

MORPHOMETRIC VARIATION AND PHYLOGEOGRAPHIC STRUCTURE IN *MACROTARSOMYS BASTARDI* (RODENTIA: NESOMYIDAE), AN ENDEMIC MALAGASY DRY FOREST RODENT

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Recent field and revisionary studies of Madagascar's endemic rodents (Muroidea: Nesomyidae: Nesomyinae) have dramatically improved our understanding of species-level diversity within this group. However, such studies have generally focused on taxa from the island's relatively well-studied eastern humid forests and have relied solely on morphometric comparisons. Herein, we undertake a study of morphometric and genetic variation within *Macrotarsomys bastardi*, a rodent endemic to Madagascar's dry forest habitats. In particular, we evaluate existing subspecific boundaries using comparisons of cranial and external measurements from 84 museum specimens. We then assess phylogeographic structure across the geographic range of the species using sequence variation in the mitochondrial cytochrome-*b* gene from a subset of these specimens. We conclude that there is little basis for recognizing established subspecies, but that molecular data reveal novel patterns of diversity and geographic structure within this species. These results, coupled with emerging patterns of diversity in other endemic Malagasy mammals, suggest that there is underestimated diversity and biogeographic structure within Madagascar's western habitats.

Key words: cranial morphometrics, cytochrome *b*, Madagascar, Muroidea, Nesomyidae, phylogeography, Rodentia

Among the 4 orders of terrestrial mammals endemic to Madagascar, the nesomyine rodents comprise a radiation of 26 recognized species in 9 morphologically distinct genera (Goodman et al., in press). This group has been characterized as relatively species-poor when compared to other island radiations of rodents (Carleton and Goodman 1998); however, new species of nesomyines are being discovered and described at a remarkable rate (Carleton 1994; Carleton and Goodman 1996, 1998, 2007; Carleton et al. 2001; Goodman et al. 2005; Goodman and Soarimalala 2005). In particular, recent surveys have added new species to many nesomyine genera, and systematic revisions of the widespread endemic genus *Eliurus* have more than tripled the number of recognized species in this group of rodents (Carleton and Goodman 2007). Such studies imply that further attention should be directed at other

nesomyine genera, with an eye toward discovering additional species-level diversity. Herein, we examine morphological and molecular variation in the nesomyine *Macrotarsomys bastardi* to evaluate whether defined subspecies might represent full species, and we explore phylogeographic structure within this taxon.

The genus *Macrotarsomys* currently contains 3 recognized species, the small-bodied *M. bastardi* and 2 larger species, *M. ingens* and the recently described *M. petteri* (Goodman and Soarimalala 2005). The genus was originally named by Milne-Edwards and Grandidier (1898) based on a single specimen collected in southwestern Madagascar by M. E. Bastard in 1897 (Milne-Edwards and Grandidier 1898 [Carleton and Schmidt (1990) corrected the published 1857 collection year to 1897]). This specimen became the type for *M. bastardi* and remained the sole record of the genus until C. S. Webb collected several individuals during his travels across the island's xeric regions between 1936 and 1940. Webb's specimens were subsequently deposited at the British Museum, and in 1949 J. R. Ellerman published his observations on Webb's collection as an appendix (Ellerman 1949) to his compendium on rodent

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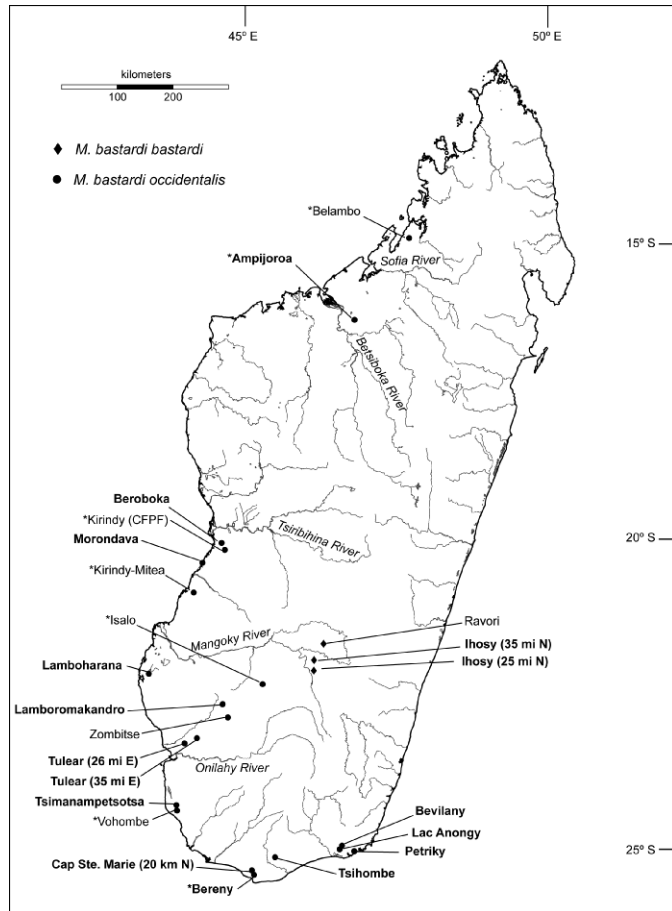


FIG. 1.—Map of Madagascar showing collecting localities of *Macrotarsomys bastardi*. Specimens from localities marked with an asterisk were used in analyses of genetic variation; those in bold have morphometric data. Two localities (Zombitse and Ravori) have associated specimens that were not used in either morphometric or molecular analyses but are shown to reflect known specimen records.

classification (Ellerman 1940, 1941, 1949). Ellerman assigned all the *Macrotarsomys* in this collection to the species *bastardi*, but he named a new subspecies *occidentalis* to encompass those that had larger bullae (“rarely below 6 mm”—Ellerman 1949:158) and longer hind feet (“25 mm and more”—Ellerman 1949:158) than typical of the nominate form. The holotype of *M. b. occidentalis* was designated as a specimen from Beroboka (Fig. 1). Ellerman apparently never examined the type specimen of *M. bastardi* in the Muséum National d’Histoire Naturelle, Paris, but he differentiated *M. b. occidentalis* from *M. b. bastardi* based on the published hind-foot measurement of the latter form (Milne-Edwards and Grandidier 1898).

In recognizing *M. b. occidentalis* as a separate form, Ellerman restricted the geographic range of *M. b. bastardi* to the interior of Madagascar, including the type locality (Ravori) and localities in the vicinity of Ihosy (Fig. 1). Consequently, *occidentalis*, the “larger western race” (Ellerman 1949:147), occurred along the southwestern rim of the island, and included collecting localities near Beroboka, Tulear [=Toliara], Tsihombe, and Bevilany (Fig. 1). Fieldwork undertaken

subsequent to Ellerman’s revision added several new localities to the distributional range of *M. bastardi*. Notably, in the late 1950s, Francis Petter collected a series of specimens of *M. bastardi* from Ampijoroa (Fig. 1), thereby extending the distributional range of this species north of the Tsiribihina River. More recently, additional specimens of *M. bastardi* were collected from Ampijoroa in the late 1990s, and the northernmost record of the species was added from Belambo in 2004 (Fig. 1). In light of these new collections and appeals for critical review of Ellerman’s subspecific categories (Carleton and Goodman 2003; Carleton and Schmidt 1990), we have undertaken a study of morphometric and genetic variation across the known distributional range of *M. bastardi*.

MATERIALS AND METHODS

Ideally, our study would compare traditional morphometric and molecular variation based on data taken from the same individuals. However, many of the specimens of *M. bastardi* that we used for our morphometric comparisons were collected well before tissue samples were routinely preserved for genetic work. Moreover, several of the specimens that we use in our study of molecular variation are juveniles, have damaged skulls, or are otherwise unavailable for morphometric analysis. Importantly, the series of individuals from localities near Ihosy that were assigned to *M. b. bastardi* were not available for molecular analyses, and our taxonomic assessment of this population is restricted to morphological comparisons. Similarly, the northernmost record, from Belambo, is a juvenile and was excluded from the morphometric analyses.

Our morphometric study is based on measurements taken from specimens housed at the following museums: The Natural History Museum, London (formerly British Museum of Natural History; BMNH); Muséum National d’Histoire Naturelle (MNHN), Paris; The Field Museum of Natural History (FMNH), Chicago; and the Département de Biologie Animale, Université d’Antananarivo (UADBA), Antananarivo. Tissue samples used in the molecular analysis and the associated voucher specimens are housed at FMNH and UADBA. Museum catalog numbers and descriptions of collecting localities for all specimens are given in Appendix I. Collecting localities were mapped based on field coordinates, information from museum records, or extrapolated from the gazetteer published by Carleton and Schmidt (1990).

External measurements were taken directly from specimen tags or field catalogs. We measured 13 of the 18 cranial and dental features described in Carleton (1994), and we added 2 measurements from the mandibular tooththrow (see Appendix II). In total, we included 84 adult specimens of *M. bastardi* from 16 localities (Fig. 1). All measurements were taken with a pair of digital calipers accurate to the nearest 0.1 mm. Descriptive statistics (mean, standard deviation, and observed range) were calculated for all specimens.

Statistical analyses of ln-transformed cranial measurements were performed using R version 2.2.1 (R Development Core Team 2005) on the subset of specimens that had a complete set of all 15 cranial measurements ($n = 54$). We assessed whether

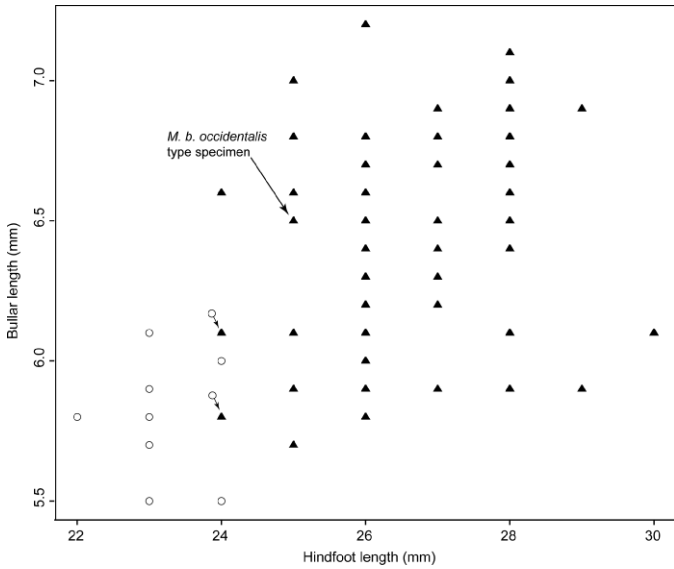


FIG. 2.—Scatterplot of hind-foot and bullar measurements for all specimens of *Macrotarsomys bastardi*. Open circles are specimens referred to *M. b. bastardi* and solid triangles to *M. b. occidentalis*.

the ln-transformed data met the assumption of multivariate normality using univariate normal quantile–quantile plots and a plot of generalized distances of each observation from the mean vector of the data versus a chi-square expectation (Everitt 2005). The statistical significance of differences due to sex was tested using Hotelling’s T^2 (Hotelling 1931), after qualitative examination of the variance–covariance matrices of males and females for uniformity. Both unsupervised (principal component) and supervised (linear discriminant function) analyses were used to assess group distinctiveness (Venables and Ripley 1999). Principal components were extracted from the variable correlation matrix by singular value decomposition, and individuals were clustered by Euclidean distances among them using arithmetic averages (unweighted pair-group method using arithmetic averages [UPGMA]—Sokal and Sneath 1963). In addition, a linear discriminant function analysis (as implemented in *lda*—Venables and Ripley 1999) was used to assess if the 2 described subspecies of *M. bastardi* (*bastardi* and *occidentalis*) were diagnosable based on morphometric comparisons. In this analysis, specimens from Ihsosy were designated a priori as *M. b. bastardi*, and all others *M. b. occidentalis*. Because so few specimens from Ihsosy were available, the data were not split into testing and training subsets. Instead, we calculated classification error using both the “plug-in” estimate with the observed class frequencies as priors (Venables and Ripley 1999:346–347) and a cross-validation estimate generated by delete-one jackknifing (Everitt 2005).

For the molecular data set, DNA was extracted from 8 specimens using a DNeasy extraction kit (Qiagen Inc., Valencia, California). These specimens included all available tissue (heart, liver, or kidney) samples and 2 recently collected prepared skins. The tissue samples yielded high-molecular-weight DNA from which the entire cytochrome-*b* (*Cytb*) gene was amplified using primers Mac05 (5’ACCATCGTTGTAA

TABLE 1.—Variable coefficients, eigenvalues, and percentage variance explained by the first 3 components of a principal component analysis (PC1–PC3) and coefficients of linear discriminant function analysis (LD1) performed on 15 ln-transformed cranial measurements from adult *Macrotarsomys bastardi* ($n = 54$). Measurement abbreviations are defined in Appendix II.

	PC1	PC2	PC3	LD1
DAB	0.319	−0.111	0.001	8.638
ONL	0.362	−0.141	−0.049	21.774
BOC	0.306	0.101	0.111	12.142
ZB	0.333	−0.137	0.107	0.370
IOB	0.045	0.300	0.551	10.737
BR	0.064	0.257	0.624	−22.310
LR	0.303	−0.034	−0.206	1.039
LIF	0.299	−0.130	0.146	−0.862
PPL	0.319	−0.212	−0.052	−12.527
BIF	0.262	−0.171	0.022	2.631
LD	0.289	−0.145	0.135	−1.958
LM ^{1–3}	0.226	0.405	−0.055	−10.834
WM ¹	0.143	0.486	−0.177	−3.305
LM _{1–3}	0.186	0.361	−0.361	−2.238
WM ₁	0.124	0.374	−0.178	1.651
Eigenvalue	6.833	1.937	1.283	
% variance	45.6	12.9	8.6	

TTCAACTATA) and Mac04 (5’CAAGACCAGGGTAATA TTTATACTA). To generate fragments of a suitable size for sequencing, this polymerase chain reaction product was used as a template in 2 subsequent reamplification reactions, 1 using primer Mac05 paired with Mac12 (5’GGTCTCCTAATA GGTCTG) and 1 using Mac13 (5’CGCAGCCCTAGCTA TAGT) paired with Mac04. Field-preserved tissues were not available from the 2 specimens from Bereny; therefore, we extracted relatively poor-quality DNA from dried museum skins of these 2 specimens. From these, we amplified the first 915 base pairs (bp) of the *Cytb* gene as a series of 3 overlapping fragments (with 35–70 bp of overlap between them) using primers Mac05 paired with Mac350R (5’TGCCRATATTTCAKG TTTCTA), Mac250F (5’GTAAACTACGGATGACTMATCC) paired with Mac700R (5’AGTCCTGAYGGGTTGTTGG), and Mac13 paired with Mac1000R (5’CGGAATATTARGCRRG GTTGTT). Sequences from the 2 specimens from Bereny were identical to each other, but none of the smaller fragments we generated from these specimens was identical to any other DNA fragment sequenced in our laboratory. Therefore, cross-contamination of these degraded samples by higher-quality DNA can effectively be ruled out. All polymerase chain reactions were performed using reaction conditions as described in Jansa et al. (2006). All fragments were sequenced in both directions, and resulting sequences have been deposited in GenBank (accession numbers EU497642–EU497648).

The resulting *Cytb* sequences were aligned by eye with reference to translated amino acid sequences. We included additional sequences from *Monticolomys koopmani* (GenBank accession numbers AF160580–AF160583) and *Hypogeomys antimena* (GenBank accession number AF160578) as out-groups to root our phylogenetic tree (Jansa and Weksler 2004). Phylogenetic analyses were performed using both maximum

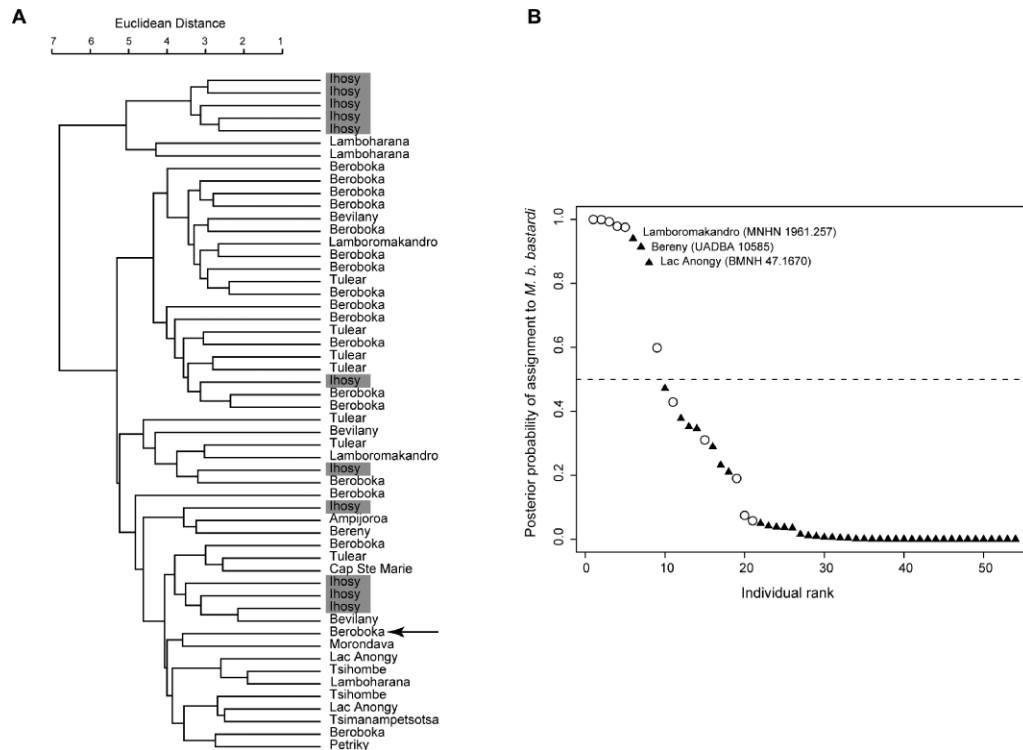


FIG. 3.—A) Results of a UPGMA analysis of Euclidean distances calculated in principal components space. Terminals are labeled by geographic locality; specimens from Ihosy (shaded) represent those currently assigned to *Macrotarsomys bastardi bastardi*; the holotype of *M. bastardi occidentalis* is indicated with an arrow. The scale bar indicates relative distance in the multivariate space defined by the principal components. B) Posterior probability of assignment to *M. b. bastardi* given prior group assignments from a linear discriminant function analysis (open circles and solid triangles indicate prior assignment to *M. b. bastardi* or *M. b. occidentalis*, respectively). The dashed line indicates assignment to either group with equal probability; points above this line have higher probability of assignment to *M. b. bastardi*. Individuals of *M. b. occidentalis* assigned to *M. b. bastardi* (posterior probability >0.5) are labeled with locality and specimen number.

parsimony and maximum likelihood as implemented in PAUP* version 4.0b10 (Swofford 2002). Tree searches using maximum parsimony were performed using the branch-and-bound algorithm. For maximum-likelihood analyses, we first identified the best-fit model of nucleotide substitution using the Akaike information criterion as employed in ModelTest version 3.6 (Posada and Crandall 1998). We also evaluated whether a molecular clock fit our data using a hierarchical log-likelihood ratio test. Parameters for the resulting best-fit model were fixed in a heuristic search using 10 replicates of random taxon addition and tree-bisection-reconnection (TBR) branch swapping. Nodal support was calculated for both maximum-parsimony and maximum-likelihood analyses using nonparametric bootstrapping (Felsenstein 1985). All bootstrap analyses employed 1,000 pseudoreplicates analyzed with heuristic searches as above. Polymorphism and divergence statistics were calculated using DnaSP version 4.10 (Rozas et al. 2003). In addition, we report maximum-likelihood-corrected (GTR+ Γ +clock) divergence values as calculated using PAUP*.

RESULTS

Macrotarsomys bastardi has been collected in a variety of xeric habitats from dry deciduous forest in northwestern Madagascar to spiny bush formations in the extreme south

and appears to be exclusively terrestrial. Several of the recently collected specimens are from sites in degraded natural forest. The majority of specimen records are from elevations below 150 m, although this species has been trapped in the inland, higher-elevation sites of Isalo (approximately 650 m above sea level), Zombitse (870 m above sea level), and near Ihosy (approximately 950 m above sea level).

Morphometric results.—Descriptive statistics for the 84 adult specimens revealed few obvious differences in either external or cranial measurements between specimens assigned to *M. b. bastardi* or to *M. b. occidentalis* (Appendix II). Our results confirm Ellerman's (1949) observation that most specimens of the more highland *M. b. bastardi* have smaller bullae and hind feet than those of *M. bastardi occidentalis*. However, a scatterplot of these 2 dimensions for our more extensive sample of individuals revealed overlap between the 2 designated subspecies in these measurements (Fig. 2).

Of the 54 specimens with complete cranial measurements, 24 were female, 25 were male, and 5 were of indeterminate sex. Multivariate comparison of the specimens for which sex was known revealed no statistically significant difference between males and females in cranial measurements (Hotelling's $T^2 = 0.42$, $d.f. = 1$, $P = 0.14$); therefore, we pooled these 54 samples for subsequent statistical analyses. We conducted a principal component analysis using the 15 ln-transformed

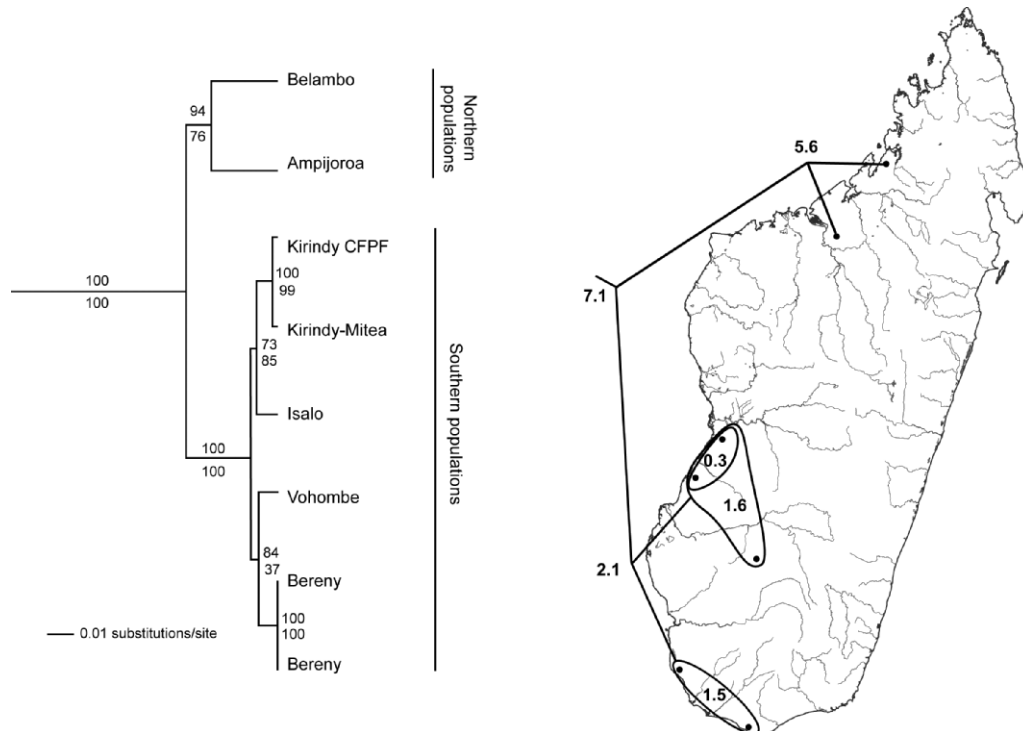


FIG. 4.—A) The maximum-likelihood tree inferred from the best-fit model of nucleotide substitution for the cytochrome-*b* data (GTR+ Γ +clock; $-\ln L = 3,471.70$). This tree differs from the single minimum-length parsimony tree (length = 418, consistency index [CI] = 0.823, retention index [RI] = 0.863) only in the placement of the specimen from Vohombe (see text). Numbers at nodes refer to maximum-likelihood bootstrap support (top) and maximum-parsimony bootstrap support (bottom). B) The tree from part A superimposed on a map of Madagascar. Numbers indicate the maximum-likelihood-corrected average percent sequence divergence within (circumscribed points) or between (nodes) groups.

cranial measurements from adults of *M. b. occidentalis* ($n = 43$) and *M. b. bastardi* ($n = 11$). The first 3 principal components had eigenvalues greater than 1 and together captured 67% of the total variance (Table 1). All variables had positive coefficients for the 1st principal component axis, which accounted for 45.6% of the total variance, suggesting that much of the variation in cranial measurements involves size. Scatterplots of individuals on the first 3 principal component axes did not reveal a clear separation between *M. b. occidentalis* and *M. b. bastardi* (not illustrated). Furthermore, UPGMA clustering of Euclidean distances between individuals on the principal component axes did not show any separation between the 2 subspecies (Fig. 3A).

To further assess whether individuals could be diagnosed based on cranial measurements, we conducted a linear discriminant function analysis with subspecies membership assigned a priori (discriminant function coefficients are given in Table 1). The misclassification rate based on the plug-in error estimate was 1 of 54 (2%); however, this method provides a best-case estimate of the true misclassification rate. A more realistic estimate provided by cross-validation suggested a misclassification rate of 8 of 54 (15%). Based on posterior probabilities of group assignment from this estimate, 5 of 11 (45%) individuals designated as *M. b. bastardi* were misclassified as *M. b. occidentalis*, and 3 of 43 (7%) of *M. b. occidentalis* were misclassified as *M. b. bastardi*. An additional 8 specimens had low posterior probability (<90%) of correct group assignment

(Fig. 3B). Therefore, even a trained analysis that attempts to sort individuals based on craniodental measurements when given prior information on subspecies designation failed to do so.

Molecular results.—We generated complete *Cytb* sequences (1,143 bp) from the 6 individuals with tissue samples and partial sequence (915 bp) from the 2 skin samples. There were 7 unique haplotypes among these 8 specimens (the 2 specimens from Bereny were identical) and nucleotide diversity (π) among these samples was 0.033. Phylogenetic analysis under both maximum-parsimony and maximum-likelihood criteria revealed 2 major clades: a northern clade containing samples from Ampijoroa and Belambo, and a southern clade comprising all other specimens (Fig. 4A). Nucleotide diversity within the northern clade was higher than within the southern clade ($\pi_{\text{north}} = 0.046$, $\pi_{\text{south}} = 0.016$), and average uncorrected divergence (p) between these 2 clades was 5.3% (corrected $p_{\text{GTR}+\Gamma+\text{clock}} = 7.1\%$). Trees resulting from maximum-parsimony and maximum-likelihood analyses differed only in the placement of the specimen from Vohombe: maximum-parsimony analysis showed this specimen as the basal-most member of the southern clade, whereas maximum-likelihood analysis placed it as sister to the population from Bereny. Although there is reasonably high bootstrap support for the arrangement recovered under maximum-likelihood analysis (84%), the alternative maximum-parsimony resolution received only negligible support (55%). We base our biogeographic analyses on the maximum-likelihood tree and note that the

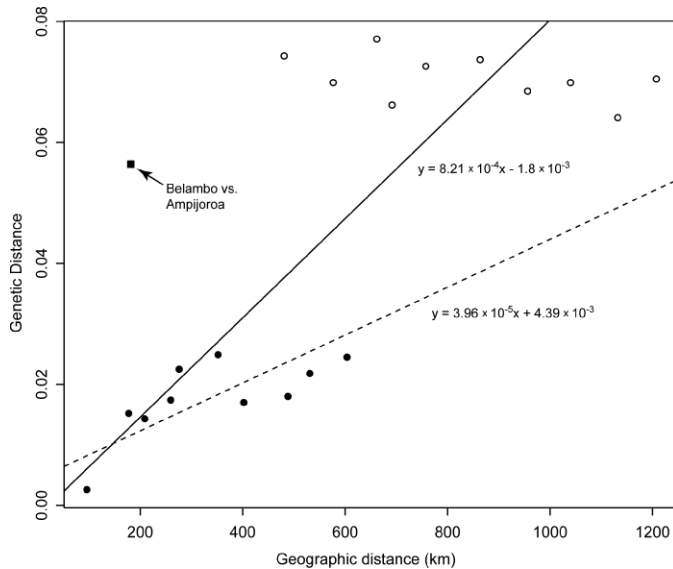


FIG. 5.—Scatterplot of maximum-likelihood-corrected (GTR+ Γ +clock) cytochrome-*b* distances versus geographic distances for pairwise comparisons among populations of *Macrotarsomys bastardi*. Solid dots represent comparisons among southern populations; open circles represent comparisons between northern and southern populations; the square represents the comparison between the 2 northernmost populations. Solid and dotted lines represent the best-fit reduced major axis regression line for all samples of *M. bastardi* ($r^2 = 0.53$) and for southern populations only ($r^2 = 0.51$), respectively.

difference between the maximum-likelihood and maximum-parsimony trees does not affect our principal conclusions.

Inspection of this tree superimposed on a map of Madagascar suggests that genetic divergence within *M. bastardi* may follow a simple isolation-by-distance model (Fig. 4B). To further test this hypothesis, we assessed whether genetic distance was positively correlated with geographic distance using reduced major axis regression and Mantel tests as implemented in IBD version 1.52 (Bohonak 2002). Genetic and geographic distances across all samples of *M. bastardi* were strongly positively correlated (Mantel test: $r = 0.727$, $P = 0.013$). However, 2 observations suggest that genetic divergence among these populations does not strictly follow an isolation-by-distance model. First, the best-fit reduced major axis regression line for all samples has a steeper slope than that for the southern populations alone (Fig. 5), suggesting that there is greater genetic divergence between the northern and southern clades than expected given their geographic separation. Second, genetic divergence between the 2 northern localities (Belambo and Ampijoroa) is notably higher ($p_{\text{uncorr}} = 4.6\%$; $p_{\text{GTR}+\Gamma+\text{clock}} = 5.6\%$) than expected given their relative proximity (Fig. 5).

DISCUSSION

When Ellerman (1949) recognized 2 distinct subspecies of *M. bastardi* based on the Webb collections, he established 2 biogeographically disjunct units: 1 restricted to midelevation

inland locales (the type locality of Ravori and those in the vicinity of Ihosy) and 1 distributed more broadly throughout southwestern Madagascar (Fig. 1). Although Ellerman (1949) noted morphometric differences in hind-foot and bullar measurements between the 2 subspecies, our analysis (which included considerably more specimens than were available to Ellerman) fails to substantiate these 2 groups based on these measurements (Fig. 2). Furthermore, we find no consistent differences between the 2 subspecies in cranial measurements (Fig. 3). We therefore suggest that subspecific epithets be abandoned for this species, because they do not appear to diagnose any meaningful biological entities. It is still possible that the inland “race” of *M. bastardi* represents a morphologically cryptic species, but at this stage we lack the necessary molecular data to test this hypothesis.

In comparison, examination of our molecular data provides a new perspective on geographic variation within *M. bastardi*. Although geographic sampling is limited, our analyses suggest that there are 2 major phylogeographic breaks within *M. bastardi* that cannot be explained under a simple isolation-by-distance model (Fig. 5). The 1st break occurs between northern and southern haplogroups (Fig. 4), and is characterized by relatively high genetic divergence (maximum-likelihood-corrected divergence = 7.1%). This fundamental division between northern and southern Madagascar is reminiscent of a pattern found for mouse lemurs of the genus *Microcebus* (Yoder et al. 2000) but differs in at least 1 important aspect. Each of the northern and the southern clades of *Microcebus* contains individuals from both eastern humid and western dry forests, suggesting that their phylogeography does not track the east-west/humid-dry ecogeography of Madagascar. In contrast, *M. bastardi* is restricted to western dry forests below 1,000 m elevation and is unknown from humid forest formations. A similar pattern of habitat specificity occurs among other nesomyine species, and suggests that nesomyines may not be as ecologically labile as mouse lemurs appear to be. Determining the role that such habitat fidelity has played in nesomyine diversification requires detailed phylogenies at multiple hierarchical levels, and such studies are one focus of ongoing research on this group.

The 2nd phylogeographic break in *M. bastardi* is characterized by unexpectedly high levels of divergence between the northernmost locales of Ampijoroa and Belambo (Fig. 4). This split is notably deeper than those among southern samples separated by comparable or greater geographic distances (Fig. 5), and is consistent with at least 2 possible explanations. First, our 2 samples from northwestern Madagascar may represent a single population that is older or larger than southern populations and therefore contains higher genetic diversity. Alternatively, these 2 localities may represent discrete populations that are separated by a past or present biogeographic barrier. Distinguishing between these 2 explanations given the present extent of geographic sampling of *M. bastardi* is difficult. However, rivers have been identified as barriers to gene flow for mouse lemur species (Olivieri et al. 2007) and also may be relevant for nesomyine species.

Given that *M. bastardi* occurs in some of the driest habitat on the island and has a largely lowland distribution, it can be hypothesized that rivers (and their associated riparian habitats) might form biogeographic barriers for this taxon. Across the known distribution of *M. bastardi*, there are several rivers with considerable discharge (Chaperon et al. 1993) that drain toward the west from the central highlands into the Mozambique Channel (Fig. 1). These rivers include, from north to south, the Sofia, which runs between the 2 sites included in the northern clade (Belambo and Ampijoroa); the Betsiboka, which lies just south of localities included in the northern clade; the Tsiribihina, occurring just north of localities in the southern clade; the Mangoky, which traverses through localities in the southern clade and has tributaries near the Ihosy region; and the Onilahy, which also runs through locations assigned to the southern clade (Fig. 1). On the basis of our molecular analyses, there is no evidence that the Onilahy and Mangoky rivers form dispersal barriers for *M. bastardi*, whereas the positions of the Tsiribihina, Betsiboka, and Sofia rivers are associated with notable genetic breaks in this taxon. Clearly, rivers may form barriers to gene flow in some taxa but not others, depending on a number of factors including relative dispersal abilities, elevational ranges, behavioral characteristics, and stochastic events to name a few (Gascon et al. 2000; Goodman and Ganzhorn 2004; Wilmé et al. 2006). Additional samples of *M. bastardi* and other nesomyine species from throughout western Madagascar will allow further tests of the generality of the riverine barrier hypotheses that are emerging as explanations for phylogeographic patterns in Madagascar's endemic fauna.

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- Macrotarsomys bastardi occidentalis*.—Province de Toliara: 5 miles E Bevilany, –25.01, 46.61 (BMNH 47.1659–47.1663, 47.1666); Lake Anongy [=Anony] area, –25.05, 46.58 (BMNH 47.1669–47.1671); Tsihombe, –25.19, 45.49 (BMNH 47.1699, 47.1700, 47.1703, 47.1704); 26 miles E Tulear [=Toliara], –23.28, 43.98 (BMNH 47.1682–47.1686, 47.1688, 47.1689); 35 miles E Tulear [=Toliara], –23.2, 44.2 (BMNH 47.1697, 47.1698); Kirindy-Mitea, 13 km W Marofihitsa, 30 m, –20.79, 44.15 (FMNH 176118); 20 km N Cap Sainte Marie, –25.48, 45.14 (MNHN 1961.272); Lamboromakandro, Forêt de Sakaraha, –22.65, 44.61 (MNHN 1961.255–1961.257); Lamboharana ou Itampolove, –22.12, 43.39 (MNHN 1912.134, 1957.787–1957.790); Bereny, 14 km N Cap Sainte Marie, –25.35, 45.14 (UADBA 10584, UADBA 10585); Parc National de Tsimanampetsotsa, –24.33, 43.85 (UADBA 19121); Petriky, –25.1, 46.82 (UADBA 46779).
- Province de Fianarantsoa: Parc National de l'Isalo, 28 km SE Berenty-Betsileo, 650 m, –22.32, 45.29 (UADBA 11575).
- Province de Mahajanga: Beroboka, 40 km N Morondava, 7 km from sea, –19.97, 44.61 (BMNH 47.1673–47.1675, 47.1677 [holotype]–47.1681; MNHN 1961.223–1961.228, 1961.230, 1961.235, 1961.238, 1961.239, 1961.243, 1961.244, 1961.248–1961.251, 1961.273–1961.276); Station Forestière d'Ampijoroa, 40 km S Marovoay, Ankarafantsika Reserve, –16.25, 46.8 (MNHN 1961.258, 1961.260–1961.262); Morondava, –20.28, 44.28 (MNHN 1973.520, 1980.291).

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APPENDIX I

Museum catalog numbers and localities for specimens of *Macrotarsomys bastardi* used in this study. Numbers following locality descriptors are geographic coordinates in decimal degree format. See text for museum acronyms.

Specimens Used in Morphometric Analyses

Macrotarsomys bastardi bastardi.—Province de Fianarantsoa: 35 miles N Ihosy, –21.91, 46.14 (BMNH 47.1626, 47.1627, 47.1629, 47.1630, 47.1632–47.1634, 47.1636, 47.1638, 47.1640, 47.1641, 47.1643, 47.1644, 47.1646); about 25 miles N Ihosy, –22.08, 46.14 (BMNH 47.1647).

Samples Used in Molecular Analysis

Macrotarsomys bastardi occidentalis.—Province de Toliara: Belambo, 7.5 km NE of Ambarijebly village, Analalava, 150 m, –14.89, 47.73 (UADBA 47006); Vohombe, 4 km NE of Vohombe village, 80 m, –24.40, 43.85 (UADBA 47007); Kirindy-Mitea, 13 km W Marofihitsa, 30 m, –20.79, 44.15 (FMNH 176118); Bereny, 14 km N Cap Sainte Marie, 30 m, –25.48, 45.15 (UADBA 10584, UADBA 10585).

Province de Fianarantsoa: Parc National de l'Isalo, 28 km SE Berenty-Betsileo, 650 m, –22.32, 45.29 (catalog number pending at UADBA [collector number FH28]).

Province de Mahajanga: Ampijoroa, Forêt d'Ankarafantsika, 160 m, –16.25, 46.80 (catalog number pending at UADBA); Kirindy (CFPF) Forest, 30 m, –20.07, 44.65 (no voucher, [Simone Summer 55]).

APPENDIX II

Summary statistics (mean \pm SD, observed range, and sample size) for morphological measurements between the subspecies of *Macrotarsomys bastardi* based on the classification of Ellerman (1949).

	<i>M. b.</i> <i>occidentalis</i>	<i>M. b.</i> <i>bastardi</i>
Length head + body	89.0 \pm 6.8 68.0–101.0 64	84.7 \pm 2.8 78.0–88.0 14
Tail length	130.9 \pm 10.0 99.0–150.0 63	114.7 \pm 5.5 104.0–123.0 14
Hind-foot length	26.6 \pm 1.3 23.0–30.0 66	23.3 \pm 0.6 22.0–24.0 14
Ear length	22.3 \pm 2.5 10.0–26.0 63	21.4 \pm 1.2 20.0–23.0 14
Depth of auditory bulla (DAB) ^a	6.5 \pm 0.4 5.7–7.2 60	5.8 \pm 0.2 5.5–6.1 14
Occipitonasal length (ONL) ^a	27.9 \pm 1.1 24.4–30.2 60	26.3 \pm 1.0 25.1–28.2 14
Breadth of occipital condyles (BOC) ^a	6.1 \pm 0.3 5.5–6.7 58	5.8 \pm 0.3 5.5–6.4 14
Zygomatic breadth (ZB) ^a	14.2 \pm 0.6 12.7–15.9 60	13.4 \pm 0.5 12.9–14.3 11
Interorbital breadth (IOB) ^a	4.5 \pm 0.2 3.9–5.0 65	4.4 \pm 0.1 4.2–4.6 14
Breadth of rostrum (BR) ^a	12.0 \pm 0.4 11.3–12.9 62	12.3 \pm 0.3 11.8–12.8 14
Length of rostrum (LR) ^a	9.7 \pm 0.5 8.4–11.3 64	9.1 \pm 0.6 8.2–10.0 14
Length of incisive foramina (LIF) ^a	4.4 \pm 0.4 3.5–5.5 65	4.1 \pm 0.2 3.7–4.5 14
Postpalatal length (PPL) ^a	9.4 \pm 0.6 8.1–10.4 54	8.9 \pm 0.4 8.3–9.7 13
Breadth of incisive foramina (BIF) ^a	1.5 \pm 0.2 1.0–1.9 62	1.2 \pm 0.1 1.0–1.4 14
Length of diastema (LD) ^a	6.9 \pm 0.5 5.2–7.8 67	6.5 \pm 0.4 5.8–7.1 14
Length of maxillary tooththrow (LM ^{1–3}) ^a	3.9 \pm 0.2 3.5–4.3 66	3.9 \pm 0.2 3.6–4.2 14
Width of upper 1st molar (WM ¹) ^a	1.1 \pm 0.1 1.0–1.3 67	1.1 \pm 0.1 1.0–1.3 14
Length of mandibular tooththrow (LM _{1–3}) ^b	3.8 \pm 0.2 3.4–4.2 66	3.7 \pm 0.1 3.6–4.0 14
Width of lower 1st molar (WM ₁) ^c	1.0 \pm 0.1 0.9–1.2 67	1.0 \pm 0.1 1.0–1.1 14

^a Measurements described in Carleton (1994).

^b The coronal length of the mandibular tooththrow recorded from the anterior border of the 1st lower molar to the posterior margin of the 3rd.

^c The crown width of the lower 1st molar across its middle lamina.