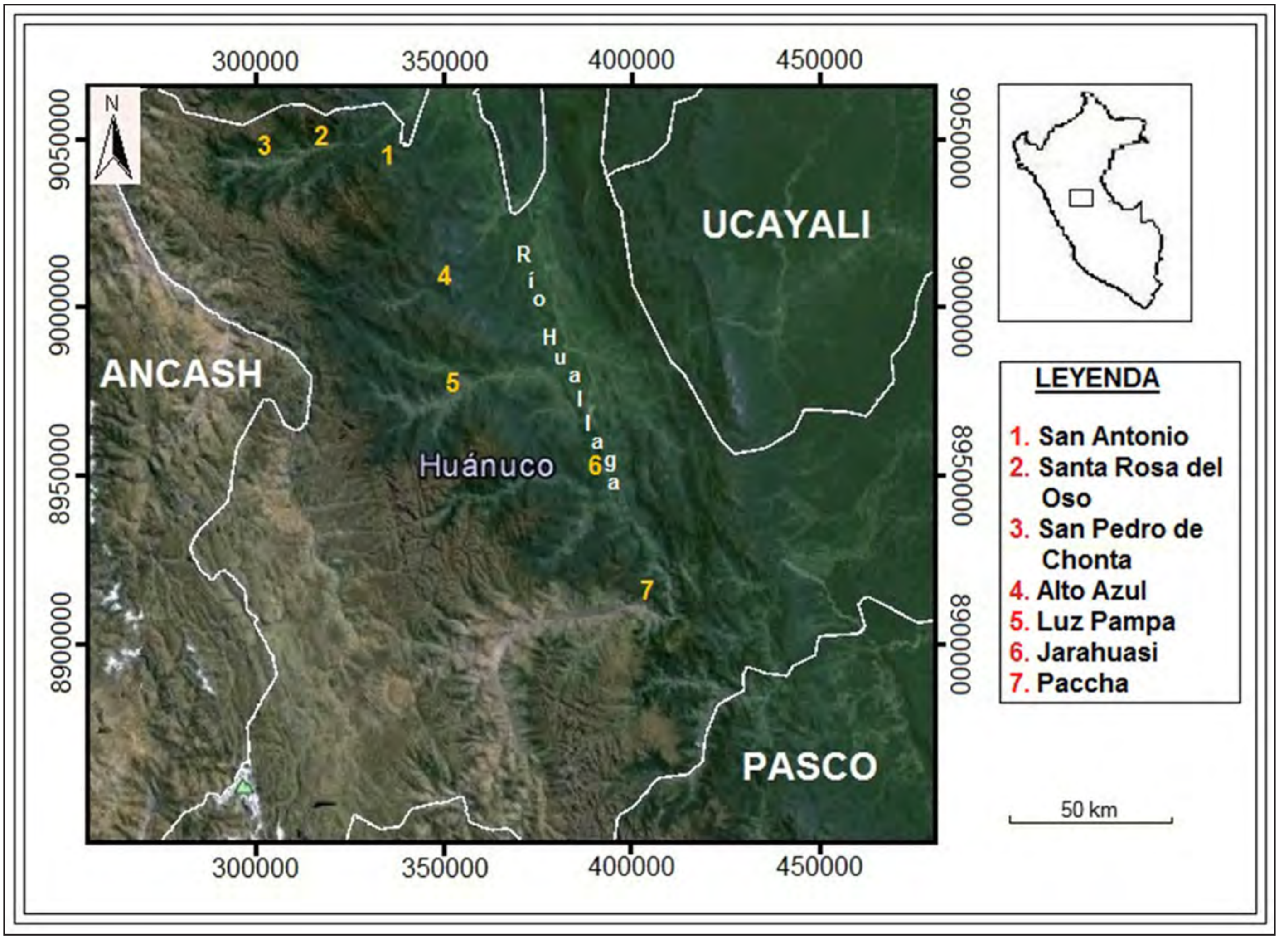

Figure 1. The survey sites for the inventory and status assessment of *L. flavicauda* and other primates.

survey site in this location was forest near to the community of Alto Azul.

Microbasin of the Río Monzón. In this sector the survey sites were placed in forest near to the community of Luz Pampa, on the right bank of the Río Monzón. There the patches of remnant forest were subject to constant timber extraction for the construction of corrals and cattle fences. The mammal fauna in general was very scarce as a result, and was mostly represented by small species such as, among the primates, the Andean saddle-back tamarin (*Leontocebus leucogenys*) and Andean night monkey (*Aotus miconax*).

Microbasin of the Río Derrepente. Access to this sector was possible only on foot and using pack mules. This survey site was near to the community of Jarahuasi, approximately 7 km from the confluence of the ríos Derrepente and Huallaga. Patches of remnant forest are what mostly remain in this sector, and primate numbers were relatively low. Some interviewees told us that there is still primary forest in good condition in the middle reaches of the microbasin, where larger primates could still be found.

Upper basin of the Río Huallaga. This survey site contained patches of primary forest near to the community of Paccha, on the left bank of the Río Alto Huallaga. There is intense deforestation for agriculture and timber extraction for local use. Wildlife is scarce due to the extent of disturbance in these forests. Some interviewees, however, claimed the presence of the spectacled bear (*Tremarctos ornatus*) and larger primates, including the red howler (*Alouatta seniculus*).

Transect censuses

The purpose of the transect censuses was to determine the relative abundance and population density of *L. flavicauda* and other primates. Censuses were conducted in primary forest near San Antonio de Padua, Santa Rosa del Oso, and Alto Azul, and in patches of remnant forest near Luz Pampa, Jarahuasi and Paccha. We used the paths and trails of farmers and hunters, and occasionally the roads of loggers. Each transect was walked up to three times. Following the guidelines established by Aquino *et al.* (2001), censuses were conducted from 07:00 to 16:00 h. Two teams of two observers walked different transects at a speed of about 0.6 km/hour. Each time a group of primates was found we noted the following: time, species, group size (complete counts when possible), perpendicular distance from the transect to the first individual seen, height (the vertical stratum of forest), activity at moment of detection, presence of infants and juveniles, and type of vegetation. The censuses were conducted on the transects in both directions (inbound and outbound). In all, we walked 402 km of transects equivalent to 663 census hours; of this total, 227 km were in the Río Chontayacu sector (Table 2).

Interviews

The main purpose of the interviews was to accumulate information about the presence of *L. flavicauda* to define the limits of its distribution in the south of Huánuco. Interviews were conducted in all survey sites and San Pedro de Chonta. To help with the identification, the interviewees were shown photographs of *L. flavicauda* and of other primates suspected to inhabit the survey sites. The questions focused on food resources (common plant names), fruit seasons, frequency of hunting, birth seasons and seasonal migratory movements, among others.

With the exception of those interviewed in San Antonio de Padua, San Pedro de Chonta and Alto Azul, all showed total ignorance of the existence of *L. flavicauda* and of other primates that inhabit their forests. Only two were able to differentiate between the howler monkey (*Alouatta seniculus*) and the so called "chocleros"—the large-headed capuchin *Sapajus macrocephalus*, and the Peruvian white-fronted capuchin *Cebus yuracus*.

Identification of threats

The activities which constitute threats were registered during the censuses and interviews. We asked about and investigated other activities related to deforestation apart from agriculture, including such as mining, logging, road construction, and hydroelectric projects. When visiting the communities, we also took the opportunity to search for primate pets, and for evidence of hunting, such as skins and skulls.

Data analysis

The number of primate groups recorded for each species in the censuses were not sufficient to calculate population density. For that reason we opted to calculate only relative

Table 1. Survey sites in montane forest of the Región Huánuco.

Survey sectors	Survey sites*	Coordinates (UTM)	Elevation (m.a.s.l.)	Human disturbance level
Río Chontayacu microbasin	1. San Antonio de Padua	334932/9047323	1650	High
	2. Santa Rosa del Oso	313423/9047511	1705	High
	3. San Pedro de Chonta	243480/9042651	2448	Very high
Río Santa Martha microbasin	4. Alto Azul	354469/9013143	1223	High
Río Monzòn microbasin	5. Luz Pampa	352080/8976150	1314	Very high
Río Derrepente microbasin	6. Jarahuasi	390646/8948682	1224	Very high
Río Huallaga upper basin	7. Paccha	403154/8919436	2415	Very high

*Numbers correspond to the points in Figure 1.

Table 2. Length (km) walked and censuses hours per survey site in the study area.

Survey sectors	Survey sites	Distance walked (km)	Effort (hours of census)
Río Chontayacu microbasin	1. San Antonio de Padua	129	215
	2. Santa Rosa del Oso	98	160
	3. San Pedro de Chonta	-	-
Río Santa Martha microbasin	4. Alto Azul	76	121
Río Monzón microbasin	5. Luz Pampa	34	57
Río Derrepente microbasin	6. Jarahuasi	38	65
Río Huallaga upper basin	7. Paccha	27	45
Total		402	663

Table 3. Survey sites and records of *L. flavicauda* and other primates.

Survey sectors	Census and interview sites	Species observed[1]	Species registered in interviews
Río Chontayacu microbasin	1. San Antonio de Padua	*Sapajus macrocephalus* (2) *Cebus yuracus* (1)	*Lagothrix flavicauda* *Aotus miconax* *Leontocebus leucogenys*
	2. Santa Rosa del Oso	*Lagothrix flavicauda* (3) *Alouatta seniculus* (2) *Sapajus macrocephalus* (3) *Cebus yuracus* (1)	*Aotus miconax*
	3. San Pedro de Chonta	No surveys carried out.	*Alouatta seniculus*
Río Santa Martha microbasin	4. Alto Azul	*Lagothrix flavicauda* (2) *Alouatta seniculus* (1) *Sapajus macrocephalus* (1) *Cebus yuracus* (2) *Aotus miconax* (2) *Leontocebus leucogenys* (3)	
Río Monzón microbasin	5. Luz Pampa	*Aotus miconax* (1) *Leontocebus leucogenys* (2)	*Sapajus macrocephalus* *Cebus yuracus*
Río Derrepente microbasin	6. Jarahuasi	*Cebus yuracus* (1) *Aotus miconax* (1) *Leontocebus leucogenys* (3)	*Alouatta seniculus* *Sapajus macrocephalus*
Upper Río Huallaga	7. Paccha	*Sapajus macrocephalus* (1) *Aotus miconax* (1)	*Alouatta seniculus* *Sapajus macrocephalus* *Cebus yuracus*

[1] Numbers in parentheses refer to the number of groups observed.

abundance—the number of groups encountered/10 km of transect.

Results

Distribution of L. flavicauda

The results obtained through direct observation and interviews conducted during this study, as well as from previous studies, allows us to affirm that the extent of occurrence of *L. flavicauda* in Huánuco comprises the montane forests from the Río Crisnejas on the boundary with the Región San Martín, southwards to the montane forests of the left bank of the Río Alto Huallaga (Fig. 2). On this particular, there is evidence to suspect that their distribution could expand towards the southeast of the Río Alto Huallaga, probably as far as the montane forests close to the boundary with the Región Pasco.

Current situation

The scarce five groups observed in this study (Table 3), and the low number of sightings (Table 4) indicate that in Huánuco *L. flavicauda* is absent over a large part of its former range. This is due to factors such as the destruction of forest for agriculture, cattle ranching, and the construction of roads and a hydroelectric dam, hunting and other activities. The few populations that still exist are mainly remnant groups and refugees in montane forests in the microbasins of the ríos Chontayacu and Monzón. There is also evidence of small groups existing in patches of primary forest in the microbasins of the ríos Chinchao and Derrepente, but they are in the process of local extinction due to the constant loss of habitat and decrease in food resources. These two factors are responsible for the migration of *L. flavicauda* from one place to another and for the appearance of *S. macrocephalus*, *C. yuracus*, *L. leucogenys* and *A. miconax* in fruit farms in search of food. These crop-raiders are repelled by shooting

Table 4. Estimated relative abundance of primates that inhabit montane forests of Huánuco.

Species	**Total length of transect walked (km)**	**Groups observed**	**Groups/10 km**	
			This study	**Aquino *et al*. (2015b)**
Lagothrix flavicauda	402	5	0.12	0.5
Alouatta seniculus	402	4	0.1	0.24
Sapajus macrocephalus	402	6	0.15	0.08
Cebus yuracus	402	5	0.12	0.1
Leontocebus leucogenys	94	8	0.85	-

them, which impacts their population numbers. According to the interviews conducted in the montane forests of Huánuco, *L. flavicauda*, *Ateles chamek*, and *A. seniculus* are the only species that do not enter farmland in search of food. In addition to migrating within a certain area, these species move between forest fragments. In order to do so they need to go to ground level, exposing themselves to dangers, including those imposed by humans.

Locations for the conservation of L. flavicauda *and threats*

The results obtained in this and previous studies indicate that the main *L. flavicauda* population is concentrated in the apparently well-conserved forests between Santa Rosa del Oso and Galicia in the Río Chontayacu microbasin and the upper reaches of the ríos Yanajanca, Santa Martha, Magdalena and Monzón. These forests are exposed to deforestation for not only agriculture and cattle ranching but also for urban and infrastructure development. A large portion of forest between Santa Rosa del Oso and San Antonio de Padua was compromised by the creation of the hydroelectric plant in the Río Chontayacu microbasin, while in the other microbasins forests are under concession to logging companies. The forests between Yanajanca and Magdalena have been logged since 2012, and, due to the resulting hunting, now have very depleted primate populations, in particular those of *L. flavicauda* (Fig. 3).

Logging concessions and the imminent construction of a hydroelectric dam impede the creation of communal conservation areas in the sectors most appropriate for protecting *L. flavicauda*. This evidently makes any such conservation project unviable, even though they would be welcomed and supported by the communities of Cocalito, Galicia and San Antonio de Padua in Chontayacu and Alto Azul in the Río Santa Martha microbasin.

Records of other primates

During our surveys, we saw 28 groups of five primates in addition to *L. flavicauda*. The highest number recorded was in Alto Azul with nine groups of five species. The lowest number of sightings was in Paccha and San Antonio de Padua; with just two and three groups, respectively, and of only two species, *S. macrocephalus* and *A. miconax* (Table 3). From our results, we deduce that the forest most seriously affected

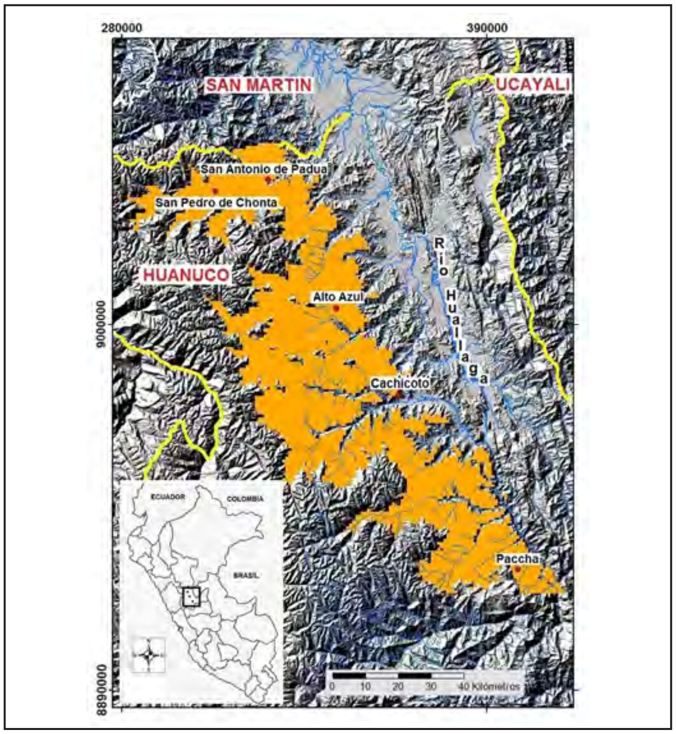

Figure 2. The distribution (orange) of *Lagothrix flavicauda* in the Región Huánuco.

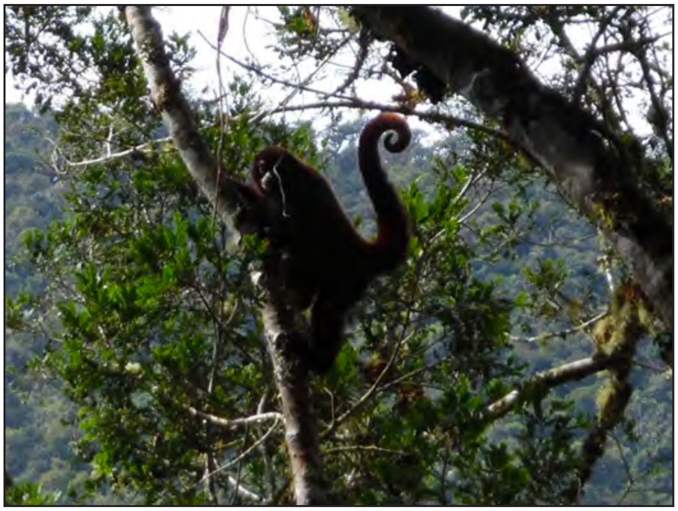

Figure 3. Adult *Lagothrix flavicauda* observed in Alto Azul forest and exposed to hunting by loggers.

by human activities is that in the vicinity of San Antonio de Padua.

Among the primates, *L. leucogenys* was the species most frequently seen, with eight groups, all below 1300 m elevation. We saw only four groups of the red howler (*Alouatta seniculus*). They were in Santa Rosa del Oso and Alto Azul, where the species shares its habitat with *L. flavicauda*. Excepting *L. leucogenys*, the sighting rate for the other species was low, and in the case of *A. seniculus* very low with 0.1 groups/10 km of transect walked (Table 4). This indicates that the populations of the majority of species in the montane forests of Huánuco are suppressed and headed to local extinction.

Discussion

The distributional limit of *L. flavicauda* in southern Huánuco considered in this study contrasts with that proposed by Aquino and Ramos (2010). Aquino and Ramos (2010) considered the probable limit the montane forests of the Río Chinchao microbasin, including Carpish near to the city of Tingo Maria. It also differs from the conclusion of Shanee (2011) that the Río Magdalena might be the limit. It is probable that the limits indicated in both of these studies were affected by unreliable information obtained during interviews with Andean migrants, whose priorities are agriculture and cattle ranching and who have little knowledge of the wildlife in the regions where they now live. We found that most interviewee farmers and cattle ranchers recognized only the "chocleros" (corneaters)—capuchin monkeys raiding their crops. In our case, the only interviewees south of the Río Chontayacu microbasin, including Paccha in Alto Huallaga, who could distinguish *L. flavicauda* from other primates were those in Alto Azul and Carpish. In addition to describing the most obvious phenotypic characteristics of *L. flavicauda*, they reported that the presence of this primate species was seasonal, from November–March. This can be interpreted as migration to procure food, coinciding as it does with the months of highest fruit production there, particularly that of *Cecropia* sp. (Cecropiaceae: locally called *tacones*) and species of Moraceae, which are important food sources for the yellow-tailed woolly monkey.

Contrary to the findings of Aquino *et al.* (2015a), who saw 13 groups of *L. flavicauda* near Cocalito and Bardales, this study found this primate species to be absent in forests close to these communities in San Antonio de Padua. This could be due to heavy hunting by communities, who traditionally hunt wildlife for food. However, groups of *L. flavicauda* were observed during this season in the forests of the Región San Martin near the boundary with Huánuco, which contradicts the belief maintained by the inhabitants of San Antonio de Padua.

The diversity of primates registered in the primary forest of Alto Azul was higher than that reported in the study of Aquino *et al*. (2015a) in Alto Miraflores, than the reports of Shanee *et al*. (2013) in pre-montane and montane forests in Monzòn and Ajenco (both in the Región Huánuco), and also higher than that recorded by Aquino *et al*. (2014) in the forests of Cajamarca. In Alto Azul, we observed six species, in Alto Miraflores just five, that included *A. chamek*, which is absent from the left bank of the Río Huallaga. Despite this, *Ateles chamek* is cited as one of six primates occurring in the Tingo Maria National Park (SERNANP 2015). It is important to confirm its presence in that protected area.

Remnant forest patches predominated at all sites, except for San Antonio de Padua, Santa Rosa del Oso and Alto Azul. These fragments, inhabited by primates, are very much disturbed and degraded due to the extraction of timber and other forest resources coveted by the local communities. For this reason the arboreal vegetation reaches a height of only 15 m with emergents of up to only 20 m. There are some fragments of well preserved primary forest on steep cliffs in the microbasins of the ríos Jarahuasi and Derrepente, and of Paccha in Alto Huallaga, because agriculture and cattle ranching are impossible there. These fragments are isolated by cultivated areas and pasture, which forces primates to descend to the ground to move between them. Contrary to the situation at these sites, primary forest can be found on both sides of the river in the Río Chontayacu microbasin, from Santa Rosa del Oso to San Antonio de Padua. The highest primate population is concentrated, however, near the communities of Cocalito, Olla and Galicia, where deforestation is increasing due to commercial logging (spurred by high timber prices), agriculture and cattle ranching in this part of Chontayacu. These activities will now be accompanied by a hydroelectric project, scheduled to begin in 2016. The imminent loss of wildlife, including *L. flavicauda* and the spectacled bear (*Tremarctos ornatus*), is probable due to the impacts of deforestation. Other primary forests which appear to be well conserved, such as those in the headwaters of the ríos Yanajanca, Santa Martha, Magdalena and Monzòn microbasins have been concessioned for high value timber extraction, underway since 2012 between Yanajanca and Magdalena. Currently small-scale logging will increase in pace and area with the construction of logging roads.

The low numbers of primates seen is not only associated with the degradation and destruction of the forests for commerce, but is also due to hunting. The capuchin monkeys *S. macrocephalus* and *C. yuracus* are shot for their crop-raiding. The remaining species are not so heavily hunted (except in San Antonio de Padua), however, because most of the people originate from the Andes, having migrated to practice agriculture and cattle ranching. This would explain the presence of *L. flavicauda* even in small patches of primary forest, and *A. seniculus* in primary and remnant forest patches. These species can evidently exist in disturbed forest if hunting pressure is low or absent. In the study area, intense deforestation is encountered in nearly all of the microbasins, and the most critical problem for the large primates is the loss of habitat. In a few places such as Cocalito, part of the Chontayacu sector, there is still moderately disturbed continuous primary forest, which explains the high population density of *L. flavicauda* (see Aquino *et al*. 2015a), and our finding of six species, including *L. flavicauda* and *A. seniculus*, in Alto Azul. However, this panorama could change in the next few years with pervasive and inexorable deforestation, and the increasing numbers of people with the construction of logging roads and the imminent construction of a hydroelectric dam.

Acknowledgments

We thank the Primate Action Fund of Conservation International and Primate Conservation Inc. for their financial support, which allowed us to continue the inventory and evaluation of *L. flavicauda* and other primates in microbasins of the Región Huánuco. We are most grateful to the Universidad Nacional Mayor de San Marcos for material support and field equipment, and to our guides in the field, from the communities of Santa Rosa del Oso, Alto Azul, Jarahuasi and Paccha, who helped us unconditionally during field activities. Finally, we thank Anthony Rylands for improving and polishing the text.

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Received for publication: 20 April 2016
Revised: 23 September 2016

Primate Conservation 2016 (30): 39–57

Mitochondrial Genetic Diversity in the Crowned Sifaka (*Propithecus coronatus*) in a Fragmented Landscape

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Abstract: *Propithecus coronatus* is an endangered, diurnal forest-dwelling lemur of northwestern Madagascar. We conducted the first extensive population genetic study for this species. We designed new primers to amplify and sequence the mitochondrial D-loop of 125 individuals from 14 localities in the northern part of the species' distribution. Our aim was to assess the genetic variability and differentiation of this species in a fragmented landscape. Compared to other lemurs, crowned sifakas have a moderate level of haplotype diversity (0.853) and a low nucleotide diversity (1.21%). Despite the considerable forest fragmentation in the region surveyed, the species does not show strong signals of genetic structure as shown by the Φ_{ST} estimates, the network of haplotypes, and the limited correlation between genetic and geographic distance. The current mtDNA estimated effective population size was relatively large (median: 11,262; 95% HPD: 5,107−20,083), in agreement with recent census estimates, suggesting that a large number of individuals is still present across the species range. Using the Extended Bayesian Skyline Plot (EBSP) approach to reconstruct the demographic history of the species, we did not detect any genetic signal of change in population size. Despite the ongoing loss and fragmentation of their habitat, the population still harbors subtantial genetic diversity, likely as a partial consequence of a taboo against hunting the crowned sifaka among the Sakalava ethnic group inhabiting the area.

Key Words: *Propithecus coronatus*, crowned sifaka, Madagascar, mitochondrial DNA, genetic diversity, demographic history, endangered species, conservation

Introduction

Madagascar has an exceptional concentration of endemic species (about 80%). Many of them are restricted to small forest patches; a result of climate change during the Pleistocene and of the fragmentation, degradation, and destruction of Madagascar's forests by humans, particularly since the 1970s. The relative contribution of each is still under debate (Virah-Sawmy *et al.* 2009; Matsumoto and Burney 1994; McConnell and Kull 2014; Scales 2014). Paleoecological data suggest that, at least for some regions, forest fragmentation has been caused by natural events (Matsumoto and Burney 1994; Virah-Sawmy *et al.* 2009; Vorontsova *et al.* 2016) that predate the arrival of humans on the island, now thought to have started around 4000 years ago (Gommery *et al.* 2011; Dewar *et al.* 2013). It is clear, however, that anthropogenic activities—slash-and-burn agriculture, charcoal production, fires for zebu cattle pasture, logging and, to a lesser extent, mining—are regarded as the major recent causes for forest loss and fragmentation. Accordingly, it has been shown that forest cover in Madagscar decreased by 50% over 60 years from 1950 to 2010 (Harper *et al.* 2007; ONE 2013; Schwitzer *et al.* 2014a, 2014b).

The lemurs are prominent among Malagasy endemics, with 110 species and subspecies currently recognized

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(Mittermeier *et al.* 2010; Schwitzer *et al.* 2013)[1], and, as a group, they are among the most threatened vertebrates in the world (Schwitzer *et al*. 2014a, 2014b). Four of the nine species of sifaka (*Propithecus*: Indriiidae) are Critically Endangered (CR)—*P. candidus*, *P. perrieri*, *P. tattersalli*, and *P. diadema*—and the remaining five are Endangered (EN)—*P. coquereli*, *P. coronatus*, *P. deckenii*, *P. edwardsi*, and *P. verreauxi* (IUCN 2014).

Our study focused on the crowned sifaka (*Propithecus coronatus*), a medium-sized (head and body length 39−45 cm), diurnal, arboreal, and folivorous species living in riparian, semi-deciduous, dry forests or in mangroves (Petter and Andriatsarafara 1987; Mittermeier *et al.* 2010). Crowned sifakas live in small social groups of 2 to 8 individuals (Ramanamisata *et al.* 2014; Razanaparany *et al.* 2014). Typically, the dominant female of a group reproduces every two years, and it is usually the males that disperse (C. Pichon and F. Bayart unpubl. data). The European Endangered Species Program (EEP) data on *P. coronatus* show that females can live to at least 20 years, and males to at least 25 years in captivity (Roullet 2014). Based on data from *P. verreauxi*, the generation time could be as high as 19.5 years (Lawler *et al.* 2009; Morris *et al.* 2011). This is a parameter for which there is little available data, however, and authors have used values of 3 to 17.5 years (Quéméré *et al.* 2012).

The geographical distribution of the species extends from the rivers Betsiboka and Mahavavy in the northwest of Madagascar (the area of the present study) to the rivers Tsiribihina and Manambolo in the southwest (Tattersall 1986; Thalmann and Rakotoarison 1994; Rasoloharijaona *et al.* 2005; Wilmé *et al*. 2006; Razafindramanana and Rasamimanana 2010; Rakotonirina *et al.* 2014; Salmona *et al.* 2014). The Betsiboka/Mahavavy river region was once believed to encompass the entire range of *P. coronatus*; their presence to the southeast was documented only recently (Tattersall 1986; Thalmann and Rakotoarison 1994; Thalmann *et al.* 2002; Rasoloharijaona *et al.* 2005; Wilmé *et al.* 2006; Razafindramanana and Rasamimanana 2010; Rakotonirina *et al.* 2014). Its range is characterized by mosaics of forest fragments surrounded by grassland and farmland (Fig. 1). The fragmentation of their habitat is believed to have led to a population decline of about 50% in the last decades, human activities being the most important cause (Mittermeier *et al.* 2010; IUCN 2014).

Surveys by Salmona *et al.* (2014) have indicated between 4,226 and 36,672 individuals in the northern part of the species' range, delimited by the Betsiboka and Mahavavy rivers. By extrapolation they estimated between 130,000 and 220,000 individuals for the species, although this is probably an overestimate because the forests between the rivers Tsiribihina and Manambolo are more fragmented than in the north of their range.

The first protected area for the species, the Nouvelle Aire Protégée of Antrema (NAP-Antrema − category VI; previously SFUM, Antrema), was established in 2001 (Gauthier *et al.* 1999) and is jointly managed by the National Museum of Natural History (MNHN) of Paris (France) and the University of Antananarivo (Madagascar). It is located in the northern coastal part of the species' range. The landscape of the NAP-Antrema includes dry forest, mangroves and grasslands; the first two being typical habitats for *P. coronatus* (Gauthier *et al.* 1999; Salmona *et al.* 2014). This particular area has attracted the interest of conservation biologists because of the remarkably high density of crowned sifakas: about 300 ind/km² in a single forest fragment (Badrala forest; Pichon *et al.* 2010) (Fig. 1).

Two other protected areas exist in the northern area delimited by the Betsiboka and the Mahavavy rivers. The NAP-Mahavavy-Kinkony (category V) was established in 2006 and is managed by the Malagasy NGO Asity (Fig. 1); it includes dry forests, a lake, a saline bay and a riparian delta ecosystem that are the home to several threatened species (Schwitzer *et al*. 2013). The NAP-Bombetoka-Belemboka (category V) was established in 2007 and was managed by the Malagasy NGO Fanamby until 2011 (Fig. 1). The three protected areas were set up as biodiversity conservation projects in collaboration with the local human populations (i.e. the *Sakalava* ethnic group) (Gauthier *et al.* 1999; FANAMBY 2008; Schwitzer *et al.* 2013; FEM 2015). The Sakalavas do not usually hunt sifakas because to do so is taboo (Harpet *et*

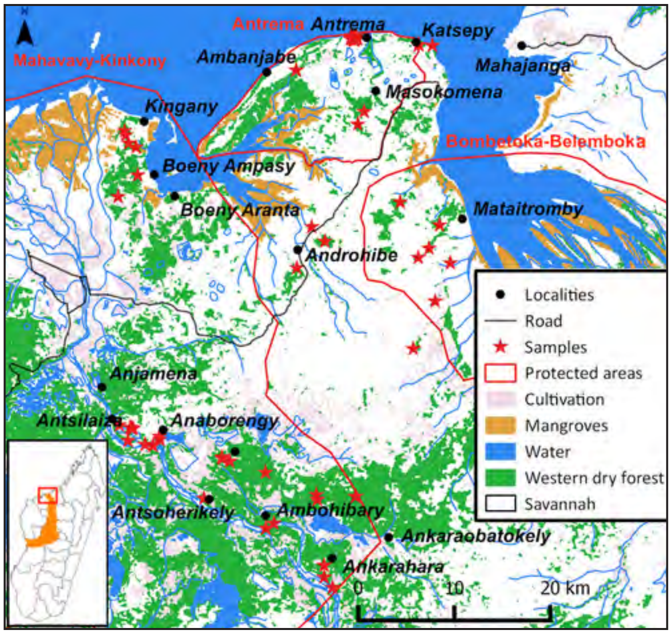

Figure 1. The study area in central western Madagascar. The study was carried out in the north of the range of *P. coronatus* (see Salmona et al. 2014). The villages named are those closest to the sampling sites. Bombetoka-Belemboka, Antrema, and Mahavavy-Kinkony are the protected areas in the study area.

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[1] The count of 103 taxa by Schwitzer *et al.* (2013) along with species described and reinstated subsequently: *Microcebus tanosi* Rasoloarison *et asl.*, 2013; *Microcebus manitatra* Hotaling *et al.*, 2016; *Microcebus ganzhorni* Hotaling *et al.*, 2016; *Microcebus boraha* Hotaling *et al.* 2016; *Cheirogaleus lavasoensis* Thiele, Razafimahatratra and Hapke, 2013; *Cheirogaleus andysabini* Lei *et al.*, 2015; and *Cheirogaleus thomasi* (Forsyth Major, 1894).

al. 2008). While this traditional taboo is widespread in the northern region, it may be more strictly respected in the NAP-Antrema because the traditional leader of the Sakalava ethnic group, Prince Tsimanendry, lives nearby.

The genetic diversity of endangered species is a useful indicator to establish effective conservation measures to diminish the level of inbreeding and to promote contact between populations that have been isolated because of habitat degradation. This is the issue we address here. To date, there have been no available population genetic studies concerning *P. coronatus*. We non-invasively collected DNA material and, as a first step, sequenced and analyzed a partial DNA sequence of the mitochondrial D-loop region of 125 individuals from the north of the species range. Mitochondria are transmitted along the female line, without recombination, and divergence between mitochondrial sequences results from mutation only.

We first measured mitochondrial diversity in the NAP-Antrema sifakas (79 individuals; AMB, ANT, KAT, MAZ sites), including one of the forests with the high population densities. We subsequently extended our assay to a wider area (~1600 km²), adding 56 samples from other sites. Female *P. verreauxi* (sister species of *P. coronatus*) are very territorial and tend not to migrate for reproduction (Richard *et al.* 1993 and references therein). Crowned sifakas are reluctant to cross grassland, preferring to move through wooded areas, although they have been observed to cross several hundred meters of open grassland (F. Bayart pers. obs.). The same is true for their sister species, *P. tattersalli* and *P. perrieri* (Quéméré *et al.* 2010a; Banks *et al.* 2007, and references therein). The increasing discontinuity in the forest canopy resulting from deforestation (ONE 2013), and direct field observations (Salmona *et al.* 2014) strongly suggest that *P. coronatus* is experiencing a marked demographic decline (IUCN 2014). We aim to contribute to the conservation of this lemur by estimating their genetic diversity, examining any geographical patterns, and inferring putative population size changes.

Methods

Fieldwork and fecal sample collection

Fecal samples were obtained from the northern part of the species range (northwestern Madagascar), the region delimited by the Mahavavy and the Betsiboka rivers (Fig. 1). Vegetation there is characterized by grassland (85%) and dry forest (13%) (data from Salmona *et al.* 2014). Sixty-nine samples came from the Antrema site and 56 were obtained from other locations in the northern part of the species' range. Fresh fecal material was collected during the day in the dry season over three years (2009−2011). Salmona *et al.* (2014) and Salmona *et al.* (2015) provide further details. Feces were picked up directly after observed defecation, leaving little chance that the same individual was sampled twice. They were stored immediately in dry conditions using silica gel (Quéméré *et al.* 2010b). Avoiding duplicate sampling of the same animal was made easy in Antrema where there was an ongoing long-term behavioural ecology project with well-identified individuals and groups. Fecal samples were obtained from 14 locations (Table 1, Fig. 1), named according to the closest village or town or to the name of the forest. DNA was extracted from a subgroup of the available fecal samples.

Laboratory procedures

A total of 125 samples were obtained, including 79 from NAP-Antrema, mostly from the Badrala forest (n = 69, Table 1; the site called "Antrema"). DNA was extracted from the outer layer of dried feces according to the 2CTAB/PCI protocol (Vallet *et al.* 2007). New primers were designed to amplify the mitochondrial Hypervariable Sequence I (HVS-1) of the D-loop. To do so, we aligned the mitochondrial D-loop sequences of 61 individuals of several lemur species (Appendix, Tab. S1) and identified two conserved regions very likely to be conserved also in *P. coronatus.* These conserved regions enabled the design of two primers: PcorL-15978 (5'-CACCTTCAGCACCCAAAGCTG-3'); PcorH-16423 (5'-TGATGGTTTCACGGAGGATGGTAG-3'). DNA was amplified in a total volume of 25 µl, containing 200 µM of each dNTP, Colorless GoTaq® Reaction Buffer 1X (1.5 mM $MgCl_2$), 100 nM of each primer, 1 Unit of GoTaq® DNA Polymerase, 0.4 µg/µl of BSA and 1 µl of DNA samples diluted 10 times. The amplification was performed by a Mastercycler® Eppendorf thermalcycler with a first denaturation at 95°C for 5 minutes, followed by forty cycles of denaturation at 95°C for 1 minute, with an annealing process at 58° for 30 seconds and extension phase at 72°C for 1 minute. The final extension occurred at 72°C for 10 minutes.

DNA double-strand sequencing was performed by Genoscreen on an Applied Biosystems 3730XL sequencer using the Sanger method. Each sequence chromatogram was visually inspected using the software BioEdit v7.2.5 (Hall 1999). To be certain that primers had amplified the target HVS-1 region, all obtained sequences were aligned against Nucleotide collection (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The sequences analyzed in this paper had a length of 395 base-pairs after the deletion of flanking conserved portions (including the primers).

Computation of the genetic diversity

The 125 sequences were aligned using BioEdit v7.2.5 (Hall 1999) with the Clustal W method (Thompson *et al.* 1994). In the general analysis, the number of samples coming from the Antrema site was too high (n = 69) to be compared to the other 13 sampling sites (total n° of individuals = 56). We therefore used a subset of 11 sequences from the Antrema site in those analyses where uneven samples were an issue. The highest probable combination of 11 sequences from a random distribution was obtained by sampling 11 individuals as shown in Figure S1 (Appendix). From these 67 D-loop sequences (56 + 11), we computed haplotype diversity (h) and nucleotide diversity (π) by using DnaSP 5.0 Software (Librado and Rozas 2009). To identify the best model of nucleotide substitution, we used jModelTest v2.1.5 (Darriba *et al.* 2012) and the Akaike Information Criterion (AIC) (Akaike 1973). By applying the jModelTest we estimated the best-fitting mutation model to be the HKY (Hasegawa *et al.* 1985), with a proportion of invariable sites of 0.86 and a kappa value of 20. The genetic diversity among lemur species was assessed on D-loop sequences retrieved from GenBank (references in Table 2).

Spatial analysis

We investigated the genetic structure using several complementary approaches. We first computed the pairwise estimator of genetic differentiation Φ_{ST} (signficance estimated by 10,000 permutations) among sampling sites (Excoffier *et al.* 1992) with ARLEQUIN v3.5.1.2 (Excoffier and Lischer 2010). We note that Φ_{ST} values were computed using the Tamura and Nei (TN93) substitution model (Tamura and Nei 1993) instead of the HKY model because ARLEQUIN v3.5.1.2 does not implement the latter.

The correlation between genetic and geographic distances was assessed by the Mantel test using GenAlex software (Peakall and Smouse 2006, 2012) with a significance established after 10,000 random permutations. Pairwise genetic distances between test-sites were computed from sequences by using the TN93 model (Tamura and Nei 1993) in MEGA v6.0 (Tamura *et al.* 2013).

To test whether grassland and farmland act as barriers to gene flow, we applied the Analysis of Molecular Variance (AMOVA) in ARLEQUIN v3.5.1.2 (Excoffier and Lischer 2010) in order to see which combination of samples, once they were put into groups, would have explained the higher fraction of the molecular variance between groups. We first computed the molecular variance (among sites in group and within sites) for all the 14 sampling sites all together, that is in one group. We then computed the same estimators for two groups as there is a large grassland area dividing the northern groups from the southern. Finally we tested the existance of four groups according to the prioximity of the sites and to the presence of grassland separating them (see details in Fig. S2; Appendix). The statistical significance of the fixation indexes was assessed using permutation procedures based on 10,000 runs.

A network of haplotypes was obtained by using TCS v1.21 (Clement *et al.* 2000), with 95% statistical parsimony. This method estimates the evolutionary steps among haplotypes, finally representing a 95% probable set of the most likely networks.

Demographic analysis

To estimate the mitochondrial DNA effective population size (mtDNA *Ne*), we used two Bayesian coalescent-based methods as implemented in the BEAST software v1.8.1 (Drummond *et al.* 2012). The first method assumes a constant population size over time. The second is the Extended Bayesian Skyline Plot (EBSP) analysis that allows population size to vary. The EBSP is a non-parametric Bayesian coalescent-based approach that estimates population-size (that is N_e multiplied by *tau*, where *tau* is the generation time in years) for each coalescent interval, without *a priori* specification of any demographic model (Heled and Drummond 2008). This analysis reconstructs the population size history over time and estimates the number of population change points, which are defined by the "demographic population size change" parameter. In this study, we considered three different values of generation time (3, 6 and 17.5 years) and we estimated mtDNA N_e, dividing the population size parameter by *tau* (i.e. generation time). We used a Markov Chain Monte Carlo (MCMC) of 1×10^8 steps. We removed the first 25% steps (burn-in) and we checked for convergence of all parameters and for acceptable Effective Sample Size values (ESS >200) in Tracer v1.6 (Rambaut *et al.* 2014). The analysis was carried out with the HKY+I evolution model. The prior for invariant sites was set as uniform with an initial value of 0.86. We applied a strict clock model, based on the hypothesis of unvaried mutation rate within species, with the clock rate sampled from a normal distribution with mean of 7.2×10^{-8} (standard deviation = 0.725×10^{-8}). To date, an accurate estimation of the D-loop mutation rate for lemurs is not available. We therefore used the mutation rate range of 11.5%−17.3% (substitutions/site) per 10^{-6} years, as estimated from the human-chimpanzee split (Vigilant *et al.* 1991). UPGMA trees were used as starting trees of the MCMC analyses. The nucleotide frequencies were estimated from a uniform prior distribution with an initial value of 0.25. A log-normal distribution was set for the prior of kappa (transversion/transition) with an initial value of 20.0. Lastly, we used a 1/X prior distribution for estimating the demographic population size and a Poisson prior distribution for the estimation of the population size changes.

In addition, to determine whether population departed from mutation-drift equilibrium we computed three summary statistics Tajima's D, Ramos R_2, and Fu's F_S (Tajima 1989; Fu 1997; Ramos-Onsins and Rozas 2002) that are sensitive to such departures. We used ARLEQUIN v3.5.1.2 to calculate these statistics. The statistical significance was determined by 10,000 coalescent simulations. Under a Wright-Fisher model without selection and population structure, positive Tajima's D and F_S values suggest population contraction, whereas in the case of a population expansion Tajima's D and F_S are negative. The R_2 statistic exhibits either a negative or a positive value when population experiences, respectively, contraction or expansion.

To overcome the confounding effect of population structure, we followed the approaches suggested by Chikhi *et al.* (2010), Heller *et al.* (2013), and Städler *et al.* (2009). Four sampling strategies were used:

1) *Local sampling,* either 69 samples ("Ant") or 11 samples ("Ant11") or seven samples ("Ant7") from the single site of Antrema;
2) *Pooled sampling*, two (or three) samples randomly picked from each site, excluding the sites with only one individual, were pooled in the same dataset ("pool_2i" or "pool_3i");

3) *Scattered sampling*, a dataset composed by pooling one randomly picked sample from each site (“scat_1i”);
4) *Structured sampling*, all samples from the surveyed area plus either 69 or 11 or seven samples from Antrema (respectively “All_Ant”, “All_Ant11”, “All_Ant7”).

Three repetitions were used for *pooled* and *scattered* sampling to test whether the results were robust.

Results

Genetic diversity and structure

We present results for the entire northernmost part of the range of *P. coronatus* shown in Figure 1; not just NAP-Antrema, even though the majority of the 125 sequences obtained came from this single area. As mentioned, we pooled 11 sequences of the 69 of Antrema and added them to the other sites for a total of 11+56 = 67 mitochondrial D-loop HVR1 sequences of a length of 395 base-pairs. The alignment of the 67 sequences showed the presence of 17 variable nucleotide positions: 16 transitions and one transversion (4.3% of the 395 bp).

Two localities of the 14 in the geographic region under investigation showed a haplotype diversity (h) of 0.5, while the diversity of the other 12 sites ranged from 0.6 to 1 (Table 1). Overall, haplotype (h) and nucleotide (π) diversity were 0.85 ± 0.022 and 1.2% ± 0.11%, respectively. The NAP-Antrema (sampling sites AMB, ANT, KAT, MAZ in Table 1) showed average genetic diversity indices; haplotype and nucleotide diversity of 0.75 ± 0.06 and 1.1% ± 0.23%, respectively.

The 67 mitochondrial partial D-loop sequences are displayed in a haplotype network representation (Fig. 2) obtained by applying a 95% statistical parsimony criterion. A total of 11 haplotypes were found and one (H3) was shared among nine sampling sites (AMB, MAT, MAZ, KIN, ANT, ANS, ANO, ANK, AND), four (H8, H9, H10, H11) were detected in one site only (ANO, KAT, ANK) (Fig. 2). From the unrooted network, the maximal number of mutational steps between the most divergent haplotypes (H10; H4) corresponds to 16. The distribution of the different haplotypes among the different test-sites showed no geographic pattern.

The 91 pairwise Φ_{ST} values for the 14 test-sites ranged from -1 to 0.63, with a mean very close to zero (mean Φ_{ST} = -0.017; see Table S2 in the Appendix). The pairwise Φ_{ST} values

Table 1. Sampling sites and measures of genetic diversity of the crowned sifaka. Each site is indicated with its complete name and its abbreviation (Abbr.). Ind. = number of individuals; Hap = number of haplotypes per site; Sites = number of variable sites; π = nucleotide diversity; h = haplotype diversity; sd = standard deviation; GPS NS = latitude; GPS EW = longitude; * = values multiplied by 100; # = decimal degree GPS data in WGS 84 coordinate reference system. Antrema_07 and Antrema_11: estimates for the Antrema sampling site using a sub-sample of 7 or 11 samples, respectively (see Methods section). Total Ant 07 or 11 = estimates for all sampling sites, with either 7 or 11 individuals from Antrema. NAP-Antrema = includes the four sampling sites placed in the protected area (AMB, ANT11, KAT, MAZ).

	Abbr.	Ind.	Hap.	Sites	π*	sd π*	h	sd h	GPS NS(#)	GPS EW(#)
Ambanjabe	AMB	3	2	3	0.51	0.24	0.67	0.31	-15.743	46.069
Ambohibary	AMO	4	2	9	1.14	0.60	0.50	0.27	-16.160	46.070
Androhibe	AND	3	2	4	0.68	0.32	0.67	0.31	-15.910	46.100
Ankarahara	ANK	4	4	5	0.76	0.20	1.00	0.18	-16.200	46.135
Antrema	ANT	69	4	9	0.96	0.04	0.67	0.02	-15.710	46.166
Antrema_07	ANT7	7	3	8	1.04	0.23	0.76	0.12	-15.710	46.166
Antrema_11	ANT11	11	3	8	0.97	0.17	0.71	0.08	-15.710	46.166
Antsilaiza	ANS	11	5	15	1.26	0.26	0.82	0.01	-16.070	45.920
Antsoherikely	ANO	3	3	14	2.36	0.67	1.00	0.27	-16.145	46.015
Boeny Ampasy	BOE	1	1	-	-	-	-	-	-15.840	45.960
Boeny Aranta	BON	1	1	-	-	-	-	-	-15.860	45.925
Katsepy	KAT	5	3	12	1.67	0.42	0.80	0.16	-15.714	46.214
Kingany	KIN	4	2	7	0.89	0.47	0.50	0.27	-15.790	45.950
Mataitromby	MAT	7	3	4	0.56	0.12	0.67	0.16	-15.880	46.260
Masokomena	MAZ	2	2	2	0.51	0.25	1	0.50	-15.760	46.175
Tsiandrarafa	TSI	8	5	14	1.3	0.31	0.89	0.01	-16.100	46.040
Total	/	125	11	17	1.12	0.07	0.80	0.02	/	/
Total Ant 07	/	63	11	17	1.22	0.11	0.86	0.02	/	/
Total Ant 11	/	67	11	17	1.20	0.11	0.85	0.02	/	/
NAP-Antrema	/	21	6	15	1.10	0.23	0.748	0.058	/	/

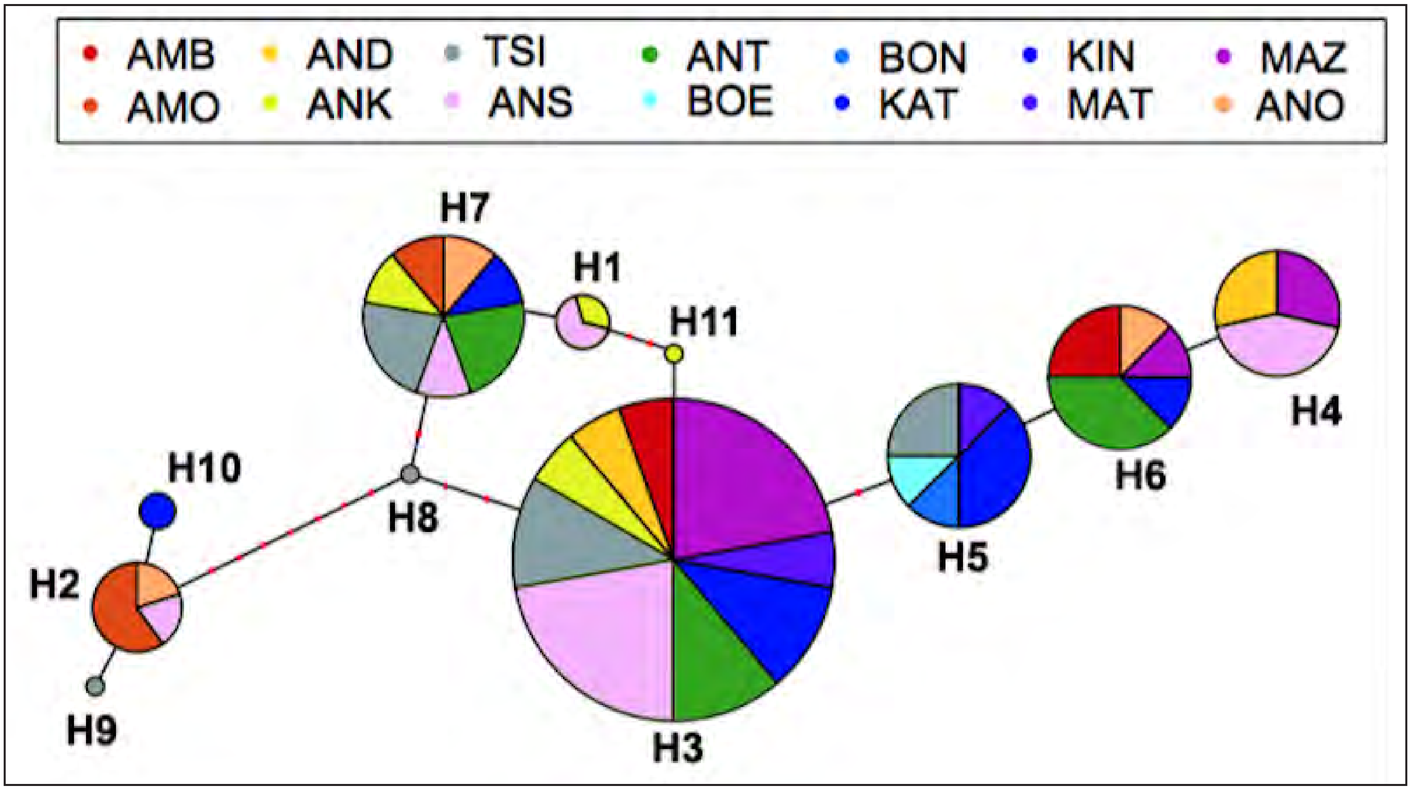

Figure 2. Haplotype network of *P. coronatus* obtained with D-loop sequences from this study. Each circle represents a single haplotype and the size is proportional to its frequency in the dataset. Small red circles represent estimated haplotypes, missing in the dataset. The 14 different colors indicate the sampling sites (see Table 1). Solid lines represent 95% parsimonious connections of single mutation between haplotypes. The loop indicates that different set of single-point mutations can give rise to the same haplotype.

exhibited a very large variance, likely due to the small number of samples per site. Indeed, only some pairwise comparisons, those for the Ambohibary and Mataitromby sites, exhibited a significant genetic differentiation (AMO-ANT; AMO-ANS; AMO-MAT; ANK-MAT) with Φ_{ST} values ranging from 0.386 to 0.659. All other values were not significant, with thirteen Φ_{ST} pairwise comparisons between 0 and 0.1 and forty-seven lower than zero. The Mantel test, comparing the pairwise matrix of genetic differentiation with the corresponding matrix of linear geographic distances, yielded a significant correlation (Rxy = 0.329; P = 0.003; R^2 = 0.11; Fig. 3).

Concerning the AMOVA analysis, it appears that when samples are put together in one single group, the differences among them explain 10.91% of the molecular variance, but this percentage falls to ~4% when we put the samples in 2 or 4 groups. The two-group hypothesis corresponds to the northern and the southern parts of the sampled area that are divided by grasslands (in white in the middle of Fig. 1), whereas the four-group hypothesis corresponds to a north/south/east/west partition of samples around the same grassland zone. The north/south partition into two groups explains more variance (~11%) than the four-group partition, suggesting that, overall, the genetic differentiation of crowned sifakas is explained mainly by the grasslands dividing northern and southern sites (Table 3 and Fig. S2 for more details).

Demographic history

Under a constant-size coalescent model, we obtained a median mtDNA effective population size of 11,883 individuals and a 95% highest posterior-density (HPD, that is the shortest interval that contains 95% of the most probable values) between 5,107 and 20,083 (assuming a generation time of six years, Table 4). Estimations using generation times of three or 17.5 years are shown in Table S3 and suggest that larger generation times will produce smaller effective size estimates, the opposite happens for shorter generiation times. This is a likely consequence of the fact that genetic drift experienced for a particular time may result in a genetic diversity in a species with long generation time and small effective population size similar to that in a species with a short generation time and large effective population size.

Very similar values were obtained for the "All_Ant" (69 samples from Antrema site plus 56 from all other sites) and "All_Ant7" (seven samples from Antrema site plus 56 from all other sites) sampling strategies (see section "Demographic analyses" in methods for details about the alternative sampling).

In nearly all cases, the four sampling strategies (*local*, *scattered*, *pooled* and *structured*) showed that most of the demographic summary statistics were not significant (Table 5), the only exception being the Antrema sample when *local sampling* was used ("Ant" and "Ant_11"; Table 5). In that case, we found positive and significant values for all the statistics.

The demographic history inference (mtDNA N_e over time), using the EBSP, was a stationary population (median mtDNA N_e: 32,172), with a large confidence interval (95% HPD: 1,471–373,933). The demographic size change estimate was zero (median; 95% HPD between 0 and 2/3), except for Antrema, for which we detected a signal of one past event of population size change (Table 5).

Discussion

Genetic diversity

Using mitochondrial DNA, we have obtained the first estimates of the genetic diversity of *Propithecus coronatus*. This diversity was addressed in geographic terms (the so-called genetic structure) for the northern part of the estimated geographic range of crowned sifaka. The nucleotide diversity detected in *P. coronatus* (π = 1.21%) was within the range of the values observed for other *Propithecus* species for which mtDNA data are available (0.42%–1.53%) (*P. candidus*, *P.*

Table 2. D-loop genetic diversity of sifakas and other lemurs. EN = Endangered; CR = Critically Endangered; Length = number of base pairs; N = number of sequences; Sites = number of variable sites; hap = number of haplotypes; *h* = haplotype diversity; *π* = nucleotide diversity expressed as a percentage; Tot. census pop. size = census population size; References for demographic data = references of the total population size estimates; /: not available

Species	IUCN status	Length	N	Sites	hap	*h*	π (%)	Tot. census pop. size	References for genetic diversity	Referencs for demographic data
Propithecus coronatus	EN	395 bp	63	17	11	0.86	1.21	130,000 to 200,000	This study	Salmona *et al.* (2014)
Propithecus candidus	CR	395 bp	7	2	2	0.29	1.15	<1000 *c.*250	Andriantompohavana *et al.* (2006) Mayor *et al.* (2002, unpubl.)	IUCN 2014
Propithecus tattersalli	CR	~560 bp	/	/	/	/	/	>10,000	Bailey *et al.* (2016)	Quéméré *et al.* (2010a)
Propithecus coquereli	EN	560 bp	82	17	12	0.76	0.66	>50,000	/	Kun-Rodrigues *et al.* (2014)
Propithecus edwardsi	EN	395 bp	13	13	4	0.71	1.53	*c.*40,000	Andriantompohavana *et al.* (2006) Mayor *et al.* (2002 unpubl.)	Irwin *et al.* (2005)
Propithecus verreauxi	EN	395 bp	7	11	5	0.91	1.35	/	Andriantompohavana *et al.* (2006)	/
Propithecus perrieri	CR	~560 bp	51	0	1	0	0	*c.*917	Bailey *et al.* (2016)	Banks *et al.* (2007)
Propithecus deckeni	EN	/	/	/	/	/	/	/	/	/
Propithecus diadema	CR	395 bp	23	15	4	0.72	0.99	/	Mayor *et al.*(2002 unpubl.) Andriantompohavana *et al.* (2006)	/
Lepilemur edwardsi	EN	496 bp	121	/	23	0.89	0.49	/	Craul *et al.* (2009)	/
Varecia variegata	CR	395 bp	186	44	24	0.84	2.67	/	Louis Jr. *et al.* (2006) Baden *et al.* (2014) Andriantompohavana *et al.* (2006)	/
Microcebus ravelobensis	EN	395 bp	113	113	26	0.94	7.91	/	Guschanski *et al.* (2006) Olivieri *et al.* 2007	/
Microcebus danfossi	EN	477 bp	6	39	6	1.00	3.55	/	Guschanski *et al.* (2006) Olivieri *et al.* (2007)	/
Eulemur cineirescens	CR	452 bp	108	39	11	0.82	2.1	<1000	Delmore *et al.* (2013) Johnson *et al.* (2008)	Vasey (1997)

Table 3. Analysis of molecular variance (AMOVA). From left to right, the three columns give the percentage of molecular variation "Among groups", "Among sites in groups" and "Within sites". The p-value is indicated in parenthesis. Details on the sites grouping are specified in the "Group composition" column (see also Table 1).

	Among groups	Among sites in groups	Within sites	Group composition
4 groups	8.55% (P= 0.053)	4.28 % (P = 0.17)	87.17 % (P = 0,04)	(AMB, ANT, MAZ, KAT) (MAT, AND) (KIN, BOE, BON) (ANS, ANO, AMO, ANK, TSI)
2 groups	11.06% (P = 0.02)	4.28% (P = 0.14)	84.67% (P = 0.03)	(AMB, ANT, MAZ, KAT, MAT, AND, KIN, BOE, BON) (ANS, ANO, AMO, ANK, TSI)
1 group	/	10.91% (P = 0.03)	89.09% /	All 14 sites

Table 4. MtDNA effective population size. Eff. Pop. Size Mean = the mean value of the mtDNA effective population size; Eff. Pop. Size Median = the median value. 95% HPD is the 95% confidence interval of the mean. These values were estimated from three different datasets. All_Ant = 56 samples + 69 from Antrema; All_Ant7 = 56 samples + 7 from Antrema; All_Ant11 = 56 samples + 11 from Antrema.

	Eff. pop. size mean	Eff. pop. size Median	95% HPD
All_ Ant	10,077	9,586	[4,132; 16,708]
All_ Ant7	12,105	11,374	[4,672; 20,640]
All_ Ant11	11,883	11,262	[5,107; 20,083]

coquereli, *P. edwardsi*, *P. verreauxi*, and *P. diadema*) (Tables 1 and 2). *Varecia variegata*, *Microcebus ravelobensis, M. danfossi* and *Eulemur cineirescens* present higher π values compared to *Propithecus*. The lower nucleotide diversity in *Propithecus* compared to the species listed in Table 2 (apart from *Lepilemur edwardsi*), might suggest either lower mutation rates or smaller effective population sizes (Nabholz *et al.* 2008). At this stage, it is difficult to identify the most likely explanation, and inter-specific comparisons will require further research. In particular, the estimation of the mutation rate across species will allow comparison of the levels of interspecific genetic diversity (Leffler *et al.* 2012), with a potential application to the assessment of extinction risks and conservation policies (see below and Akçakaya 2000).

Genetic Structure

The results illustrated in Figure 3 suggest that there is a limited, though significant, correlation between genetic differentiation and geographical distance across the region surveyed, even if the samples from different test-sites did not show a strong signal of genetic differentiation, as the majority of the Φ_{ST} values were not significant (Table S2). This suggests that the sub-populations may still be connected to each other, or have been connected until recent times. If the fragmentation of the habitat is a threat to *P. coronatus*, it seems that ancient fragmentation in this geographical region did not isolate the female populations that are, overall, quite homogeneous; only the Ambohibary and Mataitromby sample sites showed significant Φ_{ST} values (>0.36).

This absence of a clear spatial pattern of diversity is confimed both by the haplotype network (Fig. 2) that failed to reveal any phylogeographic patterns, and by the AMOVA analysis, as 89.09% of molecular variance is found within sampling sites and only 10.91% of it is explained by variance between sampling sites. We are aware, however, that more samples should be typed because in this study we are dealing with a low number of individuals in an area that is relatively large (1,600 km²)

Previous studies on other sifaka species suggest that they may not be among the species most affected by recent habitat fragmentation. Quéméré *et al.* (2010b), for instance, found that genetic differentiation in the golden-crowned sifaka, *P. tattersalli*, was influenced more by a river dividing its range

Table 5. Demographic history. The first column shows the sampling strategies tested in this study. Sampling = indicates the strategy (see Methods); n = number of individuals; hap = number of haplotypes in each strategy; sites = number of variable sites; π = nucleotide diversity; *h* = haplotype diversity; sd = standard deviation; D = Tajima's D; R2 = Ramos-Onsins & Rozas; Fs = Fu's Fs; 95% - bounds = boundaries of the 95% confidence interval; SizeChan = median demographic size changes and 95% confidence interval;° = values multiplied by 100; * = P-value <0.05; ** = P-value<0.01.

	Sampling	n	hap	sites	π °	sd (π) °	*h*	sd (*h*)	D	95% - bounds	R2	95% - bounds	Fs	95% - bounds	SizeChange
scat_1i_01	scattered	14	7	15	0.99	0.26	0.87	0.06	-0.70	[-1.76; 1.72]	0.14	[0.09; 0.22]	-0.16	[-4.48; 4.73]	0; [0. 2]
scat_1i_02	scattered	14	5	9	0.83	0.16	0.79	0.07	0.60	[-1.77; 1.74]	0.18	[0.09; 0.23]	1.37	[-3.68; 4.16]	0; [0. 2]
scat_1i_03	scattered	14	8	16	1.34	0.27	0.92	0.04	0.20	[-1.76; 1.71]	0.16	[0.09; 0.22]	-0.28	[-4.54; 4.44]	0; [0. 2]
pool_2i_01	pooled	24	9	16	1.20	0.18	0.88	0.04	0.38	[-1.69; 1.76]	0.15	[0.07; 0.20]	0.33	[-5.16; 5.0]	0; [0. 2]
pool_2i_02	pooled	24	8	16	1.22	0.19	0.87	0.03	0.43	[-1.73; 1.74]	0.15	[0.07; 0.20]	1.17	[-5.07; 4.99]	0; [0. 2]
pool_2i_03	pooled	24	9	16	1.43	0.17	0.91	0.03	1.13	[-1.73; 1.78]	0.17	[0.07; 0.20]	0.94	[-5.18; 5.02]	0; [0. 2]
pool_3i_01	pooled	30	9	16	1.32	0.15	0.87	0.03	0.99	[-1.70; 1.82]	0.16	[0.07; 0.19]	1.33	[-5.53; 5.32]	0; [0. 2]
pool_3i_02	pooled	33	10	17	1.40	0.15	0.88	0.03	1.06	[-1.71; 1.83]	0.16	[0.06; 0.19]	1.09	[-5.65; 5.24]	0; [0. 2]
pool_3i_03	pooled	33	10	17	1.54	0.12	0.90	0.02	1.51 *	[-1.70; 1.87]	0.18 *	[0.06; 0.19]	1.50	[-5.88; 5.34]	0; [0. 2]
All_Ant	structured	125	11	17	1.12	0.07	0.80	0.02	1.05	[-1.59; 1.93]	0.13	[0.04; 0.16]	2.67	[-7.44; 6.88]	0; [0. 2]
All_Ant7	structured	63	11	17	1.22	0.11	0.86	0.02	1.00	[-1.64; 1.89]	0.14	[0.05; 0.17]	1.56	[-6.68; 6.12]	0; [0. 2]
All_Ant11	structured	67	11	17	1.20	0.11	0.85	0.02	0.99	[-1.68; 1.90]	0.14	[0.05; 0.17]	1.65	[-6.69; 6.34]	0; [0. 2]
Ant	local	69	4	9	0.94	0.05	0.67	0.02	2.62 **	[-1.64; 1.97]	0.21 **	[0.04; 0.18]	7.96 **	[-5.25; 5.18]	1; [0. 3]
Ant7	local	7	3	8	1.04	0.23	0.76	0.12	1.34	[-1.58; 1.64]	0.26	[0.12; 0.31]	2.98	[-2.71; 3.64]	1; [0. 2]
Ant11	local	11	3	8	0.97	0.17	0.71	0.08	1.65 *	[-1.71; 1.76]	0.24 *	[0.1; 0.24]	4.18 *	[-3.62; 4.11]	1; [0. 3]

than by forest fragmentation. Quéméré *et al.* (2010b) also found a significant correlation between genetic differentiation and geographical distance and, in another study, they detected signals of ancient changes in population size related to climatic events rather than to the current levels of habitat fragmentation (Quéméré *et al.* 2012). Similarly, Salmona *et al.* (2015) found isolation by distance but a limited role played by grassland as a barrier to the dispersal of *P. perrieri* (one of the most endangered primates in the world). Other studies (for example, Jimenez and Vargas 2000) support the idea that grassland may not empede gene flow between neighboring forests in *P. coronatus* populations.

It is important to note, however, that habitat fragmentation is suspected to have significantly increased in recent decades across the known area of occupancy of *P. coronatus* (Rakotonirina *et al.* 2013; Ramanamisata and Razafindraibe, 2013; Razafindramanana *et al.* 2013; Salmona *et al.* 2014), though other authors have suggested an opposite trend (Andriamasimanana and Cameron, 2014). Concerning the area under investigation (see Fig. 1, Table 3), grassland divides the north and the south and this partition is the one making most sense in terms of the AMOVA analysis (11% of the variance is explained by a north/south grouping—see Table 3 and Fig. S2). We can suggest that a circulation of female individuals from the north to the south, and vice versa, is unlikely throughout the grassland itself, but becomes possible from neighboring forest fragments.

Assuming that the barriers to dispersal are the same in the three sister species (*P. coronatus*, *P. tattersalli* and *P. perrieri*), the absence of large rivers in the area investigated could also explain the weak genetic structure. Moreover, paleoenvironmental data revealed that the local landscape was characterized by a mosaic of dry forest and grassland even about 3,500 years BP, and by grassland with fire-adapted trees and ruderal herbs in the last 1000 years (Matsumoto and Burney 1994; Burns *et al.* 2016). These findings suggest that

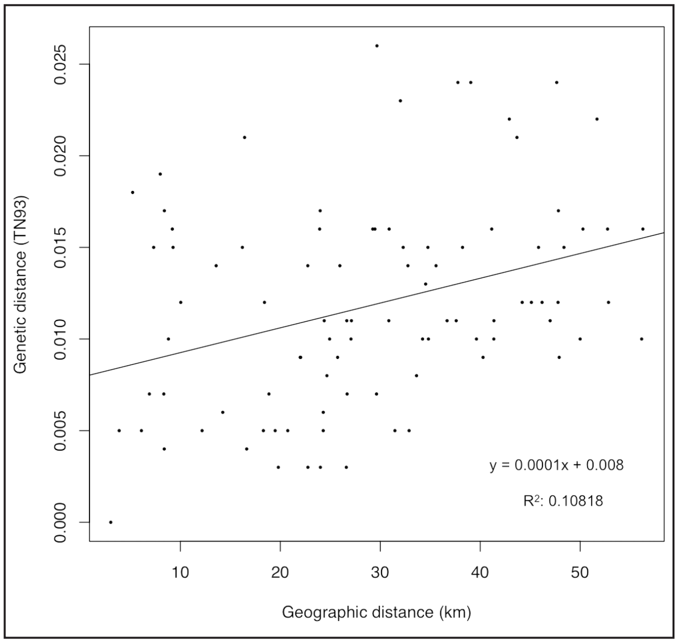

Figure 3. Isolation by distance among sampling sites. Plot of pairwise genetic distances (according to the TN93 model in Tamura and Nei (1993) versus corresponding geographic distances (kilometers). A significant linear regression is shown.

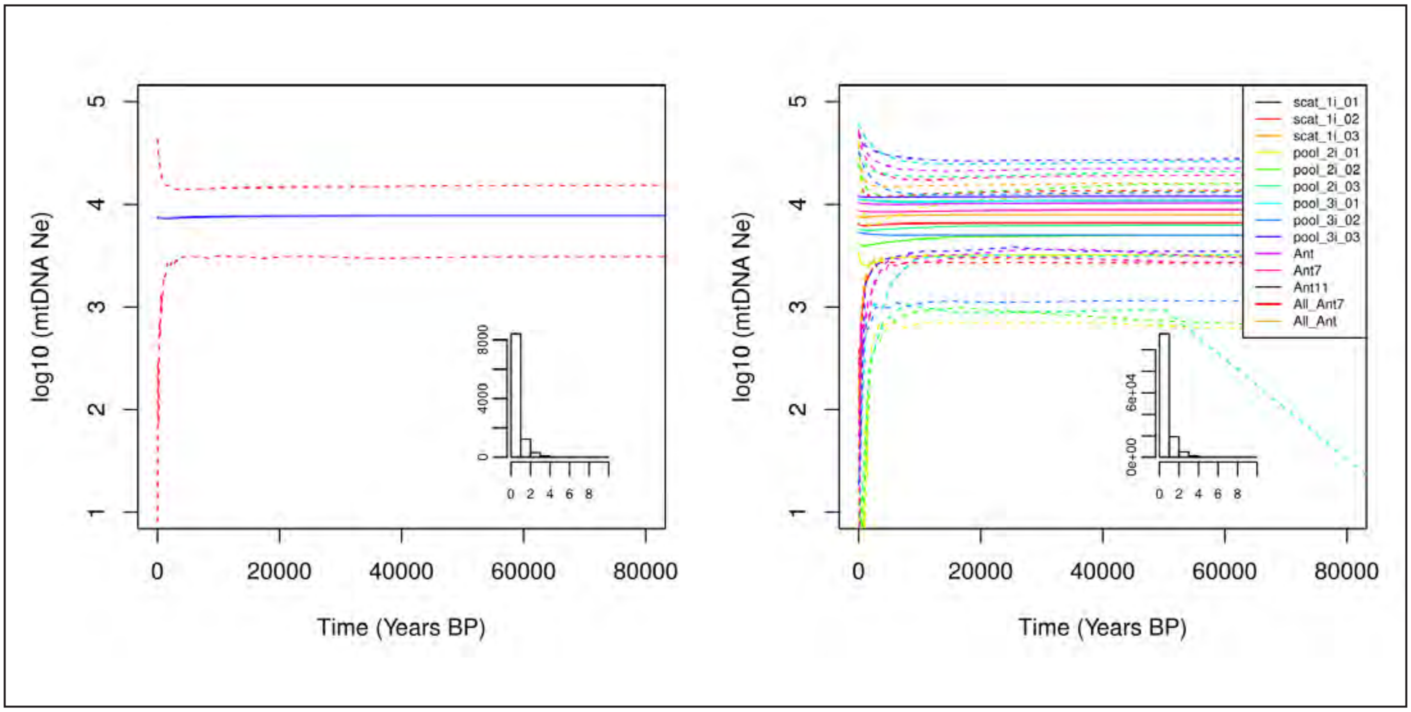

Figure 4. Extended Bayesian skyline plot using four sampling strategies. Plots of the median estimates (solid line) of the mtDNA effective population size over time obtained from D-loop sequences of *P. coronatus*. The dashed lines represent the 95% HPD boundaries. The histograms show the Posterior Size Change (PSC) values. The left graph shows the demographic trend using the"All_Ant11" dataset. The right graph shows the demographic trend of all other datasets. Scat = "scattered"; pool = "pooled."

crowned sifakas have long coped with fragmented landscapes, over at least 3,500 years, with a likely recent increase in the last decades (Harper *et al.* 2007).

The weak genetic differentiation detected may thus reflect the current or recent past abilities of females in traversing grassland and other open habitats to occupy dispersed forest patches. While female philopatry seems to be more predominant amongst *Propithecus*, female dispersal has been shown to occur under certain condition (Morelli *et al.* 2009). Although there is no record of *P. coronatus* crossing wide gaps of open habitat, other sifaka species are able to do so (Meyers and Wright 1993; Richard *et al.* 1993; Mayor and Lehman 1999), and its sister species *P. verreauxi* is known for its ability to walk on the ground (Mittermeier *et al.* 2010).

Demographic history

Most of the methods that we used to reconstruct the demographic history of *P. coronatus* implicitly or explicitly assume that there is no structure and that the samples were obtained from an isolated panmictic population. As demonstrated by several recent studies, population structure can generate spurious signals of population size change (Städler *et al.* 2009; Chikhi *et al.* 2010; Heller *et al.* 2013). To better control for the confounding effect of population structure, Städler *et al.* (2009) suggested the use of a *scattered* sampling strategy (one sample retrieved from each deme) as it gives results comparable, in terms of genealogical structure, to a panmictic population that has experienced the same temporal demographic history (for example, contraction). More recently, Heller *et al.* (2013) suggested the *pooled* sampling strategy as the best one to detect signals of population size expansion and decline. Because of this we used various sampling strategies for our demographic analyses.

Altogether the Extended Bayesian Skyline Plot (EBSP) analyses suggest a constant mtDNA N_e over the time, whichever sampling scheme was adopted. The EBSP showed wide confidence intervals, however, for mtDNA N_e and for the number of population-size-change events (Fig. 4 and Table 4). Since with confidence intervals containing one or more changes it is not possible to retain with certainty a constant population hypothesis, our results should be considered with caution (Table 5; <http://beast.bio.ed.ac.uk/tutorials>) because the lack of signal could be related to a lack of statistical power.

Under the coalescent assumption of constant population size, by using software BEAST we estimated a median mtDNA N_e of 11,883 (95% HPD: 5,107−20,083; generation time of 6 years) that falls within the range of the values obtained by EBSP (median: 32,172; 95% HPD: 1,471–373,933). This agreement was expected and suggests that, at least at the geographical scale surveyed and for mtDNA, a constant population size model is a reasonable choice for *P. coronatus*. These results were confirmed to a large extent by three summary statistics (Tajima's *D*, Fu's *F*s and R_2). With the exception of the Antrema sampling ("Ant" and "Ant11"; Table 5), which showed significant but contradictory signals between the three statistics, most of the results were not suggesting any particular departure from mutation-drift equilibrium.

In short, when using mtDNA our results suggest that there is no genetic signal of contraction or expansion in *P. coronatus* across the northern part of its distribution. It would be important to complement this study with the addition of nuclear markers such as microsatellites. Microsatellites have been developed for this species (Lei *et al.* 2008) and could therefore be used to determine whether the lack of genetic structure can be confirmed and whether recent demographic events not visible with mtDNA can be detected with nuclear markers.

It is important to link the genetic estimates for the population size of crowned sifaka with recent census data concerning the region under investigation. The population was estimated to be between 4,226 and 36,672 individuals, and most probably above 10,000, with some localities harboring high densities (>300 ind/km^2; Salmona *et al.* 2014). While these authors did not survey the southern part of the range, they suggested that there could be as many as 130,000 to 220,000 individuals across the entire species' distribution. As the authors themselves admit, these figures are disputable, though they can be regarded as an upper limit. If the current mtDNA N_e is between 5,107 and 20,083 (median: 11,262), this implies by definition (assuming that they are evolving neutrally in a panmictic population with an equal breeding sex ratio) a theoretical nuclear N_e of 20,428 to 80,332 (median: 45,048) as the effective population size of mitochondria is ¼ of nuclear genes. The estimated nuclear N_e is, therefore, rather high when compared to the census sizes computed in the northern area by Salmona *et al.* (2014), thus suggesting that a recent population decline might have occurred despite the fact that mtDNA data do not allow us to detect it with existing methods. To conclude, while mtDNA effective population size estimations (using EBSP and the constant population-size model) depend on the length of the generation time, still debated in *P. coronatus*, we note that the N_e we estimated is in agreement with the extrapolation of Salmona *et al.* (2014). Again, more samples (from the southern distribution of the species) and more genetic data accross all samples are needed to obtain more reliable estimates.

Conservation implications

In this study, we found substantial mitochondrial genetic diversity within the NAP-Antrema and along the Mahavavy riverbanks. Interestingly, these are the areas with high densities of *P. coronatus* (up to 300 ind/km^2; Salmona *et al.* 2014). While *P. coronatus* benefits from considerable conservation efforts in the NAP-Antrema, we believe that more efforts are necessary along the Mahavavy riverbanks, inside and outside the Mahavavy-Kinkony protected area.

The high population densities found by Salmona *et al.* (2014) and the substantial genetic diversity found in the region (this study) are likely a partial consequence of a taboo against hunting the crowned sifaka among the Sakalava population inhabiting the area (Harpet *et al.* 2008). Conservation measures in all areas should reinforce this traditional respect in their conservation and community outreach strategies. The NAP-Antrema community, for instance, benefits from the presence of the traditional leader of this region, Prince Tsimanendry, who lives in Antrema, in his efforts to protect one of the surviving primary coastal dry forests in northwestern Madagascar (Gauthier *et al.* 1999).

Our results reveal limited genetic structure in the northern distribution of *P. coronatus*. This suggests that crowned sifakas were recently and may still be able to move between neighboring forest fragments and, potentially, across grassland. However, since sifakas may have a long generation time and the landscape has been particularly impacted in the last 60 years (Harper *et al.* 2007; ONE 2013; Schwitzer *et al.* 2014a, 2014b), it is difficult to determine when or if the dispersal stopped between forest fragments.

Future conservation efforts should focus on re-establishing connectivity between forest fragments of the region and on maintaining current available habitat. Regular monitoring of *P. coronatus* and its habitat is also needed. Anthropogenic disturbances can be expected to increase in the near future throughout Madagascar (for example, slash-and-burn, illegal logging). Based on current human population growth rates in Madagascar (3.39% per year), Vieilledent *et al.* (2013) estimated that the popuation size may double from 21 million in 2011 to 40 million in 2045. This will undoubtedly increase significantly the intensity of deforestation to values as high as 1.17% per year by 2030 (from 1990 to 2000, it was around 0.9% per year; Harper *et al.* 2007) and may threaten *P. coronatus*' populations viability accordingly.

Acknowledgments

We thank the Direction Générale du Ministère de l'Environnement et des Forêts de Madagascar (Région Boeny), Madagascar's *Ad Hoc* Committee for Fauna and Flora and Organizational Committee for Environmental Research (CAFF/CORE), the NGO Fanamby (especially S. Rajaobelina and V. Rasoloarison, P. Ranarison, B.D. Razafindrakoto, and S. Wohlhauser). This study benefited from the continuous support of the Department of Animal Biology and Ecology, University of Mahajanga and the Department of Animal Biology, University of Antananarivo as well as from the NAP'Antrema via the Conventions cadres de Cooperation between France and Madagascar (research authorization 061/11/MEF/SG/DGF/DCB.SAP/SCB and sample export authorization 091N-EV07/MG11). The fieldwork would not have been possible without the participation of Claire Pichon, a PhD student at the NAP-Antrema. We also warmly thank the many local guides and cooks for sharing their incomparable expertise about the forest and for their help in fieldwork. Financial support for this study was provided by two LabEx grants (ANR-10-LABX-41-TULIP; ANR-10-LABX-0003-BCDiv, ref. n° ANR-11-IDEX-0004-02). Further support was provided by the Portuguese Fundação para a Ciência e Tecnologia (PTDC/BIA-BEC/100176/2008, PTDC/BIA-BIC/4476/2012 and SFRH/BD/64875/2009), by the Groupement de Recherche International (GDRI) Biodiversité et developpement durable – Madagascar and by the UMR-7206 of CNRS and the Muséum National d'Histoire Naturelle in Paris. The study was conducted in agreement with the laws of the countries of Portugal, France and Madagascar.

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Received for publication: 26 August 2015
Revised: 5 June 2016

Appendix

Table S1. Primer design based on conserved regions. Dloop (a) and tRNA (b) tables show the 61 sequences of lemur species, aligned to identify the conserved sequences in designing the primer. Using MITOMAP human project, the more conserved regions surrounding the mitochondrial Hypervariable Region 1 have been identified (c): (i) tRNA proline gene; and (ii) Conserved Domain which divides HVS-1 from HVS-2 within the Control Region.

(a)

Definition	**ID**	**Definition**	**ID**
Avahi cleesei BEMA8	DQ856036	*Avahi occidentalis* MAR46	DQ856042
Avahi cleesei BEMA9	DQ856037	*Avahi occidentalis* MAR52	DQ856043
Avahi cleesei BEMA13	DQ856039	*Avahi occidentalis* MAR54	DQ856044
Avahi cleesei BEMA14	DQ856040	*Avahi occidentalis* MAR55	DQ856045
Avahi laniger AND14	DQ856018	*Avahi occidentalis* MAR56	DQ856046
Avahi laniger AND19	DQ856019	*Avahi occidentali*s MAR60	DQ856047
Avahi laniger AND33	DQ856020	*Avahi occidentalis* MAR61	DQ856048
Avahi laniger AND34	DQ856021	*Avahi unicolor* ANT5.8	DQ856032
Avahi laniger NARA4.1	DQ856022	*Avahi unicolor* ANT5.9	DQ856033
*Avahi lanige*r NARA4.2	DQ856023	*Avahi unicolor* ANT5.10	DQ856034
Avahi laniger NARA4.10	DQ856024	*Avahi unicolor* ANT5.12	DQ856035
Avahi laniger NARA4.11	DQ856025	*Indri indri* JAR4	DQ855966
Avahi laniger NARA4.13	DQ856026	*Indri indri* MIZA5.3	DQ855967
Avahi laniger NARA4.17	DQ856027	*Homo sapiens*	NC_012920
Avahi laniger NARA4.18	DQ856028	*Pan troglodytes*	NC_001643
Avahi laniger NARA4.23	DQ856029	*Propithecus candidus* JAR14	DQ855969
Avahi laniger NARA4.31	DQ856030	*Propithecus coquereli*	NC_01105
Avahi laniger NARA4.32	DQ856031	*Propithecus coquereli* BOR2	DQ855971
Avahi laniger RANO2.10	DQ855979	*Propithecus coronatus* JAM4.7	DQ855974
*Avahi laniger*RANO66	DQ855975	*Propithecus deckeni* BEMA4	DQ855973
Avahi laniger RANO199	DQ855976	*Propithecus diadema* TAD13	DQ855970
Avahi laniger RANO319B	DQ855977	*Propithecus perrieri* ANAL10	DQ855968
Avahi laniger RANO348	DQ855978	*Propithecus tattersalli* DAR4.2	DQ855972
Avahi occidentalis MAR29	DQ856041		

(b)

Species	**ID**	**Species**	**ID**
Lepilemur hubbardorum	NC_014453	*Eulemur f. fulvus*	AB371086
Lepilemur ruficaudatus	NC_021953	*Eulemur f. mayottensis*	AB371087
Hapalemur griseus	NC_021950	*Avahi laniger*	NC_021940
Eulemur macaco	AB371088	*Propithecus coquereli*	NC_01105
Eulemur rufus	NC_021948	*Varecia variegata*	AB371089
Lemur catta	AJ421451	*Prolemur simus*	NC_021959
Eulemur mongoz	AM905040	*Cheirogaleus medius*	NC_021945

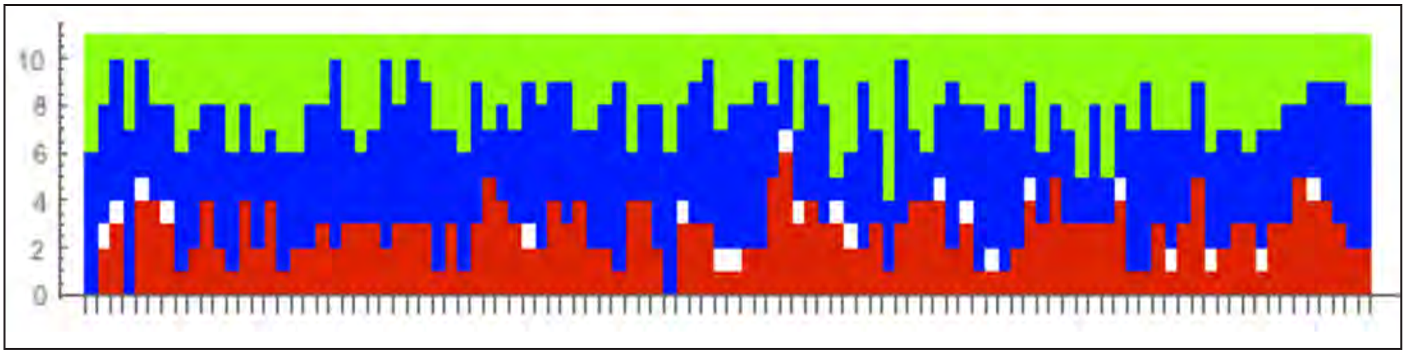

Figure S1. Random sampling of 11 individuals, among the 69 samples from the Badrala Forest. To avoid biased estimation of the genetic diversity and differentiation due to higher sample size from Antrema (n = 69) with respect to the other sampling sites (total n = 56), we built a random distribution of 11 individuals from the 69 individuals of Antrema. The most probable combination was used for further analysis. In order to visualize the probability distribution of 11 random samples, we randomly chose 100 combinations. The probability distribution is a "Multivariate Hypergeometric Distribution" with four variables, one for each haplotype detected among the 69 samples of Antrema (H3: 17; H4: 1; H6: 29; H7: 22). One hundred combinations, randomly chosen, are shown in this graph; each vertical rectangle corresponds to a combination. The colors represent the four haplotypes and the number of sampled individuals for each haplotype is indicated by the height of the colored portion of the rectangle. Red = H3; White = H4; Blue = H6; Green = H7. The most probable combination is **H3:** 3, **H4:** 0, **H6:** 5, **H7:**3, with probability of 0.068187. The probability distribution and the most probable combination were computed using Mathematica (<www.wolfram.com/mathematic>).

Table S2. Pairwise Φ_{ST} values among 14 sites. The table shows the pairwise comparisons of the genetic differentiation among the 14 sites. Almost all pairwise Φ_{ST} are not significant (* = P-value < 0.05; ** = P-value < 0.01).

	AMB	**AMO**	**AND**	**ANK**	**ANT**	**ANS**	**ANO**	**BOE**	**BON**	**KAT**	**KIN**	**MAT**	**MAZ**	**TSI**
AMB	0													
AMO	0,63	0												
AND	-0,17	0,63	0											
ANK	0,43	0,49	0,45	0										
ANT	-0,07	0,53 **	0,05	0,10	0									
ANS	0,01	0,40 **	0,02	-0,02	-0,04	0								
ANO	0,06	-0,04	0,10	-0,04	0,01	-0,08	0							
BOE	-0,51	0,52	-0,34	0,34	-0,32	-0,28	-0,56	0						
BON	-0,51	0,52	-0,34	0,34	-0,32	-0,28	-0,56	0	0					
KAT	0,13	0,10	0,18	0,19	0,17	0,05	-0,21	-0,22	-0,22	0				
KIN	-0,03	0,54	0,08	0,19	-0,07	-0,03	-0,04	-1,00	-1,00	0,13	0			
MAT	-0,14	0,66 **	-0,11	0,39 *	0,02	0,01	0,21	-0,19	-0,19	0,21	0,09	0		
MAZ	-0,20	0,55	-0,03	0,24	-0,16	-0,18	-0,14	-1,00	-1,00	-0,06	-0,31	-0,24	0	
TSI	0,13	0,30	0,19	-0,05	0,01	-0,05	-0,21	-0,21	0,01	-0,03	0,16	-0,11	-0,18	0

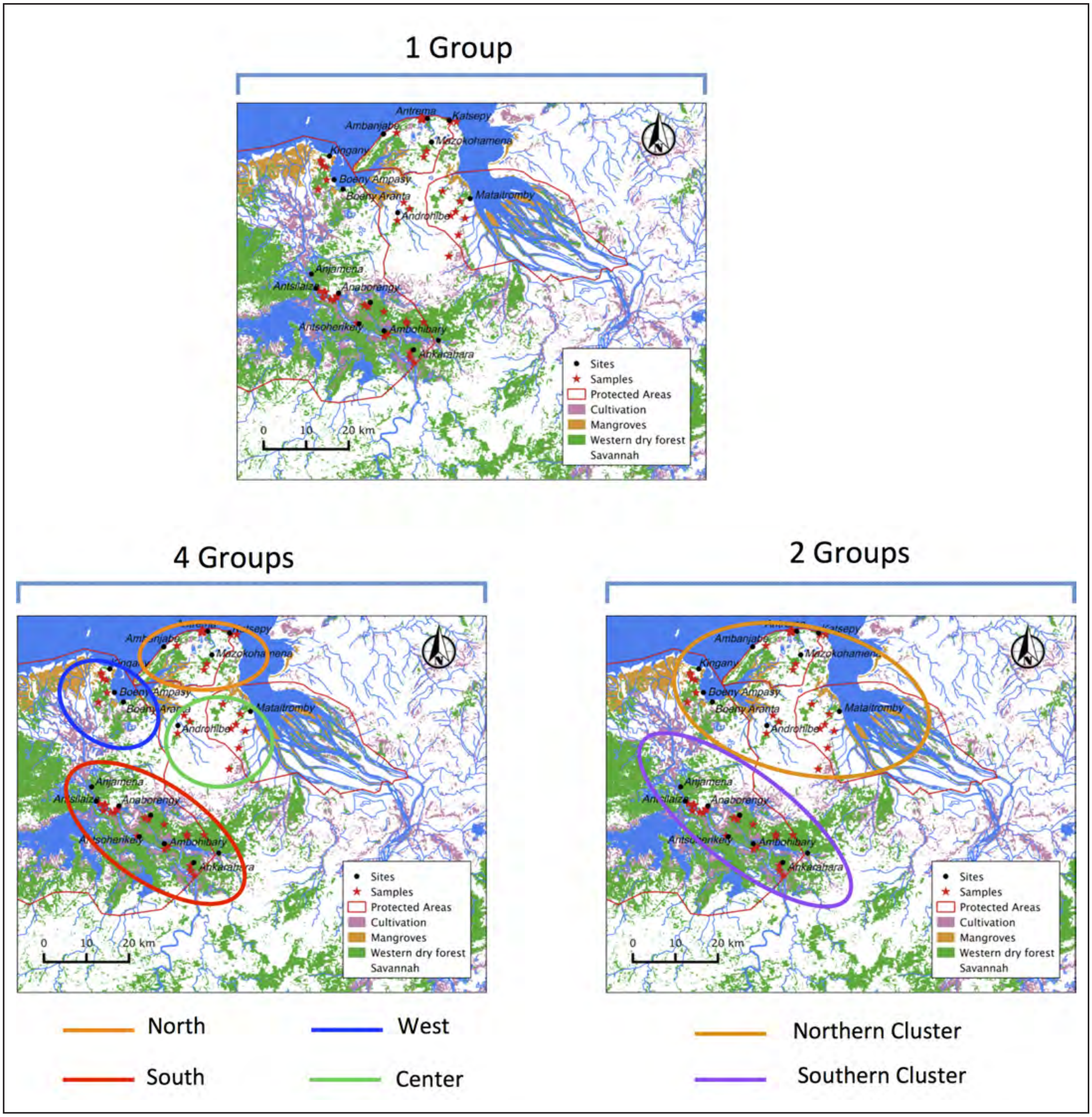

Figure S2. Visual framework of tested groupings in AMOVA analyses. Above − All the 14 sampling sites are included in one group. Below left − Four groups were defined, according to the presence of artificial barriers (e.g., main roads) and natural barriers (e.g., small rivers, savannah and cultivated lands), which would relatively isolate the four groups: North (AMB, ANT, MAZ, KAT), South (ANS, AMO, ANO, ANK, TSI), West (KIN, BOE, BON), Center (MAT, AND). Below right − The two groups were justified by the presence of a relatively large stretch of grasslands between the two groups of sites: Northern (AMB, ANT, MAZ, KAT, KIN, BOE, BON, MAT, AND), Southern (ANS, AMO, ANO, ANK, TSI). Northern and Southern cluster included; 33 and 30 individuals, respectively.

Table S3. mtDNA effective population size. Eff. Pop. Size Mean = the mean value of the mtDNA effective population size; Eff. Pop. Size Median = the median value. 95% HPD is the 95% confidence interval of the mean. These values were estimated from three different datasets. Estimations obtained assuming generation time of a) 3 years and b) 17.5 years.

a)

	Eff. pop. size mean	Eff. pop. size Median	95% HPD
All_ Ant	20,154	19,172	[8,265; 33,417]
All_ Ant7	24,209	22,747	[9,343; 41,280]
All_ Ant11	23,767	22,524	[10,213; 40,167]

b)

	Eff. pop. size mean	Eff. pop. size Median	95% HPD
All_ Ant	3,455	3,287	[1,417; 5,729]
All_ Ant7	4,150	3,899	[1,602; 7,077]
All_ Ant11	4,074	3,861	[1,751; 6,886]

Primate Conservation 2016 (30): 59–72

A New Species of Dwarf Lemur (Cheirogaleidae: *Cheirogaleus medius* Group) from the Ankarana and Andrafiamena-Andavakoera Massifs, Madagascar

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Abstract: A new species of dwarf lemur, *Cheirogaleus shethi* sp. nov., of the *C. medius* group is described from the dry and transitional forests of northern Madagascar. This species can be found along the forest corridor from Ankarana Special Reserve east to the Analamerana Special Reserve down to the Bekaraoka forest in the Loky-Manambato Protected Area. This species is genetically distinct from other members of the *C. medius* species group and is sister to a poorly known lineage in Sambava. The identification of this new species highlights the importance of northern Madagascar as a reservoir of biodiversity.

Key Words: Dwarf lemurs, primate, Strepsirrhini, taxonomy

Introduction

Dwarf lemurs (Cheirogaleidae: *Cheirogaleus*) are small, arboreal, nocturnal strepsirrhine primates endemic to the island of Madagascar (Groves 2000; Lei *et al.* 2014). The genus *Cheirogaleus* has recently been the focus of in-depth field research combined with genetic analyses that revealed greater species diversity within this genus than previously suspected (Groves 2000; Hapke *et al.* 2005; Groeneveld *et al.* 2009, 2010; Thiele *et al.* 2013; Lei *et al.* 2014, 2015). Field expeditions undertaken from the Ankarana Special Reserve (SR) east to the Analamerana SR have resulted in the identification of a distinct dwarf lemur in this region (Lei *et al.* 2014). This lineage was recognized previously (Hapke *et al.* 2005; Thiele *et al.* 2013) and is described here as a new species, although with a slightly different circumscription than initially suggested (Thiele *et al.* 2013).

Taxonomic expansion has occurred in other lemur genera, in particular *Microcebus* and *Lepilemur* (Louis *et al.* 2006a, 2006b, 2008; Rasoloarison *et al.* 2013; Hotaling *et al.* 2016). The proliferation of new lemur species has been addressed in the scientific literature, both supportively by those who view it as justified and critically by those who do not (Thalmann 2007; Tattersall 2007, 2013). A combination of factors has led to controversy about species status in dwarf lemurs. The arboreal and nocturnal habits of *Cheirogaleus* make them difficult to study in the wild. Additionally, the small number of available museum specimens and their spottily documented collection localities often limit their utility. Based on morphology, Groves (2000) recognized seven species of *Cheirogaleus*: *C. major* É. Geoffroy Saint-Hilaire, 1812; *C. medius* É. Geoffroy Saint-Hilaire, 1812; *C. crossleyi* Grandidier, 1870; *C. adipicaudatus* Grandidier, 1868; *C. sibreei* (Forsyth Major, 1896); *C. ravus* Groves, 2000; and *C. minusculus* Groves, 2000. Groeneveld *et al.* (2009) subsequently synonymized *C. ravus* into *C. major*, and *C. adipicaudatus* into *C. medius*. Groves' (2000) specific delimitation can, however, now be seen as overly conservative based on recent fieldwork and genetic research (Hapke *et al.* 2005; Groeneveld *et al.* 2009, 2010; Thiele *et al.* 2013; Lei *et al.* 2014, 2015). Lei *et al.* (2014, 2015) proposed four new species, including the recently described *Cheirogaleus andysabini* Lei *et al.*, 2015, and resurrected *C. thomasi* (Forsyth Major, 1894) (corresponding in large part to *C. adipicaudatus*). Thiele *et al.* (2013) also described a new species, *C. lavasoensis*, bringing the current number of accepted species to eight. It is likely that other species remain to be described, with an ever-growing enumeration presented in the "Species groups of *Cheirogaleus*" section of Lei *et al.* (2014; pp.17−22). Gardner and Jasper (2015) reported a possible new *Cheirogaleus* lineage from the island of Nosy Hara in northern Madagascar.

Hawkins *et al.* (1990) mentioned a dwarf lemur in Ankarana Special Reserve in northern Madagascar and referred to it as "*Cheirogaleus* ? *medius*." They suggested trapping to clarify the species' identification, as it could also have been *C. major*. Hapke *et al.* (2005) incorporated a genetic sample from Ankarana that was taken from the Collection des specimens morts, Parc Botanique et Zoologique Tsimbazaza in Antananarivo. The Ankarana species was confirmed to be a member of the *C. medius* group (designated CmeB in that study, GenBank accession number AY605904), but Hapke *et al.* (2005) hinted that this individual may be a member of an independent lineage based on genetic distance, but did not propose designating it as such. Groeneveld *et al.* (2009, 2010) expanded the dataset with additional samples from northern Madagascar, Sambava and Bekaraoka. The individuals from Sambava were placed as sister to those from Ankarana and Bekaraoka, and all were identified as *C. medius*. Thiele *et al.* (2013) also expanded the dataset with the inclusion of genetic data from dwarf lemurs in southern Madagascar. Although they did not supplement the northern dataset, additional analytical tools were applied in Thiele *et al.*'s work (2013) that also supported the sister relationship of Sambava to Bekaraoka within the *C. medius* group. The Ankarana individual (Hapke *et al.* 2005; Groeneveld *et al.* 2009, 2010) was not included, possibly due to missing data. Thiele *et al.* (2013) recognized that dwarf lemurs in the Sambava (subgroup CmeA) and Bekaraoka (CmeB) area of Madagascar represented a separate lineage and gave it the interim designation of *Cheirogaleus* sp. Bekaraoka Sambava (CmeA+CmeB).

Multiple field expeditions from the Ankarana SR east to the Analamerana SR, crossing through the Andrafiamena-Andavakoera Protected Area augmented the *Cheirogaleus* dataset (Lei *et al.* 2014). In this study, genetic data grouped individuals from Ankarana and Bekaraoka into one clade; the lineage was identified as a probable new species of dwarf lemur (*C.* sp. nov. 4, Fig. 1). Individuals from Sambava were excluded from *C.* sp. nov. 4, and were referred to as Confirmed

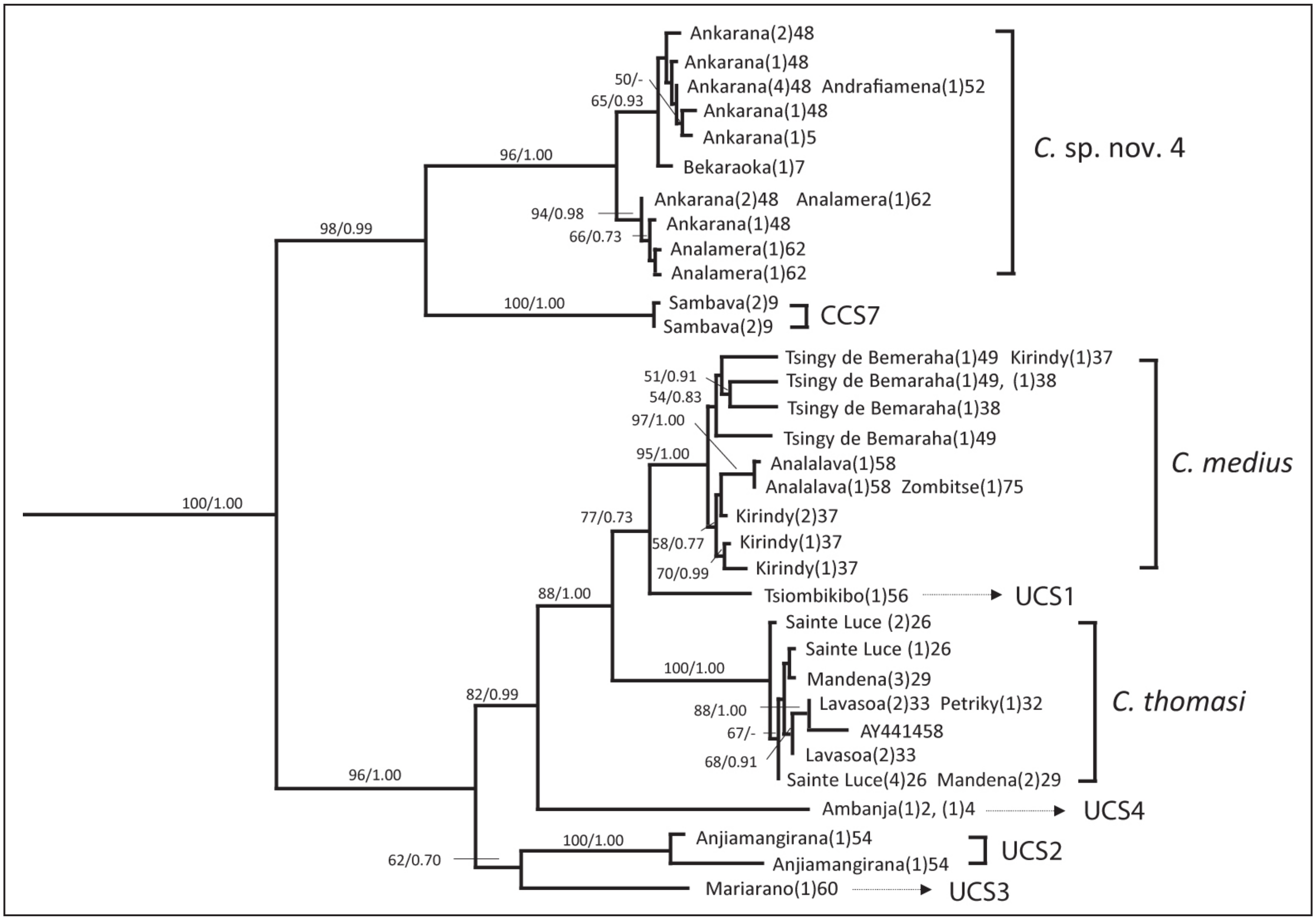

Figure 1. Subtree of the phylogenetic relationships between taxa within the *Cheirogaleus medius* group based on cytb sequence data as presented in Lei *et al.* (2014; Fig. 2). Numbers on branches represent maximum likelihood values followed by posterior probability support. Tip labels include locality,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

Candidate Species (CCS) 7, but more information is required to confidently describe the CCS7 lineage (Lei *et al.* 2014).

The Ankarana SR covers more than 18,000 ha in northern Madagascar and is about 108 km southwest of the large port city of Antsiranana. Ankarana was established in 1956, and first explored widely in the 1960s (Wilson *et al.* 1988). A plateau of limestone dating to the Jurassic Period, caves and underground rivers, and deciduous tropical forests are the most notable geographic features of the reserve (Rossi 1974a; Fowler 1989). The cave systems of Ankarana have been featured in popular writings and documentaries as a wonder of the natural world (Wilson 1990). Ankarana displays great diversity in birds (Fowler *et al.* 1989) and cave-dwelling marine life (Gurney 1984; Palacios-Vargas and Wilson 1990; Banister 1994; Wilson 1996), as well as primates (Wilson *et al.* 1995). In addition to *Cheirogaleus*, Ankarana SR is home to populations of the lemur genera *Avahi*, *Daubentonia*, *Eulemur*, *Hapalemur*, *Lepilemur*, *Microcebus*, *Phaner*, and *Propithecus* (Wilson *et al.* 1988, 1989, 1995). Subfossil remains of several extinct lemur species (*Archaeolemur*, *Babakotia*, *Megaladapis*, *Mesopropithecus*, *Pachylemur*, *Paleopropithecus*), as well as two genera still extant elsewhere in Madagascar but now extinct locally (*Indri indri*, *Prolemur simus*), have also been found in the caves of Ankarana (Wilson *et al.* 1995; Godfrey *et al.* 1996).

The limestone massif extends east through the Andrafiamena forest to the Analamerana SR (Du Puy and Moat 1996), which has nearly 35,000 ha ranging from sea level to 650 m (Mittermeier *et al.* 2010). Analamerana SR is located to the north and east of Ankarana and about 100 km southwest of Antsiranana (Mittermeier *et al.* 2010). In the 1970s this area was referred to as uninhabited by humans (Rossi 1974b), and the lemur diversity found there reflects the historically low pressure from humans. Lemur species with distributions from Ankarana to Analamerana, similar to that of *C.* sp. nov. 4, include the Critically Endangered Perrier's sifaka (*Propithecus perrieri*) and the Endangered *Lepilemur ankaranensis* (Mittermeier *et al.* 2010; Salmona *et al.* 2013, 2015a, 2015b); both of which are threatened due to human activities elsewhere within their very small geographic ranges. Fortunately, much of the area from Ankarana east to Analamerana is now protected either as special reserves or as an IUCN Category V protected area, which strives to maintain a balance between wildlife and habitation by people.

To the south of the Ankarana-Analamerana massif, the Loky River has eroded away the sandstone, creating an expansive low area prone to flooding, which has been used extensively for agriculture (Rossi 1974b). On the other side of this highly anthropogenically modified area is the Bekaraoka Forest near Daraina. This area is flanked by the Loky and Manambato rivers and is within the Loky-Manambato Category V Protected Area. Although rivers have been cited as possible barriers to dispersal, the geographic range of *Lepilmur milanoii* straddles the Loky River similar to that of *C.* sp nov. 4; *L. milanoii* is found in numerous forests of the Loky-Manambato region and the Andrafiamena Classified Forest (Mittermeier *et al.* 2010; Salmona *et al.* 2014).

Genetic samples, measurements and photographs of several *Cheirogaleus* individuals captured along the Ankarana-Andrafiamena-Analamerana corridor were obtained prior to their release (Fig. 2). The genetic samples were analyzed as part of a larger phylogenetic study of the genus *Cheirogaleus* conducted by Lei *et al.* (2014) including pre-existing data from Bekaraoka (Groeneveld *et al.* 2009). Together, they were determined to be consistently distinct from all other samples of the *C. medius* group and designated as *C.* sp. nov. 4 (Lei *et al.* 2014). Here, we describe this new species of dwarf lemur endemic to the Ankarana-Andrafiamena-Analamerana corridor and the Loky-Manambato region in northern Madagascar.

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 enumerate only those belonging to the *C. medius* group along with individuals used as outgroups from other *Cheirogaleus* species (Table 1). From the focal lineage, *C.* sp. nov. 4, ten individuals from Ankarana SR, two individuals from Andrafiamena-Andavakoera protected area, and three individuals from Analamerana SR were immobilized by the field team from the Madagascar Biodiversity Partnership (MBP; Table 2, Fig. 2). An additional individual from Ankarana was immobilized in 2015 and was incorporated into this work.

Immobilization was carried out as reported in Louis *et al.* (2006a), and genetic samples were acquired as described by Lei *et al.* (2014). Morphometric measurements taken on sedated lemurs using bone anatomical landmarks were recorded as in Louis *et al.* (2006a) and standardized according to the guidelines of Smith and Jungers (1997). All lemurs were released at their point of capture within 24 hours. 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.

Data generation and phylogenetic analyses

Methods used to identify this new species were presented in Lei *et al.* (2014). In addition, a Bayesian species delimitation analysis was performed using the bPTP webserver (http://species.h-its.org; Zhang *et al.* 2013) with 100,000 Markov Chain Monte Carlo generations as in Lei *et al.* (2015). The cytb tree was used as the starting tree as it was the most inclusive dataset allowing for the incorporation of previously published sequence data from Bekaraoka (EU825327; Groeneveld *et al.* 2009) and Ankarana SR (AY605904; Hapke *et al.* 2005). All haplotypes recovered in Lei *et al.* (2014) of *C.* sp. nov. 4 that were sampled by MBP from Ankarana SR east

Table 1. Free-ranging *Cheirogaleus* samples used in this study. IDs correspond to Table 1 (Lei *et al*. 2014), with the exception of previously published samples denoted at the bottom of this table.

ID	Species designation	Location	Latitude	Longitude
ANK5.12	*C.* sp. nov. 4	Ankarana	-12.96631	49.13808
ANK5.13	*C.* sp. nov. 4	Ankarana	-12.96631	49.13808
ANK5.14	*C.* sp. nov. 4	Ankarana	-12.96631	49.13808
ANK5.15	*C.* sp. nov. 4	Ankarana	-12.96631	49.13808
ANK5.16	*C.* sp. nov. 4	Ankarana	-12.96631	49.13808
ANK5.17	*C.* sp. nov. 4	Ankarana	-12.96631	49.13808
ANK5.18	*C.* sp. nov. 4	Ankarana	-12.96631	49.13808
ANK5.19	*C.* sp. nov. 4	Ankarana	-12.96631	49.13808
ANK5.20	*C.* sp. nov. 4	Ankarana	-12.96631	49.13808
ANK5.21	*C.* sp. nov. 4	Ankarana	-12.96631	49.13808
KAR15.1	*C.* sp. nov. 4	Ankarana	-12.95636	49.12969
FIA5.19	*C.* sp. nov. 4	Andrafiamena (Anjakely)	-12.91539	49.31956
FIA5.22	*C.* sp. nov. 4	Andrafiamena (Anjakely)	-12.91539	49.31956
MATY5.31	*C.* sp. nov. 4	Analamerana (Ampasimaty)	-12.76556	49.48358
MATY5.40	*C.* sp. nov. 4	Analamerana (Ampasimaty)	-12.76703	49.48358
MATY5.42	*C.* sp. nov. 4	Analamerana (Ampasimaty)	-12.77136	49.48303
BEMA7.19	*C. medius*	Tsingy de Bemaraha	-19.04525	44.77772
BEMA7.21	*C. medius*	Tsingy de Bemaraha	-19.04581	44.78119
BEMA7.22	*C. medius*	Tsingy de Bemaraha	-19.05383	44.78075
LAVA1	*C. medius*	Analalava	-22.59242	45.13333
LAVA45	*C. medius*	Analalava	-22.58778	45.12803
ZOM6.2	*C. medius*	Zombitse	-22.88631	44.69375
KIBO7.9	UCS1*	Tsiombikibo	-16.04886	45.81067
HIH7.3	UCS2*	Anjiamangirana	-15.21642	47.75189
HIH9	UCS2*	Anjiamangirana (Antsohihy)	-15.15692	47.73311
MAR30	UCS3*	Mariarano	-15.47992	46.69333
AMB5.27	*C. andysabini*	Montagne d'Ambre	-12.51722	49.1795
GAR8	CCS2*	Manongarivo	-14.02369	48.27233
KAL7.7	*C. lavasoensis*	Kalambatritra (Sahalava)	-23.53672	46.5335
MIZA16	*C. crossleyi*	Maromizaha	-18.97375	48.46461
TRA8.81	*C.* sp. nov. 2	Andringitra (Ambarongy)	-22.22269	47.01889
POLO5.2	*C. major*	Tampolo	-17.28989	49.40753
JOZO4.17	*C. sibreei*	Anjozorobe	-18.46789	47.94131
Roos *et al.* (2004), Hapke *et al*. (2005), Groeneveld *et al.* (2009) and Thiele *et al.* (2013)				
AY605904	*C.* sp. nov. 4	Ankarana	-12.9250	49.1250
EU825327	*C.* sp. nov. 4	Bekaraoka	-13.1047	49.7074
EU825333	*C. medius*	Bemaraha	-19.1036	44.7675
EU825325	*C. medius*	Kirindy	-20.0737	44.6757
EU825326	*C. medius*	Kirindy	-20.0737	44.6757
EU825328	CCS7*	Sambava	-14.3994	50.1739
EU825329	CCS7*	Sambava	-14.3994	50.1739
EU825323	UCS4*	Ambanja/Ambato	-13.3958	48.4705

table continued on next page

Table 1. *continued*

ID	Species designation	Location	Latitude	Longitude
AY605905	*C. thomasi*	Ste. Luce	-24.7730	47.1710
AY605906	*C. thomasi*	Ste. Luce	-24.7730	47.1710
AY605907	*C. thomasi*	Ste. Luce	-24.7730	47.1710
AY605908	*C. thomasi*	Mandena	-24.9530	46.9920
AY605909	*C. thomasi*	Petriky	-25.0610	46.8730
AY605910	*C. thomasi*	Lavasoa	-25.0840	46.7660
AY441458	*C. thomasi*	n/a	n/a	n/a

* CCS and UCS designations are from Lei *et al*. (2014).

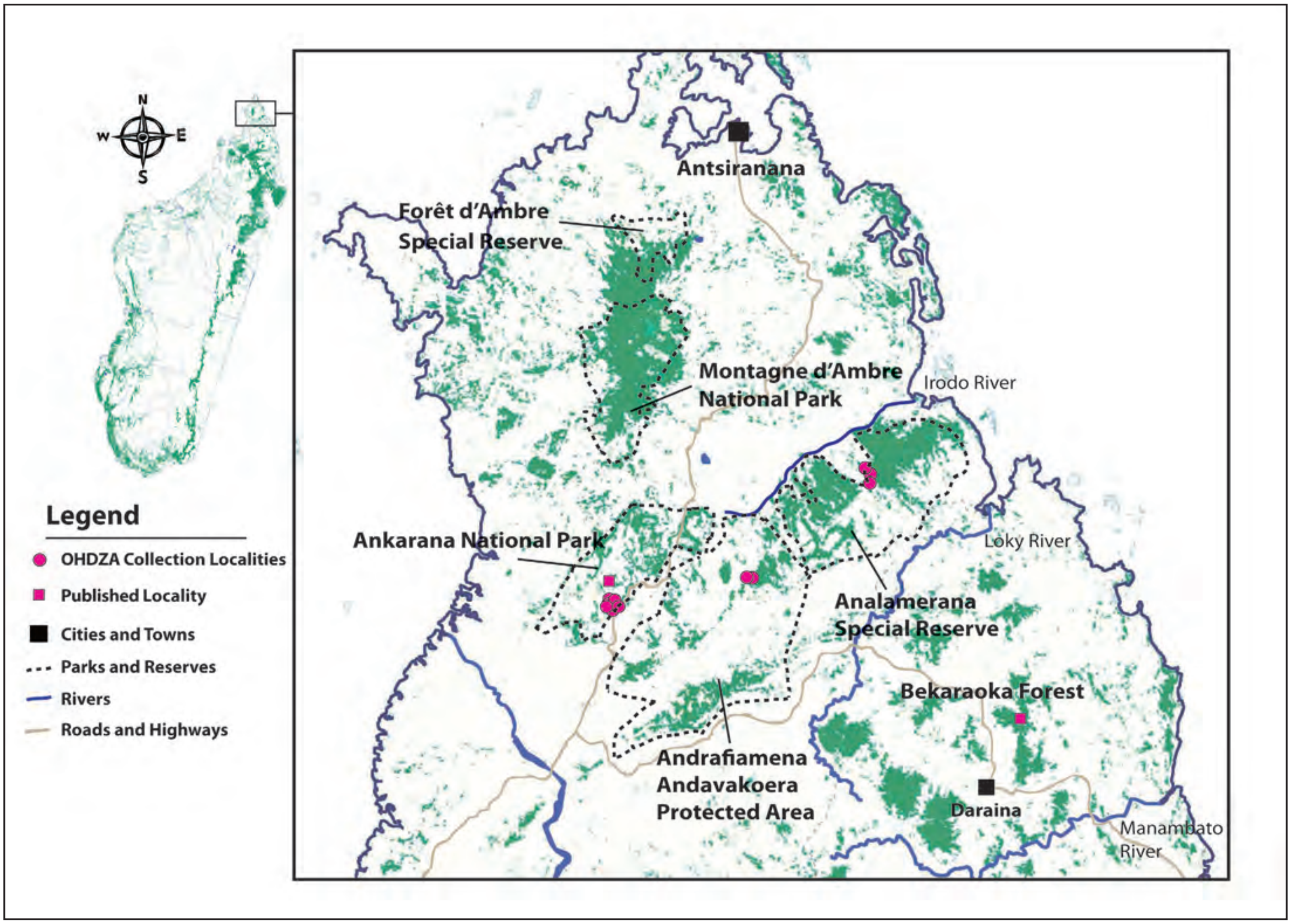

Figure 2. Map of the study locality in northern Madagascar. Circles represent collection localities of *Cheirogaleus* sp. nov. 4 individuals listed in Table 1. Pink squares represent the localities of two additional samples from other research groups.

to Analamerana SR and Bekaraoka Forest were included plus other individuals in the *C. medius* group (*C. thomasi*, UCS1-4, and CCS7). Representatives from the remaining *Cheirogaleus* species groups (*C. crossleyi*, *C. major*, and *C. sibreei*), plus *Microcebus berthae* as an outgroup, were also included in the bPTP analyses.

Additionally, a D-loop sequence was generated from a fecal sample collected in March 2015 from Ankarana SR (KAR15.1), which was identical to those from blood and tissue samples. Because of the identical nature of this sample, it was not used in the genetic analyses, but was included in the morphological analyses.

Table 2. List of dwarf lemurs, *Cheirogaleus* sp. nov. 4, from Ankarana, Andrafiamena and Analamerana examined during this study using acronyms ANK, KAR, FIA and MATY 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
ANK5.12			n/a	n/a	n/a	-12.96631	49.13808	12/04/2005
ANK5.13			n/a	n/a	n/a	-12.96631	49.13808	12/04/2005
ANK5.14			n/a	n/a	n/a	-12.96631	49.13808	12/04/2005
ANK5.15			n/a	n/a	n/a	-12.96631	49.13808	12/05/2005
ANK5.16			n/a	n/a	n/a	-12.96631	49.13808	12/05/2005
ANK5.17			n/a	n/a	n/a	-12.96631	49.13808	12/05/2005
ANK5.18			n/a	n/a	n/a	-12.96631	49.13808	12/05/2005
ANK5.19			n/a	n/a	n/a	-12.96631	49.13808	12/06/2005
ANK5.20			n/a	n/a	n/a	-12.96631	49.13808	12/07/2005
ANK5.21			n/a	n/a	n/a	-12.96631	49.13808	12/07/2005
KAR15.1			Male	480447362B	0.125	-12.95636	49.12969	03/06/2015
FIA5.19	TTU-M 122795	TK 128750	Female	442720357C	0.123	-12.91539	49.31956	12/03/2005
FIA5.22			Male	442A535373	n/a	-12.91539	49.31956	12/04/2005
MATY5.31	TTU-M 118805	TK 129243	Male	n/a	0.112	-12.76556	49.48358	11/16/2005
MATY5.40	TTU-M 118804	TK 129242	Female	n/a	0.080	-12.76703	49.48358	11/18/2005
MATY5.42	TTU-M 118806	TK 129244	Male	n/a	0.101	-12.77136	49.48303	11/18/2005

Results

Morphology

Morphological data were available for three males and two females of *C.* sp. nov. 4. One of the females (MATY5.40) was likely immature due to her lower weight and smaller linear measurements; she was excluded from the morphological analysis. In a study by Blanco and Godfrey (2013), juvenile and subadult male dwarf lemurs generally did not show visible signs of testicular development. As two of the three *C.* sp. nov. 4 males had developed testes, they are expected to be adults. The third male (KAR15.1) had small testes, but comparable linear measurements to the other adult males, so was included in the morphological analysis. This individual was caught in March, which is after the breeding season (Blanco and Godfrey 2013) and when testes are reduced in size in the closely related mouse lemurs (Perret and Aujard 2001; Wrogemann *et al*. 2001). Although the sample size was small, the combined head and body length of *C.* sp. nov. 4 was shorter than the minimum for other species of the *C. medius* group. One individual from Tsiombikibo, currently the only representative of UCS1 (Lei *et al.* 2014), was similar in length. See Table 3 for morphometric measurements.

Phylogenetic analyses

The Bayesian species delimitation analysis using the bPTP webserver provided posterior delimitation probabilities in support of our elevation of the Ankarana *Cheirogaleus* group as an independent species. We obtained a Bayesian PTP support value of 0.92 indicating a high probability that this lineage is an independent species based on the given dataset (Fig. 3). 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.

Discussion

Previous genetic studies by Lei *et al.* (2014) identified a new *Cheirogaleus* species using the following mitochondrial regions: 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); as well as the Pastorini fragment (PAST) that includes a fragment of the Cytochrome oxidase subunit III gene, NADH-dehydrogenase subunits 3, 4L, and 4 and five tRNAs (Pastorini *et al.* 2000). Three nuclear loci were also sequenced in Lei *et al.* (2014): 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). See Table 4 for GenBank accession numbers.

In the cytb sequence fragments, *C.* sp. nov. 4 differs from its closest genetic relatives, the other members of the *medius* group (*C. medius*, CCS7, CCS8, UCS 1-4), in genetic distance by 7.2%±0.7% (*C. medius*), 4.7%±0.6% (CCS7), 7.7%±0.7% (CCS8), 7.7%±0.8% (UCS10), 7.3%±0.7% (UCS2), 7.4%±0.7% (UCS3), and 8.0%±0.7% (UCS4), respectively (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. 4 segregating as a distinct lineage with a high degree of confidence (posterior probabilities equal to 1.00; Lei *et al.* 2014, Figs. 2−4). This was also supported by a Bayesian analysis based on three nuclear loci (CFTR-PAIRB, FIBA, vWF), but with less robust support (posterior probabilities ranging from 0.87 to 0.89; Lei *et al.* 2014, Figs. 5−6). The population aggregation analysis (PAA) results are congruent with those presented in Appendix II (n, q, s and t; Lei *et al.* 2014).

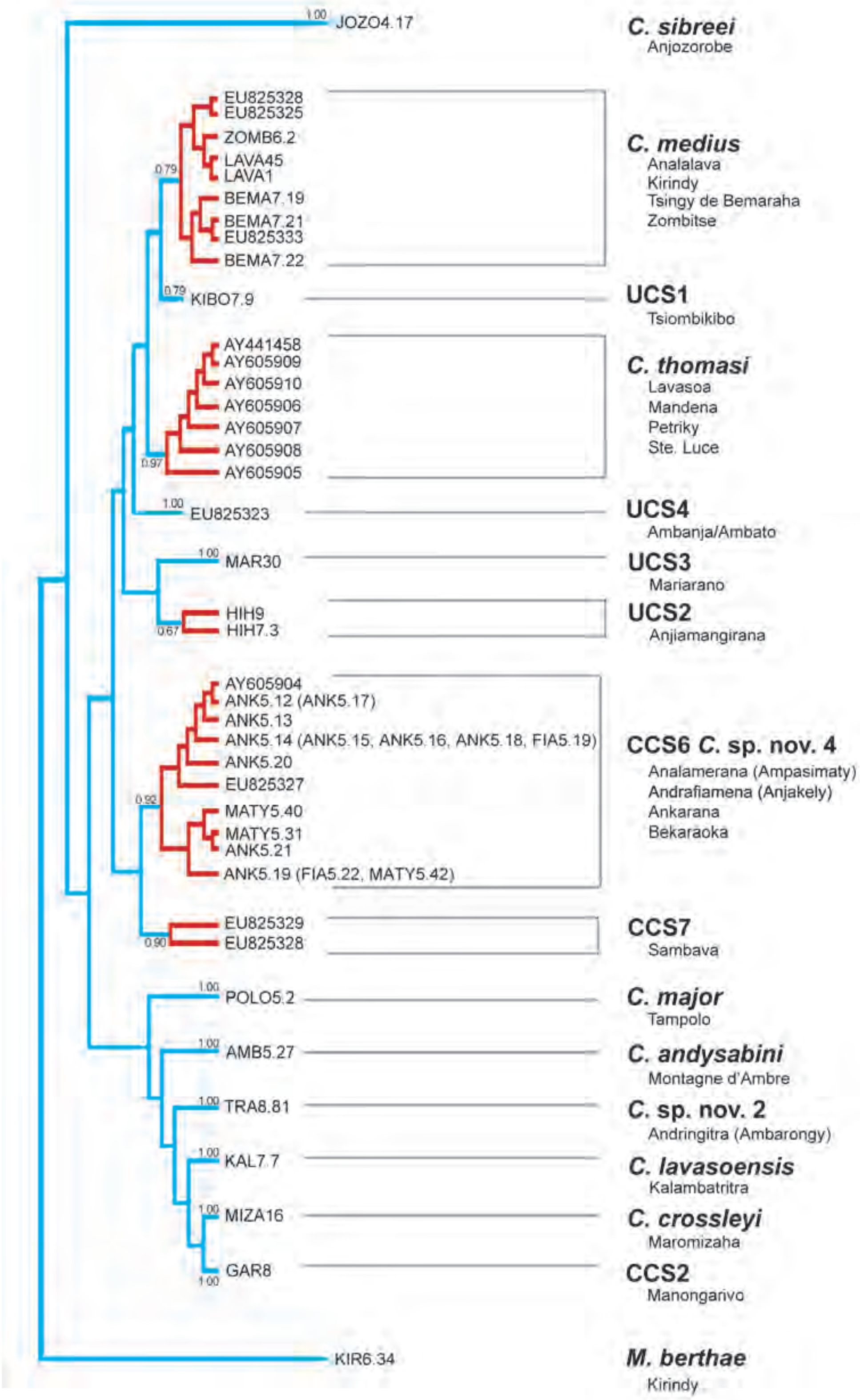

Figure 3. The Bayesian species delimitation analysis of the cytb dataset for the *Cheirogaleus medius* group using the bPTP webserver (Zhang *et al.* 2013). Maximum Likelihood phylogeny with Bayesian support values presented for all lineages recognized as species by the PTP analysis. Monophyletic groups in red indicate a single putative species as well as terminal branches in blue. Names of terminals coincide with sample IDs in Table 1; IDs in parentheses have equivalent haplotypes to their associated terminals.

Table 3. Morphological data for adult *Cheirogaleus shethi* individuals from Ankarana, Andrafiamena and Analamerana.

Class	No.	W (kg)	HC (cm)	BL (cm)	TL (cm)	EL (mm)	EW (mm)	ML (mm)	F-Tb (cm)	F-LD (cm)	F-Hd (cm)
Male	3	0.113±0.012	4.9±1.2	12.1±1.1	16.5±1.0	15.9±1.4	10.9±1.2	10.8±2.4	0.6±0.0	1.2±0.0	2.6±0.0
Female	1	0.123±0.000	4.1±0.0	12.4±0.0	15.5±0.0	14.5±0.0	9.6±0.0	8.2±0.0	0.9±0.0	1.2±0.0	2.4±0.0
Total	4	0.115±0.011	4.7±1.0	12.2±0.9	16.3±0.9	15.5±1.4	10.6±1.2	9.9±2.3	0.7±0.2	1.2±0.0	2.5±0.1

W: weight, HC: head crown, BL: body length, TL: tail length, EL: ear length, EW: ear width, ML: muzzle length, F-Tb: thumb length (forelimb), F-LD: longest digit length (forelimb), F-Hd: hand length (forelimb).

Table 3. *continued*

Class	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)	TeL-R (mm)	TeW-R (mm)	TeL-L (mm)	TeW-L (mm)
Male	3.1±0.0	3.4±0.0	1.0±0.0	1.5±0.2	4.3±0.0	4.7±0.0	4.3±0.0	2.3±0.4	2.2±0.5	8.8±1.7	5.85±1.2	8.2±0.0	4.35±0.4
Female	2.8±0.0	2.5±0.0	1.4±0.0	1.2±0.0	3.5±0.0	3.4±0.0	2.9±0.0	3.1±0.0	2.9±0.0	NA	NA	NA	NA
Total	3.0±0.2	3.0±0.6	1.2±0.3	1.4±0.2	3.9±0.6	4.1±0.9	3.6±1.0	2.6±0.6	2.4±0.6	NA	NA	NA	NA

F-UR: ulna/radius length, F-H: humerus length, H-Tb: thumb length (hindlimb), H-LD: longest digit length (hindlimb), H-Ft: foot ength (hindlimb), 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.

Cheirogaleus. sp. nov. 4 harbored five diagnostic sites for cytb, one for COII and none for both FIBA and vWF.

The small size coupled with geographic distance to other known populations of *C. medius* groups and mitochondrial genetic divergence demonstrate that this lineage is diagnosably distinct. The first factors taken alone, size or geography, provide questionable evidence of speciation. A combination of these factors with the observed genetic divergence, however, provides stronger justification for this elevation. *Cheirogaleus* sp. nov. 4 is separated from other *Cheirogaleus* populations by a significant geographical distance with the exception of the recently described Montagne d'Ambre dwarf lemur, *C. andysabini* (Fig. 4). However, *C. andysabini* is a member of the *C. crossleyi* group (Lei *et al*. 2015), while *C.* sp. nov. 4 is in the *C. medius* group. Phenotypically *C.* sp. nov. 4 individuals have less pronounced eye rings than other individuals in the *C. medius* group (Lei *et al*. 2014; Fig. 8). In addition, *C.* sp. nov. 4 individuals have a lower body weight (0.115±0.011 kg) and a shorter tail (16.3±0.9 cm) than individuals identified as *C. medius* (0.23±0.06 kg; 20.2±2.4 cm).

The species status of another possible *C. medius* population identified at Sambava (approximately 175 km from Ankarana SR) and described as CCS7 in Lei *et al.* (2014) has yet to be confirmed. This population, grouped by Thiele *et al*. (2013) into the proposed *C.* sp. Bekaraoka Sambava lineage, is sister to *C.* sp. nov. 4 (Lei *et al.* 2014).

Conservation status

Much of the corridor that includes Ankarana, Andrafiamena, and Analamerana is within Madagascar's system of protected areas. The bookends of this corridor are restrictive special reserves that afford a greater degree of protection to wildlife than the Category V protected areas (Fig. 2). Gardner (2011) acknowledged that Category V protected areas in Madagascar function differently than in Europe making it difficult to apply the same criteria globally. In Madagascar, communities rely on forests for their daily needs, and the extraction of resources at this intensity negatively affects biodiversity, contradicting one of the main tenants of IUCN's Category V definitions (Gardner 2011). Refining the objectives of Category V to reflect the reality in Madagascar was suggested (Gardner 2011).

Effective management of both the Andrafiamena-Andavakoera and Loky-Manambato Category V Protected Areas is vital to maintaining connectivity between populations of *C.* sp. nov. 4. Effective support of organizations striving to manage these sustainable use protected areas is recommended, such as FANAMBY, a Malagasy NGO that is managing both the aforementioned Category V protected areas. Additional fieldwork is necessary to establish baseline data on population density and the extent of the range to determine to which IUCN Red List category this species belongs.

***Cheirogaleus shethi* sp. nov.**

Formerly *Cheirogaleus* sp. nov. 4, also CCS6 (Lei *et al.* 2014); in part *C.* sp. Bekaraoka Sambava (Thiele *et al.* 2013). See Table 3.

Holotype: FIA5.19; adult female; Permit number 181; 4 × 2.0 mm biopsies from ear pinna and 0.02 cc of whole blood; stored and curated at the Museum of Texas Tech University (MTTU, catalog number: TTU-M 122795/ TK 128750) Genetic Resources Collection, Natural Sciences Research Laboratory (NSRL). We placed a microchip subcutaneously between the scapulae and recorded it as 442720357C; Collected by Nirina Jean de Dieu Andriamadison, Jean Aimé Andriamihaja, Jean Claude Randriamanana, Joseph Désiré Rabekinaja, François Randrianasolo, Philbert Randrianarinjaka, and Rambinintsoa Andriantompohavana on 3 December 2005.

Paratypes: ANK5.12-21, KAR15.1, FIA5.22; MATY5.31, MATY5.40, MATY5.42.

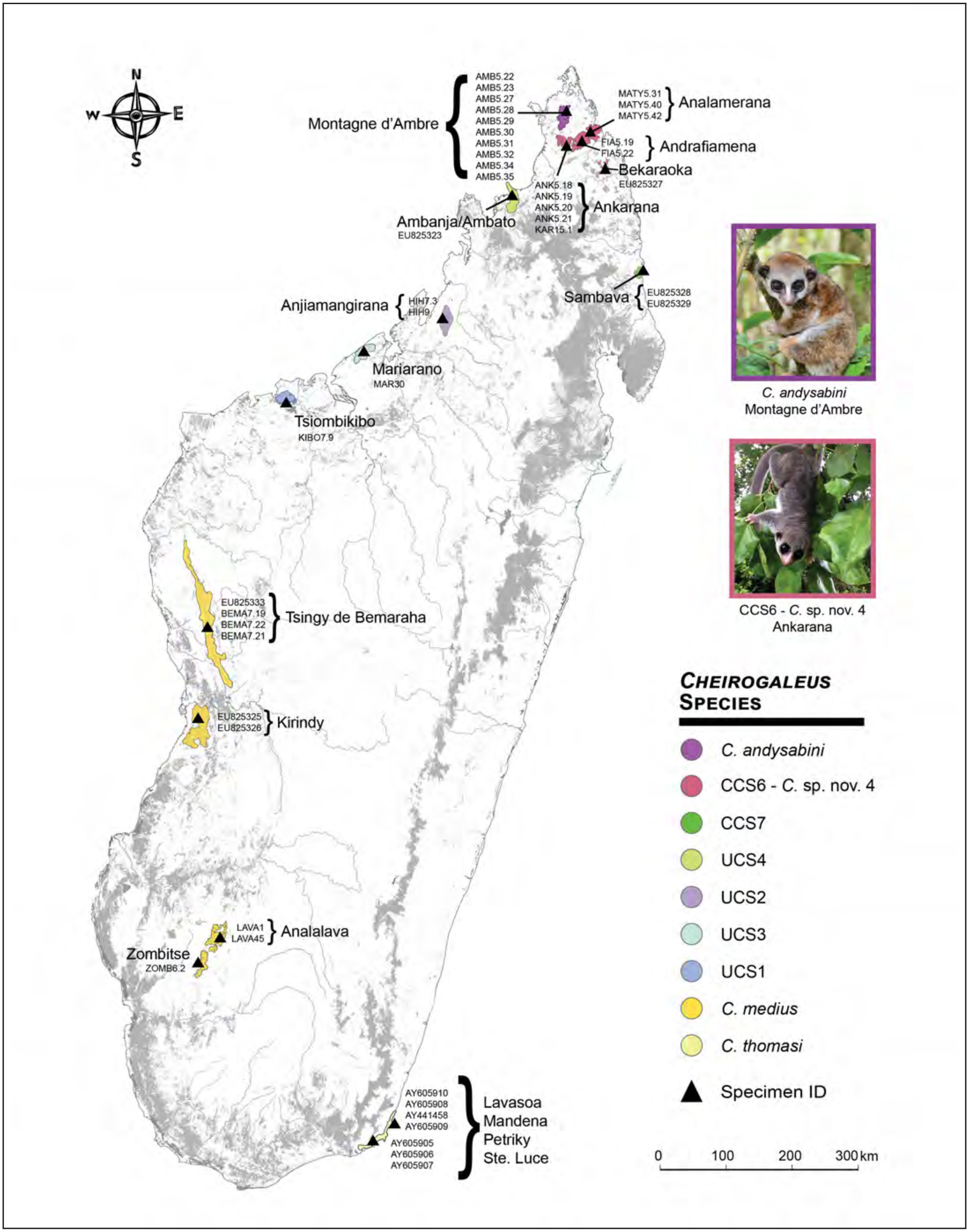

Figure 4. Map of Madagascar with the ranges of *Cheirogaleus*. sp. nov. 4 and closely related *Cheirogaleus* species highlighted to show the geographic distance between lineages. Identification numbers on the map correspond to ID numbers of animals listed in Table 1. Photographs of *C. andysabini* and *C.* sp. nov. 4 are provided to show a clear difference in pelage and the distance between the ranges of the two lineages from different species groups.

Table 4. A subset of GenBank accession numbers from Lei *et al.* (2014) for sequence data of *Cheirogaleus* individuals represented in the Bayesian species delimitation analysis.

ID	COII	Cytb	D-loop	PAST	vWF	FIBA	CFTR
AMB5.27	KM872108	KM872197	KM872288	KM872377	KM872557	KM872466	KM872648
ANK5.12	KM872119	KM872208	KM872299	KM872388	KM872568	KM872477	KM872659
ANK5.13	KM872120	KM872209	KM872300	KM872389	KM872569	KM872478	KM872660
ANK5.14	KM872121	KM872210	KM872301	KM872390	KM872570	KM872479	KM872661
ANK5.15	KM872122	KM872211	KM872302	KM872391	KM872571	KM872480	KM872662
ANK5.16	KM872123	KM872212	KM872303	KM872392	KM872572	KM872481	KM872663
ANK5.17	KM872124	KM872213	KM872304	KM872393	KM872573	KM872482	KM872664
ANK5.18	KM872125	KM872214	KM872305	KM872394	KM872574	KM872483	KM872665
ANK5.19	KM872126	KM872215	KM872306	KM872395	KM872575	KM872484	KM872666
ANK5.20	KM872127	KM872216	KM872307	KM872396	KM872576	KM872485	KM872667
ANK5.21	KM872128	KM872217	KM872308	KM872397	KM872577	KM872486	KM872668
BEMA7.19	KM872129	KM872218	KM872309	KM872398	KM872578	KM872487	KM872669
BEMA7.21	KM872130	KM872219	KM872310	KM872399	KM872579	KM872488	KM872670
BEMA7.22	KM872131	KM872220	KM872311	KM872400	KM872580	KM872489	KM872671
FIA5.19	KM872139	KM872228	KM872319	KM872408	KM872588	KM872497	KM872679
FIA5.22	KM872140	KM872229	KM872320	KM872409	KM872589	KM872498	KM872680
GAR8	AY584486	KM872230	AY584498	AY582562	KM872590	KM872499	KM872681
HIH9	KM872141	KM872231	KM872321	KM872410	KM872591	KM872500	KM872682
HIH7.3	KM872142	KM872232	KM872322	KM872411	KM872592	KM872501	KM872683
KAL7.7	KM872148	KM872238	KM872328	KM872417	KM872598	KM872507	KM872689
KIBO7.9	KM872149	KM872239	KM872329	KM872418	KM872599	KM872508	KM872690
LAVA1	KM872153	KM872243	KM872333	KM872422	KM872603	KM872512	KM872694
LAVA45	KM872154	KM872244	KM872334	KM872423	KM872604	KM872513	KM872695
MAR30	KM872156	KM872246	KM872336	KM872425	KM872606	KM872515	KM872697
MATY5.31	KM872160	KM872250	KM872340	KM872429	KM872610	KM872519	KM872701
MATY5.40	KM872161	KM872251	KM872341	KM872430	KM872611	KM872520	KM872702
MATY5.42	KM872162	KM872252	KM872342	KM872431	KM872612	KM872521	KM872703
MIZA16	KM872163	KM872253	KM872343	KM872432	KM872613	KM872522	KM872704
POLO5.2	KM872170	KM872260	KM872350	KM872439	KM872620	KM872529	KM872711
TRA8.81	KM872181	KM872272	KM872361	KM872450	KM872632	KM872541	KM872723
ZOMB6.2	KM872194	KM872285	KM872374	KM872463	KM872645	KM872554	KM872736

Other specimens: *Cheirogaleus* collected by George R. Albinac in January, 1969, in the Collection des specimens morts, Parc Botanique et Zoologique Tsimbazaza in Antananarivo (Hapke *et al.* 2005); E1055 collected from Bekaraoka (Groeneveld *et al*. 2009).

Type locality: Madagascar: Antsiranana Province, Diana Region, District Antsiranana II, Andrafiamena (Anjakely), 12°54'55"S, 49°19'10"E (S12.91539, E49.31956) at 316 m above sea level.

Measurements of holotype: Measurements recorded in field catalog: body length, 124 mm; tail length, 155 mm; head crown, 41 mm; mass, 0.123 kg.

Description: The dorsum, limbs and head are gray, with no dorsal stripe. Underside white, from interramal area to base of tail. Facial mask poorly expressed; the area around the orbits is narrowly brownish and dorsally bleeding into the gray of the forehead and crown; median strip between them is paler than rest of face. The ears are sparsely furred. Hands and feet are light colored (Fig. 5).

Diagnosis: *Cheirogaleus shethi* can be distinguished from *C. medius*, UCS1, UCS2, UCS3, CCS7, *C. thomasi* and UCS4 by eight, six, nine, nine, 11, nine and 13 diagnostic characters in the cytochrome b gene, respectively (Appendix II(k); Lei *et al.* 2014). *C. shethi* has five diagnostic sites in cytb sequence fragment (G, T, T, T and C at the positions of 387, 468, 675,

Figure 5. Illustration of *Cheirogaleus shethi* (Stephen D. Nash © Conservation International) and photographs of KAR15.1 taken at Ankarana Special Reserve (photos by Richard Randriamampionona).

886 and 1101, respectively), which differentiate *Cheirogaleus shethi* from all other species in the *C. medius* group. Furthermore, although geographically close to CCS7 from Sambava, *C. shethi* can be distinguished from CCS7 by five diagnostic characters in the COII gene (Appendix II (q); Lei *et al.* 2014). Morphometrically, *C. shethi* can be distinguished by its very small size. Head plus body length is 164−175 mm in four specimens, whereas all other members of the *C. medius* group measure >177 mm, with one individual from western Madagascar being comparable to *C. shethi*. Adult weight is 101−125 g; all other members of the *C. medius* group are >165 g. Despite being geographically close to *C. andysabini*, with the Irodo River as a barrier, *C. shethi* is distinct from *C. andysabini*, which is clustered in the *C. crossleyi* group, by nine diagnostic characters, and distinguished by differences in pelage and size.

Distribution: Known from northern Madagascar, from Ankarana east to Bekaraoka in dry and transitional forests. Found in the Ankarana Special Reserve, Andrafiamena-Andavakoera Protected Area, Analamerana Special Reserve, and Loky-Manambato Protected Area.

Etymology: This new species is named after Brian Sheth, the Chair of the Board of the NGO Global Wildlife Conservation. Brian is deeply committed to biodiversity conservation worldwide, and is a leading philanthropist for species and ecosystem conservation. He has supported many projects in Madagascar, including research and the establishment and management of nature reserves. His passion and drive to help save the diversity of life on our planet has been an inspiration to all around him.

Vernacular names: Ankarana or Sheth's dwarf lemur.

Acknowledgments

We thank Madagascar National Parks, the Ministère de l'Environnement, de l'Ecologie, de la Mer et des Forêts, and their regional offices in Diana and SAVA for sampling permission. We are most grateful to the Ahmanson Foundation, the Theodore F. and Claire M. Hubbard Family Foundation, the Primate Action Fund / Conservation International, the Margot Marsh Biodiversity Foundation, and the National Geographic Society for financial assistance. Colin Groves thanks Eileen Westwig, Judy Chupasko, Larry Heaney, Paula Jenkins, Chris Smeenk, Frieder Meier and Cecile Callou for access to museum specimens under their care. We wish to acknowledge Shannon Engberg for her helpful comments and eye for consistency as well as the helpful comments of two anonymous reviewers. We are also most grateful to the office and field staffs of the Madagascar Biodiversity Partnership for their excellence in collecting the samples safely from the *Cheirogaleus* and returning them to their forest habitat.

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Received for publication: 29 March 2016
Revised: 1 June 2016
Published: 8 December 2016

Primate Conservation 2016 (30): 73–88

Variation in Habitat and Behavior of the Northern Sportive Lemur (*Lepilemur septentrionalis*) at Montagne des Français, Madagascar

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Abstract: Understanding variation in habitat quality, diet, and behavioral patterns is fundamental for the development of conservation strategies for threatened primates. From June−August 2013, we conducted a preliminary study of four northern sportive lemurs (*Lepilemur septentrionalis*)—Critically Endangered, solitary-foraging primates living in different forest fragments in Montagne des Français, Madagascar. We sought to obtain the first systematic data on the behavior and feeding ecology of this species in the wild, and identify potential differences in habitat characteristics. The four individuals in our study are estimated to represent approximately 8% of the known population of this species, about which very little else is known. Our data showed that the variety of plant parts consumed did not differ among the four individuals despite differences in habitat as determined by measures of anthropogenic disturbance, tree density, species diversity, total tree basal area, and presence of invasive species. The data suggest, however, that individuals in areas that suffer more anthropogenic threats spent slightly more time feeding, devoted a greater percentage of feeding time to fruit than to leaves, and had larger home ranges than those in areas with fewer different anthropogenic activities. Our results suggest that the ability of the northern sportive lemur to adjust its behavior under different environmental conditions may be beneficial to their ability to persist in degraded habitats.

Key Words: Behavioral flexibility, conservation, feeding ecology, anthropogenic actions, Madagascar, sportive lemur

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Introduction

Habitat fragmentation and degradation are among the greatest threats challenging tropical primates today (Chapman *et al.* 2007; Junge *et al.* 2011; IUCN 2012). Although intermediate disturbance may favor some species, inevitably most species decline in response to forest fragmentation due to food shortage, inaccessibility of mates, or hunting (Ganzhorn *et al.* 1997; Irwin *et al.* 2010). The persistence of a species living in fragmented and degraded habitats depends on its ability to adapt to these altered landscapes (Cristobal-Azkarate and Arroyo-Rodríguez 2007).

Fragmentation has been shown to alter habitat properties that include floristic composition, habitat heterogeneity, interspecific competition, predation pressure, and population density (Marsh 2003; Irwin 2008; Gabriel 2013). In fragmented landscapes there can be variations of habitat quality, defined as the suitability of an environment to provide the necessary elements for survival (Hall *et al.* 1997; Gabriel 2013). Fragmentation and reduced habitat quality are variables that have been shown to influence the behavior and distribution of primates in several ways, including altering activity time budgets, daily path length, home range size, and population density, and, perhaps most importantly, dietary composition (Bicca-Marques 2003; Menard *et al.* 2013; Marsh *et al.* 2016). Understanding the different parameters that influence intraspecific variation in behavior patterns is vital as these are indicators of the capacity of primates to respond to rapid changes in environmental conditions (Strier 2009). Species with a greater degree of behavioral and dietary plasticity will be able to respond better to these changes, contributing to their ability to survive in degraded and fragmented habitats.

Lemurs are small to medium-sized primates, endemic to the island of Madagascar. They exhibit a variety of distinctive physiological, social, morphometric, and demographic features, often referred to as the "lemur syndrome" (Jolly 1998; Wright 1999; Kappeler and Schäffler 2008). Their unique traits include lack of sexual dimorphism, female dominance over males, even sex ratios in social groups, instances of monogamy, and cathemerality (Kappeler 1990; Jolly 1998; Wright 1999; Curtis 2006). These varying and distinguishable features of lemur biology and behavior have been suggested as adaptations for energy conservation during bouts of natural seasonal variability (Wright 1999); however, they may also allow for survival in fragmented and disturbed habitats.

Over 90% of the lemurs are now threatened with extinction; as a group, they are among the world's most endangered vertebrates (IUCN 2012; Schwitzer *et al*. 2013). Madagascar's habitats are under a wide array of anthropogenic threats from a rapidly growing human population. They include internal migration, reliance on crops, introduced invasive species, traditional cultural practices (i.e. slash-and-burn agriculture, pet trade, and ritualistic hunting), illegal logging, poaching, the bushmeat trade, and the expansion of stone and mineral mining (Barrett and Ratsimbazafy 2009; Brown and Yoder 2015). Human-induced land disturbance and the direct hunting of species are widespread, and have been linked to the extinction of at least 15 lemur species over the past few centuries (Mittermeier *et al.* 2010; Gorenflo *et al.* 2011). Although global climate change is expected to seriously affect island ecosystems such as Madagascar (Brown and Yoder 2015), the exact extent is unknown, and the impacts of widespread human actions on the island are of more immediate concern (Irwin *et al.* 2010; Dewar and Richard 2012). As a result, studies to assess the effects of habitat loss and degradation on species diversity and the ability of lemurs to cope with these pressures are of great importance (Mittermeier and Cheney 1987; Lerdaeu *et al.* 1991; Ganzhorn 1995; Marsh 2003; Dunham *et al.* 2008; Irwin *et al*. 2010; Junge *et al.* 2011; Gabriel 2013).

Lepilemuridae is a monotypic primate family of nocturnal, solitarily foraging, folivorous lemurs (Ganzhorn 1993). To date there are published studies analyzing habitat use for only six of the 26 sportive lemur species (Hladik and Charles-Dominique 1974; Warren 1997; Nash 1998; Ganzhorn *et al.* 2004; Seiler *et al*. 2014; Sawyer *et al.* 2015). The present study focused on the northern sportive lemur (also known as the Sahafary sportive lemur), *Lepilemur septentrionalis*, which was reclassified from a subspecies to a full species in 2004 (Ravaoarimanana *et al.* 2004; Rumpler 2004; Andriaholinirina *et al.* 2006). It is Critically Endangered and restricted to two small forest fragments in northern Madagascar, in the Sahafary region and Montagne des Français, an area 12 km southeast of the seaport town of Antsiranana (Ranaivoarisoa *et al.* 2013). These forest patches are under extreme anthropogenic pressure from local people who are harvesting trees to be used or sold as charcoal. Charcoal production in particular, along with other anthropogenic activities such as cattle grazing, agriculture, and development, are responsible for the alteration, fragmentation, and degradation of the northern sportive lemur's habitat in Montagne des Français.

Surveys conducted in 2007 estimated the total population of the northern sportive lemur to be 100−150 individuals; however, data collected in 2011 and early 2012 revealed the presence of only 19 known individuals (Ranaivoarisoa *et al.* 2013). The most recent survey, conducted in late 2012 and early 2013 indicated a slightly larger population of 52 individuals, with 95% of them located at Montagne des Français (Louis and Zaonarivelo 2014). As such, the northern sportive lemur is one of the rarest and least protected lemurs in Madagascar, and perhaps the one closest to extinction (Mittermeier *et al.* 2010). Understanding if the northern sportive lemur is able to adjust its behavior under different conditions in its remaining forest fragments is of vital importance to understanding its ability to adapt. Data on diet and activity budgets of this Critically Endangered species in different habitats can aid in identifying conservation priorities.

We conducted a 2-month field study of the species in Montagne des Français, Madagascar. From June to August 2013, we obtained the first systematic data on the behavior and feeding ecology of this species in the wild, with the goal of beginning to understand the extent to which individual behavior and feeding ecology varies with differences in degrees of fragmentation, degradation, and other forms of human disturbance in their habitat. We selected four previously collared individuals as study subjects. They were living in three forest fragments, each with different levels of anthropogenic pressures. We compared their diets, habitats, ranges, and activity budgets. Specifically, we investigated whether differences in these parameters resembled those found for Scott's sportive lemur (*Lepilemur scottorum*), which, although evidently tolerating selectively logged environments where there is less variety of high-quality foods, were absent from highly disturbed areas (Sawyer *et al.* 2016). Degraded forests and even agroforest ecosystems are not necessarily inimical to the survival of primates, however. Golden-headed lion tamarins (*Leontopithecus chrysomelas*), for example, thrive in old *cabruca* agroforests in Brazil. There cultivated fruit (e.g., jackfruit, mangos and figs) is abundant year round, and the diversity of microhabitats (especially their preferred bromeliad epiphytes) is sufficient for prey foraging and sleeping sites (Oliveira *et al.* 2011).

Methods

Study area

Montagne des Français (12°20'02.7"S, 49°21'21.9"E) is a small region of 5,974 ha in the far northeastern tip of Madagascar about 12 km southeast of the large seaport town of Antsiranana (Fig. 1). The area is characterized by fragmented patches of dry deciduous forest surrounding a calcareous massif. Elevation ranges from 100 to 400 m above sea level. Due to its elevation, Montagne des Français has a higher

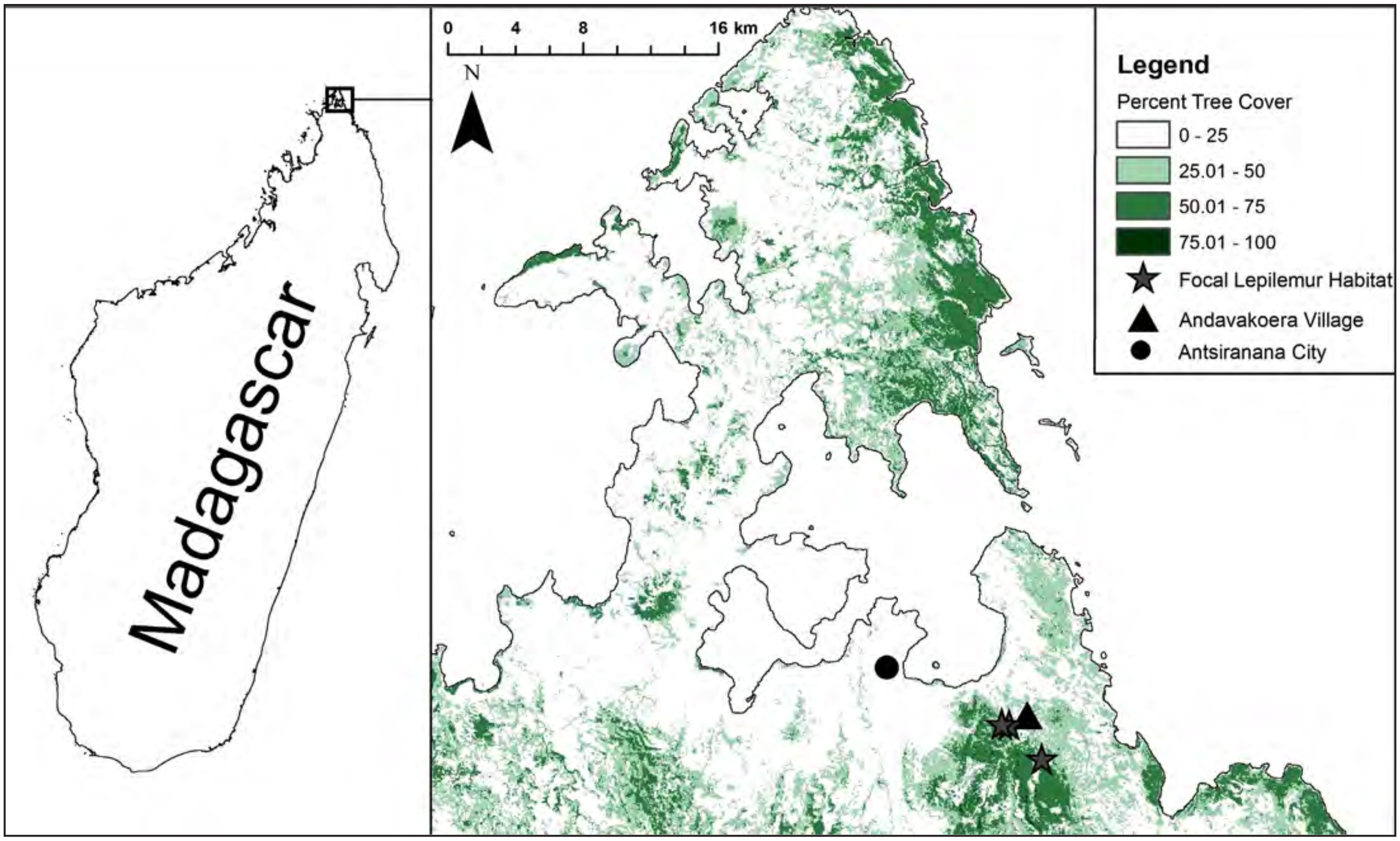

Figure 1. Location of study site in northern Madagascar showing the four habitats of focal individuals and percent of forest canopy cover. Percent forest cover data retrieved from Hansen *et al*. (2013).

annual rainfall than the town of Antsiranana, which receives a mean annual rainfall of 915 mm and nine months of the year are dry (Sabel *et al.* 2009). The wet season is from December to March, and the dry, windy season is from April to November (Robinson *et al.* 2006; Sabel *et al.* 2009). The higher rainfall in Montagne des Français helps support the wide diversity of vegetation and animal life found in the region. In 2008, Montagne des Français was added to the Ramena Special Reserve, officially listed as a government protected area. Montagne des Français is a watershed upon which both local villages and the native flora and fauna depend (Goldstein 2009).

Although protected, Montagne des Français is severely impacted by a number of anthropogenic threats. It no longer consists of contiguous, closed-canopy forest, but has become more of a patchwork of degraded, isolated forests that surround karst mountain peaks (Sabel *et al.* 2009; D'Cruze and Kumar 2011). Years of slash-and-burn agriculture have left this entire region fragmented and scarred by erosion (Ranaivoarisoa *et al.* 2013). People from Antsiranana go into the area to cut down trees for charcoal production and timber. Habitat loss from uncontrolled long-term slash-and-burn practices, regrowth of non-endemic invasive plants, desertification, and hunting are challenges that need to be addressed immediately (Goldstein 2009; Ranaivoarisoa *et al.* 2013). A variety of altered and fragmented landscapes are found at Montagne des Français as a result of these activities (D'Cruze *et al.* 2007; Sabel *et al.* 2009; D'Cruze and Kumar 2011).

Study subjects

Due to limited access and the cryptic nature of the species, only four adults were fitted with radio-telemetry collars (Edward E. Louis Jr. [unpubl. report] following methods described by Louis *et al.* [2006]). In October 2012, the four adult females (coded 'F', 'M', 'O', and 'L') were collared in three forest fragments (individuals 'F' and 'M' were in the same fragment). Beginning in October 2012, they were regularly followed by local Malagasy students to habituate them to human presence. One female ('L') was observed sleeping and associating with an infant (date of birth not known). The three forest fragments inhabited by the focal subjects differed in the extent to which they were degraded by human activities.

Behavioral sampling

Systematic instantaneous scan samples (Altmann 1974) were conducted at 5-minute intervals from 4 June to 1 August, 2013. Visibility of focal individuals varied depending on the level of degradation and canopy cover. Nonetheless, each individual was followed for five consecutive days, for a total of 10 days over the course of this study. Four days of the week, individuals were followed for six consecutive hours beginning at sundown. Scan sampling began at the first indication of movement by the focal individual in its sleep site. On the last day of the week data collection began at approximately 11:00 pm and continued for the next six hours or until the focal subject entered the sleep site for the day. Nine behaviors were recorded in the scans: feeding, resting, moving, autogrooming, social grooming, sleeping, defecating, drinking, and mating. When not seen, they were recorded as out of sight. Here we analyze just the first six behaviors. When feeding, we recorded the plant parts and the Malagasy vernacular names of the plant species. Feeding was recorded only when an individual was observed masticating a food source. Plant parts were categorized as leaves, fruit, vines, new shoots, bark, and flowers.

Ranging and sampling of human activities

At the time of each scan sample, we recorded the location of the focal individual using a handheld GPS (model: Etrex Vista HCx Garmin). GPS points were also collected if an individual moved more than 50 m between scan samples. No points were collected if the individual did not move between scans. We still obtained a GPS reading during a scan whenever the location of the individual was known, even if the animal was out of sight for behavioral observation.

Additionally, we collected GPS points at any sign of anthropogenic activity observed opportunistically while following an individual. These activities included points of logging, slash-and-burn agriculture practices, evidence of hunting, free-ranging zebu cattle grazing, human structures, and crop plantations. These observations were dependent on where the focal individual's movements led us; therefore, anthropogenic activities may have been missed.

Habitat sampling

We set up a 50 m × 20 m plot in each individual's home range to obtain comparative data on tree species composition and density (Tesfaye *et al*. 2013). The initial location of each plot was next to the most used sleep tree. Each plot was located after randomly selecting a direction from the sleep tree, and then broken down into ten, 10 m × 10 m quadrates in which we counted and recorded all trees, measuring the DBH, estimating the height, and noting the vernacular name of each. When samples of leaves, flowers or fruit could not be collected, they were only identified to the genus level. Data were collected only for trees with a DBH >5.0 cm (Irwin 2007; Olson *et al*. 2013). While the DBH cut off in such botanic inventories is generally >10.0 cm, we chose to use >5.0 cm, as *L. septentrionalis* was often observed using trees with a DBH <10.0 cm. Vernacular names were provided by a trained, local guide, and scientific names were determined subsequently. Thirty-six samples (fruits, leaves) were collected in August 2016 and identified by the Department of Botany at the Parc Botanique et Zoologique de Tsimbazaza in Antananarivo, Madagascar. No specimens of leaves or fruits were collected for trees which were easily identified by Masters students from the University of Antsiranana. We also recorded presence/absence of tickberry, *Lantana camara* (Verbenaceae), in each subplot. *Lantana camara* is an exotic invasive species that is often present in secondary forest thickets (Irwin *et al.* 2010). Secondary thickets develop when forests are disturbed and cleared and the land is cultivated and subsequently abandoned, allowing exotic shrubs to invade. Most remaining native elements are widespread species adapted to clearings and edges (Lowry *et al.* 1997; Irwin *et al.* 2010).

Data analyses: habitats

Forest fragments were characterized as more or less degraded by their species diversity, tree densities, total basal area (TBA), mean basal area (MBA), anthropogenic disturbances, and the presence of *Lantana camara*. Based on the presence and severity of each of these factors in each individual habitat, we established a habitat gradient (more degraded to least degraded). This allowed us to make comparisons in behavioral traits for those individuals found in different habitat types.

Plant diversity was determined by calculating the Shannon-Wiener diversity index (H') for each focal individual's home range (Krebs 1989; Rovero *et al.* 2012; Tesfaye *et al.* 2013). Higher values of H' were indicative of a more evenly distributed and diverse habitat. Calculations of the total basal area (TBA) (m^2/ha) and mean basal area (MBA) (m^2) for each plot indicated stand density, and were used to compare stature and biomass of different habitats. Habitats were considered to be more altered if they had a greater number of anthropogenic disturbances present, and a higher percentage of *Lantana camara* present in each habitat plot.

Data analysis: behavior

We determined the activity budget of each individual by combining all observations of a focal individual and calculating a proportion for each behavior. The behaviors collected during scan intervals were combined into five categories: feeding, resting, moving, sleeping and grooming/socializing (including autogrooming). The difficulty in seeing these cryptic lemurs and the visual limitations imposed by their nocturnal behavior meant that focal individuals were sometimes scored as "out of sight." Out of sight records, which ranged from 12.5−34.7% of observations for the four focal subjects, were excluded from the analysis so we could compare the behavior of individuals when directly observed.

To compare the composition of the diets of the individuals in different habitats, we calculated the proportions of different plant parts and species consumed by each. We also measured the food selection ratio for different food species in the diets of each focal individual (a rudimentary indicator of preference). This was calculated by dividing the percentage of time spent feeding on species *i* by the percentage species *i* was found in the plots and presumed to be available to that individual (Chapman and Chapman 2002; Tesfaye *et al*. 2013). A higher selection ratio value is indicative of a more selected species.

Individual home range areas (ha) were calculated using fixed kernel UD and kernel area tests in the R-package

AdehabitatHR. Choosing an appropriate smoothing parameter is the most important aspect when using fixed kernel methodology for analyzing home ranges (Silverman 1986; Worton 1995; Campera *et al*. 2014). While LSCVh (Least squares cross-validation) is the generally favored method, it can provide poor results with small samples sizes (Horne and Garton 2006; Campera *et al.* 2014). As such, data were analyzed using a specified, uniform smoothing parameter given by the LSCVh (h = 11.37), but were not run with LSCVh methods. Kernel area results were compared with area estimates from GPS points in ArcMap 10.3. Home range was evaluated at 95% contours while core areas were evaluated at 75% and 50% contours (Laver and Kelly 2008).

Due to the very small sample size of focal individuals (N = 4), additional statistical tests could not be conducted. Although the sample size is very small, the focal individuals represent approximately 8% of the estimated population of this species.

Ethical note

Our observational research was noninvasive and was conducted under the University of Wisconsin-Madison's Animal Care Waiver for Observational Studies and was in compliance with Malagasy law. The four focal individuals were darted and captured to equip them with radio-collars prior to this study (October 2012) and covered under a separate permit to E. E. Louis Jr. (permit no: 155/12/MEF/SG/DGF/DCB.SAP/SCB) under the Institutional Animal Care and Use Committee #12-101. Individuals were immobilized using a CO_2 remote capture MJ Model DanInject rifle and PneuDarts using 10 mg/ml of Telazol®. These darting methods and the protocol followed those described in Louis *et al.* (2006).

Results

Habitat

Tree density, tree basal area (MBA and TBA), tree diversity, and recorded disturbances varied greatly between each 50 m × 20 m plot. Habitat for individual 'O' had the lowest TBA, tree diversity, and species richness, and the highest indices of *L. camara* and other disturbances (Fig. 2; Table 1). The habitats of the remaining individuals, showed similar tree diversity, but the other parameters varied. Although the habitat of individual 'L' had the highest tree density, it had the lowest MBA. It is important to note that the last two subplots of the habitat of "L" fell slightly outside the individual's home range; these subplots had the highest tree density and lowest MBA in the overall plot. The area had no *L. camara*, and the lowest number of disturbances. It was expected that the habitat of 'F' and 'M' would be similar, as they inhabited the same forest fragment. Both these areas had two types of disturbance present (Fig. 2), and higher MBA and TBA values compared to 'L' and 'O'; however, MBA for 'M' was almost double that of 'F', and TBA was also much higher.

From 67 to 93 home range points were collected on the four focal subjects (Fig. 2). Fixed kernel area analyses indicated that individuals in areas with more anthropogenic activities had larger home ranges. Individual 'F' had the largest home range (95% contour), approximately 2× that of individuals 'L' and 'M' (Table 2). Core areas (50% contour) were more uniform among three of the individuals; however, individual 'F' still had a core area approximately 2× that of the others (Table 2). Individual home ranges varied from 1.32 to 2.90 ha.

The percentage of each tree species per individual plot was highly variable (Table 3). Although 'O' did not have the lowest density (but did have the lowest species richness),

Table 1. Habitat variables and disturbance parameters in each habitat plot in Montagne des Français, Madagascar, June−August 2013.

	Individual lemurs			
Habitat variable	**F**	**O**	**L**	**M**
Tree density (trees/ha)	2,960	2,200	6,750	2,190
Mean basal area (m^2)	0.097±0.79	0.046 ±0.083	0.04±0.13	0.19±1.09
Total basal area (m^2/ha)	288.53±40.77	100.7±2.51	219.69±11.68	411.27±67.26
Diversity (Shannon-Wiener index, H')	2.99±0.07	2.09±0.11	2.60±0.09	2.77±0.08
Tree species richness (species/ha)	40	20	29	33
Invasive (no. of subplots w/ *L. camara*)	3/10	10/10	0/10	0/10
Disturbance (no. of sign types)	2	4	1	2

Table 2. Home and core ranges (ha) of individual lemurs in Montagne des Francais, Madagascar, June−August 2013.

	Individual lemurs			
	F	**O**	**L**	**M**
95% fixed kernel range (ha)	2.90	1.94	1.32	1.49
75% fixed kernel range (ha)	1.44	0.84	0.71	0.78
50% fixed kernel range (ha)	0.68	0.35	0.39	0.34

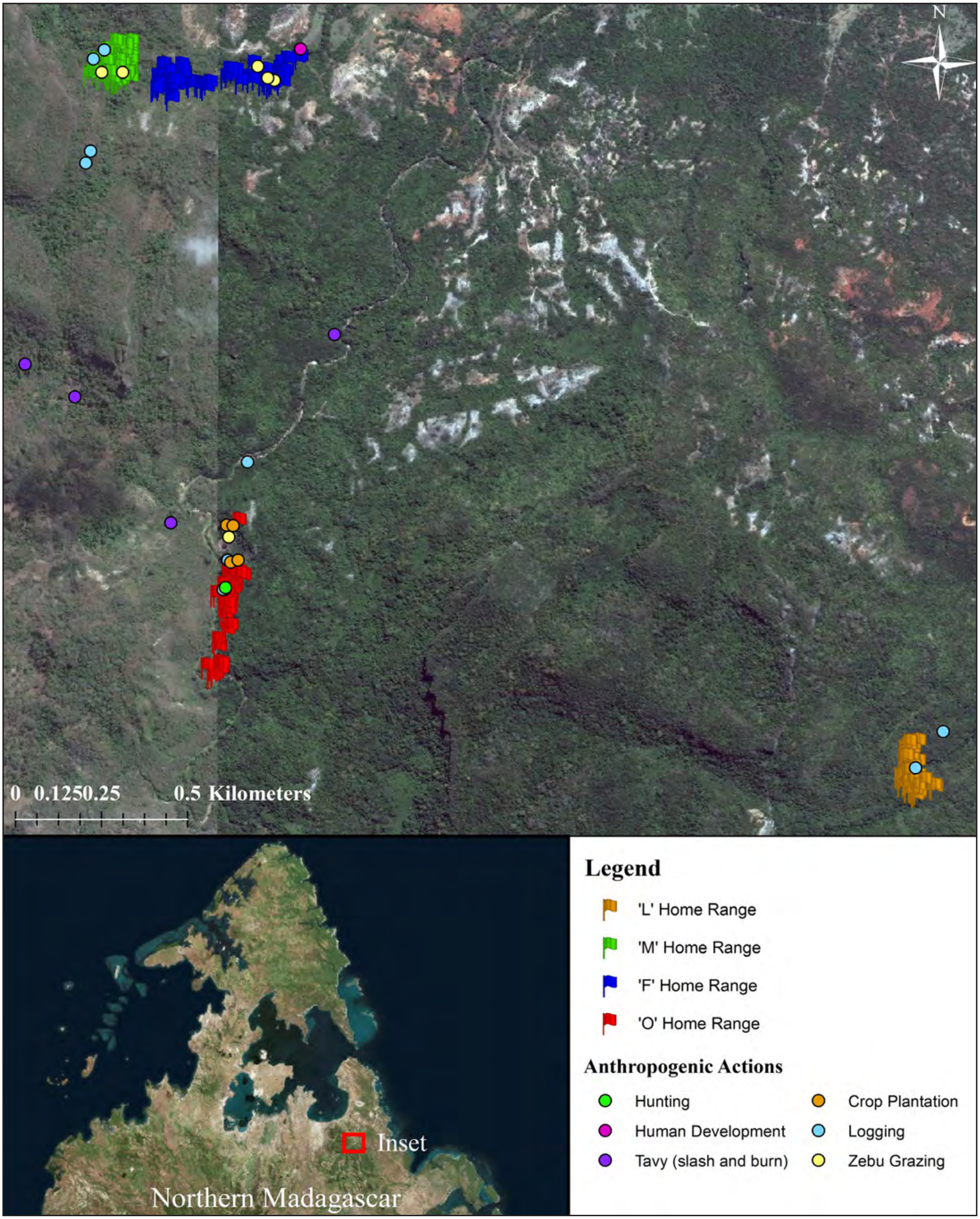

Figure 2. Anthropogenic disturbances and home range points for focal individuals in Montagne des Français, Madagascar, June−August 2013.

Table 3. Overall percentage of individual plant species within each habitat plot in Montagne des Français, Madagascar, June−August 2013.

		Individual Lemurs			
Scientific name	**Vernacular name**	**F**	**O**	**L**	**M**
Albizia lebbeck	Bonarabe	0.68	0.91	-	-
Albizia polyphylla	Hazomborogo/ Fagnamponga	0.34	-	-	-
Annona sp.	Konokono	-	0.45	-	0.91
Antidesma petiolare	Taindalitry	20.27	-	-	5.02
Artocarpus sp.	Finesy	-	-	-	2.28
Brexia sp.	Tsimiranjagna	1.35	-	-	0.46
Canarium sp.	Ndramy	2.36	-	-	9.13
Caesalpinia sp.	Ritro	1.69	3.18	0.30	0.46
Cephalocroton leucocephala	Vahimavo	0.34	-	2.67	1.83
Coffea sp.	Kafe Ala	0.34	-	0.74	0.46
Commiphora lasiodisca	Matambelona	0.34	-	1.04	2.74
Coptosperma sp.	Taolanosy	0.34	-	5.33	-
Cryptostegia madagascariensis	Lombiry	5.74	-	-	-
Delonyx sp.	Folerabonane	-	32.27	-	-
Diopsyros mapingo.	Mapingo	-	-	0.44	-
Diopsyros sp.	Jobiampototro	-	-	2.07	2.74
Dombeya sp.	Tavoa	1.83	-	-	-
Dracaena reflexa	Hasigny	-	-	-	0.46
Elaeodendron sp.	Matifioditra	-	0.91	-	-
Erytroxylum platycladum	Tapiaka	1.35	-	-	-
Fernandoa madagascariensis	Sombotsoy/ Sombitsohy	-	-	-	0.91
Ficus grevei	Mandresy	0.34	-	-	-
Ficus sp.	Voara	0.34	-	-	0.46
Grewia triflora	Sely	13.18	0.45	10.96	-
Jatrofa sp.	Valavelona	5.74	-	-	5.48
Landolphia tenuis	Vahegny	0.68	-	-	-
Leucaena leucocephala	Bonaramatsigny	0.34	20.00	-	1.37
Macpherson ia	Somtororagna	1.69	2.27	22.07	-
Mangifera indica	Manga	-	-	-	1.83
Mascarhenasia sp.	Barabonja	-	-	-	2.74
Moringa oleifera	Agnamorongo	-	0.91	-	-
Maytenus fasciculata	Vahompy	2.03	-	4.74	0.46
Maytenus linearis	Tsimboagnandrefa	0.68	-	-	-
Monanthotaxis boivinii	Fotsiavadiky	3.38	-	-	5.02
Neobeguea ankaranensis	Gavoala	-	1.36	0.89	-
Olax antsiranensis	Kombimba	-	0.45	1.48	-
Olax dissitiflora	Hazotomendry	0.34	-	2.22	-
Pentachlaena sp.	Tamenaka	-	-	-	0.46
Physena sessiliflora	Rasaonjo	-	20.00	6.37	-
Phyllanthus sp.	Mantsikiriva	9.12	-	-	1.37
Pittosporum sp.	Maimbovitsiky	0.68	-	-	-
Poupartia coffra	Sakoagna	-	-	-	0.46
Poupartia sp.	Sakoadihy	2.36	-	-	-

table continued on next page

Table 3. *continued from previous page*

Scientific name	Vernacular name	Individual Lemurs			
		F	O	L	M
Protorhus oblongifolia	Tsy maty magnota	0.34	-	-	-
Rourea orientalis	Magnary	-	-	0.15	1.83
Secamone sp.	Vahilombiry	2.36	-	0.15	-
Senna occidentalis	Sambaravatsy	4.39	8.18	-	-
Sorindea sp.	Sonjiry	2.03	-	4.44	16.44
Strychnos madagascariensis	Vakakoana	-	-	0.30	-
Syzygium sp.	Rotro	2.03	-	9.78	-
Tamarindus indica	Madiromatsiko	-	0.45	3.41	-
Tannodia sp.	Hazodomoy	-	-	1.48	-
Terminalia crenata	Mantaly	-	-	0.89	-
Thespesia gummiflua	Varoala	1.01	-	-	-
Trema sp.	Angezoko	-	0.45	-	-
Trilepisium madagascariense	Lonjo	2.03	1.36	3.11	10.50
Xanthocercis madagascariensis	Voankazomelako	-	1.82	0.30	-
	Unknown Species (multiple)	4.73	2.27	14.07	19.18
	Avocado	-	-	-	0.46
	Gavo	0.68	-	-	-
	Kiranzabmiavy	-	-	0.15	-
	Lavanana	1.69	1.36	-	-
	Mampingo	0.34	-	-	-
	Orange	-	-	-	0.46
	Paiso	-	-	-	0.91
	Pibasy	-	-	-	0.46
	Rotrobentiana	-	0.91	0.15	-
	Sarylombaka	1.01	-	-	-
	Tangegny	0.34	-	-	0.46
	Tsymagnotry	-	-	-	0.46
	Vahy	0.68	-	-	-
	Vahimalimy	-	-	0.15	-
	Vesoala	0.34	-	-	-
	Vontoka	-	-	0.15	-

three species (*Leucaena leucocephala*, *Delonyx* sp., and *Physena sessiliflora*) constituted 72.3% of total trees in the plot. This is reflected in the Shannon-Weiner Diversity index, as ‘O’ had the lowest H' value (Table 1). The other plots had a more distributed plant species composition. The top three species in the habitat of ‘F’ (*Antidesma petiolare*, *Grewia triflora*, and *Phyllanthus* sp.) constituted 42.6% of the trees. In the plot of ‘L”, the top three species (*Macpherson ia*, *Grewia* sp., and an unidentified species) made up 47.1% of total trees counted, while in habitat of ‘M’ the top three species (*Sorindea* sp., and *Trilepisium madagascariense*, and an unidentified species) made up 46.1% of all trees within the plot. This is reflected in the comparable H' diversity indices of ‘F’, ‘M’, and ‘L’ (Table 1).

Activity budget

Activity budgets were similar for all individuals (Table 4). While ‘F’ and ‘O’ spent less of their observed time feeding than ‘L’ and ‘M’, the difference was marginal. A similar result was found when comparing the percentage of time moving. Again, individuals ‘F’ and ‘O’ spent more of their time moving than ‘L’ and ‘M’, although the differences are slight. The percentage of time resting did not vary between individuals.

Dietary composition and selectivity

While the percentage of time spent feeding per individual did not differ greatly between the four individuals, the composition of what each consumed differed (Table 5). Individuals

Table 4. Overall percentage of activity budget behaviors per individual lemur in Montagne des Français, Madagascar, June−August 2013

	Individual lemurs			
Behavior	**F (N = 479)**	**O (N = 558)**	**L (N = 611)**	**M (N = 585)**
Feeding	13.36	12.01	14.73	15.55
Moving	15.45	13.62	9.98	11.45
Resting	63.68	64.51	64.48	62.91
Grooming/Socializing	6.05	5.38	5.24	6.67
Sleeping	1.46	4.48	5.57	3.42

Table 5. Overall percentage of time spent feeding on specific plant parts per individual lemur in Montagne des Français, Madagascar, June-August 2013.

	Individual lemurs			
Type of food	**F (*N* = 64)**	**O (*N* = 67)**	**L (*N* = 90)**	**M (*N* = 91)**
Leaves	78.13	77.61	87.79	81.32
Fruit	17.19	19.40	1.11	4.40
Flower	1.56	-	-	14.29
New Shoot	3.13	1.49	6.67	-
Vine	-	-	4.44	-
Bark	-	1.11	-	-

in areas with more anthropogenic activities and *Lantana camara* (individuals 'F' and 'O') consumed a lower percentage of leaves (78.1% and 77.6%, respectively) and a higher percentage of fruit (19.4% and 17.2%, respectively) than the other focal subjects (Table 5). Conversely, individuals 'L' and 'M' consumed a higher percentage of leaves (87.8% and 81.3%, respectively) and a much lower percentage of fruit (1.1% and 4.4%, respectively) (Table 5). Individual 'M' spent a higher percentage of her time consuming flowers (14.3%) compared to the other females (Table 5).

Individual 'F' consumed a total of 15 plant species, with *Ficus* sp. (34.35%), *Grewia triflora* (14.1%), and *Landolphia tenuis* (14.1%) as the top three most consumed, accounting for 62.5% of her feeding records (Table 6). 'O' consumed nine plant species in all, with *Grewia triflora* (23.9%), *Tamarindus indica* (23.9%), and *Leucaena leucocephala* (16.4%) being the top three most consumed, accounting for 64.2% of her total diet (Table 7). 'L' consumed items of a total of 12 plant species, with *Tamarindus indica* (26.7%), *Trilepisium madagascariense* (18.30%), and *Tannodia* sp. (14.4%) the top three species, accounting for 59.4% of her overall diet (Table 8). Individual 'M' consumed items from a total of 14 plant species, with *Leucaena leucocephala* (42.9%), *Mangifera indica* (24.2%), and *Tamarindus indica* (13.3%) as the top three most consumed plant species, accounting for 80.23 % of her overall diet (Table 9).

Individuals 'F' and 'O', were most selective for *Rourea orientalis* (313; Table 6) and *Cephalocroton leucocephala* (889; Table 7), respectively. Individual 'L' was most selective for *Paederia argentea* with a ratio value of 1111 (Table 8). This was the second most selected species for individual 'M' with a ratio value of 330. Individual 'M' had the highest selectivity for *Tamarindus indica* with a ratio value of 1319 (Table 9). This species also contributed greatly to the overall diet of individuals 'O' and 'L', but with lower selection ratios.

Discussion

The forest fragments inhabited by the four focal individuals differed considerably in vegetation composition and the number and types of anthropogenic activities. Yet, despite these habitat differences (number of types of disturbance, tree density, TBA, MBA, diversity, and the presence of *L. camara*) the lemurs' overall activity budgets were quite similar. Individuals in habitats with more anthropogenic activities spent less time feeding and more time moving than those in habitats with fewer anthropogenic activities, but the differences were marginal. Individuals in all forest fragments also consumed similar numbers of species.

While these individuals living in different forest patches showed similar activity budgets and types of food species consumed, they varied in the percentage of time they spent feeding on different plant parts. Individuals 'O' and 'F' spent less time eating leaves and more time eating fruit, while 'L' and 'M' spent a much lower percent of their time consuming fruit. It is not clear, however, whether these differences are due to differences in the availability of leaves versus fruits in their respective habitats, which could be more pronounced during the dry season. Individuals with the highest percentage of feeding time on fruit consumed just two species each, one of which, *Grewia triflora*, was eaten by both. The three species chosen for their fruit also contributed to a large percentage of total leaves consumed by these individuals. The selection ratio was moderate for the two species consumed by 'O' while 'F's selection ratio was high for one of the fruit species she consumed. This could suggest that lemurs that did

Table 6. Overall percentage of time spent by individual 'F' on specific plant parts (N = 64) and selection ratio for each species consumed at Montagne des Français, Madagascar, June−August 2013

Scientific name	Local name	Type	Percent of diet				Selection ratio
			Leaves	Fruit	Flowers	New shoots	
Leucaena leucocephala	Bonaramatsigny	Tree	3.13	-	-	-	3
Senna ocidentalis	Sambaravatsy	Tree	3.13	-	-	-	1
Rourea orientalis	Magnary	Tree	3.13	-	-	-	313
Ficus sp.	Voara	Tree	20.30	14.05	-	-	101
Monanthotaxis boivinii	Fotsiavadiky	Tree	1.56	-	-	-	1
Grewia triflora	Sely	Tree	10.94	3.13	-	-	1
Tamarindus indica	Madiromatsiko	Tree	1.56	-	-	-	156
Mangifera indica	Manga	Tree	-	-	1.56	-	156
Ziziphus jujuba	Lamoty	Tree	1.56	-	-	-	156
Maytenus linearis	Tsimboagnandrefa	Tree	9.38	-	-	-	6
Caesalpinia sp.	Ritro	Vine	7.81	-	-	-	5
Landolphia tenuis	Vagheny	Vine	10.94	-	-	3.13	21
Cephalocroton leucocephala	Vahimavo	Vine	1.56	-	-	-	156
Entada leptostachya	Vahintsikidy	Vine	1.56	-	-	-	156
Paederia argentea	Vahivola	Vine	1.56	-	-	-	156

Table 7. Overall percentage of time spent by individual 'O' on specific plant parts (N = 67) and selection ratio for each species consumed at Montagne des Français, Madagascar, June−August 2013

Scientific name	Local name	Type	Percent of Diet				Selection ratio
			Leaves	Fruit	Flowers	New shoots	
Morenga olifera	Agnamorongo	Tree	8.96	-	-	-	10
Leucaena leucocephala	Bonaramatsigny	Tree	14.93	-	-	1.49	1
Senna occidentalis	Sambaravatsy	Tree	1.49	-	-	-	1
Grewia triflora	Sely	Tree	8.96	14.93	-	-	53
Tamarindus indica	Madiromatsiko	Tree	19.40	4.48	-	-	53
Xanthocercis madagascariensis	Voankazomelako	Tree	2.99	-	-	-	2
Cephalocroton leucocephala	Vahimavo	Vine	7.46	-	1.49	-	895
Caesalpinia sp.	Ritro	Vine	5.97	-	-	-	2
Landolphia tenuis	Vahegny	Vine	7.46	-	-	-	746

Table 8. Overall percentage of time spent by individual 'L' on specific plant parts (N = 90) and selection ratio for each species consumed at Montagne des Français, Madagascar, June−August 2013.

Scientific name	Local name	Type	Percent of diet				Selection ratio
			Leaves	Fruit	New shoots	Vines	
Leucaena leucocephala	Bonaramatsigny	Tree	8.89	-	-	-	889
Grewia triflora	Sely	Tree	-	-	5.56	-	1
Tannodia sp.	Hazodomoy	Tree	14.44	-	-	-	10
Trilepisium madagascariense	Lonjo	Tree	17.78	-	1.11	-	6
Tamarindus indica	Madiromatsiko	Tree	25.56	1.11	-	-	8
Syzygium sp.	Rotro	Tree	1.11	-	-	-	1
Macpherson ia	Somotroragna	Tree	1.11	-	-	-	1
Strychnos madagascariensis	Vakakoana	Tree	1.11	-	-	-	4
Olax dissitiflora	Hazotomendry	Tree	1.11	-	-	-	1
Secamone sp.	Vahilombiry	Vine	4.44	-	-	3.33	52
Paederia argentea	Vahivola	Vine	11.11	-	-	-	1111
		Epiphyte	1.11	-	-	1.11	222

Table 9. Overall percentage of time spent by individual 'M' on specific plant parts (N = 91) and selection ratio for each species consumed at Montagne des Français, Madagascar, June−August 2013.

Scientific name	Local name	Type	Percent of diet			Selection ratio
			Leaves	Fruit	Flowers	
Leucaena leucocephala	Bonaramatsigny	Tree	41.76	1.10	-	31
Senna occidentalis	Sambaravatsy	Tree	3.30	-	-	2
Monanthotaxis boivinii	Fotsiavadiky	Tree	1.10	-	-	1
Grewia triflora	Sely	Tree	1.10	-	-	110
Rhopalocarpus sp.	Lombiry	Tree	2.20	-	-	220
Rhopalocarpus undulatus	Lombiry Ala	Tree	1.10	-	-	110
Trilepisium madagascariense	Lonjo	Tree	3.30	-	-	1
Tamarindus indica	Madiromatsiko	Tree	9.89	3.30	-	1319
Mangifera indica	Manga	Tree	9.89	-	14.29	13
Pentachlaena sp.	Tamenaka	Tree	1.10	-	-	110
Cephalocroton leucocephala	Vahimavo	Vine	1.10	-	-	1
Landolphia tenuis	Vahegny	Vine	1.10	-	-	110
Paederia argentea	Vahivola	Vine	3.30	-	-	330
	Vahy	Vine	1.10	-	-	110

consume more fruit were seeking species where they could gain the most nutritional benefits with the least energetic demands by selecting for species having both fruit and leaves available for consumption. Fruit consumed by these individuals included *Tamarindus indica*, a species that was also consumed for both its fruits and leaves by the other two individuals in this study. Because these data were only collected in the dry, winter months, it is possible that *Tamarindus indica* may act as a fallback food for the northern sportive lemur. Consuming *Tamarindus indica* as a fallback food source has been observed in other lemurs, especially for those living in disturbed areas during the dry months when overall food availability, especially fruit, is reduced (Sauther and Cuozzo 2009). Other species that were consumed by all individuals in both habitat types were *Leucaena leucocephala* and *Grewia triflora*, both of which were also fruiting at times during this study.

Although these data are preliminary, they suggest that the northern sportive lemur at Montagne des Français does slightly alter its diet in different forest patches. It appears that time budgets and overall plant species consumption did not vary between individuals. Those in areas with a greater number of different anthropogenic activities, however, spent more time consuming different food types, which could indicate dietary constraints. Alternatively, it is possible that the forest fragments were more similar than appeared from the data collected in this study. For example, the presence of more types of disturbances may not be as important as the severity of a single particular disturbance within a habitat. Additionally, because this species is a solitary forager, the overall level of anthropogenic disturbance in their habitat may not even be a constraining factor on their feeding ecology. A habitat with anthropogenic disturbance but that nonetheless contains some preferred species may be sufficient for a single individual to thrive.

Home range size has been reported for only four of the 26 species of sportive lemurs, but the estimated home ranges of 1.32−2.90 ha in the present study are larger than the 0.24−1.4 ha published ranges for other *Lepilemur* species (Hladik and Charles-Dominique 1974; Milton and May 1976; Warren and Crompton 1997; Zinner *et al*. 2003; Seiler 2012; Seiler *et al*. 2015). Our data show that individuals with a greater number of anthropogenic activities in their ranges and that consumed more fruit ('L' and 'M') have smaller and less distributed home ranges than the other individuals in this study ('F' and 'O'). This could indicate that although the activity budgets did not vary greatly between individuals, they may extend their home range in order to incorporate sufficient food sources, including a greater proportion of fruit, potentially compensating for a more altered environment. A similar response has been seen in *Eulemur* spp. and *Eulemur collaris* where populations living in human-altered degraded habitats had larger home ranges (Dehgan 2003; Schwitzer *et al*. 2011; Donati *et al*. 2011; Campera *et al*. 2014). It has also been suggested that long-tailed macaques (*Macaca fascicularis*) extended their home ranges in areas disturbed by drought and fire (Berenstain 1986). It is important to note that this pattern has been observed in frugivorous primates and has not been observed in other folivorous primates (Irwin 2008; Martinez 2008). More extensive data are needed to evaluate these potential factors and to determine if the northern sportive lemur does alter its behavior in other months of the year in response to varying degrees of anthropogenic activity.

It is important to note that the individual with the smallest estimated home range and lowest percentage of time consuming fruit ('L') was found in a habitat with the lowest

MBA, highest tree density, and second lowest TBA and diversity (H'), characteristics typically considered of more complex, degraded environments (Schwarzkopf and Rylands 1989; Grassi 2006). Similarly, individual 'O's habitat had the lowest TBA and diversity (H'), and a comparative MBA, also suggesting a complex, degraded environment, however, she spent a much greater percentage of time consuming fruit and had a larger home range than individual 'L'. This suggests that what appears to be a degraded habitat may not be driving individual northern sportive lemurs to alter their behavior at Montagne des Français. Instead, as previously noted variation in the lemurs' behavior could be due to the presence and degree of anthropogenic activities occurring within the home range, or alternatively, the spatial composition and abundance of significant feeding and sleeping trees within each individual home range.

The preliminary results obtained in this study are, at their most basic level, encouraging. Considering that the northern sportive lemur is currently living in different habitat fragments, at least from the behavioral and dietary comparisons quantified in this study, it seems that they are capable of adapting to the habitats with higher indicators of anthropogenic disturbances by incorporating more fruit into their diet and extending their range. Although our data show slight variations in behavior in different forest fragments, individuals are persisting and mating in varying degrees of degraded habitats at Montagne des Français. Previous studies have indicated that other folivorous primates, including lemurs, tend to have a more favorable response to forest disturbances and degradation than frugivores; certain lemur species may even increase their abundance, have more widespread and robust food resources, reduce their foraging time, and ultimately thrive in these types of environments (Irwin *et al*. 2010; Herrera 2011; Eppley *et al.* 2015, Sawyer *et al*. 2016). While it is unclear if the northern sportive lemur prefers degraded environments or intact forests, our data indicate that they are able to persist in various fragments within a degraded landscape. Nonetheless, the long-term conservation status of the northern sportive lemur is still of extreme concern. The limited distribution of this species is in direct conflict with the growing human population in the area and the easily accessible economic opportunities available to people within their habitat. Research suggests that once habitat fragmentation and degradation begin, the fragments continue to decrease in size and become less connected (Chatelain *et al.* 1996; Chapman *et al.* 2007; Tesfaye *et al.* 2013). This is of concern for the northern sportive lemur because other studies suggest that distance between trees and leaf quality are limiting constraints for *Lepilemur* spp. and they reach their limit in areas with moderate disturbance (Ganzhorn 1993). Furthermore, in cases of extreme fragmentation and isolation, native small mammal communities can disappear rapidly (Gibson *et al*. 2013). The deforestation and disturbance occurring at Montagne des Français is severe as poverty and dependence on non-timber forest products appears to be determining factors for the actions of local people.

Further studies and surveys involving the northern sportive lemur are needed in order to confirm the population size and broaden our knowledge of this Critically Endangered species. Additionally, long-term studies with a more robust sample size covering all seasons are needed in order to statistically determine if feeding ecology and home range size vary with type and frequency of anthropogenic activities. Additionally, by examining what tree species northern sportive lemurs prefer throughout the year will allow for the identification of more favorable habitats within Montagne des Français for subsequent conservation attention.

It is also imperative to evaluate the behavior of the local people and the driving forces behind their actions. Future work should identify the frequency and extent of the extraction of non-timber forest products (NTFP) by local populations. NTFPs, such as charcoal production, woodfuel, and bushmeat often support rural livelihoods as daily subsistence or supplemental income (Angelsen and Wunder 2003; Angelsen *et al*. 2014; Golden *et al.* 2014); however such a heavy reliance on NTFPs often has negative implications for the ecosystems providing these resources. Mapping the location of NTFP extraction in the area is essential in order to assess proximity to individual home ranges of northern sportive lemurs, and any associated behavioral changes. This can directly impact conservation initiatives and where selective logging may occur in the future. As habitat disturbance continues, the local people's willingness to actively participate in conservation measures (such as selective logging of only certain tree species), as well as the species' ability to withstand or adapt to these disturbances, will likely be determining factors in the persistence of northern sportive lemurs at Montagne des Français.

Acknowledgments

We thank the Ministère des Eaux et Forêts of Madagascar for issuing the permits allowing us to conduct this research. We acknowledge the people of Andavakoera village and the staff of the Madagascar Biodiversity Partnership for their assistance and in-country support. Berivo Sylvano (Tida) was of immense help as a local guide. In addition, we thank Ravelomalala Lucia Stephanie, Seva Josianne, Irene Helene, and Justin Sanamo for their help and support during the field season. Financial support was kindly provided by Conservation International's Primate Action Fund. Lisa Naughton-Treves and Rich McFarland provided helpful comments on an earlier draft of this manuscript. We are also grateful to Anthony Rylands for his insights and editorial revisions, and to the anonymous reviewers of this manuscript.

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Received for publication: 2 May 2016
Revised: 30 October 2016

Primate Conservation 2016 (30): 89–101

Going, Going, Gone…Is the Iconic Ring-tailed Lemur (*Lemur catta*) Headed for Imminent Extirpation?†

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Abstract: The ring-tailed lemur (*Lemur catta*) was once widely distributed throughout the south-central, far south, and southwest regions of Madagascar. This species is known for its marked ecological plasticity and ability to survive in a variety of habitats. Over the past decade, however, habitat destruction, forest fragmentation, hunting for subsistence or the illegal bushmeat trade, and live capture for the illegal pet trade have increased, resulting in extirpation or drastic reduction of populations throughout its geographic range. Recent mining activities in one region have resulted in further serious threats to remaining populations. In this paper, we discuss (1) population numbers and information on population extirpations, gathered over approximately the past six years, to illustrate the alarming decline of this well-known lemur, and (2) how the formerly accepted geographic range of *L. catta* now requires considerable revision. Population information was collected via on-the-ground surveys and censuses, or from reports by researchers at 34 sites where *L. catta* is or was recently present. Only three sites are known to contain populations of more than 200 animals. At 12 sites, populations number 30 or fewer individuals, and at 15 sites, *L. catta* has been recently extirpated, or populations are highly precarious and may become extinct in the very near future. Populations at three previously designated range limits have been extirpated. Many populations are surviving in small, isolated forest fragments, allowing for no male dispersal. With an estimate of just 2,000–2,400 individuals remaining in Madagascar, this iconic lemur may well become extinct in the wild in the near future, or at the very least, exist at only two or three widely dispersed sites.

Key Words: *Lemur catta*, local extinction, forest fragments, population decline, hunting, pet trade

Introduction

Populations of the iconic ring-tailed lemur (*Lemur catta*) were once widespread throughout varied habitats in southern, southwest and south-central Madagascar, largely due to this species' considerable ecological plasticity (Sauther *et al.* 1999; Sussman *et al.* 2003, 2006; Goodman 2006; Gould 2006; Gould and Gabriel 2015; Gould *et al.* 2015; Sauther *et al.* 2015). *Lemur catta* inhabits at least seven distinct habitats (Goodman *et al.* 2006; Gould 2006; Cameron and Gould 2013), and is able to adapt to local environmental extremes and natural disasters, for example, arid open spiny bush, with temperatures reaching or exceeding 45°C and with little available water (Sauther *et al.* 1999; Gould *et al.* 2011; Kelley 2011; LaFleur 2012), severe droughts (Gould *et al.* 1999), and cyclones (LaFleur and Gould 2009). However, anthropogenic habitat loss in the geographic range of *L. catta* is steadily increasing (Sussman *et al.* 2003; Harper *et al.* 2007; La Fleur 2013), and in 2003, Sussman *et al.* noted that remaining populations were unevenly distributed with very low densities. Since then, loss of forest cover throughout southern Madagascar has only increased, largely due to anthropogenic deforestation (Sussman *et al.* 2006; Bodin *et al.* 2006; Harper *et al.* 2007; Kelley *et al.* 2007; ONE *et al.* 2013a, 2013b), with little regeneration due to the xerophytic nature of much of the flora in the region (Seddon *et al.* 2000; Fenn 2003). Average rates of annual forest loss in protected areas (national parks and special reserves) in *L. catta*'s geographic range were reported as 0.28% per year from 2000–2005, and 0.22% per year from 2005–2010 (ONE *et al.* 2013a, 2013b); however, these data do not take

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† This paper is dedicated to the memory of Robert W. Sussman (1941–2016), highly respected scholar and pioneer of lemur studies.

into account the considerable amount of unprotected area in which *L. catta* has been reported. Furthermore, an increase in serious droughts in the south over the past decade has also exacerbated habitat loss (Elmqvist *et al.* 2007; Tengo and von Heland 2014). Sussman *et al.* (2006) estimated that *L. catta* habitat loss totaled 10% between 1985 and 2000, with a 20% reduction in the total population.

In 2001, Sussman *et al.* (2003) conducted a broad survey of *L. catta* in the south-western and far southern portions of its geographic range. At that time, forest fragmentation was noted as a marked threat to *L. catta* population survival. Since then, further and significant forest loss and increased fragmentation throughout southern Madagascar has been documented (Bodin *et al.* 2006; Harper *et al.* 2007; Cameron and Gould 2013). Fortunately, due to their ecological flexibility, ring-tailed lemurs in some regions are able to live, and in some cases, even thrive, in small fragments, particularly "sacred forests"—small patches containing Malagasy ancestral graves and tombs. Such forests range in size from <1 to 142 ha in the far southern Androy region (Tengo *et al.* 2007; Kelley 2011) and 2 to 53 ha in the south central area of *L. catta*'s geographic range (Gould and Andrianomena 2015; see Table 1). Sacred forests are normally undisturbed and can contain sufficient food and water resources to allow *L. catta* population persistence, reproduction and survival (Bodin *et al.* 2006; Gould and Gabriel 2014; Gould and Andrianomena 2015). However, the increasing fragmentation throughout southern Madagascar in the past decade, as well as heavy hunting for the illegal bushmeat trade and capture for the illegal pet trade, have resulted in the extirpation or drastic reduction of ring-tailed lemur populations at many sites where they are or were found (Sussman *et al.* 2006; Kelley *et al.* 2007; Cameron and Gould 2013; Sauther *et al.* 2015; Gould and Andrianomena 2015; LaFleur *et al.* 2015; Actman 2016; Reuter *et al.* 2016). As a result, the IUCN Red List status for *L. catta* was upgraded from Near Threatened to Endangered in 2012 (Andriaholinirina *et al.* 2014).

Historically, the geographic range of *Lemur catta* was broad, covering the southwestern, south-central, and southern portion of the island (Hill 1953; Tattersall 1982; Goodman *et al.* 2006). Its wide-ranging dispersal is due to its ability to survive and reproduce in areas that can be considered ecologically marginal, such as spiny bush and spiny thicket, high altitude ericoid bush, small rocky outcrop fragments, and mangroves (Goodman *et al.* 2006; Gould *et al.* 2006; Sauther *et al.* 2013). Ring-tailed lemurs are not restricted by river systems, and can survive in areas without a ground water source (Goodman *et al.* 2006). Goodman *et al.* (2006) also noted that since many river systems in southern Madagascar are without water for some months during the dry season, and since *L. catta* have been noted at gallery forest sites to cross these dry riverbeds, such ranging ability may have promoted wide geographic dispersal. However, continual anthropogenic habitat disturbance, habitat loss, and population extirpations have resulted in a much smaller geographic range than that outlined by Goodman *et al.* in 2006.

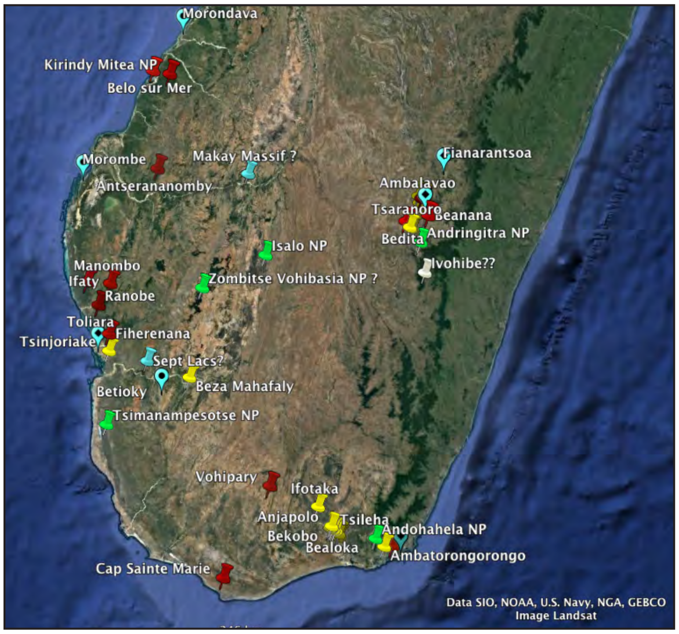
In this paper, we present *L. catta* population numbers and densities, or presence/absence information, mostly gathered over the past six years, covering 34 sites throughout this species' geographic range: south-central Madagascar, the far south, and the southwest. These sites include protected areas such as national parks, special reserves, and private reserves, as well as unprotected areas. We note the decline, and, in some cases, extirpation or likely extirpation, of the *L. catta* populations inhabiting some of these sites, and we also suggest a revision of the former boundaries of the geographic distribution of the ring-tailed lemur.

Methods

Population information was collected either via census counts by research teams, or census/survey and presence/absence reports in the literature, covering 34 sites where *L. catta* is or was recently present (Fig. 1). For the majority of sites, information was obtained within the past six years (Table 1).

South-central Madagascar

Censuses were conducted by Lisa Gould and team in 2013 in nine small (between 2 and 53 ha) forest fragments in south-central Madagascar, as part of a larger study of ecological factors driving population persistence or extinction (Gould and Cowen, in prep.; Fig. 2). The sites are situated in a 370-km^2 mountainous area and are, according to local guides with whom Gould worked, the only forest fragments containing *L. catta* in the region, outside of Andringitra National Park. Seven of the nine sites are protected by community-level



Data SIO, NOAA, U.S. Navy, NGA, GEBCO
Image Landsat

Figure 1. Known sites in the geographic range of *Lemur catta* where populations are extant, likely extirpated, or recently extinct. Red pins = extirpated or near extirpated populations, yellow pins = extant populations, green pins = national parks, where some populations are extant, others unknown and may be extinct, and blue pins = unknown, reported locally within the past 15 years but not seen during surveys.

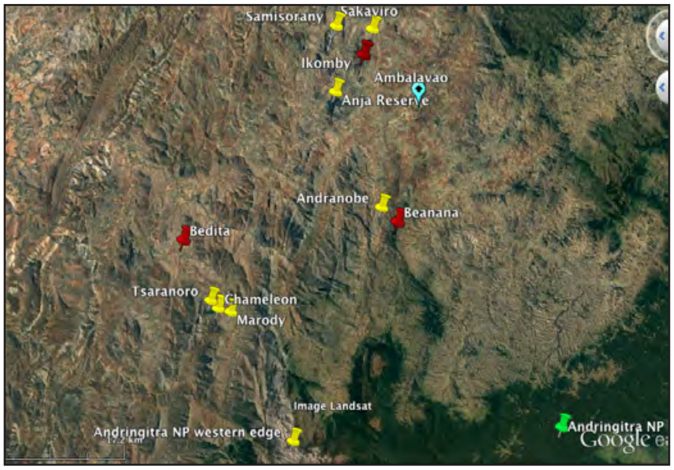


Figure 2. Sites in the south-central region of Madagascar where *Lemur catta* was surveyed and censused in 2013 by L. Gould and team.

Figure 3. Sites in the far southern region where *Lemur catta* was surveyed and censused in 2015 by Foulon *et al.* (2015).

conservation associations (Gould and Andrianomena 2015). Two of these, Anja Private Reserve and Tsaranoro Valley forest, are well-known tourist destinations. The two unprotected fragments have been seriously affected by firewood collection and forest degradation.

Census collection teams were composed of LG, as well as primary guide P. Andrianomena, and assistants J. Weir, and S. Rasoanomenjanahary. We were assisted by local guides from the above mentioned community-based conservation groups at the seven community-run sites. At each site, the census team counted all resident adult and juvenile (yearling) lemurs. Juveniles are easily determined as they are roughly half the size of adults. Animals were counted by each team member at least twice, and all counts fell within a two-animal margin of error. In smaller fragments (20 ha or fewer in size, see Table 1) all *L. catta* groups were located and members were counted over a period of one to three days. At the larger Anja and Tsaranoro forests, censuses took place over four to five days.

Andringitra and Isalo national parks are situated in south-central Madagascar. Gould and team counted two *L. catta* groups in Andringitra National Park in 2009. *Lemur catta* is also found at Isalo (Goodman *et al.*, 2006; LaFleur, pers. comm. 2016; Madagascar, MNP 2015).

Southern Madagascar

In Madagascar's far south, census data were collected on *L. catta* residing in nine forest fragments by Foulon *et al.* (2015). These fragments included the larger and well-known Berenty Reserve, 80 km from Taolagnaro (Fort-Dauphin), as well as a number of much smaller gallery forest fragments along the Mandrare River (Fig. 3). All lemurs were counted by the census team at least twice. The numbers of extant *L. catta* groups at Ifotaka-North (approximately 50 km north of Berenty), and Vohipary, a remote spiny forest fragment 55 km NW of Ifotaka, were noted in 2012, but individuals were not counted (Sterman 2012; Semel and Ferguson 2013). At Ambatotsirongorongo (approximately 25 km west of Taolagnaro), *L. catta* was censused by Razafindramanana in 2009, and at Petriky, the southeasternmost site in which *L. catta* has been recorded (Goodman 2006), individuals were counted by Malone *et al.* (2013) in 2012 (Fig. 4). Cap Sainte-Marie is the southernmost point at which *L. catta* has been recorded (Goodman *et al.* 2006). Animals were counted by Kelley (2011) during a year-long dissertation study in 2007–2008. The last census of *L. catta* at Andohahela National Park was conducted in 2003 (Rasoarimanana 2005).

Southwestern Madagascar

Individual animals have been counted at Bezà Mahafaly Reserve (Sauther and Cuozzo 2008; Sauther, 2014) and Tsinjoriake (Ravelohasindrazana 2013; Ravoavy 2013; I. A. J. Youssouf, pers. comm. 2016). Presence/absence of *L. catta* only was noted at Ifaty (I. A. J. Youssouf, pers. comm. 2016), as well as at Fiheranana-Manombo and Ranobe (Gardner *et al.* 2009), Makay forest (Dolch *et al.* 2012; Wendenbaum *et al.* 2014) and Antsiranamomby (Kelley *et al.* 2007). Historically, *L. catta* were recorded at three national parks in the southwest: Kirindy-Mitea, Tsimanampesotse, and Zombitse-Vohipary. Presence/absence, but not population counts have been reported at Kirindy-Mitea (R. J. Lewis, pers. comm. 2015) and Zombitse-Vohipary (M. Sauther, pers. obs.) and a partial population count is available for Tsimanampesotse (Sauther *et al.* 2014; M. LaFleur pers. comm.) (Fig. 5).

Area measurements of 17 fragments were calculated (1) using the polygon tool in Google Earth, and (2) polygon location points were entered into the Earthpoint tools KML Shapes program (<http://www.earthpoint.us/Shapes.aspx>) to determine actual fragment size in hectares. Areas of other sites were gathered from the literature.

Results

Table 1 lists all the sites discussed here, geographic locations, *L. catta* population counts or presence/absence estimates, population densities where possible, and forest sizes in hectares. At eight sites (Petriky, Ranobe, Kirindy-Mitea National Park, Antsiranamomby, Vohipary, Makay,

Table 1. Site names, locations, population counts or estimates, forest sizes, and population densities (where applicable) for the 34 known sites where *Lemur catta* are or were recently extant. Total population estimates are listed at the bottom of each larger region: south-central, southern, and southwestern Madagascar. n/d = no data.

Site and Location **Total N = 34 sites**	**Year of count or estimate and number of *L. catta* counted or estimated**	**Forest size and population density (where applicable)**
South-central Madagascar (N=12)		
Anja Community Reserve South-central 21°51'S, 46°50'E	2013: *c.* 210 (Gould and Andrianomena 2015)	34 ha (6.2/ha)
Sakaviro Community Reserve 21°48›S, 46°52'E	2013: *c.* 30 (Gould and Andrianomena 2015)	7.7 ha (3.8/ha)
Samisorany 21°47'S, 46°49'E	2013: 21 (Gould and Andrianomena 2015)	20.3 ha (1.03/ha)
Andranobe 21°57'S, 46°55'E	2013: 30 (Gould and Andrianomena 2015)	17.4 (1.7/ha)
Beanana 21°57'S, 46°56'E	2013: *c.* 6 (Gould and team, census) Likely extirpated in 2016	20.7 ha (0.28/ha)
Tsaranoro Valley forest 22°05'S, 46°46'E	2013: 78 (Gould and Andrianomena 2015)	53 ha (1.47/ha)
Chameleon 22°05'S, 46°46'E	2013: 28 (Gould and Andrianomena 2015)	8.1 ha (3.4/ha)
Marody 22°05'S, 46°47'E	2013: 15 (Gould and Andrianomena 2015) Precarious	2.8 ha (5.3/ha)
Ikomby 21°48'S, 46°51'E	2013: 8 (L. Gould and team, census) Likely extirpated in 2016	2 ha (4/ha)
Bedita 22°02'S, 46°43'E	2013: *c.* 15–20 (L. Gould and team, census) Precarious	Forest fragment not measured
Andringitra National Park (Western edge) 22°12' S, 46°53'E	2009: Estimate *c.* 100 (WWF guide, 2009) but likely fewer: 12 counted in 2009 (L. Gould, D. N. Gabriel and A. Cameron)	31,160 km^2
Isalo National Park 22°22'S, 45°11'E	Extant but number unknown	81,500 ha
Estimate of L. catta at known sites the south-central region	**c. 600–700 individuals**	
Southern Madagascar (N=13)		
Berenty Reserve (all parcels and environs, including gallery, scrub, spiny forests + tourist and factory areas) 25°34'S,46°18'E	2015: 562 (Foulon *et al.* 2015)	285 ha (2.0/ha)
Bealoka 24°57'S,46°16'E	2015: 163 (Foulon *et al.* 2016)	100 ha (1.63/ha)
Tsileha 24°57'S,46°14'E	2015: 39 (Foulon *et al.* 2016)	48 ha (0.81/ha)
Bekobo 24°56'S, 46°13'E	2015: 21 (Foulon *et al.* 2016)	52 ha (0.40/ha)
Analalava 24°56'S, 46°12'E	2015: 21 (Foulon *et al.* 2016)	95 ha (0.22/ha)
Anjapolo 24°55'S, 46°13'E	2015: 70 (Foulon *et al.* 2016)	104 ha (0.67/ha)
Ambinany 24°59'S, 46°18'E	2015: 65 (Foulon *et al.* 2016)	60 ha (1.08/ha)
Ambatorongorongo 25°04'S, 46°46'E	2010: *c.* 50 in 30ha (Razafindramanana 2011)	30 ha (1.5/ha)
Petriky 25°03'S, 46°52'E	2012: 4 animals in 920 ha. Likely extirpated (M. Malone, pers. comm.)	920 ha (0.004/ha)
Vohipary 24°36'S, 45°33'E	2012: 1 small group (individuals not counted) (Sterman 2012) Precarious or possibly extirpated in 2016	1250 ha
Ifotaka-North-Protected Area 24°45'S, 26°04'E	2012: 3 groups (individuals not counted) (B. P. Semel, pers. comm.) Precarious	22, 256 ha (Mahamaro 2012)
Cap Sante-Marie 25°27'S, 45°06'E	2007: 4-5 groups in 2007 (Kelley 2011, 2013) Status in 2016 unknown	*c.* 0.017/ha (Kelley 2013)

table continued on next page

Table 1, *continued*

Site and Location **Total N = 34 sites**	**Year of count or estimate and number of *L. catta* counted or estimated**	**Forest size and population density (where applicable)**
Andohahela National Park 24°59'S, 46°40'E	2003: 118 (Rasoarimanana 2005) Status unknown 2016, hunting reported (Raharivololona and Ranaivosoa 2000; Madagascar Biodiversity Fund, undated)	76,000 ha
Estimate of *L. catta* at known sites in the far southern region	*c.* 1000 individuals	
Southwestern Madagascar (N = 9)		
Beza Mahafaly Reserve All parcels 23°41'S, 44°22'E	2014: *c.* 200 (Sauther 2014)	Parcel 1: 80 ha (124 animals – 1.5/ha) All parcels: 900 ha
Tsinjoriake 23°26'S, 43°45'E	2016: 27 (I. A. J. Youssouf, pers. comm.) Precarious	25,000 ha (0.01/ha)
Fiheranana-Manombo complex and Ranobe 23°14'S, 43°52'E 22°48'S, 43°44'E	2009: (3) data deficient Likely extirpated (Gardner *et al.* 2009)	n/d
Ifaty forest 22°48'S, 43°28'E	2016: Precarious or possibly extirpated Hunting and live capture threat (I. A. J. Youssouf, pers. comm.)	n/d
Antsirananomby 21°42S, 44°8'E	2004: observed 10 times, 5 were of a lone individual (Kelley *et al.*, 2007) Status unknown likely extirpated 2016	Forest fragmented and degraded
Makay Massif 21°41'S, 45°04'E	2012: 2 surveys, none observed Possibly extirpated Dolch *et al.* (2012), Wendenbaum *et al.* (2014)	n/d
Kirindy Mitea National Park Southwest 20°03'S, 44°39'E	2012: 2 seen at Ankoatsifaka in 2012, none since. Likely extirpated in 2016 (R. J. Lewis, pers. comm. 2015).	152,000 ha (0/ha)
Tsimanampesotse National Park 24°03'S, 43°46'E	2006-2014: *c.* 80–100. 11 groups counted between 2006–2014 (Sauther *et al.* 2013; M. LaFleur, pers. comm.) Live capture threat (La Fleur *et al.* 2015)	456 km^2 (0.18/ha)
Zombitse-Vohibasia National Park 22°45'S, 44°37'E	Status unknown Likely extirpated (M. Sauther, pers. obs.)	36,308 ha (density likely 0/ha)
Estimate of *L. catta* at known sites in the southwestern region	*c.* 350 individuals	
Total population estimate based on populations at known sites	*c.* 2000–2400 individuals	

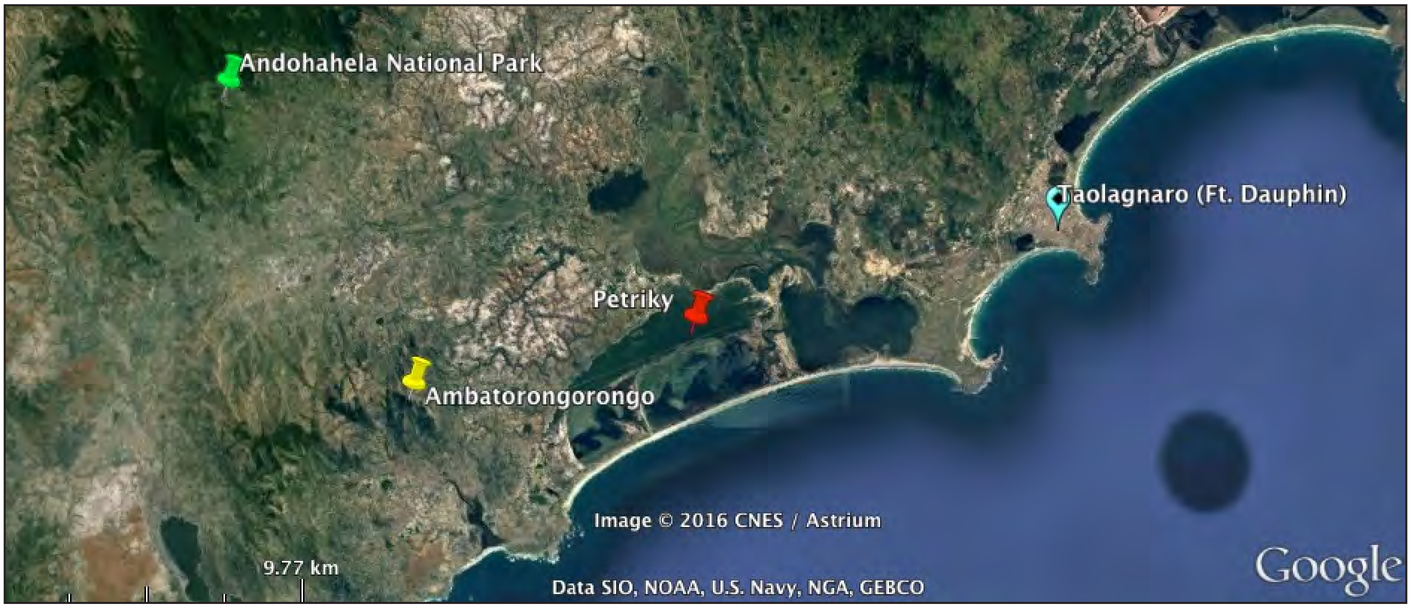


Figure 4. Sites at the far southeastern limit of the range of *Lemur catta*. Note that at Petriky, formerly the southeastern-most site where *L. catta* was found (Goodman *et al.* 2006), the population is likely now extirpated.

Fiheranana-Manombo, and Zombitse-Vohibasia National Park), ring-tailed lemurs were reported present until recently, but population sizes were very low (for example, at Petriky, only four lemurs were present in 2011, Table 1). Some of these populations are now likely extirpated. At five sites (Bedita, Beanana, Ifotaka North, Ifaty, and Tsinjoriake) populations are low and threatened by forest disturbance and/or heavy hunting pressure/live capture for the illegal pet trade. These are listed as "Precarious" or "Likely extirpated" in Table 1.

Figure 5. Sites in the southwestern region of the range of *Lemur catta* where populations are extant or recently extinct.

The former geographic distribution, proposed by Goodman *et al.* in 2006, is no longer accurate, due to recent population extirpations. Sites that were considered the northwestern (Kirindy-Mitea National Park and Belo sur Mer), northeastern (Ankafina), and southeastern (Petriky) limits of the range of *L. catta* (Goodman *et al.* 2006) no longer contain populations of ring-tailed lemurs. At Cap Sainte-Marie, the southernmost point of the geographic range of *L. catta*, four to five groups were present in 2007 (Kelley 2011), but no surveys have been conducted at this site since then.

Figures 6 and 7 reflect the old and newly proposed distributions. Population estimates of *L. catta* for each region are as follows: south-central Madagascar *c.* 600–700; southern Madagascar: *c.* 1000; and southwestern Madagascar: *c.* 350. The estimated total population of *L. catta* at known sites in the wild is *c.* 2000–2400 (Table 1).

Discussion

While this is not an exhaustive survey or census of every site inhabited by *Lemur catta*, the information in Table 1 represents all *known* populations and population extirpations as of 2016. Recent local extinctions, the known number of very small populations (*c.* 30 individuals or fewer) inhabiting small and isolated forest fragments, lack of continuous forest throughout the known range of *L. catta*, and the shrinking geographic range limits (Fig. 7) demonstrate that this well-known lemur is in rapid and alarming decline. In 2012, the IUCN Red List status of *L. catta* was upgraded from Near Threatened to Endangered, an increase of two categories. This designation was based partially on some of the data included in this paper (Andriaholinirina *et al.* 2014). Considering population

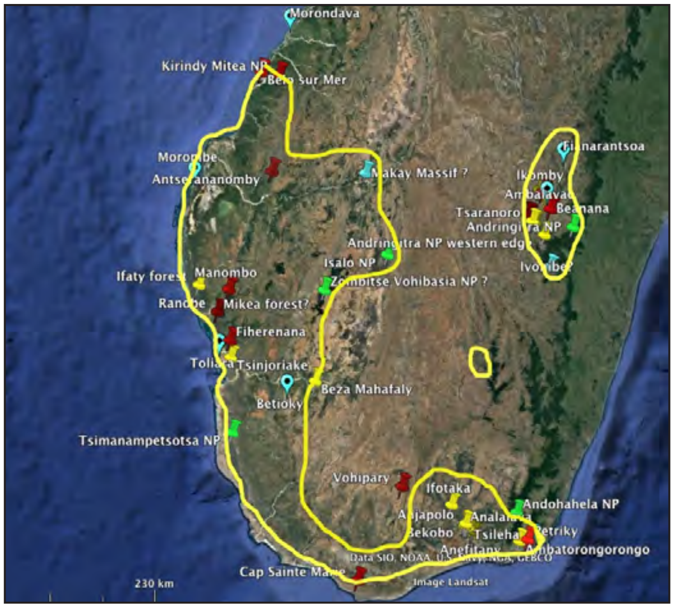


Figure 6. The former geographic distribution of *Lemur catta* (based on Goodman *et al.* 2006).

Figure 7. The current geographic distribution of *Lemur catta* based on information contained in this paper.

assessments over the past six years, and current threats to this species, it is clear that more local extinctions are on the horizon, and in August 2016, *L. catta* was placed on the list of the IUCN SSC Primate Specialist Group's "World's 25 Most Endangered Primates" (Schwitzer *et al.* in prep.).

Habitat fragmentation has been noted as a severe threat to this species since 2003 (Sussman *et al.* 2003). Since then, significant forest loss in most areas of the geographic range of *L. catta* means that more and more populations are now residing in small, isolated habitat remnants. Recent serious increases in illegal hunting for food for the black market bushmeat trade, and live capture for the illegal pet trade (Sauther *et al.* 2013; LaFleur *et al.* 2015; Reuter *et al.* 2016; I. A. J. Youssouf, pers. comm.) have decimated populations; local extinctions have occurred where *L. catta* was formerly abundant, and many remaining populations persist at extremely low numbers. These populations will likely be extirpated in the near future if they have not been already (for example, Zinner *et al.* 2001; Kelley *et al.* 2007; Gardner *et al.* 2009; Wendenbaum *et al.* 2013; Lemur Conservation Network 2015; Madagascar Biodiversity Fund, undated).

South-central Madagascar

The south-central plateau region contains no large, continuous tracts of forest, only scarce, small forest fragments dispersed throughout the landscape (Cameron and Gould 2013; Gould and Andrianomena 2015). This area contained far more forest cover just a few decades ago (J. N. Rabialahy, pers. comm.), but deforestation and fragmentation has reduced *L. catta* populations in this region to very low numbers, living in isolated patches surrounded by agriculture and villages. A survey by L. Gould and team in 2013 revealed only nine fragments within a 370-km^2 region that contained *L. catta* populations. The Anja Private Reserve and the Tsaranoro Valley forest are well-protected community operated, tourist destinations, where *L. catta* populations are the most numerous in the region (Table 1). Anja exhibits the highest population density of *L. catta* of any forest sampled; however, the 13 groups of lemurs inhabiting the reserve are crowded into a small area of 34 ha, with highly overlapping home ranges. Three smaller forests, Sakaviro, Samisorany, and Andranobe, are also community protected but without the tourism potential of the two larger sites. These smaller fragments contained small but seemingly healthy populations of ring-tailed lemurs in 2013, relative to fragment size, and in all of the above forests, food and water resources were sufficient for population survival (Gould and Cowen, in prep.). Other small fragments in the region were also surveyed. Two of these, Chameleon and Marody, were protected by villagers and each contained a very small population of *L. catta*, while the others (Ikomby, Bedita, Beanana) were unprotected, with regular tree-cutting and firewood collection. The latter three fragments contained very low numbers of lemurs in 2013, and population extirpation has likely occurred or is imminent. Six of the nine fragments surveyed in this region are too isolated to allow for male dispersal and gene flow, as they are either spaced too far from one another (for example, 7 to 18 km in the cases of Anja Reserve and Andranobe), or the landscape between fragments consists of roads, a major highway, numerous villages, agriculture, and large tracts of open savannah with no trees to allow for foraging, sleeping, or protection from predators. Such isolation is likely to result in dangerous losses of genetic variation (Davies and Schwitzer 2013; Clarke *et al.* 2015) if these populations survive. The remaining three sites, the Tsaranoro, Chameleon and Marody forests, are situated in the Tsaranoro Valley, and allow for some male dispersal, but the total *L. catta* population of the valley numbered only 121 individuals in 2013, and fires set for cattle grazing very near these small forests often burn out of control.

Both the Isalo and Andringitra national parks in this region have *L. catta* populations, but their sizes are unknown. Goodman and Langrand (1996) reported a potentially isolated group of eight high-altitude-dwelling *L. catta* at the upper elevations of the Andringitra Massif, near Pic Ivangomena (2,520 m elevation), but noted that these animals were threatened by frequent fires in the low density ericoid bush that they inhabited. In 2009, Gould and her team located two groups in the same region of the park, and counted 12 animals. Goodman and Langrand (1996) suggested, and we concur, that the number and density of this population is very low, as *L. catta* here are restricted to a small area of high-altitude ericoid bush at the western edge of the park. During observations in 2009, local people harassed the lemurs, despite the presence of WWF guides and the research team. It is unknown whether this population is still extant.

Lemur catta is also stated as present at Pic d'Ivohibe Special Reserve, 20 km south of Andringitra National Park (Travel Madagascar, undated), but again, no surveys or censuses have been conducted. No trails or tourism infrastructure exist at this site, thus it is unclear if *L. catta* are extant or protected there.

There are ring-tailed lemurs at Isalo National Park (M. LaFleur, pers. comm. 2016), but the total population number is unknown. The park is vast (81,000 ha), and there is at least one small population in one area of the park, which tourists visit and photograph.

Southern Madagascar

Madagascar's far south, stretching from Petriky and Andohahela National Park through Cap Sainte-Marie and inland, is characterized by spiny bush, occasional gallery forest, and a semi-arid climate with little annual rainfall (Bodin *et al.* 2006; Kelley 2011). In 2006, Bodin *et al.* noted that much of the far southern spiny forest ecoregion was severely fragmented and had been so for several decades, if not hundreds of years, but that many of the fragments were protected by local taboos (sacred forests). At the time of the Bodin *et al*. study, however, such traditional taboos were eroding, and the authors predicted a successive loss of forest patches in this area. Ring-tailed lemurs were found in a number of these small fragments throughout the far south, and, as seed dispersers, are or were, an essential aspect of the health of these small forests (Bodin *et al.* 2006; Tengo and von Heland 2014).

The best known of the southern sites is Berenty Reserve, a gallery forest managed by the de Heaulme family since 1936 (Jolly *et al.* 2006). The *L. catta* population there has remained relatively stable for decades (Jolly *et al.* 2006). The population of the reserve itself and surrounding environs is the largest currently known, at *c.* 560 animals (Foulon *et al.* 2015). *Lemur catta* populations of varying sizes are also found in six smaller gallery forests north of Berenty, following the course of the Mandrare River (Foulon *et al.* 2015). These forests are relatively isolated from one another, and separated by sisal plantations and agriculture (Fig. 3), but some male dispersal may be possible. The Ifotaka Community Forest is 24 km north of these fragments, and in 2012 three unprotected groups of *L. catta* were recorded there (Semel and Ferguson 2013), but were often chased by village dogs (B. P. Semel, pers. comm.). Lemur research at Ifotaka North was halted in 2013 due to the proliferation of small firearms used by, and the increased activity of, cattle thieves (*dahalo*), who burn villages and displace local people inhabiting the area; people who might otherwise protect the sacred forest patches (B. P. Semel and B. Ferguson, pers. comm.).

In 2007, two ring-tailed lemur groups, totaling only 24 individuals, were followed by Kelley at Cap Sainte-Marie (Kelley 2011), but the population fell to 17 during the year-long study. Both groups ranged outside of the Cap Sainte-Marie Special Reserve, in spiny thicket habitat, and were two of only four to five groups found in the region at that time.

Goodman *et al.* (2006) noted that the most easterly record of *L. catta* was at Petriky, a transitional zone between wet and dry forest, 32 km southwest of Taolagnaro (Fort-Dauphin) (Malone *et al.* 2013). During a survey of this forest, Malone *et al*. observed *L. catta* only twice, and only four lemurs were counted (M. Malone, pers. comm. 2012). The easternmost site where *L. catta* has been counted recently is Ambatorongorongo, 25 km west of Taolagnaro (Fort-Dauphin), where 50 animals were seen in 2009 (Razafindramanana 2011).

An extensive survey and census of *L. catta* was conducted at Andohahela National Park, north of Taolagnaro, between 1999 and 2003 (Raharivololona and Ranaivosoa, 2000; Rasoarimanana 2005). In 2003, only 118 individuals were counted. There have been no more recent surveys in the park, and hunting pressure in the area has been high for some time (Rasoarimanana 2005; Bucht 2011). *Lemur catta* was also reported to inhabit gallery forests west of Kalambatritra Massif in the southeast in the early 2000s, but information was anecdotal, and no ring-tailed lemurs were actually observed (Irwin 2001). Again, no recent surveys have been reported in this area, although a survey and action plan for the Kalambatritra Special Reserve was being planned in 2013 (Rasolofoharivelo *et al.* 2013).

South-western Madagascar

Lemur catta was once widely distributed along the west coast of Madagascar, from the Kirindy-Mitea region near Morondava, south through Tsimanampesotse National Park and Cap Sainte-Marie in the far south (Tattersall 1982; Sussman *et al*. 2003). These western forests have become severely fragmented over the past five decades, with a 10% overall loss between 1950 and the 1990s (Zinner *et al.* 2001), and the landscape has fragmented even further in the 15 years since the Zinner *et al.* study (Google Earth 2016). Much forest in this region was destroyed for maize production in the 1990s (Zinner *et al.* 2001; Kelley *et al.* 2007; Blanc-Pamard 2009), and Sussman *et al.* (2003) noted that significant deforestation had occurred in the southwest between 1985 and 2000 for grazing, small-scale farming, and charcoal production. Recent additional and significant threats to west-coast forests are the Toliara Sands mining project and oil exploration (Gardner 2009; Blanc-Pamard 2009; Huff 2016; Lemur Conservation Network 2015).

Kirindy-Mitea National Park and Belo-sur Mer were considered the north-western limits of *L. catta*'s distribution (Goodman *et al.* 2006); however, the population in this area is now considered extirpated—the last observation of *L. catta* in Kirindy-Mitea occurred in 2012, and consisted of just two individuals (R. J. Lewis, pers. comm. 2015). As far back as 1999, local people in the region outside of the national park reported that *L. catta* had not been seen in the area for years (Zinner *et al.* 2001). Similarly, Kelley *et al.* (2007) noted that the formerly lush forest of Antserananomby, east of Morombe, had, by 2004, been largely destroyed by slash-and-burn agriculture for maize crops. Only a few small fragments remained, and the gallery forest no longer existed. Heavy hunting of the remaining lemurs was apparent. In the 1960s and 1970s, Antserananomby was considered to have the highest primate density and diversity of any forest in western Madagascar (Sussman 1972; Sussman *et al.* 2006). This is the site where pioneer *L. catta* researcher Robert Sussman conducted his Ph.D. research in the early 1970s. In the 2004 survey, Kelley *et al.* observed *L. catta* only 10 times, and five of these observations were of just one individual. Furthermore, at least four lemur traps were observed during this survey.

Dolch *et al*. (2012) and Wendenbaum *et al.* (2013) found no *L. catta* in the Makay Massif, west of Morondava, even though ring-tailed lemurs had previously been reported there. Wendenbaum *et al.* (2013) suggested that heavy hunting, particularly in temporary hunting/fishing camps, as well as large-scale forest clearing for cattle grazing, will likely have reduced or extirpated any populations in the area.

Recent serious increases in hunting for the illegal bushmeat trade and live capture for the purposes of selling infant *L. catta* to hotels along the coast has severely affected ring-tailed lemur populations (Sauther *et al.* 2013; I. A. J. Youssouf, pers. comm.)—some are now extirpated and others persist at extremely low densities and are markedly threatened (Gardner *et al.* 2009; Sauther *et al.* 2013; Lemur Conservation Network, 2015). The Toliara Sands mining project seriously affects the Mikea forest, the Fiherenan-Manombo complex and the Ranobe forest. This project will have major negative impacts on local people and wildlife, including the potential destruction of sacred forests, heightened exposure to radiation from mine tailings, and the destruction of vast areas of forest (Gardner *et al.* 2009; Huff 2016; Environmental Justice Atlas 2015, Lemur Conservation Network 2015). With the addition of hunting pressure, several populations of *L. catta* in this region along the coast have been extirpated and others are now found in extremely low numbers (Gardner *et al.* 2009; Gardner and Davies 2014). Oil exploration is also occurring on the west coast and southwestern inland (Blanc-Pamard 2009; Caravel Energy Limited 2013; Environmental Justice Atlas 2015).

South of Toliara, significant deforestation occurred in 2013 to make way for new hotels (M. Sauther, pers. obs.). The Sept Lacs Region near Toliara along the Onilahy River contained key areas of gallery forest and wetland that in 2003 included numerous vulnerable species, including *L. catta*, although only presence/absence data are currently available (Emmett *et al.* 2003). The area was originally slated for ecotourism development and even as a national protected area as part of "The Durban Vision" (Tilghman *et al.* 2005). In 2004, however, an artisanal sapphire rush around the area of the village of Ifanato, near one of the lakes, brought in miners from other areas who hunted the local wildlife, including lemurs, to feed themselves (Tilghman *et al.* 2005). A 2014 report stated that while some of the habitat and lakes had been destroyed, WWF and the local community had recently worked to protect the remaining forest (Madonline 2014). Tourist circuits were developed, and local guides were trained to lead visitors through the area. It is currently unknown if or how many lemurs remain at this site.

The recently protected Tsinjoriake site is found between 15 km and 25 km south of Toliara. This site is composed of approximately 25,000 ha of limestone cliffs, southwestern dry spiny bush and coastal mangroves (Sauther *et al.* 2013). Eight ring-tailed lemur groups were reported at two locations during a survey in April, 2012, including groups using the local mangrove forests to obtain fresh water (Ravelohasindrazana 2013; Ravoavy 2013). In one area surveyed in 2016, however, just 27 individuals were counted (I. A. J. Youssouf, pers. comm. 2016), and the groups no longer used areas of the mangrove forest due to the large number of dogs in the area.

Lemur catta still occurs at the Bezà Mahafaly Reserve, a gallery forest research site inland from Toliara on the Mahafaly Plateau. The reserve, composed of two parcels, was established in the mid-1980s, and the population has remained stable since 1985, with a total of around 200 *L. catta* in both parcels of the reserve (Sauther and Cuozzo 2008; Sussman *et al.* 2012; Sauther 2014; Ranaivonasy *et al.* 2016). Due to 1) the stability of this population, 2) the large relatively intact gallery forest within the reserve, and 3) the degraded but habitable forest fragments around the reserve, the Bezà Mahafaly *L. catta* is an important source population (as per Pulliam 1988). While the population within the reserve has remained relatively constant over the years, a comparison of satellite images from 1990 and 2010 revealed that the unprotected forest surrounding the first reserve parcel had decreased by 20%, the second parcel had experienced some forest loss, and the forest contiguous to the second parcel had decreased by 50% in 20 years (Ranaivonasy *et al.* 2016). It is unknown if *L. catta* populations exist in other forest patches on the Mahafaly Plateau; during the 1990s very small, remnant, and isolated populations were observed in a few places, but the status of these in 2016 is not known. Thus, even there, there is cause for concern.

Ring-tailed lemurs were observed at Zombitse-Vohibasia National Park, north of Bezà Mahafaly Reserve, in 2001, but at a very low density, as they were heavily hunted (Sussman *et al.* 2003). It is unknown whether any remain in the park in 2016. A population of *L. catta* can still be found at the Tsimanampesotse National Park (LaFleur 2012; Sauther *et al.* 2013; LaFleur and Sauther 2015), although the total number of animals is unknown, as the park is large and, in places, inaccessible. Part of the population, containing around 80–100 animals, is continually monitored via long-term studies by M. LaFleur, and by M. Sauther and F. P. Cuozzo (Sauther *et al.* 2013; LaFleur *et al.* 2014; LaFleur and Sauther 2015). There are recent reports, however, of infants being taken for the illegal pet trade (LaFleur *et al.* 2015).

Conclusion

The information in this paper summarizes the population status at the majority of sites where *L. catta* are or have been reported, and demonstrates that this well-known lemur is in very serious and rapid decline, with a total estimation from known sites throughout their geographic range of only

Figure 8. *Lemur catta* at Tsaranoro Valley rocky outcrop forest, south-central Madagascar.

c. 2,000–2,400 animals. In most areas, populations of ring-tailed lemurs are shrinking, and numerous local extinctions in the past decade have been caused by deforestation for cattle grazing, agriculture, and charcoal production. Such deforestation has led to severe habitat fragmentation and local extinctions. Fires set in grassland areas to encourage re-growth for cattle grazing can spread to nearby small forest fragments inhabited by *L. catta* and rapidly destroy them. Hunting for the illegal bushmeat trade and live capture for the pet trade has taken its toll on many former populations, and in some regions, traditional taboos against harming ring-tailed lemurs have eroded. Additional pressures include long-term mining and oil exploration concessions.

It would be extremely useful if thorough surveys and censuses could be conducted in the national parks where *L. catta* is still known to exist: Andringitra, Tsimanampesotse, and Isalo. These parks contain large tracts of forest and shelter *L. catta* populations, but much of the terrain in these parks would be highly challenging to traverse. However, technology such as drones outfitted with thermal imaging cameras could be used to enter difficult-to-access areas. Such surveys and censuses could take several months, but it would be most beneficial to know the size of populations in these protected areas. Surveys should also be undertaken at Andohahela National Park, the Cap Sainte-Marie region, and Kalambatritra and Pic d'Ivohibe special reserves to determine if *L. catta* is still present or now extinct.

Only three known sites, relatively small reserves, contain populations of over 200 animals: Berenty Reserve, Bezà Mahafaly Reserve, and Anja Private Reserve, but Anja is a fragment with little to no possibility for male dispersal. Berenty and Bezà Mahafaly reserves do allow for male dispersal and, given the relative stability of these populations, these areas can be seen as potential source populations, which should receive conservation priority, and empirical studies should focus on source-sink dynamics (Furrer and Pasinelli 2016) in such areas.

The outlook for *Lemur catta* seems grim, particularly outside of the handful of truly protected areas in which they live. The ring-tailed lemur is a remarkably ecologically flexible species, capable of surviving and thriving even in harsh habitats and through natural disasters (for example, Sauther *et al.* 1999; Gould *et al.* 1999; Jolly 2006; Goodman *et al.* 2006; LaFleur and Gould 2009); however, populations cannot survive in completely degraded habitats or under extreme hunting pressure, the two main threats which have decimated *L. catta* populations throughout its geographic range. Given the current situation of ring-tailed lemurs in the wild, and the enormity of threats that these populations face daily, the question arises: Will we, in the next decade or two, lose the most recognized and iconic of all the lemur species?

Acknowledgments

We thank the Département des Eaux et Forêts, Madagascar, Madagascar National Parks, Community Associations Anja, Sakaviro, FI.MI.VA, Antokinihoavy-Andranobe, and Tantely for granting research permission, and to GERP (Groupe d'étude et de recherche sur les primates de Madagascar) for logistical assistance. L. Gould is grateful to the following for their much appreciated research assistance at the south-central sites: P. Andrianomena, Daniel and Raboary (Tsaranoro), S. Rasoanomenjanahary, J. S. Weir, J. N. Rabialahy. We also thank the following individuals for providing information in the form of personal communications: I. A. J. Youssouf, B. P. Semel, C. Foulon, E. A. Kelley, R. J. Lewis, B. Ferguson, and M. Malone. Funding for the population research at the south-central sites was provided by grants to LG from Conservation International's Primate Action Fund and the University of Victoria (IRG program).

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Received for publication: 5 September 2016
Revised: 11 November 2016

Primate Conservation 2016 (30): 103–111

Initiation of Genetic Demographic Monitoring of Bonobos (*Pan paniscus*) at Iyema, Lomako Forest, DRC

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Abstract: Research on wild apes is not only fundamental for elucidating human origins but for their conservation as well. Despite their relative size, apes are difficult to observe in the wild prior to habituation, limiting our ability to accurately assess demography and kin relations. Non-invasive genetic sampling provides an indirect source of this information. Here, we present findings of a pilot genetic survey of a wild community of bonobos that are in the initial stages of being studied. Fifty-three fecal samples were collected over eight days near the Iyema field site in the Lomako Forest, DRC. DNA was first extracted and quantified using a qPCR assay. Samples with a sufficient amount of DNA were genotyped at 11 microsatellite loci and sexed using an amelogenin assay. Thirty-three of 53 samples yielded a sufficient amount of DNA for complete genotyping. We identified 19 individuals, including six males and 13 females. Mean allelic richness across all loci was 5.7 and expected heterozygosity was 0.69. Estimates of population size indicate between 26 and 66 individuals are present in the study area, but more than one community may be present. These results contribute to our ongoing efforts to study and monitor the bonobos at Iyema to better understand their demography, behavior, and conservation. Our study also highlights the utility of genetic analyses in pilot and survey research.

Key Words: Non-invasive sampling, microsatellites, genetic capture-recapture, community size estimation, conservation genetics

Introduction

Bonobos (*Pan paniscus*) are classified as Endangered and are threatened by hunting and habitat loss (Fruth *et al.* 2008). Despite over forty years of research in the wild, we are still limited in our understanding of this species; especially compared to chimpanzees (*P. troglodytes*). Political instability and the difficulties of accessing bonobo habitat have both contributed to this knowledge gap. What we do know of wild bonobos is largely based on long-term data from three research areas: Lomako (Badrian and Badrian 1984; White 1992; Van Krunkelsven *et al.* 1999), LuiKotale (Hohmann and Fruth 2003), and Wamba (Kano 1992) (Fig. 1). While continued work at these established sites is important, the development of new, sustainable field sites is necessary for bonobo research and conservation. Of particular interest from new sites are demographic data, which can be difficult to obtain in non-provisioned bonobos due to their arboreality and fission-fusion social structure (Kano 1992). Non-invasive genetic sampling can help us overcome these challenges. Genetic data have already been used on wild bonobos to inform behavioral observations (for example, Gerloff *et al.* 1999; Hohmann *et al.* 1999; Surbeck *et al.* 2011), understand population structure (Kawamoto *et al.* 2013), and infer male philopatry and female dispersal (Hashimoto *et al.* 1996; Eriksson *et al.* 2004, 2006; Kawamoto *et al.* 2013). Continued use of genetic data is necessary for longitudinal monitoring to track population size trends and assess the potential effects of anthropogenic activity on genetic health. Genetic studies of wild bonobos are thus essential to their conservation and to understanding the species.

We conducted a non-invasive genetic analysis as part of our ongoing effort to reinitiate studies of, characterize, and habituate the bonobos at Iyema, in the Lomako Forest in the Democratic Republic of Congo. One community was previously studied (for example, Dupain *et al.* 2002) but has only been intermittently monitored since the Second Congo War that ended in 2003. The primary aim of this initial survey was to identify individuals for consistent long-term monitoring, future behavioral studies, and to obtain preliminary assessments on the demography of the Iyema bonobos. We developed two research objectives. The first was to identify unique individuals, determine their sex, and evaluate the genetic diversity in our sample. Our second objective was to conduct repeated sampling of individuals to estimate the size of the bonobo population in our study area using genetic capture-recapture. Previous behavioral research produced a size estimate for the main study community at this field site; however, not all of the community members were identified. Dupain *et al.* (2002) were able to visually identify 12 distinct individuals and speculated the community was composed of approximately 50 individuals. Genetic analyses can provide a second estimate, especially when differences in capture probabilities are considered. Additionally, we collected GPS data in association with fecal samples and constructed an association matrix to assess whether our collected samples represent individuals from a single community or multiple communities.

Methods

Study area and sample collection

The Lomako Forest is located between the Lomako and Yekokora rivers in the Équateur province of the Democratic Republic of Congo. The forest is approximately 3,800 km², and consists primarily of polyspecific evergreen rainforest, in addition to swamp forest and seasonally flooded forests (White 1992). There are two bonobo study sites in the Lomako Forest: Iyema and N'dele, which are separated by 15 km.

Fifty-three fecal samples were collected near the Iyema site (00°55'N, 21°06'E) in eight days in June and July 2014. Samples were collected within a 15 km² area (Fig. 2). Fecal samples were collected as part of our ongoing effort to habituate one community of bonobos at the field site. Bonobos were located in the morning before leaving their night nests. Most fecal samples were collected under these nests, and additional

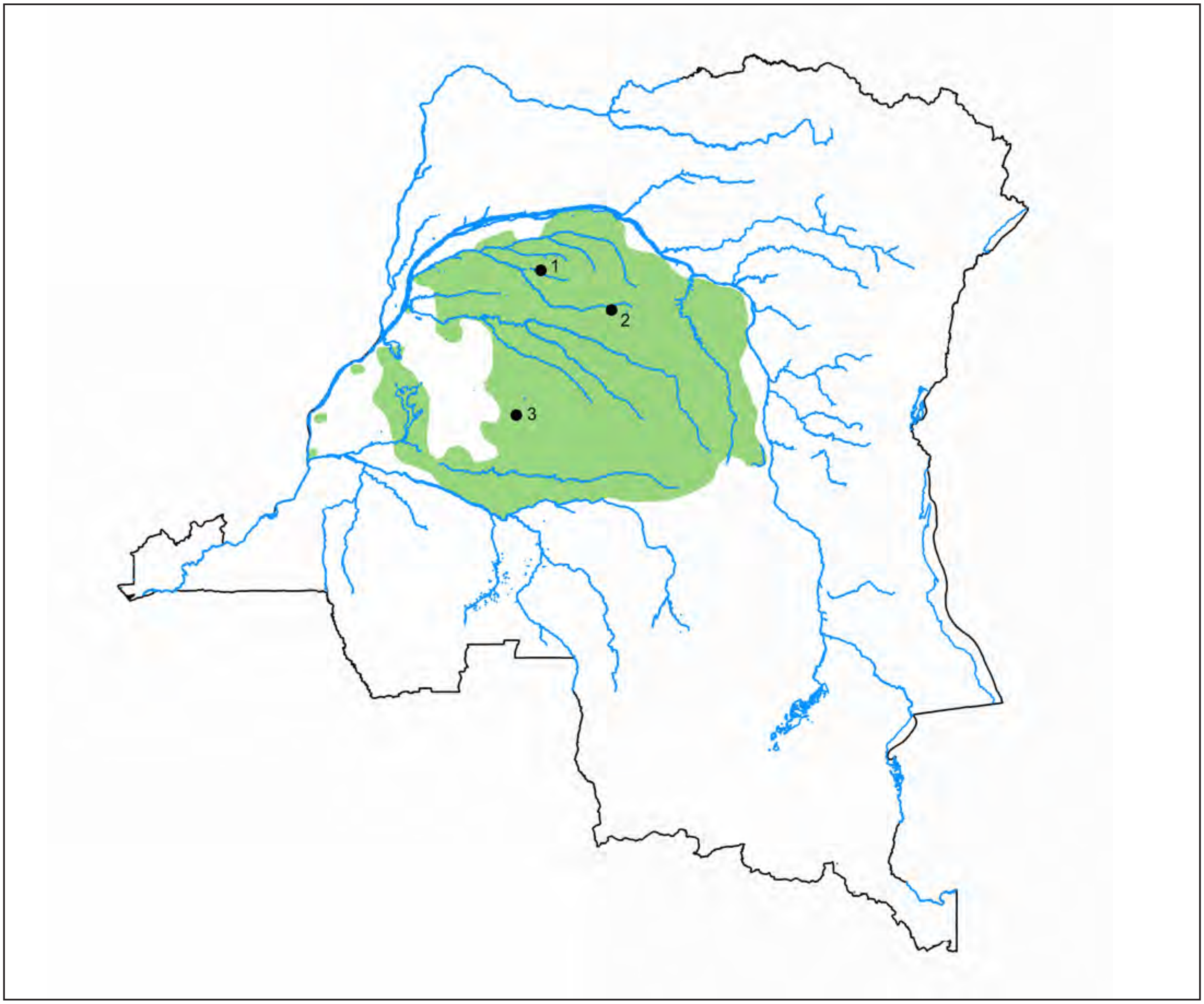

Figure 1. Distribution of *Pan paniscus* (green) and the locations of the long-term bonobo research areas: 1 - Lomako, 2 - Wamba, 3 - LuiKotale.

samples were collected opportunistically during party follows. The GPS location of each sample was recorded using a Garmin GPS unit. To minimize contamination, a mask and gloves were worn when collecting samples. Samples were placed in a 50 mL Falcon tube with 20 mL of RNAlater (Thermo Fisher Scientific, Waltham, MA, USA). All samples were stored at ambient temperature until they could be shipped to the Ting Laboratory (Molecular Anthropology Group, University of Oregon). Samples were then immediately frozen at -20°C until genomic DNA extraction.

DNA extraction and quantification

Genomic DNA was extracted using a QIAamp DNA Mini Stool Kit (Qiagen, Valencia, CA, USA). We made several modifications to the manufacturer's extraction protocol following Archie *et al.* (2003). DNA was eluted in 75 µL of buffer AE following Wikberg *et al.* (2012). Endogenous DNA extracted from noninvasive samples is often degraded, present at low concentrations, and susceptible to allelic dropout during amplification via PCR (Taberlet *et al.* 1996; Morin *et al.* 2001). We thus quantified the DNA in each sample using a quantitative PCR (qPCR) assay following Morin *et al.* (2001). Samples were amplified in 20 µL reactions containing 1X TaqMan Mastermix (Applied Biosystems, Foster City, CA, USA), 200 nM TAMRA™ probe (Applied Biosystems), 300 nM F primer, 300 nM R primer, 8 µg/mL BSA, 5.6 µL H_2O, and 2 µL of DNA. Reactions were carried out using a StepOnePlus qPCR thermocycler (Applied Biosystems). We genotyped samples that contained >0.005 ng/µL of DNA following Morin *et al.* (2001).

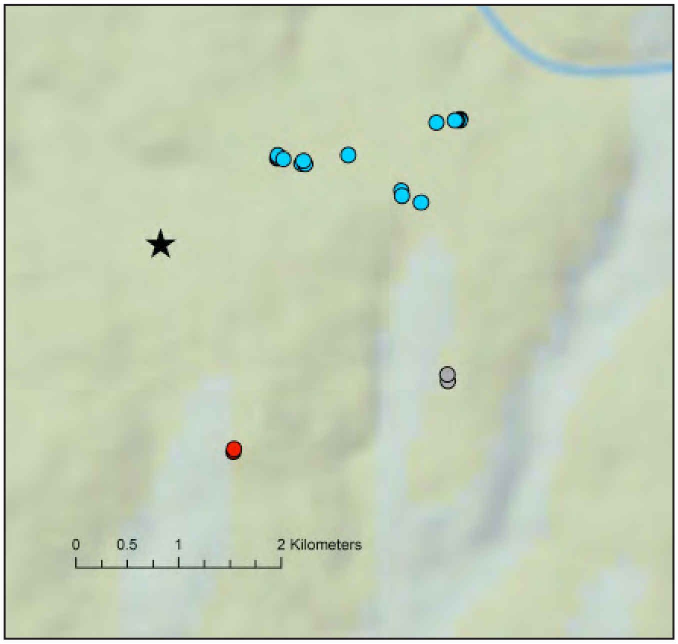

Figure 2. Map of fecal sample collection locations. Each circle represents a successfully genotyped fecal sample. Colors indicate the association network for each sample and correspond to Figure 3. The base camp is represented by a star.

Genotyping

Fifteen dinucleotide and tetranucleotide Short Tandem Repeat (STR), or microsatellite, loci were screened for amplification success and polymorphism (see Table 1, Bradley *et al.* 2000; Arandjelovic *et al.* 2009; Schubert *et al.* 2011; Wikberg *et al.* 2012; Ruiz-López *et al.* 2016). Two markers did not amplify (D1s207, D4s2408) and two (D10s611, FESPS) were found to be monomorphic in our sample. Polymorphic markers were organized into five multiplex sets consisting of two or three markers: set 1 (D5s1457, D14s306), set 2 (D3s1299, D1s548), set 3 (C19a, D6s474 D10s676), set 4 (D8s260, D11s2002), and set 5 (D3s1766, D6s311). The forward primer for each marker was fluorescently labeled with either 6-FAM, HEX or NED. Samples were amplified in 12.5 µL reactions containing 1X QIAGEN Multiplex PCR Master Mix (Qiagen, Valencia, California), 0.2 µM of each primer, 1 µg/mL BSA, 1.75 µL H_2O, and 2 µL of DNA. Initial incubation was carried out at 95°C for 15 min. Amplification was performed using 35 cycles with denaturation at 94°C for 30 sec, annealing at 58°C for 90 sec, and elongation at 72°C for 60 sec. Final extension was carried out at 60°C for 30 min. Successful amplification of each sample was verified using gel electrophoresis with a 1% agarose gel. PCR products were run with a size standard (GeneScan™ 500 Rox™, Applied Biosystems) and separated by capillary electrophoresis using a 3730 DNA sequencer (Applied Biosystems). Allele sizes were determined using the software GENEMAPPER 5.0 (Applied Biosystems) and verified by eye. To control for allelic dropout and ensure accuracy, each sample was independently amplified and genotyped at least three times at every locus. Following Morin *et al.* (2001), samples containing greater than 0.1 ng/µL of endogenous DNA were replicated three times, samples containing between 0.1 and 0.05 ng/µL of endogenous DNA were replicated four times, and samples with 0.05 and 0.005 ng/µL of endogenous DNA were replicated seven times.

Samples were determined to be heterozygous at a locus if two alleles were observed in at least two replicates, while samples were determined to be homozygous if only one allele was observed in at least three replicates. Consensus genotypes were generated using GIMLET (Valiére 2002). Unresolved consensus genotypes were determined manually using alleles with the highest frequency. GIMLET was also used to determine the allelic dropout rate and false allele rate (Supplement 1).

GENALEX 6 (Peakall and Smouse 2012) was used to compare final consensus genotypes. Samples that matched at all but one or two loci were rechecked for allelic dropout and genotyping errors. If human genotyping errors occurred, genotypes were rectified and reanalyzed for matches. This conservative approach was used to avoid identifying individuals based on erroneous genotypes.

Finally, we tested the power of our set of markers to identify unique individuals using GENALEX. We calculated the power to differentiate between random individuals P(ID) and the power to differentiate between siblings P(ID)sib (Waits *et al.* 2001). As bonobos are social primates that live in groups

Table 1. Genetic diversity measures per locus.

Marker	Motif	*Na*	*Ne*	*Ho*	*He*	*UHe*	F_{IS}	HWE
C19a	4	5	2.03	0.368	0.507	0.521	0.2981	Non-significant
D1s548	4	5	2.57	0.737	0.611	0.627	-0.1803	Non-significant
D3s1229	2	6	4.66	0.789	0.785	0.807	0.0217	Non-significant
D3s1766	4	4	2.37	0.526	0.578	0.593	0.1155	Non-significant
D5s1457	4	6	3.47	0.684	0.712	0.731	0.0659	Non-significant
D6s311	2	9	6.17	0.789	0.838	0.861	0.0847	Non-significant
D6s474	4	4	2.33	0.895	0.571	0.586	-0.5494	Non-significant
D8s260	2	6	3.76	0.789	0.734	0.754	-0.0485	Non-significant
D10s676	4	6	4.12	0.895	0.758	0.778	-0.1547	Non-significant
D11s2002	4	8	5.51	0.684	0.819	0.841	0.1903	Non-significant
D14s306	4	4	2.71	0.368	0.632	0.649	0.4388	Non-significant
Mean		5.7	3.61	0.684	0.686	0.704	0.025	

Motif, repeat motif; Na, number of alleles; Ne, number of effective alleles; Ho, Observed heterozygosity; He, expected heterozygosity; UHe, unbiased expected heterozygosity; F_{IS}, Inbreeding coefficient; HWE, results for Hardy-Weinberg equilibrium test with "Non-significant" meaning the locus did not deviate from HWE.

with related individuals, we used the more conservative measure, P(ID)sib, using a cutoff of 0.001. This approach ensures the accurate identification of unique individuals from fecal samples.

We used GENEPOP 4.2 (Raymond and Rousset 1995) to test for deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) across all markers. Fisher's exact test was used to test for a deviation from HWE across all loci. We used the program's Markov chain algorithm to determine departures from HWE and LD for each marker using 100 batches and 1000 iterations. Significance levels were adjusted using a Bonferroni correction. MICROCHECKER (Van Oosterhout *et al.* 2004) was used to test for evidence of null alleles. We used GENALEX (Peakall and Smouse 2012) to determine various measures of genetic diversity: allelic richness, number of effective alleles, observed heterozygosity, and unbiased expected heterozygosity. We also calculated the inbreeding coefficient (F_{IS}) following Weir and Cockerham (1984) for each locus using GENEPOP (Raymond and Rousset 1995).

Sex identification

Following the identification of distinct individuals within our sample, the sex of each individual was assessed using an amelogenin assay (Bradley *et al.* 2001) modified for visualization via gel electrophoresis. Extracted DNA was amplified in 15 µL reactions containing 1X GoTaq® (Promega, Madison, WI, USA), 200 µM F primer, 200 µM R primer, 12 µg/mL BSA, 2.7 µL H_2O, and 3 µL of DNA. We electrophoresed DNA using a 4% agarose gel at 100 volts for 2.5 hrs. Validation of the methodology was conducted on human samples of known sex (see Supplement 2), and each bonobo sample was amplified and visualized on a gel twice to ensure correct sex identification.

Community size estimation

We used two approaches to estimate the number of individuals at Iyema: the software Capture (Otis *et al.* 1978) and the R Package Capwire (Miller *et al.* 2005). We used a comparative approach to contrast a traditional capture-recapture method (Capture) with a program developed specifically for genetic capture-recapture (Capwire). Traditional methods, such as Capture, estimate population size based on the occurrence of captures per session. We used the m(h) model from Chao (1989), which accounts for heterogeneity in individual capture probabilities. We also used both models from the R Package Capwire to estimate the number of individuals: 1) the Equal Capture Model (ECM), that assumes that all individuals in a population have an equal probability of being sampled (Miller *et al.* 2005); and 2) the Two-Innate Rates Model (TIRM) which assumes that two classes of individuals exist—individuals that are easy to capture and those that are difficult to capture (Miller *et al.* 2005). We also used a likelihood ratio test to determine which model best fit the data. Finally, we generated 95% confidence intervals for both ECM and TIRM models using maximum population estimates of 50, 200, 500, and 1000 individuals. We report the confidence intervals for a maximum population estimate of 200 because the intervals varied little for maximum population sizes greater than this value.

Association analysis

We created an association matrix to estimate how many bonobo communities were represented in our sample. Dyads were scored as "0" if fecal samples were not found in association and scored as "1" if fecal samples were collected from individuals in the same party (nests within a 30-m radius) on the same day (McCarthy *et al.* 2015). We used the software NetDraw 2.155 (Borgatti 2002) to visualize associations.

Results

Thirty-eight samples yielded a sufficient quantity of DNA for genotyping, and 33 samples were genotyped at all 11 loci. Our sample success rate was thus 62% (33/53). Probability of identity analyses revealed that at least nine loci are needed to identify unique individuals. P(ID) was 1.8×10^{-8} and P(ID)sib

Table 2. Sample capture and recaptures.

Times Captured (N)	Individuals (N)
1	12
2	4
3	1
4	1
6	1

was <0.001 at 9 loci. Of the 33 samples collected, we identified 19 distinct individuals (13 females and six males).

Microsatellite diversity

Allelic richness ranged from four to nine different alleles for each locus (Table 1). Mean allelic richness across all markers was 5.7. Our sample deviated significantly from Hardy-Weinberg equilibrium across all loci (χ^2 = 47.27, df = 22, P <0.01). However, subsequent tests for heterozygote deficiency and heterozygote excess yielded no significant results (p-value = 0.08 and p-value = 0.92, respectively), and individually, all 11 loci conformed to Hardy-Weinberg equilibrium (Table 1). No loci showed evidence of linkage disequilibrium. The presence of null alleles was detected in one marker: D14s306 (Oosterhaut = 0.1934). Mean expected heterozygosity was 0.69 and expected unbiased heterozygosity was 0.70 (Table 1). F_{IS} ranged from -0.18 to 0.44, averaging 0.025 across all markers.

Population size estimation

Out of 19 individuals, 12 were captured once, four individuals were captured twice, one individual was captured three times, one individual four times, and one individual six times (Table 2). Using the m(h) model in Capture, the program estimated 36 individuals were present in this area (95% CI: 24−81). The equal capture model (ECM) indicated approximately 26 individuals were present (95% CI: 19−36), whereas the two innate rates model (TIRM) indicated approximately 38 individuals present (95% CI: 26−66). Finally, we tested both Capwire models and found TIRM to better fit our data (LR = 8.72, bootstraps = 500, P <0.05). While these models generated both point estimates and confidence intervals, we focus on the latter for the remainder of the paper.

Patterns of association

Associations between dyads are displayed in Figure 3. Thirteen individuals were found to form the largest association (blue nodes). Six individuals formed two smaller, separate networks; one containing four individuals (red nodes) and the other containing two individuals (gray nodes). Individuals in these smaller networks were never found in association with individuals in the larger network.

Discussion

These results highlight our ability to obtain genetic information using non-invasive sampling from this population of bonobos. Our success rate (62%) for sample viability was comparable to other studies of African apes (Arandjelovic *et al.* 2010; Basabose *et al.* 2015; McCarthy *et al.* 2015; Moore and Vigilant 2014). Additionally, our microsatellite panel yielded a sufficient number of loci to confidently identify individuals. Taken together, this study represents the beginning of long-term bonobo monitoring at the Iyema site, with the identification of unique individuals and the first genetic capture-recapture population size estimates.

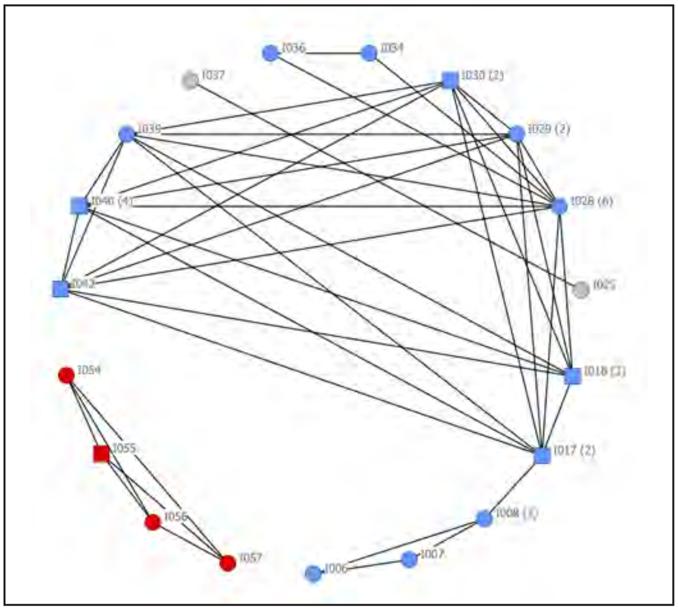

Figure 3. Association Network. Each node is an individual; circular nodes are female and square nodes are male. Numbers in parentheses indicate the number of times an individual was captured for individuals that were sampled more than once. Lines connecting two nodes indicate the pair of individuals was found in association at least once. Individuals with blue nodes are members of the main study community and were collectively sampled on six days. Individuals with gray nodes were sampled on a different day as were individuals with red nodes. These individuals (gray and red nodes) may represent individuals who are not members of the main study community.

While some markers exhibited relatively high rates of allelic dropout and false alleles (Supplement 1), only one marker (D14s306) showed evidence of null alleles. Null alleles describe mutations in the regions that flank target nucleotide sequences that can prevent primers from properly annealing (Chapuis and Estoup 2007). While null alleles typically do not impact basic population analyses, they can affect accurate assessment of relatedness and paternity analyses (Dakin and Avise 2004). As we hope to use microsatellite data to determine infant paternity in the future, we will exclude this marker in future analyses.

The genetic diversity represented by our sample is similar to wild-born captive bonobos and other wild bonobo populations. Reinartz *et al.* (2000) examined the autosomal diversity in 14 wild-born captive bonobos (founders). Across 28 polymorphic microsatellite loci, mean allelic richness was 5.2 and mean expected heterozygosity was 0.58. Schubert *et al.* (2011) analyzed genetic diversity at 19 autosomal markers among five groups of wild bonobos around LuiKotale and found a mean allelic richness of 7.3 and mean expected heterozygosity of 0.75. Our study of 19 wild bonobos resulted in similar findings to both studies (mean allelic richness: 5.7; mean expected heterozygosity: 0.69). These results highlight the genetic diversity at Iyema and are very encouraging from a conservation standpoint, although more work needs to be done to assess threats to bonobos in this area since taxa with long generation times can be affected and threatened by human disturbance prior to visible declines in genetic diversity (e.g., see Ruiz-López *et al.* 2016).

All three mean estimates of population size fell near the previous estimate based on behavioral observations (approximately 50 individuals). Indeed, the population size estimate and confidence intervals from the m(h) model in Capture and the TIRM estimate in Capwire are very similar (24–81 and 26–66, respectively). These estimates are also consistent with known maximum bonobo community sizes: Lomako (Bakumba): 36; Lomako (Eyengo): 21; LuiKotale: 35; Wamba (E1): ~28; Wamba (E2): ~45 (Kano 1992, Surbeck *et al.* 2011, White and Wood 2007). While these samples may represent a single community, our analyses cannot exclude the possibility that more than one community was sampled. Of the two Capwire models, TIRM fit the data better than the ECM; a result that is congruent with previous research (Arandjelovic *et al.* 2010). TIRM is a better model for estimating population or community size in bonobos than ECM because it accounts for heterogeneity in capture probabilities largely due to the fission-fusion social structure of bonobos. While consistent estimates were generated, our small sample size resulted in large confidence intervals further supporting that more accurate census measures require repeated sampling of nearly all individuals present in a community (Basabose *et al.* 2015). As the main bonobo community is not fully habituated, it is highly unlikely that all individuals were sampled. Additionally, heterogeneity of capture probabilities can greatly influence size estimates and confidence intervals. Basabose *et al.* (2015) noted that the two-innate rates model accounted for this heterogeneity, as did the m(h) model in Capture. One particular demographic group that exhibited a higher probability of avoiding genetic capture was infants, who nest with their mothers for several years (Fruth and Hohmann 1994), may not defecate outside of the nest, and whose feces are smaller and more difficult to find. Collectively, these factors can result in infrequent infant fecal sampling, and thus result in inaccurate and underestimated population sizes.

One challenge of using genetic capture-recapture approaches to estimate community size rather than population size is the possibility of sampling more than one community. Prior to habituation, distinguishing between different communities of bonobos and chimpanzees can be difficult. Both bonobos and chimpanzees fission-fusion and form parties that may last several days (Goodall 1986; Kano 1992). Particular parties or individuals may be more difficult to sample. Basabose *et al.* (2015) used a nest sharing analysis to overcome the challenge of distinguishing communities. While chimpanzees are generally considered to maintain strictly defined territories, bonobo ranges appear to frequently overlap (Kano 1992; Waller 2011). This makes it difficult to discern whether or not unhabituated animals belong to a particular community. Our analysis of association revealed the possibility that more than one community was sampled. The samples that constitute the two smaller networks (gray and red) were all collected on two separate days that did not involve any sampling of the main network (blue). Additionally, these six individuals were sampled much farther away from the remaining samples. We are unsure, therefore, whether or not these individuals are members of the same community from which the other 13 individuals were sampled. The association network illustrates three separate networks; however, this may reflect fission-fusion dynamics, especially considering the small sampling period. These results highlight the difficulty of determining the number of communities sampled when collecting bonobo fecal samples over a short time period.

While our genetic survey is preliminary, this study is an important first step for resuming longitudinal bonobo research in the Lomako Forest. We identified 19 individuals and estimated the population size in the study area to be between 26 and 66 individuals. Continued non-invasive sampling will enable us to identify and monitor specific individuals in addition to assessing the number of bonobo communities present at Iyema. Future research will also use this study as a starting point for the use of relatedness data to better understand bonobo social organization and community membership, the effects of kinship on social behavior, and bonobo reproductive strategies.

Acknowledgments

We gratefully acknowledge Alexana Hickmott, Jef Dupain, Freddy Makengo Lusendi, Papa Mangue, Dipon Bomposo, and all of the African Wildlife Foundation staff in Kinshasa and Iyema for assistance in the field. CMB deeply appreciates the guidance of Stephanie Fox in the lab and Diana Christie for her insight about R and Capwire. CMB also thanks Elisabeth Goldman and Jessica Stone for providing human DNA samples for validating the visualization of the amelogenin assay. We kindly thank Mimi Arandjelovic and one anonymous reviewer whose comments greatly improved this manuscript. Field research was supported by the Northern Kentucky University College of Arts and Science, awarded to MLW. Additional support came from NSF grants BNS-8311252, SBR-9600547, BCS-0610233, and from the Leakey Foundation awarded to FJW. Funding for the genetic analyses was provided by the Office of the Vice President for Research and Innovation, University of Oregon awarded to FJW, as well as NIH grant TW009237 awarded to NT as part of the joint NIH-NSF Ecology of Infectious Disease program and the UK Economic and Social Research Council. Finally, we thank the Department of Anthropology, University of Oregon, and the Department of Sociology, Anthropology, and Philosophy of Northern Kentucky University. All research reported in this study was non-invasive and complied with all standards set forth by the University of Oregon Institutional Animal Care and Use Committee (IACUC) (#12-09). This research also adhered to the legal requirements of the Democratic Republic of Congo and was approved by the Institut Congolais pour la Conservation de la Nature (#0492). We dedicate this manuscript in memory of Papa Ikwa Nyamanolo (Bosco).

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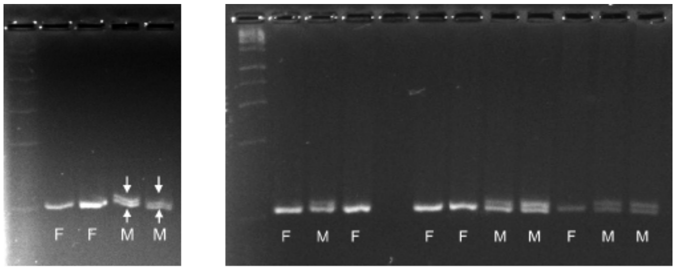
Received for publication: 12 April 2016
Revised: 21 June 16

Supplement 1. Dropout and False Allele Rate per Locus.

Marker	Allelic Dropout Rate	False Allele Rate
C19a	0.217	0.054
D1s548	0.097	0.303
D3s1229	0.077	0.253
D3s1766	0.155	0.085
D5s1457	0.080	0.293
D6s311	0.139	0.133
D6s474	0.125	0.310
D8s260	0.068	0.293
D10s676	0.077	0.221
D11s2002	0.191	0.365
D14s306	0.136	0.082

Supplement 2. Amelogenin Assay Visualization Validation



Amplified products from the amelogenin assay (Bradley *et al.* 2001). The gel on the left displays four human control samples and the gel on the right displays multiple bonobo samples. The presence of one band indicates female, while two bands indicates male. The sex of each sample is identified below the product.

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