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# Illumination of cryptic species boundaries in long-tailed shrew tenrecs (Mammalia: Tenrecidae; *Microgale*), with new insights into geographic variation and distributional constraints

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The increasing use of mitochondrial DNA (mtDNA) to explore and test species limits among morphologically similar species is potentially compromised by phenomena poorly reflective of organismal history and speciation, including (but not limited to) stochastic lineage sorting and gene flow. In situations where molecular data are only available from a single gene or linkage partition (e.g. mtDNA), corroboration of suspected species boundaries should be sought from independent lines of evidence, such as morphology. Recent attempts to delimit species using mtDNA and morphology have either implicitly or explicitly ignored the possibility that distinct species can occur in direct sympatry throughout much of their range, presumably because such situations are believed to be rare. We examined phylogenetic relationships within the long-tailed shrew tenrecs (Mammalia: Tenrecidae; *Microgale* spp.) from Madagascar. Current taxonomy recognizes two broadly sympatric species, though as many as six have been described. Given that alpha taxonomy within shrew tenrecs has been controversial, and that patterns of morphological variation can be especially difficult to assess for this group, some authors have suggested that additional cryptic species may exist. To examine this possibility, we conducted a phylogenetic study using the mitochondrial NADH dehydrogenase subunit 2 gene and a morphometric analysis of 29 craniodental, postcranial, and external measurements from a broad geographical sample of long-tailed shrew tenrecs. The two data sets were nearly perfectly congruent in identifying four groups that can be classified as species, thereby doubling the currently recognized number of species. We present previously unrecognized distributional evidence consistent with our conclusions and provide an empirical example of how a revised understanding of species limits alters inferences of geographic variation and species coexistence, particularly with respect to fine-scale habitat partitioning. The results of this study suggest that certain species pairs, previously assumed to be single species occupying broad elevational ranges, are actually reproductively isolated units that are partitioning their environment along elevational lines.  *Biological Journal of the Linnean Society*, 2004, **83**, 1–22.

ADDITIONAL KEYWORDS: Madagascar – mitochondrial DNA – morphometry – phylogeography – species limits.

## INTRODUCTION

Madagascar is renowned for its high levels of faunal endemism (Mittermeier & Mittermeier, 1997; Myers *et al*., 2000), yet new species (e.g. Rasoloarison, Goodman & Ganzhorn, 2000) and ecological zones (e.g. Goodman *et al*., 1996a) are being described at an ever-increasing rate. This refinement of taxonomic classification and ecological categories is not simply an academic exercise. The proper understanding of evolutionary and historical units is crucial for determining conservation priorities and strategies in this and any other threatened environment. Thus, among many other reasons, informed delineation of species

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boundaries can and should have real world impact. Unfortunately, however, the definition of a species remains one of the most contentious issues in biology (e.g. Otte & Endler, 1989; Hey, 2001; Pigliucci, 2003). Of the more than 20 species concepts described to date (Mayden, 1997), none has met with universal acceptance. The difficulties relate to a range of issues from biological, to conceptual, to operational. Among the various species concepts, morphological distinctiveness remains one of the most operationally feasible and hence widely applied criterion proposed for species recognition, particularly for field applications. One shortcoming of the morphological criterion, however, is the potential for 'cryptic' species, i.e. the existence of distinct species, as recognized by historical or biological criteria, for which no discriminating morphological characters have been identified. Such situations can result in the underestimation of species richness and, consequently, the overestimation of intraspecific variability and dispersal abilities.

This problem is not unique to adherents of morphology-based species concepts. The ability to differentiate species morphologically, regardless of the conceptual framework, is desirable to the practitioners of multiple biological subdisciplines (and necessary for many). A number of studies have demonstrated the utility of mitochondrial DNA (mtDNA) sequence variation in illuminating potential species boundaries between morphologically similar or non-differentiable taxa (Avise, 2000). A major problem with this approach is that processes unrelated to speciation (e.g. deep coalescence) may be responsible for patterns such as reciprocal monophyly of mtDNA haplotype lineages within a species as well as for patterns of non-monophyly in reproductively isolated taxa (Avise *et al*., 1983; Maddison, 1997). Ideally, taxonomic delineations between closely related species suggested by mtDNA variation should be considered hypotheses to be tested with additional data, whether genotypic or phenotypic (e.g. Ballard, Chernoff & James, 2002).

Given the complexity of the species problem, and the fact that our primary focus is the empirical investigation of the evolutionary history and biogeography of a specific group of organisms, we will not belabour the conceptual and theoretical issues relating to species recognition. Nonetheless, as we are attempting to recognize meaningful evolutionary and biological boundaries within long-tailed shrew tenrecs, a brief statement of our philosophical framework for this investigation is warranted. A restatement of the 'general lineage concept' of species (de Queiroz, 1998) best expresses our view of the problem, just as it justifies the operational grounds for recognizing the species-level taxonomy proposed in this study. This articulation simultaneously recognizes the complexity of the problem while providing a conceptual framework for unifying an approach for identifying species in nature. In short, de Queiroz (1998: 64) accomplishes this by defining species as an outcome of the speciation process. 'At some point during divergence, the lineages cross a threshold beyond which their separation becomes irreversible: they can no longer fuse.' Although he goes on to show that species can defencibly be recognized at a variety of points along this process, prior to the endpoint, we prefer to take a conservative approach and recognize as species only those lineages that have passed this point of no return. Our operational criteria for doing so include assessments of reproductive isolation (as inferred from sympatric haplotype clades and the ability to differentiate their members morphologically) and obvious periods of evolutionary isolation (as inferred through branch length comparisons within the mtDNA phylogeny).

## Organismal background

Shrew tenrecs (Tenrecidae, Oryzorictinae, genus *Microgale*) comprise the most speciose genus of mammal on Madagascar (Jenkins, 2003). All are endemic to the island, and most of the 18 currently recognized species are broadly distributed along the eastern forests of Madagascar and are readily collected when appropriate techniques are employed (Raxworthy & Nussbaum, 1994; Goodman, Raxworthy & Jenkins, 1996b). Distributional data for shrew tenrecs are being increasingly utilized in studies investigating both temporal (Goodman *et al*., 1997) and latitudinal (Lees, Kremen & Andriamampianina, 1999) variation in species richness and the effects of habitat fragmentation on community structure (Goodman & Rakotondravony, 2000). As a consequence of this research activity, the alpha taxonomy within the genus has undergone substantial change in recent years. Since MacPhee's (1987) revision of the genus, in which 12 of the 22 previously described forms were subsumed, increased field collecting and the concomitant growth of comparative material in museum collections has resulted in both the discovery of new species and the resurrection of others from synonymy (e.g. Jenkins, 1992; Jenkins, Raxworthy & Nussbaum, 1997; Jenkins & Goodman, 1999). A number of confounding factors make species delimitation difficult in shrew tenrecs. First, like many closely related small mammals, shrew tenrec species are typically distinguished from one another based on variation in one or more linear measurements of the skull and/or external body, with few differences observed in qualitative characters (Olson, 1999; Jenkins, 2003). Furthermore, the use of quantitative continuous linear measurements for delimiting species of shrew tenrec is often severely compromised by the difficulty in discriminating adults

from juveniles (MacPhee, 1987). Finally, although the effect of latitude on species richness in shrew tenrecs (and other taxa) has been studied (Lees *et al*., 1999), a similar effect on morphological variation has not been investigated. Ecogeographic variation in body size has been well documented in similarly distributed Malagasy primates (Albrecht, Jenkins & Godfrey, 1990), suggesting that comparable phenomena may be present in tenrecs. High levels of intraspecific variability were cited by MacPhee (1987) in his decision to synonymize numerous nominal species of shrew tenrec, yet many of these have since been resurrected. The potential influence of unrecognized geographic variation on attempts to identify morphological species boundaries suggests that future efforts must take intraspecific geographic variation into account (e.g. Puorto *et al*., 2001).

Nowhere are these issues and their confounding influence on taxonomy better illustrated than in the long-tailed shrew tenrecs, so named for their inordinately long tails, which can exceed twice their head and body length (e.g. Goodman & Jenkins, 1998). Long-tailed shrew tenrecs are currently divided into two species, though as many as six have been described (Table 1). Goodman & Jenkins (1998) recently suggested the occurrence of a bimodal body size distribution in the lesser long-tailed shrew tenrec (*Microgale longicaudata*), with smaller forms conforming to Thomas's (1918) original description of *M. majori* as a diminutive counterpart of the former. Unfortunately, as is frequently the case with shrew tenrecs, sample sizes for adult specimens from the localities presented were insufficient to assess this potential revision based on morphology alone. *Microgale longicaudata* is the widest ranging member of the genus, known to occupy the drier western forests of Madagascar in addition to the humid eastern forests to which most shrew tenrecs are confined (Ade, 1996; S. M. Goodman, unpubl. specimens in Field Museum of Natural History). For this reason, *M. longicaudata* is believed to be exceptionally vagile among shrew tenrecs, having been collected over a broad geographic range in forested habitats as well as in isolated forest fragments as small as 0.64 ha (Goodman & Rakotondravony, 2000). It is distinguished from the greater long-tailed shrew tenrec (*M. principula*) by its slightly smaller body and skull sizes. It is often difficult to distinguish the two species, however, in that linear measurements in these species are either contiguous (MacPhee, 1987; Jenkins, 2003) or broadly overlapping (Garbutt, 1999). *M. principula* is less frequently collected than is *M. longicaudata* for unknown reasons and is believed to be patchily distributed across its known range (Goodman & Jenkins, 2000), which encompasses much of the latitudinal extent of the island's eastern rain forests (Goodman, Jenkins & Pidgeon, 1999). Although these two species occupy broadly sympatric ranges, and have been reported to occur in syntopy, recent evidence suggests that some degree of elevational separation exists, with the larger *M. principula* occurring in greater numbers at lower elevations relative to *M. longicaudata* (Goodman & Jenkins, 1998; Goodman *et al*., 1999).

## Objectives of this study

Renewed fieldwork over the past two decades has dramatically increased the pace of species discovery and description for Madagascar's endemic mammal fauna (e.g. Jenkins, 1992, 1993; Carleton, 1994; Carleton & Goodman, 1996; Jenkins *et al.*, 1996; Jenkins *et al*., 1997; Goodman & Jenkins, 1998; Zimmermann *et al*.,

**Table 1.** Taxonomic history of long-tailed shrew tenrecs

| Described species of long-tailed *Microgale* | Morrison-Scott, 1948 | Heim de Balsac, 1972 | MacPhee, 1987[3] | Goodman & Jenkins, 1998 |
|---|---|---|---|---|
| *M. longicaudata* Thomas, 1882 | *M. longicaudata* | *M. longicaudata* | *M. longicaudata* | *M. longicaudata* |
| *M. majori* Thomas, 1918 | (*majori*) | *M. l. principula* | (*majori*) | *M. majori*?[4] |
| *M. principula* Thomas, 1926 | (*principula*) | *M. l. sorella* | (*prolixacaudata*) | |
| *M. sorella* Thomas, 1926 | (*sorella*) | *M. major* | *M. principula* | *M. principula* |
| *M. decaryi*[1] Grandidier, 1928 | | *M. paramicrogale decaryi* | (*sorella*) | |
| *M. prolixacaudata* Grandidier, 1937 | (*prolixacaudata*) | *M. prolixacaudata*[2] | (*decaryi*) | |

Parentheses indicate junior synonymy as proposed by each author; trinomina indicate proposed subspecies.[1]Subsequently *Paramicrogale decaryi* (Grandidier & Petit, 1931). The type specimen of *M. decaryi* was found in a cave associated with a bone assemblage. It is unclear if the remains are modern or date from an earlier geological period, presumably Holocene.[2]Incorrectly referred to as '*amplexicaudata*' throughout Heim de Balsac (1972).[3]The current accepted taxonomy (e.g. Hutterer, 1993; Jenkins, 2003).[4]No formal resurrection was proposed.

1998; Rasoloarison *et al*., 2000), yet only recently have scientists begun to elucidate patterns of genetic variation (Yoder *et al*., 2000). Clarifying species richness within shrew tenrecs is of fundamental importance not only from a conservation standpoint, but also for better understanding the evolutionary history of a spectacular diversification within one of the world's most threatened biotas (Mittermeier & Mittermeier, 1997). Our aim was to determine whether phylogenetic analysis of mtDNA sequence variation supports the two-species hypothesis for long-tailed shrew tenrecs. We identified lineages (*sensu* de Queiroz, 1998) provisionally as mtDNA haplotype clades and considered these as hypotheses of species boundaries to be tested with additional data.

While we recognize the potential for non-monophyletic gene trees to be embedded within reproductively cohesive species due to incomplete lineage sorting (Pamilo & Nei, 1988) or recurrent gene flow (Slatkin & Maddison, 1989), mtDNA haplotypes are expected to coalesce much more rapidly relative to nuclear markers due to their smaller effective population size (Moore, 1995). They should therefore serve as relatively early indicators of reproductive isolation. We tested whether divergent clades suggested by mtDNA data were supported by both a priori and posthoc morphometric analyses at several levels of comparison. Wiens & Penkrot (2002) recently proposed a similar strategy for using mtDNA and morphology to test species limits, but their method, as outlined, assumes that species are allopatric or parapatric, whereas we were interested in testing for species that are believed to be widely sympatric. We showed that morphometric analyses corroborate species limits suggested by mtDNA variation, supporting the existence of cryptic species of long-tailed shrew tenrecs. Finally, we found consistent but previously unrecognized patterns of elevational segregation among haplotype clades representing broadly sympatric 'cryptic' species and demonstrated contrasting patterns of clinal variation, both of which have ecological and conservational implications. We conclude that the addition of a molecular perspective on such patterns has advantages over traditional morphological approaches due to the numerous and interrelated factors confounding taxonomic investigations of shrew tenrecs. Furthermore, we argue that distributional evidence can and should be included in studies of species limits and that a priori assumptions of non-sympatry among morphologically similar species can be misleading.

## MATERIAL AND METHODS

A total of 120 museum specimens was included in the molecular component of this project (Appendix 1) from a wide variety of sites spanning the complete length of the eastern humid forest of Madagascar and numerous localities in the central highlands and western dry forest (Fig. 1).

One of the authors (L.E.O. or S.M.G.) verified the identification of all adult and most juvenile specimens as either *M. principula* or *M. longicaudata* using comparative museum collections or published keys (MacPhee, 1987; Goodman & Jenkins, 1998, 2000; Goodman *et al*., 1999); juveniles unidentifiable to species level were still easily assignable to the *M. principula*/*M. longicaudata* species complex based on relative tail length. We employ the two-species nomenclature throughout this paper but do not assume monophyly at any level.

### DNA SEQUENCE GENERATION AND ANALYSIS

We sequenced the entire mitochondrial NADH dehydrogenase subunit 2 gene (ND2 hereafter) from each specimen available for sequencing. Genomic DNA was extracted from frozen or buffered tissue (spleen, muscle or kidney) using the animal tissue protocol in the PureGene kit (Gentra Systems, Inc.). For some individuals, ~4 $mm^2$ skin samples were excised from museum specimens and subjected to the same extraction procedure, with proteinase-K digestion extended until little, if any, solid material was visible. Numerous primer combinations were employed depending on the preservation of each individual (see Appendix 2 for a complete list of primers). For most specimens, the entire ND2 gene was amplified using primers Met-1 and Trp-2. PCR amplifications were performed in 20-μL reactions (1–5 μL unquantified template DNA, 1 × amplification buffer and 0.4 U Taq polymerase [Gibco], 0.5 μM each primer, 80 μM each dNTP). Additional $MgCl_2$ was added in some cases to a final concentration of between 2.0 and 3.0 mM, depending on the template. Thermal cycling parameters included 2 min at 94°C followed by 30 cycles of 20 s at 94°C, 15 s at 50°C, and 60 s at 72°C, with a final 2 min extension at 72°C. Aliquots of the initial reactions were electrophoresed and visualized by UV spectroscopy on 1.5% TBE agarose gels. Bands of the appropriate size were excised and melted in 50–500 μL $sdH_20$. Aliquots (1–3 μL) of these were then used in a second round of PCR employing nested primers of overlapping (= 50 bp minimum overlap) portions of the gene (generally using primers ND2-LOR2 and ND2–3TX). Reamplification conditions were similar to initial amplifications except shorter cycling times were used. Reamplification products were purified using the GeneClean protocol (Bio101) following the manufacturer's instructions. Unquantified aliquots of purified PCR product (1–5 μL) were cycle-sequenced for both strands using either the ABI PRISM dRhodamine Terminator Cycle Sequencing Ready

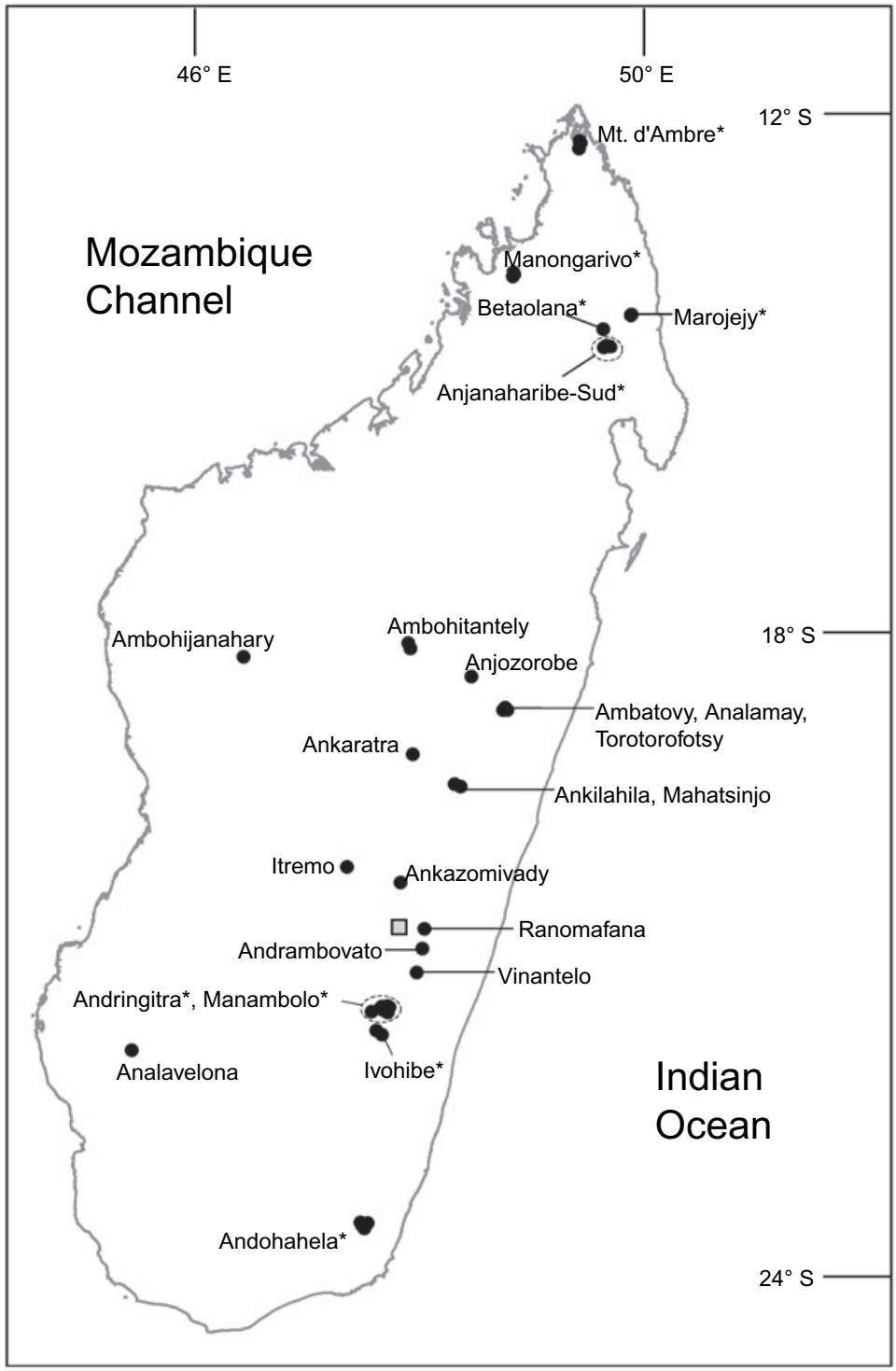



**Figure 1.** Map of Madagascar, showing collecting localities for specimens included in this study (see Appendix 1 for site descriptions and coordinates). Names marked with an asterisk denote localities from which specimens were collected at multiple locations along an elevational transect and therefore subsume multiple, but closely situated, individual sites. The shaded box represents the type locality of Ankafina for *Microgale longicaudata* and *M. majori* discussed in the text.

Reaction Kit (with AmpliTaq DNA Polymerase, FS; Perkin-Elmer) or BigDyes, version 1 (Perkin-Elmer), according to the manufacturer's directions, except using 10-μL reaction volumes with reagents scaled down accordingly. Sequencing reactions were purified using ethanol/sodium acetate precipitation. Samples were then electrophoresed on an ABI 377 automated sequencer (Applied Biosciences, Perkin-Elmer). The resulting sequence output was imported and contigs were aligned using Sequencher 3.0 (Genecodes, Ann Arbor, MI). All sequences in this study have been deposited in GenBank under accession numbers AY193297–AY193416.

Sequences from the entire ND2 gene were aligned by eye with reference to the translated amino acid sequence using MacClade 4.0 (Maddison & Maddison, 2000). Phylogenetic and bootstrap analyses were carried out using PAUP* 4.0 (Swofford, 2002) under the criterion of maximum parsimony (MP) with equally weighted characters. Heuristic tree searches were conducted using stepwise addition (100 random addition sequences) and the tree bisection–reconnection (TBR) branch-swapping algorithm. Branch length estimates were obtained under the maximum likelihood criterion constrained on the strict consensus parsimony tree. A best-fit likelihood model of sequence evolution was estimated based on likelihood-ratio tests of increasingly general models using Modeltest 3.0 (Posada & Crandall, 1998, 2001). Nodal support was estimated by bootstrap resampling under MP with 1000 pseudoreplicates employing TBR branch swapping and ten random-addition replicates. Tree topology was also estimated with a Markov chain Monte Carlo approach as employed in MrBayes (version 2.01; Huelsenbeck & Ronquist, 2002). Four Markov chains (three heated, one cold) were allowed to run for 500 000 generations using random starting trees and the same model employed in branch length estimates, with trees saved every 100 generations. Trees saved prior to the attainment of parameter stationarity in ln likelihood sums (assessed visually using the 'sump' command) were discarded. This process was repeated four times, and the saved trees from each of the five analyses were used to calculate posterior probabilities (Larget & Simon, 1999). To determine whether the Markov chain was mixing sufficiently and had run a sufficient number of generations to accurately estimate the joint posterior distribution, we compared the nodal posterior probabilities obtained in each of the five runs to determine the associated variance (M. Alfaro, pers. comm.). Multiple specimens of *M. pusilla*, supported as the closest outgroup to long-tail shrew tenrecs by morphological and molecular data (Olson, 1999; Olson & Goodman, 2003), were used to root all trees.

### Morphometry

Twenty-one craniodental and six postcranial skeletal measurements were taken using digital calipers accurate to 0.1 mm by one of the authors (L.E.O.). An additional three external measurements were taken from collectors' field notes or specimen tags. A list of these measurements and their definitions is provided in Appendix 3 and the raw data are provided online in Table S1 (see Supplementary material section). Some measurements were recorded for comparison with published studies but were not included in morphometric analyses for reasons given in their definitions. Only adults, defined by the presence of fully erupted permanent dentition (e.g. Jenkins *et al.*, 1996), were included. While we are aware of the potential for morphological variation to be influenced by habitat-related factors (e.g. Patton & Brylski, 1987), this has yet to be demonstrated in shrew tenrecs and we therefore assumed that such variation is primarily due to genetic factors, pending any evidence to the contrary.

We calculated descriptive statistics and conducted univariate and bivariate analyses using StatView version 4.5. We used JMP version 3.1.6 to perform principal components analysis (PCA) and multiple-group discriminant function analysis (DFA) on log-transformed (base 10) measurement data. PCA was conducted on the covariance matrix to identify patterns of variation without a priori allocation of individuals to a haplotype clade. DFA was used to test whether morphology could correctly predict haplotype clade membership. We performed Student's *t*-tests on all measurements to test for sexual dimorphism. Because the number of measurements possible for a given individual was dependent on specimen preparation and condition, DFA was conducted separately for skull (20 measurements); skull + external (22 measurements); and skull + external + skeletal (28 measurements) characters. Only adults were included in the *t*-tests and morphometric analyses. Abbreviations presented in parentheses after each measurement in Appendix 3 are used in subsequent text and tables.

Potential patterns of morphological variation associated with patterns of clinal and elevational distribution were assessed using correlation *Z*-tests (alpha level of $P < 0.05$ corrected for multiple comparisons) at multiple hierarchical levels.

## RESULTS AND DISCUSSION

### Phylogeny and genetic variation

The use of mtDNA sequence data to test and revise taxonomic boundaries has become a widespread practice in evolutionary biology (Avise, 2000; Puorto *et al.*, 2001; Wiens & Penkrot, 2002 and references cited therein). Results derived from a single molecular

marker (or multiple linked markers) must be interpreted with caution, however, due to the potential for gene tree–species tree incongruence (Goodman *et al*., 1979; Avise *et al*., 1983; Pamilo & Nei, 1988; Maddison, 1997; Hudson & Coyne, 2002). In the absence of information from multiple unlinked markers, falsifiability of single-locus hypotheses can be sought using morphological, ecological and distributional evidence. We took such a synthetic approach towards testing species boundaries and clarifying the patterns of diversification in a group suspected of harbouring 'cryptic' species-level entities.

All *Microgale* ND2 sequences generated for this study were identical in length (1044 bp) to that of the lesser Madagascar hedgehog tenrec *Echinops telfairi* (Mouchaty *et al*., 2000). Visual inspection of translated amino acid sequences failed to reveal a need to invoke any insertion/deletion events and the alignment was therefore considered to be unambiguous. Unlike *Echinops*, all *Microgale* ND2 sequences possessed a methionine start codon rather than an isoleucine. Of the 120 sequences generated, 91 represented unique haplotypes (80 in specimens referred to *M. longicaudata,* seven in *M. principula*, and four in *M. pusilla*). An alignment file in Nexus format is provided online in Table S2 (see Supplementary material section).

The heuristic search recovered 848 equally parsimonious trees (944 steps, consistency index = 0.55, retention index = 0.95), the strict consensus of which is shown in Figure 2.

Long-tailed shrew tenrecs were found to be a well-supported monophyletic assemblage relative to the outgroup *M. pusilla*, a finding corroborated by previous morphological and molecular studies limited to interspecific relationships (Olson, 1999; Olson & Goodman, 2003). Likelihood-ratio tests of successively general models of sequence evolution (as implemented by Modeltest) resulted in a model incorporating unequal transition rates (A–G = 22.0144; C–T = 11.5143), estimated base frequencies (A = 0.3680, C = 0.3013, G = 0.0746, T = 0.2561), and among-site rate variation approximated by the gamma distribution (shape parameter = 1.85), with the proportion of invariant sites estimated to be 0.5015. Posterior probabilities for the clades shown in Figure 2 were associated with SD < 1% among the five separate runs, suggesting that the Markov chain was adequately approximating the joint posterior distribution.

Both the MP and Bayesian analyses identified five divergent haplotype lineages within long-tailed shrew tenrecs, each with optimal statistical support as measured by both parsimony bootstrap and Bayesian posterior probability (Fig. 2). Specimens identified as *M. principula* comprised a monophyletic sister group to the remaining long-tailed specimens, most of which had been identified as *M. longicaudata*, but some of which conformed to published definitions of *M. principula* by virtue of their relatively large size (some juvenile specimens were not confidently identified as either *M. principula* or *M. longicaudata*). Within putative *M. longicaudata*, four clades were identified that, based on general elevational and/or latitudinal distribution patterns (Table 2), are referred to hereafter as North (restricted to northern latitudes), Highland (restricted to elevations 1300 m and above), Widespread (broadly distributed), and Mid (recovered at middle latitudes) clades. Monophyly of the *M. longicaudata* clade with respect to *M. principula*, however, was only weakly supported by parsimony bootstrapping (45%) and had a posterior probability of slightly less than 0.95.

These five clades were separated by long internal branches with comparatively short terminal branches within each clade. Uncorrected pairwise sequence divergence estimates between the morphologically distinctive *M. pusilla* and long-tailed shrew tenrecs ranged from 14.3% to 18.5%. This was comparable to the range observed in comparisons of *M. principula* to the four named *M. longicaudata* haplotype clades (14.5–16.9%) as well as that between each of the two basal *M. longicaudata* clades (North + Highland and Widespread + Mid; 14.6–16.6%). Divergences between each of the two terminal sister clades in *M. longicaudata* were slightly lower, ranging from 11.3% to 13.2% in comparisons of North and Highland haplotypes and 10.6–12.9% between Widespread and Mid specimens. All of these pairwise distance measures fall within the range of values for similar comparisons between sister species of oryzorictines (shrew and mole tenrecs) for ND2 (10.6–16.6%; Olson, 1999). Maximum uncorrected divergences within each of the recognized terminal clades were highest in *M. principula* (6.6%), followed by Widespread (5.2%), Highland (3.7%), North (3.4%) and West (0.2%).

Based on the striking difference between the long internal and short terminal branches within the *M. longicaudata* clade, the questionable monophyly of *M. longicaudata* with respect to *M. principula*, and the levels of divergence observed between major haplotype clades within *M. longicaudata*, we considered each of the four *M. longicaudata* haplotype clades (North, Widespread, Mid and Highland) as potential species in subsequent investigations of morphological boundaries.

### Morphometric variation

Of the *M. longicaudata* and *M. principula* specimens sequenced, 49 were judged to be dental adults (Fig. 3), 57 were subadults, and nine could not be confidently

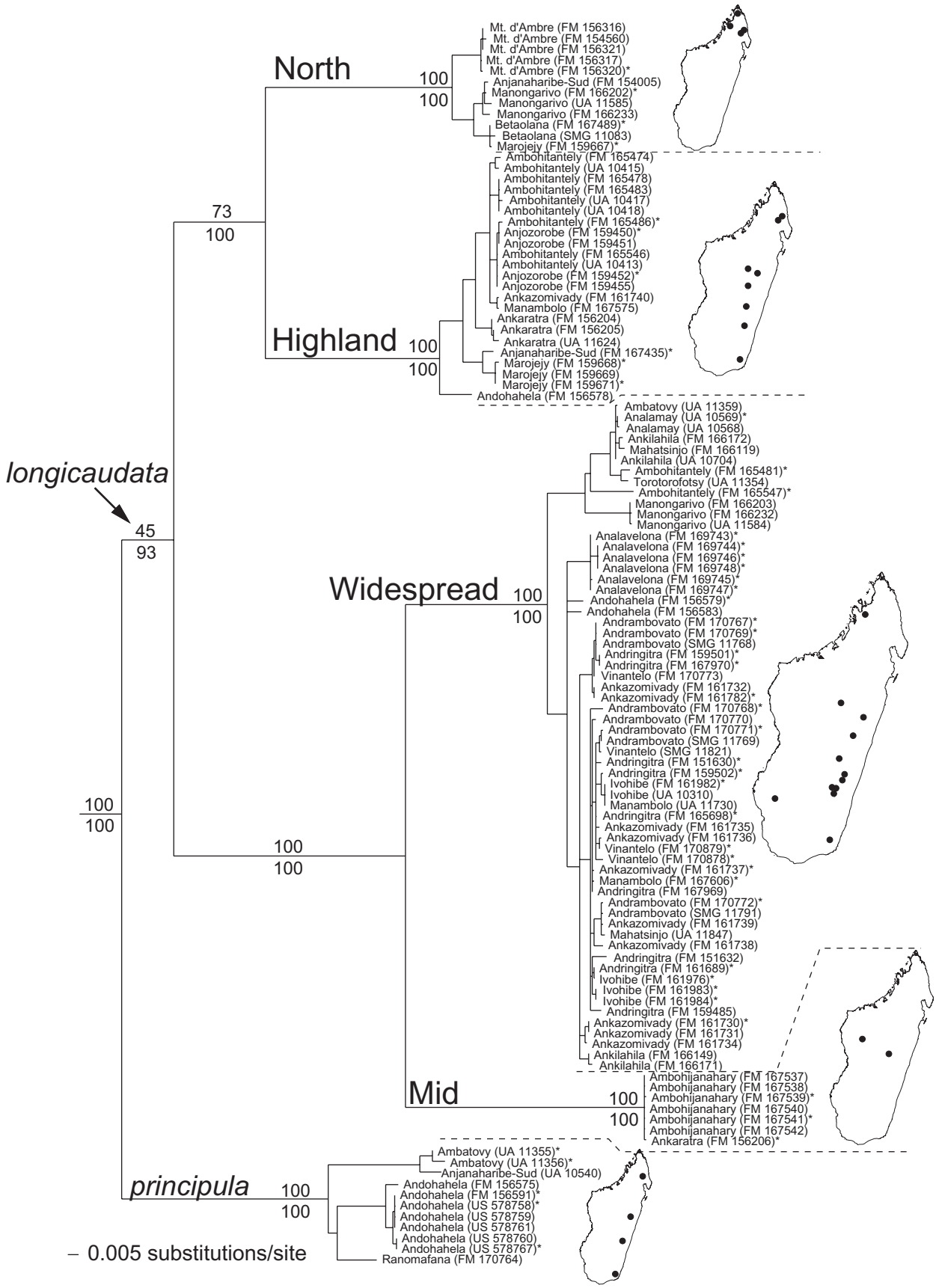
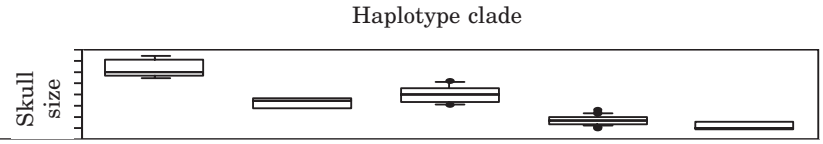

North
100
100
Mt. d'Ambre (FM 156316)
Mt. d'Ambre (FM 154560)
Mt. d'Ambre (FM 156321)
Mt. d'Ambre (FM 156317)
Mt. d'Ambre (FM 156320)*
Anjanaharibe-Sud (FM 154005)
Manongarivo (FM 166202)*
Manongarivo (UA 11585)
Manongarivo (FM 166233)
Betaolana (FM 167489)*
Betaolana (SMG 11083)
Marojejy (FM 159667)*
73
100
Highland
100
100
Ambohitantely (FM 165474)
Ambohitantely (UA 10415)
Ambohitantely (FM 165478)
Ambohitantely (FM 165483)
Ambohitantely (UA 10417)
Ambohitantely (UA 10418)
Ambohitantely (FM 165486)*
Anjozorobe (FM 159450)*
Anjozorobe (FM 159451)
Ambohitantely (FM 165546)
Ambohitantely (UA 10413)
Anjozorobe (FM 159452)*
Anjozorobe (FM 159455)
Ankazomivady (FM 161740)
Manambolo (FM 167575)
Ankaratra (FM 156204)
Ankaratra (FM 156205)
Ankaratra (UA 11624)
Anjanaharibe-Sud (FM 167435)*
Marojejy (FM 159668)*
Marojejy (FM 159669)
Marojejy (FM 159671)*
Andohahela (FM 156578)
longicaudata
45
93
Ambatovy (UA 11359)
Analamay (UA 10569)*
Analamay (UA 10568)
Ankilahila (FM 166172)
Mahatsinjo (FM 166119)
Ankilahila (UA 10704)
Ambohitantely (FM 165481)*
Torotorofotsy (UA 11354)
Ambohitantely (FM 165547)*
Manongarivo (FM 166203)
Manongarivo (FM 166232)
Manongarivo (UA 11584)
Analavelona (FM 169743)*
Analavelona (FM 169744)*
Analavelona (FM 169746)*
Analavelona (FM 169748)*
Analavelona (FM 169745)*
Analavelona (FM 169747)*
Widespread
100
100
Andohahela (FM 156579)*
Andohahela (FM 156583)
Andrambovato (FM 170767)*
Andrambovato (FM 170769)*
Andrambovato (SMG 11768)
Andringitra (FM 159501)*
Andringitra (FM 167970)*
Vinantelo (FM 170773)
Ankazomivady (FM 161732)
Ankazomivady (FM 161782)*
Andrambovato (FM 170768)*
Andrambovato (FM 170770)
Andrambovato (FM 170771)*
Andrambovato (SMG 11769)
Vinantelo (SMG 11821)
Andringitra (FM 151630)*
Andringitra (FM 159502)*
Ivohibe (FM 161982)*
Ivohibe (UA 10310)
Manambolo (UA 11730)
Andringitra (FM 165698)*
Ankazomivady (FM 161735)
Ankazomivady (FM 161736)
Vinantelo (FM 170879)*
Vinantelo (FM 170878)*
Ankazomivady (FM 161737)*
Manambolo (FM 167606)*
Andringitra (FM 167969)
Andrambovato (FM 170772)*
Andrambovato (SMG 11791)
Ankazomivady (FM 161739)
Mahatsinjo (UA 11847)
Ankazomivady (FM 161738)
Andringitra (FM 151632)
Andringitra (FM 161689)*
Ivohibe (FM 161976)*
Ivohibe (FM 161983)*
Ivohibe (FM 161984)*
Andringitra (FM 159485)
Ankazomivady (FM 161730)*
Ankazomivady (FM 161731)
Ankazomivady (FM 161734)
Ankilahila (FM 166149)
Ankilahila (FM 166171)
100
100
100
100
Mid
Ambohijanahary (FM 167537)
Ambohijanahary (FM 167538)
Ambohijanahary (FM 167539)*
Ambohijanahary (FM 167540)
Ambohijanahary (FM 167541)*
Ambohijanahary (FM 167542)
Ankaratra (FM 156206)*
principula
100
100
Ambatovy (UA 11355)*
Ambatovy (UA 11356)*
Anjanaharibe-Sud (UA 10540)
Andohahela (FM 156575)
Andohahela (FM 156591)*
Andohahela (US 578758)*
Andohahela (US 578759)
Andohahela (US 578761)
Andohahela (US 578760)
Andohahela (US 578767)*
Ranomafana (FM 170764)
0.005 substitutions/site

**Figure 2.** Strict consensus tree of 848 equally parsimonious trees (944 steps, consistency index = 0.55, retention index = 0.95) obtained from heuristic search with all characters equally weighted. Branch lengths are shown as optimized under the maximum likelihood criterion on the strict parsimony consensus tree. Numbers above select nodes indicate bootstrap support; those below represent Bayesian posterior probabilities estimated with a Markov chain Monte Carlo approach. Individual specimens are identified by museum catalogue number (FM, FMNH; UA, UADBA; US, USNM; SMG, uncatalogued specimens collected by S. M. Goodman housed at UADBA; see Appendix 1) and collecting localities as shown in Figure 1. An asterisk denotes adults included in discriminant function analysis. Haplotype clades as discussed in the text are labelled above their respective ancestral branches and their distributions are shown on the right.

**Table 2.** Sympatric occurrences of haplotype clade members




| Locality | Elevation (m) | *principula* | North | Highland | Widespread | Mid |
|---|---|---|---|---|---|---|
| Andohahela | 440 | 0,1 | | | | |
| | 725 | 2,3 | | | | |
| | 810 | 1,0 | | | 1,0 | |
| | 1200 | | | | 0,1 | |
| | 1875 | | | 0,1 | | |
| Anjanaharibe-Sud | 875 | 0,1 | | | | |
| | 1260 | | 0,1 | | | |
| | 1600 | | | 1,0 | | |
| Marojejy | 1225 | | 1,0 | | | |
| | 1625 | | | 1,0 | | |
| | 1875 | | | 1,1 | | |
| Ambatovy | 1164 | 2,0 | | | 0,1 | |
| Ambohitantely | 1450 | | | 0,7 | 1,0 | |
| | 1500 | | | 1,1 | 1,0 | |
| Ankaratra | 2000 | | | 0,3 | | 1,0 |
| Ankazomivady | 1675 | | | 0,1 | 1,7 | |
| Manambolo | 1300 | | | | 1,0 | |
| | 1600 | | | 0,1 | 0,1 | |
| | | | | | 0,1 | |
| Manongarivo | 785 | | | | | |
| | 1240 | | 0,2 | | 0,1 | |
| | 1600 | | 1,0 | | 0,1 | |
| Elevation ranges (m), entire distribution | | 440–1164 | 1000–1600 | 1300–2000 | 720–1990 | 1150–2000 |
| Latitudinal ranges, entire distribution (°S) | | 14.76–24.63 | 12.52–14.75 | 14.44–24.56 | 13.98–24.59 | 18.26–19.35 |

Numbers of adult and juvenile specimens, respectively, are shown for each clade at each locality where more than one clade occurs. A boxplot of relative skull size (condylopremaxillary length, adults only) is given above (scale ranges from 17 to 25 mm). Elevational and latitudinal (all south latitude) ranges for each clade over its entire distribution are also provided.

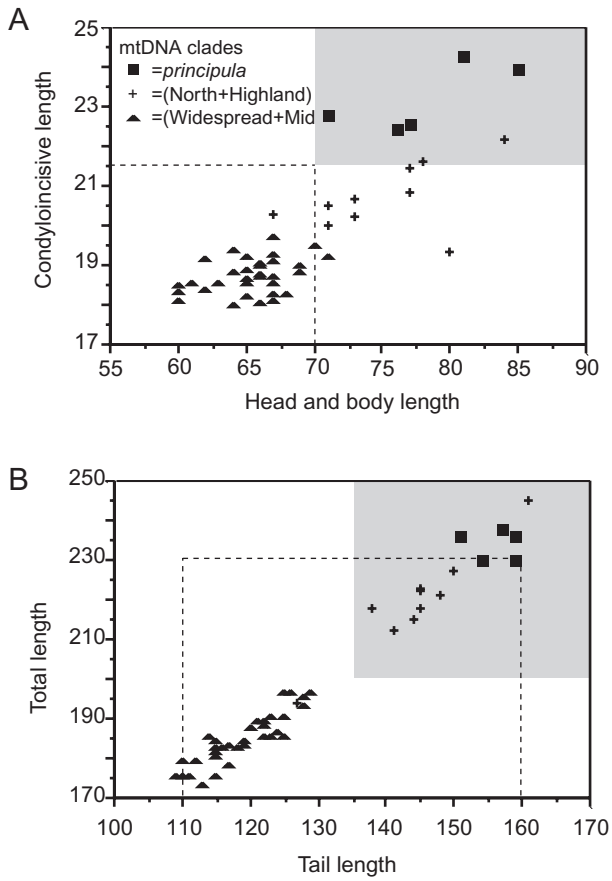



**Figure 3.** Previous hypotheses of morphological species boundaries as they apply to specimens sampled in this study. Bivariate plots of adult *Microgale longicaudata* and *M. principula* specimens sequenced and measured in this study. Haplotype clades: ■ = *M. principula*; + = (North + Highland); ▲ = (Widespread + Mid). Species boundaries for *M. principula* (grey) and *M. longicaudata* (dashed line) are shown as proposed by Jenkins (2003; A) and Garbutt (1999; B). All measurements in mm. See Appendix 3 for measurement descriptions.

aged (either skulls were left intact in fluid specimens or the presence of a full set of adult dentition was equivocal). Among adults, skull length as measured between the occipital condyles and the anterior premaxillaries (condylopremaxillary length) ranged from 17.5 mm to 24.4 mm. With respect to haplotype clade and skull size, three general size classes were evident, comprising small-sized (Widespread and Mid), medium-sized (Highland and North), and large-sized (*M. principula*) adults (Table 2), although some overlap was observed among clades. We noted that some individuals with *M. longicaudata* haplotypes (as identified here) fell in the general size range of *M. principula* as delimited by previous authors (Fig. 3).

Bivariate plots of the first two axes of the PCA for skull and skin measurements showed no overlap among any clusters (as identified by haplotype) with the exception of Widespread and Mid (Fig. 4A). Thus, the overlap in size seen between *M. longicaudata* and *M. principula* for select individual measurements (Fig. 3) disappeared when multiple measurements were subjected to ordination.

Sample sizes were insufficient to test for sexual dimorphism within all but the Widespread clade (number of males/total number of individuals = 5/6 for *M. principula*, 5/6 for Highland, 4/4 for North and 3/3 Mid). Within that clade, no significant difference was found for any measurement between the 20 males and 12 females in the adults sampled. With the acknowledgement that increased sampling may reveal sexual dimorphism in the remaining clades, we proceeded with the DFA on all adult specimens of both sexes.

Based on the results of the molecular phylogenetic analysis and the PCA, we chose to perform DFA at two hierarchical levels, one employing the *M. principula* and each of the basal two *M. longicaudata* clades as grouping classes, and a second considering *M. principula* and each of the four haplotype clades within *M. longicaudata*. For each level we conducted separate analyses on the skull, skull + external, and skull + external + skeletal measurements, which permitted sample sizes of 49, 48, and 37 adult individuals, respectively. Results are summarized in Table 3 and Figure 4B and C.

*M. principula* and the two basal *M. longicaudata* clades were readily discriminated by all three sets of measurements as illustrated in the plot of canonical axes 1 and 2 (Fig. 4B). When all four *M. longicaudata* haplotype subclades were defined as separate grouping variables, two specimens bearing Widespread haplotypes were misclassified as Mid based on skull measurements (mirroring the PCA results). Of these two individuals, one was correctly classified when external characters were included and both were correctly classified when skeletal measurements were included.

Morphometric variation thus corroborates molecular phylogenetic evidence of multiple cryptic species within *M. longicaudata.* Skull morphology alone distinguished each of the two broadly sympatric basal haplotype clades within *M. longicaudata* (Table 3), supporting recent speculation that at least two species exist within *M. longicaudata* (Goodman & Jenkins, 1998). Despite overlap among clades in several individual measurements, DFA nonetheless discriminated each of the four subclades within *M. longicaudata* when postcranial skeletal characters were included. Although sample sizes for adults were small for some clades, we consider the possibility that four evolutionarily independent lineages exist within

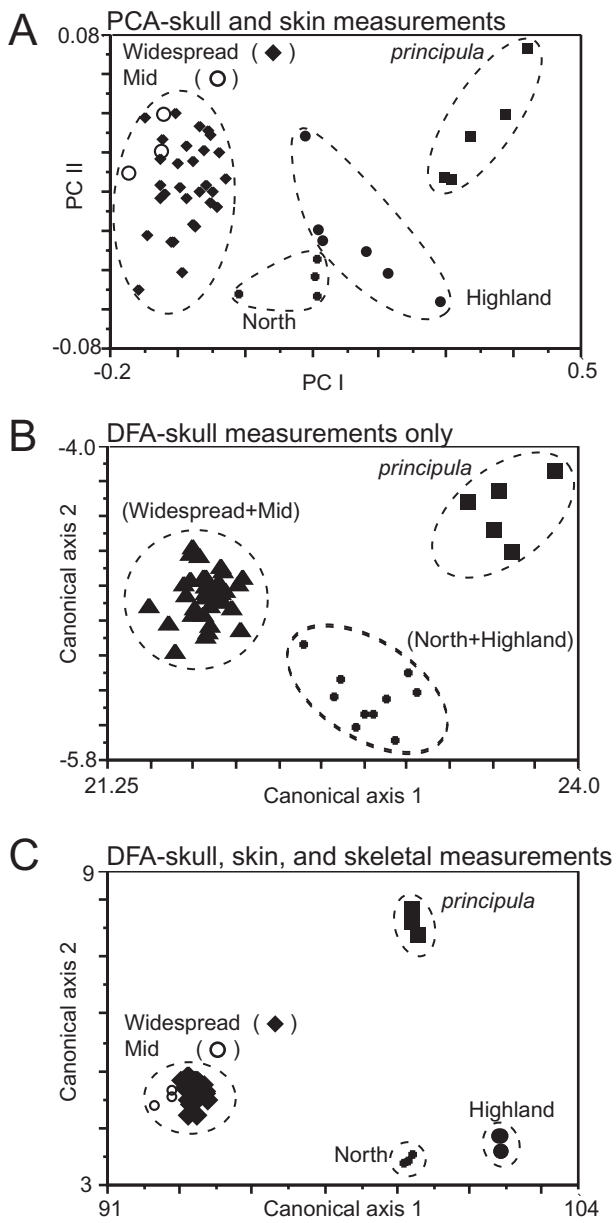



**Figure 4.** Results of select principal components (PCA) and discriminant function (DFA) analyses. A, Plot of first and second principal components for all long-tailed specimens, skull and skin measurements only. B, Plot of canonical axes 1 and 2 for 20 craniodental measurements used to differentiate (North + Highland), (Widespread + Mid) and *Microgale principula* haplotype clades. C, DFA of the expanded set of measurements incorporating an additional two external and six postcranial skeletal measurements used to discriminate Mid, Highland, Widespread, North and *M. principula* clade members. For DFA, axes 1 and 2 readily discriminate the three basal haplotype clades based on skull characters alone (B) while the inclusion of additional measurements allows for the correct classification of all five basal haplotype clades with a slightly smaller sample size (C; Widespread and Mid specimens overlap in the plot of canonical axis 1 against axis 2 but are clearly separated by axis 4, which is not shown). Ellipses drawn around clusters are provided for visual purposes only. Table 3 summarizes the results for all DFAs conducted.

*M. longicaudata* and here consider additional lines of evidence.

### Distributional patterns as evidence of distinct species

Inspection of the geographical distributions of each of the five major haplotype lineages (North, Highland, Widespread, Mid and *M. principula*) revealed varying degrees of sympatry and geographical range size among clades (Fig. 2 and Table 2). The Highland, Widespread, and *M. principula* haplotypes were all widely distributed latitudinally, occurring from Andohahela in the south to Manongarivo (Widespread clade), Marojejy (Highland clade) and Anjanaharibe-Sud (*M. principula*) in the north. In addition, specimens from the Widespread clade were recovered from Analavelona, the western-most locality in this study. The remaining two clades occupied much smaller ranges confined to either the five northernmost localities (North clade) or two mid-latitude localities (Mid clade).

Sympatric occurrence of at least two haplotype lineages was observed at nine of the sites shown in Figure 1, with as many as three occurring at Andohahela and Anjanaharibe-Sud (Table 2). Consideration of elevational occurrences at localities where elevational transects were conducted showed some combinations of broadly sympatric clades to be parapatric rather than syntopic. For example, the ranges of the North and Highland clades would appear to overlap at Anjanaharibe-Sud and Marojejy, yet in neither locality were individuals from the two clades recovered from the same elevation. For both localities, specimens bearing North haplotypes occurred at lower elevations relative to Highland haplotype individuals. Similarly, *M. principula* was broadly sympatric with Highland at Andohahela and both Highland and North at Anjanaharibe-Sud, yet in all cases, *M. principula* only occurred at lower elevations where neither Highland nor North were collected. Of the remaining possible pairwise combinations of sympatry, only Widespread and Mid clades were never observed at the same locality.

As shown in Table 2, the broad sympatry observed among some haplotype clades could be subdivided at finer scales when elevation was taken into account.

**Table 3.** Discriminant function analysis (DFA) results, showing the number of adult specimens from each haplotype clade correctly classified by DFA (# correctly classified/total number adults in that haplotype clade for which measurements were available) for all three sets of measurements

| Haplotype clade membership | Proportion adult specimens in each haplotype clade correctly assigned to that clade by DFA | | |
|---|---|---|---|
| | Skull | Skull & skin | Skull, skin & skeleton |
| North | 4/4 | 4/4 | **3/3** |
| Highland | 6/6 | 6/6 | **4/4** |
| Widespread | 30/31[1] | 30/30[2] | **24/24[2]** |
| Mid | 3/3 | 3/3 | **3/3** |
| *Microgale principula* | 5/5 | 5/5 | **3/3** |
| (North + Highland) | **10/10** | 10/10 | 7/7 |
| (Widespread + Mid) | **34/34** | 33/33 | 27/27 |
| *M. principula* | **5/5** | 5/5 | 3/3 |
| Sample size | 49 | 48 | 37 |

Bold type depicts the analyses corresponding to Fig. 4B, C. Separate analyses were conducted on each of three sets of measurements (see text).

[1]Misclassified specimen predicted as Mid.

[2]Sample includes specimen misclassified as Mid based on skull measurements only.

Specifically, certain combinations were never found at the same altitude. These included: *M. principula* with North or Highland; North with Highland; Mid with Widespread. Furthermore, elevational segregation with respect to these combinations was consistent, with North always occurring at lower elevations compared with Highland (at both Marojejy and Anjanaharibe-Sud) and *M. principula* likewise occurring at lower elevations compared with Highland (at Andohahela and Anjanaharibe-Sud). While replacement of *M. principula* by *M. longicaudata* with increasing elevation has been reported previously for Anjanaharibe-Sud (Goodman & Jenkins, 1998), these two species were found to broadly overlap at elevations ranging from 440 m to 1200 m at Andohahela by Goodman *et al*. (1999). However, as our results have shown (Fig. 3 and below), several individuals previously identified as *M. principula* may represent large-bodied *M. longicaudata* specimens instead. This appears to be the case with the *M. principula* specimens reported from elevations above 810 m in Goodman *et al*. (1999) (L. Olson, unpubl. data). While sample sizes from these localities were small, our results nonetheless suggest elevational replacement of *M. principula* by large-bodied *M. longicaudata* clades (North and Highland) where the two occur in parapatry (Table 2). Furthermore, their respective elevational distributions correspond to habitat differences observed at these localities (Rakotondrainibe & Raharimalala, 1998; Helme & Rakotomalaza, 1999), with *M. principula* occupying lowland forest and large-bodied *M. longicaudata* restricted to montane forest habitats above 1200 m. Whether habitat differences underlie the observed elevational segregation of the North and Highland haplotype clades is less clear, as is the potential role of competitive exclusion among the three large-bodied morphs (*M. principula*, North and Highland).

In contrast to the lack of syntopy observed between *M. principula*, North and Highland clades, members of the small-bodied clade (Widespread + Mid) were found together with one of the three larger morphs at nine localities throughout much of their range (Table 2). Evidence from two of these sites further demonstrates their ability to disperse and coexist in direct sympatry. Our specimens from Ambohitantely came from five isolated forest fragments, ranging in size from 1250 to 0.64 ha, surveyed by Goodman & Rakotondravony (2000), who reported a nested distribution of small mammal species with respect to fragment area. Only two species were captured in all fragments, one of which was *M. longicaudata*. Based on their findings and on previously published accounts of *M. longicaudata* occurring outside of forested zones, it was concluded that this species is an adept disperser relative to most small mammals in Madagascar (Goodman & Rakotondravony, 2000). Our original molecular sample of specimens from these forest fragments included 11 specimens, two that have now been identified as belonging to the Widespread clade, and the remainder identified as belonging to the Highland clade

(Fig. 2). Subsequent sequencing of additional specimens from the Ambohitantely forest patches confirmed that both clades are present in even the smallest fragment (0.64 ha) (L. Olson, S. Goodman & A. Yoder, unpubl. data). The second noteworthy locality, Ankaratra, was represented by four specimens from the Highland and Mid clades. These were collected from a planted, effectively monospecific, forest of a native tree, largely isolated from bigger blocks of natural forest and surrounded by anthropogenically degraded habitats (Goodman *et al*., 1996a). As with Ambohitantely, this suggests that the small-bodied *M. longicaudata* clades (Widespread and Mid) are not in direct competition with their larger counterparts and that both general size morphs of *M. longicaudata* are resilient under the effects of habitat degradation and isolation.

### How many species of long-tailed shrew tenrec?

Our results support the taxonomic status of *M. principula* as distinct from *M. longicaudata* just as they strongly suggest the existence of multiple cryptic species within *M. longicaudata.* We agree with de Queiroz's (1998) notion of species as lineages united by gene flow (for sexually reproducing organisms). Our operational definition of lineages as reciprocally monophyletic mtDNA haplotype clades suggests five such groups corresponding to four divergent (and monophyletic) clades within *M. longicaudata*, along with a monophyletic *M. principula*. Under the genealogical species concept (Baum & Shaw, 1995; Shaw, 1998), each of these five clades would potentially warrant recognition as a distinct species since each is both 'basal' (i.e. there is no evidence of further subdivision within a clade) and 'exclusive' (monophyletic in the case of a single marker phylogeny). Because of the tendency for mtDNA to attain reciprocal monophyly far more rapidly than do nuclear genes, Hudson & Coyne (2002: 1563) recently cautioned against recognizing genealogical species based on mtDNA data alone 'unless population divergence is very ancient.' As we have noted, branch lengths and sequence divergence separating each of the clades are on a par with those observed in other closely related shrew tenrecs (Olson, 1999). This suggests that sufficient time has elapsed for assessing reciprocal monophyly in the context of genealogical species recognition.

Rather than rely exclusively on molecular data, however, we believe it essential to incorporate non-molecular data into our investigation of species boundaries. Both PCA and DFA corroborate the molecular results, suggesting multiple distinct morphological clusters within *M. longicaudata* (Fig. 4, Table 3). The latter analyses correctly classify all adult specimens by haplotype group when external and skeletal measurements are included (Fig. 4C, Table 3). We interpret these results as evidence of morphological (and, by extension, nuclear DNA) divergence corresponding to the mtDNA phylogeny, which serves as further evidence of evolutionary isolation among haplotype lineages. We restricted our morphological investigation to quantitative variation, however, and did not survey qualitative characters; as such, our results are not amenable to interpretation under the phylogenetic species concept (e.g. Nixon & Wheeler, 1990)

The geographic distributions of each haplotype clade lend further support to the hypothesis that multiple cryptic species are present. Extensive sympatry observed between the Highland and Widespread clades suggests ample opportunities for interbreeding, yet these remain morphologically distinct throughout their range, suggesting complete reproductive isolation. The same is true of Widespread and *M. principula* clades. Because they are not observed in direct sympatry, the same argument does not apply to the Widespread and Mid haplotype clades. Moreover, individuals from these two clades overlap in morphospace (Fig. 4A) and are not consistently differentiated by DFA (Table 3). In the case of the North and Highland clades, however, there is evidence of elevational and habitat segregation (Table 2 and above discussion). Furthermore, though these clades were not observed in direct sympatry (syntopy), they were collected less than 4 km from each other at Anjanaharibe-Sud and less than 0.5 km apart at Marojejy (along elevational transects). While proponents of the biological species concept (Mayr, 1963) would therefore presumably recognize at least the *M. principula*, (Widespread + Mid), and (Highland + North) clades as separate species, we believe the evidence from molecular divergence, morphological distinctiveness, elevational segregation and local parapatry justifies recognition of the North and Highland clades as separate species as well.

Our interpretation and application of de Queiroz's (1998) unified species concept thus conservatively recognizes four distinct species of long-tailed shrew tenrec corresponding to the North, Highland, (Mid + Widespread) and *M. principula* haplotype clades (lineages), with molecular, morphological, and distributional evidence either supporting or failing to refute their reproductive isolation.

### Revised taxonomy of long-tailed shrew tenrecs

Confident assignment of nomenclatural epithets to these various taxa in accordance with the rules of taxonomic seniority requires a re-evaluation of the type material for the named forms listed in Table 1. Junior synonymy of *M. sorella* Thomas 1926 and *M. decaryi* Grandidier 1928 with respect to *M. principula*

Thomas 1918 has been advocated by MacPhee (1987) and adopted by subsequent authors (e.g. Hutterer, 1993).

Comparison of published measurements of the former (Thomas, 1926) with our results (see below) support *M. sorella* as a junior synonym of *M. principula*, but the status of *M. decaryi* is less certain in light of our revised morphological boundaries for *M. principula* and the fragmentary nature of the type material of *M. decaryi* (see MacPhee, 1987). We advocate continued recognition of *M. principula* as a distinct species but note that external measurements alone do not reliably differentiate it from large-bodied *M. longicaudata* (see below and Fig. 3).

With respect to epithets currently synonymized with *M. longicaudata* (Table 1) data collected from the holotypes of *M. longicaudata* (BM[NH] 82.3.1.15) and *M. majori* (BM[NH] 82.3.1.17), together with external measurements published in their respective descriptions (Thomas, 1882, 1918) and the type locality as subsequently determined (MacPhee, 1987; Carleton & Schmidt, 1990), provide some insight into the reconciliation of our results with these original descriptions. The type specimens of both *M. longicaudata* and *M. majori* were collected from the same locality, Ankafina, indicated in Figure 1. This locality falls within the bounded ranges of both Highland and Widespread haplotype clades (Fig. 2). Thomas's (1918) differentiation of *M. majori* from *M. longicaudata* relied primarily on the former's smaller body size. Both type specimens are adults, and limited craniodental measurements available for these individuals suggest that the holotype of *M. longicaudata* corresponds to the large-bodied (Highland) clade and that of *M. majori* belongs to the small-bodied (Widespread) clade (Fig. 5).

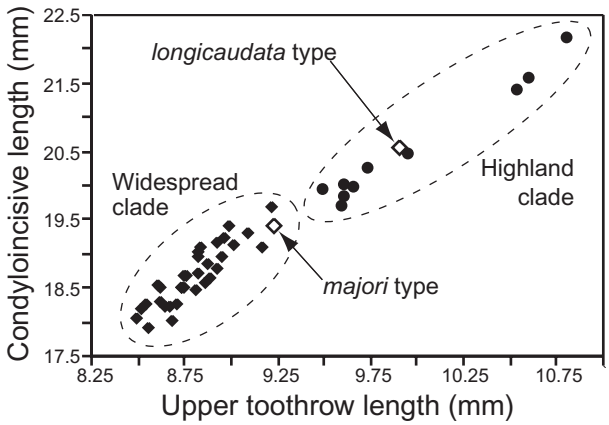



**Figure 5.** Bivariate plot of condyloincisive length against upper toothrow length for the adult specimens in the Highland and Widespread haplotype clades. Values for the holotypes of *Microgale longicaudata* and *M. majori* are indicated.

The type locality of *M. prolixacaudata* Grandidier 1937 has not been firmly established but is believed to be in the vicinity of Antsiranana (MacPhee, 1987), approximately 40 km north north-east of Mt. d'Ambre and farther north than any of the samples included in this study. Given the distribution observed for North haplotype specimens, and the absence of any other haplotypes north of Manongarivo (Figs 1, 2), it seems likely that this clade includes the type specimen of *M. prolixacaudata*. This specimen (MCZ 45035) is a subadult undergoing tooth replacement and is thus not amenable to morphometric comparisons with the adult sample from our study (the paratype, MCZ 46020, is an intact alcohol-preserved specimen of indeterminate age). We believe it to be premature to formally assign *M. prolixacaudata* to the North clade until samples from the type specimens can be included in a molecular analysis.

We do, however, believe our results justify the formal resurrection of *M. majori* Thomas 1918, which includes the Widespread and Mid haplotype clades, from synonymy with *M. longicaudata* (Highland clade) for reasons explained above. Recognition of *M. majori* as a distinct species makes it the most widely distributed shrew tenrec known (Fig. 2). We propose the provisional key below for discriminating among adults of the three species of long-tailed shrew tenrecs formally recognized here and will await a more thorough investigation of the *M. prolixacaudata* type material to distinguish it from the Highland haplotype clade.

PROVISIONAL KEY FOR SPECIES OF LONG-TAILED SHREW TENRECS

1. Mandible height >6.2 mm; condylopremaxillary length >22 mm; greatest skull length >23 mm; total length >225 mm ..... *M. principula*
   Mandible height <6.0 mm; condylopremaxillary length <22.5 mm; greatest skull length <23 mm ..... 2
2. M3 width generally >6.0 mm; total length >190 mm; if M3 < 6.0 mm due to excessive wear, total length generally >210 mm; tail length >125 mm ..... *M. longicaudata* (including North haplotype clade)
   M3 width <6.0 mm; total length <200 mm; tail length <135 mm ..... *M. majori*

### A REVISED PERSPECTIVE ON GEOGRAPHIC VARIATION: IMPLICATIONS FOR MORPHOLOGICAL SPECIES BOUNDARIES IN SHREW TENRECS

The observation of cryptic species within *M. longicaudata* permits a novel perspective on patterns of geographic variation in long-tail shrew tenrecs. Given the broad elevational and latitudinal distributions of the three widely distributed clades (*M. principula,* Highland and Widespread) (Table 2), we investigated whether either of these factors was significantly associated with variation in body size, using condylopremaxillary length as proxy for overall size. In addition, we explored whether inferences of geographic variation based on the assumption of a single species of *M. longicaudata* differed from those incorporating the hypothesis of multiple cryptic species. In no case was elevation significantly associated with body size. However, both *M. principula* and *M. longicaudata* (all haplotype clades combined) showed a negative correlation between body size and distance from the equator (Fig. 6), though this result was not significant for *M. principula* when corrected for multiple comparisons. When the two wide-ranging *M. longicaudata* clades were considered individually, contrasting patterns were observed, with a significant negative correlation between size and increasing latitude found in Highland and a significant positive relationship in Widespread. Although the latter finding is consistent with expectations based on Bergmann's Rule (Bergmann, 1847; reviewed in Ashton, Tracy & de Queiroz, 2000), detailed climatic data for these localities are generally lacking.

Furthermore, there appears to be a historical component to this pattern. In each of the three wide-ranging clades (Highland, Widespread and *M. principula*), the basal-most split separated northern and southern populations (with sympatry among these fundamental groups observed in the Widespread clade at Mahatsinjo). Within the Highland clade, there was a primary bifurcation between the single Andohahela specimen (a juvenile) and a clade subsequently branching into a second, nested north–south dichotomy. Student's *t*-tests (correcting for multiple comparisons, i.e. $P < 0.05/3$) conducted on each of these three north–south population samples likewise showed significantly larger-bodied individuals at lower latitudes in *M. principula* ($P = 0.0092$) and Highland ($P < 0.0001$) and the opposite relationship in Widespread ($P = 0.003$). Disentangling the relative influences of historical and environmental factors on body size variation will require additional sampling and analysis. However, it is clear that geographic variation should be more seriously considered in future studies of shrew tenrecs, particularly in groups suspected to contain cryptic species. For example, previous authors

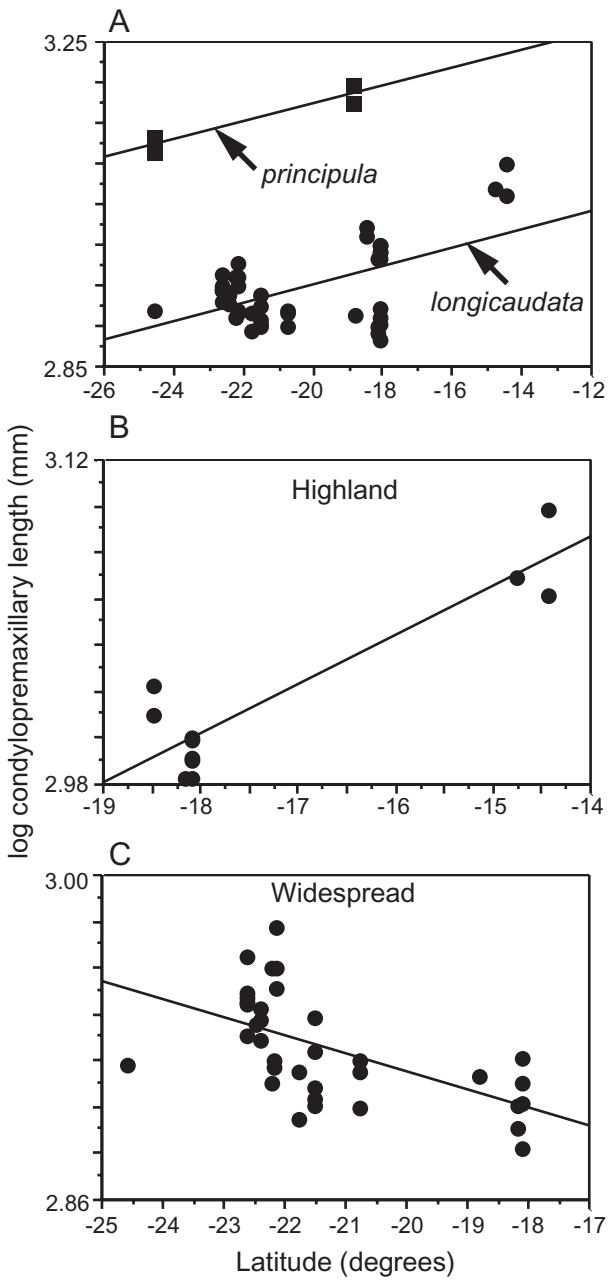



**Figure 6.** Correlation between condylopremaxillary length and latitude. Least squares regression lines are shown. A, *Microgale principula* and all specimens currently referred to *M. longicaudata*, both of which show a negative correlation between size and distance from the equator ($r = -0.96$ and $-0.55$; $P = 0.006$ and $<0.0001$; and $R^2 = 0.924$ and 0.323, respectively; the former is not significant when corrected for multiple comparisons). B, Highland haplotype clade individuals only ($r = -0.912$, $P < 0.0001$, $R^2 = 0.831$). C, Widespread haplotype clade members, which show a contrasting positive correlation with distance from the equator ($r = 0.580$, $P = 0.0002$, $R^2 = 0.336$).

have noted that subadults of other species of *Microgale* frequently exceed average adults in external and cranial measurements (MacPhee, 1987; Jenkins *et al*., 1996), an as-yet unexplained phenomenon that MacPhee (1987: p3) attributed to an 'almost reptilian propensity' for individual size variation. In fact, this phenomenon may be best explained by an alternative hypothesis of sympatric cryptic species. As we have shown, the possibility of cryptic shrew tenrec species occurring in sympatry is a reality, with juveniles of *M. longicaudata sensu stricto* collected from the same localities as were adult *M. majori* (Fig. 2). The potential for confusion can be exacerbated when contrasting patterns of clinal variation coincide with sympatric occurrences of cryptic species. Finally, the widespread sympatry and syntopy observed among different haplotype clades and size morphs, together with the contrasting patterns of clinal variation in body size, suggest that the morphological variation between these size morphs is primarily genetic rather than habitat-related.

We believe this study demonstrates the utility of molecular data to test and refine taxonomic boundaries among morphologically similar and closely related taxa, particularly when such taxa are broadly sympatric. Despite the numerous vagaries confounding attempts to identify morphological boundaries among long-tail shrew tenrecs species, including a preponderance of juvenile specimens, contrasting patterns of geographic variation, and widespread sympatry at broad spatial scales, our results suggest a relatively straightforward picture of habitat segregation and possible ecological separation among similarly sized taxa. While we readily acknowledge the inferential limitations due to small sample sizes for some clades, the strategy employed here has provided new insight into the patterns of diversity in Malagasy tenrecs. The revised taxonomic framework developed here, which has doubled the number of species of long-tailed shrew tenrecs, suggests the need for similar studies on the remaining species of tenrec, such that a more accurate picture of species diversity, historical diversification, geographic variation and ecological interactions can emerge. Finally, this study highlights the urgent need for continued collecting of voucher specimens for the rigorous documentation and interpretation of biological diversity. Seemingly well-intentioned attempts to circumvent the collection of complete specimens, such as photographing and releasing individuals (Smith *et al*., 1991), severely compromise the ability to pursue this fundamental goal of biological inquiry (Peterson & Lanyon, 1992; Goodman & Lanyon, 1994). Certainly, many of the conclusions reached in this study would not have been possible with genetic data only and would have left us with tantalizing but untestable hypotheses.

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## SUPPLEMENTARY MATERIAL

The following material is available from: http://www.blackwellpublishing.com/products/journals/suppmat/BIJ/BIJ366/BIJ366sm.htm

Table S1. Raw measurement data.

Table S2. ND2 alignment.

## REFERENCES

**Ade M. 1996.** Morphological observations on a *Microgale* specimen (Insectivora, Tenrecidae) from Western Madagascar. In: Ganzhorn JU, Sorg J-P, eds. *Ecology and economy of a*

*tropical dry forest in Madagascar. Primate report, special issue*. Göttingen: Deutsches Primatenzentrum, 251–255.

**Albrecht GH, Jenkins PD, Godfrey LR. 1990.** Ecogeographic size variation among the living and subfossil prosimians of Madagascar. *American Journal of Primatology* **22:** 1–50.

**Ashton KG, Tracy MC, de Queiroz A. 2000.** Is Bergmann's rule valid for mammals? *American Naturalist* **156:** 390–415.

**Avise JC. 2000.** *Phylogeography*. Cambridge: Harvard University Press.

**Avise JC, Shapiro JF, Daniel SW, Aquadro CF, Lansman RA. 1983.** Mitochondrial DNA differentiation during the speciation process in *Peromyscus*. *Molecular Biology and Evolution* **1:** 38–56.

**Ballard JWO, Chernoff B, James AC. 2002.** Divergence of mitochondrial DNA is not corroborated by nuclear DNA, morphology, or behavior in *Drosophila simulans*. *Evolution* **56:** 527–545.

**Baum DA, Shaw KL. 1995.** Genealogical perspectives on the species problem. In: Hoch PC, Stephenson AG, eds. *Experimental and molecular approaches to plant biosystematics*. St. Louis: Missouri Botanical Garden, 289–303.

**Bergmann C. 1847.** Uber die verhaltnisse der warmeokonomie der thiere zu ihrer grosse. *Göttinger Studien* **1:** 595–708.

**Carleton MD. 1994.** Systematic studies of Madagascar's endemic rodents (Muroidea: Nesomyinae): revision of the genus *Eliurus*. *American Museum Novitates* **3087:** 1–55.

**Carleton MD, Goodman SM. 1996.** Systematic studies of Madagascar's endemic rodents (Muroidea: Nesomyinae): a new genus and species from the Central Highlands. In: Goodman SM, ed. *A floral and faunal inventory of the eastern slopes of the Réserve Naturelle Intégrale d'Andringitra, Madagascar: with reference to elevational variation. Fieldiana: Zoology, N.S., 85: (1–319).* Chicago: Field Museum of Natural History, 231–256.

**Carleton MD, Schmidt DF. 1990.** Systematic studies of Madagascar's endemic rodents (Muroidea: Nesomyinae): an annotated gazetteer of collecting localities of known forms. *American Museum Novitates* **2987:** 1–36.

**Garbutt N. 1999.** *Mammals of Madagascar*. Sussex: Pica Press.

**Goodman M, Czelusniak J, Moore GW, Romero-Herrera AE, Matsuda G. 1979.** Fitting the gene lineage into its species lineage, a parsimony strategy illustrated by cladograms constructed from globin sequences. *Systematic Zoology* **28:** 132–163.

**Goodman SM, Ganzhorn JU, Olson LE, Pidgeon M, Soarimalala V. 1997.** Annual variation in species diversity and relative density of rodents and insectivores in the Parc National de la Montagne d'Ambre, Madagascar. *Ecotropica* **3:** 109–118.

**Goodman SM, Jenkins PD. 1998.** The insectivores of the Réserve Spéciale d'Anjanaharibe-Sud Madagascar. In: Goodman SM, ed. *A floral and faunal inventory of the Réserve Spéciale d'Anjanaharibe-Sud, Madagascar: with reference to elevational variation. Fieldiana: Zoology, N.S., 90: 1–246.* Chicago: Field Museum of Natural History, 139–162.

**Goodman SM, Jenkins PD. 2000.** Tenrecs (Lipotyphla: Tenrecidae) of the Parc National de Marojejy, Madagascar. In: Goodman SM, ed. *A floral and faunal inventory of the Parc National de Marojejy, Madagascar: With reference to elevational variation. Fieldiana: Zoology, N.S., 97: 201–229.* Chicago: Field Museum of Natural History.

**Goodman SM, Jenkins P, Pidgeon M. 1999.** The Lipotyphla (Tenrecidae and Soricidae) of the Réserve Naturelle Intégrale d'Andohahela, Madagascar. In: Goodman SM, ed. *A floral and faunal inventory of the Réserve Naturelle Intégrale d'Andohahela, Madagascar: with reference to elevational variation. Fieldiana: Zoology, N.S., 94: 187–216.* Chicago: Field Museum of Natural History.

**Goodman SM, Lanyon SM. 1994.** Scientific collecting. *Conservation Biology* **8:** 314–315.

**Goodman SM, Rakotondravony D. 2000.** The effects of forest fragmentation and isolation on insectivorous small mammals (Lipotyphla) on the Central High Plateau of Madagascar. *Journal of Zoology (London)* **250:** 193–200.

**Goodman SM, Rakotondravony D, Schatz G, Wilmé L. 1996a.** Species richness of forest-dwelling birds, rodents and insectivores in a planted forest of native trees: a test case from the Ankaratra, Madagascar. *Ecotropica* **2:** 109–120.

**Goodman SM, Raxworthy CJ, Jenkins PD. 1996b.** Insectivore ecology in the Réserve Naturelle Intégrale d'Andringitra. In: Goodman SM, ed. *A floral and faunal inventory of the eastern slopes of the Réserve Naturelle Intégrale d'Andringitra, Madagascar: With reference to elevational variation. Fieldiana: Zoology, N.S., 85: 1–319.* Chicago: Field Museum of Natural History, 218–230.

**Grandidier G. 1928.** Description de deux nouveaux mammifères insectivores de Madagascar. *Bulletin du Museum National d'Histoire Naturelle, Paris, Sér. 2* **34:** 63–70.

**Heim de Balsac H. 1972.** Insectivores. In: Battistini R, Richard-Vindard G, eds. *Biogeography and ecology in Madagascar*. The Hague: W. Junk, 629–660.

**Helme NA, Rakotomalaza PJ. 1999.** An overview of the botanical communities of the Réserve Naturelle Intégrale d'Andohahela, Madagascar. In: Goodman SM, ed. *A floral and faunal inventory of the Réserve Naturelle Intégrale d'Andohahela, Madagascar: with reference to elevational variation. Fieldiana: Zoology, N.S., 94.* Chicago: Field Museum of Natural History, 25–50.

**Hey J. 2001.** *Genes, categories, and species: the evolutionary and cognitive causes of the species problem*. New York: Oxford University Press.

**Hudson RR, Coyne JA. 2002.** Mathematical consequences of the genealogical species concept. *Evolution* **56:** 1557–1565.

**Huelsenbeck JP, Ronquist FR. 2002.** Mr Bayes: a program for the Bayesian inference of phylogeny. Version 2.01. Rochester, NY.

**Hutterer R. 1993.** Order Insectivora. In: Wilson DE, Reeder DM, eds. *Mammal species of the world, a taxonomic and geographic reference*, 2nd edn. Washington D.C.: Smithsonian Institution Press, 69–130.

**Jenkins PD. 1992.** Description of a new species of *Microgale* (Insectivora: Tenrecidae) from eastern Madagascar. *Bulletin of the British Museum of Natural History (Zoology)* **58:** 53–59.

**Jenkins PD. 1993.** A new species of *Microgale* (Insectivora: Tenrecidae) from eastern Madagascar with an unusual dentition. *American Museum Novitates* **3067:** 1–11.

**Jenkins PD. 2003.** *Microgale*, shrew tenrecs. In: Goodman SM, Benstead JP, eds. *The natural history of Madagascar*. Chicago: The University of Chicago Press, 1273–1278.

**Jenkins PD, Goodman SM. 1999.** A new species of *Microgale* (Lipotyphla, Tenrecidae) from isolated forest in southwestern Madagascar. *Bulletin of the British Museum of Natural History (Zoology)* **65:** 155–164.

**Jenkins PD, Goodman SM, Raxworthy CJ. 1996.** The shrew tenrecs (*Microgale*) (Insectivora: Tenrecidae) of the Réserve Naturelle Intégrale d'Andringitra, Madagascar. In: Goodman SM, ed. *A floral and faunal inventory of the eastern slopes of the Réserve Naturelle Intégrale d'Andringitra, Madagascar: With reference to elevational variation. Fieldiana: Zoology, N.S., 85: (1–319).* Chicago: Field Museum of Natural History, 191–217.

**Jenkins PD, Raxworthy CJ, Nussbaum RA. 1997.** A new species of *Microgale* (Insectivora, Tenrecidae), with comments on the status of four other taxa of shrew tenrecs. *Bulletin of the British Museum of Natural History (Zoology)* **63:** 1–12.

**Larget B, Simon D. 1999.** Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* **16:** 750–759.

**Lees DC, Kremen C, Andriamampianina L. 1999.** A null model for species richness gradients: bounded range overlap of butterflies and other rainforest endemics in Madagascar. *Biological Journal of the Linnean Society* **67:** 529–584.

**MacPhee RDE. 1987.** The shrew tenrecs of Madagascar: Systematic revision and Holocene distribution of *Microgale* (Tenrecidae, Insectivora). *American Museum Novitates* **2889:** 1–45.

**Maddison WP. 1997.** Gene trees in species trees. *Systematic Biology* **46:** 523–536.

**Maddison DR, Maddison WP. 2000.** *MacClade 4. Analysis of phylogeny and character evolution.* Version 4.0. Sunderland, MA: Sinauer Associates.

**Mayden RL. 1997.** A hierarchy of species concepts: the denouement in the saga of the species problem. In: Claridge MF, Dawah HA, Wilson MR, eds. *Species: the units of biodiversity*. New York: Chapman & Hall, 381–424.

**Mayr E. 1963.** *Animal species and evolution*. Cambridge: Harvard University Press.

**Mittermeier RA, Mittermeier CG. 1997.** Madagascar. In: Mittermeier RA, Gil PR, Mittermeier CG, eds. *Megadiversity. Earth's biologically wealthiest nations*. Monterrey, Mexico: CEMEX, 209–223.

**Moore WS. 1995.** Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution* **49:** 718–726.

**Morrison-Scott TCS. 1948.** The insectivorous genera *Microgale* and *Nesogale* (Madagascar). *Proceedings of the Zoological Society of London* **118:** 817–822.

**Mouchaty SK, Gullberg A, Janke A, Arnason U. 2000.** Phylogenetic position of the tenrecs (Mammalia: Tenrecidae) of Madagascar based on analysis of the complete mitochondrial genome sequence of *Echinops telfairi*. *Zoologica Scripta* **29:** 307–317.

**Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J. 2000.** Biodiversity hotspots for conservation priorities. *Nature* **403:** 853–858.

**Nixon KC, Wheeler QD. 1990.** An amplification of the phylogenetic species concept. *Cladistics* **6:** 211–223.

**Olson LE. 1999.** *Systematics, evolution, and biogeography of Madagascar's tenrecs (Mammalia: Tenrecidae). Committee on Evolutionary Biology*. Chicago: University of Chicago, 345.

**Olson LE, Goodman SM. 2003.** Phylogeny and biogeography of tenrecs. In: Goodman SM, Benstead JP, eds. *The natural history of Madagascar*. Chicago: The University of Chicago Press, 1235–1242.

**Otte D, Endler J**, eds. **1989.** *Speciation and its consequences*. Sunderland, MA: Sinauer.

**Pamilo P, Nei M. 1988.** Relationships between gene trees and species trees. *Molecular Biology and Evolution* **5:** 568–583.

**Patton JL, Brylski PV. 1987.** Pocket gophers in alfalfa fields: Causes and consequences of habitat-related body size variation. *American Naturalist* **130:** 493–506.

**Peterson AT, Lanyon SM. 1992.** New bird species, DNA studies and type specimens. *Trends in Ecology and Evolution* **7:** 167–168.

**Pigliucci M. 2003.** Species as family resemblance concepts: the (dis-) solution of the species problem? *Bioessays* **25:** 596–602.

**Posada D, Crandall KA. 1998.** Modeltest: testing the model of DNA substitution. *Bioinformatics* **14:** 817–818.

**Posada D, Crandall KA. 2001.** Selecting the best-fit model of nucleotide substitution. *Systematic Biology* **50:** 580–601.

**Puorto G, Salomão MG, Theakston RDG, Thorpe RS, Warrell DA, Wüster W. 2001.** Combining mitochondrial DNA sequences and morphological data to infer species boundaries: phylogeography of lanceheaded pitvipers in the Brazilian Atlantic forest, and the status of *Bothrops pradoi* (Squamata: Serpentes: Viperidae). *Journal of Evolutionary Biology* **14:** 527–538.

**de Queiroz K. 1998.** The general lineage concept of species, species criteria, and the process of speciation: a conceptual unification and terminological recommendations. In: Howard DJ, Berlocher SH, eds. *Endless forms. Species and speciation*. New York: Oxford University Press, 57–75.

**Rakotondrainibe F, Raharimalala F. 1998.** The pteridophytes of the Réserve Spéciale d'Anjanaharibe-Sud, Madagascar: Floristic analysis and altitudinal distribution. In: Goodman SM, ed. *A floral and faunal inventory of the Réserve Spéciale d'Anjanaharibe-Sud, Madagascar: with reference to elevational variation. Fieldiana: Zoology, N.S., 90: 1–246.* Chicago: Field Museum of Natural History, 17–38.

**Rasoloarison RM, Goodman SM, Ganzhorn JU. 2000.** Taxonomic revision of mouse lemurs (*Microcebus*) in the western portions of Madagascar. *International Journal of Primatology* **21:** 963–1019.

**Raxworthy CJ, Nussbaum RA. 1994.** A rainforest survey of amphibians, reptiles and small mammals at Montagne d'Ambre, Madagascar. *Biological Conservation* **69:** 65–73.

**Shaw KL. 1998.** Species and the diversity of natural groups. In: Howard DJ, Berlocher SJ, eds. *Endless forms: species and speciation*. Oxford: Oxford University Press, 44–56.

**Slatkin M, Maddison WP. 1989.** A cladistic measure of gene flow infered from the phylogenies of alleles. *Genetics* **123:** 603–613.

**Smith EFG, Arctander P, Fjeldså J, Amir OG. 1991.** A new species of shrike (Laniidae: *Laniarius*) from Somalia, verified by DNA sequence data from the only know individual. *Ibis* **133:** 227–235.

**Swofford DL. 2002.** *PAUP*: phylogenetic analysis using parsimony (*and other methods).* Version 4.0. Sunderland, MA: Sinauer Associates.

**Thomas O. 1882.** Description of a new genus and two new species of Insectivora from Madagascar. *Journal of the Linnean Society of London* **16:** 319–322.

**Thomas O. 1918.** On the arrangement of the small Tenrecidae hitherto referred to *Oryzorictes* and *Microgale*. *Annals and Magazine of Natural History* **1:** 302–307.

**Thomas O. 1926.** On some small mammals from Madagascar. *Annals and Magazine of Natural History* **17:** 250–252.

**Wiens JJ, Penkrot TA. 2002.** Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (*Sceloporus*). *Systematic Biology* **51:** 69–91.

**Yoder AD, Rasoloarison RM, Goodman SM, Irwin JA, Atsalis S, Ravosa MJ, Ganzhorn JU. 2000.** Remarkable species diversity in Malagasy mouse lemur (primates, *Microcebus*). *Proceedings of the National Academy of Sciences, USA* **97:** 11325–11330.

**Zimmermann E, Cepok S, Rakotoarison N, Zietemann V, Radespiel U. 1998.** Sympatric mouse lemurs in north west Madagascar: a new rufous mouse lemur species (*Microcebus ravelobensis*). *Folia Primatologica* **69:** 106–114.

## APPENDIX 1

### SPECIMENS EXAMINED AND COLLECTING LOCALITIES

In most cases, locality data were taken directly from collectors' field catalogues. Names in capital letters refer to the localities as identified in Figure 1, which in some cases include multiple sampling sites too closely situated to differentiate at the scale illustrated. FMNH, Division of Mammals, Field Museum of Natural History; SMG, Uncatalogued specimens collected by S. M. Goodman housed at UADBA; UADBA, Département de Biologie Animale, Université d'Antananarivo; USNM, Division of Mammals, United States National Museum.

*MONTAGNE tD'AMBRE*

*Antsiranana Province, Montagne d'Ambre National Park*

5.5 km SW Joffreville (Ambohitra) 12°31′S, 49°10′E, 1000 m: *M. longicaudata* FMNH 156316, 156321; near Station des Roussettes, 5.5 km SW Joffreville, 12°31′38′S, 49°10′18′E, 1000 m: *M. longicaudata* FMNH 154560; Grande Lac, 12 km SW Joffreville (Ambohitra) 12°35.8′S, 49°09.6′E, 1325 m: *M. longicaudata* FMNH 156317, 156320.

*MANONGARIVO*

*Antsiranana Province, Manongarivo Special Reserve*

12.8 km (228°) SW Antanambao, 13°58.6′S, 48°25.4′E, 785 m: *M. majori* FMNH 166232; 14.5 km (220°) SW Antanambao, 14°0.0′S, 48°25.7′E, 1240 m: *M. longicaudata* FMNH 166233, UADBA 11585, *M. majori* UADBA 11584; 17.3 km (218°) SW Antanambao, 14°01.3′S, 48°25.1′E, 1600 m: *M. longicaudata* FMNH 166202, *M. majori* FMNH 166203.

*MAROJEJY*

*Antsiranana Province, Marojejy National Park*

Along tributary of Manantenina River, 11 km NW Manantenina, Antranohofa, 14°26.2′S, 49°44.5′E, 1225 m: *M. longicaudata* FMNH 159667; 10.5 km NW Manantenina, along tributary at head of Andranomifototra River, 14°26.4′S, 49°44.5′E, 1625 m: *M. longicaudata* FMNH 159668, 159669, 159671.

*BETAOLANA*

Antsiranana Province, Betaolana Forest, 11.0 km NW Ambodiangezoka, 14°36.6′S, 49°25.5′E, 1200 m: *M. longicaudata* FMNH 167489, SMG 11083.

*ANJANAHARIBE-SUD*

*Antsiranana Province, Anjanaharibe-Sud Special Reserve*

9.2 km WSW Befingitra, 14°44.7′S, 49°27.7′E, 1260 m: *M. longicaudata* FMNH 154005; 6.5 km SSW Befingitra, 14°45.3′S, 49°30.3′E, 875 m: *M. principula* UADBA 10540.

Mahajanga Province, western slope of Anjanaharibe-Sud, 13.0 km SW Befingotra, 14°45.9′S, 49°25.9′E, 1600 m: *M. longicaudata* FMNH 167435.

*AMBOHITANTELY*

*Antananarivo Province, Ambohitantely Special Reserve*

28 km NNE Ankazobe, 18°06.4′S, 47°15.1′E, 1500 m: *M. longicaudata* FMNH 165483, 165486; 24 km NE Ankazobe, 18°10.1′S, 47°16.6′E, 1450 m: *M. longicaudata* FMNH 165474, 165478, 165546, UADBA 10413, 10415, 10417, 10418, *M. majori* FMNH 165481, 165547, *M. pusilla* FMNH 165489.

*AMBOHIJANAHARY*

*Mahajanga Province, Ambohijanahary Special Reserve*

Ankazotsihitafototra Forest, 18°15.7′S, 45°25.2′E, 1150 m: *M. majori* FMNH 167537, 167538, 167539, 167540, 167541, 167542.

*ANJOZOROBE*
Antananarivo Province, 2 km NNE Andranomay, 13 km SE Anjozorobe, 1300 m, 18°28.8′S, 47°57.3′E: *M. longicaudata* FMNH 159450, 159451, 159452, 159455.

*ANALAMAY, AMBATOVY & TOROTOROFOTSY*
Toamasina Province, Analamay, 18°49.7′S, 48°19.9′E, 969 m: *M. longicaudata* UADBA 10568, *M. majori* UADBA 10569; Ambatovy, 18°51.1′S, 48°18.5′E, 1164 m: *M. majori* UADBA 11359, *M. principula* UADBA 11355, 11356; Torotorofotsy, 18°51.3′S, 48°21.3′E, 980 m: *M. majori* UADBA 11354.

*ANKARATRA*
Antananarivo Province, Ankaratra Massif, Nosiarivo Forest, 2 km NNW (by air) Manjakatompo Station, 19°20.7′S, 47°18.2′E, 2000 m: *M. longicaudata* FMNH 156204, 156205, 156206, UADBA 11624.

*MAHATSINJO & ANKILAHILA*
Antananarivo Province, 10 km SE Tsinjoarivo, Mahatsinjo Forest, Andasivodihazo, 19°40.8′S, 47°46.2′E, 1550 m: *M. majori* FMNH 166119, UADBA 11847, *M. pusilla* FMNH 166123, 166124; 16.2 km SE Tsinjoarivo, Ankilahila Forest, along Andrindrimbolo River, 19°42.4′S, 47°50.1′E, 1400 m: *M. majori* FMNH 166149, 166171, 166172, UADBA 10704.

*ITREMO*
Fianarantsoa Province, Ianasana Forest, 7 km W Itremo, 20°36.1′S, 46°34.3′E, 1630 m: *M. pusilla* FMNH 166040.

*ANKAZOMIVADY*
Fianarantsoa Province, 28 km SSW Ambositra, 5 km SW Ambalamanakana, Ankazomivady Forest, 20°46.5′S, 47°10.1′E, 1675 m: *M. longicaudata* FMNH 161740, *M. majori* FMNH 161730, 161731, 161732, 161734, 161735, 161736, 161737, 161738, 161739, 161782.

*RANOMAFANA*
Fianarantsoa Province, Ranomafana National Park, Vatoharanana, 4.0 km SW Ranomafana (village), 21°17.4′S, 47°26.0′E, 1025 m: *M. principula* FMNH 170764.

*ANDRAMBOVATO*
Fianarantsoa Province, 2 km W. Andrambovato, along Tatamaly River, 21°30.7′S, 47°24.6′E, 1075 m: *M. majori* FMNH 170767, 170768, 170769, 170770, 170771, 170772, SMG 11768, 11769, 11791.

*VINANTELO*
Fianarantsoa Province, Vinantelo Forest, at foot of Ambodivohitra, 15.5 km SE Vohitrafeno, 21°46.6′S, 47°20.8′E, 1100 m: *M. majori* FMNH 170773, 170878, 170879, SMG 11821.

*MANAMBOLO*
Fianarantsoa Province, Manambolo Forest, Ambavafatra, along Andohabatotany River, 17.5 km SE Sendrisoa, 22°8′58′S, 47°1′25′E, 1300 m: *M. majori* FMNH 167606, *M. pusilla* FMNH 167619; 19.5 km SE Sendrisoa, 22°9.8′S, 47°2.5′E, 1600 m: *M. longicaudata* FMNH 167575, *M. majori* UADBA 11730.

*ANDRINGITRA*
*Fianarantsoa Province, Andringitra National Park*
Anjavidilava, 8.5 km SE Antananifotsy, 22°09.5′S, 46°57.6′E, 1990 m: *M. majori* FMNH 159485, 159501, 159502; 8.5 km SE Antanifotsy, Andohan 'Ambolo encampment, 22°10.273′S, 46°56.756′E, 1960 m: *M. majori* FMNH 161689, 165698; ~38 km S. Ambalavao, on ridge east of Volotsangana River, 22°11'39′S, 46°58′16′E, 1625 m: *M. majori* FMNH 151632; Forêt de Ravaro, 12.5 km SW Antanifotsy, 22°12.7′S, 46°50.7′E, 1500 m: *M. majori* FMNH 167969, 167970.

Fianarantsoa Province, ~45 km S Ambalavao, east bank Iantara River, along Ambalamanenjana-Ambatomboay trail, edge of Andringitra National Park, 22°13′20′S, 47°01′29′E 720 m: *M. majori* FMNH 151630.

*IVOHIBE*
Fianarantsoa Province, 8 km NE Ivohibe, 5.5 km SE Angodongodona, 22°25.3′S, 46°53.9′E, 1200 m: *M. majori* FMNH 161982, 161983, 161984, UADBA 10310.

Fianarantsoa Province, exterior northern limit Ivohibe Special Reserve, along Hefitany River, ~7.5 km ENE Ivohibe, 22°28.2′S, 46°57.6′E, 900 m: *M. majori* FMNH 161976.

*ANALAVELONA*
Toliara Province, Analavelona Forest, near source of Manasay River, 16.5 km NW Andranoheza, 22°38.5′S, 44°10.3′E, 1250 m: *M. majori* FMNH 169743, 169744, 169745, 169746, 169747, 169748.

*ANDOHAHELA*
Toliara Province, Andohahela National Park, parcel 1, 20 km SE Andranondambo 24°33.7′S, 46°43.3′E, 1875 m: *M. longicaudata* FMNH 156578; 12.5 km NW Eminiminy, 24°35.6′S, 46°44.3′E, 810 m: *M. majori*

FMNH 156579, 156583, *M. principula* FMNH 156591; 8 km NW Eminiminy, 24°37.55′S, 46°45.92′E, 440 m: *M. principula* FMNH 156575.

Toliara Province, Marosohy Forest, at edge of Andohahela National Park, 16 km WNW Ranomafana du Sud, 24°34′S, 46°48′E, 725 m: *M. principula* USNM 578758, 578759, 578760, 578761, 578767.

## APPENDIX 2

### PRIMERS

The following primers were used for both amplification and/or sequencing in various combinations (depending on sample). Numbers in parentheses represent the position of the 3′ nucleotide on the heavy strand of the human mitochondrial genome (GenBank accession number V00662). The first three letters of each primer refer to its location by gene (Met, tRNA-methionine; 16S, 16S rRNA; ND2, NADH dehydrogenase subunit 2; Trp, tRNA-Trp; CO1, cytochrome oxidase subunit I). Standard IUB codes are used for degenerate positions.

*Forward primers*
Met-1: CTAATAAAGCTTTCGGGCCCATAC (4436)
16S-F1: ACGACCTCGATGTTGGATCA (3000)
ND2-3TX: TAGCMCCATTYCACTTCTGA (4811)
ND2-3TY: ACTAGGCATAGCCCCATTCCACTT (4809)
ND2-3TZ: YCAAATCCACCMATCACT (4915)
ND2-4TX: TAATATCHATAGGAGG (5227)
ND2-LTF1: CCCCCGAACAACTGAAGCAG (4635)
ND2-LTF2: TACCAAATCCACCMATCACT (4915)
ND2-LTF3: GCACACATGGGTTGAATAGCMGCA (4971)
ND2-LTF4: ACTATTCTAATAAACTGCTCCTC (5140)
ND2-LTF5: CAGTAACAATAGCAGTAATAGC (5326)

*Reverse primers*
Trp-2: TTCTACTTAAGGCTTTGAAGGC (5540)
Trp-2M: GGGCTATGAAGGCTCTTGG (5534)
Trp-2T: GCTTTGAAGGCTCTTGGTC (5532)
ND2-4: ACTTCTGGTACTCAGAAGTGGAA (4800)
CO1-R1: GTTCCRATATCTTTGTGGTT (5934)
ND2-LOR2: GAGTAGGCTATGATTTTDCGTA (4993)
ND2-LTR1: ATTATTGAGGCTGAGGCTTG (4656)
ND2-LTR2: TGGTGCCTTGGGTTACTTCTGG (4815)
ND2-LTR3: CTCAACCTCCAATTAGRATTGA (4950)
ND2-LTR4: GAATAGTAAACATTGCTAGTG (5104)
ND2-LTR5: TGGTTATTTCATGTYATGGATA (5158)
ND2-LTR6: TTGGGGATAAATCCTGATAG (5238)
ND2-LTR7: GAGGATGCGTAGATTAGTCG (5352)
ND2-MR1: TACTGCTGCYATTCATCC (5033)
ND2-MR2: GGTATATGATTGAAAGGGGGGCTAG (4877)
ND2-MR3: ATTGATAAAACAGCGGATGTTA (4933)

## APPENDIX 3

### MEASUREMENTS

Bilateral measurements marked with an asterisk (*) were taken on both sides and averaged for all analyses unless one side was damaged, in which case only the undamaged side was measured; the remainder were taken from the left side only (or right side only when the left side was damaged). Measurements denoted with a dagger (†) were recorded for comparison with published studies but were not included in morphometric analyses for reasons given in their definitions. All cranioskeletal measurements were taken as minimum distance between landmarks, i.e. no attempt was made to hold calipers parallel or orthogonal to a given axis (e.g. the midline of the skull or femoral shaft). Only adults, defined by the presence of fully erupted permanent dentition (e.g. Jenkins, Goodman & Raxworthy, 1996), were included. Dental loci are identified by tooth type (I, incisor; C, canine; P, premolar; and M, molar) and position, e.g. I3 refers to the third upper incisor of the permanent dentition (only upper teeth are considered here). We follow the dental nomenclature of MacPhee (1987).

*Condyloincisive length (CIL)†:* Posterior-most (caudal) surface of occipital condyle to anterior-most (rostral) surface of $I^1$. CPM (see below) was used instead of the more commonly employed CIL as a measure of skull length because CIL is sensitive to the presence/absence of $I^1$ on a given specimen as well as its looseness within the alveolus when measured with calipers.
*Condylopremaxillary length (CPM)*:* Posterior-most (caudal) surface of occipital condyle to anterior-most (rostral) surface of the premaxilla.
*Greatest skull length (GSkL)†:* Greatest distance between rostral and caudal surfaces of skull (without attempting to measure along an axis parallel to palate or tooth row). This measurement does not rely on explicit landmarks and was recorded only for comparison with MacPhee (1987). We use CPM (above) as a measure of skull length in the morphometric analyses.
*Condylo-I2 length (CI2)*:* Posterior-most (caudal) surface of occipital condyle to anterior-most (rostral) surface of $I^2$.
*Condylo-P3 length (CP3)*:* Posterior-most (caudal) surface of occipital condyle to anterior-most (rostral) surface of $P^3$.
*Condylo-entoglenoid length (CEG)*:* Posterior-most (caudal) surface of occipital condyle to anterior-most (rostral) surface of the entoglenoid process of the squamosal.
*Paroccipital process to entoglenoid length (PEG)*:* Rostral surface of entoglenoid process of squamo-

sal to caudal surface of paroccipital process of basioccipital.

*Greatest breadth across M3 (M3B):* Greatest breadth across $M^3$, as measured from lateral surface of distostyle.

*Greatest breadth across M2 (M2B):* Greatest breadth across $M^2$, as measured from lateral surface of distostyle.

*Greatest posterior width across M1 (M1WP):* Greatest breadth across $M^1$ as measured from lateral surface of distostyle.

*Greatest anterior width across M1 (M1WA):* Greatest breadth across $M^1$ as measured from lateral surface of anterior ectostyle and/or mesiostyle.

*Greatest width across P4 (P4W):* Greatest breadth across $P^4$.

*Greatest width across P3 (P3W):* Greatest breadth across $P^3$.

*Greatest width across I2 (I2W):* Greatest breadth across $I^2$.

*Premaxillary to zygomatic length (PZ)*:* Rostral surface of premaxilla to caudal surface of zygomatic process of maxilla.

*Upper toothrow length (UTR)*:* Rostral surface of $I^1$ to caudal surface of $M^3$.

*C1 to I1 length (C1I1)*:* Rostral surface of $I^1$ to caudal surface of posterior accessory cusp of $C^1$.

*M3 to P3 length (M3P3)*:* Rostral surface of $P^3$ to caudal surface of $M^3$.

*Braincase breadth (BB):* Greatest cranial breadth, as measured across squamosals.

*Greatest breadth across petrosals (PB):* Greatest breadth across petrosals as measured from lateral surface of prominence of lateral semicircular canal.

*Height of mandible (MH)*:* Greatest distance between coronoid and angular processes of mandible.

*Mandibular condyle width (MCW)*:* Greatest breadth across buccal and labial surfaces of mandibular condyle.

*Femur, lateral condyle to greater trochanter (FGT):* Distal surface of lateral condyle to proximal surface of greater trochanter.

*Femur, medial condyle to head (FMH):* Distal surface of medial condyle to proximal surface of femoral head.

*Femur, medial condyle to lesser trochanter (FLT):* Distal surface of medial condyle to proximal surface of medial condyle.

*Greatest length humerus (HGL):* Proximal surface of humeral head to distal surface of trochlear.

*Humerus, head to capitulum length (HHC):* Proximal surface of humeral head to distal surface of capitulum.

*Humerus, greatest distal condylar width (HCW):* Greatest breadth across distal condyles.

*Total length (TL):* Tip of rostrum to tip of tail.

*Tail length (TV):* External length of tail.

*Head and body length (HB)†:* Tip of nose and caudal point of the body (at base of tail). Counterintuitively, this is often unequal to the calculated difference between TL and TV. HB was not recorded in the field for several specimens included in this study and cannot be accurately measured on prepared skins or fluid-preserved carcasses. As such this measurement was recorded for comparison with published studies but was not included in morphometric analyses. HB was calculated as TL-TV for Figure 3 to include the maximum number of specimens; similar comparisons on the subset of specimens for which traditional field measurements of HB were available reveals similar patterns with respect to large-bodied *Microgale longicaudata* specimens falling in the range previously proposed for *M. principula* (not shown).