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Mitogenomic Phylogeny, Diversification, and Biogeography of South American Spiny Rats

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Abstract

Echimyidae is one of the most speciose and ecologically diverse rodent families in the world, occupying a wide range of habitats in the Neotropics. However, a resolved phylogeny at the genus-level is still lacking for these 22 genera of South American spiny rats, including the coypu (Myocastorinae), and 5 genera of West Indian hutias (Capromyidae) relatives. Here, we used Illumina shotgun sequencing to assemble 38 new complete mitogenomes, establishing Echimyidae, and Capromyidae as the first major rodent families to be completely sequenced at the genus-level for their mitochondrial DNA. Combining mitogenomes and nuclear exons, we inferred a robust phylogenetic framework that reveals several newly supported nodes as well as the tempo of the higher level diversification of these rodents. Incorporating the full generic diversity of extant echimyids leads us to propose a new higher level classification of two subfamilies: Euryzygomatomyinae and Echimyinae. Of note, the enigmatic *Carterodon* displays fast-evolving mitochondrial and nuclear sequences, with a long branch that destabilizes the deepest divergences of the echimyid tree, thereby challenging the sister-group relationship between Capromyidae and Euryzygomatomyinae. Biogeographical analyses involving higher level taxa show that several vicariant and dispersal events impacted the evolutionary history of echimyids. The diversification history of Echimyidae seems to have been influenced by two major historical factors, namely (1) recurrent connections between Atlantic and Amazonian Forests and (2) the Northern uplift of the Andes.

Key words: **biogeography, diversification, Echimyidae, mitogenomics, Neotropics, nuclear DNA, fast-evolving gene, *Carterodon*.**

Introduction

Thanks to a unique geological history and ecology, the Neotropical biota together composes the richest realm of biodiversity in the world. The formation of complex riverine systems and the uplift of the Andean Cordillera has, together with climatic changes, driven several spectacular biological radiations (Hoorn et al. 2010; Patterson and Costa 2012). Based on geology and zoogeography (Morrone 2014), the tropical Americas can be subdivided into six major biomes (fig. 1): Amazon Basin, Central Andes and Guyana Shield (AM), the trans-Andean region plus Chocó (CH), the Northern Andes and northern coastal dry forests in Venezuela and Colombia (NA), Caatinga, Chaco, Cerrado and the Pantanal (CC), Atlantic Forest (AF), and the insular West Indies (WI). These biomes are characterized by several large forested regions (Amazon Basin, Atlantic Forest, Chocó, Orinoco watersheds and West Indies), open habitats (CC: Cerrado + Caatinga, and Chaco; NA: Pantanal + Llanos), and the Andean mountain range (Central + Northern Andes). The major barriers currently separating the forested biomes were formed following climate aridification and/or mountain uplift during the Miocene (Hoorn et al. 2010), and have substantially contributed to biotic divergence and endemism in the Neotropics. The Amazon Basin forms the largest Neotropical biome and is surrounded to the west by the Chocó wet forest (across the Northern Andes), both the Orinoco watershed and the northern coastal dry forests in Venezuela and Colombia, and the coastal Atlantic Forest of Brazil and Paraguay. Disjunct distributions of terrestrial vertebrate species among the Amazon Basin and the Atlantic Forests reflect a common geoclimatic divergence structuring in South American biotas (da Silva and Patton 1993; Patton et al. 2000; Batalha-Filho et al. 2013). Biotas of those two rainforests are among the most diverse globally, and are separated by a diagonal region of xeric habitats comprising the

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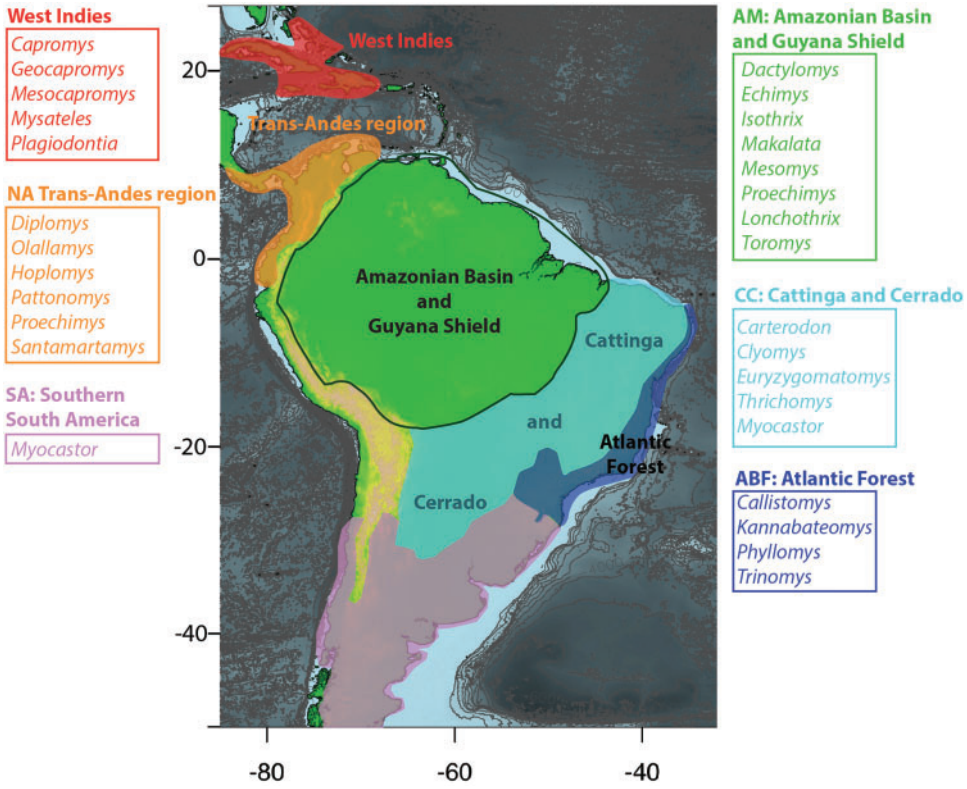

FIG. 1. Distribution map for all genera of Echimyidae and correspondence between their ranges and the biogeographical provinces of the Neotropics.

Cerrado and Caatinga biomes (Ab'Sáber 1977). Geological and paleontological data (Hoorn et al. 2010) suggest that areas of the Amazon Basin and Atlantic Forest have undergone successive separations and connections since the Miocene, including: (1) expansion of foreland hydrologic basins of the West Amazon basin (23–7 Ma; see Lundberg et al. 1998; Wesselingh and Salo 2006; Wesselingh et al. 2006); (2) potential watershed shifts between the Paraná Sea and the Amazon River (12–7 Ma; Hoorn 1996; Räsänen and Linna 1996; Lundberg et al. 1998; Roddaz et al. 2006); and, (3) more recent Plio-Pleistocene climatic events (Costa 2003). Xeric habitats appear to have represented more labile barriers to gene flow than previously hypothesized (Patton et al. 2000) as demonstrated by the relatively young phylogeographic structure in birds and mammals of the northern Neotropics (Batalha-Filho et al. 2013; Nascimento et al. 2013). North of the Amazon Basin, the uplift of the Northern Andes and emergence of the Orinoco watershed has also shaped patterns of biodiversity, both as a vicariant barrier between Mesoamerica and coastal areas north of the Amazon Basin, and as a heterogeneous region within which species can be isolated, diverge, and later disperse to other regions (i.e., a "species pump") (Cracraft and Prum 1988; Ron 2000; Patterson and Costa 2012).

In this paleo-geographical context, South American spiny rats (Mammalia: Rodentia: Echimyidae) have radiated across multiple biomes, and encompass today a vast array of life histories and ecomorphological adaptations, including capacities for semi-fossorial, arboreal, and semi-aquatic lifestyles (see review by Emmons et al. 2015). Understanding the process of higher level diversification of Echimyidae is at the core of several major biogeographical questions. Historical biogeography has been posited (Leite and Patton 2002; Galewski et al. 2005) as a main driver of echimyid rodent divergence, with two major regions involved in alternating vicariance and dispersal through time: (1) eastern South America, including the Atlantic Forest, and the open formations of the Cerrado and Caatinga; and, (2) the Amazon Basin, including the cis-Andean highland regions and the forests of the Southern Orinoco Basin and Guiana Shield. The lowland rainforest of the Amazon Basin supports most of the extant echimyid diversity, with eight arboreal genera (*Dactylomys*, *Echimys*, *Isothrix*, *Makalata*, *Mesomys*, *Lonchothrix*, *Toromys*, *Pattonomys*) and the speciose terrestrial genus *Proechimys* (at least 22 species; Emmons et al. 2015). The Atlantic Forest biome also harbors a diverse assemblage of echimyid species grouped into three arboreal genera (*Kannabateomys*, *Phyllomys*, *Callistomys*), one terrestrial genus (*Trinomys*), and one semi-fossorial genus (*Euryzygomatomys*). In between the Amazon Basin and Atlantic Forest, echimyids also diversified in: (1) grasslands, as semi-fossorial specialists in the Cerrado and Caatinga biomes (*Carterodon*, and *Clyomys*) and, (2) within open vegetation of the Caatinga, Cerrado, Chaco and Pantanal with the ground-dwelling terrestrial genus *Thrichomys* (Borodin et al. 2006; D'Elía and Myers 2014). This pattern suggests the influence of vicariant processes between the Amazonian and Atlantic forests (Galewski et al. 2005; Fabre, Galewski et al.

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2013), as well as the influence of past Andean uplift (Patterson and Velazco 2008; Upham et al. 2013), offering two important drivers for the genus-level diversification of echimyid species.

Previous phylogenetic results suggest the initial diversification of Echimyidae during the Early Miocene (Galewski et al. 2005; Upham and Patterson 2012; Fabre, Galewski, et al. 2013), including independent originations of arboreality in the Amazon and presumably again in the Atlantic Forest on the lineage leading to *Callistomys* (Loss et al. 2014). The main arboreal clade of echimyids, which includes taxa in both Amazonian and Atlantic rainforest areas, indicates that multiple transitions have been made among these regions (Upham and Patterson 2012; Fabre et al. 2013). If these obligate arboreal taxa could not have crossed the dry biomes of Caatinga and Cerrado without a forest corridor, then Miocene introgressions of freshwater (e.g., Pebas, or Acre Systems) could have played a role in the arboreal diversification of mammals, as has been suggested for *Chaetomys* (Vilela et al. 2009) and other vertebrates (Martini et al. 2007; Pellegrino et al. 2011; Fouquet et al. 2012). As this pattern is not well documented in mammalian lineages (but see Costa 2003 and Costa and Leite 2012), additional comparisons with phylogenetic, biogeographical and geological results will offer a better understanding of isolation processes in this area through the Miocene.

The role of the Northern Andes as a barrier between the lowland rainforests of the Chocó and Orinoco watersheds, and those of the Amazon Basin are overlooked in all current biogeographical models. Since the late Miocene, Andean orogeny has been an active force in major faunal separation between these areas (Cracraft and Prum 1988; Ron 2000; Delsuc et al. 2004; Hoorn et al. 2010; Patterson and Costa 2012; Patterson et al. 2012). This mountain uplift has led to: (1) cis- (eastern slope) and trans-Andean (eastern-to-western slope) vicariant separations of several mammalian groups (Ron 2000; Patterson and Costa 2012); and, (2) south-to-north divergences between the Amazon Basin and Orinoco watershed. At least five echimyid genera are now endemic to the Northern Andes, including *Olallamys*, *Hoplomys* and *Diplomys* in trans-Andean Chocó rainforest and Central America, and *Pattonomys* and *Santamartamys* in cis-Andean north of the Cassiquiare canals (we exclude Amazonian *Pattonomys occasius*, a species pending revision; Emmons LH and Fabre P-H, personal communication). The trans-Andean regions include the basins between the separate branches of the northern Andes, the lowland Pacific coastal region, the very wet Chocó forest, and the extension of that biome into southern Central America—the barriers between which may have encouraged alternating events of vicariance and dispersal for echimyids.

The picture of phylogenetic, ecomorphological and biogeographical evolution of echimyids is complicated by Capromyidae, the family comprising several extinct and extant genera of hutias endemically distributed in the West Indies (Dávalos and Turvey 2012). Capromyids also display various life history traits and adaptations, including terrestrial, scansorial and arboreal taxa. Because capromyids have been considered as a distinct family closely related to echimyids (Woods and Kilpatrick, 2005; Upham and Patterson 2012), or even nested within a paraphyletic echimyid assemblage (Galewski et al. 2005; Upham and Patterson 2012, 2015; Fabre, Galewski et al. 2013, Fabre et al. 2014), taxa from both families should be sampled to understand the evolution of these taxa.

Beyond biogeographical patterns, the Echimyidae have been the focus of recent taxonomic debates (see Carvalho and Salles 2004; Emmons 2005; Patton et al. 2015 for an overview). Pioneering taxonomies were primarily based on external characters and teeth occlusal patterns that are now considered homoplasic (Candela and Rasia 2012; Fabre et al. 2013). Recent molecular studies (Lara et al. 1996; Leite and Patton 2002; Galewski et al. 2005; Fabre et al. 2013; Upham et al. 2013) have indicated that the terrestrial spiny rats *Proechimys* and *Trinomys*, long considered congeneric, belong to different echimyid clades and are not closely related, despite overall morphological similarities. Within the main arboreal clade, there is a lack of phylogenetic signal at deeper nodes so that many basal relationships remain unresolved (Galewski et al. 2005; Fabre, Galewski, et al. 2013; Upham et al. 2013). The sampling of new molecular markers, as well as hitherto unsequenced genera (*Carterodon*, *Callistomys*, *Diplomys*, *Pattonomys*, *Santamartamys*) therefore promises to enhance our understanding of echimyid systematics and biogeographic history (Upham and Patterson 2015).

The rise of next-generation DNA sequencing (NGS) methods (e.g., Illumina; Meyer et al. 2008) has made access to mitochondrial (mito) genomes and nuclear genes from fresh tissues readily available, and in an increasingly time- and cost-effective manner for museum skins (Rowe et al. 2011; Guschanski et al. 2013). High-throughput sequencing methodologies have for example increased the number of available complete mitogenomes of mammals (Horner et al. 2007; Horn et al. 2011; Fabre, Jønsson, et al. 2013), including two recent studies on Caviomorpha, the lineage of South American rodents to which echimyids belong (Tomasco et al. 2011; Vilela et al. 2013). Moreover, the availability of probabilistic mixture models of sequence evolution (Lartillot 2004; Lartillot et al. 2007) allow us to maximally extract phylogenetic information from mitochondrial sequences, nuclear genes, and a combination thereof.

Here, we used an Illumina shotgun sequencing approach (Tilak et al. 2015) to obtain 38 new mitogenomes of Echimyidae (including members of the Capromyidae and Myocastorinae) and up to five slower-evolving nuclear exons from key taxa. Our approach has, for example, generated the first complete mitogenomes from rare museum specimens of endemic species in the genera *Santamartamys*, *Olallamys*, and *Mesocapromys*. After combining these newly sequenced mitogenomes and nuclear markers with publically available sequences, we used model-based approaches (maximum likelihood, Bayesian inference) to infer the phylogeny of Echimyidae + Capromyidae and to estimate a timeframe for their diversification. Then we explored their Neogene evolutionary history addressing the following questions:

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(1) Does the addition of new mitogenomic and nuclear gene data, plus enhanced sampling of arboreal taxa, help to improve the node support of the higher level echimyid and capromyid phylogeny? (2) What has been the timeframe of the Echimyidae + Capromyidae diversification? (3) To what extent are geo-climatic historical events reflected in the higher level composition of echimyid communities within Neotropical biomes?

Materials and Methods

Taxonomic Sampling

We sampled all 26 extant genera (Emmons 2005; Woods and Kilpatrick 2005) in the clade composed of Echimyidae (23/23; including Myocastorinae) and Capromyidae (5/5). Biological samples were obtained of both museum and fresh tissue collections from the American Museum of Natural History (AMNH: New York, NY), the Field Museum of Natural History (FMNH: Chicago, IL), the Museum of Comparative Zoology (MCZ: Cambridge, MA), the Museum of Vertebrate Zoology, University of California (MVZ: Berkeley, CA), the U.S. National Museum of Natural History (USNM-Smithsonian: Washington DC), the Nationaal Natuurhistorisch Museum (Naturalis: Leiden, The Netherlands), the Royal Ontario Museum (ROM: Toronto, Canada), the Universidade Federal do Espirito Santo—Animal Tissue Collection (UFES-CTA: Vitória, Brazil) and the University of Montpellier (UM-ISEM: Montpellier, FR) (see supplementary table S1, Supplementary Material online). In total, we obtained complete mitogenomes for 38 taxa spanning all extant members of Echimyidae and Capromyidae. In addition, we searched NCBI (see also supplementary table S1, Supplementary Material online) for mitogenomic and nuclear sequences documented by voucher specimens. We incorporated representatives of the octodontoid families Ctenomyidae and Octodontidae, their closest Caviomorpha relatives (Upham and Patterson, 2015), to serve as outgroups. We also included one member of the families Caviidae, Erethizontidae, and Chinchillidae, to represent the other three superfamilies of caviomorphs, as more distant outgroups to stabilize the Octodontoidea relationships.

Sequencing and Assembly of Complete Mitochondrial Genomes

Eleven museum skin samples were stored in Eppendorf tubes, a subset of which was processed in the "Degraded DNA" facility of the University of Montpellier, France (dedicated to processing low quality/quantity DNA tissue samples). Alcohol-preserved samples of fresh tissues were available for 27 other taxa (table 1 and supplementary table S1, Supplementary Material online); the DNA from these samples was extracted in a dedicated room of the laboratory to reduce contamination risks. An additional DNA extract of *Santamartamys rufodorsalis* was included in the preparation of genomic libraries, derived using methods described in Upham *et al.* (2013). DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's instructions, with a final elution in water. The 11 oldest samples were extracted in small batches (of 3 samples maximum), and a negative control was included in each batch to monitor possible contamination. The DNA was fragmented by sonication using an ultrasonic cleaning unit (Elmasonic). The 3′-ends of the obtained fragments were then repaired and double-strand filled before being ligated with adaptors and tagged according to a cost-effective protocol for Illumina library preparation (Meyer and Kircher 2010; Tilak et al. 2015). Tagged DNA libraries were pooled and sequenced as single end reads on Illumina HiSeq 2000 lanes at the GATC–Biotech company (Konstanz, Germany).

Raw 101-nt reads were imported in Geneious R6 (Kearse et al. 2012) and adaptor fragments were removed by the "trim ends" utility. Then, a mapping of the reads on the phylogenetically closest available mitochondrial genome was performed for each species. The following mapping parameters were used in the Geneious read mapper: a minimum of 24 consecutive nucleotides (nt) perfectly matching the reference, a maximum 5% of single nt mismatch over the read length, a minimum of 95%-nt similarity in overlap region, and a maximum of 3% of gaps with a maximum gap size of 3-nt. Iterative mapping cycles were performed in order to elongate the sequence until recovery of the complete mitogenome. A high-quality consensus was generated and the circularity of the mitogenome was verified by the exact 100-nt sequence superimposition at the assembly extremities. The number of satellite repeats in the control region remained underestimated, as there were Illumina reads consisting entirely of repeats, making it difficult to determine the exact copy number. New mitochondrial genomes are available under accession numbers KU762015, and KU892752 to KU892788.

Sequencing and Assembly of Nuclear Markers

To complement the mitochondrial information with nuclear DNA markers, we used the protocol of Fabre et al. (2014: cf., Supplementary Methods in the Data Supplement, Supplementary Material online) to capture five partial nuclear exons for *Carterodon* and *Callistomys* (*apoB*, *GHR*, *IRBP*, *RAG1*, *vWF*). In brief, nine primer sets from the literature were used to amplify nuclear DNA baits from modern genomic extracts of *Capromys pilorides*. Bait amplicons were sheared into ~300–500 bp fragments. All purified amplicons were built into blunt-end libraries. Captured libraries were amplified before being pooled equimolarly and sequenced on one lane of a Illumina MiSeq (50 bp) single-read run. Another capture using just the nuclear DNA baits was also sequenced on one lane of a HiSeq2000 run. Newly acquired nuclear exons were combined with previously published ones (Fabre et al. 2014). With the exception of two taxa (*Lonchothrix emiliae* and *Santamartamys rufodorsalis*), we obtained nuclear gene sequences for all examined species. New nuclear exons are available under accessions KY303652 to KY303660.

DNA and Protein Supermatrices

We compared our newly obtained mitogenomes and nuclear data with sequences available from public databases (Lara et al. 1996; Lara and Patton 2000; Leite and Patton 2002; Galewski et al. 2005; Patterson and Velazco 2008;

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Table 1. List of the Species Treated in This Study and GenBank Accession Numbers of Sequences.

Taxa	Collection	Tissue_number	Publication	Accession mitogenome	Tissues
Ctenomys rionegrensis	—	—	Tomasco et al. (2011)	HM544130.1	Fresh tissue
Octodon degus	—	—	Tomasco et al. (2011)	HM544134.1	Fresh tissue
Spalacopus cyanus	—	—	Tomasco et al. (2011)	HM544133.1	Fresh tissue
Tympanoctomys barrerae	—	—	Tomasco et al. (2011)	HM544132.1	Fresh tissue
Proechimys longicaudatus	—	—	Tomasco et al. (2011)	HM544128.1	Fresh tissue
Trinomys dimidiatus	—	—	Voloch et al. (2013)	JX312694.1	Fresh tissue
Callistomys pictus	UFES-CTA	RM-233	*This paper*	KU892754	Fresh tissue
Capromys pilorides	MVZ Mammals	MVZ-191417	*This paper*	KU892766	Fresh tissue
Carterodon sulcidens	UFES-CTA	LGA-735	*This paper*	KU892752	Fresh tissue
Clyomys laticeps	UFES-CTA	MCNM-2009	*This paper*	KU892753	Fresh tissue
Dactylomys dactylinus	UFES-CTA	UFROM-339	*This paper*	KU762015	Fresh tissue
Diplomys labilis	USNM-Smithsonian	USNM-335742	*This paper*	KU892776	Dry study skin
Echimys chrysurus	UM-ISEM	T-3835	*This paper*	KU892781	Fresh tissue
Euryzygomatomys spinosus	UFES-CTA	CTA-1028 (YL-53)	*This paper*	KU892755	Fresh tissue
Geocapromys browni	Naturalis-Leiden	ZMA.MAM.23884	*This paper*	KU892767	Dry study skin
Geocapromys ingrahami	USNM-Smithsonian	USNM-395696	*This paper*	KU892768	Dry study skin
Hoplomys gymnurus	MVZ Mammals	MVZ-225082	*This paper*	KU892779	Fresh tissue
Isothrix sinnamariensis	UM-ISEM	T-4377	*This paper*	KU892785	Fresh tissue
Kannabateomys amblyonyx	UFES-CTA	MBML-3001	*This paper*	KU892775	Fresh tissue
Lonchothrix emiliae	FMNH	FMNH 140821	*This paper*	KU892786	Dry study skin
Makalata didelphoides	UM-ISEM	T-5023	*This paper*	KU892782	Fresh tissue
Mesocapromys melanurus	MCZ-Harvard	MCZ-34406	*This paper*	KU892769	Dry study skin
Mesomys hispidus	UM-ISEM	T-6523	*This paper*	KU892787	Fresh tissue
Mesomys stimulax	Naturalis-Leiden	RMNH.MAM.21728	*This paper*	KU892788	Dry study skin
Myocastor coypu	UM-ISEM	T-0245	*This paper*	KU892780	Fresh tissue
Mysateles prehensilis gundlachi	MCZ-Harvard	MCZ-17090	*This paper*	KU892770	Dry study skin
Olallamys albicauda	USNM-Smithsonian	USNM-241343	*This paper*	KU892774	Dry study skin
Pattonomys carrikeri	ROM	ROM-107955	*This paper*	KU892783	Fresh tissue
Phyllomys blainvillii	UFES-CTA	CTA-1205	*This paper*	KU892756	Fresh tissue
Phyllomys dasythrix	UFES-CTA	AC-628	*This paper*	KU892757	Fresh tissue
Phyllomys lundi	UFES-CTA	CTA-881	*This paper*	KU892758	Fresh tissue
Phyllomys mantiqueirensis	UFES-CTA	CTA-912	*This paper*	KU892759	Fresh tissue
Phyllomys pattoni	UFES-CTA	CTA-984	*This paper*	KU892760	Fresh tissue
Plagiodontia aedium	Naturalis-Leiden	RMNH.MAM.3865	*This paper*	KU892771	Dry study skin
Proechimys cuvieri	UM-ISEM	T-5765	*This paper*	KU892778	Fresh tissue
Proechimys roberti	UFES-CTA	CTA-1524	*This paper*	KU892772	Fresh tissue
Santamartamys rufodorsalis	AMNH	AMNH-34392	*This paper*	KU892777	Dry study skin
Thrichomys apereoides	MVZ Mammals	MVZ-197573	*This paper*	KU892773	Fresh tissue
Toromys grandis	FMNH	FMNH 92198	*This paper*	KU892784	Dry study skin
Trinomys albispinus	UFES-CTA	AL-3054	*This paper*	KU892761	Fresh tissue
Trinomys iheringi	UFES-CTA	ROD-156	*This paper*	KU892762	Fresh tissue
Trinomys paratus	UFES-CTA	CTA-588	*This paper*	KU892763	Fresh tissue
Trinomys setosus	UFES-CTA	YL-710	*This paper*	KU892764	Fresh tissue
Trinomys yonenagae	UFES-CTA	PEU-880027	*This paper*	KU892765	Fresh tissue

NOTE.—Voucher numbers of the newly sequenced taxa used in this study are given in gray shades. AMNH, American Museum of Natural History, New York, NY; FMNH, Field Museum of Natural History, Chicago, IL; MCZ, Museum of Comparative Zoology, Harvard University, Cambridge, MA; MVZ, Museum of Vertebrate Zoology, Berkeley, CA; Naturalis, Nationaal Natuurhistorisch Museum, Leiden, The Netherlands; ROM, Royal Ontario Museum, Toronto, Ontario, Canada; UFES-CTA, Animal Tissue Collection, Universidade Federal do Espírito Santo, Brazil; UM, University of Montpellier collections, Montpellier, France; USNM, National Museum of Natural History, Smithsonian Institution, Washington, DC. Most of these samples are vouchered and archived in accessible collections. Additional information could be retrieved via the following database webpages: http://vertnet.org/.

Meredith et al. 2011; Fabre, Galewski, et al. 2013; Upham et al. 2013). Complete mitogenomes and nuclear exons were aligned with MUSCLE (Edgar 2004), as implemented in SEAVIEW v4.5.2 (Gouy et al. 2010), and with MACSE version 1.01b (Ranwez et al. 2011). Ambiguous regions of the alignments were cleaned with trimAl version 1.4 (Capella-Gutiérrez et al. 2009).

We combined the mitochondrial and nuclear sequences into three DNA supermatrices: (1) the complete mitogenomes (47 taxa and 15,896 sites; 0.7% of missing character states, i.e., calculated on the taxon x site basis), (2) the concatenated nuclear exons (45 taxa and 5,415 sites, 32% of missing character states), and (3) the complete mitogenomes combined with nuclear exons that maximize our character sampling (47 taxa and 21,311 sites; 11% of missing character states). We also concatenated the 13 mitochondrial and 5 nuclear protein-coding genes translated into amino acid (AA) sequences in a super-protein dataset (47 taxa and 5,614 sites, partition "AA"; 15% of missing character states).

Phylogenetic Analyses

Individual gene trees for the 13 mitochondrial protein-coding genes and the 5 nuclear exons were built using maximum likelihood (ML) with parameters and topologies estimated

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under PAUP* (Swofford 2003), version 4b10. A neighbor-joining (NJ) starting topology allowed to optimize the parameter values of the GTR + Γ + invariable sites model of NT evolution. The optimal topology was then identified after a heuristic search under tree bisection–reconnection (TBR) branch swapping. Node bootstrap percentages were computed by ML after 100 replicates using previously estimated parameters, NJ starting tree and TBR branch swapping.

Bayesian inferences were then conducted on all of the concatenated datasets. To account for potential heterogeneity of substitution patterns within and between mitochondrial and nuclear partitions, we used the CAT mixture model (Lartillot 2004), implemented in PHYLOBAYES (Lartillot et al. 2009), version 3.3f. Relative exchangeabilities between NT and AA were described under the general time reversible (GTR) model (Rodriguez et al. 1990). To account for among-site heterogeneity in the NT and AA substitution rates, we used a Gamma distribution with four discrete categories (Γ_4). For each dataset, two Markov chain Monte Carlo analyses were run with PHYLOBAYES for 10,000 cycles (ca., 8,000,000 generations) with trees sampled every five cycles after discarding the first 1,000 as burn-in. Convergence was ensured when the maximum difference in bipartition frequencies as estimated by the two chains was <0.1. Node support was estimated by posterior probabilities (PP) computed from samples of 9,000 post-burn-in trees.

To estimate the average rate of evolution among mitochondrial and nuclear partitions at the DNA level, we used a backbone topology (the highest PP topology inferred under the CAT model for the mitochondrial + nuclear DNA concatenation). PAUP* (Swofford 2003) was used to estimate branch lengths with the same backbone topology under a GTR + Γ + invariable sites model of NT evolution across all 22 partitions (13 mitochondrial protein-coding genes, 2 ribosomal RNAs, the control region, all tRNAs combined into a single partition, and the 5 nuclear exons). The super distance matrix (SDM) approach (Criscuolo et al. 2006) was used to estimate the 22 average rates of evolution of the DNA partitions through the use of the resulting trees converted into additive distance matrices by computing the path-length between each pair of taxa (see Ranwez et al. 2007 for details). The resulting SDM rates were standardized into relative evolutionary rates (RER) with respect to the slowest-evolving marker, set to RER = 1.

Molecular Dating Analyses

Divergence times were estimated from the mitochondrial + nuclear NT and AA supermatrices to provide a temporal framework of the echimyid radiation. A Bayesian relaxed molecular clock method with rate autocorrelation along branches (Thorne et al. 1998; Thorne and Kishino 2002; Lepage et al. 2007) was used to estimate divergence dates while accounting for changes in evolutionary rate over time and allowing for mixture models of sequence evolution to account for heterogeneities in patterns of evolution among mitochondrial and nuclear partitions. We again used PHYLOBAYES (Lartillot et al. 2009) to estimate the divergence dates within South American spiny rats using the Bayesian consensus topology obtained from DNA data (see also fig. 2). The best previously estimated Bayesian topology was used to run the molecular dating analysis under the CAT + GTR + Γ mixture model for both NT and AA datasets. To calibrate the molecular clock, we selected five fossil constraints already considered in previous studies on rodents (Upham et al. 2013; Patterson and Upham 2014). First, the most recent common ancestor (MRCA) of Cavioidea–Erethizontoidea with an age range between 31.3 and 45.9 Ma. The minimum bound was based on the fossil *Andemys termasi* (voucher: SGOPV 2933), which is conservatively the oldest stem Cavioidea representative, and which could even be considered as a stem Dasyproctidae, (31.3–33.6 Ma) from the Tinguiririan SALMA, late Eocene-early Oligocene (Fields 1957; Vucetich et al. 1993, 2010; Bertrand et al. 2012; Dunn et al. 2013; Verzi 2013). The older bound was assumed to be no younger than the oldest stem members of Caviomorpha (*Canaanimys* and *Cachiyacuy*, Barrancan SALMA, middle Eocene ~41 Ma [40.9–45.9 Ma]; Antoine et al. 2012).

Secondly, the Octodontidae MRCA was constrained using the oldest crown fossil *Pseudoplateomys innominatus* (MACN 8663) from the Huayquerian SALMA, late Miocene (6.8–9.0 Ma). We set a soft maximum (upper 95%) prior age of 11.8 Ma for this octodontid node, based on oldest occurrence of stem octodontids such as *Acarechimys* from the Laventan SALMA, late Miocene (Candela and Noriega 2004; Verzi 2008).

We subsequently used three ingroup calibrations. We constrained the divergence between *Trinomys* and *Clyomys* + *Euryzygomatomys* to range from 6.0 to 11.8 Ma, as based on *Theridomysops parvulus* (MACN-Pv8379), the oldest stem taxon to *Euryzygomatomys* + *Clyomys*, from the Huayquerian SALMA, late Miocene (Hussain et al. 1978; Wyss et al. 1993; Schultz et al. 2002; Verzi 2008). We then used the oldest arboreal echimyid *Maruchito trilofodonte* (MLP 91-IV-1-22) to date the MRCA of the arboreal clade (Echimyini; Fabre et al. 2014) with a range of 15.7–24.2 Ma. This fossil from the Colloncuran SALMA ~15.7 Ma, middle Miocene, is the oldest stem taxon between *Phyllomys* and *Echimys* in cladistic studies (Vucetich et al. 1993; Macphee 2005; Verzi 2008; Dunn et al. 2013). Finally, we calibrated the MRCA of *Thrichomys* and the clade including *Hoplomys*, *Proechimys*, *Callistomys*, and *Myocastor* using the fossil *Pampamys emmonsae* (GHUNLPam 2214) from the Huayquerian SALMA, late Miocene and the age range of 6.0–11.8 Ma (Olivares et al. 2012; Verzi 2013; Verzi et al. 2015).

Biogeographical Analyses

We used LAGRANGE C++ to elucidate ancestral biogeographical patterns using Dispersal Extinction Cladogenesis + X (DECX) historical biogeography models (Ree et al. 2005; Ree and Smith 2008; Matzke 2014). This approach allowed us to infer the number of dispersal/vicariance events among genera of Echimyidae throughout the Neogene. Probabilistic biogeographic analyses using DECX (Ree et al. 2005, Beeravolu and Condamine 2016) optimize reconstructed ancestral areas upon internal nodes. LAGRANGE C++ calculates maximum likelihood estimates of the ancestral states (range inheritance scenarios) at speciation events by modelling transitions

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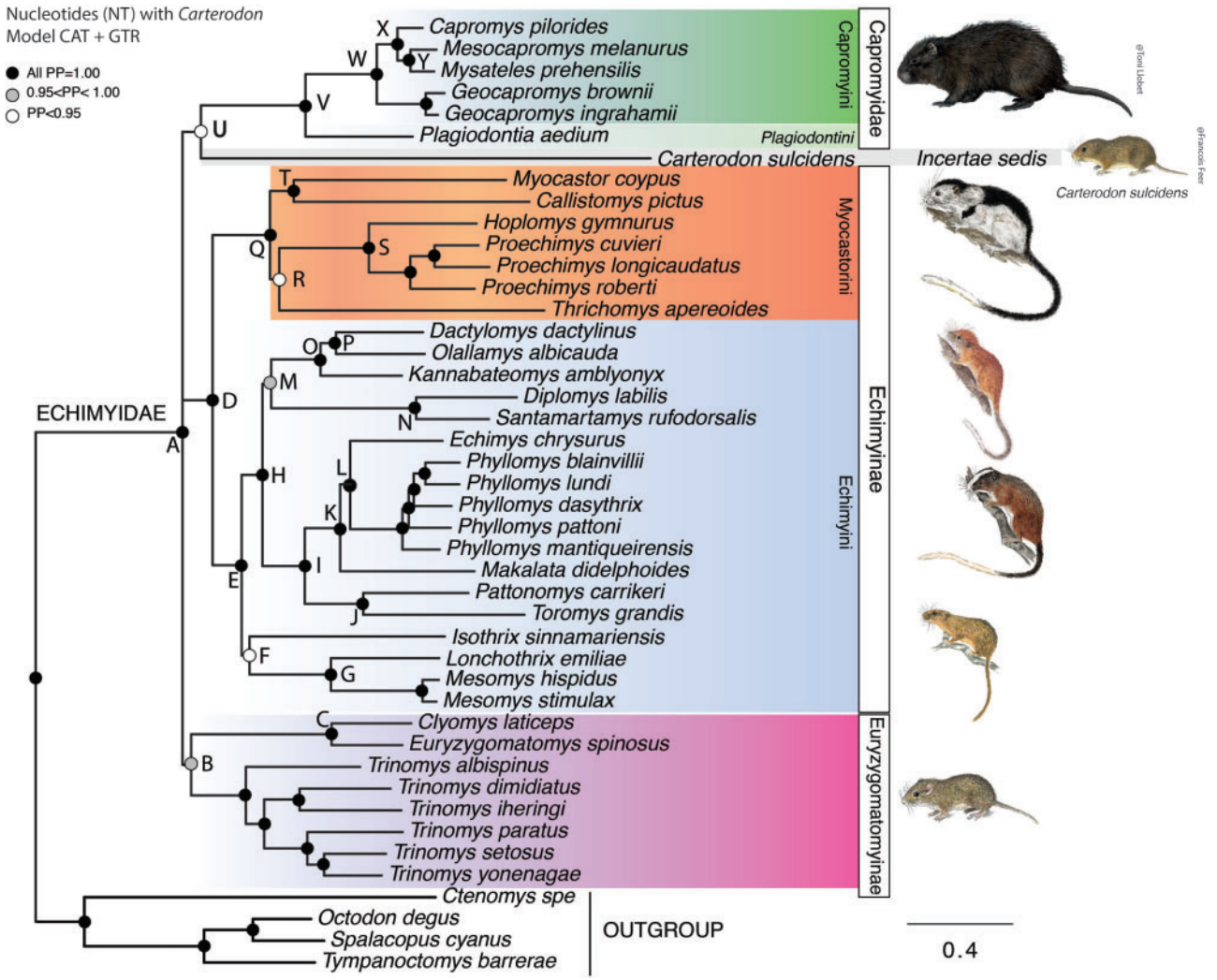

FIG. 2. Bayesian topology obtained from the nucleotide dataset (NT) concatenation of the 13 protein-coding, and the 5 nuclear genes. Posterior probabilities as computed under the CAT + GTR + Γ mixture model by Phylobayes are indicated on each node. See figure 3 for deeper exploration of the results. The tree is rooted by Caviomorpha taxa: Ctenomyidae + Octodontidae (outgroup), and *Cavia porcellus*, *Coendou* sp. and *Chinchilla lanigera* (not shown). Systematic names, clade colors, and letter labels for the major echimyid clades are given along the phylogeny. @ Illustrations of *Plagiodontia aedium* by Toni Llobet from: Wilson et al. (2016). @ Illustrations of Echimyidae by Francois Feer from Emmons and Feer (1997).

between discrete states (biogeographic ranges) along phylogenetic branches as a function of time. In addition to DEC's two free parameters, *d* (dispersal) and *e* (extinction), DECX is characterized by two additional parameters: vicariance (VI) and rapid anagenetic change (RAC), that were not considered in the previous DEC model (Ree et al. 2005) and DEC + J model (Matzke 2013). VI is a standard parameter, whereas RAC contrasts the rapid cladogenetic change model implemented in DEC + J. The distribution of echimyid ranges from South and Central America were defined based on recent revisions available in Patton et al. (2015). We subsequently divided regions into smaller biogeographic entities to obtain higher resolution in the ancestral area of origin. Due to our genus-level approach, we joined together open habitat areas into a single region as well as Amazonia and the Guiana shield. Using tectonic inferences, notably the evolution of past Amazonian landscapes (Hoorn et al. 2010), we assigned six areas for the DEC analysis: Amazon Basin, Central Andes and Guiana Shield (AM), the trans-Andean region plus Chocó (CH), the Northern Andes and northern coastal dry forests in Venezuela and Colombia (NA), Caatinga, Chaco, Cerrado and the Pantanal (CC), Atlantic Forest (AF), and the West Indies (WI).

We inferred echimyid areas of origin and the direction and sequence of colonization among these areas on the chronogram inferred from AA under the CAT mixture model. One great advantage of the DECX model (e.g., over parsimony-based models such as DIVA; Ree et al. 2005) is the ability to model transition rates and connectivity scenarios. We acknowledge that such a constrained model might be skewed, especially if transition rates and connectivities between areas are not estimated properly. In consequence, two models were considered in the present study. First, a simple ("unconstrained") model was computed under equal exchange

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probabilities between areas. Second, to test whether transitions among regions were correlated with major geological events, we used a "stratified" biogeographic scenario that allowed for: (1) limited dispersal between unconnected areas; and, (2) connectivity changes between major geological events. Movement between unconnected areas is prohibited in this model by setting the dispersal parameter *d* (probability of dispersal between nonadjacent areas) equal to 0. We set $d = 1$ between all adjacent areas. Next, we stratified the model based on the Neotropical geology data presented in Hoorn et al. (2010), employing five transition matrices based on successive geological time-intervals. The West Indies has been separated from mainland South America during the entire Neogene (Iturralde-Vinent and Macphee 1999). We allow connectivity between the Amazon Basin and Atlantic Forest during the period between 12 and 7 Ma, due to the potential presence of savannah corridors and the Paraná sea (Hoorn et al. 2010). Connectivity between the Amazon Basin and Northern South America ended between 12 Ma and the present due to major Andean uplift events, which isolated the trans- and cis-Andean regions from each other, and the Orinoco watershed from the cis-Andean and Southern Amazon basin fauna (Hoorn et al. 2010). The widespread Neotropical genus *Proechimys* was coded as AM + NA due to our limited species sampling for this genus. Beeravolu and Condamine (2016) reported that DECX outperforms DEC + J in the case of stratified models. The methodology of its cousin DEC + J is usually better at dealing with nonstratified models. However, in our case DECX performed better than DEC + J both on unconstrained and stratified models (supplementary table S3, Supplementary Material online). With our AIC and Akaike weight results, the differences between DECX and DEC + J were not statistically different for the unconstrained and significant under the stratified model (supplementary table S3, Supplementary Material online). We chose not to use DEC + J, instead implementing a stratified model that takes into account geological history and because the J parameter (jump dispersal) is more suited to island systems (Beeravolu and Condamine 2016).

Ecomorphological Analyses

We mapped the ecomorphological character of locomotion lifemode onto the phylogeny for all Echimyidae genera based on account in Patton et al. (2015). Coding for locomotion lifestyle was as follows: (1) semi-arboreal, scansorial, or arboreal (*Callistomys*, *Capromys*, *Dactylomys*, *Diplomys*, *Echimys*, *Isothrix*, *Kannabateomys*, *Lonchothrix*, *Mesocapromys*, *Makalata*, *Pattonomys*, *Plagiodontia*, *Mesomys*, *Mysateles*, *Olallamys*, *Phyllomys*, *Santamartamys*, *Toromys*); (2) semi-fossorial (*Clyomys*, *Euryzygomatomys*, *Carterodon*); (3) terrestrial (*Proechimys*, *Geocapromys*, *Hoplomys*, *Thrichomys*, *Trinomys*); and (4) semi-aquatic (*Myocastor*). Ancestral character state reconstructions were performed in the phytools package in the R programming language (R Core Team 2016) using the "make.simmap" function (Bollback 2006; Revell 2012). This stochastic mapping approach iteratively reconstructs states at all ancestral nodes in the phylogeny using random draws from a k-state Markov model (Mk + Γ), as conditioned on the best-fitting model of transition rates among states, and integrates over the uncertainty in observed nodal states. We used maximum-likelihood (ML) to estimate the best-fitting model with the function "ace" in the ape package (Paradis et al. 2004), and then simulated 1000 stochastic maps on the maximum clade credibility tree from PHYLOBAYES. Plotting of the SIMMAP results were performed using the function plotSimmap in R.

Results and Discussion

Mitogenomes Help to Resolve a Thorny Star-Phylogeny

We screened the new DNA sequences for potentially anomalous assemblies and/or contamination by building phylogenetic trees on individual mitochondrial genes and nuclear exons. We did not detect any well-supported ($1 > PP > 0.95$) topological conflict among the inferred mitochondrial and nuclear gene trees (table 2 and supplemental table S2, Supplementary Material online). For this reason, and because both concatenation and coalescence analyses using sophisticated models of sequence evolution and in-depth tree search routines generally yield concordant phylogenetic hypotheses for mammals (Gatesy et al. 2016), we conducted all subsequent inferences on mitochondrial and/or nuclear supermatrices, and we did not use coalescent-based investigation. We relied on the combination of faster-evolving mitochondrial sequences for reconstructing relationships among species and genera, with slower-evolving nuclear exons for deeper nodes of the echimyid and capromyid tree. We therefore approximated the species phylogeny through the combination of gene alignments. Thanks to cumulative signal potentially contributed by all individual genes, the supermatrix approach here appeared to be the best way to provide support for the trickiest nodes of the spiny rat phylogeny. This distinction is emphasized because higher level phylogenetic relationships within Echimyidae have been consistently enigmatic using both single and combined mitochondrial and nuclear markers, but never evaluated using mitogenomes and all extant genera (Lara et al. 1996; Leite and Patton 2002; Galewski et al. 2005; Fabre et al. 2013; Upham et al. 2013).

With more than 21,000 unambiguously aligned mitochondrial plus nuclear positions, our analysis provides improved support for the phylogenetic position of several echimyid genera. First, we confirmed that *Myocastor* belongs within the Echimyidae (*contra* Woods and Kilpatrick 2005). Combined analysis of all markers show *Myocastor* nested within Echimyinae, a subfamily comprised of an arboreal tribe (Echimyini) and a terrestrial tribe (Myocastorini) (fig. 2). This well-supported Myocastorini tribe unexpectedly includes *Callistomys* as sister taxa to *Myocastor* (Loss et al. 2014). Both taxa are the sister group to a moderately supported group containing *Thrichomys* and *Hoplomys* + *Proechimys* (Node R). This position for *Myocastor* is compatible with the classification of Echimyidae including *Myocastor* (sensu Woods et al. 1992; Galewski et al. 2005), giving further support to the McKenna and Bell (1997), who recognize

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Table 2. Phylogenetic Support Values of the Major Clades Identified from the Mitochondrial and/or Nuclear Supermatrix Analyses Under Probabilistic Approach for Nucleotides.

Nodes	PP			Congruence	Genes
	Mitonuc	Mito	Nuclear		
A	1.00	1.00	1.00	5 vs. 0	V A I G R
B	0.99	0.95	0.29	1 vs. 4	v a i G r
C	1.00	1.00	1.00	3 vs. 1	V _ I G r
D	1.00	1.00	1.00	3 vs. 2	V A I g r
E	1.00	1.00	0.99	3 vs. 2	v A I G r
F	0.94	0.98	0.10	3 vs. 0	v _ _ g r
G	1.00	1.00	?	?	?
H	1.00	1.00	0.77	2 vs. 1	v _ I G r
I	1.00	1.00	1.00	3 vs. 0	V _ _ G R
J	1.00	1.00	1.00	2 vs. 0	V A _ _ _
K	1.00	1.00	0.74	3 vs. 2	V A I g r
L	1.00	1.00	0.89	3 vs. 2	V A I g r
M	0.99	0.98	0.96	1 vs. 1	v _ _ G _
N	1.00	1.00	?	?	?
O	1.00	1.00	0.99	1 vs. 1	V _ _ g _
P	1.00	1.00	0.48	1 vs. 1	v _ _ G _
Q	1.00	1.00	1.00	5 vs. 0	V A I G R
R	0.86	0.45	0.08	2 vs. 2	v _ I G r
S	1.00	1.00	1.00	4 vs. 0	V _ I G R
T	1.00	1.00	0.76	4 vs. 1	V A i G R
U	0.91	0.69	0.10	1 vs. 2	v _ _ G r
V	1.00	1.00	1.00	5 vs. 0	V A I G R
W	1.00	1.00	1.00	3 vs. 1	v A _ G R
X	1.00	1.00	1.00	3 vs. 2	v a I G R
Y	1.00	1.00	0.46	1 vs. 2	v _ I _ r

NOTE.—Nodes are labelled with letters (see figs. 2 and 3).
PP, posterior probabilities computed under the CAT mixture model by Phylobayes; Mitonuc, mitonuclear DNA supermatrix; Mito, mitogenome DNA supermatrix; Nuclear, nuclear DNA supermatrix.
The column "congruence" indicates the number of nuclear gene trees that, respectively, recover versus do not recover the corresponding clade of the mito-nuclear supermatrix tree. Gene names: V, von Willebrand Factor exon 28 (vWF), A, apolipoprotein B exon 26 (APOB), I, Interphotoreceptor retinoid-binding protein exon 1 (RBP3), G, Growth hormone receptor exon 10 (GHR), and R, Recombination activating protein 1 gene (RAG1). The upper case letter indicates congruence with the CAT + GTR + Γ (Phylobayes) mito-nuclear topology while the lower case indicates a different topology. A question mark indicates that genes are absent for the taxa under focus, precluding the recognition of the corresponding clade.

Myocastorinae within Echimyidae. We confirm three well-supported major clades: (1) the Capromyidae, consisting of West Indian endemics, and closely related to Echimyidae (Fabre et al. 2014); (2) the East Brazilian old endemics, here referred to as Euryzygomatomyinae; and (3) the Echimyini (i.e., all arboreal echimyids except *Callistomys*) branching with the tribe Myocastorini. We confirm that *Trinomys* is placed within Euryzygomatomyinae, closely related to the fossorial *Clyomys* and *Euryzygomatomys* genera (Lara and Patton 2000). Thus, despite its prior synonymy with *Proechimys* over most of the 20th century, *Trinomys* is only distantly related to the Myocastorini, contradicting previous classifications.

The arboreal Echimyini clade (node E) is always recovered and we propose the best-supported hypothesis to date for the genus-level branching patterns within this radiation. We recovered several previously suggested subclades: (1) the bamboo rats (*Dactylomys*, *Olallamys* and *Kannabateomys*; node O), (2) the Northern Andean endemics *Santamartamys* and *Diplomys* (*Diplomys* also occurs in trans-Andean areas); node N), (3) the Amazonian *cis*-Andean clade *Toromys* and *Pattonomys* (node J), (4) the three remaining Echimyinae taxa (*Echimys*, *Makalata*, *Phyllomys*, node K), and the *Mesomys*/*Lonchothrix* clade (Node G). Our comparative analysis of mitogenomes provides strong statistical support for (1) the sister relationship between *Dactylomys* and *Olallamys* (node P) to the exclusion of *Kannabateomys*, (2) a sister relationship between *Phyllomys* and *Echimys* (node L), and (3) the association of Nodes J and K into a subclade that includes the most diverse arboreal genera (*Makalata* and *Phyllomys*) (Node I), followed by their branching with bamboo rats + *Diplomys* and *Santamartamys* (node H). Within this Echimyini clade, we also found weak support—though consistently recovered by different markers—for the association of the isolated lineage *Isothrix* with *Lonchothrix* + *Mesomys* (node F, but see also fig. 3).

Regarding the gene sampling, our expanded set of molecular markers appeared to be central to reconstructing several aspects of Echimyidae and Capromyidae phylogeny (fig. 3). Individual mitochondrial genes such as the widely used cytochrome *b* marker in mammals or the cytochrome oxidase I barcode can show limited phylogenetic resolution, particularly at deeper nodes. While several mitochondrial genes can be concatenated to form a "supergene", the minimal number of genes necessary to significantly resolve the phylogenetic structure remains contentious, with an average of three to five mitochondrial genes seeming sufficient to reach a resolution similar to that derived from the analysis of the complete mitogenome (Duchêne et al. 2011; Havird and Santos 2014). Because the selection of genes serving as a proxy for the mitogenomic tree can vary with the particular clades of interest, our use of complete mitochondrial genomes for the echimyids and capromyids allowed us to assemble comparable data across taxonomic groups while avoiding a highly "gappy" supermatrix of characters. In the present study, the combination of complete mitogenomes with nuclear exons yields a 21,311-site supermatrix with only 11% taxon-by-site missing data.

The estimation of the relative evolutionary rates among the 22 mitochondrial and nuclear partitions (table 3) indicated that (1) the slowest-evolving markers are the nuclear exons *apoB* and *RAG1*, followed by the 2-fold faster-evolving *GHR*, *IRBP*, and *vWF* (RER = 2.0–2.3); (2) the mitochondrial tRNAs and rRNAs evolved 5–6 times faster; (3) the 13 mitochondrial protein-coding genes evolved ca., 22–45 times faster (RER = 22.3, 22.8, 42.4 and 44.6, respectively, for *atp8*, *cytb*, *cox1* and *nd4l*); and (4) the control region evolved 37.3-fold faster (see Supplementary Material online). These estimates show that our mitochondrial and nuclear DNA supermatrix of ca., 21,000 unambiguously aligned sites contains partitions with contrasting evolutionary dynamics. This combination of slower- and faster-evolving markers therefore provided phylogenetic resolving power at the species level by the faster-evolving sequences (e.g., within *Phyllomys* and *Trinomys*, or among closely related genera of the Node E), as well as at deeper taxonomic levels by the slower-evolving genes (e.g., Capromyidae, Echimyinae, and Euryzygomatomyinae).

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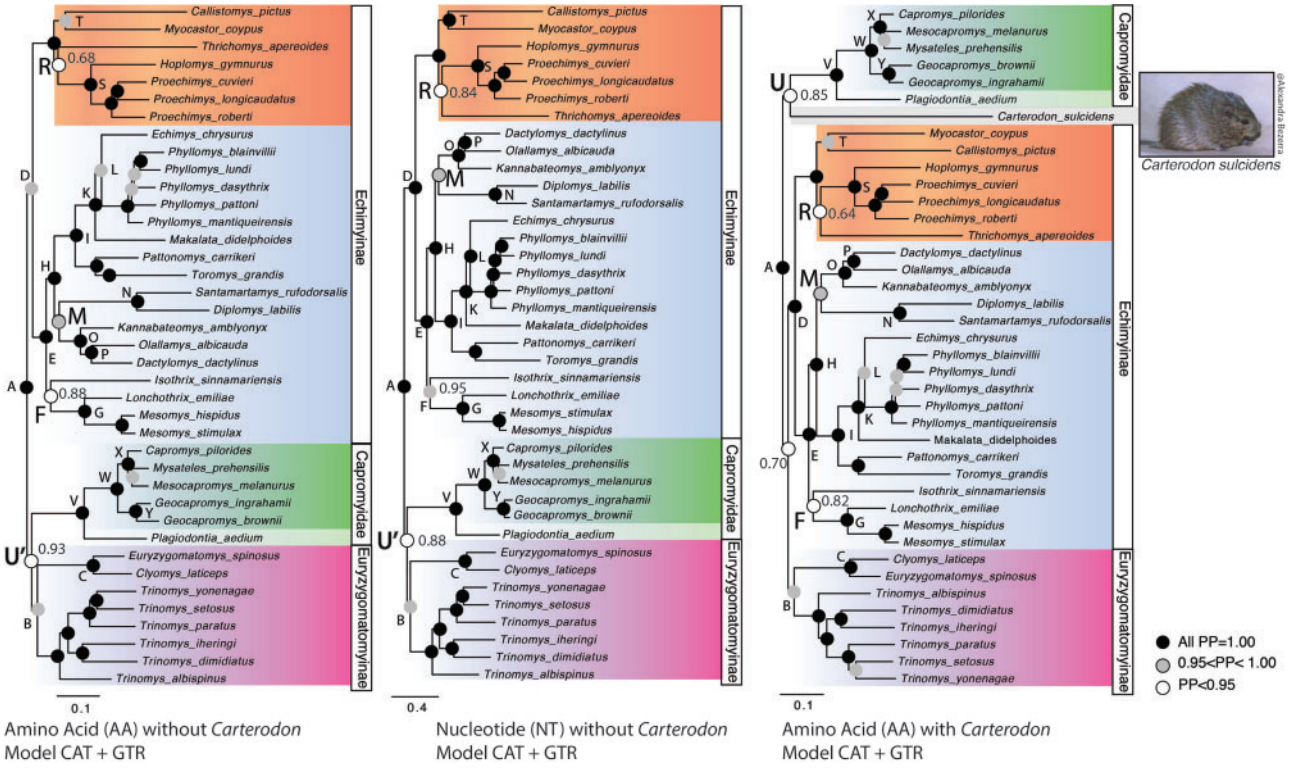

Fig. 3. Bayesian topologies obtained from the nucleotide (NT) and amino acid (AA) concatenations of the 13 protein-coding, and the 5 nuclear genes. Results without and with *Carterodon* are highlighted herein. Posterior probabilities as computed under the CAT + GTR + Γ mixture model by Phylobayes are indicated on each node. See figure 2 for nucleotide results including *Carterodon*. The tree is rooted by caviomorph taxa that were pruned from the trees in order to produce these figures. Taxonomic names, clade colors, and letter labels for the major echimyid clades are given along the phylogeny. U′ represents the alternative relationships for the node U: Capromyidae + Euryzygomatomyinae.

Table 3. DNA Substitution Rates among the 22 Mitochondrial and Nuclear Partitions.

Genome	Markers	Sites	Cumul sites	SDM rates	Scaled rates	Remarks
mtDNA	12S	880	880	1.23	6.1	—
mtDNA	16S	1406	2286	1.24	6.2	—
mtDNA	ND1	960	3246	6.54	32.5	—
mtDNA	ND2	1041	4287	5.84	29,0	—
mtDNA	CO1	1545	5832	8.52	42.4	—
mtDNA	CO2	684	6516	6.68	33.2	—
mtDNA	AT8	192	6708	4.48	22.3	—
mtDNA	AT6	681	7389	6.35	31.6	* 31 nt AT8-AT6 overlap
mtDNA	CO3	783	8172	6.76	33.6	* 1 nt overlap AT6-CO3
mtDNA	ND3	345	8517	7.04	35,0	—
mtDNA	ND4L	297	8814	8.96	44.6	* 7 nt overlap NDL-ND4
mtDNA	ND4	1380	10194	4.69	23.3	—
mtDNA	ND5	1812	12006	5.74	28.6	—
mtDNA	ND6	534	12540	7.20	35.8	* 4 nt overlap ND5-ND6
mtDNA	CYB	1140	13680	4.59	22.8	—
mtDNA	CR	765	14445	7.49	37.3	—
mtDNA	tRNA+OL	1494	15939	1.03	5.1	—
nucDNA	apoB	1173	17112	0.20	1.0	—
nucDNA	GHR	806	17918	0.39	2.0	—
nucDNA	RAG1	1071	18989	0.20	1.0	—
nucDNA	IRBP	1207	20196	0.39	2.0	—
nucDNA	vWF	1153	21349	0.46	2.3	—

Note.—Genome: Mitochondrial (mt) or nuclear (nuc). Markers: Name of the partition (CR, control region; tRNA + OL, 22 tRNAs and the L-strand origin of replication). Sites: Number of sites of the corresponding alignment. Cumul sites: Cumulative number of sites in the DNA supermatrix. SDM rates: Relative substitution rates among markers as measured by SDM. Scaled rates: Rates scaled with respect to the slowest partition (*apob*, set to 1.0).

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Regarding the sampling of DNA markers, we built trees from the mitochondrial-only or nuclear-only supermatrix under the CAT + GTR + Γ model. The matrilineal (mitochondrial DNA) topology produced the same four major clades: Capromyidae (PP = 1.00), Euryzygomatomyinae (PP = 0.95), Myocastorini (PP = 1.00) and Echimyini (PP = 1.00), with the latter two strongly grouped together (PP = 1.00) and comprising Echimyinae. Deeper relationships are not resolved, with all Echimyidae except *Carterodon* branching together (PP = 0.64), as the sister-group of all Capromyidae plus *Carterodon* (PP = 0.69). The biparental (nuclear DNA) topology also evidenced major clades: Capromyidae (PP = 1.00), Euryzygomatomyinae + *Carterodon* (PP = 0.87), Myocastorini (PP = 1.00) and Echimyini (PP = 0.99), with the latter two strongly grouped together (PP = 1.00). Deeper relationships are not resolved with a trifurcation involving Capromyidae, Euryzygomatomyinae + *Carterodon*, and Myocastorini + Echimyini. The mitogenomic tree and the nuclear exon tree are congruent, apart from the phylogenetic position of *Carterodon* (but see "Discussion" below).

Regarding the molecular character sampling, we cannot rule out the possibility that mitogenomic sites reached substitution saturation for resolving the echimyid phylogeny because of the fast evolutionary rates of the mitochondrial protein-coding sequences. However, when conducting the same inferences at the AA level, we recovered a topology virtually identical to the one reconstructed from the NT supermatrix, suggesting that substitution saturation had little (if any) impact on our findings (see figs. 2 or 3). The only topological difference at the protein level is the modification of the basal trifurcation of Echimyidae, with Capromyidae + *Carterodon* branching as the sister group of all other genera, although with a low PP value. Other differences involve weaker PP supports at the protein level for the few nodes that were not fully supported at the DNA level (e.g., the PP value decreases for node R). This might reflect the lower percentage of variable sites available for the probabilistic inference in the AA versus NT supermatrices: at the DNA level, 49.1% and 38.1% of the sites are, respectively, variable and parsimony-informative, whereas these proportions decrease to 42.0% and 27.5% at the protein level.

Regarding taxon sampling, we sampled all of the recognized genera of Echimyidae and Capromyidae and analysed these in the most rigorous molecular phylogenetic framework to date (but see also Upham and Patterson 2015). Sampling all major lineages with at least one species per genus enables us to better understand the evolution of the spiny rat family by minimizing the number of isolated—and potentially longer—branches in the resulting phylogeny. This phylogeny therefore benefits from an improved detection of multiple substitution events leading to a more accurate estimation of both topology and branch lengths, which could otherwise hinder the delineation of evolutionary signal versus noise (Poe 2003). Our sampling reduced this systematic error in order to produce a more stable phylogenetic picture with exhaustive generic coverage (Delsuc et al. 2005).

Regarding the inference model, an inherent difficulty in mitochondrial phylogenetic analyses results from differing substitution patterns due to different functions of DNA (protein-coding, ribosomal RNA, tRNA, and noncoding), as well as different NT and AA compositions (e.g., 12 H-strand protein-coding genes versus the single L-strand ND6). This difficulty is here compounded by the further concatenation of genetically unlinked nuclear exons that also display different NT or AA compositions, substitution patterns, and rates of replacement. Mixture models like the CAT model implemented in PHYLOBAYES circumvent these problems by dynamically reconstructing independent NT profiles that individually fit subsets of similarly evolving sites (Lartillot et al. 2007). Moreover, they avoid the need to remove supermatrix partitions that would otherwise violate model assumptions. We also note that the timetree estimation relies heavily upon accurate branch length measurement under a specified model of sequence evolution. Distribution by the CAT mixture model of the alignment columns into different categories accounts for site-specific NT and AA preferences (Lartillot 2004). Branch lengths estimated under the CAT model will therefore be less affected by saturation because of a better ability to detect multiple hits, and will integrate the substitution pattern heterogeneity exhibited by mitochondrial and nuclear loci (Lartillot et al. 2007).

As a result of the improved sampling of mitochondrial and nuclear markers, molecular characters, and taxa, and through the use of the CAT mixture model, most nodes of the Echimyidae + Capromyidae phylogeny are now strongly supported. Exceptions are four nodes that are unresolved despite the use of ca., 21,000 sites (figs. 2 and 3). Genera involved in these weaker supported nodes are *Carterodon* (node U), *Thrichomys* (node R), *Isothrix* (node F), and to a lesser extent the *Diplomys* + *Santamartamys* + the three bamboo rat genera (node M). Three genera are represented in the phylogeny as isolated branches (*Carterodon*, *Thrichomys*, and *Isothrix*). A fourth branch—the one subtending *Diplomys* + *Santamartamys*—also behaves similarly as its subdivision is not very deep: the branch lengths from node N to terminal taxa are of roughly similar length to the internal one between nodes M and N (figs. 2 and 3). Phylogenetic placement of isolated branches might be difficult, but would benefit from further improvements to taxon sampling. Future studies might therefore incorporate a richer species sampling within *Thrichomys* (five species are recognized) and *Isothrix* (six species are recognized), but, to our knowledge, the branch leading to *Diplomys labilis* could only be subdivided by adding *D. caniceps*, whereas *Santamartamys rufodorsalis* is monotypic. It is noteworthy that the analysis of Upham (2014) and Upham and Patterson (2015), based on ca., 5,000 nucleotides but with enhanced taxon sampling (e.g., all 6 *Isothrix*), recovered stronger support for some of these same groupings (nodes M and R), but not others (nodes F and U).

The placement of *Carterodon* is perhaps the most problematic as this monotypic genus exhibits the longest branch in the analysis of complete mitogenomes and the *apoB*, *GHR*, *IRBP*, *RAG1*, and *vWF* exons. In the tree inferred from the mito-nuclear DNA supermatrix (cf., figs. 2 and 3), the median distance from the Echimyidae + Capromyidae MRCA to the tips is 0.89 NT substitution per site whereas it is ca., twice as

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long for *Carterodon* (1.61). At the AA level, a similar contrast is observed with a MRCA-to-tips median distance of 0.28 AA substitution per site versus 0.49 for *Carterodon*. Moreover, both analyses of either the matrilineal or the biparental supermatrices again yield trees with the longest branch leading to *Carterodon*. Regarding the phylogenetic position of *Carterodon*, we caution that saturation could hamper its accurate placement and possibly impact the branching pattern with its closest relatives. We note that the 13 mitochondrial and the five nuclear protein-coding sequences sampled for *Carterodon* do not display premature stop codons in the open reading frames, suggesting that pseudogenes were not incorporated in the alignments and are not involved in the long-branch attribute of this taxon. However, we can postulate that several, nonexclusive adaptive and neutral processes may have increased the evolutionary rate of the mitochondrial and nuclear markers here under focus. For example, animal mitochondrial genomes have been shown to be recurrently impacted by episodes of positive selection (James et al. 2016) and by life-history traits driven mutational biases (Jobson et al. 2010). Positive selection, neutral processes, or a combination thereof, may therefore lead to the inference of long branches in the phylogenetic tree. Investigations at the population level would allow these hypotheses to be explored, but the task would be complicated by the need for samples from several individuals of the rare *Carterodon sulcidens*.

Topologies reconstructed from all mitochondrial plus nuclear characters agree to link *Carterodon* with Capromyidae (PP = 0.91 and 0.85 with NT and AA, respectively), whereas the relationships among the three clades of Echimyidae + Capromyidae are either unresolved (trifurcation with NT: cf., fig. 2) or receive moderate support for an association of Euryzygomatomyinae with Echimyinae to the exclusion of *Carterodon* + Capromyidae. After excluding *Carterodon* from additional inferences, we observed that the phylogenetic position of Capromyidae shifted to a branching with Euryzygomatomyinae with increased support (PP = 0.88 with NT and 0.93 with AA; fig. 3), whereas Myocastorini remained tightly linked to Echimyini (PP = 1.00 and 0.99, respectively). The same trend is also observed when *Carterodon* is removed from either the matrilineal-only or the biparental-only analyses: Capromyidae cluster with Euryzygomatomyinae (PP = 0.85 and PP = 0.62, respectively). Clearly, without *Carterodon*, the resolution of the phylogeny is improved in a consistent manner, and even provides increased support, compared with previous studies (however PP < 0.95 is still viewed cautiously). Since the studies of Woods and Howland (1979) and Woods et al. (2001), hypotheses of evolutionary affinities between hutias and the ancestral stock of spiny rats have been debated on molecular grounds. Galewski et al. (2005) analysed four markers (three mitochondrial, one nuclear) and found weak signal for a branching of their single capromyid *Capromys* with *Clyomys* + *Euryzygomatomys* and *Trinomys* (ML bootstrap = 47%). Upham and Patterson (2012) analysed four markers (one mitochondrial, three nuclear) and found the sister relationship of *Capromys* to all Echimyidae, but again with low support (ML bootstrap = 43%). Fabre et al. (2013) analysed eight markers (three mitochondrial, five nuclear) and found increased support for *Capromys* + Euryzygomatomyinae (ML bootstrap = 72%), albeit with reduced taxon sampling in this hutia + East Brazilian clade. Fabre et al. (2014) used an expanded taxon sampling within hutias with the same eight markers and found increased support for Capromyidae + Euryzygomatomyinae (ML bootstrap = 76% and PP = 1.00). Upham and Patterson (2015) analysed five markers (two mitochondrial, three nuclear) for all genera but again found an unresolved relationship among Capromyidae, Euryzygomatomyinae, and *Carterodon* (PP = 0.28; ML bootstrap = 41% for a *Carterodon* + Capromyidae relationship).

In the present study, and for the first time, analysis of the complete mitogenomes together with five nuclear markers for all genera of hutias and spiny rats—but excluding *Carterodon*—supports the above-mentioned Capromyidae + Euryzygomatomyinae relationships. However, including the long *Carterodon* branch destabilized the topology (fig. 3) and prevents any taxonomic conclusions from being made. We suspect that the deepest relationships among Echimyidae and Capromyidae are impacted by long-branch attraction phenomena involving the long branch of *Carterodon* as well as the long branches of the close Ctenomyidae + Octodontidae outgroups, and/or the more distant Caviidae + Erethizontidae outgroups. When *Carterodon* is incorporated in the mitochondrial + nuclear DNA analyses, Echimyidae would therefore be paraphyletic unless the hutias are included as a subfamily within Echimyidae, an arrangement previously advocated by Ellerman (1940). We hope that future work including additional nuclear and slowly evolving markers will offer gain a more favourable signal/noise ratio profiting from the virtues of a phylogenomic approach (Philippe et al. 2005). Additionally, efforts to sequence DNA from recently extinct and allied subfossil taxa (e.g., *Boromys*, *Brotomys*) could prove fruitful for parsing the long branch of *Carterodon*. For now, and until definitive phylogenetic evidence to the contrary can be ascertained, we conservatively recommend maintaining the family-level status of hutias within Capromyidae.

Transitions between the Atlantic Forest and Amazon Basin: "Busy" Corridors in the Miocene

Our molecular dating and biogeographic analyses (table 4 and fig. 4) suggest that geographic opportunities constitute the primary driver of echimyid higher level divergences, with the most likely biogeographic reconstruction including at least 1 event of vicariance and 14 dispersals explaining the current distribution of genera (DECX: ln$L$ = −62.2). Our biogeographic inferences reveal transitions among eastern Neotropical regions of Atlantic Forest, Caatinga, and Cerrado throughout the Neogene (fig. 4), plus Atlantic Forest–Amazon Basin splits among echimyid lineages (nodes O, L, T) from 4.6 to 11.3 Ma (mean = 7.6 Ma). This scenario suggests that rainforest corridors would have recurrently appeared throughout the Miocene to allow for dispersals and/or vicariances events leading to genus-level divergences.

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Table 4. Molecular Dating Results from the Concatenated Mitochondrial + Nuclear Dataset, for Both Nucleotides (NT) and Amino Acids (AA).

Nodes	Nucleotides (NT)	Amino acids (AA)
A	13.6 [15.7–12.1]	15.2 [17.1–13.6]
B	12.7 [14.7–11.7]	11.3 [13.9–11.6]
C	4.8 [5.9–3.9]	4.8 [6.3–3.6]
D	13.3 [13.9–10.9]	14.4 [15.1–12.3]
E	12.0 [12.6–9.8]	13.7 [13.1–10.3]
F	10.4 [12.1–9.1]	12.0 [13.4–10.6]
G	6. 3[7.6–5.1]	7.3 [8.9–5.8]
H	9.8 [10.3–8.7]	11.6 [13.1–10.3]
I	7.9 [9.2–6.8]	9.2 [10.7–7.9]
J	5.3 [6.5–4.4]	7.1 [8.6–5.7]
K	6.2 [7.5–5.2]	7.1 [8.5–5.8]
L	5.6 [6.7–4.6]	6.4 [7.9–5.2]
M	9.4 [10.8–8.3]	11.1 [12.7–9.8]
N	3.3 [4.1–2.6]	4.5 [5.7–3.5]
O	6.6 [7.8–5.5]	7.8 [9.2–6.5]
P	5.6 [6.7–4.7]	6.2 [7.6–4.9]
Q	9.7 [11.3–8.7]	11.3 [12.2–10.2]
R	9.4 [10.9–8.3]	10.9 [11.8–9.8]
S	5.5 [6.7–4.6]	7.4 [8.6–6.1]
T	8.8 [10.3–7.8]	10.2 [11.3–8.8]
U	12.7 [14.73–11.4]	14.4 [16.4–12.6]
V	7.6 [9.1–6.3]	8.8 [10.6–7.1]
W	3.5 [4.6–2.7]	4.5 [6.3–3.2]
X	2.1 [2.9–1.5]	2.9 [4.2–2.0]
Y	1.4 [2.0–1.0]	2.9 [3.5–1.6]

NOTE.—Letters refer to the nodes in figure 1. The mean age of each node is given in million years ago (Ma), together with the lower and upper bounds of the 95% credibility intervals derived from the Bayesian relaxed molecular clock analysis. The fossil constraints are indicated in the Material and Methods.

Caatinga + Cerrado and the Atlantic Forest were involved sequentially as a corridor and/or a barrier to dispersal. This area of high endemism includes several divergent echimyid lineages from both open (e.g., *Carterodon*) and closed habitats (e.g., *Trinomys*, *Callistomys*) that are not found elsewhere in the Neotropics. Echimyids in both Atlantic Forest and Caatinga + Cerrado have a higher frequency of endemism than those in the Amazon Basin, northern Neotropics, and West Indies. Relative to the Amazon, the Atlantic Forest has two arboreal lineages that evolved arboreality independently (*Callistomys* and the Echimyini).

The open-country corridors of Caatinga and Cerrado meanwhile have triggered uncommon echimyid specializations to fossoriality, but they have also preserved ancient "surviving" forest and savanna lineages found nowhere else in the Amazon Basin source pool (e.g., *Carterodon* and some *Trinomys*). With only two inferred Miocene dispersals (Nodes O and U), Caatinga and Cerrado open habitats have also been involved in two genus-level divergences of echimyids (Nascimento et al. 2013). These historical events co-occurred with locomotory shifts from terrestriality to: (1) fossoriality (Nodes C and V; see *Clyomys*, *Euryzygomatomys*, and *Carterodon*); and, (2) rock and bush-climbing capacities (Node R; *Thrichomys*). The majority of echimyid species are forest adapted, and some competition with their octodontoid sister group (families Ctenomyidae and Octodontidae, which are mostly open-country and montane forms in southern South America) has been suggested to possibly explain this differential success between open and closed habitats (Upham and Patterson 2012; Nascimento et al. 2013; Upham 2014; Arnal and Vucetich 2015).

Geographic isolation, extinctions, and habitat fragmentation have shaped the modern diversity of the Atlantic Forest (Costa 2003; Batalha-Filho et al. 2013; Leite et al. 2016). The evolutionary relationships among Atlantic Forest echimyid genera are particularly scattered across their family topology (fig. 4). The composition of the echimyid communities in the Caatinga, Cerrado, and Atlantic Forest, as well as their deep divergence within the topology, provide evidence of ancient and recurrent links among these biomes. Based on the scarce palynological record, the Caatinga and Cerrado biomes originated in the early or mid-Miocene (Pascual and Jaureguizar 1990; Hoorn et al. 2010), and have acted ever since as a semi-permeable barrier between the two major rainforest biomes. This poorly documented botanical transition (Colinvaux and De Oliveira 2001) occurs at the end of the middle Miocene climatic optimum and the start of the ensuing cooling event (Zachos et al. 2001), and is then followed by major changes in the placental mammal fauna (Flynn 1998; Croft et al. 2009; Antoine et al. 2016). The Caatinga and Cerrado dry corridor might thus be seen as an east–west transition zone for Neotropical biota. However, the fauna of the Atlantic Forest biome is closely tied to Caatinga and Cerrado, as evidenced by some members of the Atlantic Forest terrestrial spiny rats (*Trinomys*), which also occur in the Caatinga and Cerrado (i.e., *T. albispinus and T. yonenagae*; Attias et al. 2009; Pessôa et al. 2015). Our results converged with Quaternary fossil record from Lagoa Santa in Minas Gerais, that documented faunal shifts between Cerrado and Atlantic Forest faunas (ref: documented by Peter Lund; Pardiñas et al. 2016). Small mammals from these caves document *Callistomys* as well as several sigmodontines such as *Blarinomys*, *Brucepattersonius*, and *Castoria*, both Atlantic forest endemic, and *Pseudoryzomys* chiefly a Cerrado endemic (Voss and Myers 1991; Emmons and Vucetich 1998; Pardiñas et al. 2016). As *Thrichomys* and *Carterodon* sister-relationships are poorly supported (figs. 2 and 3), potential links between these open habitat taxa and other echimyid from Amazon Basin and or Atlantic Forest cannot be confirmed for these lineages.

These eastern Neotropical biomes have an old isolation history compared with Northern South America, with sporadic episodes of connections. The pathway to and from Amazon and Atlantic Forests are usually divided into: (1) an ancient pathway on the southern margin of Cerrado; and, (2) a young Plio-Pleistocene pathway through NE Brazil (Caatinga and Cerrado) (Costa et al. 2000; Batalha-Filho et al. 2013; Leite et al. 2016). Different events might be responsible for these faunal exchanges within these regions. Firstly, the edge of the Brazilian Shield might have been connected throughout the Miocene from the northern Chaco in Mato Grosso (Brazil) to western Bolivia and Paraguay, and from Rondônia southeast to Paraná (Brazil). Also, the axial groove of the western Amazon Basin caused repeated inland freshwater and marine expansions (Webb 1995; Hoorn et al. 2010). Throughout the Miocene, marine incursions occurred

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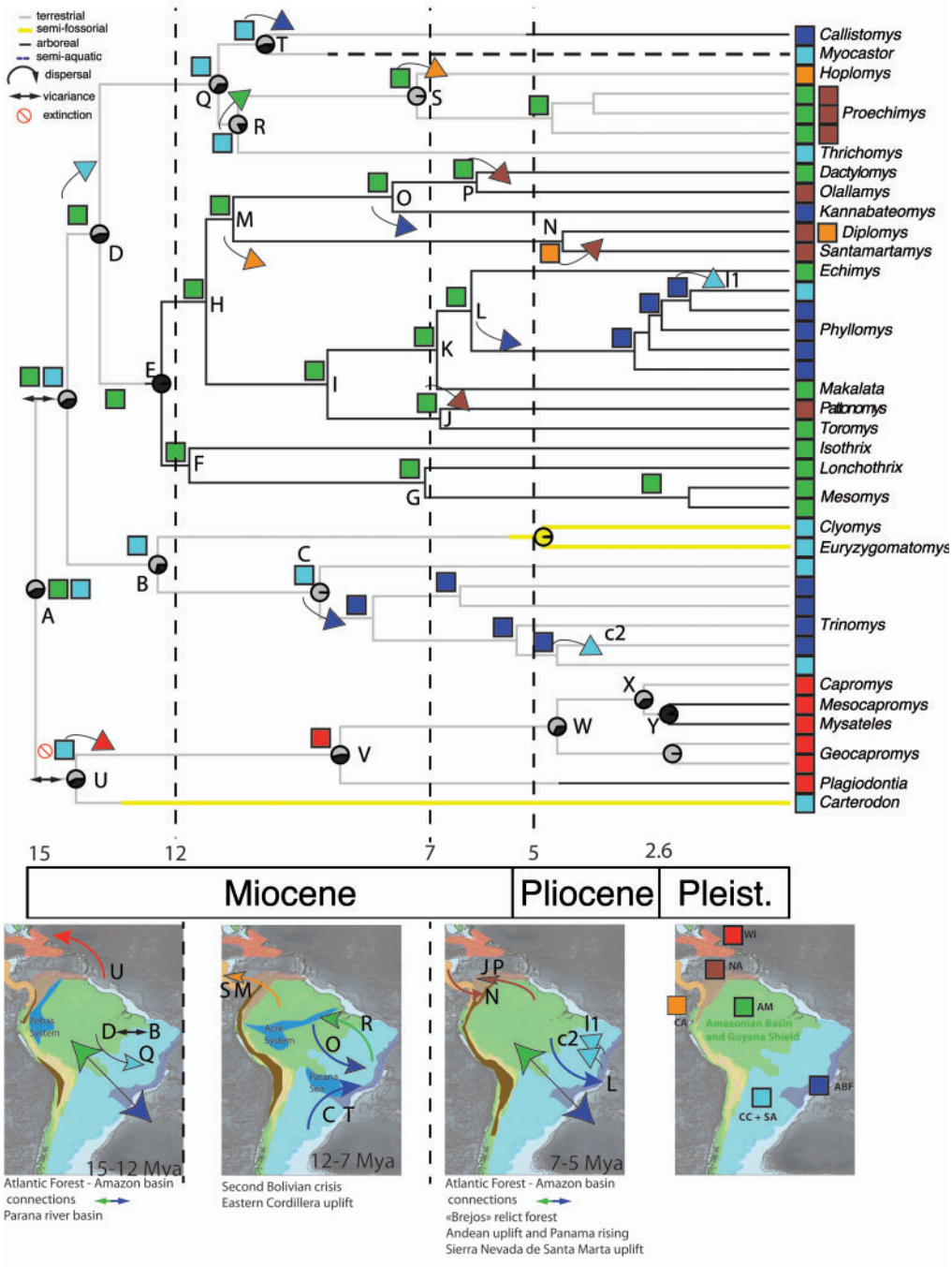

Fig. 4. Ancestral-area reconstructions of Neotropical Echimyidae. The tree is a chronogram (relaxed molecular clock) based on a PHYLOBAYES Markov Chain Monte Carlo (MCMC) analysis of the combined data set (see also table 4). The rodent outgroups are not shown (full tree provided as supplementary figures S1 and S2, Supplementary Material online). Maximum-likelihood ancestral area reconstructions were conducted from this chronogram. The result from our DECX stratified and constrained model is presented here (see "Materials and Methods" for details). The employed geographical distribution for each taxon is presented to the right of the taxon names. Colored squares indicate the geographical origin of a given node. Bidirectional arrows indicate vicariance and single arrows dispersal. The map indicates the inferred dispersal and colonization routes of members of the echimyids as well as the different biogeographical hypotheses of our DECX model. The branch colors indicate the results of the stochastic mapping results for the life-mode characters (see legends and "Materials and Methods", but see also supplementary figure S3, Supplementary Material online). Taxonomic names, and lettered labels for the major echimyid clades are given along the phylogeny.

both in the south along the Paraná basin (Cozzuol 1996) and in the north along the Amazon palaeobasin (Räsänen et al. 1995; Hoorn 1996). These recurrent shifts in the watershed of Amazonia as well as the emergence of inland seas might have connected or isolated Amazonian and Atlantic rainforest via dispersal corridors such as flooded savannah and rainforests (Roddaz et al. 2006; Wesselingh and Salo 2006; Wesselingh et al. 2006). Any of these complex water ingressions could

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have driven the generic splits of arboreal taxa *Callistomys*, *Kannabateomys*, *Phyllomys* and the isolation of terrestrial node C (*Trinomys*).

A Recent Vicariant Barrier to the North: The Late Miocene Andean Wall

Constituting a long and high physical barrier separating tropical lowland biotas, the Northern Andean uplift has been the most recent orogenic event in the Neotropics (Hoorn et al. 2010). This major event resulted in the separation of echimyids from trans- (West) and cis- (East) Andean Amazonian region, as well as from the Carribean coastal region. Most of the trans-Andean echimyid genera are arboreal and have a northern Amazonian genus counterpart, thus highlighting their common evolution related to the uplift of the Andes (e.g., *Olallamys* and *Dactylomys*, Node O). With some few highland exceptions (Upham et al. 2013; Patton et al. 2015), arboreal echimyids are lowland specialists that are more speciose and abundant at lower elevations. Due to their limited dispersal abilities across highland and unforested habitats, arboreal echimyids constitute potential candidates for Amazonian rainforest vicariance similar to some other placental mammals (Cortés-Ortiz et al. 2003; Delsuc et al. 2004; Patterson and Costa 2012; Gibb et al. 2015). The Northern Andean uplift has mainly triggered the provincialism of Carribean coastal areas north of the Amazon Basin + trans-Andean among arboreal lineages, as revealed by sister-relationships between arboreal genera (figs. 2 and 4). We identified only one genus-level divergence among terrestrial lineages, *Hoplomys* and *Proechimys* (cf., Node S; Myocastorini clade), compared with three splits within the main arboreal clade (Node E). If the Andes have generated such generic diversity, it might also be a diversity pump in the eastern part of cis-Andean region at shallower taxonomic levels (Upham et al. 2013), but that idea awaits further studies at a finer geographic scale involving of *Dactylomys*, *Makalata*, *Proechimys* and *Isothrix* from the same region. Several species (e.g., *Isothrix barbarabrownae* and *Dactylomys peruanus*) occur at altitudes >2000 m in mossy or montane rainforest in this area, where elevation and isolation associated with montane habitat heterogeneity have been hypothesized to drive divergence and speciation (Bates and Zink 1994; Patterson and Costa 2012; Upham et al. 2013).

Our analysis confirms an Amazon Basin origin for most of these genera with several cis—trans Andean + Northern Andes pairs, with Chocó *Hoplomys*—Amazonian *Proechimys*, trans Andean *Diplomys* + northern Andes *Santamartamys* clade—Amazonian ancestors of node M, including northern Andes *Olallamys*—Amazonian *Dactylomys*, and Northern Carribean coastal *Pattonomys*—Amazonian *Toromys*. We already noted the exception of the West Amazonian *Pattonomys occasius*, which represents a lineage closely related to East Amazonian *Toromys* (Emmons LH and Fabre P-H, personal communication). Northern Neotropical echimyid assemblages are more recently derived from their Amazon Basin ancestors compared with taxa inhabiting eastern biomes. The strength of the Amazon riverine barrier, as well as greater proximity and similarity of rainforest habitats might explain this close faunistic pattern compared with the older and more phylogenetically clustered eastern biomes. This endemism is also recovered and paralleled in bats (Koopman 1982) and sigmodontine rodents (Weksler 2009; Parada et al. 2013; Salazar-Bravo et al. 2013).

From the Late Oligocene, northern South America has experienced major tectonic events related to the Andes uplift including the first (Late Oligocene and Early Miocene) and second (Late Miocene) Bolivian crises as well as some recent Pliocene uplifts (Sempere et al. 1990; Lovejoy et al. 2006; Hoorn et al. 2010). North Andean echimyid splits (nodes J, P, N, S) range from 8.6 to 2.6 Ma (mean = 5.6 Ma), which is a rather short time frame. This brief time window and multiple generic splits likely reflect a significant impact of the second Bolivian crisis as a major driver of vicariance. All these genera might have been widespread prior to the start of Northern Andean orogeny. Emergence of the Northern Andes and resulting trans-Andean isolation might have triggered the divergence of some of the rarest montane endemics (*Santamartamys rufodorsalis* and *Olallamys edax*) from events like the uplift of the Sierra Nevada de Santa Marta (~8 Ma; Hoorn et al. 2010), but a recent study pointed out an Oligocene age with some recent uneroded Pleistocene uplifts (Villagómez et al. 2011). The rise of these montane rainforests between the Magdalena and Pacific Slope ridges, along with the isolation of the arid Maracaibo and Orinoco Basins might have also played a major role on species level diversification of echimyids west of the Andes and in isolated northern coastal regions. Looking at species diversity patterns, the following genera have been isolated since the second Bolivian crisis and speciated into three areas of endemism: the Chocó and Central American forest to the west (*Diplomys*, *Olallamys albicaudus*, *Proechimys*, and *Hoplomys*) and the Northern Andean and Carribean coastal area (*Olallamys edax*, *Santamartamys*, *Proechimys*, *Pattonomys*). The highly divergent genera of *Proechimys*, *Makalata*, and *Mesomys* represent target model organisms for a future look at recent dispersal along the Northern Andes, and to test for the role of more recent Andean orogenic events.

Conclusion. Echimyid Higher Level Diversification: Biogeographic Events and Adaptive Divergence

The family Echimyidae is considered as a model biological radiation given the rapid and early diversification of spiny rat lineages and their dietary and life-mode diversity (Leite and Patton 2002; Patton et al. 2015). From our ancestral state reconstruction (fig. 4, branch colours), only one ecological shift from terrestrial to arboreal lifestyles (Node E) is involved in the early diversification of major lineages. A second, more recent acquisition of the arboreal lifestyle is observed for *Callistomys*, an event that coincides with a simultaneous shift into semi-aquatic adaptation in the southern Neotropics for its sister genus, *Myocastor* (Emmons and Vucetich 1998). Flooded systems might have favoured such a divergence between semi-aquatic (*Myocastor*) and arboreal (*Callistomys*) habits. Arboreal adaptations have been hypothesized to coincide with the first occurrence of flooded forests during the

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Table 5. Proposed Higher Level Classifications for Echimyids, Based on Our Phylogenetic Results.

Classification					
Hypothesis 1	**Hypothesis 2**	**Molecular tribes**	**Genera**	**N species**	**GenBank**
	Echimyidae				
Capromyidae	**Capromyinae**	**Plagiodontini**	***Plagiodontia***	**1**	**1 (100%)**
		Capromyini	***Capromys, Geocapromys, Mesocapromys, Mysateles***	**9**	**8 (88%)**
Incertae sedis family	***Incertae sedis subfamily***		***Carterodon***	**1**	**1 (100%)**
Echimyidae	**Euryzygomatomyinae**		***Clyomys, Euryzygomatomys, Trinomys***	**12**	**10 (83%)**
	Echimyinae	**Myocastorini**	***Callistomys, Hoplomys, Myocastor, Proechimys, Thrichomys***	**30**	**12 (40%)**
		Echimyini	***Dactylomys, Diplomys, Echimys, Isothrix, Kannabateomys, Lonchothrix, Makalata, Mesomys, Olallamys, Pattonomys, Phyllomys, Santamartamys, Toromys***	**46**	**35 (76%)**

NOTE.—N species represent the number of species described in Wilson and Reeder (2005) and Patton et al. 2015; GenBank represent the number of species available in GenBank; Percentage represents the proportion of species included in our study.

Miocene, likely due to the presence of inland flooded habitats during episodes of the Pebas or Acre Systems, or even the Paraná sea (Galewski et al. 2005; Hoorn et al. 2010). Despite its morphological distinctiveness (see Emmons and Vucetich 1998), the arboreal, soft-furred *Callistomys* was previously allied with other spiny tree rats in Echimyini (Carvalho and Salles 2004; Candela and Rasia 2012; Verzi 2013; Verzi et al. 2015). They also share high-crowned hypselodont molars, suggesting a shared herbivorous ancestry (Emmons and Vucetich 1998). However, our mitogenomic analysis confirms the result of Loss et al. (2014) that *Callistomys* lies outside the arboreal clade, and also strongly supports a sister-group relationship of *Callistomys* with *Myocastor*. Both taxa join the Myocastorini clade together with terrestrial *Proechimys*, *Hoplomys*, and *Thrichomys*. Moreover, the monophyly of *Callistomys* + *Myocastor* is reinforced by a synapomorphic feature of their chromosomes, as both genera share the $2n = 42$ karyotype (González and Brum-Zorrilla 1995; Ventura et al. 2008). Evident homoplasies of dental occlusal patterns hampered previous attempts to recover affinities among arboreal echimyids (Carvalho and Salles 2004; Candela and Rasia 2012). Further analyses taking into account our new phylogenetic background will likely help clarify hypotheses of primary homology among cheekteeth characters across the diversity of extant and extinct echimyid rodents. For example, entirely laminar molar occlusal patterns appear to have arisen independently in *Phyllomys* and *Diplomys* lineages, and of the latter, *Santamartamys* alone shares joined, wishbone-shaped lophs with the sister-group of dactylomyines (Node O), suggesting that it retains a more plesiomorphic occlusal pattern (Emmons 2005).

Historical opportunities from geographic vicariance and dispersal appear to play a primary role in the diversification among echimyid genera, perhaps to a greater extent than their subsequent acquisition of locomotory adaptations. We inferred that several echimyid ancestors from the Amazon Basin, Central Andean region, and Guyana Shield experienced either repeated vicariant events or independent dispersals between Atlantic Forest, Cerrado, Caatinga, and the Orinoco watershed and trans-Andean region (fig. 4). This pattern is well illustrated by the bamboo rat genera *Dactylomys*, *Kannabateomys*, and *Olallamys*, which occur in each of the tropical rainforest areas (figs. 2 and 4). Ecological and morphological convergences also appear to be involved as illustrated by the aforementioned dual acquisition of arboreality and some remarkably convergent cheekteeth patterns in *Diplomys* and *Phyllomys* (Emmons 2005; Candela and Rasia 2012). Despite their ecological diversity of diet and locomotion, echimyids seem to be a morphologically conservative lineage. Plesiomorphies in both arboreal and terrestrial taxa formerly allocated to Eumysopinae (e.g., *Trinomys* and *Proechimys*), and even within Echimyini (e.g., *Makalata* and *Toromys*; but see Candela and Rasia 2012; Fabre et al. 2013), have hampered past efforts to reconstruct the evolutionary history of the family using morphology.

Mirroring the ecological diversification of the Echimyidae as a whole, the mid-Miocene colonization of the West Indies triggered an insular radiation of hutias (Fabre et al. 2014). Present-day hutias do not represent their past diversity, and the fossil record harbours at least 21 extinct species belonging to extant hutia genera and 3 extinct genera (Dávalos and Turvey 2012), along with other extinct rodents that may be closely related (*Boromys*, *Brotomys*, and *Heteropsomys*). These extinct and extant capromyids display a wide range of life histories and ecomorphological adaptations, including both scansorial (*Geocapromys*) and terrestrial (*Isolobodon*) forms; they appear to also document independent acquisitions or loss of arboreal lifestyles (fig. 4; *Plagiodontia* and *Mysateles* + *Mesocapromys*). This island diversity has been greatly impacted by extinctions related to human activities (Turvey et al. 2007), hindering our view of their past diversity (Dávalos and Turvey 2012).

Future directions include the need to improve the species-level sampling in phylogenetic analyses of Echimyidae and Capromyidae. Thanks to the availability of priceless specimens from natural history collections and high-throughput

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DNA sequencing methodologies, under-sampled and/or rare lineages (table 1) are likely to be sequenced soon for both nuclear and mitochondrial genes. Compared with previous higher level classifications, the initial star-phylogeny (Lara et al. 1996; Leite and Patton 2002) has now been completely scrutinized leaving few open questions at this taxonomic level (but see "Discussion"). The Miocene radiation of Echimyidae and Capromyidae is rather young (<20 Ma) when compared with older splits of other ctenohystrican rodent families (Patterson and Upham 2014), but is comparable in age to their sister clade Octodontidae + Ctenomyidae (16.4 Ma [13.7–18.9]). In view of our phylogenetic results showing short internal branches for the initial diversification of Echimyidae and Capromyidae, their taxonomic status at the family or subfamily level needs further evaluation with larger sets of nuclear DNA markers. We therefore propose two alternative classifications (table 5), which summarize our evolutionary hypotheses, and which should provide a refined phylogenetic framework for subsequent in-depth studies of their genomics, molecular evolution, and macroevolution.

Taxonomy

Euryzygomatomyinae new subfam.

Genus content. *Clyomys*, *Euryzygomatomys*, *Trinomys*

Definition. Euryzygomatinae, members of which all share an origin in the eastern part of Brazil, close to the Atlantic Forest. All of them are spiny, have lower deciduous premolar 4 with 5 lophids; 4 (*Trinomys*) or 3 (*Clyomys*, *Euryzygomatomys*) lophids on the lower molar 1; well-connected lophs are present on the cheek teeth; 3 molar roots anchor the upper molars; elongate lower and upper incisor roots; reduced zygomatic arch with a slightly concave dorsal margin, a ventrally expanded jugal with much reduced, scarcely salient inferior process. Molecular synapomorphies include the amino-acid Proline at position homologous to site 294 of the human GHR (protein accession AAI36497.1); the amino-acid Leucine at position homologous to site 1318 of the human VWF (protein accession AAH22258.1).

Myocastorini new tribe

Genus content. *Callistomys*, *Hoplomys*, *Myocastor*, *Proechimys*, *Thrichomys*

Definition. Myocastorini, members of which all share long upper incisor roots (except *Callistomys*); mid- to long-sized lower incisor roots; all of them share 4 (*Callistomys*, *Thrichomys*) or 5 (*Hoplomys*, *Myocastor*, *Proechimys*) lophids on the lower deciduous premolar 4; 3 roots anchor the upper molars; well-connected lophs characterize the cheek teeth. Members exploit a diverse array of lifestyles including terrestrial (*Hoplomys*, *Proechimys*, *Thrichomys*), arboreal (*Callistomys*), and amphibious (*Myocastor*) adaptations.

Supplementary Material

Supplementary data are available at *Molecular Biology and Evolution* online.

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