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ORIGINAL PAPER

Phylogeny of the Rodent Genus *Isothrix* (Hystricognathi, Echimyidae) and its Diversification in Amazonia and the Eastern Andes

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Abstract Efforts to clarify the affinities of the torós or brush-tailed rats (*Isothrix*) and document the radiation of these distinctive echimyids have been limited. The discovery of a new Andean species prompted a reanalysis of *Isothrix* and its relatives. Prior morphological analyses of skulls, mandibles, teeth, and external characters permitted robust diagnosis but offered little resolution of within- or between-group relationships. Analyses of mitochondrial cytochrome *b* sequences (798 bp), which are available for numerous echimyids, confirm the monophyly of recognized genera, including *Isothrix*, and resolve a number of interspecific relationships. Strikingly, the Andean toró (*Isothrix barbarabrownae*) is consistently recovered as sister to the remaining species. These are allied into three clades: *I. sinnamariensis* + *I. pagurus* in the lower Amazon Basin and Guianan Shield, *I. orinoci* + *I. negrensis* in the Rio Negro and Río Orinoco drainages, and *I. bistriata* across much of the western and southern Amazon Basin. However, the addition of a new basal taxon does not aid in identifying the sister taxon of *Isothrix*. These relationships are confirmed in combined analyses of cyt-*b* with sequence variation in the mitochondrial control region (D-loop; 450 bp) and in the nuclear RAG1 gene (1,072 bp). Analyses identify the Andes, or proto-Andes, as an important theater for the group's evolution and may offer an explanation for the luxuriant fur of this genus. However, neither the biogeographic history of *Isothrix* nor the remarkable pelage evolution of the Echimyidae can be understood until the deeper nodes within the arboreal spiny rats (Echimyinae) are more fully resolved.

Keywords Andes · Amazonia · Distribution · mtDNA · Neotropics · Nuclear DNA · Phylogeny · Pelage · Sequence analysis

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Introduction

The richest faunas and floras on Earth inhabit tropical rainforests (Myers et al. 2000; Myers 2003). The tropics are thought to generate species faster (Wright et al. 2006) and to harbor species longer (Wiens et al. 2006) than temperate areas, therefore qualifying as both "cradle" and "museum" (McKenna and Farrell 2006). A complex historical interplay of lineages, climates, geography, and adaptation shapes present-day biodiversity patterns, and phylogenies offer primary documentation of their effects (Brooks and McLennan 1991). Phylogenetic patterns of lineages that have limited dispersal abilities and are diversified among regions of endemism can be particularly instructive in reconstructing regional histories (Nelson and Platnick 1981).

Understanding the complex radiations of tropical organisms is improved by continuing species discovery, deepened knowledge of group relationships, and expanded genetic surveys. The recent discovery and description of a new species of *Isothrix* (Rodentia: Echimyidae) in Peruvian cloud forests (Patterson and Velazco 2006), together with better generic diagnoses (Emmons 2005) and improved phylogenetic understanding of Echimyidae (Galewski et al. 2005), invited phylogenetic and biogeographic reconstructions of these rodents. The genus *Isothrix* is widely distributed across northern South America and its species are distributed across the Guianan Shield, lower Amazon, upper Amazon, Rio Negro, and Río Orinoco drainages (Fig. 1). The contiguously allopatric distribution of these species offers insight into the historical relationships of the faunal regions they occupy.

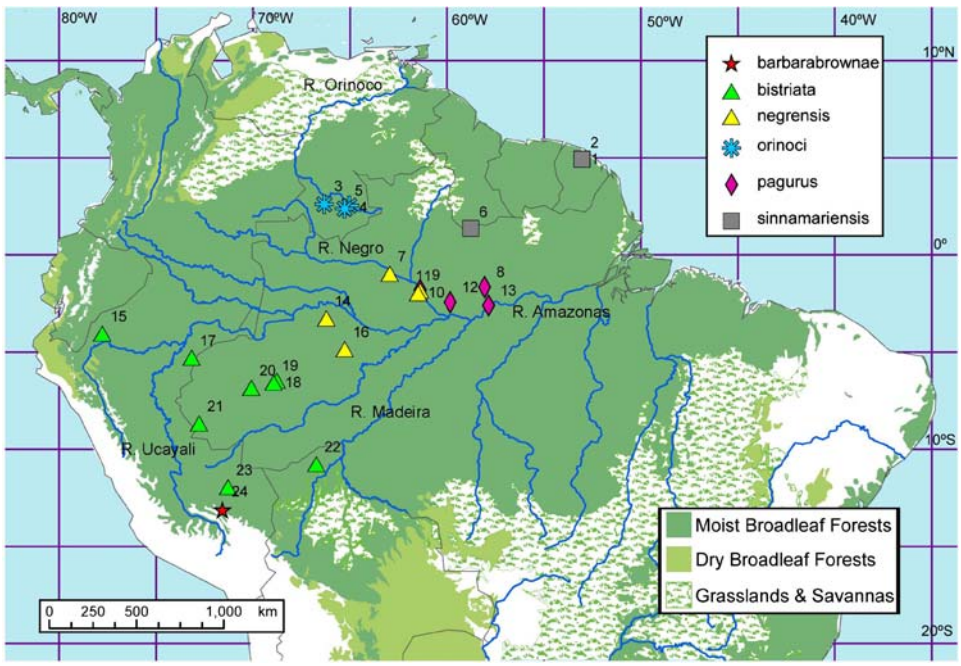


Fig. 1 Map showing the distribution of *Isothrix* species and locations of 31 genetic samples used in molecular analyses (see Table 2). South American distributions of Moist tropical broadleaf forests, Dry tropical broadleaf forests, and Grassland, savannah and shrubland ecoregions from Olson et al. (2001).

Taxonomic context and background

The Neotropical family Echimyidae is one of 17 families in the Infraorder Hystricognathi (Woods and Kilpatrick 2005). Except for the Pan-American Erethizontidae and paleotropical Hystricidae, the remaining hystricognath families are continental endemics. Three small families (Bathyergidae, Petromuridae, and Thyronomyidae) are African, while a dozen others are restricted to the Neotropics. Except for a few fossil taxa, the infraorder's membership is non-controversial (McKenna and Bell 1997). However, the origins and biogeography of its disjunct members have long been disputed, because these Southern Hemisphere groups are too young to have diverged with the breakup of Gondwana. Rodents first appeared in South America during its Tertiary isolation, in the late Eocene Santa Rosa Formation of Amazonian Peru (Frailey and Campbell 2004), and are represented by taxa allocated to Echimyidae. Additional echimyids are known from the Deseadan Formation (late Oligocene) of Bolivia (Patterson and Wood 1982), obvious products of a Paleogene radiation.

South American radiations of Echimyidae took place long before the late Miocene-earliest Pliocene arrival of myomorphs (rats and mice) and sciuromorphs (squirrels) as 'heralds' and 'legions' of the Great American Biotic Interchange with formation of the Panamanian isthmus (Woods 1982; McKenna and Bell 1997). Absence of potential competitors may explain the impressive array of ecological niches exploited by echimyid genera (Mares and Ojeda 1982; Galewski et al. 2005): in addition to semi-fossorial (*Clyomys*, *Carterodon*, *Euryzygomatomys*), terrestrial (*Hoplomys*, *Proechimys*, *Trinomys*), scansorial (*Thrichomys*), and semi-aquatic (*Myocastor*) genera, many forms are mainly arboreal, moving like squirrels on trunks, branches and vines but feeding on leaves and fruits (*Callistomys*, *Diplomys*, *Echimys*, *Isothrix*, *Makalata*, *Mesomys*, *Lonchothrix*, *Pattonomys*, *Phyllomys*, *Santamartamys*, and *Toromys*) or mostly restricted to bamboo (*Dactylomys*, *Kannabateomys*, and *Olallamys*). Most of the arboreal genera are poorly sampled by traditional collecting methods, so that many taxa remain incompletely known. Inadequate sampling has clouded knowledge of echimyid diversity, artificially circumscribed their geographic ranges, and constrained taxon sampling in phylogenetic analyses, but the situation is improving. In the last decade, four new arboreal genera (*Callistomys*, *Pattonomys*, *Santamartamys*, and *Toromys*) have been erected – three in 2005 alone – to reflect new understanding of group relationships.

Early phylogenetic analyses of echimyids using cytochrome-*b* (cyt-*b*) sequences underscored the marked differentiation of genera and suggested their near-simultaneous divergence. Lara et al. (1996) aptly termed the resulting tree structure "the star phylogeny," comprised of long, distinctive terminal branches (mainly genera and species groups) and short, poorly supported internodes for higher-order relationships. They provided evidence that Atlantic Forest rats previously allocated to *Proechimys* deserved generic distinction as *Trinomys,* and showed close relationships between *Proechimys* (*sensu stricto*) and *Hoplomys*. A subsequent analysis employing more taxa and three mitochondrial genes (cyt-*b*, 12S, and 16S) offered more in-group resolution and showed that both *Myocastor* and *Capromys* belonged in Echimyidae (Leite and Patton 2002). The climbing rats *Lonchothrix* and *Mesomys* were removed from the Eumysopinae, which then formed a sister clade to the arboreal forms. However, neither *Isothrix* nor *Lonchothrix* + *Mesomys* clustered with remaining arboreal genera grouped into either Dactylomyinae or Echimyinae, reaffirming the star-phylogeny.

Recently, Galewski et al. (2005) added exon 28 of the nuclear gene von Willebrand Factor (vWF) to the earlier data set, generating more resolution for some terrestrial members. They uncovered three clades of echimyids: (*Myocastor*, *Thrichomys* (*Proechimys*, *Hoplomys*)); a second of ((*Euryzygomatomys*, *Clyomys*) *Trinomys*)); and a third of the arboreal genera. However, the positions of *Capromys* and the interrelationships of the arboreal genera were

unresolved. Using a relaxed molecular clock, they estimated that most modern genera date to the middle Miocene (14.4 Mya); radiations of species of arboreal genera were dated to the Pliocene (2.3–2.7 Mya). Both interpretations coincide with the group's scant fossil record. As in other analyses, *Isothrix* failed to cluster with any other genus.

Morphology permits confident diagnosis for most echimyid genera, but like the molecular data set it offers weak support for most suprageneric groupings. Employing 50 dental and cranial characters on 54 taxa (23 of them extinct), Carvalho and Salles (2004) found that subfossorial forms *Carterodon*, *Clyomys*, and *Euryzygomatomys* formed a clade with fossil *Pampamys* and *Theridomysops* at the base of crown-group Echimyidae; they also associated *Trinomys* with *Proechimys* and *Hoplomys* (thereby reforming the eumysopines) and allied as sister all the arboreal genera plus *Myocastor* in a poorly resolved clade. Analyzing the arboreal genera using 47 external, cranial, and dental characters, Emmons (2005) recovered *Echimys*, *Isothrix*, *Makalata*, *Pattonomys*, and *Phyllomys* as monophyletic, reasonably well-supported genera, but found weak or equivocal support for generic, tribal, and subfamilial groupings. Adding three additional *Isothrix* taxa to the morphological analysis brought no additional resolution either to the affinities of *Isothrix* or inter-relationships of its species (Patterson and Velazco 2006).

To obtain additional resolution, we gathered available gene sequences for echimyids and generated new ones for recently collected forms and new genetic systems. We studied variation in both mitochondrial (cyt-*b* and D-loop fragments) and a fragment of the nuclear recombination activating gene (RAG1). Cyt-*b* has been widely used in studies of rodent interrelationships, including Echimyidae (Patton et al. 1994; Lara and Patton 2000), so that analyses using cyt-*b* offer the most comprehensive taxon sampling. To enhance the possibility that sequence variation would be informative across the taxonomic hierarchy, we also studied the generally faster-evolving, non-coding mitochondrial D-loop (Moscarella et al. 2003; Matisoo-Smith and Robins 2004; Rivera et al. 2007) and the slower-evolving, nuclear-coding sequence RAG1 (Baker et al. 2004; Steppan et al. 2004, 2005). Our primary goal was to clarify relationships among species of *Isothrix*, and of *Isothrix* with other echimyids.

Materials and methods

Data acquisition and sequence alignment

Genomic DNA was isolated from a small (~0.05 g, wet weight) portion of liver, heart, or muscle samples that were preserved frozen, in lysis buffer, or in ethanol. DNA was extracted with the Qiagen DNAeasy kit (Qiagen, Inc., Valencia, CA). For *Echimys chrysurus* [Field Museum of Natural History (FMNH) 93267], *Isothrix barbarabrownae* [Museo de Historia Natural, Universidad de San Marcos (MUSM) 16819], *Isothrix orinoci* [US National Museum of Natural History (USNM) 406370, USNM 406375, USNM 415193], *Isothrix pagurus* (USNM 555639), *Lonchothrix emiliae* (FMNH 140821), and *Toromys grandis* (FMNH 92198), total DNA was extracted from dried skins, using Rauri Bowie's modification of the Qiagen DNAeasy protocol, which involves four steps prior to the Qiagen DNAeasy protocol for animal tissues: 1) place skin into 1 ml 95–100% EtOH, vortex at high speed for 30 s; 2) remove fluid, add 1 ml 70% EtOH, vortex at high speed for 30 s; 3) remove fluid, add 1 ml dH_2O, vortex for 30 s; 4) remove fluid, add 1 ml dH_2O, soak for 30–45 min, afterwards excise a small piece.

Aliquots of genomic DNA isolates were used as templates for polymerase chain reaction (PCR) to amplify double-stranded DNA products from one mitochondrial gene (cyt-*b*), one mitochondrial regulatory region (D-loop), and one nuclear gene (RAG1). Each PCR had a reaction volume of 25 μl and contained 1 μl of DNA stock, 2.5 μl 10× reaction buffer, 2.5 μl

of 8 mM premixed deoxynucleotide triphosphates, 15 μl of double-distilled H2O, 2.0 μl of FMNH Taq, and 1 μl of each oligonucleotide, each at 10 μM concentration. For the skin samples, we replaced the FMNH Taq with 0.15 μl AmpliTaq Gold™ DNA Polymerase (Applied Biosystems, Foster City, CA), and added 2 μl of Bovine Serum Albumin (BSA, Applied Biosystems, Foster City, CA) and 4 μl of $MgCl_2$, and 4 μl of DNA stock, reducing the volume of ddH_2O to reach a 25 μl solution.

Cyt-*b* was amplified using primer combinations MVZ05–MVZ16, MVZ05–MVZ04, MVZ45–MVZ16, and MVZ127–MVZ16; D-loop was amplified using primer combination L0-E3; RAG1 was amplified using primer combinations RAG1F1705–RAG1R2864, RAG1F1705–FMNH1b, FMNH2a–FMNH2b, FMNH3a–RAG1R2864. Primers are given in Table 1.

PCR profiles included an initial denaturation step at 94–95°C for 2–3 min, followed by 30–35 cycles of PCR. The cycles involved denaturation at 94–95°C for 30 s, annealing at 50–65°C for 30–90 s, polymerization at 68–72°C at 2 min, and a final extension at 72°C at 5–8 min. The PCR bands were cut, and intact DNA was melted at 70°C for 10 min, and then 1.5 μl of gelase was added and incubated for at least 2 h at 45°C. The PCR products were cycle-sequenced using ABI PRISM Big Dye version 3.1 (Applied Biosystems, Foster City, CA). The cycling protocol used involved an initial denaturation step at 96°C for 60 s, followed by 25 cycles of denaturation at 96°C for 10 s, annealing at 50°C for 5 min, and extension at 60°C for 4 min. Cycle-sequencing products were purified through an EtOH–EDTA precipitation protocol and run on an ABI PRISM 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA) using the amplification primers. Sequences were edited and compiled using Sequencer 4.6 software (Gene Codes). Base-calling ambiguities between strands were resolved by choosing the call on the cleanest strand or using the appropriate IUB ambiguity code if both strands showed the same ambiguity. All molecular sequences presented in this study have been deposited in GenBank (EU313204–EU313338, Table 2).

Phylogenetic analyses

Two different data sets were analyzed independently. The first comprised the first 798 bp of cyt-*b* of 104 samples from 17 echimyid genera (including *Capromys pilorides* and *Myocastor*

Table 1 Primers and primer sequences used for amplification and sequencing in this study

Gene	Primer name	Primer sequence	Source
cyt-*b*	MVZ05	5′-CGA AGC TTG ATA TGA AAA ACC ATC GTT G-3′	Smith and Patton (1993)
	MVZ04	5′-GCA GCC CCT CAG AAT GAT ATT TGT CCT C-3′	Smith and Patton (1993)
	MVZ127	5′-TRY TAC CAT GAG GAC AAA TAT C-3′	Leite and Patton (2002)
	MVZ16	5′-AAA TAG GAA RTA TCA YTC TGG TTT RAT-3′	Smith and Patton (1993)
	MVZ45	5′-ACJ ACH ATA GCJ ACA GCA TTC GTA GG-3′	Smith and Patton (1993)
D-loop	L0	5′-CCC AAA GCT GAA ATT CTA CTT AAA CTA-3′	Douzery and Randi (1997)
	E3	5′-ATG ACC CTG AAG AAA SAA CCA G-3′	Huchon et al. (1999)
RAG1	RAG1F1705	5′-GCT TTG ATG GAC ATG GAA GAA GAC AT-3′	Teeling et al. (2000)
	FMNH1b	5′-CTT GAA GGT CCT GGG RAT GCC TCC C-3′	This study
	FMNH2a	5′-GAG AGG GAA GCC ATG AAG AGC AGY GA-3′	This study
	FMNH2b	5′-TTA TAC ACC TCC CCT ATC TCK AGC-3′	This study
	FMNH3a	5′-GGC AAT GCH GCY GAA TTC TAC AAG AT-3′	This study
	RAG1R2864	5′-GAG CCA TCC CTC TCA ATA ATT TCA GG-3′	Teeling et al. (2000)

Table 2 Genetic sampling for this study, showing GenBank registration numbers for the cyt-*b*, D-loop and RAG1 sequences we employed. Sequences prefixed by "EU" were newly generated for this study or were shared by Dr. J. L. Patton and are newly registered (unique cyt-b sequences). Leading numbers for *Isothrix* specimens identify provenience on map (see Fig. 1)

	Taxon	Museum voucher	Collector #	Cyt-*b*	D-loop	RAG1
	Ctenomyidae					
	Ctenomys maulinus		MHG 1151	AF370703		
	Octodontidae					
	Octodon degus		T-1052	AM407929		
	Abrocomidae					
	Abrocoma bennettii			AF244387		
	Caviomorpha incertae sedis					
	Capromys pilorides		T-2120	AF422915		
	Echimyidae					
	Clyomys laticeps		CIT1235	AF422918		
	Dactylomys boliviensis	FMNH 175249	BDP 3942	EU313204	EU313257	EU313298
	Dactylomys boliviensis	FMNH 175250	SS 2225	EU313205	EU313258	EU313299
	Dactylomys dactylinus	LHE 878	L23337	EU313259	EU313300	
	Dactylomys dactylinus	USNM 549842	LHE 607	L23336	EU313260	EU313301
	Dactylomys peruanus	MUSM 13052	LHE 1374	EU313206		
	Dactylomys peruanus	USNM 582148	LHE 1398	EU313207		
	Echimys chrysurus		FG 9	EU313211		
	Echimys chrysurus		FG 12	EU313208		
	Echimys chrysurus		FG 18	EU313209		
	Echimys chrysurus		FG 23	EU313210		
	Echimys chrysurus	FMNH 93267	HAB 128	EU313212	EU313261	EU313302
	Echimys chrysurus	MVZ 194303	MNFS 984	L23344		
	Echimys chrysurus	ROM 111578		EU313213	EU313262	EU313303
	Echimys chrysurus	USNM 549594	LHE555	L23341		
	Euryzygomatomys spinosus		SU73	U34858		
	Hoplomys gymnurus	MVZ 162309	JLP 9692	AF422922		
24	*Isothrix barbarabrownae*	MUSM 16819	BDP 3878	EU313214	EU313263	EU313304
15	*Isothrix bistriata*	MVZ 157974	JLP 8336	L23347	EU313267	EU313308
17	*Isothrix bistriata*	AMNH 272808	RSV 2292	EU313215	EU313264	EU313305
18	*Isothrix bistriata*	MVZ 190625	MNFS 797	L23351	EU313268	EU313309
18	*Isothrix bistriata*	MVZ 191299	MNFS 833	L23352	EU313270	EU313311
19	*Isothrix bistriata*		MNFS 901	L23354		
19	*Isothrix bistriata*	MVZ 190626	MNFS 893	L23353	EU313269	EU313310
20	*Isothrix bistriata*	INPA 2905	MNFS 471	L23349		
20	*Isothrix bistriata*	INPA 3888	MNFS 500	L23350		
21	*Isothrix bistriata*	INPA 2903	MNFS 1273	L23346		
21	*Isothrix bistriata*	INPA 2904	MNFS 1411	L23345		
22	*Isothrix bistriata*		LHE 881	EU313216	EU313265	EU313306
23	*Isothrix bistriata*	KU 144527	VPT 735	EU313218		
23	*Isothrix bistriata*	MUSM 13305	RSV 2293	EU313217	EU313266	EU313307
7	*Isothrix negrensis*		MN-CBR 2432	AY386321		

Table 2 (continued)

	Taxon	Museum voucher	Collector #	Cyt-*b*	D-loop	RAG1
10	*Isothrix negrensis*	INPA	MNFS 2111	EU313221		
10	*Isothrix negrensis*	INPA	MNFS 2122	EU313222		
11	*Isothrix negrensis*	INPA	JLP 16749	EU313220		
14	*Isothrix negrensis*	INPA 2901	JUR 545	EU313219		
16	*Isothrix negrensis*	INPA	MNFS 97	L23355		
3	*Isothrix orinoci*	USNM 415193	SVP	EU313225		
4	*Isothrix orinoci*	USNM 406370	SVP	EU313223		
5	*Isothrix orinoci*	USNM 406375	SVP	EU313224		
8	*Isothrix pagurus*	INPA 2463		L23348		
9	*Isothrix pagurus*	INPA	MNFS 2126	AY745733		
12	*Isothrix pagurus*	USNM 555639	LHE 141	EU313227		
13	*Isothrix pagurus*	INPA 2462	PIT 46	EU313226		
1	*Isothrix sinnamariensis*	MNHN 1995.1321	9461	AY745731		
1	*Isothrix sinnamariensis*	T 4377		EU313228	EU313272	EU313313
2	*Isothrix sinnamariensis*	MNHN 1995.1322	94100	AY745732		
6	*Isothrix sinnamariensis*	ROM 106624		AY745734	EU313271	EU313312
	Kannabateomys amblyonyx		CTX2942	AF422917		
	Kannabateomys amblyonyx		YL182	AF422916		
	Lonchothrix emiliae	FMNH 140821	AMO 53	EU313229		
	Lonchothrix emiliae	INPA 2472		AF422921		
	Makalata didelphoides		ECH 4	EU313230	EU313273	EU313314
	Makalata didelphoides		TTS 380	EU313233	EU313276	EU313317
	Makalata didelphoides	MVZ 197569	LPC 700	EU313231	EU313274	EU313315
	Makalata didelphoides	MVZ 198121	LPC 716	EU313232	EU313275	EU313316
	Makalata didelphoides	USNM 549593	LHE 600	L23363	EU313277	EU313318
	Makalata didelphoides	USNM 549852	LHE 554	L23361	EU313279	EU313320
	Makalata didelphoides	USNM 549853	LHE 632	L23364	EU313280	EU313321
	Makalata didelphoides	USNM 549837	LHE 595	L23362	EU313278	EU313319
	Makalata didelphoides	USNM 581981	TTS 383	EU313234		
	Makalata macrura	MUSM 15327	DWF 466	EU313235	EU313282	EU313323
	Makalata macrura	MVZ 153636	JLP 7191	L23358	EU313283	EU313324
	Makalata macrura	MVZ 153637	JLP 7197	EU313236	EU313284	EU313325
	Makalata macrura	MVZ 190621	JLP 15394	L23357	EU313285	EU313326
	Makalata macrura	MVZ 190622	MNFS 1717	EU313237	EU313286	EU313327
	Makalata macrura	MVZ 194324	JLP 15214	L23356	EU313287	EU313328
	Makalata macrura	MVZ 194325	MNFS 465	L23360	EU313288	EU313329
	Makalata macrura	MVZ 194326	MNFS 894	EU313238	EU313289	EU313330
	Mesomys hispidus	MVZ 194391/INPA 2974	MNFS 745	L23395	EU313281	EU313322
	Mesomys occultus	MVZ 194396/INPA 2690	JUR 501	L23388	EU313290	EU313331
	Myocastor coypus		NUTRIA289	AF422919		

Table 2 (continued)

Taxon	Museum voucher	Collector #	Cyt-*b*	D-loop	RAG1
Phyllomys blainvillii		LPC 227	EU313239		
Phyllomys blainvillii		LPC 290	EU313240		
Phyllomys blainvillii	MNRJ 43810	LMP27	U35412		
Phyllomys brasiliensis		AP 48	EU313241		
Phyllomys dasythrix		AC 632	EU313242		
Phyllomys dasythrix		NSV 160599	EU313243		
Phyllomys lamarum		LC 73	EU313244		
Phyllomys lundi	MNRJ 62392		EU313245		
Phyllomys mantiqueirensis	MNRJ 62393		EU313246		
Phyllomys nigrispinus		FS 12–03	EU313247		
Phyllomys pattoni	MNRJ 62391		EU313248		
Proechimys amphichoricus		ALG 14040	U35413		
Proechimys simonsi	FMNH 175264	BDP 3996	EU313249	EU313291	EU313332
Proechimys simonsi	FMNH 175283	BDP 3986	EU313250	EU313292	EU313333
Thrichomys apereoides	MVZ 197572	JLP 16981	EU313252	EU313293	EU313334
Thrichomys apereoides	MVZ 197573	JLP 16983	EU313253	EU313294	EU313335
Toromys grandis	FMNH 92198		EU313256	EU313295	EU313336
Trinomys albispinus		AL 3054	EU313251		
Trinomys dimidiatus		MAM 10	U35169		
Trinomys eliasi		ML141	U35166		
Trinomys iheringi	FMNH 141667	BDP 2831	EU313254	EU313296	EU313337
Trinomys iheringi	FMNH 141668	BDP 2864	EU313255	EU313297	EU313338
Trinomys mirapitanga	MNRJ 31459		U35173		
Trinomys paratus		YL34	U35165		
Trinomys setosus denigratus	MNRJ 31441		AF422923		
Trinomys setosus elegans	MNRJ 31448		AF422924		
Trinomys setosus setosus		AL3072	U34856		
Trinomys yonenagae		PEU88027	U35172		

Collection acronyms are: AMNH, American Museum of Natural History; FMNH, Field Museum of Natural History; INPA, Instituto Nacional de Pesquisas da Amazônia (Manaus); KU, Museum of Natural History, University of Kansas; MNHN, Muséum National d'Histoire Natural (Paris); MNRJ, Museu Nacional de Historia Natural, Rio de Janeiro; MUSM, Museo de Historia Natural, Universidad de San Marcos (Lima); MVZ, Museum of Vertebrate Zoology, University of California, Berkeley; ROM, Royal Ontario Museum; USNM, National Museum of Natural History

coypus, per Galewski et al. 2005). Representatives of the other three families of Octodontoidea (*Octodon degus*, *Abrocoma bennettii*, and *Ctenomys maulinus*) were selected as outgroups (Table 2). Some of the sequences of cyt-*b* were obtained from GenBank and others were kindly provided by Dr. James L. Patton. To assess possible effects of substitutional saturation on the topology, we re-ran the analyses using only base-pairs at the first and second (coding) positions.

The second data set includes the first 798 bp of cyt-*b* concatenated with ~440 bp of D-loop and 1,072 bp of the nuclear RAG1, for a total of 2,301 bp. Because D-loop and RAG1

sequences were unavailable for many terrestrial echimyids and the outgroups, we used the echimyid *Thrichomys apereoides* to root trees that included these sequences. In prior analyses (Lara et al. 1996; Leite and Patton 2002; Galewski et al. 2005), *Thrichomys* has typically been recovered as a member of the terrestrial clade that includes *Proechimys* and *Hoplomys*.

The three partitions of the second data set were analyzed in all possible combinations: (1) individual sequences, (2) three pairs of concatenated sequences, and (3) all three sequences concatenated together. Differences in phylogenetic signal between sequence combinations were tested using the incongruence length difference (ILD) test (Farris et al. 1995), implemented in PAUP* v. 4.0b10 (Swofford 2002) with 1,000 replicates. Although ILD was developed to evaluate parsimony-based analyses, it is also useful in evaluating trees constructed using alternative methodologies (Darlu and Lecointre 2002).

For maximum likelihood (ML) and Bayesian inference (BI) analyses, the Akaike information criterion (AIC -Akaike 1974; Hasegawa 1990) implemented in MODELTEST, version 3.7 (Posada and Crandall 1998) was used to identify the model of nucleotide substitution that best fit the data (Table 3) as assessed by increases in the log-likelihood scores.

ML analyses were conducted using Garli v. 0.951 (Zwickl 2006). Searches were run five times for 5,000,000 generations each to examine likelihood scores and trees for variation. Likelihood scores were saved every 100 generations. To gauge clade support, we ran 1,000 bootstrap replicates. BI analyses were used to estimate a phylogeny applying different models of molecular evolution for each partition. BI was conducted using MrBayes v. 3.1.2 (Ronquist and Huelsenbeck 2003), with random, unconstrained starting trees; four simultaneous Markov chains were run for 10,000,000 generations, and trees were sampled every 100 generations. Log-likelihood scores of sample points were plotted against generations to determine when the Markov chain reached stationarity, and sample points prior to "burn-in" were discarded. A 50% majority rule consensus tree calculated from the 98,000 remaining trees was constructed, and the percentage of samples recovering a given clade was used to estimate that clade's posterior probability.

Results

Cytochrome-*b* analyses

The analyses included 104 individuals representing 41 ingroup taxa and three outgroups. Genetic distances were generally high, averaging 16.57% over all taxa and individuals. The

Table 3 Models of molecular evolution selected for each molecular data set, with associated rate matrix parameters (*R*-matrix), shape parameter (α), and proportion of invariant sites (*I*)

Data	Model	*R*-matrix	α	*I*
cyt-*b* "104 samples"	K81uf + I + Γ	1.0, 14.6, 1.2, 1.2, 14.6	0.7824	0.4155
cyt-*b* "41 samples"	GTR + I + Γ	2.7, 15.5, 3.2, 0.2, 34.7	1.7393	0.5181
D-loop	TrN + I + Γ	1.0, 3.6, 1.0, 1.0, 5.5	0.8441	0.4511
RAG1	TIM + I + Γ	1.0, 2.8, 0.7, 0.7, 6.4	0.7566	0.5725
cyt-*b* + D-loop	GTR + I + Γ	1.0, 6.0, 1.2, 0.3, 9.5	1.3333	0.5112
cyt-*b* + RAG1	GTR + I + Γ	2.9, 6.3, 2.7, 0.6, 26.3	0.6522	0.5389
D-loop+RAG1	GTR + I + Γ	1.4, 3.2, 2.2, 0.6, 7.3	0.6318	0.5848
3 genes combined	GTR + I + Γ	2.0, 5.3, 2.4, 0.5, 15.6	0.7954	0.5626

ML and BI tree topologies were identical and are shown in Fig. 2. Clades strongly supported in the ML analysis were also recovered with high posterior probabilities in the BI tree.

The analysis recovered a monophyletic Echimyidae. Confirming earlier analyses, the tree supports the monophyly of all genera, as well as the affinities of *Hoplomys* + *Proechimys*, *Lonchothrix* + *Mesomys*, *Clyomys* + *Euryzygomatomys*, and *Echimys* + *Phyllomys*. Although most suprageneric groupings lacked support, all four dactylomyine species appear as a well-supported clade, with *Kannabateomys* sister to the three species of *Dactylomys*, and *D. peruanus* sister to *D. dactylinus* + *D. boliviensis*. Each of these groupings received strong nodal support (Fig. 2). The distinctive echimyine *Toromys* appears as the basal member of Echimyidae, although that node has little support.

The monophyly of *Isothrix* was well supported, and *Isothrix barbarabrownae* was recovered as its most basal element. The remaining species were grouped together with high posterior probability and bootstrap support into three clades. *I. pagurus* and *I. sinnamariensis* formed a well supported group, but all four sequences of *I. sinnamariensis* were nested within *I. pagurus*. Another clade contained all *I. negrensis* and *I. orinoci*, while the last contained all 13 samples of *I. bistriata*. As with *I. pagurus–I. sinnamariensis*, all three samples of *I. orinoci* were nested within *I. negrensis* (Fig. 2). Genetic distances between these species pairs scarcely exceeded the intraspecific variation of the paraphyletic member (Table 4).

We re-ran the cyt-*b* data set using only substitutions at the first and second base-pair positions. Genetic distances remained high, averaging 11.2%. There were very minor changes to the topology of the tree. *Isothrix* remained monophyletic, again with *I. barbarabrownae* appearing basal to the others, and those appearing as essentially a trichotomy: *I. pagurus* + *I. sinnamariensis*, *I. negrensis* + *I. orinoci*, and the various *I. bistriata*. Although most nodes retained high posterior-probability support, bootstrap values were noticeably weaker, many less than 50%, reflecting fewer characters in the rarified data set. Other groupings were as specified in the full cyt-b analysis: *Kannabateomys* + *Dactylomys*, *Hoplomys* + *Proechimys*, *Lonchothrix* + *Mesomys, Clyomys* + *Euryzygomatomys*, and *Echimys* + *Phyllomys*.

Mitochondrial and nuclear sequences

Sequences for cyt-*b*, D-loop, and RAG1 were obtained for 41 echimyid individuals representing 14 taxa, a subset of the previous data set. Analyses of cyt-*b* variation over the reduced set of taxa affirmed strong support (100% bootstrap) for monophyly of both *Isothrix* and *Makalata* and high posterior probability but low bootstrap for their sister-group relationship. Again, with high nodal support, *I. barbarabrownae* was basal to *I. sinnamariensis* and *I. bistriata*, and both were monophyletic, but specimens of *I. negrensis, I. orinoci*, and *I. pagurus* were not included. Average sequence divergence among individuals in this analysis was 16.01%.

Analyses of D-loop variation failed to support monophyly for several genera, including *Isothrix*, *Makalata*, and *Mesomys*. Some *Makalata macrura* were included in a modestly supported clade (73%) that contained specimens of *Isothrix*, while others were nested within a clade of *Makalta didelphoides.* Within the cluster containing *Isothrix*, only the interspecific grouping of *I. bistriata* + *I. sinnamariensis* showed any nodal support, and *I. bistriata, I. sinnamariensis*, *M. macrura*, and *M. didelphoides* lacked monophyly. Average D-loop sequence divergence between individuals was 14.9%.

Analyses of RAG1 variation affirmed the monophyly of both *Isothrix* and *Makalata*. *Isothrix* was sister to *Dactylomys*, and *Makalata* to *Echimys* + *Toromys*, but these intergeneric groupings were weakly supported. Although *M. macrura* and *M. didelphoides* were reciprocally monophyletic, *Isothrix* species were not, and only *I. sinnamariensis* and a pair

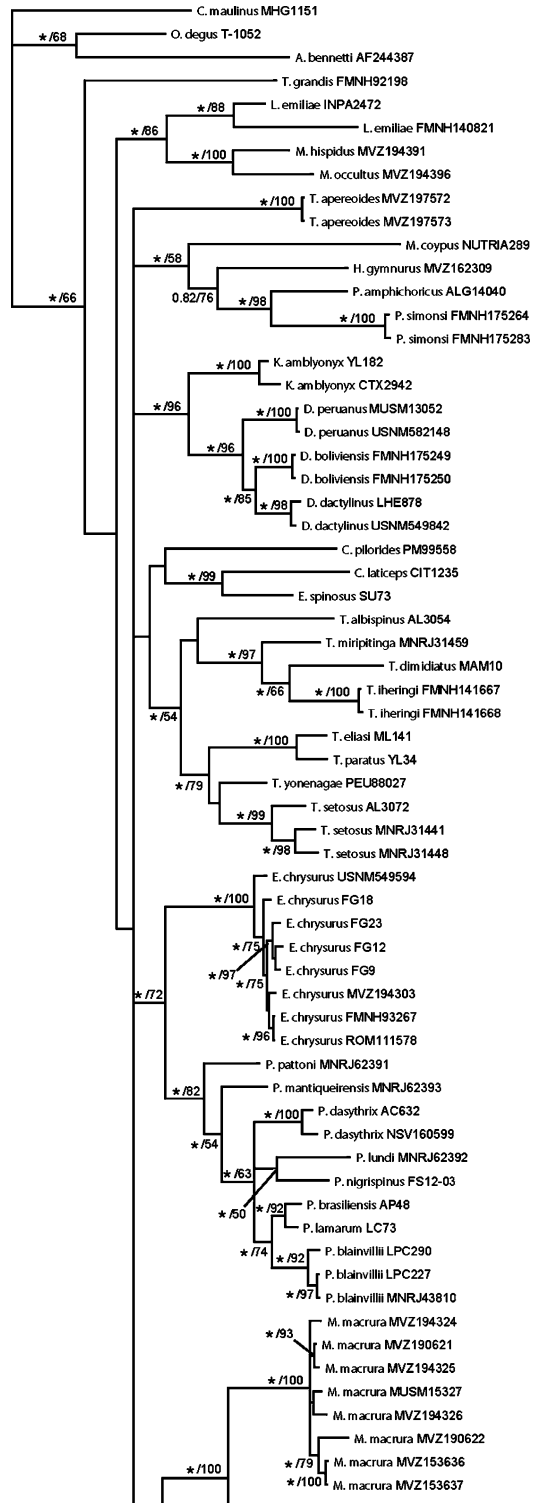
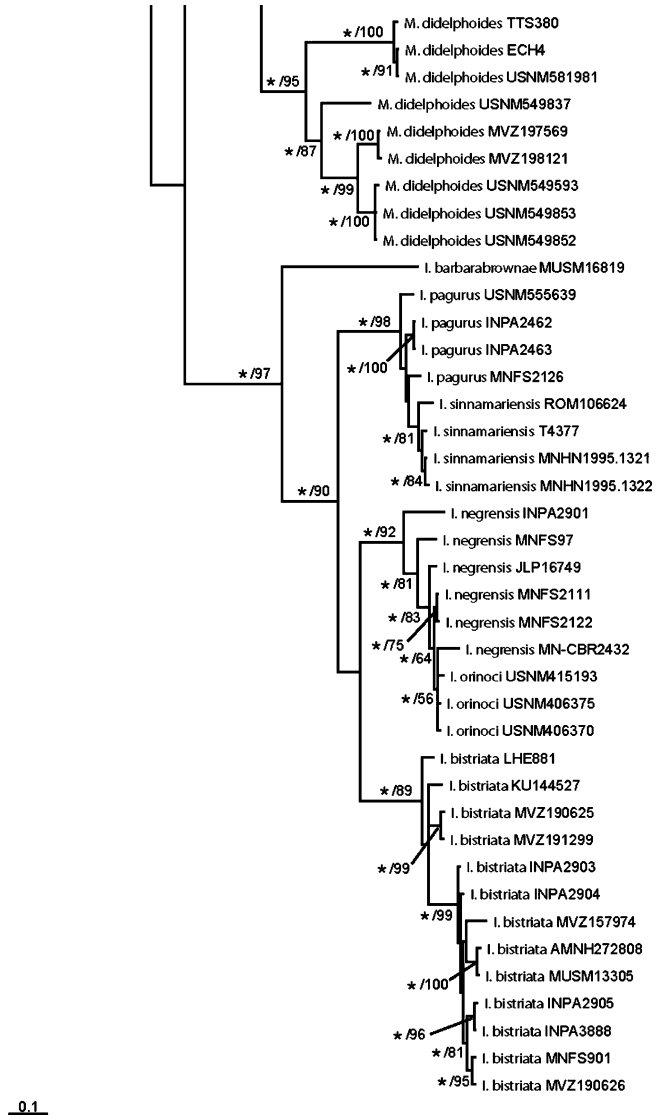


Fig. 2 Tree for cyt-*b* sequences for 104 individuals, rooted with *Octodon degus* (Octodontidae), *Abrocoma bennetti* (Abrocomidae), and *Ctenomys maulinus* (Ctenomyidae). *Asterisks* (*) at nodes denotes BI posterior probabilities ≥0.95; numbers are bootstrap values >0.5 for the ML analysis.

Fig. 2 (continued)



of *I. bistriata* (LHE881 and MUSM 13305) emerged as recognizable subgroupings. Average RAG1 sequence divergence between individuals was 2.85%.

The Incongruence Length Difference test demonstrated that all sequences – cyt-*b*, D-loop, and RAG1 – could be combined ($p = 0.993$). Combining mtDNA sequences (cyt-*b* + D-loop), most clades were strongly supported, including the monophyly of *Isothrix*. Again, *Isothrix barbarabrownae* was recovered as the most basal element of *Isothrix*, sister to *I. bistriata* and *I. sinnamariensis*. *Makalata* appeared to be paraphyletic, with *M. macrura* recovered as sister to *Isothrix* and *M. didelphoides* found to be sister to this group. *Toromys grandis* was recovered as sister to *Echimys chrysurus*.

The topology of trees based on coding gene sequences (cyt-*b* + RAG1) was similar. Most clades were strongly supported, including the monophyly of *Isothrix*, and again *Isothrix barbarabrownae* was recovered as the most basal element of *Isothrix*, sister to the clade

Table 4 Pairwise genetic distances (%) within and among species of *Isothrix*, based on 798 bp of cyt-*b*

	barbarabrownae	*bistriata*	*negrensis*	*orinoci*	*pagurus*	*sinnamariensis*
I. barbarabrownae	–					
I. bistriata	14.73	3.31				
I. negrensis	14.95	10.80	3.68			
I. orinoci	14.75	10.33	2.44	0.50		
I. pagurus	14.97	10.94	11.03	10.61	1.25	
I. sinnamariensis	14.54	11.16	10.77	10.38	2.69	1.27

I. bistriata + *I. sinnamariensis*. Contrary to the mtDNA analyses, *Makalata* was recovered as monophyletic, but *Toromys grandis* was recovered as the sister taxon to *Echimys chrysurus*.

The topology of trees recovered from D-loop and RAG1 sequences is as follows. *Dactylomys* is recovered as sister to an echimyine clade, which includes *Toromys*, *Mesomys hispidus*, and three well-supported clades: two specimens of *Echimys chrysurus*, *Makalata macrura* plus three species of *Isothrix*, and the third containing other *Makalata macrura* individuals with *M. didelphoides;* included within the latter is *Mesomys occultus.*

Combining all three sequences in a single combined analysis yields a topology consistent with most analyses as well as current taxonomy (Fig. 3). All genera were strongly supported as monophyletic, including *Isothrix, Mesomys*, and *Makalata*, which were inconsistently recovered in the preceding analyses. In fact, *Proechimys, Trinomys, Dactylomys*, *Isothrix*, and *Echimys* were all recovered as monophyletic in 100% of the bootstrapped analyses. *Mesomys* (94%) and *Makalata* (73%) were less well supported in the combined analysis, and *Toromys grandis* grouped with *Echimys chrysurus* in 65% of bootstraps. *I. barbarabrownae* was unambiguously placed basally to other *Isothrix* species.

Discussion

Analyses of mitochondrial and nuclear sequences corroborate the allocation of the Andean toró to the genus *Isothrix* on morphological grounds (Patterson and Velazco 2006). Virtually all mitochondrial, nuclear, and combined analyses show the genus to be monophyletic, and it is robustly diagnosed on cranial, dental and external grounds (Emmons 2005; Patterson and Velazco 2006). The unexpected appearance of some *Makalata macrura* within *Isothrix* in analyses involving the D-loop is almost certainly due to homoplasy in this non-coding sequence. Other peculiar relationships indicated in this same analysis include the polyphyly of *Makalata macrura*, *Isothrix sinnamariensis*, and the genus *Mesomys*. All of these relationships are recovered as monophyletic using other sequences. On the basis of both mitochondrial and combined analyses (Figs. 2 and 3), *Isothrix* includes six known species: *I. barbarabrownae*, *I. bistriata, I. negrensis, I. orinoci, I. pagurus,* and *I. sinnamariensis.*

Isothrix barbarabrownae is repeatedly and robustly recovered as basal within the genus. The cyt-*b* analyses included all recognized species of *Isothrix,* and recovered these in geographic groupings. Andean *I. barbarabrownae* was recovered as the sister to the remaining five species, which fell into three well-supported clades. Guianan *I. sinnamariensis* joined with (and was actually included within) *I. pagurus* of the lower Amazon Basin. *I. orinoci* of the Río Orinoco drainage joined with (and was actually included within) *I. negrensis* of the adjacent Rio Negro drainage. Various samples of *I. bistriata* comprise the final, most variable species.

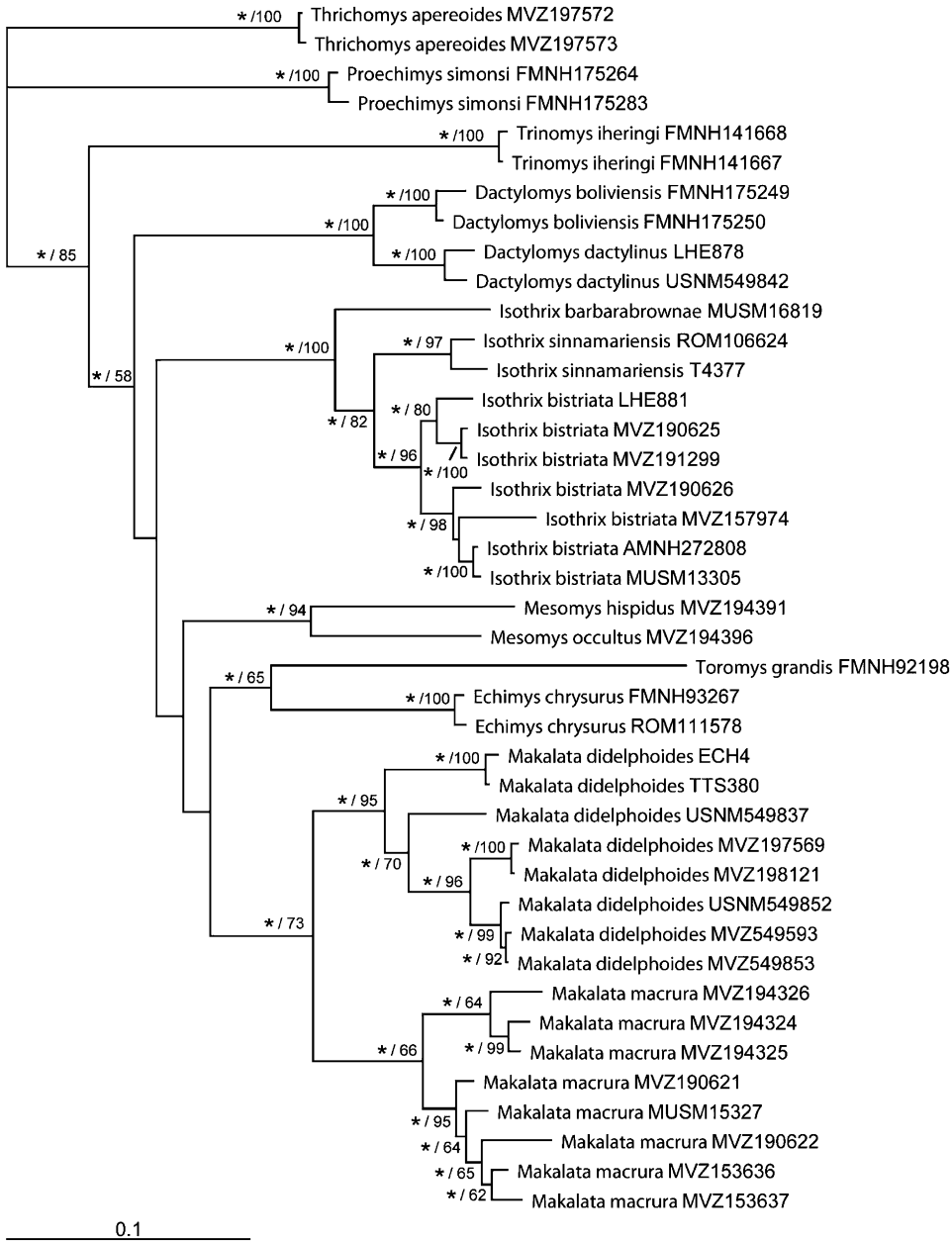


Fig. 3 Tree for cyt-*b*, D-loop, and RAG1 genes rooted with *Thrichomys* (Echimyidae). *Asterisks* (*) at nodes denote BI posterior probabilities ≥0.95; numbers are bootstrap values >0.5 for the ML analysis.

These relationships extend those identified by Lim et al. (2006), which did not include *I. barbarabrownae* or *I. orinoci*.

Although several *Isothrix* species appear not to be reciprocally monophyletic on sequence grounds, we do not suggest nomenclatural revisions at this time. One related dyad,

I. sinnamariensis and *I. pagurus*, are so distinctive morphologically that they can be distinguished at 10 m in the field; moreover, they are known to have distinctive karyotypes, the former with $2n = 28$ and the latter with $2n = 22$ (Vie et al. 1996). Another dyad includes *I. orinoci*, which Patton and Emmons (1985) found was highly distinctive in cranial measurements, but has not been characterized karyotypically; *I. bistriata* and *I. negrensis* are known to differ in fundamental number (Bonvicino et al. 2003). Decisions on species limits are better made from coordinated studies of multiple character systems, not from phylogenetic analyses such as these.

The paraphyly documented here for *I. pagurus–I. sinnamariensis* and *I. orinoci–I. negrensis* would be expected in recently radiating lineages that had not yet lost ancestral polymorphisms (e.g., Maddison and Knowles 2006). The genetically nested positions (Fig. 2) of the northernmost and easternmost members of the genus suggest that the speciation events giving rise to them accompanied range expansion of *Isothrix* into the Orinoco Basin (in the case of *I. orinoci*) and the Guianas (in the case of *I. sinnamariensis;* see Fig. 1). The small genetic distances involved (Table 4) suggest that these speciation events at least have been relatively recent.

Sampling limitations and weak nodal support enable few other systematic conclusions from these analyses. However, the cyt-*b* analyses provide the first evidence of species relationships within the widespread bamboo rats *Dactylomys*. The lowland species *D. boliviensis* and *D. dactylinus* are sisters to the montane species *D. peruanus*. As in Galewski et al. (2005), this group clusters with the Atlantic forest bamboo rat, *Kannabateomys*. It confirms the integrity of the large-toothed bamboo rat group previously named "Dactylomyinae;" samples should be sought to expand and test this hypothesis by inclusion of the north Andean forms currently allocated to *Olallamys*.

Although the combined-analysis topology clusters *Echimys* + *Phyllomys* and *Makalata* + *Isothrix*, there is little support for either pairing. The absence of numerous related genera, including *Callistomys, Diplomys*, and *Santamartamys* among others, renders these pairings meaningless.

Historical biogeography

Various centers of endemism have been recognized in the Neotropical Realm (Cracraft 1985; Fjeldså 2000). Some, like the temperate *Nothofagus* forests of southern Chile and Argentina, support highly distinctive faunas whose endemism rivals that of New Guinea (Patterson 1992b). Others, like the Andean Altiplano or arid Chaco and Caatinga, are ecologically sharply differentiated from their neighbors (Reig 1981, 1986). For tropical rainforest organisms, the principal endemic regions of South America include (1) Atlantic Forests of southeastern Brazil, eastern Paraguay, and northern Argentina, (2) greater Amazonia, including the Guianan Shield, and (3) the Pacific Chocó and adjacent Central America (Stotz et al. 1996).

A fourth tropical rainforest region blankets the eastern Andean slopes and harbors one of South America's richest biotas, but it is usually not specified in analyses of lowland faunas. Cracraft and Prum (1988) developed a generalized area cladogram for Neotropical forest biotas that is broadly descriptive, despite the heterogeneity of most areas and groups (e.g., Costa 2003). The Atlantic Forests harbor some of South America's oldest and most distinctive lineages (Patton and da Silva 1997), and their inhabitants often occupy basal positions on cladograms. "Trans-Andean" forests of the Chocó in Pacific Colombia and adjoining Central America, which became isolated from other South American forests by the rising Andes, form the next-most-basal element (Hackett 1996; Van Den Bussche et al. 1998; Albert et al. 2006). Finally, many of the species distributed in the Guianas and Amazonia, some of which extend

into the cerrado, Atlantic Forest, and east Andean slopes, were generated in the latest wave of diversification events (Cracraft and Prum 1988; Lim et al. 2004).

These last regions – Guianan Shield, Amazonia proper, and the lower Andean slopes – include some of the world's most speciose biotas, and patterns of diversification are still not well understood. Although "refuge theory" was originally proposed for this region (Haffer 1969; Vanzolini and Williams 1970) and supporting genetic data can be marshaled (Eberhard and Bermingham 2004), many Amazonian species are simply too old to be products of Pleistocene climate-induced vicariance in refugia (e.g., Patton et al. 2000). The antiquity of the Guianan Shield, one of three extensive highlands that escaped Cenozoic inundation (Nores 1999), makes it a likely locus for regional endemics (Hall and Harvey 2002). On the other hand, the still-rising Andes have been colonized repeatedly by species from the adjacent lowlands (Vuilleumier and Monasterio 1986; Bates and Zink 1994). While such colonizations have sometimes led to explosive speciation in the Andes (Fjeldså and Rahbek 2006; Hughes and Eastwood 2006), Andean species may occasionally reinvade the lowlands, which could trigger a new radiation (Burns and Naoki 2004; Brumfield and Edwards 2007).

The phylogenetic patterns documented here raise the possibility that the Andes or perhaps the proto-Andes were included in the ancestral range of the most-recent common ancestor of living *Isothrix*. Andean *I. barbarabrownae* is sister to all of the remaining lowland species of *Isothrix*. On distributional grounds invoking the "progression rule," (Hennig 1966) it is as likely for this radiation to have originated in the Andean theater occupied by *I. barbarabrownae* as in the lowland area occupied by the other five species in the genus. This topology of an Andean clade basal to an Amazonian radiation is repeated in Fig. 2, by the bamboo rats *Dactylomys*. Andean *D. peruanus* is sister to the grouping formed by *D. boliviensis* of southwestern Amazonia and *D. dactylinus* in areas north and east. Like *I. barbarabrownae*, *D. peruanus* is restricted to cloud forests in southern Peru between 1,000–2,000 m (Woods and Kilpatrick 2005). However, showing that either genus originated in the Andes or proto-Andes would require identification of an Andean sister group. The Colombian dactylomyine *Olallamys*, which has not yet been included in molecular analyses, may prove to fill this phylogenetic position (between *Dactylomys* and *Kannabateomys* in Fig. 2), but the sister-group for *Isothrix* remains elusive despite numerous analyses.

Galewski et al. (2005) used a relaxed molecular clock to estimate divergence times for the echimyid radiation. Living members are supposed to have originated in the early Miocene and most of its radiation dates to middle and late Miocene times (10–15 Mya). This includes the divergence of arboreal genera (14.4 Mya) and the *Dactylomys–Kannabateomys* split (9.5 Mya). Galewski and colleagues estimated various species-level divergences within genera to have taken place 2.3–5.5 Mya, and dated the *I. sinnamariensis–I. bistriata* bifurcation at 2.7 Mya (late Pliocene). Clearly, only the latest divergences (*I. sinnamariensis–I. pagurus* and *I. orinoci–I. negrensis*) could have been triggered by Pleistocene climatic changes in accordance with refuge theory. In contrast, divergence of *I. barbarabrownae* must have predated these other splits, dating to late Miocene or early Pliocene times.

Although the Andes are young, tectonically active, and continue to uplift, they have developed via irregular accretion over the past 25 My (Ramos and Aleman 2000). By the middle Miocene (9–15 Mya), a proto-Andean block was already in place, creating the semi-arid to arid climate that characterized the Central Andes for most of the Miocene and Pliocene. This landform and associated climate changes helped to trigger the profusion of hypsodonty in Cenozoic faunas (Croft 2001) and would have been a major landscape feature during the time-period in which echimyids diversified. We suggest that the Andean components of both *Isothrix* and *Dactylomys* evolved not in the Andes *per se* but rather in this proto-Andean block. The topology in Fig. 2 cannot resolve whether these lineages subsequently colonized the

lowlands from this montane center, or whether highland forms were isolated from lowland sisters by vicariance during uplift—the highland lineage simply diverged prior to the radiation of lowland forms. By the progression rule, both topologies identify the Andes as a possible theater for the group's basal diversification.

The antiquity of this proto-Andean region, 10–15 My old, contributes to the obvious heterogeneity of eastern versant faunas. Some are clearly descended from Amazonian stocks while others may be basal to or coeval with them. In a similar fashion, the Atlantic Forest harbors some of South America's most distinctive biotas, which are basal to those in remaining forests, but certain taxa are obviously recently derived from Amazonian clades (Costa et al. 2000).

Pelage evolution in Echimyidae

The Echimyidae exhibits tremendous variation in the texture of dorsal pelage. Genera like *Callistomys*, *Diplomys*, and *Isothrix* have long, lax hairs on the back and rump, while others depart sharply from this condition. Dorsal pelage tends to be moderately soft in *Thrichomys*, coarser in *Toromys*, and may be spiny or even quilled in *Echimys*, *Lonchothrix* and *Hoplomys* (see

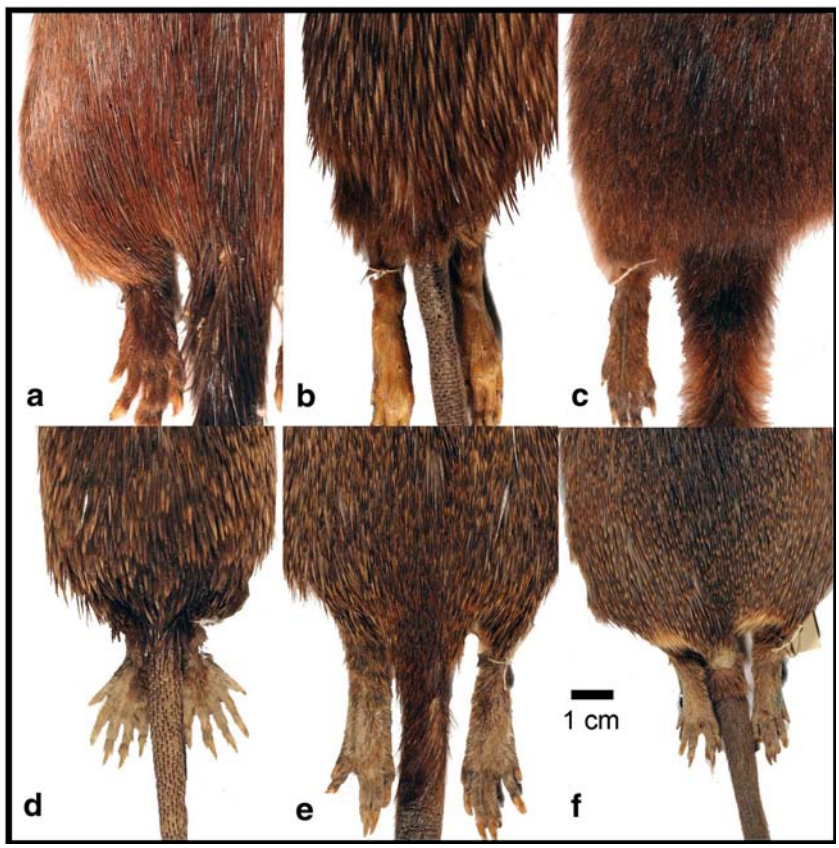


Fig. 4 Pelage of echimyid rodents (rump, hind feet and tail) included in this analysis, all to same scale (visible in **f**): **a** *Echimys chrysurus* (FMNH 93267); **b** *Hoplomys gymnurus* (FMNH 90122); **c** *Isothrix barbarabrownae* (MUSM 16819; image inverted); **d** *Lonchothrix emiliae* (FMNH 52374); **e** *Makalata didelphoides* (FMNH 71123); **f** *Mesomys hispidus* (FMNH 71124).

Fig. 4). Within certain genera, for example *Makalata* (Patterson 1992a; Emmons 1993), *Phyllomys* (Leite 2003), and *Proechimys* (da Silva 1998), there is substantial interspecific variation in the coarseness of pelage. However, the functional significance of both intergeneric and interspecific variation in pelage remains unknown (Hoey et al. 2004). Even the spiniest members of the family are not as heavily fortified as porcupines (Erethizonidae), where the defensive function of the pelage is well established. Without a resolved phylogeny, it is impossible to document the evolution of this striking pelage diversity, rendering both its phylogenetic and adaptive significance unknown. Nevertheless, some cursory observations are possible.

The spiniest members of the family Echimyidae are found in tropical lowland forests. And many of the softest-haired members of the family range into high elevations or latitudes in the Andes (*Isothrix, Dactylomys*, *Diplomys*) or the cerrado (*Thrichomys*). However, exceptions abound (e.g., soft-furred *Callistomys* in coastal Brazil) in this diverse radiation. Long, lax hair has obvious adaptive value for thermoregulation in cool and damp habitats. Fooden and Aimi (2005) showed hair density of *Macaca fuscata* populations increased in colder climates. In keeping with these observations, the Andean species like *Isothrix barbarabrownae* and *Dactylomys peruanus* have longer and denser pelage than their lowland congeners. In fact, the entire radiations of both forms exhibit relatively soft fur, even in habitats where most "spiny rats" are coarsely furred or spiny.

Another spiny rat shows parallel variation in apparently adaptive pelage variation. Like *Isothrix* and *Dactylomys*, the spinose genus *Mesomys* contains a single highland species in the Peruvian Andes (*Mesomys leniceps*), neighboring three others in western, central, and eastern Amazonia (*M. hispidus, M. occultus,* and *M. stimulax*, respectively). Unlike those genera, *Mesomys* is predominantly spiny. In keeping with a thermoregulatory interpretation of hirsuteness, the Andean "woolly-headed spiny rat" is distinctly longer-haired and woollier than lowland forms of *Mesomys* (Thomas and St. Leger 1926), but *M. leniceps* retains its spiny overcoat. More complete phylogenetic analyses of echimyid diversity and more detailed study of variation in guard hairs, aristiforms, and undercoats will be needed to understand this morphological diversity.

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