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Genetic tests of the taxonomic status of the ring-tailed lemur (*Lemur catta*) from the high mountain zone of the Andringitra Massif, Madagascar

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Abstract

A recent survey of the high-mountain zone of the Madagascar Parc National (PN) d'Andringitra revealed the presence of an apparently isolated troop of the ring-tailed lemur *Lemur catta*. These animals display phenotypic and ecological characteristics that are unusual for the monotypic genus *Lemur*, thus raising the possibility that they are members of a different undescribed species. We present analyses of two mitochondrial genes to test the hypothesis that *L. catta* from Andringitra should be considered a distinct species. The results indicate that taxonomic revision is not warranted under the expectations of the phylogenetic, coalescent, or biological species concepts. Rather, the genetic patterns observed among the Andringitra and lowland mitochondrial haplotypes are consistent with those expected for a single species.

Key words: species identification, mtDNA, Madagascar, primates, Strepsirrhini, *Lemur catta*

INTRODUCTION

The ring-tailed lemur *Lemur catta* is the most intensively studied and best known species of the family Lemuridae. It has been the subject of numerous studies on population dynamics, ecology and social interactions (Jolly, 1966; Budnitz & Dainis, 1975; Petter, Albignac & Rumpler, 1977; Sussman, 1977, 1991, 1992; O'Connor, 1987; M. L. Sauther, 1991; Jolly *et al*., 1993; M. Sauther, 1993; Gould, 1996; Nakamichi & Koyama, 1997) and continues to be of interest because of its unique ecology. Perhaps the most remarkable aspect of this lemur's behaviour is that it is the only one of >40 extant species of the primate suborder Strepsirrhini to spend an appreciable amount of time on the ground. Indeed, certain populations have been described as baboon analogues (Mittermeier *et al*., 1994). Typically, the species is characterized as inhabiting areas of dry deciduous forest, gallery forest, and spiny thorn scrub in southern and south-western Madagascar (Sussman, 1977; Tattersall, 1982; Harcourt & Thornback, 1990; Mittermeier *et al*., 1994). Reports have also suggested that this species may occur in the interior highlands of the Andringitra Massif to the south of Ambalavao (Petter *et al*., 1977; Tattersall, 1982; Mittermeier *et al*., 1994).

During a 1995 mission to the summital zone of the Parc National (PN) d'Andringitra (formerly classified as a Réserve Naturelle Intégrale), investigators discovered an isolated troop of *L. catta* that exhibited phenotypic variation unusual for the species. Goodman & Langrand (1996) noted that the individuals they observed had pelage characteristics unlike typical lowland *L. catta*, including thicker and distinctly darker fur and fewer rings on the tail. Moreover, the climate and environment of the upper portions of Andringitra Massif are considerably more severe than the typical lower-lying habitat of *L. catta*. From these geographic, morphological, and ecological distinctions, the question was raised as to whether this population might represent a previously undescribed species of *Lemur* (Goodman & Langrand, 1996). The purpose of our paper is to employ genetic data to investigate the hypothesis that a taxonomic revision is warranted.

The recognition, indeed the very definition, of species is one of the most controversial aspects of evolutionary biology. Despite the problematic nature of the exercise, identifying species remains an important function of phylogenetics and population genetics, both for the significance that the species unit has for evolutionary studies and for the political weight that it carries in the formulation of conservation policies. Species concepts

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have been recently summarized and critiqued (Adachi & Hasegawa, 1995; Baum & Donoghue, 1995; Baum & Shaw, 1995; Mallet, 1995; Davis, 1996; Avise & Wollenberg, 1997; Davis, 1997; Maddison, 1997; Sites & Crandall, 1997) and it is not our intent to summarize the reviews. None the less, Sites & Crandall's (1997) point is well-taken: any study that proposes to recognize a new species should also be prepared to provide an explicit, testable hypothesis of species status. Historically, species-level taxonomy has not been based on an explicit hypothesis-testing methodology. Rather, species designations have been based on a complex (and by no means universal) mix of morphological, ecological, and geographic distinctions that taxonomists have inferred to be the result of evolutionary isolation. More recently, genetic-distance patterns have also been employed for identifying new species (e.g. Arnason & Gullberg, 1993; Mallett, 1995; Xu & Arnason, 1996). The concept of a universal genetic distance test of species status is impracticable, however, in that it immediately raises two unanswerable questions: (1) what genetic region should be examined? (2) what level of divergence reflects a species distinction? None the less, within the limits of a particular study, comparative genetic distances as measured by branch lengths can yield valuable information, so long as the genetic marker shows equivalent rates of molecular evolution within the desired phylogenetic context.

Two commonly employed criteria for species recognition are the phylogenetic species concept (PSC) and the biological species concept (BSC). In the PSC (also called the 'diagnostic approach' by Baum & Donoghue, 1995), a population may be considered a species as long as that population shows a diagnostic character or suite of characters to define it (Cracraft, 1983, 1989; Nixon & Wheeler, 1990, 1992; Davis & Nixon, 1992). Although the PSC was originally developed with reference to morphological characters, its application has been extended to genetic data and made operationally feasible with the formulation of population aggregation analysis (PAA). The PAA method holds that the 'occurence of a unique fixed character combination in an extended genealogical population . . . is evidence that this population has diverged from other such populations' and thus 'involves a search for fixed differences among local populations' (Davis & Nixon, 1992: 421). By this criterion, all individuals within a phylogenetic species must carry a unique and fixed combination of characters (i.e. attributes). Therefore, a hypothesis of species status can be falsified in a number of ways: (1) the character may be fixed but not unique (i.e. it can be found as a trait in another population), (2) the character may be unique (i.e. not found in other populations) but not fixed (i.e. not characteristic of all individuals in the population) or (3) the character may be neither unique nor fixed (i.e. it can be found in multiple populations with different frequencies of occurrence in each). Although the PAA method for defining species has intuitive appeal because of its amenability to a hypothesis-testing framework, it is weak because of its sensitivity to sample size. One can easily imagine a case wherein a given population sample shows a genetic character or suite of characters that appear to meet the criteria of an attribute, but upon increased sampling of individuals, the same characters are found to be either not unique or not fixed. Thus, the PAA is poorly suited for studies in which populations are poorly sampled.

The BSC (Dobzhansky, 1935; Mayr, 1942), on the other hand, is less sensitive to sample size but is far more difficult to test as it requires that the putative species occur in sympatry. Although it is conceivable that representatives of the two populations could be tested for reproductive isolation in a laboratory setting, this is usually impractical and perhaps not definitive (e.g. two populations might produce viable offspring in the lab that would not otherwise be produced in nature). A third and intermediate alternative to the PSC and BSC has recently been proposed. In what Avise & Wollenberg (1997) describe as a 'multilocus coalescent theory of speciation', species are identifiable by non-intersecting 'genealogical transmission pathways'. By this criterion, species identification relies on determining for the populations in question the transmission pathways for multiple alleles (defined as 'a length of DNA that has been free of recombination within it during the ecological or evolutionary time under consideration'). Species integrity is recognized in cases where all observed allelic genealogies show a pattern of reciprocal monophyly. These data can be employed to support or reject a hypothesis such as 'populations A and B are representatives of different species'. If any of the allelic genealogies do not show reciprocal monophyly of the putative species, then the 'different species' hypothesis must be rejected. Thus, any single gene has the capacity to cause the rejection of a species hypothesis, but many (though the actual number is not defined) must be examined before a species hypothesis can be taken as well-supported. Mitochondrial DNA (mtDNA), although not alone sufficient for species identification, is ideal as a first approximation of coalescent patterns beause of its sensitivity to both population structure and effective population size (Birky, Fuerst & Maruyama, 1989; Avise, 1995; Moore, 1995). Under the coalescent criterion, support for the different-species hypothesis in the present study would be derived from a result in which lowland representatives of *L. catta* together formed a clade that excluded the Andringitra specimens. Conversely, if the Andringitra samples nested within an inclusive *L. catta* clade, the hypothesis would be falsified.

We analyse two mtDNA markers for *L. catta* from several geographic localities. These data are employed to investigate patterns of character distribution, genealogical descent and genetic distance among and between *L. catta* individuals from several populations, other representatives of the family Lemuridae, and two Malagasy primate outgroups. To test the hypothesis that the Andringitra population represents a species distinct from lowland populations, analyses were conducted to identify potentially diagnostic sites and to

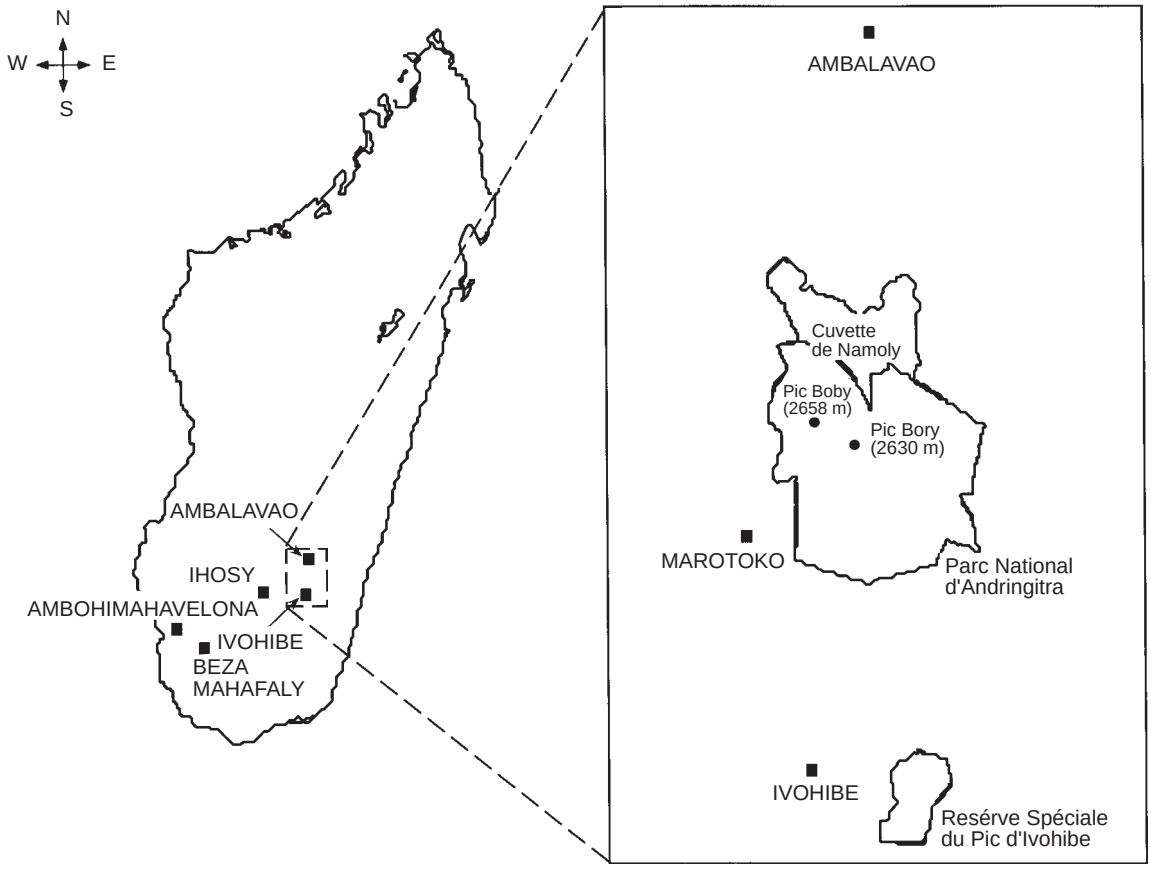

Fig. 1. Map of Madagascar with enlargement of Parc National d'Andringitra. The lowland sites of Beza Mahafaly and Ambohimahavelona are also illustrated as two of the comparison *Lemur catta* sequences derive from individuals native to these localities.

determine the branching patterns and genetic distances for cytochrome *b* and HV1 haplotypes.

BACKGROUND INFORMATION

The Andringitra Massif lies at the eastern side of the Central High Plateau, centred around Pic Boby (2658 m) and Pic Bory (2630 m), respectively the second and fourth highest peaks on Madagascar (Fig. 1). The eastern flank of massif descends into the eastern humid forest and the western into dry deciduous forest and subsequently further west into spiny thorn scrub forest. Tree-line on the mountain is between 1900 and 2000 m, and the alpine zone is composed largely of sclerophyllous forest which grades into a mixture of ericoid scrub, open anthropogenic savanna, and exposed rock. The main crystalline ridge forms the divide between the wet east and the dry west. Along this divide, a biogeographic mix of vertebrates from the east and west occur along with those restricted the alpine zone (Goodman, 1996; Raxworthy & Nussbaum, 1996). The 1995 observations of *L. catta* were made in the Cuvette de Pic Boby at about 2500 m (Goodman & Langrand, 1996). More recently, one of us (SR) has observed these animals in various areas of the massif within a elevational range of 1100–2500 m.

Populations of phenotypically standard *L. catta* are known from lower-lying areas near Ambalavao, further west towards Ihosy, and south towards Pic Ivohibe (Fig. 1). Based on information of the elevational and geographical context of animals occurring in the alpine zone of the Andringitra Massif, it seems that this population is isolated from typical populations in neighbouring lowland areas. People living in the Cuvette de Namoly at 1500–1600 m, an extensive valley below and north of the expansive summital zone of the Andringitra massif have, however, reported movements of *L. catta* into the high elevational zone of the massif. These movements may therefore provide opportunities for genetic exchange between the highland and lower-lying populations.

The climate in the high mountain zone of the PN d'Andringitra is one of the most extreme on Madagascar (Paulian *et al.*, 1971; Goodman & Langrand, 1996). During the cold season, temperatures drop to below $-12\,^{\circ}C$, small streams freeze over, and snow falls on rare occasions. Most of the plants that are typical of the diet of *L. catta* in other areas of the island (O'Connor, 1987; M. Sauther, 1993) are not found in

Table 1. List of specimens included in the study

Binomila	Common name	Specimen no.[a]	Genbank	Accession no.
			cyt *b*	HV1
Lemuridae				
Lemur catta (Andringitra)	Ring-tailed lemur	A18f	AF17954	AF175500
Lemur catta (Andringitra)	Ring-tailed lemur	A19m	AF17953	AF175499
Lemur catta	Ring-tailed lemur	DUPC 5738m	U53575[c]	AF081031[d]
Lemur catta	Ring-tailed lemur	DUPC 6621m	AF17955	AF175501
Lemur catta	Ring-tailed lemur	DUPC 582m	AF17956	AF175502
Lemur catta	Ring-tailed lemur	DUPC 6530f	AF17957	AF175503
Lemur catta	Ring-tailed lemur	DUPC 6268m	U38271[b]	NA
Lemur catta	Ring-tailed lemur	DUPC 6271m	AF17958	AF175504
Lemur catta (Beza Mahafaly)	Ring-tailed lemur	UA	AF17959	AF175505
Lemur catta (Ambohimahavelona)	Ring-tailed lemur	FMNH 85134	AF17960	AF175506
Hapalemur griseus	Gentle lemur	DUPC 6043m	U53574[c]	AF081030[d]
Eulemur fulvus collaris	Collared lemur	DUPC 561m	U53576[c]	AF081032[d]
Eulemur fulvus rufus	Red-fronted brown lemur	DUPC 6341f	U53577[c]	AF081033[d]
Eulemur fulvus albifrons	White-fronted brown lemur	DUPC 3511m	AF081048[d]	AF081034[d]
Eulemur rubriventer	Red-bellied lemur	DUPC 6120m	AF081052[d]	AF081038[d]
Eulemur mongoz	Mongoose lemur	DUPC 6132m	AF081051[d]	AF081037[d]
Eulemur macaco macaco	Black lemur	DUPC 6406m	AF081049[d]	AF081035[d]
Eulemur macaco flavifrons	Sclater's black lemur	DUPC 6146f	AF081050[d]	AF081036[d]
Varecia variegata rubra	Red ruffed lemur	DUPC 5874f	U53578[c]	AF081028[d]
Varecia variegata variegata	Black and white ruffed lemur	DUPC 6178m	AF081047[d]	AF081029[d]
Lemuriform outgroups				
Microcebus murinus	Grey mouse lemur	DUPC 846f	U53572[c]	AF081026[d]
Propithecus tattersalli	Tattersall's sifaka	DUPC 6196m	U53573[c]	AF081027[d]

[a] DUPC = Duke University Primate Center; UA = Université d'Antananarivo; FMNH = Field Museum of Natural History.
[b] Cyt *b* sequence from Collura & Stewart (1995), not sequenced for D loop.
[c] Sequences from Yoder, Ruvolo & Vilgalys (1996).
[d] Sequences from Yoder & Irwin (1999).

the summital zone of the Andringitra Massif, thus necessitating a significantly different dietary regime for the highland population (Goodman & Langrand, 1996; Rakotoarisoa, pers. obs.). Compared to the low-lying areas of the dry south-western portion of the island, the ecological conditions of the upper slopes of Andringitra Massif are considerably different and therefore contribute to the sense of the Andringitra population's unique status.

MATERIALS AND METHODS

DNA samples were extracted from ear clippings made from 2 captured and released individuals from the Andringitra population. One individual was an adult female and the second a sub-adult male, both members of the same troop. The 2 lemurs were trapped at 2100 m on the western side of the massif, south of the Marotoko Cuvette (see Fig. 1). Ear clippings were taken after the application of EtOH. DNA for 6 *L. catta* examined in this study, and for all lemurid outgroups (Table 1), derives from tissues (liver, spleen, kidney, muscle) of animals that died of natural causes at the Duke University Primate Center (DUPC). All DUPC *L. catta* show phenotypic characteristics expected for lowland forms although precise collecting localities for founding females are unknown. Two *L. catta* of known origin were also included in the study. In the first, DNA was extracted from skin fragments of an animal found dead in the Réserve Spéciale de Beza Mahafaly (specimen housed in the Département de Paléontologie et d'Anthropologie Biologique, Université d'Antananarivo [UA] collections). In the second case, DNA was extracted from pieces of connective tissue taken from a skeletal specimen in the collections of the Field Museum of Natural History. This specimen (FMNH 85134) was collected in 1948 by Hoogstraal & Alison near the village of Ambohimahavelona. The positions of the Beza Mahafaly and Ambohimahavelona localities relative to Andringitra can be seen in Fig. 1.

Total genomic DNA was extracted with a standard phenol/chloroform technique after digesting overnight in a SDS-based extraction buffer. The polymerase chain reaction (PCR) was used to generate a double-stranded template of the entire 1140 bp cytochrome *b* gene and *c*. 500 bp of the mitochondrial control region homologous with the hypervariable 1 (HV1) region found in humans. For cytochrome *b*, PCR and sequencing conditions are as reported in Yoder, Ruvolo & Vilgalys (1996). HV1 was amplified and sequenced with primers L15926 (TCA AAG CTT ACA CCA GTC TTG TAA ACC), L16540 (CCA TCG TGA TGT CTT ATT TAA GGG GAA CGT), and H16498 (CCT GAA GTA GGA ACC AGA TG). For the FMNH and UA specimens, however, a PCR strategy contrived to amplify old

and damaged DNA was employed (Yoder, Rakotosamimanana & Parsons, 1999). In this case, primers specific to *L. catta* were designed to amplify targets of no more than 250 bp in length. In all other respects, PCR conditions were identical to those used for modern samples. All PCR products were cycle sequenced using a dye terminator sequencing kit (Applied Biosystems, Foster City, CA) and then analysed by gel electrophoresis with an Applied Biosystems automated DNA sequencer model 377. These sequences were edited and compiled with AutoAssembler 1.3.0 (Applied Biosystems). The complete gene sequences are the consensus of at least 2 independent double-stranded PCR amplifications for which both strands were sequenced.

Cytochrome *b* sequences were easily aligned by eye because of the lack of insertions and deletions (indels). HV1 sequences within the Lemuridae were also aligned by eye although CLUSTAL (Higgins & Sharp, 1988; Thompson, Higgins & Gibson, 1994) was used for a first approximation of the alignment of the mouse lemur *Microcebus murinus* and Tattersall's sifaka *Propithecus tattersalli* outgroup sequences. The HV1 sequences show multiple indels among the different taxa compared. Gaps resulting from the indels were treated as missing data rather than scored as a new state, for both the phylogenetic and phenetic analyses. Sequences for both genes and alignment for HV1 are available from GenBank (see Table 1). The branch-and-bound algorithm in PAUP* 4.0b1 (Swofford, 1998) was used for all parsimony analyses. One hundred bootstrap replicates were run with 10 replicates of the random addition option selected from the heuristic search menu. PAUP* was also used for the calculation of uncorrected pairwise distances ('p') and for distance analysis of HKY85-corrected data. For the latter, the weighted least squares (inverse-squared weighting, power = 2) was selected from the objective functions submenu. All other options were set to default and trees were calculated with the branch-and-bound algorithm. Sequences for the 2 mitochondrial regions were analysed both separately and in combination. A likelihood ratio test of the combined data was conducted using the program PUZZLE 4.0.1 (Strimmer & von Haesler, 1996) to test for a molecular clock. We employed MacClade 3.01 (Maddison & Maddison, 1992) in a search for diagnostic sites in the Andringitra specimens using PAA (after Wyner *et al*., 1999).

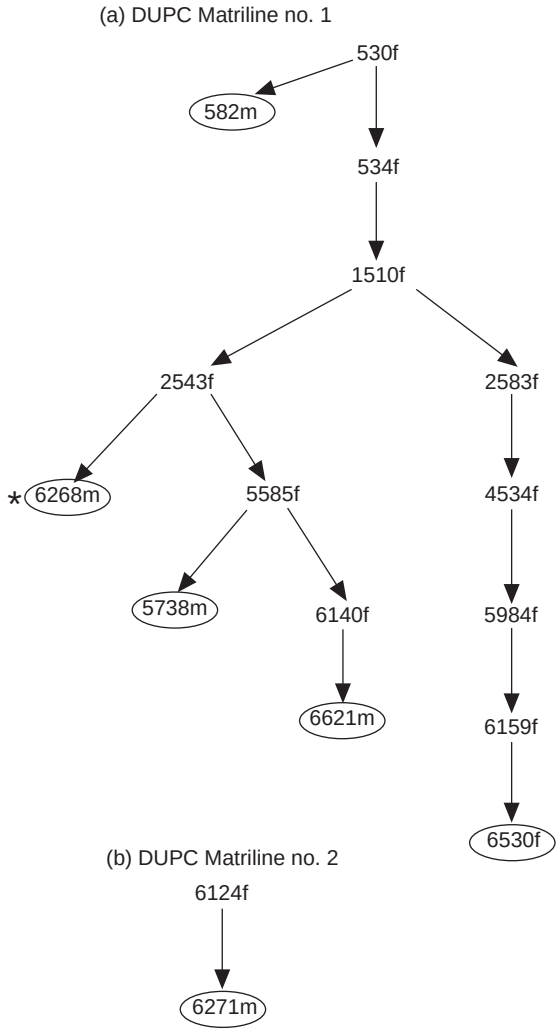

Fig. 2. Diagram of matrilineal relationships among select Duke University Primate Center (DUPC) *Lemur catta*. Circled numbers indicate individuals included in this study. 6268m (asterisk) indicates a sample for which a cytochrome *b* sequence was taken from Genbank (Accession no. U38271). D-loop sequence for this specimen is therefore not included in the study. Arrows, mother–offspring genetic transmission.

RESULTS AND DISCUSSION

Five of the six DUPC individuals showed identical sequences for both cytochrome *b* and for HV1, as do the two Andringitra individuals. Figure 2 shows that all but one of the sampled DUPC individuals are related through a single matriline (Fig. 2a); the exception is DUPC 6271m which is the offspring of an individual from a presumably independent matriline acquired from the Detroit Zoo (Fig. 2b). Within the first matriline, only 14 independent generational events are being sampled thus making it unlikely that even one nucleotide substitution would be observed, even assuming the most rapid mutation rate to have been proposed for mtDNA (Parsons *et al*., 1997). The patterns of genetic identity within the DUPC matrilines emphasize the point that mtDNA can potentially yield highly skewed views of intra-population genetic diversity when there are strong matrilineal affiliations. Also, as only five mtDNA haplotypes were sampled, our interpretation of the PAA is severely limited.

Assuming that the Andringitra haplotype has diverged from the other *L. catta* haplotypes to any significant degree, it is to be expected that it will show unique character states at several to many positions in the mtDNA data set. Even so, without further sampling

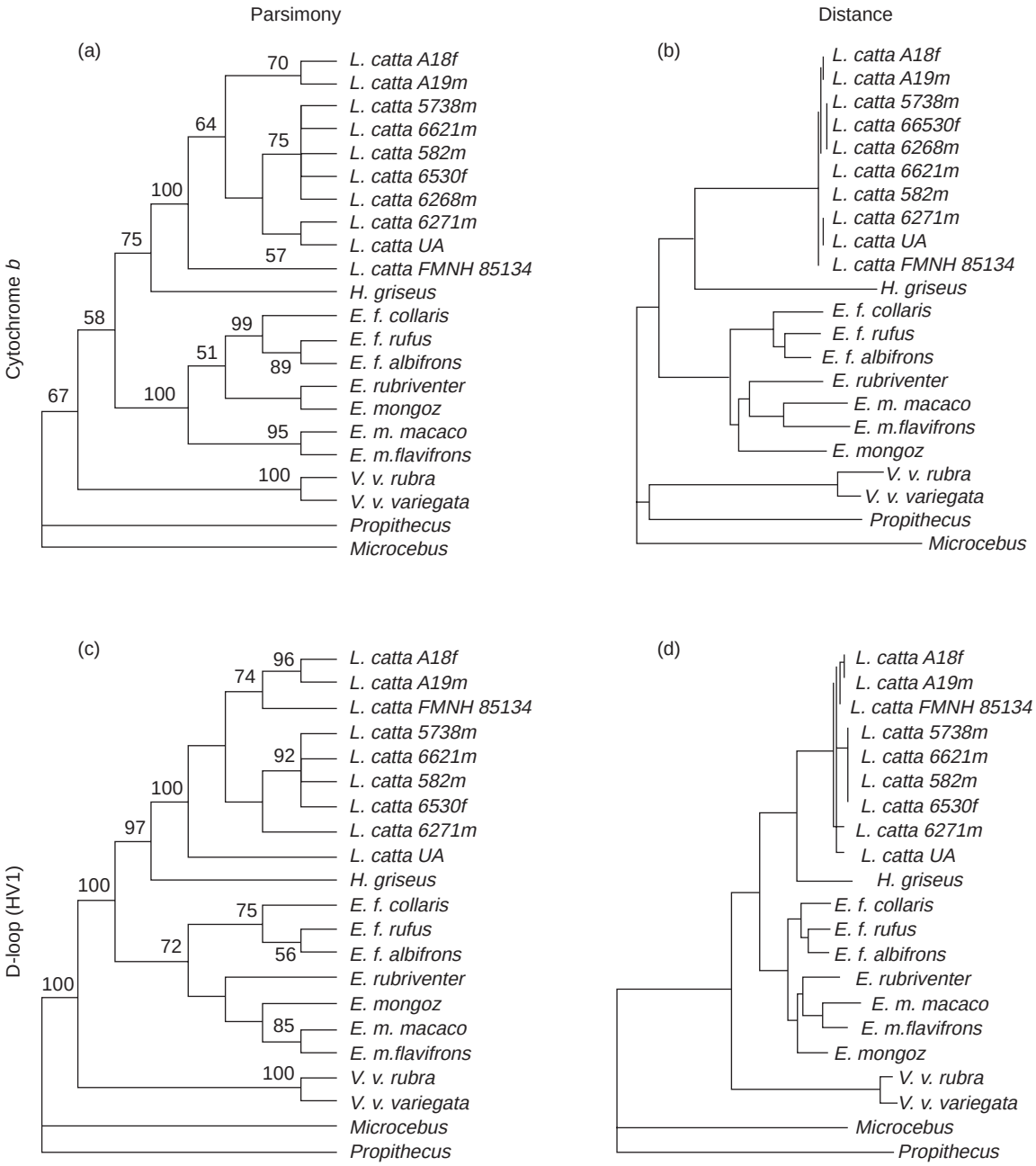

Fig. 3. Parsimony and distance analyses for cytochrome *b* and HV1. See Table 1 for explanation of taxon abbreviations. (a) Strict consensus of six equally parsimonious trees derived from cytochrome *b* data. Trees are 952 steps; CI = 0.576, RI = 0.726. Numbers on branches indicate bootstrap support for node above that branch. (b) One of two phylograms of cytochrome *b* data derived from weighted least squares analysis of HKY85-corrected distance matrix. Tree score = 0.254 (average %SD = 3.323). (c) Single most-parsimonious tree derived from HVI data. Tree is 662 steps; CI = 0.757, RI = 0.789. Numbers on branches indicate bootstrap support for node above that branch. (d) One of three phylograms of HV1 data derived from weighted least squares analysis of HKY85–corrected distance matrix. Tree score = 0.448 (average %SD = 4.631).

of the population, it is impossible to determine if the unique sites are attributes (i.e. fixed and different) or traits (i.e. different but not fixed). Despite the limitations of the analysis for the current haplotype sample, it is worth reporting the PAA results. The Andringitra haplotype was found to differ uniquely from the other *L. catta* haplotypes at only three sites for the data analysed: positions 305 (showing a C rather than a T) and 416 (showing a G rather than an A) in the HV1 alignment and position 196 (showing an A rather than a G) for the cytochrome *b* alignment. Thus, there are potentially three sites out of the 1680 bp sampled that could define the Andringitra population as a phylogenetic species. We do not consider this to be persuasive

evidence of species-level distinction for two reasons: (1) most of the other haplotypes examined (both within and outside of *L. catta*) were also observed to show unique sites at one or more positions; (2) even if the three putative attributes were upheld through further sampling, the biological underpinnings of these differences do not offer compelling evidence of significant evolutionary divergence.

The analysis of haplotype genealogy does nothing to refute these conclusions. For both genes, the Andringitra individuals nest securely among the lowland representatives (Fig. 3). In fact, for the more rapidly evolving HV1 data, the Andringitra individuals are shown to group with the FMNH specimen from Ambohimahavelona (Fig. 3c,d) which, relative to Andringitra, is near the opposite extreme of the geographic distribution of *L. catta*. This result should not be considered of particular historical significance as it is probably an artifact of intraspecific mtDNA allelic variation confounded by poor sampling. It does suggest, however, that genetic exchange throughout the geographic range of *L. catta* has been persistent and pervasive.

The genetic distance tests add further support to the conclusions derived from the parsimony analyses. When branch lengths are compared for the HKY85–corrected distance trees, for the two genes individually (Fig. 3b,d) and in combination (Fig. 4), it seems that relatively little evolutionary time has elapsed since all of the *L. catta* mtDNA haplotypes coalesce to a common ancestor. In comparison, the branch lengths distinguishing various outgroup subspecies from one another (e.g. the three *Eulemur fulvus* and the two *Varecia variegata* subspecies) are considerably longer than are those distinguishing the various *L. catta* individuals. The likelihood ratio test indicates that rates of evolution are not significantly different among the lemurid ingroup and non-lemurid outgroup sequences (with the exception of the two *Eulemur macaco* sequences). Thus, branch-length comparisons should convey equivalent temporal information. The branch-length results are further supported by the uncorrected pairwise comparisons ('p'). This measure was tallied among *L. catta* samples as well as among various non-*L. catta* lemurids (Table 2). Again, both genes show that genetic distances for *L. catta* are below even the subspecies level, given current lemurid taxonomy.

In summary, the PAA, coalescent patterns, and genetic distance data do not lend support to the hypothesis that the lowland and Andringitra populations are distinct evolutionary units. The control region data in particular offer one of the most sensitive genetic tests available for both genetic divergence and population structuring, and these data, as well as the cytochrome *b* data, indicate that genetic exchange between highland and lowland populations has occurred on an evolutionary scale expected for a single species. Although the data set examined in this study is limited, both for numbers of individuals and alleles sampled, it is doubtful that more complete population sampling or the addition of nuclear markers would contradict the

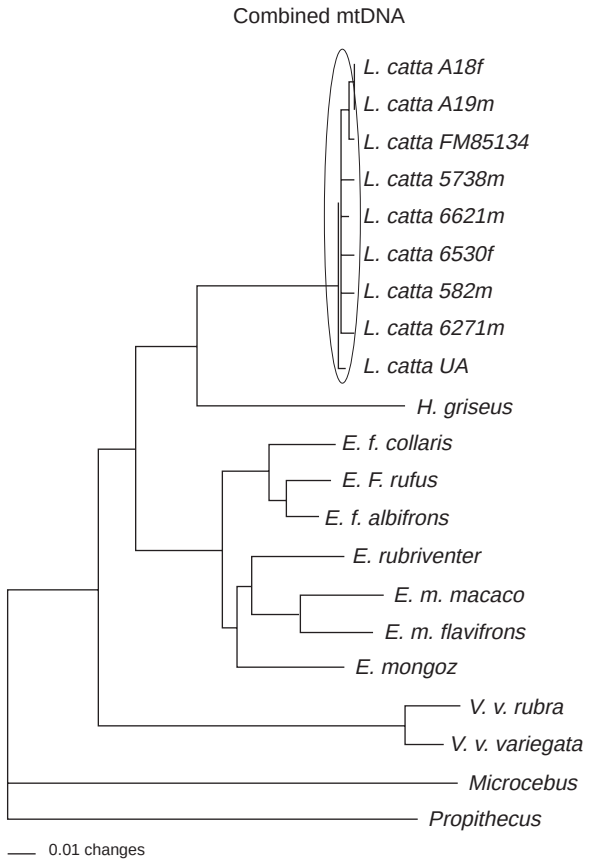

Fig 4. Phylogram of combined HV1 and cytochrome *b* data derived from weighted least squares analysis of HKY85-corrected distance matrix. Tree score = 0.161 (average %SD = 2.779). Oval highlights short branches among *Lemur catta* haplotypes.

results. This is especially true for *L. catta* as it is males that transfer among troops, with females remaining in their natal troop for life (Jones, 1983; Sussman, 1991, 1992). The most structured coalescent patterns would therefore be expected for the mitochondrial allele.

These results are compatible with recent field observations. Subsequent to the publication of Goodman & Langrand (1996), much field work has been conducted on *L. catta* living the Andringitra Massif and surrounding areas. Although it was originally noted that individuals of one troop in the high mountain zone showed distinct pelage characteristics, more thorough observations of the local Andringitra population reveal a complete range of lowland and highland phenotypes with continuous variation in pelage characteristics (S. V. Rakotoarisoa, pers. obs.). Further, from direct observation and discussion with local villagers, one of us (SR) has determined that there are seasonal movements of *L. catta* along the Andringitra slopes. During the warm months (September–January) groups move towards lower elevations (1100–2100 m) on the massif, and during the cold months (June–August), they generally are found on the higher slopes (1650–2500 m). At the lower elevations, the population seasonally occurring on the upper slopes would presumably have the

Table 2. Pairwise comparisons for Fig. 3

Reference no.	Comparison	'p' (cyt *b*)	'p' (HV1)
I. *L. catta* comparisons			
1	*L. catta* A19m–*L. catta* A18f	0.000	0.000
2	*L. catta* A19m–*L. catta* DUPC 6530f	0.004	0.024
3	*L. catta* A19m–*L.catta* DUPC 6271m	0.003	0.017
4	*L. catta* A19m–*L. catta* UA	0.004	0.022
5	*L. catta* A19m–*L. catta* FMNH 85134	0.004	0.009
II. Subspecies comparisons			
6	*E. f. rufus–E. f. albifrons*	0.025	0.030
7	*E. f. rufus–E. f. collaris*	0.042	0.054
8	*E. f. albifrons–E. f. collaris*	0.037	0.049
9	*E. m. macaco–E. m. flavifrons*	0.058	0.054
10	*V. v. variegata–V. v. rubra*	0.031	0.038
III. Species comparisons			
11	*E. mongoz–E. m. macaco*	0.092	0.081
12	*E. mongoz–E. f. collaris*	0.078	0.088
13	*E. mongoz–E. rubriventer*	0.072	0.082
14	*E. rubriventer–E. f. collaris*	0.072	0.091
15	*E. rubriventer–E. m. macaco*	0.064	0.086

potential for contact with groups occurring in lower-lying areas. Thus, there exists among populations a mechanism for continuing genetic exchange and likely falsification of the different-species hypothesis by the criteria of the BSC.

CONCLUSIONS

For both of the chosen mitochondrial markers, there seems to be a correlation between genetic distance and established taxonomy within the Lemuridae. Genetic comparisons reveal that the level of divergence within *L. catta* falls securely within the range of intraspecific variation. None the less, although *L. catta* from Andringitra are clearly not members of a distinct *Lemur* species, the PAA analysis indicates that incipient evolutionary divergence may be underway. Population isolation abetted by anthropogenic environmental destruction, may lead to the earliest stages of a vicariant speciation. Ironically then, habitat degradation could sometimes be viewed as a mechanism for taxonomic diversity. Any such sundering, however, of natural mechanisms for genetic exchange among populations will ultimately curtail the evolutionary diversity and potential for adaptive change that should be the hallmark of the species unit.

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