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Tenrec Phylogeny and the Noninvasive Extraction of Nuclear DNA

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Abstract.—Due in part to scarcity of material, no published study has yet cladistically addressed the systematics of living and fossil Tenrecidae (Mammalia, Afrotheria). Using a noninvasive technique for sampling nuclear DNA from museum specimens, we investigate the evolution of the Tenrecidae and assess the extent to which tenrecids fit patterns of relationships proposed for other terrestrial mammals on Madagascar. Application of several tree-reconstruction techniques on sequences of the nuclear growth hormone receptor gene and morphological data for all recognized tenrecid genera supports monophyly of Malagasy tenrecids to the exclusion of the two living African genera. However, both parsimony and Bayesian methods favor a close relationship between fossil African tenrecs and the Malagasy *Geogale*, supporting the hypothesis of island paraphyly, but not polyphyly. More generally, the noninvasive extraction technique can be applied with minimal risk to rare/unique specimens and, by better utilizing museum collections for genetic work, can greatly mitigate field expenses and disturbance of natural populations. [Afrotheria; DNA; fossils; Madagascar; museums; phylogeny; Tenrecs.]

With the exception of a single genus of shrew (*Suncus*), insectivoran-grade mammals from Madagascar are members of the family Tenrecidae (Eisenberg and Gould, 1970; Olson and Goodman, 2003). This group of placental mammals consists of eight genera endemic to Madagascar and two from equatorial Africa and is remarkably diverse, occupying terrestrial, semiarboreal, fossorial, and semiaquatic niches. Other Malagasy groups are similarly diverse; previous morphological investigations of its primates (Cartmill, 1975; Yoder, 1992), carnivorans (Veron, 1995), and rodents (Ellerman, 1940, 1941), as well as its tenrecs (Butler, 1984; Asher, 1999, 2000), have indicated multiple sister-group relationships with mainland taxa within each group.

Given the absence of modern taxa from the Malagasy Cretaceous and the isolation of Madagascar from other landmasses over the past ca. 80 to 90 million years (Krause, 2003), dispersal has become the primary hypothesis for explaining the arrival of many of Madagascar's inhabitants (cf. Raxworthy et al., 2002; Zakharov et al., 2004). Phylogeny can further illustrate the biogeographic history of a given group. Monophyly (Fig. 1A) and paraphyly (Fig. 1B) of island taxa are compatible with a single dispersal event leading to island colonization, whereas polyphyly (Fig. 1C) implies multiple colonization events.

The aforementioned morphological studies noting the diversity of Malagasy mammalian groups have to varying degrees implied island polyphyly (Fig. 1C); i.e., that each of the modern groups has undergone multiple dispersal events across water barriers in order to colonize the island. In contrast, recent molecular phylogenies of terrestrial Malagasy mammals have supported island monophyly (Fig. 1A) for living primates (Yoder et al., 1996), carnivorans (Flynn et al., 2005), tenrecs (Olson and Goodman, 2003), and possibly rodents (Jansa and Weksler, 2004; Steppan et al., 2004; see discussion below).

Many tenrecid species are rare and/or endangered (Vogel, 1983; Benstead and Olson, 2003) and are difficult to obtain for research purposes. For example, the semiaquatic *Limnogale mergulus* is known from barely over a dozen museum specimens in Europe and North America. Destructive sampling of such material (e.g., for DNA sequencing) is generally not possible. Because it is so difficult to obtain tissues, most molecular studies sampling this group (e.g., Emerson et al., 1999; Mouchaty et al., 2000; Douady et al., 2002; Malia et al., 2002) have included between one and five of the over two dozen species. Olson and Goodman (2003) described a much better sample and were the first to publish a study with representatives of all living tenrecid genera, including sequences from one nuclear (vWF) and three mitochondrial (12S, tRNA-Valine, ND2) genes. However, as of this writing (August 2005), their DNA sequences and alignments are unavailable from public sources (e.g., GenBank). No published study has yet cladistically analyzed the three recognized fossil tenrecids, *Erythrozootes*, *Protenrec*, and *Parageogale* (Butler, 1984; McKenna and Bell, 1997). Jacobs et al. (1987) named a fourth fossil genus, *Ndamathaia*. However, we follow Morales et al. (2000) in regarding this taxon as a non-tenrecid.

In this article, we provide new DNA sequence data from the nuclear growth hormone receptor (GHR) gene using a noninvasive procedure applied to museum specimens. We also include a morphological data set, enabling us to sample all recognized living and extinct tenrecid genera. To reconstruct phylogenetic trees, we apply both maximum parsimony (MP) and a Markov *k* (Mk) model (Lewis, 2001) in a Bayesian framework (Nylander et al., 2004). Using these data we estimate the fit of living and fossil tenrecs to phylogenetic and biogeographic patterns proposed for other Malagasy groups.

MATERIALS AND METHODS

The Noninvasive Extraction Method

We obtained between 756 and 855 base pairs from exon 10 of the growth hormone receptor (GHR) gene from crania accessioned at the Zoologisches Museum Berlin (ZMB), Harvard Museum of Comparative Zoology (MCZ), and the Department of Ecology and Evolution, University of Lausanne (IZEA). Specifically, we used skulls of *Hemicentetes semispinosus* (ZMB 71599),

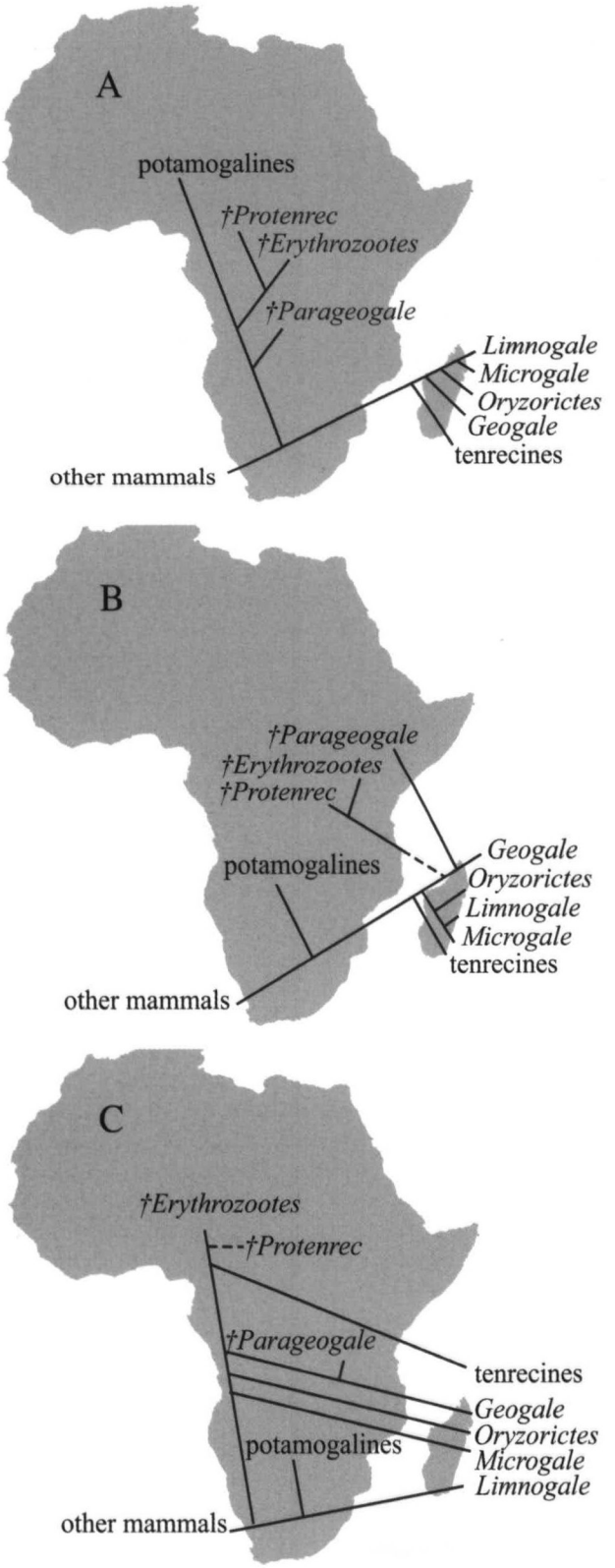

FIGURE 1. Biogeographic implications of (A) monophyly, consistent with a single dispersal event to colonize Madagascar (cf. Eisenberg, 1975; Olson and Goodman, 2003); (B) paraphyly, consistent with a single dispersal event coupled with limited back-migration from Madagascar to Africa (cf. Butler, 1985); and (C) polyphyly, consistent with multiple dispersal events between Africa and Madagascar (cf. Asher 2000: fig. R1-12). Dotted lines in B and C indicate uncertainty in the positions of *Erythrozootes* and *Protenrec*.

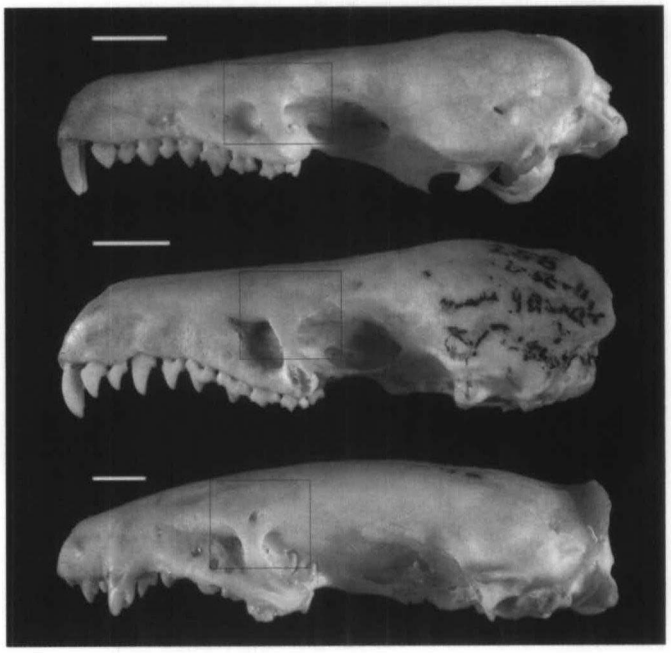

FIGURE 2. Lateral view of crania in *Micropotamogale* (top, IZEA 4975), *Limnogale* (middle, ZMB 35258), and *Setifer* (bottom, ZMB 44586), used for noninvasive extraction of nuclear GHR sequences. Images were taken after DNA extraction. Boxes highlight patent lacrimal foramen in *Setifer*, and absence thereof in *Micropotamogale* and *Limnogale*.

Limnogale mergulus (ZMB 35258; Fig. 2), *Potamogale velox* (ZMB 46588), *Setifer setosus* (ZMB 44586; Fig. 2), *Geogale aurita* (MCZ 45044), and *Micropotamogale lamottei* (IZEA 4975; Fig. 2). New sequences were aligned with previously published GHR sequences (Malia et al., 2002; Adkins et al., 2001; Pantel et al., 2000; van Garderen et al., 1999; Zogopoulos et al., 1999; Wang et al., 1995; Adams et al., 1990; Baumbach et al., 1989; Smith et al., 1989; Leung et al., 1987). Table 1 shows GenBank accession numbers for extant taxa, including DQ202287 to DQ202292, for our new sequences.

Expanding upon the method of Rohland et al. (2004) for mitochondrial DNA, we obtained nuclear GHR sequences from museum crania, leaving the treated specimens completely intact. We incubated either lower jaws or rostra in 20 mL of a buffer containing 5 M guanidinium isothiocyanate, 50 mM Tris, pH 8.0, 25 mM NaCl, 1.3% Triton-X, 20 mM EDTA, and 50 mM DTT. To minimize the possibility of damage, we incubated the specimens at room temperature and rotated them in near-vertical tubes that permitted flow of the buffer but kept specimens stationary. DNA was then eluted from the buffer and the specimens washed and dried as described in Rohland et al. (2004). The DNA was eluted in a final volume of 200 μL 1×TE. PCR amplification was done using 2 units of Taq Gold and 60 cycles under the conditions described in Hofreiter et al. (2002). Depending on the taxon, we used seven to nine primer pairs to amplify GHR sequences (Tables 2, 3). When possible, we designed at least one primer per primer pair that selected against human GHR sequence to avoid amplification of contaminating

TABLE 1. Taxon sample and accession numbers of taxa used in our sample of nuclear GHR sequences. Boldface indicates new GHR sequences; daggers indicate extinct taxa. For nomenclature we follow Nowak (1999) and Asher (2005).

High-level clade	Supra-generic clade	Genus	Accession number
Didelphimorphia	Didelphidae	*Monodelphis*	AF238491
Artiodactyla	Bovidae	*Bos*	X70041
	Capridae	*Ovis*	M82912
	Suidae	*Sus*	X54429
Carnivora	Canidae	*Canis*	AF133835
	Ursidae	*Ursus*	AF392879
Chiroptera	Phyllostomidae	*Artibeus*	AF392895
	Pteropodidae	*Pteropus*	AF392893
	Vespertilionidae	*Myotis*	AF392894
Hyracoidea	Procaviidae	*Procavia*	AF392896
Lipotyphla	Erinaceidae	*Echinosorex*	AF392887
		Erinaceus	AF392882
	Soricidae	*Blarina*	AF392880
		Crocidura	AF392884
		Sorex	AF392881
		Suncus	AF392888
	Talpidae	*Parascalops*	AF392883
Macroscelidea	Macroscelididae	*Elephantulus*	AF392876
Perissodactyla	Equidae	*Equus*	AF392878
Primates	Cercopithecidae	*Macaca*	U84589
		Papio	AF150751
	Hominidae	*Homo*	X06562
Proboscidea	Elephantidae	*Elephas*	AF332013
		Loxodonta	AF332012
Rodentia	Geomyidae	*Geomys*	AF332028
	Muridae	*Mus*	M33324
		Rattus	X16726
Scandentia	Tupaiidae	*Tupaia*	AF540643
Sirenia	Trichechidae	*Trichechus*	AF392891
Tenrecoidea	Chrysochloridae	*Chrysospalax*	AF392877
	Geogalinae	†*Parageogale*	—
		Geogale	**DQ202287**
	Oryzorictinae	*Limnogale*	**DQ202289**
		Microgale	AF392885
		Oryzorictes	AF392886
	Potamogalinae	*Micropotamogale*	**DQ202290**
		Potamogale	**DQ202291**
	Protenrecinae	†*Erythrozootes*	—
		†*Protenrec*	—
	Tenrecinae	*Echinops*	AF392889
		Hemicentetes	**DQ202288**
		Setifer	**DQ202292**
		Tenrec	AF392890
Tubulidentata	Orycteropodidae	*Orycteropus*	AF392892
Xenarthra	Myrmecophagidae	*Myrmecophaga*	AF392875

TABLE 3. Primers used to obtain tenrec GHR sequences.

Primer fragment	Sequence
F1g	G AAT TCAACAATGATGACT CTT GG
R1g	GAATGT CAG GTT CATAAC AAC TGG TAC
R1gap	AT CAT CAT CCT TTG CCC CA
F2	GCT TCTAAS CAT TGA CCT GC
F2a	CTAAGC ATT GAC TYD CAAAAATCA CT
R2	TG GTC AAG GCA CAA GAG ATC TA
F2g	G ATC RGA CAC AGA CAG RCT TCTAA
R2g	TTGATT CTT CTG GTC AAG GCA C
R2.2s right	GGT CAA GGC ACAAGA GAT CA
F3	GTGACATGT GTGATG GTACCT CAG AGG TG
R3	A KGA GCT GAC TCA GAY CCA
F3g	ACA RAG GTT RAAAGG GGAAG
R3g	TG GGC ATAAAA GTC GAT GTT TG
F4	TAG CTTACT GTC TMYWGAYRC TG
R4	CGG GGAAAG GAC CAC ACT C
R4gap1	GG GAC ATC CCT GCT TTAAG
F4gapg	CAG GTAAGC GAGATTACA CCA G
F4.1g	CAATAC CAC TTC TTAATG GTG GAT C
R4.1s right	CA CTG GAATAT CCC TGC TTTAAG
F5	GAC TTT TAT GCC CAG GTAAGC GAG
F5g	GC AGG GAG TGT GGT CCT TTC
R5g	CT GTG GTG ATG TAA CTG TCT TCC TG
R5gap	TGG TAA GGC TTT CTG TGG TGA
F6	A CCT GGC CAA GCC AAC TTCA
R6	GC ATC TCG GAG CTV GGK GCT
F6g	AC TTC TGT GAG GCA GAT GCC A
R6g	TGGACT ATATGGATG GAG GTATAG TCT G
F6gap	CAG ATG CCAAAAAGT GCATTG
R6gap	AGC TCG GGG CTC CTT CTG
F7	AGAAAG CCT TAC CAC TAC TGC TGT
R7	T GTT CAG TTG GTC TGT GCT CAC
F7gap	CA CCA CAG AAA GCC TTACCA CTA
F7gap	CA CCA CAG AAA GCC TTACCA CTA
R7 short a	T GTT CAG TTG GTC TGT GCT C

human DNA, ubiquitous in the environment (Hofreiter et al., 2001). Amplification of human sequences occurred regularly when it was not possible to select against human DNA, showing that not only mitochondrial but also nuclear human DNA is an abundant contaminant. Due to the variability of the GHR sequences, different primer pairs were used for the different species for some of the amplified fragments (Table 3). Amplification products were cloned using the TOPO TA cloning kit (Invitrogen, The Netherlands) and multiple clones sequenced.

TABLE 2. Primer pairs for the amplification of the seven fragments used to determine GHR sequences in the six tenrecid species. Primer sequences are listed in Table 3. The length of the products is given in base pairs, including primers. n.p.: no product obtained.

	1	2	3	4	5	6	7
Potamogale velox	n.p.	F2a/R2g 201	F3/R3 200	F4.1g/R4.1s 175 F4gapG/R4gap1 73	F5g/R5g 223	F6g/R6g 205	F7gap/R7shorta 195
Limnogale mergulus	F1g/R1g 181	F2/R2 199 F2a/R2g 201	F3g/R3g 225	F4/R4 217	F5/R5gap 264	F6g/R6g 205 F6/R6 206	F7/R7 189
Geogale aurita	F1g/R1g 181	F2/R2 199 F2a/R2g 201	F3g/R3g 225	F4/R4 217	F5/R5gap 264	F6g/R6g 205	F7gap/R7 195
Micropotamogale lamottei	n.p.	F2g/R2 209	F3g/R3g 225	F4.1g/R4.1s 175 F4gapG/R4gap1 73	F5g/R5g 223	F6g/R6g 205	F7gap/R7 195
Hemicentetes semispinosus	F1g/R1gap 147	F2/R2 199	F3/R3 200	F4/R4 217	F5/R5gap 264 F5g/R5g 223	F6gap/R6gap 150	F7/R7 189
Setifer setosus	F1g/R1gap 147	F2/R2 199	F3/R3 200	F4/R4 217	F5/R5gap 264 F5g/R5g 223	F6gap/R6gap 150	F7/R7 189

The challenges confronting ancient DNA studies (Hofreiter et al., 2001; Olson and Hassanin, 2003) are relevant to this work, as we used museum specimens collected nearly 100 years ago. Hence, we used appropriate laboratory techniques at a dedicated ancient DNA facility at the Max Planck Institut for Evolutionary Anthropology, Leipzig.

Figure 3 shows sequence overlap between adjacent fragments for all taxa. Except for *Potamogale*, which has a 6-bp gap between fragments 3 and 4, all species have continuous sequences when the amplified fragments are concatenated after trimming the primers. Table S1 details sequence overlap across our amplified fragments, all of which are identical within species, both between different fragments and alternative amplicons that span homologous regions. Moreover, each amplified fragment excluding primers is unique in our data set, making it highly unlikely that our sequences are chimaeric (see Olson and Hassanin, 2003). When compared to the available sequences in GenBank by Blast searches (Table S2), all fragments show closest matches to members of the Afrotheria, and 38 out of 47 fragments are closest to published GHR sequences of the Tenrecidae. Given the occasionally short length of the fragments, slightly closer matches to other members of Afrotheria are not surprising. Finally, except for two fragments from *Setifer setosus* (which match corresponding sequences from *Echinops* AF392889), all others differ slightly from previously published sequences available in GenBank.

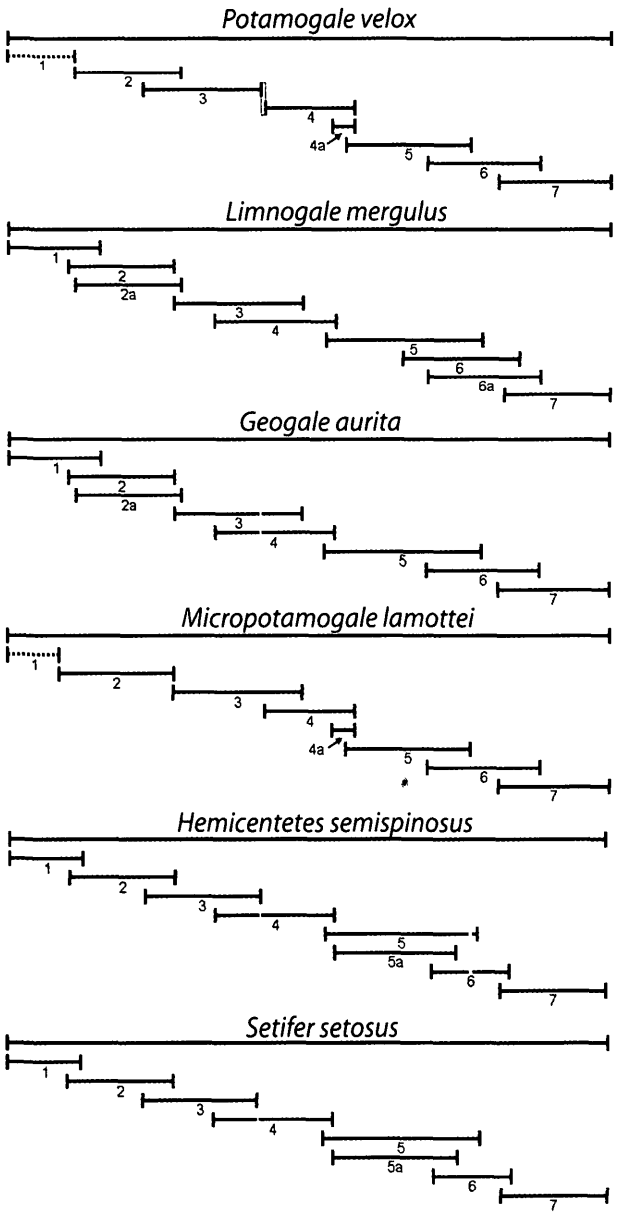

FIGURE 3. Schematic view of PCR fragments used to reconstruct GHR sequences (see also Tables 2 and 3). Dashed lines for *Potamogale* and *Micropotamogale* indicate missing sequences due to nonamplification of the first PCR fragment. A 6-bp gap between fragments 3 and 4 in the sequence data for *Potamogale* is indicated by the box. Deletions are shown as gaps in the sequence but do not represent missing data. The length of overlap among fragments is shown to scale.

Sequence Alignment

Using MacClade 4.07 (Maddison and Maddison, 2000) and Clustal X (Thompson et al., 1997), we concatenated GenBank files, added new sequences, and constructed alignments that preserved reading frames and contained few indels. GHR shows several conserved regions that facilitate a priori homology assessment. Nevertheless, some ambiguity remains regarding the positions of certain indels and adjacent nucleotides. Exploration of alignment ambiguity has occasionally (e.g., Messenger and McGuire, 1998), but not always (e.g., Douady et al., 2003), led to revised phylogenetic interpretations. For this reason, we explore a limited number of alternative alignments, differences across which are summarized in Table S3. Confidence indices mentioned in the text, as well as statistical comparisons of alternative topologies, are based on the first alignment (with the addition of the morphological partition, as indicated below), unless stated otherwise. Topological results were not significantly altered by using the other three alignments.

Each series of internal (i.e., not leading or trailing), contiguous gap characters was assumed to represent a single insertion and/or deletion event (indel). For all of our analyses including sequence data, we coded indels for each alignment, adding them as binary characters following the aligned nucleotides. Actual gap characters interspersed among the aligned nucleotides were treated as missing data. In Bayesian analyses, indels were treated using the binary (restriction site) model without assuming that all presence/absence characters have been observed (MrBayes command "LSET CODING=VARIABLE"). Sequence alignments and other supplementary data are available online at http://systematicbiology.org.

Morphological Data Collection

We used an anatomical dataset consisting of 126 characters, 20 of which are from the soft-tissues of the rostrum and cranial arterial supply, 46 from the cranium, 30

from the jaw and dentition, and 30 from the postcranial skeleton. Morphological characters were based on Asher (2000) and coded using specimens noted in Appendix 1. A nexus file with the morphological data is available at www.treebase.org (accession S1460).

Olson and Goodman (2003) questioned two coding decisions made by Asher (1999, 2000): occurrence of the fenestrate basioccipital in *Microgale* and morphology of the nasolacrimal duct (also known as the "lacrimal canal") in *Limnogale*. Olson and Goodman stated that Asher (1999) coded both as absent, whereas they noted that a fenestrate basioccipital occurs in some species of *Microgale* (Asher [1999] sampled only *M. talazaci*) and stated that *Limnogale* possesses a nasolacrimal duct. As of this writing, *M. talazaci* remains the only species of *Microgale* with nuclear DNA sequences available to us (Malia et al., 2002). Hence, we still have a limited sample of this genus, but accept Olson and Goodman's (2003) observation and code the genus *Microgale* as polymorphic for the fenestrate basioccipital (character no. 35) in this study.

Concerning the presence of a nasolacrimal duct in *Limnogale*, this was in fact not the character cited as a potential semiaquatic tenrec synapomorphy by Asher (1999, 2000). Rather, absence of an external lacrimal foramen (character no. 53) was coded in both studies, as depicted here in Figure 2. There is a clear osteological difference in the expression of a single, conspicuous lacrimal foramen at the anterior margin of the orbit, dorsal to the infraorbital canal, in most tenrecs (e.g., *Setifer*, Fig. 2). This region is smooth and without a major foramen in both potamogalines and *Limnogale* (Fig. 2). Hence, we retain the coding of Asher (1999, 2000) for the present study (see also Sánchez-Villagra and Asher, 2002). Expression of a nasolacrimal duct was coded separately from the lacrimal foramen in Asher (2000) based on observations of soft tissue anatomy in histologically prepared anatomical sections (Asher, 2001). To our knowledge, no histological preparation of *Limnogale* has ever been made, so we cannot compare the patent, partly soft tissue nasolacrimal duct in most tenrecs and other mammals with any such structure in *Limnogale*. Hence, we code this character (no. 15) "missing" for *Limnogale* in our morphological data matrix. Coding these two characters (duct, foramen) independently is justified by the variable expression of the lacrimal foramen in taxa with a patent nasolacrimal duct (e.g., Frahnert, 1999).

Phylogenetic Inference

The search strategies described below were applied to each of the four alignments summarized in Table S3 using a 43-taxon data set sampling only GHR and a 23-taxon data set sampling GHR plus morphology.

MP analyses were undertaken with PAUP 4.0b10 (Swofford, 2002); Bayesian algorithms were applied with MrBayes 3.1 (Huelsenbeck and Ronquist, 2001). For the full GHR taxon sample, our MP analyses searched heuristically with at least 100 random addition replicates with TBR branch swapping, multiple states treated as polymorphic, and branches with a zero length under any optimization collapsed. For our Bayesian and likelihood bootstrap analyses, we used the HKY+I+G model for the full GHR taxon sample (Fig. 4) and GTR+I+G as the optimal model for the smaller GHR sample (Fig. 5), as indicated by the AIC in MrModeltest 2.1 (Nylander, 2004). We used the default PAUP commands given in MrModeltest ("DSet distance=JC objective=ME base=equal rates=equal pinv=0 subst=all negbrlen=setzero; NJ showtree=no breakties=random") to obtain an initial tree, used by PAUP to estimate maximum likelihood (ML) parameters for the likelihood bootstrap analysis of the 43-taxon GHR data set (which resulted in the values "Lset Base=[0.2547 0.3078 0.2293] Nst=2 TRatio=2.1473 Rates=gamma Shape=1.7732 Pinvar=0.1990"). In addition, the ML bootstrap analysis excluded indels, used 143 pseudoreplicates of an "as-is" addition sequence with TBR branch swapping, and obtained starting trees with stepwise addition.

Bayesian analyses of the larger GHR dataset were based on at least four independent runs, each using a random starting tree and 1,000,000 generations with one cold and three heated chains, sampling trees every 100 generations. Bayesian runs of the 43-taxon GHR and the 23-taxon combined morphology-GHR data sets both reached stationarity between approximately 10,000 to 14,000 generations, as determined by visually inspecting asymptotic graphs of likelihood scores across generations. Our phylogenetic conclusions are based on only succeeding generations, starting at 15,000, discarding the first 14,900 (sampling every 100th generation) as "burn-in." Each run of 1,000,000 generations converged on a single, consistent result.

The taxon sample for the smaller, combined GHR-morphology data set was chosen based on availability of GHR sequences as well as osteological and soft tissue data from Asher (2000, 2001; see also Appendix 1). This sample included all genera of tenrecs, including fossils, plus *Orycteropus afer*, *Procavia capensis*, *Elephantulus brachyrhynchus*, a composite golden mole (*Chrysospalax trevelyani* GHR, *Chrysochloris stuhlmanni* and *C. asiatica* morphology), *Erinaceus europaeus*, three soricids, and *Canis latrans*, and was rooted with a composite didelphid (*Monodelphis domestica* GHR, *Didelphis* sp. morphology). Most soft tissue characters remain missing for two of the extant tenrecs: *Limnogale* and *Oryzorictes*.

Combined and GHR-only MP analysis of the smaller dataset used the same search parameters as described above, leaving DNA entries missing for the three fossil taxa. MP bootstrap support values were generated with 1,000 pseudoreplicates, each with 10 random addition replicates and TBR branch swapping. Bayesian search parameters for the smaller GHR dataset were as described above. In the combined Bayesian analysis of morphology and sequences we used different models for each partition: the GTR+I+G for sequences following the AIC in MrModelTest (Nylander 2004), the binary (restriction site) model for indels (with LSET CODING=VARIABLE), and Mk for morphology following Lewis (2001) and Nylander et al.

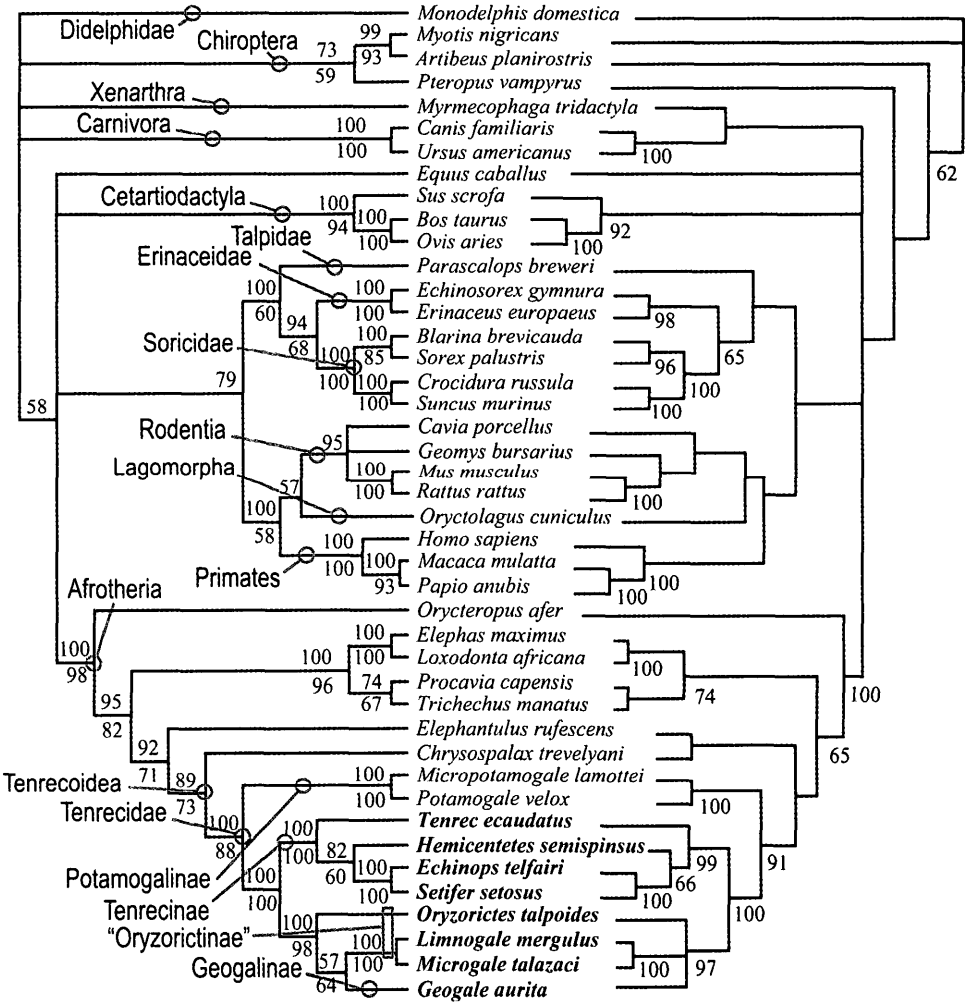

FIGURE 4. Phylogenetic trees based on GHR sequences. Bayesian tree (left) is a majority rule consensus of 9,850 trees (1,000,000 generations sampling every 100), excluding the first 150 as burn-in, from alignment 1 (Table S3) using the HKY+I+G substitution model. MP tree (right) is strict consensus of six trees, each with 2389 steps. Numbers above and below nodes at left indicate, respectively, Bayesian posterior probability values and ML bootstrap support values. (The latter exclude gap characters.) Numbers below nodes at right indicate MP bootstrap support values. Malagasy tenrecs are shown in boldface. Branch lengths do not represent divergences.

(2004). We undertook multiple runs using both LSET CODING=VARIABLE and LSET CODING=ALL commands for the morphological partition. We also ran our morphological partition with (Mk+G) and without (Mk) gamma-shaped rate variation (LSET RATES=GAMMA).

RESULTS

Affinities of Living Tenrecs

Parsimony, likelihood, and Bayesian methods applied to our GHR data consistently supported the monophyly of Malagasy tenrecs to the exclusion of the two living African genera with high support indices (Fig. 4). In each case, regardless of the alignment (Table S3) or algorithm used, and in agreement with Olson and Goodman (2003), *Limnogale* was closely related to *Microgale*, and potamogalines were reconstructed as the sister group to other living tenrecs. The extant Malagasy tenrec clade consisted of two radiations: spiny tenrecs (Tenrecinae) and soft tenrecs (Oryzorictinae plus *Geogale*). Less clear were the positions of *Geogale* and *Oryzorictes* within the soft-tenrec clade, and of *Hemicentetes* and *Tenrec* within the spiny tenrec clade.

Bayesian analysis of sequence data alone favors *Oryzorictes* at the base of a soft-tenrec clade, contradicting oryzorictine monophyly (Fig. 4). However, Bayesian support for a soft-tenrec clade excluding *Oryzorictes* ranged from 54 to 58 across the four alignments; and trees produced by MP for each of the four alignments left *Oryzorictes* and *Geogale* unresolved at the base of this clade (Fig. 4). Furthermore, we cannot statistically reject a monophyletic Oryzorictinae with *Geogale* as its sister taxon (Table 4). In contrast, statistical comparisons based on the GHR-only and combined datasets reject any sister-group relation between the semiaquatic Malagasy *Limnogale* and African potamogalines (Table 4).

Application of MP to the morphological dataset yields optimal trees similar in some regards to those generated by sequences alone, such as monophyly of tenrecids and potamogalines, support for a spiny tenrec clade, and a

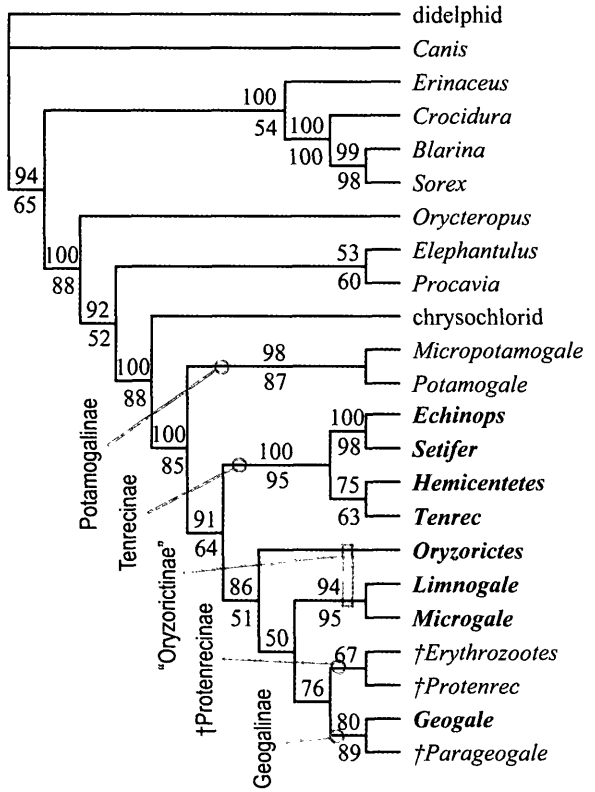

FIGURE 5. Single topology supported by both Bayesian and MP algorithms using combined GHR and morphological data. Bayesian tree is a majority rule consensus as described in Figure 4, using the GTR+I+G substitution model for GHR and Mk (Lewis, 2001) for morphology. This tree was generated without gamma-distributed rate variation (Mk+G) and uses LSET CODING=ALL for the morphological partition; optimal trees from additional runs with Mk+G and LSET CODING=VARIABLE were compatible. MP with all character changes equally weighted supports a single best tree with 1550 steps. Numbers above nodes indicate Bayesian posterior probabilities; numbers below nodes indicate MP bootstrap support values. Malagasy tenrecs are shown in boldface, fossils with a dagger.

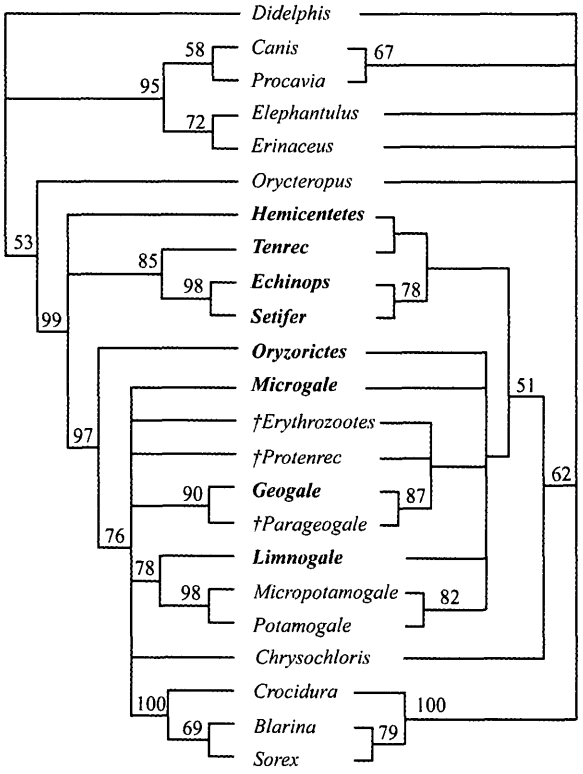

FIGURE 6. Phylogenetic trees based on morphological data. Bayesian tree (left) is a majority rule consensus as described in Figure 4, using Mk (Lewis, 2001) and LSET CODING=VARIABLE. MP tree (right) is a strict consensus of six trees, 382 steps, all morphological character changes treated equally. Numbers above nodes indicate Bayesian posterior probabilities (left) or MP bootstrap support values (right). Malagasy tenrecs are shown in boldface, fossils with a dagger.

sister taxon relationship between *Echinops* and *Setifer*. However, in contrast to the GHR signal, morphological data support the position of African potamogalines near *Limnogale* (Fig. 6). This relationship appears in most of the optimal trees in the combined MP analysis, but is unresolved in the strict consensus. Nevertheless, a *Limnogale*-potamogaline clade is supported by MP applied to the living taxa alone with a bootstrap value of 71 (not figured; see also Asher, 1999), and by Mk applied to the morphological dataset including fossils (Fig. 6). Using the morphological dataset alone, the alternative topologies summarized in Table 4, including variants that preserve monophyly of Malagasy tenrecs and a *Limnogale-Microgale* clade, are rejected by Templeton and winning sites tests.

Affinities of Extinct Tenrecs

Application of MP to the combined dataset including the three fossil tenrecs, regardless of alignment (Table S3) or analysis parameters, supports a *Parageogale-Geogale* clade with relatively high confidence, with MP-bootstrap support values (89) comparable to that for potamogalines (87; see Fig. 5). Bayesian analyses of the combined dataset also supported this clade, but with a posterior probability (80) weaker than that for potamogalines (98). *Erythrozootes* and *Protenrec* are also reconstructed together, in turn adjacent to *Geogale-Parageogale*, regardless of alignment or tree-building technique. However, support indices for this clade are much lower (posterior probability 67, MP bootstrap below 50), as are the supports for a clade joining the three fossil taxa with *Geogale* (posterior probability 76, MP bootstrap below 50; see Fig. 5).

Several morphological characters support a *Parageogale-Geogale* clade, which, following Butler (1984) may be referred to the Geogalinae. First, the reduction of its upper molar metacone (character no. 73, state 2), protocone (no. 74, state 1), and of the lower molar talonid (no. 85, state 1) favor its placement with other dentally zalambdodont taxa (i.e., in this sample, tenrecs and golden moles; see Asher and Sánchez-Villagra [2005] for a definition of anatomical zalambdodonty). *Parageogale* and *Geogale* share a highly reduced maxillary process of the zygoma (no. 59, state 1; also present in soricids). Geogalines also possess a broad

TABLE 4. Tests of alternative topologies.

(A) One-tailed Shimodaira-Hasegawa test using 1,000 RELL bootstrap replicates, applied to the 43-taxon GHR dataset (Fig. 4), using HKY+I+G likelihood model in PAUP. The completely bifurcating tree with the highest likelihood score from alignment 1, run 1 (generation no. 478,200, see supporting data) was used for comparisons. Alternatives are the same except as indicated. Abbreviations are as follows: Ec, *Echinops*; Er, Erythrozootes, FT, fossil tenrecs; Ge, *Geogale*; He, *Hemicentetes*; Li, *Limnogale*; Mi, *Microgale*; MT, Malagasy tenrecs; On, Oryzorictinae; Or, *Oryzorictes*; Pa, *Parageogale*; Pn, Potamogalinae; Pr, *Protenrec*; Se, *Setifer*; Te, *Tenrec*; Tn, Tenrecinae.

Tree	−ln L	Diff −ln L	SH-test P
best Bayesian tree, align1, run1	12,034.29976	—	—
(Ge(Or(Li,Mi)))	12,043.75843	9.45867	0.484
(He,Te)	12,046.09929	11.79953	0.394
((Li,Pn)(Tn,(Or(Ge,Mi))))	12,163.30139	129.00163	0.000*
(Tn(Or(Ge(Mi(Li,Pn)))))	12,141.31100	107.01123	0.000*

(B) Templeton and winning sites tests applied to 23-taxon combined data set (Fig. 5) using MP in PAUP. The completely bifurcating tree from Figure 5 was used for comparisons; alternatives are the same except as indicated.

Tree	Length	Rank Sums*	*N*	*z*	Templeton *P*	Counts	Winning Sites *P*
As in Fig. 5	1550	(best)	—	—	—	—	—
((Or(Li,Mi))((Er,Pr)(Pa,Ge)))	1551	16.0	7	−0.3780	0.7055	4	
		−12.0				−3	1.0000
(Pn(Er,Pr)(Pa(MT)))	1557	25.0	7	−1.9332	0.0532	6	0.1250
On paraphyletic		−3.0				−1	
(Pn(Er,Pr)(Pa(MT)))	1557	77.0	14	−1.6977	0.0896	10	0.1796
On monophyletic		−28.0				−4	
(Pn((Er,Pr)Pa)(MT))	1556	21.0	6	−2.4495	0.0143*	6	0.0313*
On paraphyletic		−0.0				0	
(Pn(Pa((Er,Pr)(MT))))	1558	21.0	6	−2.2711	0.0231*	6	0.0313*
On paraphyletic		−0.0				0	
((Li,Pn)(Tn,(Or((Ge,FT),Mi))))	1592	1638.0	62	−5.3340	<0.0001*	52	<0.0001*
		−315.0				−10	
(Tn(Or((Ge,FT)(Mi(Li,Pn)))))	1582	1254.0	56	−4.2762	<0.0001*	44	<0.0001*
		−342.0				−12	
(Ge((Er,Pr)Pa))	1552	3.0	2	−1.4142	0.1573	2	0.5000
		−0.0				0	

gap between the anterior central incisors (character no. 126, state 1), a condition also seen in *Erinaceus* and in some specimens of *Setifer* (here coded as polymorphic). They also have two premaxillary teeth (no. 67, state 2; also present in some tenrecines and *Erythrozootes*). In contrast to the other nine tenrecid genera, fossil tenrecs plus *Geogale* possess a relatively long infraorbital canal (no. 60, state 0).

Templeton and winning sites tests based on MP reject alternative hypotheses placing all three fossils either outside of living Tenrecidae or together as the sister-clade to African potamogalines (Table 4). However, another alternative, placing *Parageogale* as the sister-taxon to a (potamogaline (*Protenrec Erythrozootes*)) clade, again with all African tenrecs outside of the Malagasy radiation (Fig. 1A), cannot be rejected.

Additional Tests of Fossil Tenrec Phylogeny

All three fossil tenrec genera were first described from the Kenyan Miocene (Butler and Hopwood, 1957) and remain known only from a few craniodental fragments (Butler, 1984). Published reviews including these taxa have generally supported their affinity to modern tenrecids (Butler, 1969, 1978, 1984, 1985; McKenna and Bell, 1997; Mein and Pickford, 2003; but see Poduschka and Poduschka, 1985). As is the case for other fossils over 1 million years in age, sequence data cannot be obtained from these specimens (Hofreiter et al., 2001). Of the 126 characters sampled in our morphological matrix, *Erythrozootes* and *Protenrec* are 24% complete and *Parageogale* is ca. 18% complete. Nevertheless, the most poorly known taxon in this study, *Parageogale*, shows a relatively well-supported position, consistent with the hypothesis originally presented by Butler and Hopwood (1957) that it is the sister-taxon to the living *Geogale aurita*, and contradicting the monophyly of the Malagasy radiation (Fig. 1A).

To test the hypothesis that the 22 characters sampled for *Parageogale* can accurately reconstruct its phylogeny, we used these same characters to reconstruct the phylogeny of other tenrecs in our study. That is, for each of the 10 living tenrecid genera, we replaced GHR data and all morphological characters, except for the 22 known for *Parageogale*, with missing entries and ran the modified morphology+GHR dataset using MP, as described above in Materials and Methods. Stated differently, if a living tenrecid genus had gone extinct in the early Miocene, and were known only from cranial fragments similar to those of *Parageogale*, would we be able to accurately (as defined by the full-data sample depicted in Fig. 5) reconstruct its phylogenetic position? If the respective extant taxon sampled only for the 22 *Parageogale* characters appears in a different part of the tree relative to its position in the full analysis, we would have less confidence in the placement of *Parageogale*.

In fact, the reduced dataset did not greatly change the position of any extant tenrec (Fig. 7). Out of the 10

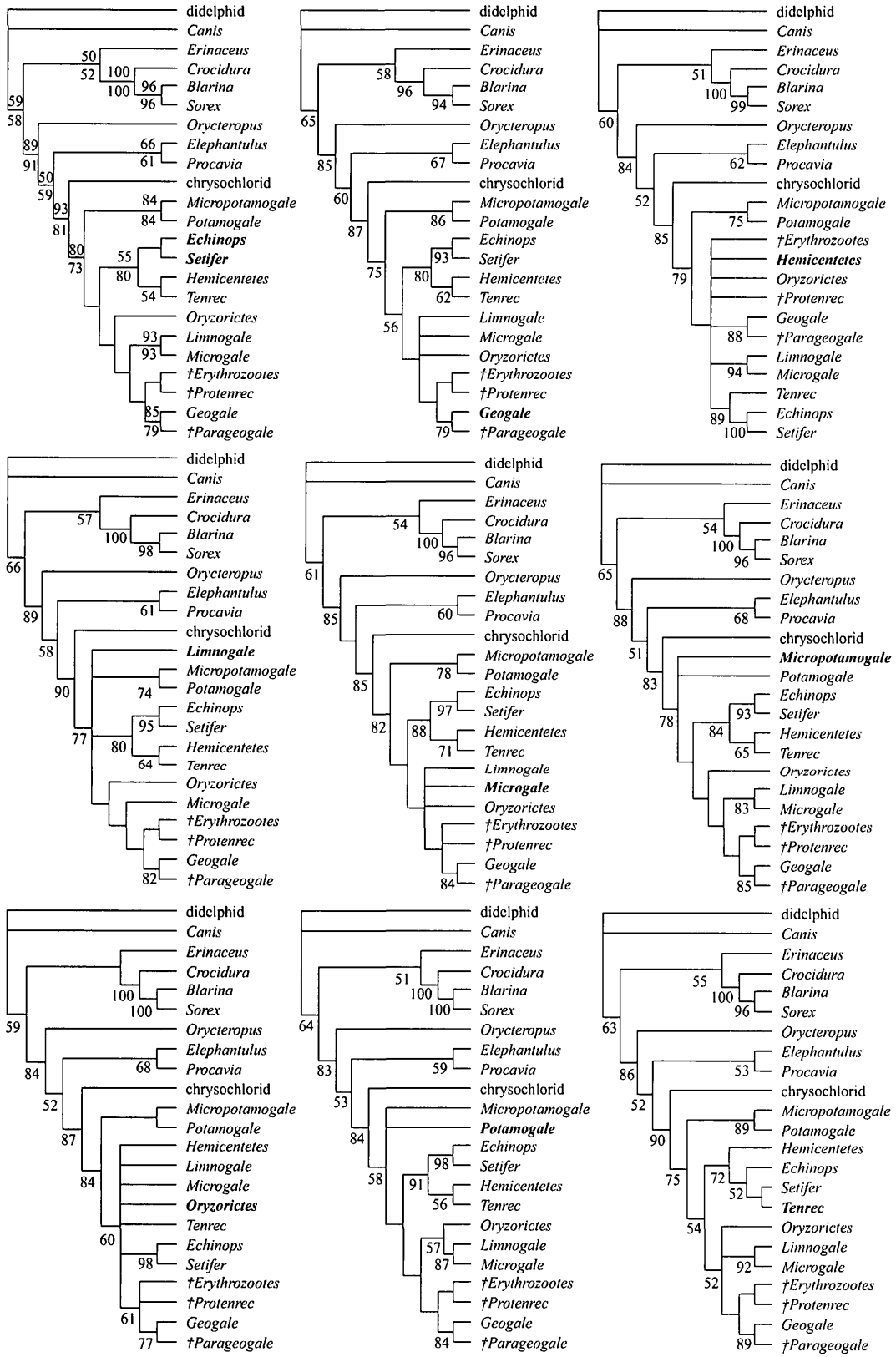

FIGURE 7. Results of reduced-character MP analyses of each of the 10 living tenrecid genera (as identified in boldface), with all GHR sequences and morphological characters, except for the 22 known for *Parageogale*, coded as missing. Matrices with either *Echinops* or *Setifer* coded in this fashion yield the same topology (top left), also identical to the combined-data topology depicted in Figure 5. Each tree represents either a strict consensus or a single, most-parsimonious result, as follows: *Echinops* 1 tree 1,539 steps, *Geogale* 3 trees 1,491 steps, *Hemicentetes* 4 trees 1,517 steps, *Limnogale* 3 trees 1,521 steps, *Microgale* 4 trees 1,521 steps, *Micropotamogale* 1 tree 1,520 steps, *Oryzorictes* 7 trees 1,515 steps, *Potamogale* 1 tree 1,511 steps, *Setifer* 1 tree 1,543 steps, *Tenrec* 2 trees 1,522 steps. Numbers adjacent to nodes represent MP bootstrap support values (100 pseudoreplicates of a simple addition sequence). Bootstrap values in tree at top left for *Echinops* are listed above nodes, *Setifer* below.

modified datasets (1 for each living tenrecid genus), 2 (*Echinops* and *Setifer*) yielded the same tree as the full sample, and 6 of the remaining 8 yielded varying degrees of nonresolution in multiple shortest trees, consensuses of which (Fig. 7) were still compatible with the topology supported by the full dataset. Only two cases (*Tenrec* and *Potamogale*) yielded optimal trees with a slightly different topology. The former altered relations within spiny tenrecs (supporting *Tenrec-Setifer* rather than *Tenrec-Hemicentetes*), and the latter reconstructed *Oryzorictes* adjacent to *Microgale-Limnogale* to the exclusion of *Geogale*, preserving oryzorictine monophyly. However, *Tenrec* bootstrap resampling still supports a spiny-tenrec clade with a value of 79; and the *Tenrec-Setifer* clade has an MP bootstrap support value under 50. Similarly, for the run using a reduced sample for *Potamogale*, oryzorictine monophyly is supported with an MP bootstrap of just 57, and the unmodified, combined-data sample cannot reject this hypothesis (Table 4). In these and other cases, bootstrap resampling generally yielded lower support values compared to the full sample (cf. Fig. 5 versus Fig. 7), but in no case did a clade produced by a reduced-sample analysis contradict a well-supported clade in the full sample.

Discussion

Data Combination and Tenrec Phylogeny

A previous morphology-based investigation of tenrecid phylogeny published by one of us (Asher, 1999) argued for a clade of semiaquatic tenrecs, placing Malagasy *Limnogale* as the sister-taxon to continental African potamogalines. Character support for this clade was primarily from the skull, including a fenestrate basioccipital (no. 35 in this study), a shortened frontal bone (no. 61), and a reduced lacrimal foramen (no. 53). Importantly, none of these character states are consistently found in nontenrecid, semiaquatic, faunivorous, small mammals (Sánchez-Villagra and Asher, 2002), a factor that had previously led Asher to view the "semiaquatic" tenrec clade with increased confidence.

As discussed above, morphological data analyzed alone still yield some support for a semiaquatic clade, although recoding fenestration in the basioccipital to account for polymorphism in *Microgale* (as recommended by Olson and Goodman, 2003) has eliminated this character from optimizing unambiguously as a *Limnogale*-potamogaline synapomorphy. Furthermore, compared to the study of Asher (1999), the larger number of characters and sampled tenrecs in this study yields reduced support for a semiaquatic tenrec clade (Fig. 6).

However, the key reason for the nonrecovery of a semiaquatic clade in this study is the very strong sequence-based signal favoring a *Limnogale-Microgale* clade. Indeed, with their sample of different loci for multiple species of *Microgale*, Olson and Goodman (2003) found that *Limnogale* actually nests within that genus, comprising the sister-taxon to an *M. dobsoni–M. talazaci* clade to the exclusion of other *Microgale* species. The strength of the signal supporting a *Microgale-Limnogale* clade in our study (100 MP bootstrap, 100 ML bootstrap, and 100 Bayesian posterior probability in the GHR-only analysis [Fig. 4]; 95 MP bootstrap and 94 Bayesian posterior probability in the combined analysis [Fig. 5]) has convinced both of us that the previous interpretation of the morphological signal as indicative of a semiaquatic tenrec clade (Asher, 1999) is incorrect. Due to this unambiguous support from GHR sequences, which is considerably stronger than that from morphology alone for a semiaquatic clade and which prevails in the combined analysis, the cranial characters supporting the "semiaquatic" clade cited above must be reinterpreted as homoplastic.

If the morphological data used here are misleading regarding a semiaquatic tenrec clade, why do we then combine them with our GHR data? The most important reason for retaining morphology in our dataset is one of principle: most individual datasets are not in their entirety either "true" or "false"; but are themselves mosaics of variable character-data that may provide resolution at different levels in any given tree (Gatesy et al., 2003). Combined data sets enable recognition of phylogenetic signals that would remain obscure with the analysis of subdivisions thereof (Gatesy et al., 1999, 2005). Furthermore, including morphological data in the combined analysis remains the best means to sample fossil tenrecs. We cannot be completely sure that the morphology known for these fossils enables us to accurately understand their phylogenetic history. However, as discussed above, when used in simulations to replace the complete morphology-GHR dataset for each of the 10 living tenrecid genera, the morphological characters known for the most incomplete of the fossils (*Parageogale*) yield results that are largely congruent with the combined-data topology.

Character Assessment and Hindlimb Function in Potamogale

One recent study of hindlimb characters (Salton and Szalay, 2004) has also argued for the inclusion of *Limnogale* within the Malagasy radiation. By assessing characters of the tarsal complex in an "ecological and evolutionary framework," Salton and Szalay proposed to identify phylogenetically informative characters: "traits with clear species-specific adaptations are a potential interference in cladistic analyses and cannot be meaningfully used without ecology-based character assessment" (Salton and Szalay, 2004:73). In regards to the "semiaquatic" clade, their procedure resulted in the identification of anatomical differences (e.g., astragalar neck-head transition) and similarities (e.g., medially directed tibial-fibular malleoli) between *Limnogale* and *Potamogale* (they did not include *Micropotamogale* in their analysis). In their opinion, the former comprise phylogenetic data in support of the "family level distinction" of *Potamogale* from other tenrecs, and the latter are interpreted as homoplastic.

However, we are concerned that Salton and Szalay (2004) did not identify a replicable optimality criterion (e.g., MP, ML) by which they reached their conclusions

on homology. Furthermore, we believe that Salton and Szalay have not fully appreciated the function of the hindlimb in *Potamogale*. Regarding its locomotion, Salton and Szalay refer to its "heavy foot thrusts" (p. 90), and note that "heavy loading in the UAJ [upper ankle joint]... and UAJ stabilization plays an important role... in the aquatic locomotion of *Potamogale*" (p. 86). In regards to calcaneal morphology, Salton and Szalay state that "*Potamogale* has an extremely long and narrow calcaneus with a long tuber, appropriate for strong, dorsolateral aquatic propulsion" (p. 93). In fact, these inferences of locomotion run counter to published descriptions of locomotor behavior in *Potamogale* (e.g., DuChaillu, 1860; Kingdon, 1974), which indicate that it uses its massive tail, not its feet, for aquatic propulsion. As in the other two potamogaline species (*Micropotamogale lamottei* and *M. ruwenzorii*), digits II and III of the hindfoot in *Potamogale* are syndactyl, and their use in grooming has been documented (Nicoll, 1985; Kingdon, 1997). As summarized by Nowak (1999), the relatively small, nonwebbed pes of *Potamogale* is tucked under its pelvic region during swimming and is not used for propulsion. Dobson (1883:97–98) infers from its anatomy that during locomotion, "the sole [of the foot] lies so evenly against the [pelvic ventrum] as to present the least possible projection and interfere in the least degree with the rapid passage of the body through the water, propelled by the powerful tail.... [The tail] is doubtless the sole organ of propulsion." Based on field observations, Kingdon observed that in the water, "the animal is propelled entirely by lateral movements of the back and tail" (Kingdon, 1974:15). In contrast, *Limnogale* (the "web-footed" tenrec), has been observed to use its hindlimbs for semi-aquatic propulsion (Benstead and Olson, 2003:1272). Despite this, and without presenting new behavioral data for either taxon, Salton and Szalay (2004:100) propose the opposite: "... the tarsal complex indicates that [*Limnogale's*] hind limbs are less important for propulsion than those of *Potamogale*."

Hence, we remain skeptical about Salton and Szalay's method for distilling phylogenetically informative data from their morphological observations. Although we agree with them that *Limnogale* is not more closely related to *Potamogale* than to other Malagasy tenrecs, contra Asher (1999), we do not believe they presented in their paper a basis for reaching this conclusion, independent of the sequence data analyzed by Olson and Goodman (2003), and confirmed with additional data in this article.

Tenrec Biogeography

Considering the living radiation alone, Malagasy tenrecs show substantial morphological diversity, yet are recognized as a single radiation by sequence data, as observed for primates (cf. Yoder, 1992, versus Yoder et al., 1996) and carnivorans (cf. Veron, 1995, versus Flynn et al., 2005). Similarly, our results support a cohesive Malagasy radiation and argue against Malagasy tenrec polyphyly. However, the living tenrecid radiation is not a complete picture of this group's diversity. Although its paleontological record is meager, fossil African tenrecids appear to have a close relationship with living *Geogale*. This relationship makes the Malagasy tenrec radiation paraphyletic (Figs. 1B, 5).

A similar phylogenetic scenario was presented by Jansa et al. (1999) for Malagasy nesomyine rodents. Based on cytochrome *b* sequences for multiple representatives of all genera of this group, Jansa et al. (1999) disputed previous interpretations of polyphyly (Ellerman, 1940, 1941), but argued that two mainland African genera (*Steatomys* and *Tachyoryctes*) nested within the Malagasy radiation. They suggested that this phylogenetic pattern would be consistent with colonization of Madagascar by nesomyines via a single founder event, followed by dispersal to Africa from Madagascar. The inclusion of mainland African muroids within the Malagasy radiation has subsequently been questioned (Steppan et al., 2004; Jansa and Weksler, 2004); and nesomyine monophyly remains possible. A definitive conclusion must await a study that synthesizes the taxon and character samples discussed by Jansa et al. (1999), Jansa and Weksler (2004), and Steppan et al. (2004).

Monophyly of Malagasy tenrecs is also possible. We have at present no way of knowing how the missing GHR nucleotides for *Parageogale*, or characters from its still unknown skeleton, would affect our estimate of its relationships. Some uncertainty regarding our results supporting paraphyly (Fig. 1B) is reflected in the nonrejection of at least one alternative topology that preserves Malagasy tenrec monophyly (Table 4); and we eagerly anticipate how this result is affected by future discoveries of better-preserved fossil tenrecid material. Nevertheless, the current hypothesis of a *Parageogale-Geogale* clade has support from both MP and Bayesian methods (Fig. 5). Furthermore, as discussed above, the limited morphological sample available for *Parageogale* appears to perform fairly well when these same characters are used to reconstruct the phylogeny of each of the ten living tenrecid genera, a result that slightly increases our confidence in the placement of this fossil.

As stated in the introduction, the absence of modern mammalian orders from Madagascar (and elsewhere) during the Mesozoic (Krause, 2003), during which time land connections existed with mainland Africa (until the late Jurassic) and India (until the early Late Cretaceous), has led many to favor dispersal as the prime mechanism by which modern mammals colonized Madagascar (e.g., Olson and Goodman, 2003; Yoder et al., 2003). Repeated monophyly of Madagascar's endemic radiations is consistent with dispersal, as individual colonization events are hypothesized to be rare, and a previously unpopulated island may have open adaptive zones into which a founder can radiate into a diverse clade.

Nonmonophyly is also compatible with dispersal, but requires more (potentially unparsimonious) crossings of a geographic barrier, in this case the Mozambique channel. No one will ever know exactly how or why the tenrec crossed the channel; but based on our phylogeny we can estimate how often such an event took place. Given the combined-data tenrec phylogeny presented in

Figure 5, we hypothesize that a single founder event of Madagascar by the common ancestor of Malagasy tenrecs took place at some point after the Maastrichtian, during which time a diverse vertebrate fauna shows no sign of Madagascar's modern inhabitants (Krause, 2003). Prior to the Miocene, when fossil tenrecs were present in east (Butler, 1984) and southwest (Mein and Pickford, 2003) Africa, an additional dispersal of an animal related to the geogaline common ancestor took place from Madagascar to continental Africa. The position of *Erythrozootes* and *Protenrec* (in the Protenrecinae of Butler [1984]) as sister taxa to *Geogale-Parageogale* implies that a protenrecine relative made this back-migration yet again.

However, we note that a *Protenrec-Erythrozootes* clade to the exclusion of geogalines has an unimpressive MP bootstrap value below 50 and a Bayesian posterior probability of 67 (Fig. 5). An alternative hypothesis, placing protenrecines as the sister clade to *Parageogale* within Geogalinae, would require just a single Madagascar-Africa dispersal event postdating the initial Madagascar colonization. This alternative is just two steps longer in MP analyses and cannot be statistically rejected (Table 4). As stated above, we are cognizant of yet another alternative that preserves Malagasy tenrec monophyly (Fig. 1A), also statistically unrejected in Table 4. This scenario would require only a single colonization event of Madagascar by tenrecs, with no back-migration, again at some point after the Late Cretaceous.

Nevertheless, the optimal explanation of the data presented in this article supports paraphyly of Malagasy tenrecs relative to their mainland relatives (Figs. 1B, 5), not monophyly (Fig. 1A) or polyphyly (Fig. 1C). DNA sequence data for extinct, pre-Pleistocene tenrecs will probably never be available; and even for certain living taxa, in particular *Geogale* and *Limnogale*, such data are very difficult to obtain. Our technique for sequencing nuclear DNA from museum specimens without damaging them eases this constraint, can be applied to other groups, and greatly reduces fieldwork expense and disturbance of living populations otherwise necessary for obtaining research material. This highlights yet further the value of museum collections for basic science (Suárez and Tsutsui, 2004).

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APPENDIX 1

List of osteological specimens examined. The geographic provenance of specimens is listed in parentheses following the taxon name. Crosses denote extinct taxa; asterisks denote taxa sampled for soft tissue characters using an uncataloged collection of histological specimens (see table 2 of Asher, 2001). Institutional abbreviations are as follows:

AMNH, American Museum of Natural History, New York
BMNH, The Natural History Museum, London
FMNH, Field Museum of Natural History, Chicago
IZEA, Institut de Zoologie et d'Ecologie Animale, Lausanne
MCZ, Museum of Comparative Zoology, Harvard University
MNHN, Museum Nationale d'Histoire Naturelle, Paris
USBA, University at Stony Brook, Department of Anatomical Sciences
USNM, United States National Museum, Washington
ZIUT, Zoologisches Institut, Universität Tübingen
ZMB, Zoologisches Museum Berlin

1. **Didelphis* sp. (Mexico, Nicaragua, USA): AMNH 28408, 28962, 29255, 70082, 145630, 146551, 148959, 201327; USBA MMr1, MMr4, MMr5
2. **Blarina brevicauda* (USA): AMNH 95256, 95297, 98912, 144485, 144486, 144487, 144488, 207017, 207018, 207019, 207020, 207754, 207755, 212504; FMNH 108390, 121194
3. **Canis latrans* (USA): AMNH 5392, 99653, 131833, 131865, 208363, 208367, 208371; USBA MCn28
4. **Chrysochloris stuhlmani* (Zaire, Uganda, South Africa, Burundi): AMNH 82399, 167615, 167963, 180909, 180911, 180912, 180913, 236000; USNM 49896; FMNH 26352, 26353, 26355, 127361, 148200, 148201, 148917
5. **Crocidura olivieri* (Zaire, Malawi, Burundi, Cameroon, Ghana): AMNH 48490, 48491, 48497, 161848, 236229, 239320, 239321, 239326; FMNH 137591, 137592
6. **Echinops telfairi* (Madagascar): AMNH 31270, 100751, 100753, 100760, 100767, 100808, 170602, 170605, 170606, 170607, 170608, 170599, 170609, 170610, 170611, 207717, 207718, 212918, 212919; USNM 464980
7. **Elephantulus branchyrhynchus* (Kenya): AMNH 86554, 86555, 86556, 86557, 86577, 86578, 86580, 86581, 86582, 86583, 86584; ZIUT 3860, 3861
8. **Erinaceus europaeus* (France, Italy, England, Germany): AMNH 3770, 42561, 42563, 57219, 70613, 140469, 140470, 160470, 201230, 215299; USNM 251763, 251764, ZIUT M140
9. †*Erythrozootes chamerpes* (Kenya): BMNH M14314, M21831; Butler, 1969, 1984
10. **Geogale aurita* (Madagascar): MCZ 37807, 45496, 46274; FMNH 151947, 156551, 15652, 156553, 159732, 159733; MNHN 1912-110A, 1912-110B, 1962-2518, 1962-2519, 1981-1374, 1987-110, 1991-1450
11. **Hemicentetes semispinosus* (Madagascar): AMNH 90421, 100777, 100780, 100781, 100783, 206755, 207711, 207712, 207713, 207714, 212921, 212922, 212935, 212938, 212940; USNM 83658; ZMB 71599
12. *Limnogale mergulus* (Madagascar): AMNH 100688, 100689, MCZ 45050, 45054, 45055; BMNH 35.1.8.255, 35.1.8.256, 48.89, 48.90, 97.9.1.161; MNHN 1962-2511, 1962-2513, 1984-521; ZMB 35258
13. **Microgale talazaci* (Madagascar): AMNH 100708, 100709, 100714, 100799, 119216, 207003, 207077; USNM 520881; FMNH 154582, 154583, 154584, 154585, 154586, 154587, 154588, 154589; MNHN 1961-198, 1977-42, 1983-898, 1984-856, 1984-857
14. **Micropotamogale* sp. (Congo, Ivory Coast, Liberia): IZEA 4942, 4975; BMNH 67.213, 73.170; MNHN 1970-514, 1976-397, 1980-52, 1980-53, 1980-57
15. *Oryzorictes* sp. (Madagascar): AMNH 31243, 31257; USNM 578789; FMNH 5637, 5639, 5640, 5641, 156226; MNHN 1897-520, 1912-111A, 1912-112A, 1941-34, 1962-2501, 1984-523, 1984-525, 1987-108
16. **Orycteropus afer* (Zaire): AMNH 34866, 51370, 51372, 51374, 51905, 51906, 51907, 51908, 51909, 51010, 70036, 70189
17. †*Parageogale aletris* (Kenya): BMNH M33046, Butler, 1984
18. **Potamogale velox* (Zaire, Cameroon, Congo, Gabon): AMNH 51161, 51162, 51164, 51165, 51319, 51322, 51324, 51334, 51344, 51348, 51368, 55203, 55204, 120250, 240968; USNM 266897, MCZ 35321, 35322; FMNH 72831, 25973; BMNH 26.11.1.62; MNHN 1892-2064, 1892-2065, 1898-1576, 1947-864, 1947-865, 1947-866, 1962-2520; ZMB 46588
19. †*Protenrec* sp. (Kenya, Uganda) BMNH M34149, M34150, M33036, M34153, M43551, M43552
20. **Procavia capensis* (Kenya, Zaire, Central African Republic, South Africa): AMNH 53777, 53781, 53784, 53785, 83411, 83412, 80997, 80998, 80999, 88418; USBA Mhy1, Mhy4, Mhy5
21. **Setifer setosus* (Madagascar): AMNH 100749, 100750, 100762, 170532, 170533, 170534, 170535, 170537, 170540, 170547, 170548, 170579, 170581, 170582, 170612, 207005, 207076; USNM 578790; BMNH 93.12.6.8; ZMB 44586
22. **Sorex* sp. (Canada, Finland, England, Sweden): AMNH 126007, 126990, 126991, 141626, 148521, 115593, 115594, 115595, 115597
23. **Tenrec ecaudatus* (Madagascar): AMNH 100729, 100732, 100733, 100735, 100738, 100809, 170502, 170511, 212913; USNM 19361, 577051; BMNH 70.3.10.5

NOTE: We wish to acknowledge the recent study of Poux et al. (2005), published after the completion of this paper, on the colonization of Madagascar by terrestrial mammals. Poux et al. sampled most Recent genera of tenrecids (except Potamogale and Geogale), plus a large sample of other endemic Malagasy genera, and report a tenrecid phylogeny congruent with that discussed here and by Olson and Goodman (2003), for example in supporting a Limnogale-Microgale clade. However, they did not address the phylogeny of fossil taxa.