

Unexpected phylogenetic relationships of the painted tree rat *Callistomys pictus* (Rodentia: Echimyidae)[§]

Relações filogenéticas inesperadas do rato-do-cacau *Callistomys pictus* (Rodentia: Echimyidae)

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Abstract *Callistomys pictus* is an arboreal echimyid rodent, and the only living species in this genus. It is endemic to a very small Atlantic Forest region in the state of Bahia, east Brazil. Here we used DNA sequences from 4 genes to infer the phylogenetic position of *Callistomys* within Echimyidae. The results show that *Callistomys* forms a clade with the semi-aquatic coypu (*Myocastor*) from the grasslands in the southern South America and terrestrial spiny rats (*Proechimys*) from the Amazon forest, but the relationships among these three genera are uncertain. This clade is sister to *Thrichomys*, a terrestrial rat from the dry lands of central South America. These clades are unexpected, given the contrasting morphology, ecology, and geographic ranges of its members. The resulting echimyid phylogeny indicates that *Callistomys* is not closely related to the other arboreal echimyids, and suggest that arboreal habits evolved more than once in this family.

Keywords: Atlantic Forest; endemism; evolution; molecular phylogeny.

Resumo *Callistomys pictus* é um roedor equimídeo arborícola e o único representante vivo do seu gênero. É endêmico de uma região restrita na Mata Atlântica, no estado da Bahia, leste do Brasil. No presente trabalho utilizamos sequências de 4 genes para inferir a posição filogenética de *Callistomys* na família Echimyidae. Os resultados mostram que *Callistomys* forma um clado com o rato-dobanhado semi-aquático (*Myocastor*) que ocorre nas regiões abertas no cone sul da América do Sul e com o rato terrestre *Proechimys* com ocorrência na Amazônia, mas a relação filogenética entre esses três gêneros é incerta. Esse clado é irmão de *Thrichomys*, um equimídeo terrestre que ocupa as áreas áridas do centro da América do Sul. O agrupamento encontrado é inesperado, uma vez que seus membros apresentam aspectos morfológico, ecológicos e distribuição geográfica distintos e contrastantes. A filogenia resultante para a família Echimyidae indica que *Callistomys* não é proximamente relacionado

aos outros equimídeos arborícolas e sugere que o hábito arborícolas evoluiu mais de uma vez nessa família.

Palavras-chaves: endemismo; evolução; filogenia molecular; Mata Atlântica.

Introduction

Neotropical spiny rats in the family Echimyidae represent the major radiation among Caviomorpha, with approximately 23 genera and 90 living species (Fabre *et al.* 2013). These rodents are distributed throughout several Neotropical habitats, from wet forested regions (e.g., Atlantic Forest, Amazon) to dry open areas (e.g., Caatinga, Cerrado), showing adaptations to different modes of life, such as semi-fossorial (e.g., *Euryzygomatomys*, *Clyomys*), terrestrial (e.g., *Trinomys*, *Proechimys*), arboreal (e.g., *Callistomys*, *Echimyis*) and semiaquatic (*Myocastor*).

The painted tree rat *Callistomys pictus* Pictet, 1843, is the only living species in this genus (Emmons and Vucetich 1998). It is restricted to a very small area of Atlantic Forest in south Bahia (Vaz 2002, Ventura *et al.* 2008), and listed as endangered by the IUCN (International Union for Conservation of Nature and Natural Resources; Moura and Fonseca 2008). The genus is classified in the subfamily Echimyinae (Emmons 2005), which includes arboreal echimyid genera distributed across different forest regions of South and Central America. The other three echimyid subfamilies are: Dactylomyinae (bamboo rats), Eumysopinae (terrestrial or semi-fossorial spiny rats), and Heteropsomyinae (recently extinct Antillean rats) (Woods and Kilpatrick 2005). However, several phylogenetic analyses not only failed to recover the monophyly of most of these subfamilies, but also found that rodents traditionally placed in their own families — the semi-aquatic coypu

(Myocastoridae) and the Caribbean hutias (Capromyidae)—often fall inside the echimyid clade (Leite and Patton 2002, Carvalho and Salles 2004, Galewski *et al.* 2005, Fabre *et al.* 2013).

Phylogenetic analyses of morphological characters recovered *Callistomys* in a clade with other arboreal species, including bamboo rats (Carvalho and Salles 2004, Emmons 2005). Recent phylogenies inferred from DNA sequence data confirmed the close relationship of arboreal echimyids forming a monophyletic group (Fabre *et al.* 2013), but *Callistomys* has not yet been included in any molecular phylogeny. Therefore, our goal was to infer the phylogenetic position of *Callistomys* using DNA sequence data, and discuss the evolutionary and biogeographical significance of the results.

Methods

One female *Callistomys pictus* was captured by R. Moura (field number RM 233) at Fazenda Norma, Rio do Braço, municipality of Ilhéus, Bahia, Brazil (39°16'30" W, 14°40'56" S), and radio-tracked until it was found dead in October 2003. The genetic material was isolated using the salt protocol for total DNA extraction (SDS / NaCl / Proteinase K) described by Bruford *et al.* (1992) from a liver sample. The data set used to reconstruct the phylogenetic hypothesis consisted of four gene fragments: the mitochondrial cytochrome b (CytB), exon 28 of the von Willebrand factor (vWF), exon 10 of the growth hormone receptor (GHR), and recombinase activator gene 1 (RAG1), totalling 3,810 base pairs (bp) from each of 14 Echimyidae genera: *Callistomys*, *Capromys*, *Clyomys*, *Dactylomys*, *Echimyis*, *Euryzygomatomys*, *Isotbrix*, *Makalata*, *Mesomys*, *Myocastor*, *Phyllomys*, *Proechimys*, *Thrichomys*, *Trinomys*. *Octodon* and *Ctenomys* were used as outgroups. Specimens used in the present study and corresponding GenBank accession numbers are listed in Appendix 1. Fragments of interest were amplified by Polymerase Chain Reaction (PCR) *in vitro* cloning using the following primer combinations: MVZ05 and MVZ16 (Smith and Patton 1993) for CytB; V10, W13 (Galewski *et al.* 2005.), V2 and W1 (Huchon *et al.* 1999.) for vWF; GHREND, GHR50F (Adkins *et al.* 2001), GHR10F, GHR11F and GHR14R (Upham *et al.* 2013) for GHR and RAG1F1705, RAG1R2864 (Teeling *et al.* 2000), and FMNH2b FMNH3a (Patterson and Velazco 2008) for RAG1.

Samples were sequenced in both directions using the automated DNA sequencer ABI 3500 (Applied Biosystems Inc., Foster City, California, USA) following the manufacturer's protocol for the sequencing reaction. The sequences were aligned in Geneious 5.6 (Biomatters, Auckland, New Zealand) and substitution saturation in different codon position for coding regions was detected using Xia *et al.* (2003) test performed in DAMBE5 (Xia 2013). Appropriate models of evolution for each gene partition were determined in jModelTest 2.1.4 (Guindon and Gascuel 2003, Darriba *et al.* 2012), allowing three substitutions schemes and using the corrected Akaike information criterion (AICc).

We found saturation only in the third position of the CytB and set two data partitions: first and second codon positions, and

third position. The evolutionary models selected for each partition were: 1) GTR+I+G for first and second CytB positions, and vWF; 2) GTR+G for the third position of the CytB and GHR; and 3) TRN+I+G for RAG1. We used a matrix with all combined data to build the phylogenetic tree using Bayesian inference in MrBayes 3.2 (Ronquist *et al.* 2012), through 10^6 generations and sampling every 500 generations, which resulted in 2×10^3 trees. The first quarter was discarded as burn-in for building the final consensus tree. Independent analyses were repeated five times to avoid local optima. Only groups showing Bayesian posterior probability (BPP) values equal to or greater than 95% were considered significant.

Alternative phylogenetic scenarios regarding the phylogenetic position of *Callistomys* and its closest relatives were tested using the approximately unbiased test proposed by Shimodaira (2002) implemented in the software CONSEL (Shimodaira and Hasegawa 2001). For this purpose, the maximum likelihood values of each site were calculated in PAUP* 4.0b10 (Swofford 2002) using the evolutionary model appropriate for each gene partition.

Results

Callistomys was recovered in a well-supported clade with the semi-aquatic *Myocastor* and the terrestrial *Proechimys*, forming a polytomy (Figure 1). The topology test did not reject any of the three alternative hypotheses of relationship: *Callistomys* closer to *Myocastor* or to *Proechimys*, or *Proechimys* closer to *Myocastor*. The *Callistomys* + *Myocastor* + *Proechimys* clade is sister to the terrestrial *Thrichomys*, forming a more inclusive group sister to another clade comprising the remaining arboreal taxa, including tree rats (paraphyletic "Echimyinae") and bamboo rats (Dactylomyinae) (Figure 1).

Discussion

The most recent molecular phylogeny of Echimyidae (Fabre *et al.* 2013) has three major clades: terrestrial and semi-aquatic genera (*Thrichomys*, *Proechimys*, *Hoplomys*, and *Myocastor*); arboreal genera (*Mesomys*, *Lonchothrix*, *Makalata*, *Phyllomys*, *Echimyis*, *Isotbrix*, *Kannabateomys* and *Dactylomys*); semi-fossorial, terrestrial and scansorial taxa (*Euryzygomatomys*, *Clyomys*, *Trinomys* and *Capromys*). The recurring discrepancy found between morphological and molecular phylogenies may be due to the abundance of plesiomorphic characters in morphological data (Fabre *et al.* 2013). In addition, the diversification of the family may have been primarily driven by distinct habitat occupations, followed by morphological divergence related to the occupied habitat, and finally an adaptive radiation giving rise to the arboreal lineage (Fabre *et al.* 2013). Among the 23 genera of Echimyidae, 14 are arboreal, and the

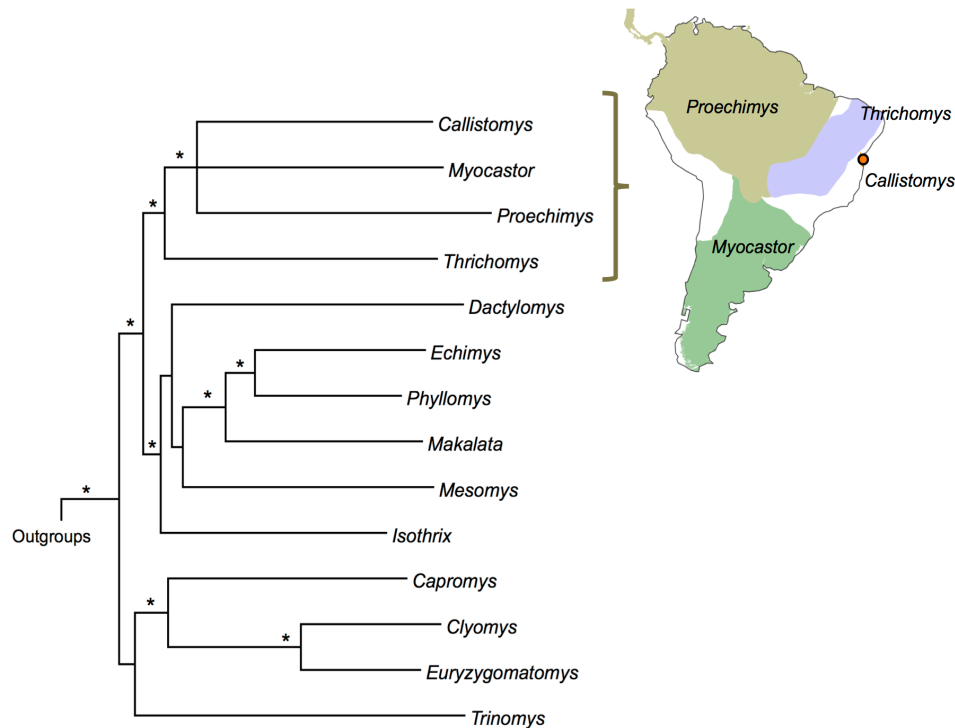


Figure 1 Bayesian phylogeny of echimyid rodents based on four molecular markers (CytB, vWF, GHR and RAG1). Asterisk indicates node with significant statistical support (Bayesian posterior probabilities $\geq 95\%$). Map shows range of *Callistomys* and closely related genera.

phylogenetic hypotheses based on molecular data proposed for the family indicated the monophyly of arboreal genera (Galewski *et al.* 2005, Fabre *et al.* 2013, Upham *et al.* 2013). Phylogenetic trees constructed using morphological characters recovered *Callistomys* within a clade of arboreal echimyids (Carvalho and Salles 2004, Candela and Rasia 2012). The phylogenetic position of *Callistomys* found in the present study, outside this arboreal clade and grouped with terrestrial and semi-aquatic genera, indicates that arboreal echimyids are paraphyletic, and suggests that arboreal habits and corresponding morphological adaptations evolved more than once during the evolutionary history of this family (contra Carvalho and Salles 2005). Taxonomic classifications considered *Callistomys* as a member of the Echimyinae subfamily (e.g., Woods and Kilpatrick 2005), but Emmons and Vucetich (1998) had already called attention for the fact that *Callistomys* has morphological features intermediate between Eumysopinae and Echimyinae. The genetic data available indicate that both are paraphyletic (Upham and Patterson 2012, Fabre *et al.* 2013), a result confirmed in our molecular study including *Callistomys* for the first time.

The four genera closest to *Callistomys* in our analysis show distinct ecological characteristics and almost non-overlapping geographic ranges (Figure 1). The arboreal *Callistomys* is restricted to the Atlantic Forest of eastern Brazil; the semi-aquatic *Myocastor* occurs in open areas of southern South America, ranging from Bolivia to Tierra del Fuego; the terrestrial *Proechimys* is widely distributed in lowland Amazon rainforests and adjacent habitats, extending into Central America; the terrestrial *Thrichomys* occurs in dry forests and open areas of Caatinga and Cerrado in central Brazil, and adjacent parts of

Paraguay and Bolivia. Although very restricted today, the range of *Callistomys* extended more than 700 km to the southwest in the late Pleistocene to early Holocene (Emmons and Vucetich 1998). The estimated diversification of the clade *Callistomys* + *Myocastor* + *Proechimys* is estimated at about 15 million years ago (Fabre *et al.* 2013), and range discrepancies among taxa and displacement over time illustrate that current geographical distribution does not necessarily reflect the historical diversification of the group, and may explain little about the habitat occupied by ancestral lineages, especially ancient ones. One of the main limitations of evolutionary interpretations based on molecular phylogenies is the absence of fossil taxa, including extinct genera closely related to *Callistomys* and *Myocastor*, such as *Maruchito* and *Tramyocastor* (Carvalho and Salles 2004, Candela and Rasia 2012). Despite such limitations, our results indicate that the closest living relatives of *Callistomys* are not in the Atlantic Forest, and show very distinct morphological characters and ecological adaptations, highlighting the dynamic nature of echimyid evolution and biogeography.

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Appendix 1.

GenBank accession numbers of sequences used in the present study. Numbers correspond to CytB, GHR, RAG1 e vWF sequences respectively.

Echimyidae: *Callistomys pictus* [KJ742659, KJ742627, KJ742677, KJ742614]; *Capromys pilorides* (AF422915, AF433950, JX515322, AJ251142); *Clyomys laticeps* [AF422918, JX515326, KJ742679, AJ849306]; *Dactylomys boliviensis* [L23339, JX515334, EU313298, AJ849307]; *Echimys chrysurus* [L23341, JX515333, EU313303, AJ251141]; *Euryzygomatomys spinosus* [EU544667, JX515327, KJ742680, AJ849319]; *Isotrrix bistrata* [L23355, JX515336, EU313305, AJ849308]; *Makalata didelphoides* [EU302692, JX515332, EU313315, AJ849311]; *Mesomys bispidus* [L23385, JX515335, EU313322, AJ849305]; *Myocastor coypus* [EU544663, AF520662, AY011892, AJ251140]; *Phyllomys blainvillii* [EF608180, JX515331, JX515323, AJ849314]; *Proechimys simonsi* [EU313249, JX515324, EU313332, AJ849320]; *Thrichomys apereoides* [EU544668, JX515325, EU313334, AJ849315]; *Trinomys iberingi* [EU544664, KF590695, EU313337, KF590677].

Outgroups*: *Octodon* [AM407929, AF520650, XM_004627038, AJ238386]; *Ctenomys* [AF370703, AF520656, KM514671, AJ251138].

* Outgroup sequences used to build the data matrix were from different species within each genera, resulting in chimeric operational taxonomic units (OTUs). For *Octodon* we used CytB and RAG1 sequences from *O. degus*, and GHR and vWF sequences from *O. lunatus*. For *Ctenomys*, we used CytB and vWF sequence from *C. maulinus*, GHR sequences from *C. steinbachi*, and RAG1 sequence from *C. bicolor*.