Diversification of South American spiny rats (Echimyidae): a multigene phylogenetic approach

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We investigated the phylogenetic relationships of 14 Echimyidae (spiny rats), one Myocastoridae (nutrias) and one Capromyidae (hutias) genera based on three newly sequenced nuclear genes (*APOB*, *GHR* and *RBP3*) and five previously published markers (the nuclear *RAG1* and *vWF*, and the mitochondrial cytochrome *b*, *12S* rRNA and *16S* rRNA). We recovered a well-supported phylogeny within the Echimyidae, although the evolutionary relationships among arboreal echimyid taxa remain unresolved. Molecular divergence times estimated using a Bayesian relaxed molecular clock suggest a Middle Miocene origin for most of the extant echimyid genera. Echimyidae seems to constitute an example of evolutionary radiation with high species diversity, yet they exhibit only narrow skull morphological changes, and the arboreal and terrestrial taxa are shown to retain numerous plesiomorphic features. The most recent common ancestor of spiny rats is inferred to be a ground-dwelling taxon that has subsequently diverged into fossorial, semiaquatic and arboreal habitats. The arboreal clade polytomy and ancestral character estimations suggest that the colonization of the arboreal niche constituted the keystone event of the echimyid radiation. However, biogeographical patterns suggest a strong influence of allopatric speciation in addition to ecology-driven diversification among South American spiny rats.

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Introduction

Understanding the causes of adaptive radiations constitutes one of the most important challenges for modern systematics. Following Schluter (2000), the 'adaptive radiation' is defined as a rapid ecological and phenotypical diversification through the evolutionary history of a given lineage. In other words, the partitioning of the environment, by the use of different ecological niches and by phenotypical differentiation, allows for the coexistence of closely related species inside the same habitat. Many taxonomic groups like birds, lepidosaurians or angiosperms display adaptive radiation (Ricklefs 2003, 2004; Davies *et al.* 2004; Grant & Grant 2008; Losos 2009). High speciation rates and morphological homoplasy have led to difficulties in resolving the corresponding phylogenetic relationships, and most taxa within a radiation are characterized by unresolved (star) phylogenies. Based on these studies, the definition of radiation has shifted from an ecological sense to a macroevolutionary one (Simpson 1953; Givnish & Sytsma 1997). Phylogenetic studies of radiation patterns within numerous taxonomic groups have correlated these events to high speciation rates (Losos 2009; Rabosky & Glor 2011), morphological convergences (Gaubert *et al.* 2005) and key historical events (Moore & Donoghue 2007, 2009).

Among placental mammals, South American spiny rats (Echimyidae, Rodentia) are a prime example of evolutionary and adaptive radiation. The family Echimyidae, comprising more than 90 extant species and 19 genera (Woods & Kilpatrick 2005), constitutes an example of a major evolutionary radiation within the hystricognath rodents. Echimyidae display a wide range of life histories and ecomorphological adaptations including fossorial, scansorial, arboreal and semiaquatic capacities. These small to medium-sized rodents (Emmons & Feer 1997) have spread over most of South America including Caribbean Islands (hutias), where they occupy several terrestrial ecosystems (Eisenberg & Redford 1989, 1992, 1999; Emmons & Feer 1997; Redford *et al.* 2000). Most of the extant echimyid diversity occurs in the Amazon Basin where many arboreal (*Echimys, Isothrix, Makalata, Mesomys, Lonchothrix, Pattonomys*) and terrestrial (*Proechimys*) genera live in the lowland rainforest. *Kannabateomys, Phyllomys, Callistomys* and *Trinomys* are found in tropical forest and drier woodlands of the Atlantic Forest. Semifossorial taxa are found in grassland of Cerrado and Caatinga (*Carterodon* and *Clyomys*) or near the Atlantic Forest biome (*Euryzygomatomys*). The ground-dwelling *Thrichomys* is found in the Cerrado and Pantanal areas within bushland habitats (Borodin *et al.* 2006). The remaining genera *Olallamys, Hoplomys* and some *Proechimys* species are endemic to the Chocó rainforest situated west of the central Andes Cordillera (Central America to northern regions of Venezuela, Colombia and Ecuador).

Many classifications (see Carvalho & Salles 2004 for an overview) have been proposed for the Echimyidae since Geoffroy St. Hilaire (1838, 1840). Based on morphological characters, Echimyidae was traditionally splits into three subfamilies: (i) the Eumysopinae (*Carterodon, Clyomys, Euryzygomatomys, Hoplomys, Mesomys, Lonchothrix Proechimys, Thrichomys, Trinomys*), (ii) the Dactylomyinae (*Dactylomys, Kannabateomys, Olallamys*) and (iii) the Echimyinae (*Callistomys, Echimys, Isothrix, Makalata, Phyllomys* and *Toromys*). Although morphological and molecular phylogenetic analyses have been conducted to understand the complex systematics and diversification of this family (e.g. Patton & Reig 1989; Lara *et al.* 1996; Leite & Patton 2002; Carvalho & Salles 2004; Emmons 2005; Galewski *et al.* 2005; Patterson & Velazco 2006), there has not been a consensus about the relationships among these taxa. Previous molecular (Lara *et al.* 1996; Leite & Patton 2002; Emmons 2005) and morphological (Carvalho 1999; Carvalho & Salles 2004; Emmons 2005) works failed to resolve the deepest part of the phylogeny of Echimyidae, leading to the use of the star phylogeny to characterize the Echimyidae radiation. These studies suggested that the unresolved polytomies are likely because of high speciation rate, high level of homoplasy in morphological characters and heter-ogeneous rates of molecular evolution. Recently, a molecular phylogeny based on the *vWF* nuclear gene provided support to unsuspected ecological clades (Galewski *et al.* 2005). This study has revealed three ecological assemblages with, respectively, (i) a fossorial clade (*Euryzygomatomys, Clyomys*), (ii) a terrestrial clade (*Proechimys, Hoplomys, Thrichomys* and *Myocastor*) and (iii) an arboreal clade (*Dactylomys, Echimys, Isothrix, Kannabateomys, Lonchothrix, Makalata, Mesomys* and *Phyllomys*). In addition, it has been shown that the highly derived Capromyidae and Myocastoridae families are actually part of the Echimyidae radiation (Woods 1985, 1989; Leite & Patton 2002; Galewski *et al.* 2005). Moreover, the recent revisions of echimyid taxonomy (Emmons 2005; Woods & Kilpatrick 2005; Leite 2008) combined with the discovery of new phylogenetic relationships involving the central American *Callistomys* (Emmons & Vucetich 1998), the Amazonian *Pattonomys*, the *Santamartamys* from the Sierra Nevada de Santa Marta (Emmons 2005) and the Amazonian *Toromys* (Iack-Ximenes *et al.* 2005) further illustrate the gaps in our knowledge. The discrepancies among initial classifications and molecular phylogenies (Lara *et al.* 1996; Leite & Patton 2002; Galewski *et al.* 2005; Upham & Patterson 2012) highlight the difficulties for understanding the echimyid adaptive radiation based on limited sets of characters.

Resolving rodent phylogenies from molecular data is challenging, and the need for a combined use of multiple mitochondrial and nuclear markers has been stressed (e.g. Huchon *et al.* 2002, 2007; Steppan *et al.* 2004). To provide a better understanding of their systematics, we present an updated phylogenetic hypothesis for 16 genera (51% of genus diversity) of all major Echimyidae clades, based on the combination of three mitochondrial and five nuclear DNA markers. Using this phylogenetic framework coupled to a molecular dating approach, we explored the systematics and macroevolution of the spiny rats throughout the Neogene. Based on published matrices of morpho-anatomical characters (Carvalho & Salles 2004; Emmons 2005), we subsequently compared the arboreal and terrestrial lineages through the quantification of their relative degree of apomorphies versus plesiomorphies. Using ecomorphological and biogeographical informations, we discuss the ecological evolution and the major vicariance/dispersal events in South American biomes for Echimyidae.

Material and methods

Taxon sampling

To study the phylogenetic relationships among genera of Echimyidae, 23 species representing 16 of the 28 extant genera listed by Woods & Kilpatrick (2005) were sampled,

including Myocastoridae – a monospecific family – and Capromyidae – only one genus (*Capromys*) available among the six extant ones – as part of the Echimyidae *sensu lato* (Galewski *et al.* 2005). All subfamilies of echimyids as well as the three main ecomorphological groups (fossorial, ground dwelling and arboreal) were represented by several genera in our sampling (Table S1). The corresponding voucher numbers are provided in Table S2. We incorporated the closest Caviomorpha outgroup, that is, representatives of Abrocomidae, Octodontidae and Ctenomyidae families. We also included one representative of each Ctenohystrica families as more distant outgroup to stabilize Octodontidae relationships.

DNA amplification and sequencing
Ethanol-preserved samples were obtained from the mammalian tissue collection of the 'Institut des Sciences de l'Evolution de Montpellier' (Catzeflis 1991). Total DNA extractions were performed using the QIAamp DNA mini kit (Qiagen, Crawley, UK). Fragments from the following four nuclear genes were amplified and sequenced: apolipoprotein B exon 26 (*APOB*), growth hormone receptor exon 10 (*GHR*), interstitial retinol binding protein exon 1 (*RBP3*) and recombination activating protein 1 gene (*RAG1*). These DNA markers were chosen for the following reasons: (i) several sequences are already available for those genes, especially within Ctenohystrica, (ii) they provide adequate phylogenetic signal within Rodentia and (iii) they are genetically unlinked. The genes were amplified and sequenced using the following primers: (i) for *APOB* (Amrine-Madsen *et al.* 2003): 187F (5′ GTGCCAGGTT-CAATCAGTATAAGT 3′), J1R (5′ CCAGCAAAATT TTCTTTTACTTCAA 3′), F60 (5′ GGAGAAGCCAA YCTGGATTTCYT 3′), R1280 (5′ AGCTTCACYGAA GABCGWGYVCCCTTGG 3′), (ii) for *GHR* (Galewski *et al.* 2006): GHR5 (5′ GGCRTTCATGAYAACTAC AAACCTGACYTC 3′), GHR6 (5′ GAGGAGAG GAACCTTCTTTTTWTCAGGC 3′), GHR3 (5′ GACT TTATGCYCARGTRAG 3′), GHR4 (5′ CTYACY TGRGCATAAAAGTC 3′), (iii) for *RBP3* (Poux & Douzery 2004): I1 (5′ ATGGCCAAGGTCCTCTTGGATAA CTACTGCTT 3′), J1 (5′ CCACTGCCCTCCCA TGTCTG 3′), I2 (5′ ATCCCCTATGTCATCTCCTAC YTG 3′), J2 (5′ CGCAGGTCCATGATGAGGTGCTC CGTGTCCTG 3′), I5 (5′ GCCCTGGACCTCCAG AAGCTGAGGATMGG 3′), J5 (5′ CARGGTCCAGA TCTCYGTGGT 3′), (iv) for *RAG1* (Teeling *et al.* 2003; Patterson & Velazco 2008), RAG1F1705 (5′ GCTTT GATGGACATGGAAGAAGACAT 3′), FMNH1b (5′ CTTGAAGGTCCTGGGRATGCCTCCC 3′), FMNH 2a (5′ GAGAGGGAAGCCATGAAGAGCAGYGA 3′), FMNH2b (5′ TTATACACCTCCCCTATCTCKAGC

3′), FMNH3a (5′ GGCAATGCHGCYGAATTC- TACA-AGAT 3′), RAG1R2864 (5′ GAGCCATCCCTCTCAAT-AATTTCAGG 3′). PCR conditions were 95 °C 5 min, followed by 95 °C 30 s, 61 °C 30 s, 72 °C 1 min (5 times), then 95 °C 30 s, 59 °C 30 s, 72 °C 1 min (5 times), followed by 95 °C 30 s, 57 °C 30 s, 72 °C 1 min (5 times), then 95 °C 30 s, 55 °C 30 s, 72 °C 1 min (5 times) and then 95 °C 30 s, 53 °C 30 s, 72 °C 1 min (20 times), with a final extension at 72 °C 5 min. PCR products for all genes were purified from 1% agarose gel using Amicon Ultrafree-DNA columns (Millipore) and sequenced on both strands using automatic sequencing (Big Dye Terminator cycle kit) on an ABI 310 (PE Applied Biosystems, Warrington, UK).

Alignment and construction of DNA supermatrices
We combined the newly obtained *APOB*, *GHR* and *RBP3* sequences with all mitochondrial (cytochrome *b*, 12S and 16S rRNAs) and other nuclear (*vWF* and *RAG1*) sequences available from public databases (Lara *et al.* 1996; Lara & Patton 2000; Leite & Patton 2002; Galewski *et al.* 2005; Patterson & Velazco 2008). We focused on one accession per Echimyidae and other representative Ctenohystrica genera. To improve the nuclear and mitochondrial gene coverage, we built a genus-level DNA matrix with several taxonomic chimeras (see Table S1). Non-overlapping sequences (*i.e.* sequences available for different species of the same genus) were concatenated in the final multigene matrices. As an example, to solve the non-overlap of genetic sampling for the two *Euryzygomatomys* species, a chimera has been drawn with *E. spinosus* and *E. guiara* as indicated in Table S1. Reciprocal genus monophyly for the chimeras was defined using published phylogenies and systematic results. Within Echimyidae, five chimeras were built: (i) *Dactylomys* from *Dactylomys boliviensis* + *Dactylomys dactylinus* (Leite & Patton 2002), (ii) *Isothrix* from *Isothrix bistriata* + *Isothrix sinnamariensis* (Upham & Patterson 2012), (iii) *Euryzygomatomys* from *Euryzygomatomys spinosus* + *Euryzygomatomys guiara* (Woods & Kilpatrick 2005), (iv) *Proechimys* from *Proechimys guyannensis* + *Proechimys simonsi* (Leite & Patton 2002; Galewski *et al.* 2005; Upham & Patterson 2012) and (v) *Trinomys* from *Trinomys iheringi* + *Trinomys paratus* (Lara & Patton 2000). The ingroup monophyletic genera (*Echimys*, *Phyllomys*, *Makalata*, *Isothrix*, *Clyomys*, *Mesomys*, *Proechimys*, *Thrichomys*, *Trinomys*, *Myocastor*, *Capromys*) are represented for the eight genes. Sequences were aligned with the ED editor of the MUST package (Philippe 1993). For the 12S rRNA and 16S rRNA alignments, ambiguous positions were discarded using the GBLOCKS program (version 0.91b; Castresana 2000) with the following options: a minimum of half the number of sequences for a conserved position and for a flank position, a maximum of eight contiguous

non-conserved positions, a minimum of two sites for the block length after gap cleaning and all gap positions can be selected. From these individual alignments, we built three supermatrices with, respectively, mitochondrial (32 taxa and 2445 sites; 14% of missing character states), nuclear (31 taxa and 5395 sites; 26% of missing data) and nuclear + mitochondrial (32 taxa and 7840 sites; 25% of missing data) sequences.

Phylogenetic analyses on the individual and concatenated genes

Phylogenetic trees were reconstructed using maximum likelihood (ML) and Bayesian inference for the single nuclear gene matrices, the concatenated mitochondrial dataset, the concatenated nuclear dataset and the nuclear + mitochondrial concatenated datasets. The MOD-ELTEST 3.07 software (Posada & Crandall 1998) was used to determine the best-fit ML model of DNA sequence evolution under the Akaike Information Criterion. Maximum likelihood parameters and topologies were estimated by PAUP* (Swofford 2002), version 4b10 using a loop approach. The ML parameter values were firstly optimized on a neighbor-joining (NJ) topology issued from ML distances obtained using the best-fit model. An ML heuristic search was subsequently applied with tree bisection-reconnection (TBR) branch swapping to identify the optimal topology. Then, the highest likelihood parameter values were re-estimated on this new topology. A new heuristic tree search was run under these new parameters. The loop procedure was performed until stabilization of both topology and parameters. The robustness of nodes was estimated by ML bootstrap percentages after 100 replicates using previously estimated parameters, NJ starting tree and TBR branch swapping.

To account for differences in DNA substitution patterns among genes, we applied a Bayesian model-partitioned strategy for the analyses of the three supermatrices. Bayesian analyses used MRBAYES 3.1.2 (Ronquist & Huelsenbeck 2003), which allows different models for each gene partition. Best-fit models for the Bayesian analyses were identified using MODELTEST, and those with the highest Akaike Information Criterion were implemented. All parameters except topology were unlinked across partitions, and two independent runs with 4 Markov chain Monte Carlo (MCMC) were computed simultaneously, each with one cold and three heated chains. The MrBayes analyses were run for 10×10^6 generations with sampling every 100 generations. In all cases, stability of log likelihood and model parameters had been reached by the end of the analysis. Majority rule consensus was constructed after a burn-in of 5×10^5 generations. Clade support was calculated by posterior probabilities.

To account for the potential differences in DNA substitution patterns among sites whatever gene boundaries, Bayesian analyses were performed under the CAT + Γ_4 mixture model (Lartillot & Philippe 2004) using Phyloba-yes 3 (Lartillot *et al.* 2009). For each supermatrix, two chains were run for 100 000 cycles and trees were sampled every 100 cycles after the first 25 000 cycles. Convergence was achieved when the maximum difference of each bipartition frequency between the two chains was below 0.1.

Testing alternative topologies

The topologies found in the various analyses as well as a number of alternative phylogenetic hypotheses taken from previous works were tested under the nuclear + mitochondrial supermatrix using the approximately unbiased test (AU – Shimodaira 2002) as implemented in CONSEL (Shimodaira & Hasegawa 2001). PAUP* version 4.0b10 (Swofford 2002) was used to calculate the site likelihoods for each of the test topologies with the gene partitioning scheme assumed and the appropriate model for each partition specified using the output from MODELTEST. The CONSEL analyses employed 10 batches of 10^6 bootstrap replicates.

Molecular dating

Divergence times were estimated from the mitochondrial + nuclear supermatrix to provide a temporal framework of echimyid radiation. A Bayesian relaxed molecular clock method was used to estimate divergence dates while accounting for changes in evolutionary rate over time and allowing for independent models of sequence evolution for each gene partition. We used BEAST v1.6.2 (Drummond *et al.* 2002; Drummond & Rambaut 2007) to estimate the divergence dates within South American spiny rats while accounting for phylogeny uncertainty. We assumed a Yule speciation process for the tree prior, an uncorrelated log-normal distribution for the molecular clock relaxation model (Ho *et al.* 2007), and used default prior distributions for all other parameters. Clock models were unlinked across gene partitions to take into account missing data (Lemmon *et al.* 2009). We ran MCMC chains for 200 million generations with a 20% burn-in period, and parameters sampled every 1000 generations. We applied the best fitting model as estimated by MODELTEST to each of the gene partitions. We performed the analyses four times to check for the convergence of model parameter estimates, and the program TRACER (Rambaut & Drummond 2007) was used to assess convergence diagnostics. After removing 15% of each run as burn-in, trees from the four independent runs were combined into a maximum clade credibility tree with mean height for each node calculated using TreeAnnotator.

To calibrate the molecular clock, we selected four fossil constraints already considered in previous studies on rodents (Galewski *et al.* 2005; Opazo 2005; Huchon *et al.* 2007; Upham & Patterson 2012). To take into account uncertainties in the phylogenetic position of hystricognath fossils (Vucetich *et al.* 1999; Carvalho & Salles 2004; Marivaux *et al.* 2004; Sallam *et al.* 2009; Antoine *et al.* 2012), all constraints were set as minimum hard bounds with a soft upper bound using a lognormal prior, as suggested by recent palaeontological studies (Donoghue & Benton 2007; Benton *et al.* 2009; Parham *et al.* 2012). Firstly, the most recent common ancestor (MRCA) among Caviomorpha (Node A) was assumed to be no younger than the oldest stem Caviomorpha (*Canaanimys*, Middle Eocene [latest Lutetian: 48.6–40.4 million years ago (MYA)]; Antoine *et al.* 2012) and crown Caviomorpha (*Cachiyacuy*; Antoine *et al.* 2012), with the prior adjusted for a median of 44 MYA (upper 95%: 41.3–56.7 MYA). Due to uncertainty of the sister taxa of the fossils used to calibrate this node, we chose either to use or not use the 'IncludeStem' option. When the 'IncludeStem' is used, the parent node of the MRCA is also considered within the constraints (the node of the stem leading to that clade). Secondly, we incorporated the stem echimyid fossils from the Deseadan of SALMA (late Oligocene: 29–24.5 MYA; Vucetich *et al.* 1999; Flynn *et al.* 2003) to constrain node I (MRCA of Ctenomyidae, Octodontidae and Echimyidae + Myocastoridae + Capromyidae; upper 95%: 24.5–37.4 MYA). Thirdly, the Echimyidae + Myocastoridae + Capromyidae MRCA was constrained using the *Paradelphomys* fossil (Carvalho & Salles 2004) from the early Miocene (21–17.5 MYA). We set a median prior at 20 MYA (upper 95%: 17.5–30.4 MYA). Finally, for the Octodontidae + Ctenomyidae clade (Node J), we used the late Miocene (10–9 MYA from the Chasicoan of SALMA) crown ctenomyid *Xenodontomys* (Verzi *et al.* 2010) and a soft maximum prior extended to the Deseadan (upper 95%: 9.0–24.5 MYA).

Subsequently, we performed analyses using (i) all the fossil constraints (with or without the 'IncludeStem' option for the Caviomorpha constraint) and (ii) a leave-one-out approach to test for the compatibility among the different constraints. The leave-one-out approach uses three (all but one) fossil constraints. For the 'excluded constraint' and the 'all constraints' approaches, the molecular and fossil estimated divergence dates were compared. This was carried out for each of the four constraints.

Ecomorphological ancestral character estimation
We coded the ecomorphology of Echimyidae as follows: arboreal (*Lonchothrix*, *Mesomys*, *Isothrix*, *Kannabateomys*, *Dactylomys*, *Makalata*, *Echimys*, *Phyllomys*), fossorial (*Clyomys*,

Euryzygomatomys), scansorial (*Capromys*), semiaquatic (*Myocastor*) and terrestrial (*Trinomys*, *Proechimys*, *Hoplomys*, *Thrichomys*) (Eisenberg & Redford 1989, 1992, 1999; Emmons & Feer 1997; Nowak & Wilson 1999; Redford *et al.* 2000). Analyses allowing free rates of exchange between the four life mode types were conducted under the BayesMultiState model (Pagel *et al.* 2004). Ancestral ecomorphologies were estimated for the Echimyidae topology issued from the Bayesian analysis. The Multistate model (Pagel *et al.* 2004) was computed using the BAYESTRAITS software. This model estimates the instantaneous forward and backward rates among two states to compute probabilities of a state change along a given branch. BayesMultiState was selected as model of evolution and MCMC as the method of analysis. The rate deviation was set to 10, and a hyperprior approach was used with an exponential prior with a seed issued from a uniform prior (interval 0–10). The acceptance rates ranged between 20% and 40%. Two MCMC chains were run for 10 000 000 generations and were sampled every 10 000 generations for rate parameters.

Morphological ancestral character estimation
The characterization of plesiomorphies and apomorphies in the arboreal and terrestrial lineages and clades was conducted with the help of the morphological matrices from Carvalho & Salles (2004) and Emmons (2005). Using a *Capromys pilorides* skull from 'Institut des Sciences de l'Evolution de Montpellier' collections (UM/524N), the plates and descriptions of Woods & Howland (1979) and Chapman (1901), we coded 81 cranio-dental characters within both matrices [matrix data are available as Data S1] following Carvalho & Salles (2004) and Emmons (2005) character descriptions. Keeping only binary state characters, we performed an ancestral character state reconstruction using the highest posterior probability Bayesian topology inferred from the mitochondrial + nuclear dataset. This ancestral reconstruction approach was used to count the number of symplesiomorphies and synapomorphies within the different ecomorphological lineages (genera) and clades (nodes L, M, V, W) of Echimyidae recognized by our molecular phylogenetic inferences (see Discussion). Ancestral character states were computed to test if arboreal (see nodes S, T, V: Fig. 1) and terrestrial-like lineages (nodes L, W) are more characterized by plesiomorphic or apomorphic character states as compared with the echimyid root (node K). For each node and terminal taxon, we computed its degree of apomorphy as the percentage of apomorphies relative to the total number of plesiomorphies and apomorphies. We included in this analysis only genera for which both morphological and molecular characters were available. The outgroup was

Fig. 1 Maximum likelihood (ML) phylogram issued from the 8-gene mitochondrial-nuclear supermatrix analysis. Clades are labelled with acronyms: ARB = arboreal, HEB = hutias + East Brazilian echimyids, TER = terrestrial. The ecology of the Echimyidae is also indicated. Nodes are labelled with a letter. Bootstrap percentages (top), posterior probabilities computed by MrBayes (PP1: middle) and posterior probabilities computed by Phylobayes (PP2: down) are indicated near the nodes (see Table 1 for support values). A star indicates the highest support.

pruned from the molecular topology. The Multistate model (Pagel *et al.* 2004) was also computed in Bayes-Traits software. We followed the methods of Aigoin *et al.* (2009) to perform the ancestral character state reconstruction analysis. To contrast the morphological hypothesis among arboreal and terrestrial groups, we used a local approach (Pagel 1999) and compared state reconstruction at each local node of interest (node K = Echimyidae root,

node W = clade TER, node M = East Brazilian Forest Eumysopinae, node S = Dactylomyinae, node T = *Echimys* + *Phyllomys* + *Makalata* clade). In this way, we constrained each of the MRCA at one of the two potential states of the character under focus. An MCMC was used to explore the sample of 10 000 trees generated by the MrBayes analysis and the space of rate parameter values. Because we have no information about the rates of

changes among characters states, priors on uniform distribution were set between 0 and 100. The ratedev parameters (rate of parameter changes) were set at the start of each run. By this way, the acceptance rate of the proposed transition ranges between 20% and 40%. The MCMC chain was run for 10 000 000 generations and was sampled every 10 000 generations for rate parameters. Two chains were run independently to sample rate parameters and to derive overall likelihoods of the reconstructions when the node of interest was constrained at its alternative state. Bayes factors were then calculated to determine the support for alternative state at each node of interest.

Results

Molecular phylogenetics

The different probabilistic analyses of the combined dataset with single or multiple partitions yield similar phylogenetic results. Topology, node supports and congruence with each single-gene tree are displayed in Fig. 1 and Table 1. Hystricognathi (Fig. 1) splits into Hystricidae (Old World porcupines) and two well-supported clades: Bathyergidae [ML bootstrap (BP=92), MrBayes posterior probability (PP1 = 1), Phylobayes posterior probability (PP2 = 0.99)] and Caviomorpha (node A; BP=100, PP1 = 1, PP2 = 0.99). Phiomorpha (Old World hystricognaths) are paraphyletic with Bathyergidae being more closely related to Caviomorpha (South American hystricognaths) than to Hystricidae. Within Caviomorpha, we recovered a dichotomy between Erethizontidae + Cavioidea (node B; BP=90, PP1 = 1, PP2 = 0.74) and Chinchillidae + Octodontidae (node F; BP=100, PP1 = 1, PP2 = 1). Echimyidae is nested within the octodontoid clade (node H; BP=100, PP1 = 1, PP2 = 1), and our dataset suggests a sister clade relationship between *Octodon* + *Ctenomys* and the Echimyidae family (node I; BP=84, PP1 = 1, PP2 = 0.76). Monophyly of Echimyidae is supported by the supermatrix (node K; BP=100, PP1 = 1, PP2 = 1) and all single-gene analyses. Within Echimyidae, three monophyletic groups are evidenced: (i) a clade comprising the semiaquatic genus *Myocastor* and the three terrestrial echimyid genera *Thrichomys*, *Proechimys* and *Hoplomys* (node W; BP=100, PP1 = 1, PP2 = 1), (ii) an arboreal echimyid clade (node P; BP=100; PP1 = 1, PP2 = 1) comprising *Dactylomys*, *Kannabateomys*, *Isothrix*, *Echimys*, *Makalata*, *Phyllomys*, *Mesomys* and *Lonchothrix* and (iii) Capromyidae (hutias) and the three East Brazilian echimyid genera *Trinomys*, *Clyomys* and *Euryzygomatomys* (node L; BP=71, PP1 = 0.99, PP2 = 0.79). Moreover, we obtained strong support for a sister clade relationship between the clade TER (Node W) and the arboreal clades (node O; BP=100, PP1 = 1, PP2 = 1). Our data suggest the inclusion of *Capromys* within Echimyidae with moder-

ate support values (node L). Topological tests significantly reject a relationship between *Capromys* and clades TER + ARB (Table S3, $P_{AU} = 0.01$), but do not reject the *Capromys* + Echimyidae ($P_{AU} = 0.06$) and *Capromys* + (*Clyomys*, *Euryzygomatomys*) hypotheses.

Within the hutias + East Brazilian echimyid clade (HEB), the monophyly of the semifossorial genera (*Clyomys*, *Euryzygomatomys*) is well supported (node N; BP=100, PP1 = 1, PP2 = 1). A sister clade relationship between *Trinomys* and the semifossorial genera is also suggested (node M; BP=44, PP1 = 1, PP2 = 0.99). However, topological tests do not reject the alternative relationships of *Trinomys* with clades ARB + TER, of *Capromys* with *Trinomys* and the exclusion of *Trinomys* from clade HEB (Table S3).

Among the arboreal clade (clade ARB; node P), three strongly supported subclades emerged from our analysis: (i) a bamboo spiny rats clade grouping together *Dactylomys* + *Kannabateomys* (node S; BP = 100, PP1 = 100, PP2 = 100), (ii) a *Makalata* + *Phyllomys* + *Echimys* clade (node T; BP = 100, PP1 = 100, PP2 = 100) and (iii) a *Mesomys* + *Lonchothrix* clade (node V; BP = 100, PP1 = 100, PP2 = 100). Moderate node support values suggested a relationship between Dactylomyinae and the *Makalata* + *Phyllomys* + *Echimys* subclade (node R; BP = 73, PP1 = 0.99, PP2 = 0.96). Nevertheless, relationships among these three clades relative to *Isothrix* remain unclear (node Q; BP = 45, PP1 = 0.64, PP2 = 0.60). Moreover, topological tests (Table S3) do not reject the alternative hypotheses of Echimyinae monophyly (*Echimys*, *Isothrix*, *Makalata*, *Phyllomys*; $P_{AU} = 0.14$), of monophyly of (*Makalata*, *Phyllomys*, *Echimys*) + (*Mesomys*, *Lonchothrix*) ($P_{AU} = 0.10$), of a sister clade relationship between (*Mesomys*, *Lonchothrix*) and *Isothrix* ($P_{AU} = 0.18$), and of the grouping of Dactylomyinae + *Isothrix* ($P_{AU} = 0.18$). Only two topological hypotheses are rejected: (i) a grouping of (*Isothrix*, Dactylomyinae) + (*Lonchothrix*, *Mesomys*) ($P_{AU} = 0.005$) and (ii) a (*Isothrix*, (*Lonchothrix*, *Mesomys*)) + Dactylomyinae relationship ($P_{AU} = 0.01$). A (*Phyllomys* + *Echimys*) clade is supported by moderate support values (node U; BP=67, PP1 = 0.98, PP2 = 0.94). Topological tests do not reject the (*Phyllomys* + *Makalata*) and (*Makalata* + *Echimys*) alternatives (Table S3).

Within the *Myocastor* clade (clade TER; node W), a *Proechimys* + *Hoplomys* relationship is highly supported (node Y; BP=100, PP1 = 1, PP2 = 1). However, the relationship between (*Proechimys* + *Hoplomys*), *Thrichomys* and *Myocastor* remains poorly supported although all analyses yield a (*Myocastor* + (*Proechimys*, *Hoplomys*)) grouping (node X; BP = 48, PP2 = 0.52). The gene-partitioned MrBayes analysis yields to a polytomy between *Thrichomys*, *Myocastor* + (*Proechimys*, *Hoplomys*). Topological tests do not reject the *Thrichomys* + (*Proechimys*, *Hoplomys*) and *Myocastor* + (*Proechimys*, *Hoplomys*) alternatives (Table S3).

Gene congruence and nuclear/mitochondrial corroboration

Node support, congruence among genes and multigene analyses are displayed in Table 1 and Table S4. The Table S5 shows the distribution of bootstrap values among nuclear, mitochondrial and combined datasets. Mitochondrial datasets provided lower bootstrap support than the nuclear genes and the combined dataset. We distinguished two categories of nodes following their among-gene congruence and statistical support. Firstly, we found nine nodes (K, N, O, P, S, T, V, W and Y) for which (i) more than half of the single-gene trees provided identical topologies, and (ii) single-gene support was high. All single-gene analyses suggested the monophylies of Echimyidae (including Capromyidae), *Euryzygomatomys + Clyomys*,

arboreal Echimyidae, *Makalata + Phyllomys + Echimys*, *Mesomys + Lonchothrix* and terrestrial taxa + *Myocastor*. Secondly, six nodes displayed moderate to low support: three nodes (L, R and U) have moderate bootstrap values (65 < BP < 75) and three nodes (M, Q and X) have low bootstrap values (BP < 50). Even with the five nuclear genes dataset, nodes L, Q, R and X are characterized by low support values and among-gene topological differences. About nodes U and Q, discrepancies between nuclear and mitochondrial genes lead to different branching patterns and lower BP values for the combined datasets. For the *Phyllomys + Echimys* clade (node U), nuclear DNA data provide higher support (BP = 84) compared with the combined mitochondrial + nuclear data (BP = 67). By contrast, the mitochondrial DNA alone poorly supports a *Makalata + Phyllomys* clade (BP = 52 and PP = 0.67).

Molecular dating

Based on the relaxed molecular clock Bayesian analysis, a time scale for the evolution of the Hystricognathi here sampled is depicted in Table 2. The initial radiation of Echimyidae (node K), subtending the split of the three main echimyid lineages (clade TER and clade ARB + HEB), was estimated to have occurred 20.5–18.8 MYA [95% credibility interval (CI) 17.5–23.1] depending on the fossil constraint settings. The split between arboreal Echimyidae (clade ARB) and *Myocastor* (clade TER) was dated at 17.1–19.0 MYA (node O, 16.9–22.7). The Caribbean capromyines were estimated to have split from East Brazilian sister clade (node L) 17.8–19.8 MYA (CI 16.3–22.1). The primary diversification of the arboreal echimyids (clade ARB) was estimated to have occurred from 15.3 to 17.1 MYA (node P, CI 13.8–19.2) to 14.8 to 16.5 MYA (node Q, CI 13.4–19.0) and 13.9 to 15.5 MYA (node R, CI 12.4–18.5). Secondary diversifications among Dactylomyinae and among the *Echimys + Phyllomys + Makalata* clade were estimated to have begun, respectively, between 9.2–10.2 MYA (node S, CI 7.0–12.6) and 9.8–11.0 MYA (node T, CI 8.4–12.8). The initial divergence between *Thrichomys*, *Myocastor* and (*Proechimys*, *Hoplomys*) was estimated at 15.1–19.8 MYA (node W, 13.6–22.1).

We also used the upper and the lower bounds around each fossil calibration, and the 95% credibility interval of the Bayesian dating estimates to distribute the calibrations into four categories of compatibility: (i) the molecular interval is included in the palaeontological one; (ii) the palaeontological interval is included in the molecular one; (iii) the two intervals overlap; and (iv) the two intervals are distinct. Three fossil calibrations used within our analyses belong to categories (i) or (iii) (Table 2), indicating that these calibrations are in full or partial agreement with

Table 1 Phylogenetic support values issued from the mitochondrial-nuclear supermatrix analyses under maximum likelihood and Bayesian approaches

Nodes	BP	PP1	PP2	Congruence	Genes
A	*	*	0.99	6 vs. 0	VRGIAM
B	91	*	0.74	3 vs. 3	VRgiaM
C	*	*	*	6 vs. 0	VRGIAM
D	87	0.99	0.95	3 vs. 1	VRG–m
E	*	*	*	5 vs. 0	V-GIAM
F	99	*	*	5 vs. 1	VRgIAM
G	*	*	*	4 vs. 0	V-GI-M
H	*	*	*	4 vs. 1	V-GIaM
I	84	*	0.76	3 vs. 2	v-GIaM
J	97	*	0.99	4 vs. 1	v-GIAM
K	*	*	*	6 vs. 0	VRGIAM
L	72	0.98	0.79	3 vs. 2	v-GiAM
M	74	*	0.99	3 vs. 2	V-GiAm
N	*	*	*	4 vs. 0	VRGIAM
O	*	*	*	5 vs. 1	V-GI-M
P	*	*	*	5 vs. 1	VRgIAM
Q	48	0.64	0.6	2 vs. 4	vrgiAM
R	65	0.83	0.96	1 vs. 5	vrgiAm
S	*	*	*	2 vs. 0	V–M
T	*	*	*	2 vs. 0	VRGIAM
U	74	0.98	0.94	1 vs. 4	V-giam
V	*	*	*	3 vs. 0	–GI-M
W	*	*	*	4 vs. 1	VRGi-M
X	53	-	0.52	3 vs. 3	vRgiAM
Y	*	*	*	2 vs. 0	–G–M

Nodes are labelled with letters (see Fig. 1).

BP, Bootstrap proportion; PP1, posterior probabilities computed by MrBayes; PP2, posterior probabilities computed by Phylobayes.

*BP = 100% or PP = 1. The column 'congruence' indicates the number of gene trees in agreement *versus* in disagreement with the 8-gene supermatrix tree. Gene names: V = von Willebrand Factor exon 28 (*vWF*), R = Recombination activating protein 1 gene (*RAG1*), G = Growth hormone receptor exon 10 (*GHR*), I = Interphotoreceptor retinoid-binding protein exon 1 (*RBP3*), A = apolipoprotein B exon 26 (*APOB*) and M = mitochondrial concatenate (cytochrome *b* + 12S and 16S rRNAs). The upper case letter indicates congruence with the ML mito-nuclear topology while the lower case indicates a different topology. A dash indicates that the gene is absent for the taxa under focus.

Table 2 Cross-validation of the fossil constraints through molecular dating issued from the concatenated mitochondrial + nuclear dataset

Nodes	ALL (IncludeStem) Age	ALL Age	Without CA 41.3–56.7 MYA Age	Without OC_CT_EC 24.5–37.4 MYA Age	Without OC_CT 9.0–24.5 MYA Age	Without EC 17.5–30.4 MYA Age
A	**37.4 [34.8–40.3]**	**42.1 [40.8–44.1]**	37.4 [34.8–40.2]	42.5 [40.8–45.2]	42.2 [40.8–44.5]	42.4 [40.8–44.8]
I	**25.1 [24.1–26.5]**	**27.1 [24.8–29.4]**	25.1 [24.1–26.5]	**27.7 [25.1–30.6]**	27.4 [25.0–29.8]	27.5 [24.9–30.1]
J	**20.1 [18.7–23.2]**	**22.6 [19.7–25.4]**	21.0 [18.5–23.2]	23.1 [19.9–26.1]	**23.0 [20.2–25.9]**	22.9 [19.9–25.9]
K	**18.8 [17.5–20.2]**	**20.5 [18.6–22.5]**	18.9 [17.6–20.3]	20.9 [18.4–23.1]	20.7 [18.8–22.7]	**20.9 [18.9–23.1]**
L	17.8 [16.3–19.4]	19.4 [17.5–21.4]	17.9 [16.5–19.5]	19.8 [17.7–22.1]	19.6 [17.5–21.6]	19.8 [17.6–22.0]
M	16.1 [14.4–17.8]	17.6 [15.5–19.7]	16.2 [14.6–17.8]	18 [15.8–20.3]	17.7 [15.6–19.8]	17.9 [15.7–20.2]
N	6.6 [5.0–8.3]	7.3 [5.5–9.1]	6.6 [5.1–8.2]	7.4 [5.6–9.4]	7.3 [5.6–9.2]	7.4 [5.6–9.2]
O	17.1 [15.7–18.5]	18.6 [16.8–20.4]	17.2 [15.8–18.7]	19.0 [17.1–21.2]	18.8 [16.9–22.7]	19.0 [17.1–21.0]
P	15.3 [13.8–16.7]	16.7 [14.9–16.5]	15.4 [13.9–16.4]	17.1 [15.1–17.4]	16.8 [15.1–18.7]	17.0 [15.1–19.2]
Q	14.8 [13.4–16.3]	16.2 [14.5–18.0]	14.9 [13.5–16.4]	16.5 [14.6–19.0]	16.3 [14.6–18.1]	16.5 [14.6–18.5]
R	13.9 [12.4–15.4]	15.1 [13.4–16.9]	13.9 [12.5–15.5]	15.5 [13.6–18.5]	15.3 [13.5–17.1]	15.5 [13.5–17.4]
S	9.2 [7.2–11.5]	10.1 [7.8–12.3]	9.2 [7.0–11.4]	10.2 [7.9–12.6]	10.1 [7.8–12.4]	10.2 [7.9–12.6]
T	9.8 [8.4–11.4]	10.8 [9.13–12.4]	9.9 [8.4–11.4]	11.0 [9.3–12.8]	10.8 [9.2–12.5]	11.0 [9.2–12.7]
U	9.6 [7.1–10.0]	9.4 [7.8–11.0]	8.7 [7.2–10.2]	9.6 [7.9–11.3]	9.4 [7.9–11.1]	9.6 [7.9–11.3]
V	8.8 [6.7–11.9]	9.6 [7.3–11.9]	8.9 [6.8–11.1]	9.9 [7.5–12.2]	9.7 [7.6–11.9]	9.8 [7.5–12.3]
W	15.1 [13.6–16.6]	16.5 [14.7–18.3]	15.2 [13.7–16.8]	19.8 [17.7–22.1]	16.6 [14.8–18.5]	16.8 [14.9–18.9]
X	14.4 [12.9–16.1]	15.7 [13.9–17.6]	14.5 [12.8–16.1]	17.6 [15.8–20.3]	15.9 [14.0–17.7]	16.1 [14.1–18.2]
Y	8.5 [6.6–10.5]	9.3 [7.1–11.5]	8.7 [6.7–10.6]	7.5 [5.6–9.4]	9.4 [7.2–11.6]	9.5 [7.2–11.8]

Letters referred to the nodes in Fig. 1. The mean age of each node is given in million years ago (MYA), together with the lower and upper bounds of the 95% credibility intervals issued from the Bayesian relaxed molecular clock analysis. The fossil constraints are as follows: ALL = all 4 calibrations (CA, OC_CT_EC, OC_CT, EC); CA = Most recent common ancestor (MRCA) among Caviomorpha (node A); OC_CT_EC = MRCA of Ctenomyidae, Octodontidae and Echimyidae + Myocastoridae + Capromyidae (node I); OC_CT = MRCA Octodontidae + Ctenomyidae clade (node J); EC = MRCA Echimyidae + Myocastoridae + Capromyidae (node K). Values pertaining to the cross-validation are in bold.

each other. The Ctenomyidae/Echimyidae/Octodontidae (Node I) and Echimyidae (Node K) divergences belong to category (i), with credibility intervals for molecular date estimates contained within those of fossil dates (see Table 2). This result is reassuring with respect to the ability of Bayesian methods to handle rate heterogeneity and multiple fossil constraints in the Octodontidae lineages. One divergence belongs to category (iii), with the palaeontological dating interval overlapping with the molecular one: the Octodontidae/Ctenomyidae (Node J; 20.2–25.9 MYA for the molecules, and 9.0–24.5 MYA for the fossils). The aforementioned fossil constraints can be considered as cross-validated: 75% of the molecular credibility intervals were contained within the palaeontological one. The Caviomorpha split belongs to category IV, because the palaeontological interval does not overlap with the molecular one (34.8–40.2 MYA for the molecules versus 41.3–56.7 MYA for the fossils). This result suggests a palaeontological versus molecular discrepancy, even if the estimated divergence dates nearly overlap (<1.1 MYA of difference). This observation would indicate that the placement of the caviomorphs found within the Yahuarango formation (Middle Eocene, Peru; Antoine *et al.* 2012) is uncertain and justifies the use of the 'IncludeStem' option for the corresponding calibration. When the caviomorph fossil constraint is excluded, more congruent leave-one-out results are obtained (Table 2). Therefore, the divergence

of extant caviomorphs seems to be more recent than the palaeontological bounds defined by Antoine *et al.* (2012). This suggests that the corresponding fossils might be linked to extinct stem lineages without direct relation to crown caviomorphs. Alternatively, there is the possibility that extensive substitution rate variations have occurred among the eight nuclear and mitochondrial markers along the caviomorph branch, leading to difficulties in the dating estimation under the relaxed molecular clock model.

Ecological ancestral character state estimations

Ancestral ecomorphologies were estimated for the five nodes defining higher-taxa clades (nodes K, L, O, P and W) of the maximum posterior probability Bayesian topology (Fig. S1, pie charts). For the root of Echimyidae (node K), the analysis suggests a terrestrial origin (probability of this ecomorphological character state $P = 0.57$), followed by less likely hypotheses (arboreal $P = 0.20$, scansorial $P = 0.10$ or semifossorial $P = 0.09$). For the clade HEB (node L), we inferred a terrestrial ($P = 0.55$), semifossorial ($P = 0.20$) or scansorial ($P = 0.18$) origin. The ancestral life mode ranges of the clade ARB + TER (node O) are reconstructed as either terrestrial ($P = 0.56$) or arboreal ($P = 0.38$). On the next-most distal bifurcations (node P), the ancestral habitat types are reconstructed as arboreal with high probability ($P > 0.95$). The ancestral habitat ranges of the clade TER (node W) suggest terrestrial
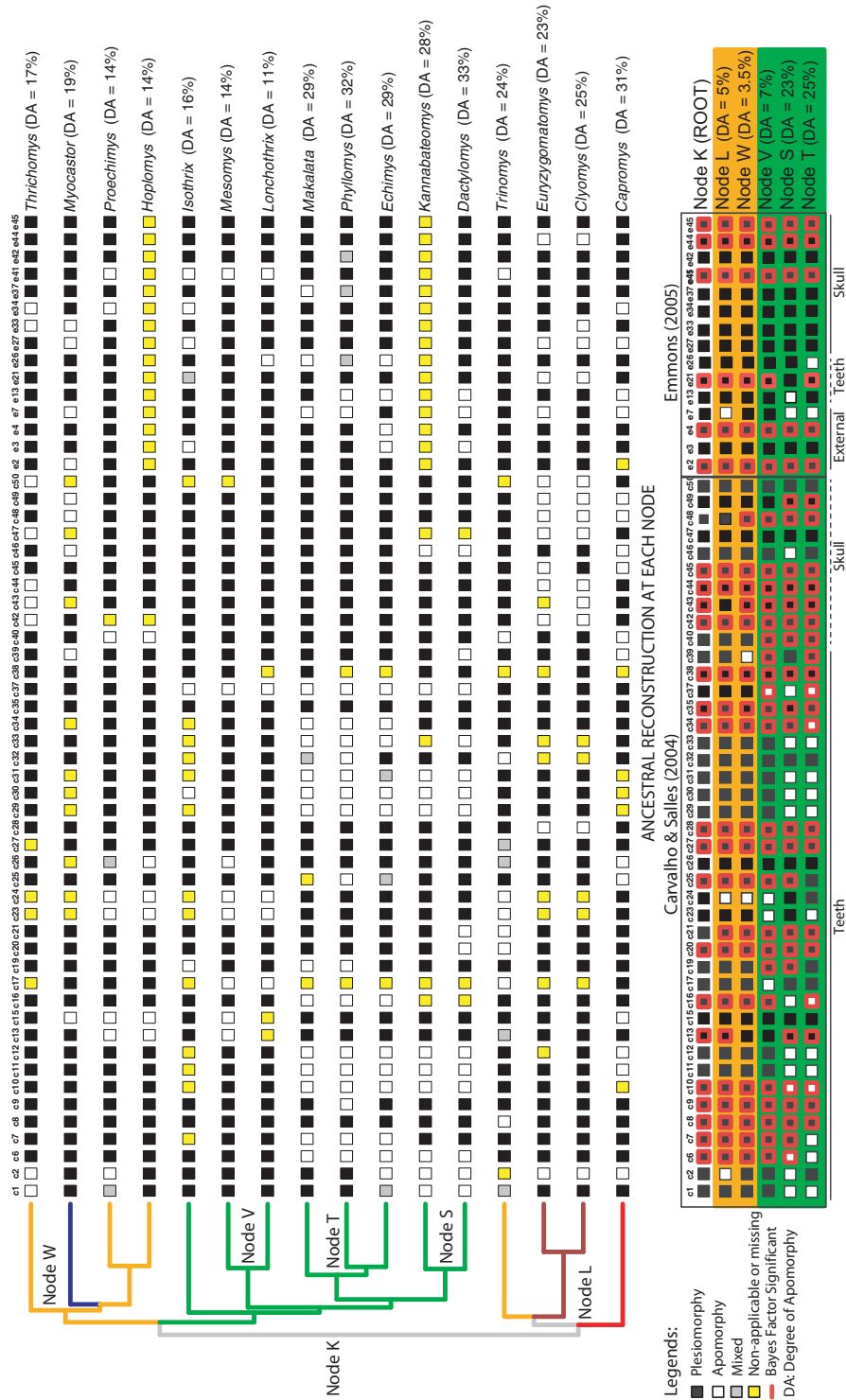
Fig. 2 Chronogram and morphological ancestral character state estimation for 16 Echimyidae taxa. Ancestral state reconstruction at the Echimyidae root (node K) and 5 internal nodes (L, S, T, V and W) are also represented in the lower part. Binary cranio-dental characters are labelled, respectively, with 'c' for Carvalho & Salles (2004) and 'e' for Emmons (2005). Apomorphies were labelled with white squares for each character along lineages and nodes of interest (L, S, T, V, W), whereas black squares indicated plesiomorphic character states. Degrees of apomorphy (DA) are indicated for each lineage and node of interest. Bayes factors (BF) measure twice the difference between the log of the harmonic means (HM) inferred by the model when the MRCA is fixed at its two possible states. The state returning the highest marginal log likelihood is (i) indicated with regular outline if BF is not significant or (ii) in red bold outline if BF is significant

ecotypes ($P = 0.60$) or the less likely arboreal ($P = 0.24$) and semiaquatic ones ($P = 0.20$).

Morphological ancestral character state estimations

The results of the ancestral morphological character state reconstruction at each node of interest of the Echimyidae phylogeny are summarized in the Table S6 and Fig. 2. For each node of interest (nodes L, S, T, V and W), we have constrained the character state to be identical to the root of Echimyidae (node K). Using our phylogeny, we identified two terrestrial clades (nodes L and W) and three arboreal ones (Node S, T and V). For the MRCA of the following clades, the marginal log likelihood of the ancestral reconstructions was statistically significant for a various number of characters (Fig. 2: red squares): 24 characters for Echimyidae (node K), 24 for the East Brazilian clade (node L), 25 for the terrestrial clade (node W), 29 for *Lonchothrix* + *Mesomys* (node V), 27 for Dactylomyinae (node S) and 27 for Echimyinae (node T). A comparison of taxa with different ecomorphologies (Fig. 2 and Table S6) shows that arboreal lineages display more apomorphic characters [see degree of apomorphy (DA) for nodes V = 7%, S = 23% and T = 25%] compared with terrestrial ones (DA for nodes L = 5% and W = 3.5%). The Eumysopinae taxa also show more plesiomorphies than the Echimyinae ones (see Fig. 2). The node P (subtending the ARB clade) displays a single apomorphy (character Carvalho & Salles (2004) [c] 37: *unilateral hypsodonty in upper molariform teeth = [0] absent*). The node R (subtending nodes T [*Makalata* + *Phyllomys* + *Echimys*] + S [*Kannabateomys* + *Dactylomys*]) shows five potential apomorphies (characters Carvalho & Salles (2004) c6: *lower dP4 sulcus between the protoconid region and the anterolophid in little-worn teeth = [1] deep*; c10: *lower dP4 lingual opening of the anteroflexid in little-worn teeth = [1] deep*; c29: *upper dP4-M3 labial opening of paraflexus in little-worn teeth = [1] deep*; c31: *upper dP4-M3 labial opening of metaflexus in little-worn teeth = [1] deep*; c33: *upper dP4/M3 sulcus between the lingual end of the protoloph and the posterior portion of the tooth in little-worn teeth = [1] deep*). The clade *Makalata* + *Phyllomys* + *Echimys* (node T) displayed two apomorphies (c16: *lower M1–3 ectolophid = [1] poorly developed and not posteriorly orientated*; c34: *upper dP4-M3 sulcus between the hypocone region and the posteroloph in little-worn teeth = [1] deep*).

Discussion

Molecular systematics and taxonomy of Echimyidae

Our multigene analysis suggested a close affinity between Octodontidae and Chinchillidae in agreement with previous supermatrix analyses (Huchon *et al.* 2007; Blanga-Kanfi *et al.* 2009; Sallam *et al.* 2009; Meredith *et al.* 2011;

Upham & Patterson 2012) and retroposon studies (Churakov *et al.* 2010). Within Octodontoidea, we found a first split between Abrocomidae and the other families. The closest relatives of Echimyidae are the Octodontidae and Ctenomyidae as previously evidenced in multigene analyses (Honeycutt *et al.* 2003; Huchon *et al.* 2007; Blanga-Kanfi *et al.* 2009; Meredith *et al.* 2011; Upham & Patterson 2012). The close affinity of Echimyidae with Capromyidae on the one hand and Myocastoridae on the other hand is proposed by our inferences (node K, Fig. 1), in agreement with Galewski *et al.* (2005). Inclusion of Capromyidae within Echimyidae is moderately supported by our multigene analyses with five congruent nuclear genes and moderate to strong bootstrap and posterior probability supports. In addition, *GHR*, *APOB* and the mitochondrial supermatrix suggest the placement of *Capromys* as sister lineage to the *Trinomys* + *Clyomys* + *Euryzygomatomys* clade. With more echimyid taxa but fewer genes, Upham & Patterson (2012) found a different set of relationships, as their supermatrix analysis recovered a Capromyidae + Echimyidae (including *Myocastor*) clade (BP = 43, PP = 0.4). These discrepancies highlight the difficulties of resolving this part of the Octodontidae/ Echimyidae phylogeny. Sampling of other capromyids (e.g. *Geocapromys*, *Mysateles*, *Plagiodontia*) in a phylogenetic framework is required to provide additional information about the affinities of the West Indian hutias.

Our multigene data strongly support the inclusion of *Myocastor* within Echimyidae. Five markers (*APOB*, *RBP3*, *RAG1*, *vWF* and the mitochondrial supermatrix) include *Myocastor* within the ARB + TER clade with high support (Fig. 1). In addition, four markers (*APOB*, *GHR*, *RAG1* and mitochondrial supermatrix) include the nutria within the terrestrial clade (*Thrichomys*, *Hoplomys*, *Proechimys*). Our results are therefore compatible with the monophyly of the Echimyidae (sensu Woods *et al.* 1992; Leite & Patton 2002; Galewski *et al.* 2005; Upham & Patterson 2012), giving further support to the classification of McKenna & Bell (1997), which includes Myocastorinae and Capromyidae within Echimyidae. This affiliation is also supported by the retention of the deciduous premolar character state within these three families (Patterson & Pascual 1968; Patterson & Wood 1982). Some studies on Capromyidae endoparasites have also concluded to their inclusion within echimyids (Price & Timm 1997). Moreover, Woods *et al.* (1992) described the subfamily Adelophomyinae, which contains echimyid-like relatives of the Myocastoridae from the Oligocene of South America. The wide spectrum of the echimyid adaptive radiation is emphasized by the Capromyidae and Myocastorinae highly derived characters, which reflect their peculiar ecology (respectively scansorial and semiaquatic).

Among Echimyidae, we evidenced three well-supported arboreal, terrestrial and arboreal + terrestrial clades. The monophyly of the East Brazilian group (clade HEB, node M) is corroborated by three nuclear genes (*vWF*, *GHR* and *APOB*). This result confirms that *Trinomys* is not closely related to *Proechimys* and *Hoplomys* (Lara & Patton 2000; Galewski *et al.* 2005; Monteiro *et al.* 2005) contrary to previous suggestions (Woods 1993; McKenna & Bell 1997; Carvalho & Salles 2004). The fossorial *Clyomys* and *Euryzygomatomys* genera are closely related as suggested by morphological analyses (Carvalho & Salles 2004; Emmons 2005). However, we cannot reject a closer relationship between *Capromys* and the fossorial clade or between *Capromys* and *Trinomys*. Within the terrestrial clade (clade TER), *Proechimys* and *Hoplomys* are closely related although the affinities of *Myocastor* and *Thrichomys* remain unresolved. The arboreal clade (clade ARB, node P) is corroborated by five markers (*APOB*, *RBP3*, *RAG1*, *vWF* and mitochondrial supermatrix). Within this clade, we found an isolated genus (*Isothrix*) and three highly supported subclades: (i) *Lonchothrix* + *Mesomys* (arboreal Eumysopinae, node V), (ii) the bamboo rats (*Dactylomys* and *Kannabateomys*, node S) and (iii) three other Echimyinae taxa (*Echimys*, *Makalata*, *Phyllomys*, node T). The relationships among these four arboreal lineages remain unresolved despite the concatenation of, respectively, five nuclear and three mitochondrial genes. However, sampling of the unsequenced genera *Olallamys*, *Callistomys* and *Diplomys* could potentially provide some clues about the evolutionary affinities among arboreal echimyids. The morphological works of Carvalho & Salles (2004) and Emmons (2005) suggested a close relationship of these genera with the Echimyinae and Dactylomyinae, confirming the monophyly of the arboreal clade. Regarding the branching pattern among *Echimys*, *Phyllomys* and *Makalata* (node T), the combined nuclear genes (Table S4) highly support a close relationship between *Echimys* and *Phyllomys*, but the concatenation of mitochondrial and nuclear markers drastically decreased the support values, possibly because of heterogeneity in the mitochondrial evolutionary rate or incomplete lineage sorting within these echimyid taxa.

The concatenation of five nuclear and three mitochondrial genes has provided new insights regarding echimyid systematics – e.g., the paraphyly of Eumysopinae and Echimyinae (Fig. S1) and improved support for phylogenetic hypotheses suggested by previous analyses (Galewski *et al.* 2005; Patterson & Velazco 2008; Upham & Patterson 2012). However, even if the combined use of slowly evolving nuclear genes and faster-evolving mitochondrial ones has challenged the star phylogeny hypothesis of relationships among Echimyidae (Lara *et al.* 1996; Leite & Patton 2002), it seems that the resolution of the evolutionary

affinities among the arboreal (nodes Q and R) and Caribbean + East Brazilian (node L) taxa will remain a difficult systematic topic till new data are available. Future directions to explore the trickiest parts of the echimyid phylogeny would be to (i) increase the taxon sampling with additional capromyines and echimyines to reduce the number of isolated, potentially long branches, (ii) increase the sampling of DNA characters with additional mitochondrial and nuclear markers and/or (iii) search for rare genomic changes (e.g. indels and retroposons).

Quantifying the degree of apomorphies versus plesiomorphies in comparative morphology

Our 8-marker phylogeny is a reasonably corroborated estimate of higher-level echimyid phylogeny available for a comparative morphology analysis. We inferred ancestral states of the MRCA of Echimyidae (node K) and its five main clades (nodes L, S, T, V and W) to test which of the discrete cranio-dental characters are either plesiomorphic or apomorphic. We identified few character state changes along the deepest branches of the Echimyidae radiation. Most of them occurred in the arboreal Echimyidae (nodes S and T) for which we identified two and four synapomorphies, respectively (see Results). Our analyses suggest that the discrepancies between phylogenies based on morphological and molecular characters are mainly due to plesiomorphies rather than to convergences (Results: Table S6 and Fig. 2) and to a lack of structure of the phylogenetic signal within morphological characters (see also Wenzel & Siddall 1999; Gaubert *et al.* 2005). This is illustrated by the recognition of Eumysopinae in morphological inferences. Eumysopinae appears to be an artificial assemblage of terrestrial taxa that have retained many plesiomorphies (Table S6 and Fig. 2; Lara & Patton 2000; Monteiro *et al.* 2005). This feature has previously led to the grouping of the three unrelated genera *Proechimys*, *Hoplomys* and *Trinomys* in the same taxonomic (McKenna & Bell 1997) or phylogenetic unit (Carvalho & Salles 2004). Eumysopinae was defined on the basis of similar narrow-fold molar patterns (Patton & Reig 1989), which is recovered in most of the Oligocene octodontoid taxa (Vucetich *et al.* 1997). This retention of plesiomorphic characters has also led to classification difficulties in the echimyid fossil record (Vucetich & Kramarz 2003; Carvalho & Salles 2004). Our ancestral reconstruction analysis mainly relies on cranio-dental and a few external/postcranial characters, which could have limited the number of apomorphies identified. Moreover, recent landmark analyses based on the skull and the mandible did not find a clear differentiation between arboreal and terrestrial taxa (Perez *et al.* 2009). In complement of using cranio-dental characters, postcranial characters and morphometric data (e.g. Monteiro &

Nogeira 2011) are required for a better understanding of the evolution of morphological disparity within Echimyidae.

We here demonstrated that Echimyidae have retained a large number of plesiomorphic teeth characters (35), skull characters (18) and some external structures (4). Despite their ecological versatility, species diversity and deep, early Miocene origin (see below), terrestrial echimyid taxa are characterized by a low morphological divergence of the skull. An ecological shift with little cranio-dental character variations is therefore one striking feature of the Echimyidae diversification. Conversely, members of the arboreal clade have evolved higher morphological disparity than their terrestrial counterparts as indicated by a larger number of genera and a higher amount of inferred apomorphies (Fig. 2 and Table S6). The lack of discrete character state differentiation in terrestrial genera of echimyids is an interesting feature to understand adaptive radiations within other diversified rodent lineages. Rodents exhibit numerous adaptive radiations (see Mercer & Roth 2003; Steppan *et al.* 2004; Lecompte *et al.* 2008; Rowe *et al.* 2008) and a vast range of morphologies, and they have colonized most terrestrial habitats on Earth. Nevertheless, many evolutionary radiations within Rodentia are associated to a narrow range of morphological variations either with or without clear ecomorphological divergences. Actually, several highly diversified terrestrial groups are characterized by plesiomorphic patterns (e.g. Rattini, Oryzomyini, Praomyini) and high speciation rates (Rowe *et al.* 2011). Rodents then represent a suitable model to identify those factors that have enhanced morphological diversity or ecological disparity during either adaptive or non-adaptive radiations.

Diversification of South American spiny rats through the Neogene

Molecular dating analyses on the 8-gene mitochondrial + nuclear dataset suggest an Early Miocene radiation of echimyids (Node K; Table S2). These results are similar to those of Upham & Patterson (2012) (18.8 MYA), younger than those of Galewski *et al.* (2005) based on relaxed molecular clock (22.4 MYA) and older than previous local and global molecular clock estimates (Huchon & Douzery 2001; Leite & Patton 2002). Differences among studies are linked to the different gene sampling, taxon sampling, fossil constraints and molecular dating methodologies.

Our phylogenetic analysis suggests an Amazonian rainforest + East Brazilian forest origin for Echimyidae. This implies that a vicariant event may have led to the differentiation of the East Brazilian and arboreal + terrestrial clades during the Early Miocene (ca. 23–16 MYA). This chronostratigraphic transition is a crucial event in South America with the end of the Miocene climatic optimum and the beginning of a cooling event. This transition has been followed by major changes in mammalian fauna (Flynn & Wyss 1998; Vucetich *et al.* 1999). During the Middle Miocene period (ca. 16–12 MYA), many Atlantic Forest endemic taxa started to diverge as suggested by phylogenetic arguments (Patton *et al.* 1997; Patton & Costa 1999; Costa 2003; Vilela *et al.* 2009; Upham & Patterson 2012). Within Echimyidae, the East Brazilian clade (node M), the *Thrichomys* lineage and possibly *Callistomys* (Emmons 2005) emerged between 15.1 and 17.8 MYA (Nodes L, P, W; Table 2). These three lineages occurred in different biotopes like humid rainforest (*Trinomys* and *Callistomys*), savannah (*Euryzygomatomys*, *Clyomys*, *Carterodon*) or steppe (*Thrichomys*). A recent phylogenetic analysis of echimyid fossils (Olivares *et al.* 2012) revealed a Late Miocene origin (i) of the *Trichomys* lineage, which is related to *Pampamys* (Verzi *et al.* 1995), and (ii) of the fossorial clade with *Theridomysops* (Montalvo *et al.* 1995; Vucetich 1995), which seems to be closely related to *Euryzygomatomys* and *Clyomys*. Our molecular dating results indicate an early split for *Thrichomys*, which predates the first occurrence of *Pampamys*. Our results also indicate that the Late Miocene divergence of *Euryzygomatomys* and *Clyomys* is congruent with the first occurrence of *Theridomysops*.

The split between East Brazilian forest and Amazonian rainforest taxa could be correlated with the expansion of open habitats like savannah (cerrado) or steppe (caatinga) during the Middle Miocene in South America. However, there is a lack of palaeobotanical evidence for the opening of the habitat in these regions (Hoorn 1994a; Colinvaux & De Oliveira 2001). Moreover, another split between Amazonian rainforest and East Brazilian taxa occurred subsequently (node S: 9.2 MYA and node U: 9.6 MYA; Table 2) within the arboreal clade. The inherently arboreal taxa (clade ARB; Emmons & Feer 1997) cannot have crossed the caatinga and cerrado biome without a forest corridor. To explain this recent split inferred from our phylogeny, we should assume that the cooling event must have been pursued throughout all the Late Miocene till the Early Pliocene (Zachos *et al.* 2001). Correlated with this climatic cooling, other events could have played a major role in shaping the echimyid evolution. Galewski *et al.* (2005) correlated these vicariant patterns in echimyids with potential continental sea inundations, which have led to isolation of different northern South American landmasses during Miocene (Webb 1995; Nores 1999). The arboreal clade polytomy suggests that colonization of the arboreal habitat triggered the major ecological radiation within the Echimyidae. Galewski *et al.* (2005) have also suggested that initiation of the Amazonian hydrographical sys-

tem around 14.5 MYA (Hoorn 1993, 1994a,b; Hoorn *et al.* 1995) and the appearance of a vast inundated forest in Amazonian basin (Hoorn *et al.* 1995; Hoorn 1996) could have driven this explosive diversification. Throughout the Miocene, marine incursions (Haq *et al.* 1987) have occurred in the north along the Amazon palaeobasin (Räsänen *et al.* 1995; Paxton & Crampton 1996) and in the south along the Parana basin (Cozzuol 1996). These transgression events could have played a role in the diversification of spiny rats. Our results on Echimyidae clearly suggest multiple vicariant events between East Brazilian and Amazonian rainforest areas. As this East Brazilian/Amazonian vicariance pattern is well documented in mammalian lineages, additional comparisons with climatic and geological data will allow us to provide a better understanding of isolation process in this area through the Miocene.

Our results also suggest a potential colonization of West Indies by Capromyidae around 18.8–17.8 MYA (Table 2). This molecular dating estimate is then incongruent with an early Oligocene arrival of extant capromyid lineages in West Indies as suggested by geological data (Iturralde-Vinent & MacPhee 1999). Our results are younger but congruent with the Galewski *et al.* (2005) and Upham & Patterson (2012) dating and with the palaeontological hypotheses of MacPhee & Iturralde-Vinent (1995) and Woods (1989). However, the phylogenetic position of Capromyidae needs to be refined to provide better insights into these dating estimates and the arrival of echimyids into the West Indies. Another range expansion event has occurred within the terrestrial clade with the differentiation of the nutria (11.5 MYA) in the southern South America. *Myocastor* is the only extant echimyid relative in the wide range of habitat of the southern South America (e.g. Valvidian and Magellan rainforest, Humid Chaco, Pampas). Compared with other Octodontidae groups, Echimyidae has not experienced speciation success in open habitats. In fact, burrowing-lifestyle adaptation has led to only five extant echimyid taxa, as compared with the greater diversity of tuco-tucos (Parada *et al.* 2011). Vucetich *et al.* (1997) and Vucetich & Verzi (2002) have suggested an exclusive competition hypothesis with other Octodontidae taxa to explain this absence of echimyids in the arid habitat of southern South America. In addition to habitat shifts, East Brazilian biogeographical patterns, competition with other octodontoids and also climate changes could have impacted the radiation of echimyids within this ecological niche.

Conclusion and perspectives

About the Echimyidae systematics, three monophyletic groups emerged from our molecular analysis: (i) an East Brazilian terrestrial Echimyidae + Capromyidae clade, (ii)

an arboreal clade and (iii) a *Myocastor* + *Hoplomys* + *Proechimys* + *Thrichomys* clade. Moreover, mitochondrial and nuclear gene corroboration and combined analyses provide strong support for the branching of arboreal echimyids with *Myocastor* + *Hoplomys* + *Proechimys* + *Thrichomys*. Our data also support the monophyly of Dactylomyinae, of *Hoplomys* + *Proechimys*, of *Euryzygomatomys* + *Clyomys*, of *Lonchothrix* + *Mesomys* and of *Makalata* + *Echimys* + *Phyllomys*. Additional molecular markers – complete mitochondrial genomes and nuclear exons and introns – are required to further refine the echimyid phylogeny and to solidify the taxonomic reassessment of 'spiny rats' to include Myocastorinae and Capromyidae.

We here provide a new insight into the diversification of Echimyidae by discussing their systematics, their adaptive radiation and their biogeographic history. The echimyid radiation appears to have started with habitat divergence, subsequently followed by morphological divergence, and finally, the arboreal lineage has undergone a burst of speciation linked to a key adaptation like nocturnal arboreality. Moreover, the adaptive radiation has not been followed by high morphological diversification in terrestrial taxa as compared with arboreal taxa and Capromyidae. Evidence for adaptive radiation without marked morphological differentiation over periods of time that span millions of years are phenomena rarely observed in mammals. As Rodentia explosive radiations are often associated with a narrow range of morphological variation, either with or without clear ecological divergence among species, more comparisons between lineages that exhibit adaptive radiation will be needed to understand the diversification process and morphological evolution within mammalian lineages.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Ecological ancestral character state estimation for Echimyidae.

Table S1. Taxon sampling and loci used in this study.

Table S2. Voucher numbers of the newly sequenced taxa used in this study.

Table S3. Results of likelihood-based tests of alternative topologies within Echimyidae.

Table S4. Bootstrap supports issued from the maximum likelihood single-gene analyses and gene concatenated analyses.

Table S5. Number of nodes supported by a given range of bootstrap percentages (BP) within single-gene and gene concatenated analyses.

Table S6. Ancestral state reconstruction at the most recent common ancestor (MRCA) of the Echimyidae (node K), the terrestrial clade (nodes L, V, W) and the arboreal clade (nodes S, T, *Isothrix* lineage).

Data S1. Morphological matrix data from Carvalho & Salles (2004) and Emmons (2005).

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