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RESEARCH ARTICLE

# Does *Eulemur cinereiceps* Exist? Preliminary Evidence From Genetics and Ground Surveys in Southeastern Madagascar

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Although appearing in the literature as early as 1890, the brown lemur form *Eulemur cinereiceps* has recently resurfaced as a potentially valid taxon, distinct from neighboring, presumably closely related species such as white-collared lemurs (*Eulemur albocollaris*). We propose two scenarios for the potential separation of *E. cinereiceps* and *E. albocollaris*: (1) coastal and interior populations represent two distinct taxa and (2) the coastal population north of the Manampatrana River (the locality for purported museum specimens of *E. cinereiceps*) represents a distinct species from *E. albocollaris* found south of the river and in the interior escarpment forests. We tested these hypotheses using data from ground surveys and genetic sampling. Surveys were conducted in coastal forest fragments both north and south of the Manampatrana River in July–August 2006. Genetic samples were collected at two coastal sites and one interior forest. We used maximum parsimony, maximum likelihood, and neighbor-joining analyses on mitochondrial DNA regions to determine if populations from different sites clustered into diagnosable clades. Results from field surveys confirmed the presence of forms commonly referred to as *E. albocollaris* at the two southern coastal forests; no consistent phenotypic differences across sites were observed. All genetic analyses yielded identical results: coastal and interior populations do not cluster into separate groups, thus rejecting the first hypothesis. *Eulemur* species and all other day-active lemurs have apparently been extirpated from coastal forests north of the Manampatrana. Owing to the absence of lemurs from the northern coastal localities, we could not conclusively support or reject the second scenario. However, based on examination of the original plates and museum specimens, as well as the biogeographic patterns typical of this region, we strongly suspect that all populations from this area belong to a single species. We conclude with remarks regarding the apparent priority of *E. cinereiceps* for this taxon. Am. J. Primatol. 70:372–385, 2008. 

**Key words: *Eulemur cinereiceps*; *Eulemur albocollaris*; white-collared lemur; mitochondrial DNA; biogeography; taxonomy; southeastern Madagascar**

## INTRODUCTION

The brown lemurs (*Eulemur fulvus* and related species) are among the most widespread primates in Madagascar [Johnson, 2006; Mittermeier et al., 2006b; Tattersall, 1982]. However, anthropogenic disturbance in last two millennia has resulted in the presently discontinuous range. The central plateau, which likely contained a mosaic of forest and more open habitats before humans [Godfrey et al., 1997], has been largely deforested; remaining brown lemur habitats are confined to the periphery of Madagascar [Tattersall, 1982; Tattersall & Sussman, 1998], with some taxa having discontinuous ranges incorporating both eastern and western forests.

The taxonomy of this diverse group is contentious. Previously, brown lemurs were considered a single polytypic species (*E. fulvus*), with six recognized subspecies: *E. fulvus fulvus* (the common brown lemur), *E. f. rufus* (the red-fronted lemur), *E. f. albocollaris* (the white-collared lemur, WCL), *E. f. collaris* (the collared lemur), *E. f. sanfordi*

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(Sanford's lemur), and *E. f. albifrons* (the white-fronted lemur) [Mittermeier et al., 1994]. However, recent cytogenetic and molecular genetic evidence has supported the elevation of *E. albocollaris* and *E. collaris*, the only two brown lemur taxa that cannot produce fertile hybrids, to full species [Djlelati et al., 1997; Wyner et al., 1999a]. Groves [2001], noting distinct phenotypic appearance and craniodental features [Tattersall & Schwartz, 1991] among brown lemur taxa, suggested further splitting, with species-level designation for all recognized subspecies.

There is some evidence that such extensive reclassification is not yet warranted. Tattersall [1993] noted that homoplasy plagues phylogenetic analyses based on anatomical characters in this group. Moreover, no genetic analyses have thus far been able to sort *E. f. fulvus*, *E. f. rufus*, *E. f. albifrons*, and *E. f. sanfordi* into diagnosable clades; Wyner et al. [1999a] found no markers to distinguish among these subspecies and Pastorini et al. [2000] identified clades that cross-cut the recognized subspecies. It should be noted that both of these studies found *E. albocollaris* and *E. collaris* were sister taxa, separated from other brown lemurs, but they disagreed over whether to elevate these taxa to species.

Recently, there has been discussion concerning a possible seventh brown lemur taxon [Groves, 2001; Mittermeier et al., 2006b]. *E. cinereiceps* was described (as *Lemur* (*Prosimia*) *macaco cinereiceps*) by Groves [1974] as a distinct ''white-cheeked'' variety from the Farafangana region, separated geographically from neighboring *E. collaris*. The name is based on plates from Milne-Edwards and Grandidier [1890], but unfortunately no text accompanied these plates. Schwarz [1931] concluded that two mounted specimens from the Paris National Museum were the individuals depicted by Milne-Edwards and Grandidier [1890]. The localities for these specimens were Farafangana and Salohy [north of Farafangana; Schwarz, 1931]. Schwarz [1931] included *E. cinereiceps* among the synonyms for *E. collaris*. Shortly after Groves' [1974] publication, Rumpler [1975] proposed *E. albocollaris* (as *Lemur fulvus albocollaris*) based on karyoptypic divergence ($2N = 48$) from *E. collaris* ($2N = 50$, 51, 52) individuals of known capture locations.

Despite the apparent priority of *E. cinereiceps* over *E. albocollaris* in nomenclature, a considerable debate ensued [Groves, 1974, 2001; Tattersall, 1979, 1982]. Tattersall [1979, 1982] rejected *E. cinereiceps* as the senior designation, suggesting the Milne-Edwards and Grandidier [1890] plates did not resemble the WCL of southeastern Madagascar. In addition, the individuals depicted in the plates and the mounted specimens were females, which are not diagnostic for southeastern brown lemurs [white-collared and collared lemur females are largely indistinguishable; Tattersall, 1982]. Thus, Tattersall [1982] supported Rumpler's [1975] description of *E. albocollaris* as the brown lemur taxon in the region in question. Groves [2001] disagreed and continued to support the seniority of *E. cinereiceps*, but also suggested that *E. cinereiceps* and *E. albocollaris* could represent two distinct taxa. He described *E. cinereiceps* separately and explicitly contrasted it with *E. albocollaris*: ''[c]ompared with females of *E. collaris* (and so presumably of *E. albocollaris*) they are much lighter and redder; the cheeks are light gray, with no trace of orange or white whiskers; the muzzle is very light, not black [Groves, 2001:78].'' Subsequently, Mittermeier et al. [2006b] reported a captive female observed in 2005 in Farafangana that closely resembled the Milne-Edwards and Grandidier [1890] plate and Paris specimens. They reserved judgment as to the validity of a separate *E. cinereiceps*, but recommended further surveys in the vicinity of Farafangana to evaluate its status.

Field surveys and genetic sampling over the past 10 years have clarified the geographic distribution of WCL [Irwin et al., 2005; Johnson & Overdorff, 1999; Johnson & Wyner, 2000; Wyner et al., 1999a, 2002]. They are found in the eastern escarpment rain forest corridor from the Mananara River in the south (the boundary with *E. collaris*) to the Andringitra Massif in the north, where they form a hybrid zone with the more northerly *E. fulvus rufus* [Irwin et al., 2005]. North of Andringitra, populations with *E. fulvus rufus* phenotypes are found at least as far south as Ankopakopaka [Goodman et al., 2001; Fig. 1]. Within the WCL range, the remaining forest is highly fragmented, and coastal populations in the fragments south of Farafangana are separated from the interior corridor by approximately 40 km of deforested area (Fig. 1). The Manampatrana River does not serve as a barrier to WCL in the interior [Johnson & Wyner, 2000; Fig. 1]. There are no recent surveys describing coastal *Eulemur* populations immediately north of Farafangana (i.e., north of the mouth of the Manampatrana River and the locality for at least one of the Paris specimens).

The objective of this study is to investigate the potential separation of WCL into *E. cinereiceps* and *E. albocollaris*. Specifically, we evaluate two possible scenarios for their division:

(1) *Coastal and interior populations represent two distinct taxa*. In this case, coastal populations should have claim to *E. cinereiceps* based on the Paris specimens' localities (Farafangana region). We suggest that distinct interior populations would retain the binomial *Eulemur albocollaris*, as this term has prevailing usage in the literature [Johnson, 2006; Mittermeier et al., 2006b; Overdorff & Johnson, 2003; Wyner et al., 2002].
(2) *Coastal populations north of Farafangana (i.e., north of the Manampatrana River mouth)*

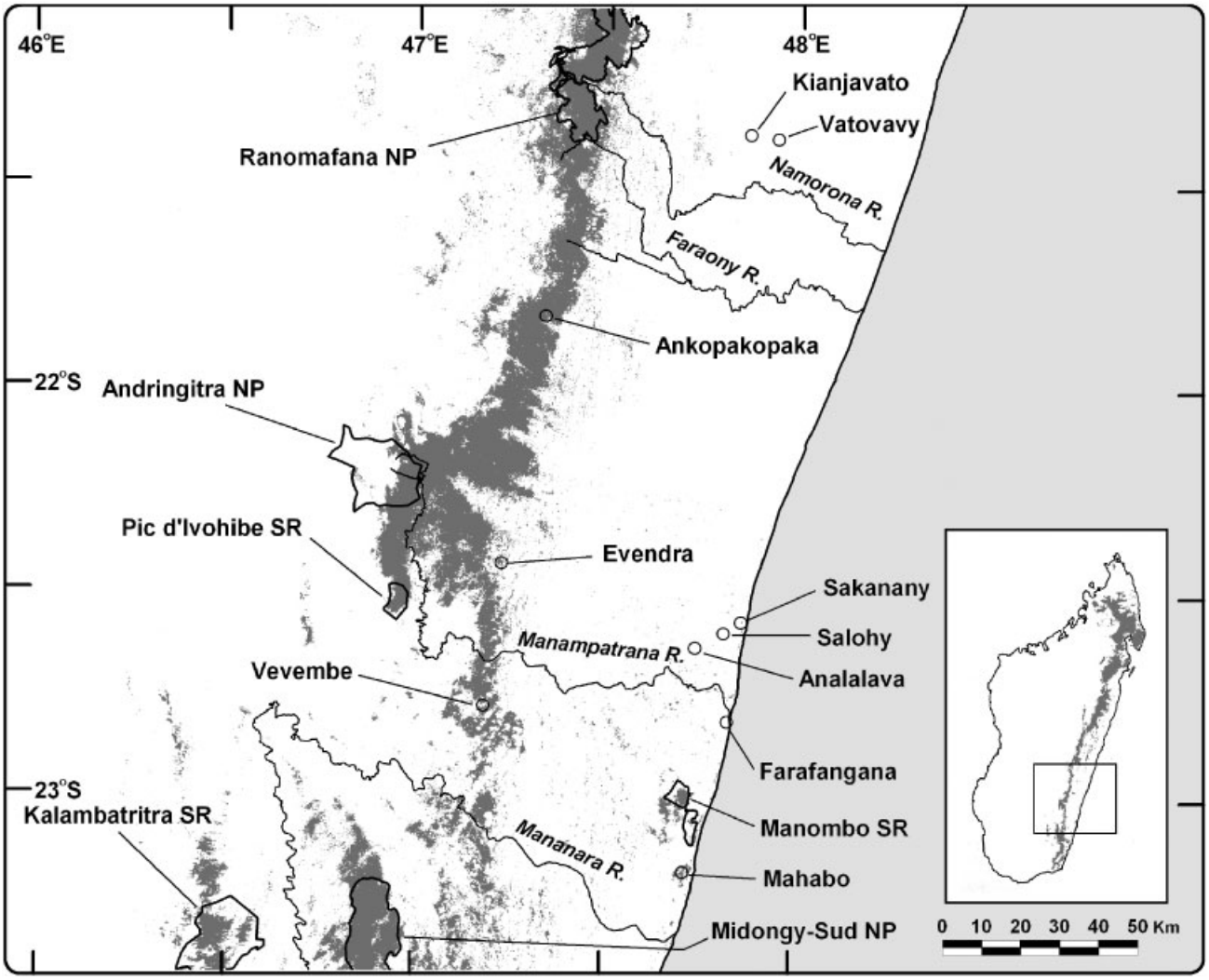


Fig. 1. Study region. Ground survey sites include: Sakanany, Analalava, Manombo, and Mahabo. Genetic sampling sites for the present analysis include: Vevembe, Manombo, and Mahabo. Additional localities include: Evendra [white-collared lemurs; Johnson & Wyner, 2000], Andringitra [white-collared × red-fronted lemur hybrids; Wyner et al., 2002], Ankopakopaka, Ranomafana, Kianjavato, and Vatovavy [red-fronted lemurs; Goodman et al., 2001; Wyner et al., 1999a].

*represent a distinct taxon*. In this case, populations north of Farafangana would represent *E. cinereiceps* and *E. albocollaris* would be located south of Farafangana (e.g., Manombo) as well as in the interior corridor (e.g., Vevembe). This possibility is at least potentially supported by the locality information for the Paris specimens: one is found at Salohy, north of Farafangana and the Manampatrana River [Schwarz, 1931].

To test these hypotheses, we present data from recent ground surveys and genetic sampling of brown lemurs in the region in question. If scenario 1 holds, we anticipate phylogenetic analyses will indicate that individuals from interior and coastal populations can be sorted consistently into distinct clades based on mitochondrial DNA (mtDNA) sequences. If scenario 2 holds, we expect that coastal populations north and south of the Manampatrana River will cluster in separate clades, even if southern coastal populations are not found to differ from interior populations. If no consistent differences among populations are found, this would suggest that all belong to the same species. As the distinctions between taxa are not presently well established, we will refer to brown lemur populations from the region in question collectively as WCL.

In examining the question of divergence among WCL populations, we adopt the phylogenetic species concept, in which species are defined as the smallest cluster of individuals that are diagnosably distinct from other taxa [i.e., share apomorphic characters; Cracraft, 1983]. The phylogenetic species concept is by no means conservative in assigning populations as distinct taxa, particularly in comparison with other

species definitions relying on reproductive isolation, such as the biological species concept [Mayr, 1942]. In this first attempt to examine potential species-level distinctions in WCL, we present phylograms based on mtDNA sequence data of wild-caught individuals from target populations. However, we caution that phylograms based on limited character sets (e.g., mtDNA) may not be sufficient for describing species diversity [Rubinoff, 2006]. If the study populations can be sorted into distinct clades by the present techniques [commonly employed in lemur phylogenetics; Pastorini et al., 2000; Wyner et al., 1999a], we recommend further analyses, including sampling multiple genetic loci, quantitative analysis of morphological characters, and field studies of contact zones to further assess the validity of assigning WCL to multiple taxa.

There are significant conservation implications for determining the relationships among these populations. WCL, with their limited range and ongoing threats from anthropogenic disturbance, are presently listed among the most endangered primates in the world [Mittermeier et al., 2006a]. If this species should be divided into separate taxa, each form would certainly be critically endangered and would require distinct management plans.

## METHODS

### Ground Surveys

We conducted ground surveys in the Farafangana region in July–August 2006. Sites south of Farafangana included: Mahabo (S 23° 11.175′ E 47° 43.095′; altitude = 18 m) and Manombo (S 23° 01.697′ E 47° 43.838′; altitude = 36 m; Fig. 1). Sites north of Farafangana included: Analalava (S 22° 38.183′ E 47° 44.526′; altitude = 88 m) and Sakanany (S 22° 34.341′ E 47° 51.747′; altitude = 18 m; Fig. 1).

Mahabo contains approximately 1,500 ha of degraded littoral rain forest. Manombo Special Reserve and adjacent classified forest contains 15,730 ha of a mosaic of anthropogenic matrix, lowland rain forest, and littoral rain forest. Deforestation and hunting continue at both sites. The two northern sites are the closest remaining forests to Salohy, the locality for one of the Paris specimens [Schwarz, 1931; Fig. 1]. Analalava, near the village of Andramena, consists of approximately 70 ha of highly degraded lowland rain forest. Sakanany is a thin strip of littoral rain forest (ca. 200 ha). The forest is heavily and actively degraded, with virtually no large trees (>10 cm diameter at breast height) remaining (i.e., there are few remaining potential food sources for frugivorous lemurs).

All sites were surveyed during daylight hours, using existing trails where present to minimize disturbance. Survey effort varied depending on the size of the forest fragment; however, most fragments were small enough to investigate a majority of the forested area. The northern sites were investigated for one to three days by three to five observers searching independently or in teams of two to three individuals. Survey effort was estimated in person hours per hectare of forest. We surveyed the southern sites of Manombo and Mahabo to confirm the presence of WCL, examine phenotypic variation among populations, and to collect DNA samples (see below). These populations are currently subjects for ongoing behavioral ecology research. Searches at these sites were therefore nonrandom, as known social groups were targeted. Pelage characters were assessed visually and compared qualitatively with published descriptions of WCL phenotypes [Tattersall, 1982] and previous observations elsewhere [Johnson & Overdorff, 1999; Johnson & Wyner, 2000]. Brown lemur taxa are generally easily distinguished by facial pelage, particularly in males [Tattersall, 1982]. Phenotypic characters were not included in quantitative phylogenetic analyses.

### Genetic Sampling and Analysis

Samples were collected at Manombo ($N = 10$ individuals) and Mahabo ($N = 6$) in April 2006 (Table I). This sample augmented previous collections at Manombo in 2000 ($N = 2$; Table I). Samples ($N = 11$) were also collected at Vevembe (S 22° 47.065, E 47° 11.110′) in 2000 (Fig. 1; Table I). This interior corridor forest is 65 km west of Farafangana (Fig. 1). Outgroups include *Eulemur* and other lemur taxa from across Madagascar (Table I). On the basis of the null hypothesis of no population differences among WCL, we refer to test individuals from all sites as *E. albocollaris* in all figures and tables, recognizing that different nomenclature may need to be adopted for different populations depending on results.

Study animals (all adults) were immobilized with a $CO_2$ projection rifle with 10 mg/kg of Telazol (Fort Dodge, Overland Park, Kansas). DNA was extracted from 2.0-mm biopsies using a phenol-chloroform extraction [Sambrook et al., 1989]. We analyzed the following regions of the mtDNA: the displacement loop or control region [D-loop; Baker et al., 1993; Wyner et al., 1999b] and a fragment of the cytochrome oxidase subunit III gene, NADH-dehydrogenase subunits 3, 4L, and 4 (ND3, ND4L, and ND4) as well as the $tRNA^{Gly}$, $tRNA^{Arg}$, $tRNA^{His}$, $tRNA^{Ser}$, and partial $tRNA^{Leu}$ genes [subsequently referred to as the PAST fragment; Pastorini et al., 2000]. Using 50 ng of genomic DNA, the D-loop (552–555 bp) and the PAST (2388 bp) fragments were amplified using the following conditions: 94°C for 4 min, 94°C for 30 sec, 47°C for 45 sec, 72°C for 45 sec for 35 cycles, 72°C for 10 min. The samples were electrophoresed on a 1.2% agarose gel to verify

**TABLE I. Samples (29 *Eulemur albocollaris* and 41 outgroups total) Used in the Present Genetic Analyses**

| ID# | Site | Taxon | Sex | PAST GenBank accession no. | D-loop GenBank accession no. |
|---|---|---|---|---|---|
| HABO6.1 | Mahabo | *Eulemur albocollaris* | F | EF552610 | EF552658 |
| HABO6.2 | Mahabo | *Eulemur albocollaris* | F | EF552611 | EF552659 |
| HABO6.3 | Mahabo | *Eulemur albocollaris* | M | EF552612 | EF552660 |
| HABO6.4 | Mahabo | *Eulemur albocollaris* | M | EF552613 | EF552661 |
| HABO6.9 | Mahabo | *Eulemur albocollaris* | F | EF552614 | EF552662 |
| HABO6.10 | Mahabo | *Eulemur albocollaris* | M | EF552615 | EF552663 |
| M151 | Manombo | *Eulemur albocollaris* | F | EF552616 | EF552664 |
| M152 | Manombo | *Eulemur albocollaris* | M | EF552617 | EF552665 |
| MBO6.2 | Manombo | *Eulemur albocollaris* | M | EF552618 | EF552666 |
| MBO6.3 | Manombo | *Eulemur albocollaris* | M | EF552619 | EF552667 |
| MBO6.4 | Manombo | *Eulemur albocollaris* | F | EF552620 | EF552668 |
| MBO6.5 | Manombo | *Eulemur albocollaris* | M | EF552621 | EF552669 |
| MBO6.6 | Manombo | *Eulemur albocollaris* | F | EF552622 | EF552670 |
| MBO6.7 | Manombo | *Eulemur albocollaris* | M | EF552623 | EF552671 |
| MBO6.8 | Manombo | *Eulemur albocollaris* | M | EF552624 | EF552672 |
| MBO6.9 | Manombo | *Eulemur albocollaris* | F | EF552625 | EF552673 |
| MBO6.10 | Manombo | *Eulemur albocollaris* | M | EF552626 | EF552674 |
| MBO6.11 | Manombo | *Eulemur albocollaris* | M | EF552627 | EF552675 |
| VVEV1 | Vevembe | *Eulemur albocollaris* | M | EF552628 | EF552676 |
| VVEV2 | Vevembe | *Eulemur albocollaris* | F | EF552629 | EF552677 |
| VVEV3 | Vevembe | *Eulemur albocollaris* | M | EF552630 | EF552678 |
| VVEV4 | Vevembe | *Eulemur albocollaris* | F | EF552631 | EF552679 |
| VVEV5 | Vevembe | *Eulemur albocollaris* | M | EF552632 | EF552680 |
| VVEV6 | Vevembe | *Eulemur albocollaris* | M | EF552633 | EF552681 |
| VVEV7 | Vevembe | *Eulemur albocollaris* | M | EF552634 | EF552682 |
| VVEV8 | Vevembe | *Eulemur albocollaris* | M | EF552635 | EF552683 |
| VVEV9 | Vevembe | *Eulemur albocollaris* | M | EF552636 | EF552684 |
| VVEV10 | Vevembe | *Eulemur albocollaris* | M | EF552637 | EF552685 |
| VVEV11 | Vevembe | *Eulemur albocollaris* | F | EF552638 | EF552686 |
| RANO261 | Ranomafana | *Avahi laniger* | M | AY582559 | AY584496 |
| RANO67 | Ranomafana | *Avahi laniger* | M | AY582558 | AY584495 |
| ANK33 | Ankarafantsika | *Avahi occidentalis* | F | AY582560 | AY584497 |
| RANO229 | Ranomafana | *Cheirogaleus major* | F | AY582563 | AY254050 |
| GAR8 | Manongarivo | *Cheirogaleus medius* | M | AY582562 | AY584498 |
| DOG8 | Midongy du Sud | *Eulemur collaris* | F | EF552591 | EF552639 |
| AND25 | Andohahela | *Eulemur collaris* | F | EF552592 | EF552640 |
| MER16 | Analamera | *Eulemur coronatus* | F | EF552608 | EF552656 |
| ANKA3 | Ankarana | *Eulemur coronatus* | F | EF552609 | EF552657 |
| BET31 | Betampona | *Eulemur fulvus albifrons* | M | EF552593 | EF552641 |
| JAR11 | Anjanaharibe-Sud | *Eulemur fulvus albifrons* | F | EF552596 | EF552644 |
| ZAH19 | Zahamena | *Eulemur fulvus fulvus* | M | EF552594 | EF552642 |
| ANK3 | Ankarafantsika | *Eulemur fulvus fulvus* | — | EF552595 | EF552643 |
| RANO45 | Ranomafana | *Eulemur fulvus rufus* | M | AY582561 | AY585738 |
| ISA2.3 | Isalo | *Eulemur fulvus rufus* | M | EF552599 | EF552647 |
| ANAL4 | Analamera | *Eulemur fulvus sanfordi* | M | EF552597 | EF552645 |
| MER12 | Analamera | *Eulemur fulvus sanfordi* | M | EF552598 | EF552646 |
| LOKO4.10 | Lokobe | *Eulemur macaco macaco* | M | EF552604 | EF552652 |
| LOKO4.25 | Lokobe | *Eulemur macaco macaco* | M | EF552605 | EF552653 |
| MIT40 | Antrema | *Eulemur mongoz* | F | EF552602 | EF552650 |
| MIT39 | Antrema | *Eulemur mongoz* | F | EF552603 | EF552651 |
| RANO25 | Ranomafana | *Eulemur rubriventer* | F | EF552600 | EF552648 |
| MERY9 | Marojejy | *Eulemur rubriventer* | F | EF552601 | EF552649 |
| LAZA5.02 | Sahamalaza (Ankarafa) | *Euleumur macaco flavifrons* | F | EF552606 | EF552654 |
| LAZA5.08 | Sahamalaza (Ankarafa) | *Euleumur macaco flavifrons* | F | EF552607 | EF552655 |
| RANO351 | Ranomafana | *Hapalemur aureus* | M | AY582549 | AY584489 |
| RANO352 | Ranomafana | *Hapalemur aureus* | M | AY582550 | AY254048 |
| RANO61 | Ranomafana | *Hapalemur griseus griseus* | — | AY582551 | AY584490 |
| RANO62 | Ranomafana | *Hapalemur griseus griseus* | F | AY582552 | AY584491 |
| ANAL2.23 | Analamera | *Hapalemur griseus occidentalis* | M | AY582554 | AY584493 |

**TABLE I. Continued**

| ID# | Site | Taxon | Sex | PAST GenBank accession no. | D-loop GenBank accession no. |
|---|---|---|---|---|---|
| GAR9 | Manongarivo | *Hapalemur griseus occidentalis* | M | AY582553 | AY584492 |
| JAR4 | Anjanaharibe-Sud | *Indri indri* | F | DQ855969 | DQ856049 |
| MIZA5.3 | Maromizaha | *Indri indri* | F | DQ855967 | DQ856050 |
| ANAL5 | Analamera | *Lepilemur septentrionalis* | F | AY582564 | AY769363 |
| ANK7 | Ankarafantsika | *Microcebus ravelobensis* | F | AY582545 | AY159695 |
| RANO250 | Ranomafana | *Microcebus rufus* | M | AY582546 | AY159722 |
| KIAN124 | Kianjavato | *Prolemur simus* | F | AY582548 | AY584488 |
| RANO338 | Ranomafana | *Prolemur simus* | F | AY582547 | AY254049 |
| RANO332 | Ranomafana | *Propithecus edwardsi* | M | AY582556 | AY585739 |
| MOR68 | Beroboka | *Propithecus verreauxi verreauxi* | — | AY582557 | AF354712 |
| FAN21 | Fandriana | *Varecia variegata variegata* | F | AY582555 | AY584494 |

Mitochondrial DNA sequence data for each sample are available from GenBank under the listed accession numbers.

the polymerase chain reaction (PCR) product and purified using QIAquick PCR purification kit (QIAGEN cat. no. 28106, Valencia, CA). The cleaned products were cycle sequenced using a big dye-terminator sequencing kit (Applied Biosystems, Foster City, CA). The sequences were analyzed by capillary electrophoresis with an Applied Biosystems Prizm 3100 genetic analyzer. A suite of internal sequencing primers from Pastorini et al. [2000, 2001] was used to generate the PAST fragment. The sequence fragments were aligned to generate a consensus sequence using Sequencher (Gene Corp, Ann Arbor, MI), and the consensus sequences were aligned using Clustal X [Thompson et al., 1997]. All sequences have been deposited in GenBank and the sequence data and information are available from the referenced accession numbers (Table I).

Maximum parsimony (MP), maximum likelihood (ML), and neighbor-joining (NJ) analyses were performed for the phylogenetic study of the combined D-loop and PAST fragments sequence data with PAUP* Version 4.0b10 software [Swofford, 2001]. The trees described in this study are all consensus trees except for the bootstrap analysis (all trees are presented as phylograms for presentation purposes only). Bootstrap analyses were accomplished with 4,000 replicates, with ten random additional heuristic searches per replicate. Only nodes with greater than 50% support were reported. The NJ tree was generated using the Tamura–Nei model [Tamura & Nei, 1993]. A stepwise addition was selected for MP and ML analyses, and corrections for nucleotide sequence data suggested by Kimura [1980] were used with the NJ analyses. Gaps were considered as a fifth character in MP analyses, whereas gaps were treated as missing data in the NJ analyses. The ML trees were estimated via the heuristic search. For the substitution model, the transition/transversion ratios were estimated in MacClade [Maddison & Maddison, 1992], and a discrete approximation to $\gamma$ distribution was estimated for among site rate variation. The default settings were maintained for all other settings, thus yielding the equivalent of the HKY model [Hasegawa et al., 1985]. In addition to character-based phylogenetic analysis of DNA sequences, PAUP software [Swofford, 2001] and MEGA Version 3.1 [Kumar et al., 2004] were used to calculate genetic distance.

All research procedures complied with protocols approved by Institutional Animal Care and Use Committee (US) and Animal Care Committee (Canada), and adhered to the legal requirements of the Government of Madagascar.

## RESULTS

### Surveys

WCL presence was confirmed at Manombo and Mahabo forests (Fig. 1). Three social groups with six to 11 individuals were identified at Mahabo. Two social groups of four to eight individuals were recorded at Manombo. We detected no evident differences in coat patterns or facial markings between these populations, nor did either differ from interior WCL populations [e.g., Vevembe; S.E.J., personal observation]. Analalava was searched for 3.25 person hours (ca. 0.05 hr/ha). Search time at Sakanany forest was approximately 95 person hours (ca. 0.48 hr/ha). No *Eulemur* or other lemur taxon was observed in either forest, nor was there any sign of lemur activity (feeding remains, feces). Local informants believed *Eulemur* was still present but could not consistently identify lemur species in photographs.

### Genetic Analyses

mtDNA sequence data were completed for D-loop and PAST fragments for 29 WCL (Table I). Relationships among species or genera were consistent in all analyses (Figs. 2–4). Generally, there was very high support in both MP and NJ analyses with

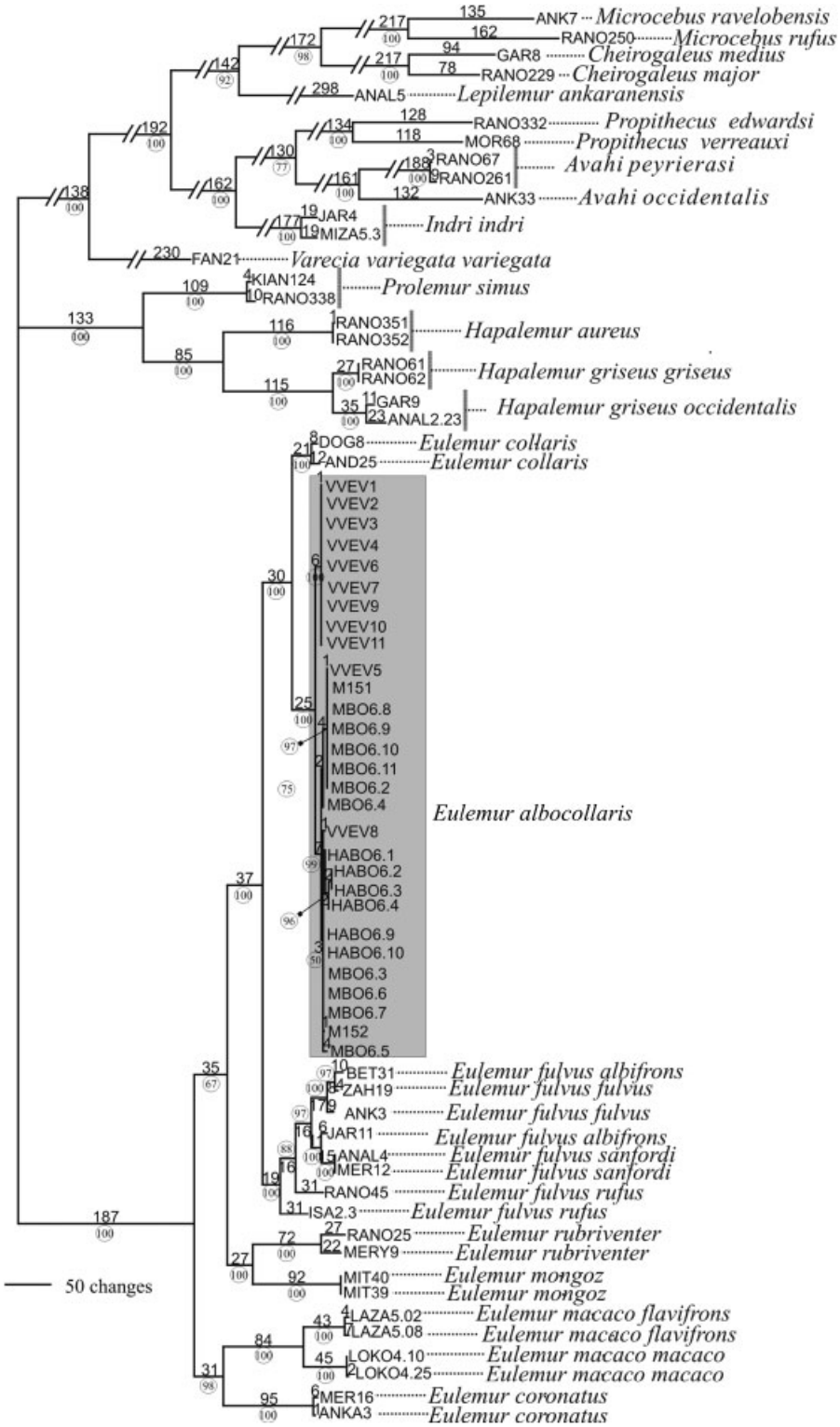


Fig. 2. Neighbor-joining phylogram derived from the D-loop and PAST fragment combined DNA sequence data from the 29 *Eulemur albocollaris* individuals with 41 outgroup taxa. Values above branches indicate the number of changes between nodes. Values within circles indicate support of bootstrap pseudoreplicates.

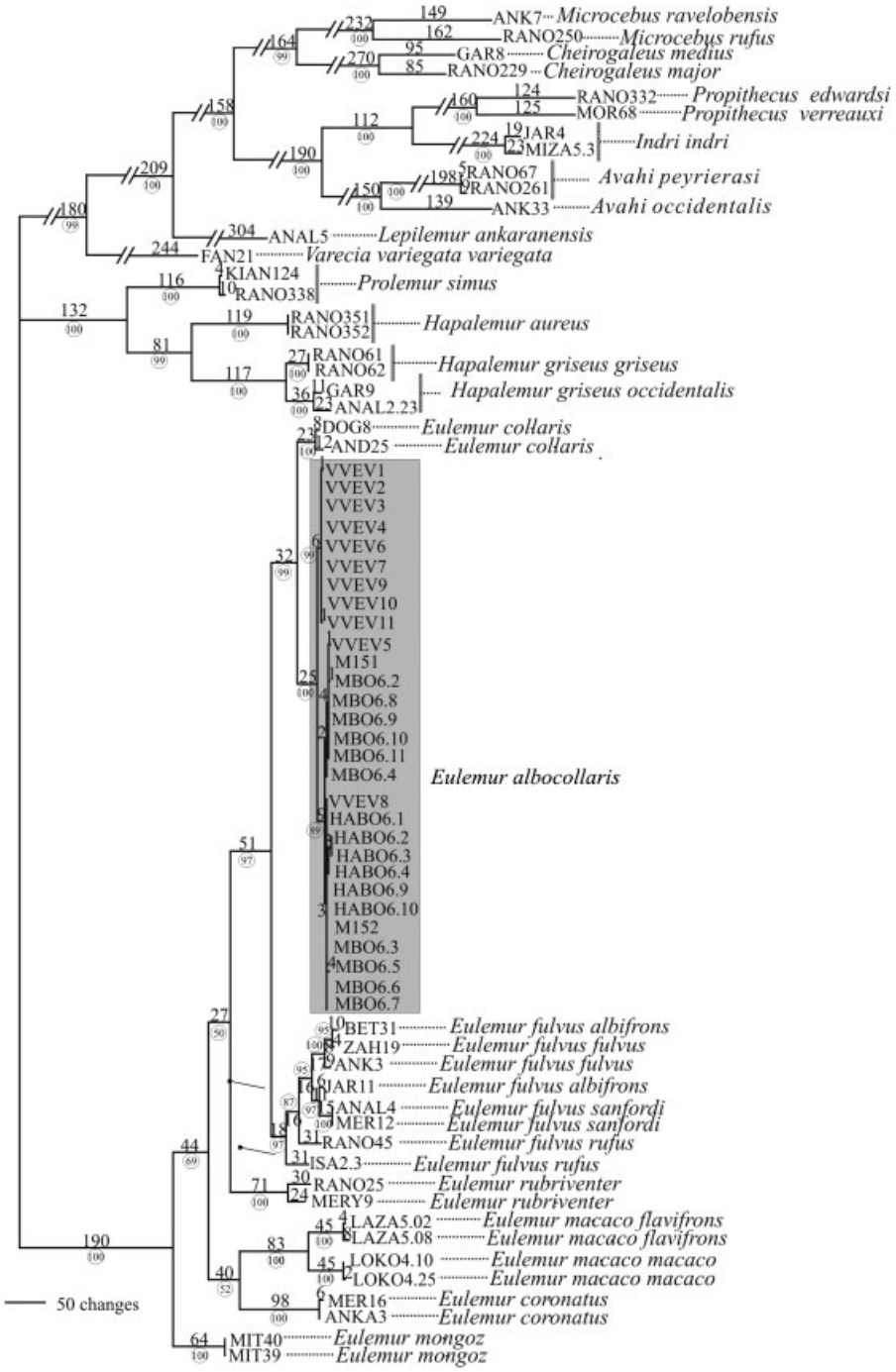


Fig. 3. Maximum parsimony phylogram derived from the D-loop and PAST fragment-combined DNA sequence data from the 29 *Eulemur albocollaris* individuals (one of six most parsimonious trees). Values above branches indicate number of changes between nodes. Values within circles indicate support of bootstrap pseudoreplicates. Length = 5,574; Consistency index (CI) = 0.4760; Retention index (RI) = 0.7875; Rescaled consistency index (RC) = 0.3748; Homoplasy index (HI) = 0.5240.

respect to the branching order of genera and species (Figs. 2–4). On the basis of phylogenetic inferences of the NJ, MP, and ML analyses of the sequence alignments, WCL individuals from the three different sites were clustered together with 100% bootstrap support. The absolute distance and the Kimura two-parameter distance measures are presented in Tables II and III. The absolute distances among WCL individuals are 2–9 bp for D-loop and 1–11 bp for PAST (Tables II and III).

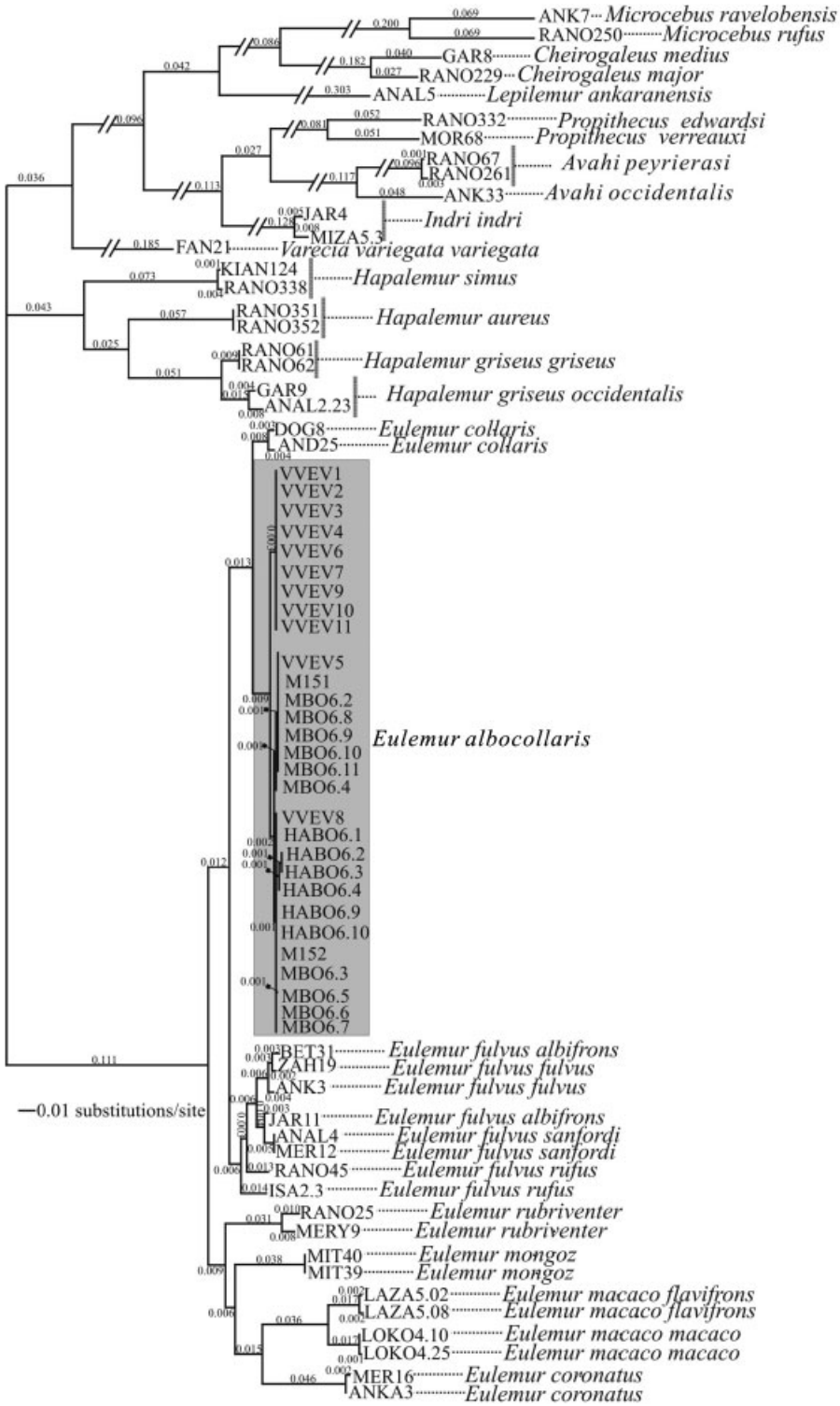


Fig. 4. Maximum-likelihood phylogram derived from the D-loop and PAST fragment-combined DNA sequence data from the 29 *Eulemur albocollaris* individuals. The phylogram presented with branch lengths proportional to the number of changes (values specified on the branches). We obtained the Maximum-likelihood phylogram (-ln likelihood = 26,784.84) from the PAST alignment from a transition/transversions ration of 4.54 ($\kappa = 9.72$) and $\gamma$ shape parame 0.38.

**TABLE II. Kimura Two-Parameter Distance (under the diagonal) and Absolute Distance (above the diagonal) Matrices Derived From the D-loop Sequence Data Set, With Gaps Treated as Missing Data**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | 11 | 27 | 25 | 24 | 26 | 24 | 21 | 26 | 40 | 42 | 42 | 49 | 50 | 48 | 42 | 37 | 37 | 19 | 19 | 21 | 18 |
| 2 | 0.022 | | 32 | 30 | 25 | 29 | 23 | 22 | 27 | 37 | 42 | 41 | 46 | 47 | 43 | 40 | 36 | 34 | 26 | 26 | 28 | 25 |
| 3 | 0.057 | 0.068 | | 5 | 10 | 13 | 14 | 19 | 20 | 39 | 39 | 37 | 49 | 50 | 42 | 34 | 36 | 36 | 24 | 24 | 22 | 27 |
| 4 | 0.052 | 0.063 | 0.010 | | 9 | 12 | 13 | 16 | 19 | 36 | 36 | 36 | 46 | 47 | 39 | 31 | 33 | 33 | 22 | 22 | 20 | 25 |
| 5 | 0.050 | 0.052 | 0.020 | 0.018 | | 11 | 14 | 17 | 20 | 35 | 35 | 33 | 43 | 44 | 38 | 30 | 30 | 30 | 22 | 22 | 20 | 25 |
| 6 | 0.054 | 0.061 | 0.027 | 0.025 | 0.022 | | 9 | 16 | 17 | 40 | 40 | 32 | 48 | 49 | 41 | 33 | 34 | 32 | 23 | 25 | 21 | 26 |
| 7 | 0.050 | 0.048 | 0.029 | 0.027 | 0.029 | 0.018 | | 13 | 14 | 39 | 41 | 31 | 45 | 46 | 40 | 34 | 35 | 33 | 27 | 27 | 25 | 28 |
| 8 | 0.043 | 0.046 | 0.039 | 0.033 | 0.035 | 0.033 | 0.027 | | 15 | 38 | 42 | 35 | 46 | 47 | 41 | 35 | 34 | 32 | 22 | 22 | 22 | 25 |
| 9 | 0.054 | 0.057 | 0.042 | 0.039 | 0.042 | 0.035 | 0.029 | 0.031 | | 39 | 43 | 37 | 47 | 48 | 44 | 36 | 37 | 35 | 29 | 31 | 29 | 30 |
| 10 | 0.086 | 0.079 | 0.083 | 0.076 | 0.074 | 0.086 | 0.083 | 0.081 | 0.083 | | 23 | 39 | 39 | 40 | 45 | 38 | 39 | 40 | 37 | 39 | 39 | 36 |
| 11 | 0.090 | 0.090 | 0.083 | 0.076 | 0.074 | 0.085 | 0.088 | 0.090 | 0.092 | 0.048 | | 36 | 37 | 36 | 41 | 41 | 37 | 38 | 40 | 42 | 40 | 39 |
| 12 | 0.091 | 0.088 | 0.079 | 0.077 | 0.070 | 0.068 | 0.065 | 0.074 | 0.079 | 0.083 | 0.076 | | 40 | 41 | 36 | 30 | 28 | 29 | 41 | 43 | 41 | 43 |
| 13 | 0.106 | 0.099 | 0.106 | 0.099 | 0.092 | 0.103 | 0.096 | 0.099 | 0.101 | 0.083 | 0.079 | 0.085 | | 1 | 21 | 25 | 38 | 37 | 52 | 54 | 50 | 52 |
| 14 | 0.108 | 0.101 | 0.108 | 0.101 | 0.094 | 0.106 | 0.099 | 0.101 | 0.103 | 0.085 | 0.076 | 0.087 | 0.002 | | 22 | 26 | 39 | 38 | 53 | 55 | 51 | 53 |
| 15 | 0.104 | 0.092 | 0.090 | 0.083 | 0.080 | 0.087 | 0.085 | 0.087 | 0.094 | 0.097 | 0.088 | 0.076 | 0.044 | 0.046 | | 11 | 32 | 31 | 43 | 45 | 41 | 46 |
| 16 | 0.090 | 0.085 | 0.072 | 0.065 | 0.063 | 0.069 | 0.072 | 0.074 | 0.076 | 0.081 | 0.088 | 0.063 | 0.052 | 0.054 | 0.022 | | 26 | 25 | 40 | 42 | 38 | 41 |
| 17 | 0.079 | 0.076 | 0.076 | 0.070 | 0.063 | 0.072 | 0.074 | 0.072 | 0.078 | 0.083 | 0.078 | 0.059 | 0.081 | 0.083 | 0.067 | 0.054 | | 3 | 37 | 39 | 36 | 39 |
| 18 | 0.079 | 0.072 | 0.076 | 0.070 | 0.063 | 0.067 | 0.070 | 0.067 | 0.074 | 0.086 | 0.081 | 0.061 | 0.078 | 0.081 | 0.065 | 0.052 | 0.006 | | 37 | 39 | 36 | 39 |
| 19 | 0.039 | 0.055 | 0.050 | 0.046 | 0.046 | 0.048 | 0.057 | 0.046 | 0.061 | 0.079 | 0.085 | 0.088 | 0.113 | 0.115 | 0.092 | 0.085 | 0.079 | 0.079 | | 2 | 4 | 5 |
| 20 | 0.039 | 0.055 | 0.050 | 0.046 | 0.046 | 0.052 | 0.057 | 0.046 | 0.066 | 0.083 | 0.090 | 0.093 | 0.118 | 0.120 | 0.097 | 0.090 | 0.083 | 0.083 | 0.004 | | 6 | 7 |
| 21 | 0.044 | 0.059 | 0.046 | 0.042 | 0.042 | 0.044 | 0.052 | 0.046 | 0.061 | 0.083 | 0.085 | 0.088 | 0.108 | 0.111 | 0.087 | 0.081 | 0.076 | 0.076 | 0.008 | 0.012 | | 9 |
| 22 | 0.037 | 0.052 | 0.057 | 0.052 | 0.052 | 0.055 | 0.059 | 0.052 | 0.063 | 0.076 | 0.083 | 0.093 | 0.113 | 0.115 | 0.099 | 0.088 | 0.083 | 0.083 | 0.010 | 0.014 | 0.018 | |

1, DOG8; 2, AND25; 3, BET31; 4, ZAH19; 5, ANK3; 6, JAR11; 7, ANAL4, MER12; 8, RANO45; 9, ISA2.3; 10, RANO25; 11, MERY9; 12, MIT39, MIT40; 13, LOKO4.10; 14, LOKO4.25; 15, LAZA5.02; 16, LAZA5.08; 17, MER16; 18, ANKA3; 19, HABO6.1, HABO6.9, HABO6.10, M152, MBO6.3, MBO6.4, MBO6.6, MBO6.7, VVEV8; 20, HABO6.2, HABO6.3, HABO6.4; 21, MBO6.2, MBO6.5, MBO6.8, MBO6.9, MBO6.10, MBO6.11, M151, VVEV5; 22, VVEV1, VVEV2, VVEV3, VVEV4, VVEV6, VVEV7, VVEV9, VVEV10, VVEV11.

**TABLE III. Kimura two-parameter distance (under the diagonal) and Absolute Distance (above the diagonal) Matrices Derived From the PAST Sequence Data Set, With Gaps Treated as Missing Data**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | 9 | 91 | 90 | 90 | 83 | 87 | 89 | 81 | 152 | 144 | 157 | 183 | 186 | 187 | 199 | 195 | 45 | 47 | 48 | 44 | 45 | 44 | 43 | 41 | 44 | 43 | 41 |
| 2 | 0.004 | | 88 | 87 | 85 | 76 | 80 | 82 | 78 | 149 | 141 | 156 | 182 | 185 | 186 | 196 | 192 | 42 | 44 | 45 | 41 | 42 | 41 | 40 | 38 | 41 | 40 | 38 |
| 3 | 0.040 | 0.039 | | 9 | 17 | 32 | 38 | 57 | 56 | 151 | 147 | 156 | 193 | 195 | 196 | 185 | 181 | 92 | 94 | 95 | 91 | 92 | 91 | 90 | 90 | 91 | 90 | 90 |
| 4 | 0.039 | 0.038 | 0.004 | | 12 | 31 | 37 | 54 | 57 | 147 | 143 | 155 | 192 | 194 | 195 | 192 | 188 | 91 | 93 | 94 | 90 | 91 | 90 | 89 | 89 | 90 | 89 | 89 |
| 5 | 0.039 | 0.037 | 0.007 | 0.005 | | 31 | 37 | 54 | 57 | 147 | 143 | 153 | 193 | 193 | 194 | 189 | 185 | 89 | 91 | 92 | 88 | 89 | 88 | 87 | 87 | 88 | 87 | 87 |
| 6 | 0.036 | 0.033 | 0.014 | 0.013 | 0.013 | | 12 | 47 | 56 | 142 | 137 | 148 | 185 | 190 | 191 | 189 | 185 | 84 | 86 | 87 | 83 | 84 | 83 | 82 | 82 | 83 | 82 | 82 |
| 7 | 0.038 | 0.035 | 0.016 | 0.016 | 0.016 | 0.005 | | 51 | 60 | 142 | 137 | 148 | 186 | 192 | 193 | 194 | 190 | 90 | 92 | 93 | 89 | 90 | 89 | 88 | 88 | 89 | 88 | 88 |
| 8 | 0.039 | 0.036 | 0.025 | 0.023 | 0.023 | 0.020 | 0.022 | | 60 | 144 | 136 | 155 | 192 | 188 | 189 | 197 | 193 | 91 | 93 | 94 | 90 | 91 | 88 | 87 | 88 | 88 | 89 | 88 |
| 9 | 0.035 | 0.034 | 0.024 | 0.025 | 0.025 | 0.024 | 0.026 | 0.026 | | 143 | 137 | 151 | 188 | 184 | 185 | 187 | 183 | 82 | 84 | 85 | 81 | 82 | 81 | 80 | 76 | 81 | 80 | 76 |
| 10 | 0.068 | 0.067 | 0.068 | 0.066 | 0.066 | 0.064 | 0.064 | 0.065 | 0.064 | | 24 | 151 | 184 | 192 | 193 | 192 | 188 | 149 | 151 | 152 | 148 | 149 | 150 | 149 | 146 | 150 | 147 | 146 |
| 11 | 0.065 | 0.063 | 0.066 | 0.064 | 0.064 | 0.061 | 0.061 | 0.061 | 0.061 | 0.010 | | 147 | 182 | 186 | 187 | 192 | 188 | 145 | 147 | 148 | 144 | 145 | 144 | 143 | 140 | 144 | 143 | 140 |
| 12 | 0.071 | 0.070 | 0.071 | 0.070 | 0.069 | 0.067 | 0.067 | 0.070 | 0.068 | 0.068 | 0.066 | | 195 | 187 | 186 | 194 | 190 | 151 | 153 | 154 | 150 | 151 | 150 | 149 | 151 | 150 | 151 | 152 |
| 13 | 0.083 | 0.083 | 0.088 | 0.088 | 0.088 | 0.084 | 0.085 | 0.088 | 0.086 | 0.084 | 0.083 | 0.090 | | 70 | 71 | 190 | 187 | 186 | 188 | 189 | 185 | 186 | 189 | 188 | 180 | 189 | 184 | 180 |
| 14 | 0.085 | 0.084 | 0.089 | 0.089 | 0.088 | 0.087 | 0.088 | 0.086 | 0.084 | 0.088 | 0.085 | 0.086 | 0.030 | | 1 | 184 | 181 | 181 | 183 | 184 | 180 | 181 | 184 | 183 | 181 | 184 | 181 | 181 |
| 15 | 0.085 | 0.085 | 0.090 | 0.089 | 0.089 | 0.087 | 0.088 | 0.086 | 0.085 | 0.088 | 0.085 | 0.085 | 0.031 | 0.000 | | 185 | 182 | 182 | 184 | 185 | 181 | 182 | 185 | 184 | 181 | 185 | 182 | 182 |
| 16 | 0.091 | 0.090 | 0.084 | 0.088 | 0.086 | 0.086 | 0.089 | 0.090 | 0.085 | 0.088 | 0.088 | 0.089 | 0.087 | 0.084 | 0.084 | | 4 | 204 | 206 | 207 | 203 | 204 | 205 | 204 | 202 | 205 | 202 | 202 |
| 17 | 0.089 | 0.088 | 0.082 | 0.086 | 0.084 | 0.084 | 0.087 | 0.088 | 0.083 | 0.086 | 0.086 | 0.087 | 0.085 | 0.082 | 0.083 | 0.002 | | 200 | 202 | 203 | 199 | 200 | 201 | 200 | 198 | 201 | 198 | 198 |
| 18 | 0.019 | 0.018 | 0.040 | 0.040 | 0.039 | 0.037 | 0.039 | 0.040 | 0.036 | 0.067 | 0.065 | 0.068 | 0.085 | 0.082 | 0.083 | 0.094 | 0.092 | | 2 | 3 | 1 | 2 | 7 | 6 | 12 | 7 | 2 | 10 |
| 19 | 0.020 | 0.019 | 0.041 | 0.041 | 0.040 | 0.038 | 0.040 | 0.041 | 0.037 | 0.068 | 0.066 | 0.069 | 0.086 | 0.083 | 0.084 | 0.095 | 0.093 | 0.001 | | 1 | 3 | 4 | 9 | 8 | 14 | 9 | 4 | 12 |
| 20 | 0.021 | 0.019 | 0.042 | 0.041 | 0.040 | 0.038 | 0.041 | 0.041 | 0.037 | 0.068 | 0.066 | 0.069 | 0.086 | 0.084 | 0.084 | 0.095 | 0.093 | 0.001 | 0.000 | | 4 | 5 | 10 | 9 | 15 | 10 | 5 | 13 |
| 21 | 0.019 | 0.018 | 0.040 | 0.039 | 0.039 | 0.036 | 0.039 | 0.039 | 0.035 | 0.066 | 0.065 | 0.068 | 0.084 | 0.082 | 0.082 | 0.093 | 0.091 | 0.000 | 0.001 | 0.002 | | 1 | 6 | 5 | 11 | 6 | 1 | 11 |
| 22 | 0.019 | 0.018 | 0.040 | 0.040 | 0.039 | 0.037 | 0.039 | 0.040 | 0.036 | 0.067 | 0.065 | 0.068 | 0.085 | 0.082 | 0.083 | 0.094 | 0.092 | 0.001 | 0.002 | 0.002 | 0.000 | | 5 | 6 | 12 | 7 | 2 | 12 |
| 23 | 0.019 | 0.018 | 0.040 | 0.039 | 0.039 | 0.036 | 0.039 | 0.039 | 0.035 | 0.067 | 0.065 | 0.068 | 0.086 | 0.084 | 0.084 | 0.094 | 0.092 | 0.003 | 0.004 | 0.004 | 0.003 | 0.002 | | 1 | 11 | 2 | 7 | 11 |
| 24 | 0.018 | 0.017 | 0.039 | 0.039 | 0.038 | 0.036 | 0.039 | 0.038 | 0.035 | 0.067 | 0.064 | 0.067 | 0.086 | 0.083 | 0.084 | 0.094 | 0.092 | 0.003 | 0.003 | 0.004 | 0.002 | 0.003 | 0.000 | | 10 | 1 | 6 | 10 |
| 25 | 0.018 | 0.016 | 0.039 | 0.039 | 0.038 | 0.036 | 0.039 | 0.039 | 0.033 | 0.066 | 0.063 | 0.068 | 0.082 | 0.082 | 0.082 | 0.093 | 0.091 | 0.005 | 0.006 | 0.006 | 0.005 | 0.005 | 0.005 | 0.004 | | 11 | 10 | 2 |
| 26 | 0.019 | 0.018 | 0.040 | 0.039 | 0.039 | 0.036 | 0.039 | 0.039 | 0.035 | 0.067 | 0.065 | 0.068 | 0.086 | 0.084 | 0.084 | 0.094 | 0.092 | 0.003 | 0.004 | 0.004 | 0.003 | 0.003 | 0.001 | 0.000 | 0.005 | | 7 | 11 |
| 27 | 0.018 | 0.017 | 0.039 | 0.039 | 0.038 | 0.036 | 0.039 | 0.039 | 0.035 | 0.066 | 0.064 | 0.068 | 0.084 | 0.082 | 0.083 | 0.093 | 0.091 | 0.001 | 0.002 | 0.002 | 0.000 | 0.001 | 0.003 | 0.003 | 0.004 | 0.003 | | 10 |
| 28 | 0.018 | 0.016 | 0.039 | 0.039 | 0.038 | 0.036 | 0.039 | 0.039 | 0.033 | 0.066 | 0.063 | 0.069 | 0.082 | 0.082 | 0.083 | 0.093 | 0.091 | 0.004 | 0.005 | 0.006 | 0.005 | 0.005 | 0.005 | 0.004 | 0.001 | 0.005 | 0.004 | |

1, DOG8; 2, AND25; 3, BET31; 4, ZAH19; 5, ANK3; 6, JAR11; 7, ANAL4, MER12; 8, RANO45; 9, ISA2.3; 10, RANO25; 11, MERY9; 12, MIT39, MIT40; 13, LOKO4.10, LOKO4.25; 14, LAZA5.02; 15, LAZA5.08; 16, MER16; 17, ANKA3; 18, HABO6.1; 19, HABO6.2; 20, HABO6.3; 21, HABO6.4, HABO6. 9,HABO6.10, MBO6.3, MBO6.4, MBO6.6, MBO6.7; 22, M152; 23, MBO6.2; 24, MBO6.5, MBO6.8, MBO6.9, MBO6.10, MBO6.11, M151; 25, VVEV1, VVEV2, VVEV3, VVEV4, VVEV6, VVEV7, VVEV9, VVEV10; 26, VVEV5; 27, VVEV8; 28, VVEV11.

## DISCUSSION

Our ground surveys confirmed the presence of WCL at the coastal forests south of the Manampatrana River: Manombo and Mahabo. We found no consistent pattern of phenotypic variation between these populations, or between these groups and interior populations, unlike the established brown lemur species and subspecies [Mittermeier et al., 2006b; Shedd & Macedonia, 1991]. These results were expected based on recent research at Manombo [Johnson & Overdorff, 1999; Ratsimbazafy, 2002] and other published observations from Mahabo [Mittermeier et al., 2006b].

Genetic analyses indicate the monotypy of WCL populations. Using multiple mtDNA regions, every analysis yielded identical results: all WCL from two coastal (Manombo and Mahabo) and one interior site (Vevembe) clustered together as a single species, with high bootstrap support and very short genetic distances relative to recognized lemur species and subspecies (Figs. 2–4). Moreover, none of these populations formed a distinct lineage within this species. Specifically, Manombo and Mahabo individuals formed mixed clades. Individuals from the interior site Vevembe could be expected to cluster more exclusively, owing to the relative geographic isolation of this population. Nonetheless, two individuals from this site (VVEV5 and VVEV8) consistently clustered with the coastal populations. One possibility is that these individuals represent recent migrants from the coastal region. Both individuals are adult males (Table I), the sex that commonly disperses in brown lemurs [Overdorff et al., 1999]. However, this is unlikely given the substantial distance between the coastal forests and Vevembe ($>50$ km) and the lack of suitable habitat between these sites (Fig. 1). Thus, we suggest that the lack of distinct geographic clustering among populations from the coast south of the Manampatrana River and the interior forests indicates gene flow across this region before isolation caused by anthropogenic disturbance. Further research with larger sample sizes and additional loci may show additional substructure and biased directionality in gene flow (suggested as east to west here).

The two fragments we surveyed north of the Manampatrana River, Analalava and Sakanany, appeared to be the most likely locations for lemurs to persist in a region severely impacted by habitat loss. They are also very near Salohy, a locality reported for the mounted specimens of what Schwarz [1931] referred to as *E. cinereiceps* (Fig. 1). However, we did not detect *Eulemur* or any other day-active lemur species at these sites. Therefore, it seems that brown lemurs have been extirpated from the coastal plain between Manombo (south of the Manampatrana) and the lowland forests of Vatovavy and Kianjavato, within the range of *E. fulvus rufus* [Mittermeier et al., 2006b; Tattersall, 1982] (although we note our surveys were brief). As a consequence, we were unable to investigate directly whether the Manampatrana River divides distinct forms of WCL on the coastal plain. However, previous studies of interior WCL found shared diagnostic characters between populations found to the south (Vevembe) and to the north (Evendra) of the Manampatrana River [Johnson & Wyner, 2000]. Thus, this river is not a boundary for brown lemur taxa in the eastern escarpment forests.

With the evidence gathered from ground surveys and genetic sampling, we may now evaluate the two scenarios for the separation of the two possible forms of WCL: *E. albocollaris* and *E. cinereiceps*. The first hypothesis suggests an east–west separation, with *E. albocollaris* found in the escarpment rain forests and *E. cinereiceps* found in the littoral and lowland rain forests of the east coast. On the basis of the lack of distinct clustering among individuals from Vevembe (interior) or Manombo/Mahabo (coast), we can provisionally reject this hypothesis.

The second scenario suggests a north–south geographic division at the Manampatrana River. As noted, we are unable to test directly this hypothesis with the data presented here, as northern populations may have been extirpated. We may, however, be able to draw inferences from other evidence. First, we have suggestions of what *E. cinereiceps* would have looked like from the original plates and the Paris specimens [Milne-Edwards & Grandidier, 1890; Schwarz, 1931]. There is also the photograph of the captive female of unknown provenance in Farafangana from 2005 [Mittermeier et al., 2006b]. In describing the female mounted specimens, Groves [2001] noted that the body pelage and muzzle are relatively light in color, and the cheeks are not white (a WCL trait) or red (a collared lemur trait). However, in examining photographs of these specimens [Mittermeier et al., 2006b], we cannot identify traits that suggest important differences from WCL at known extant localities (e.g., Vevembe, Manombo). The body pelage of the museum specimens appears to have faded substantially *postmortem*, so we do not feel coat color (hue) is a valid distinction. Moreover, the gray muzzle is consistent with extant WCL females. Finally, white or red cheeks are traits found only in male WCL or collared lemurs, respectively [Tattersall, 1982], so their absence is expected for female WCL specimens. As for the plate [Milne-Edwards & Grandidier, 1890] and Farafangana captive female [Mittermeier et al., 2006b], again we find no clear differences between the individuals depicted and WCL females observed during our surveys and captures. In other words, all sources seem to depict the same species. However, females are not particularly useful in diagnosis of southeastern brown lemurs as WCL and collared

lemur females are largely indistinguishable [Mittermeier et al., 2006b; Tattersall, 1982].

Patterns of lemur biogeography offer additional lines of evidence. In recent geological history, lemur populations in coastal areas have been subject to severe climatic fluctuations and likely range contractions [Goodman & Ganzhorn, 2004]. Lemur species may have maintained high elevational ranges owing to the relative stability of vegetational zones in mid-high elevation forests [Goodman & Ganzhorn, 2004]. Indeed, there is not a single species that is confined to lowland habitats in eastern Madagascar; all species found in the coastal forests maintain broad ranges that include higher elevation interior forests [Goodman & Ganzhorn, 2004]. Therefore, it seems unlikely that the WCL that occupied the coastal forests north of Farafangana at least until the late 19th century (i.e., the Paris specimens) would be a distinct taxon from WCL still currently found in adjacent forests such as Evendra [Johnson & Wyner, 2000]—particularly in light of the absence of clear pelage differences.

From the available evidence, we may tentatively conclude that *E. cinereiceps* does not exist as a separate taxon from *E. albocollaris*. Moreover, if a distinct brown lemur species once existed in a narrow coastal plain north of the Manampatrana River, this animal would now likely be extinct. This evidence suggests a response to the question posed in the title. However, we believe that the issue of priority in nomenclature for WCL warrants revisiting and *E. cinereiceps* may be retained. As discussed above, the *cinereiceps* designation first appeared as a plate [Milne-Edwards & Grandidier, 1890]. According to the International Commission on Zoological Nomenclature (ICZN), names accompanied only by illustrations may be valid for taxa proposed before 1931 [Article 12.2.7; ICZN, 1999]. Such a precedent can be reversed if the senior name has not been used since 1899 and the junior synonym [in this case, *E. albocollaris*; Rumpler, 1975] has been in common use in the previous 50 years [Article 23.9.1; ICZN, 1999]. However, both Schwarz [1931] and Groves [1974] used *cinereiceps* to refer to what we can infer are WCL by their descriptions of localities and phenotypes, respectively. Accordingly, we conclude that, if indeed WCL represent a single species, priority should be given to the senior synonym, *E. cinereiceps* [Milne-Edwards & Grandidier, 1890].

The reemergence of *E. cinereiceps* as a valid taxon would perhaps be more dramatic if it represented a truly ''new'' species. However, the available evidence suggests that there is a single WCL species, recently connected by gene flow. Our findings also underscore the ''critically endangered'' conservation status of WCL, as they seem to have been extirpated from large areas of their already limited range. Future research should examine more closely the relationships among the presently fragmented populations. In addition, management plans should consider immediate steps to maintain the viability of all remaining individual populations as well as ensuring the connectivity of these populations to preserve genetic diversity.

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