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Rodents of the Caribbean: origin and diversification of hutias unravelled by next-generation museomics

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The Capromyidae (hutias) are endemic rodents of the Caribbean and represent a model of dispersal for non-flying mammals in the Greater Antilles. This family has experienced severe extinctions during the Holocene and its phylogenetic affinities with respect to other caviomorph relatives are still debated as morphological and molecular data disagree. We used target enrichment and next-generation sequencing of mitochondrial and nuclear genes to infer the phylogenetic relationships of hutias, estimate their divergence ages, and understand their mode of dispersal in the Greater Antilles. We found that Capromyidae are nested within Echimyidae (spiny rats) and should be considered a subfamily thereof. We estimated that the split between hutias and Atlantic Forest spiny rats occurred 16.5 (14.8–18.2) million years ago (Ma), which is more recent than the GAARlandia land bridge hypothesis (34–35 Ma). This would suggest that during the Early Miocene, an echimyid-like ancestor colonized the Greater Antilles from an eastern South American source population via rafting. The basal divergence of the Hispaniolan *Plagiodontia* provides further support for a vicariant separation between Hispaniolan and western islands (Bahamas, Cuba, Jamaica) hutias. Recent divergences among these western hutias suggest Plio-Pleistocene dispersal waves associated with glacial cycles.

1. Introduction

Ever since the beginning of naturalist exploration, insular communities have played an important role in the development of ecological and evolutionary theories [1,2]. The Caribbean islands are no exception and have experienced a series of spectacular adaptive radiations within terrestrial lizards, snakes, sloths, primates and rodents [3,4]. This exceptional diversity, together with the geographical location of the archipelago in between both American continental masses, has promoted local vertebrate assemblages as biological models. The Caribbean fauna has, thus, greatly contributed to the advancement of our knowledge of adaptive radiation, biogeography and community assembly [2].

Rodents represent the richest terrestrial mammalian component of the West Indies, comprising four hystricognath families and one muroid subfamily. These rodents are also known for the Holocene extinctions of species belonging to Capromyidae (Hexolobodontinae and Isolobodontinae [5]) and Chinchilloidea (Heptaxodontidae [6]), likely following human arrival [7]. Capromyidae hystricognaths, also called hutias, certainly experienced the largest radiation.
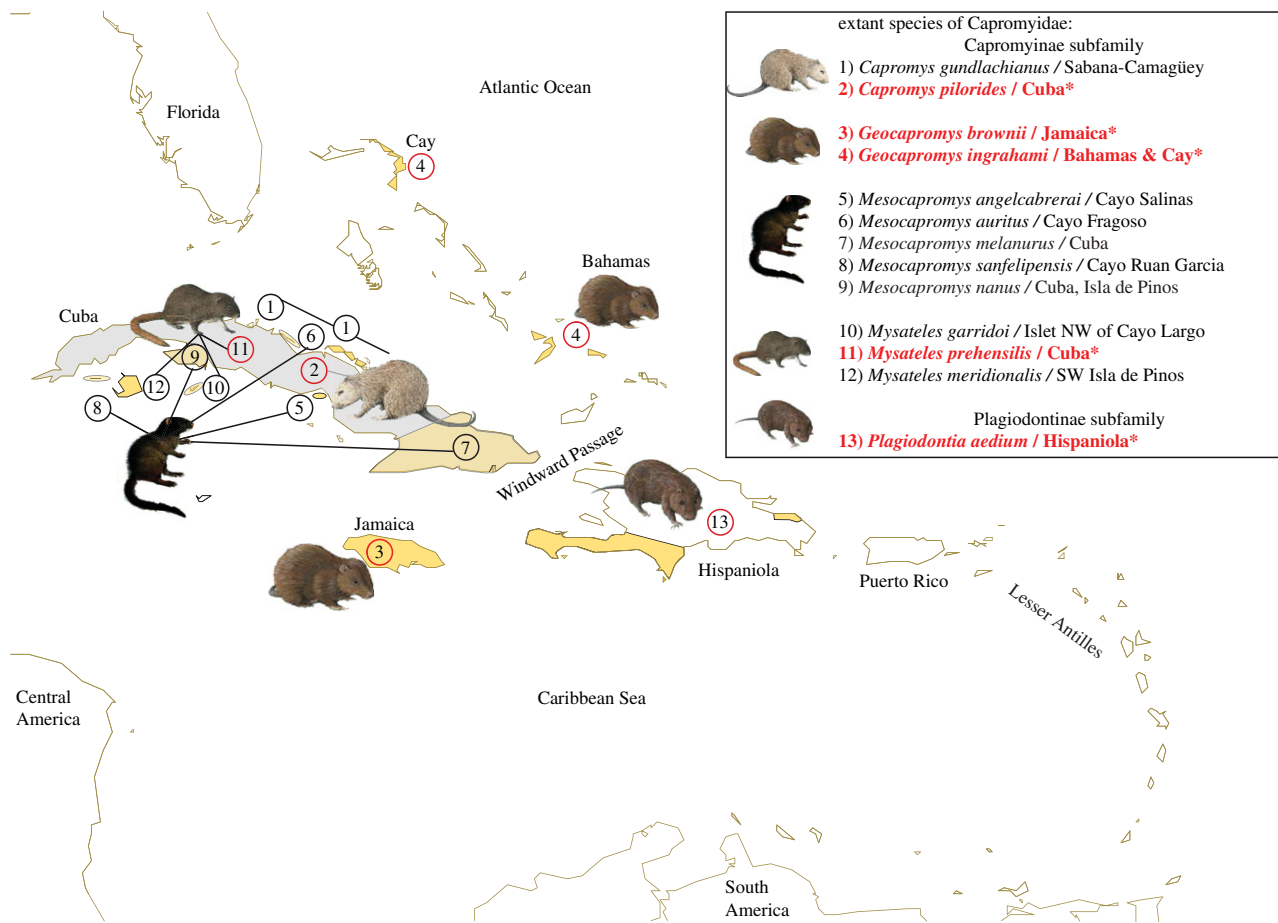
Figure 1. Map of the West Indies and the Bahamas archipelagos indicating contemporary islands, seas, and hutia fauna. Major islands and the Windward Passage are labelled. Lineages of hutias present on each island are also indicated. Species sampled in this study are indicated in the key by an asterisk and highlighted in bold (red). (Online version in colour.)

With eight genera and 32 described species, hutias constitute the most diversified terrestrial endemic group. These small- to large-sized rodents have spread throughout all of the Greater Antilles archipelago (Puerto Rico, Hispaniola, Jamaica, Cuba), the Bahamas and surrounding islands (figure 1). Hutias display a wide range of life histories and ecomorphological adaptations, including scansorial (*Geocapromys*) and arboreal (*Plagiodontia*) abilities.

Hutias belong to Southern American caviomorphs and are part of the highly diversified Octodontoidea superfamily [8]. Their exact affinity within octodontoids still remains debated, precluding our understanding of their biogeographic origins. Morphological characters support hutias as a subfamily of their own within Echimyidae, or as a group closely related to the Myocastoridae (nutria) family [9]. However, both hypotheses are poorly supported by molecular evidence, with mitochondrial and nuclear DNA suggesting Capromyidae either (i) as most closely related to the Echimyidae [10], or (ii) as nested within an Echimyidae paraphyletic assemblage [11].

The oldest capromyid, *Zazamys*, suggests an Early Miocene hutia diversification in Cuba *ca* 23–16 Ma [12]. This post-dates by at least 11 million years the Greater Antilles and Aves Ridge (GAARlandia) event from the Early Oligocene (35–34 Ma), where a land bridge or island chain connected the Greater Antilles to South America [13]. Therefore, the palaeobiogeographic scenario, consisting of a first terrestrial colonization event from the continent followed by later vicariance events, often proposed for explaining the

origin of non-flying mammals in these islands [14,15], seems inadequate for hutias.

The vicinity of Hispaniola and Puerto Rico relative to the South American landmass combined with the richness of their hutia fossil record has suggested these islands as the possible centre of capromyid diversification [16], with later dispersal events driving further adaptive radiations. The early split between Plagiodontinae from Hispaniola and other hutias as proposed by Woods *et al.* [16] would provide compelling support for this hypothesis, if confirmed with stronger phylogenetic support and a more extensive sampling of Caribbean capromyids.

In this study, we used target enrichment approaches coupled with next-generation sequencing (NGS) to generate a new DNA dataset consisting of *ca* 9000 bp, with 2000 and 6800 bp of mitochondrial and nuclear genes, respectively. Our sample includes five species from five genera obtained from museum collections and thus far represents the most extensive taxonomic survey of hutias. This allows us to unravel the phylogenetic relationships and colonization history of hutias by inferring a highly resolved molecular phylogeny and computing divergence dates between major clades (figure 1). More specifically, we use our phylogenetic and dating framework to address the following questions: (i) What are the systematic affinities of hutia lineages in the context of octodontoid relationships? (ii) Is GAARlandia a valid hypothesis for the arrival of extant hutia lineages in the Caribbean? and (iii) Which dispersal scenario best explains the hutia phylogenetic and biogeographic patterns?
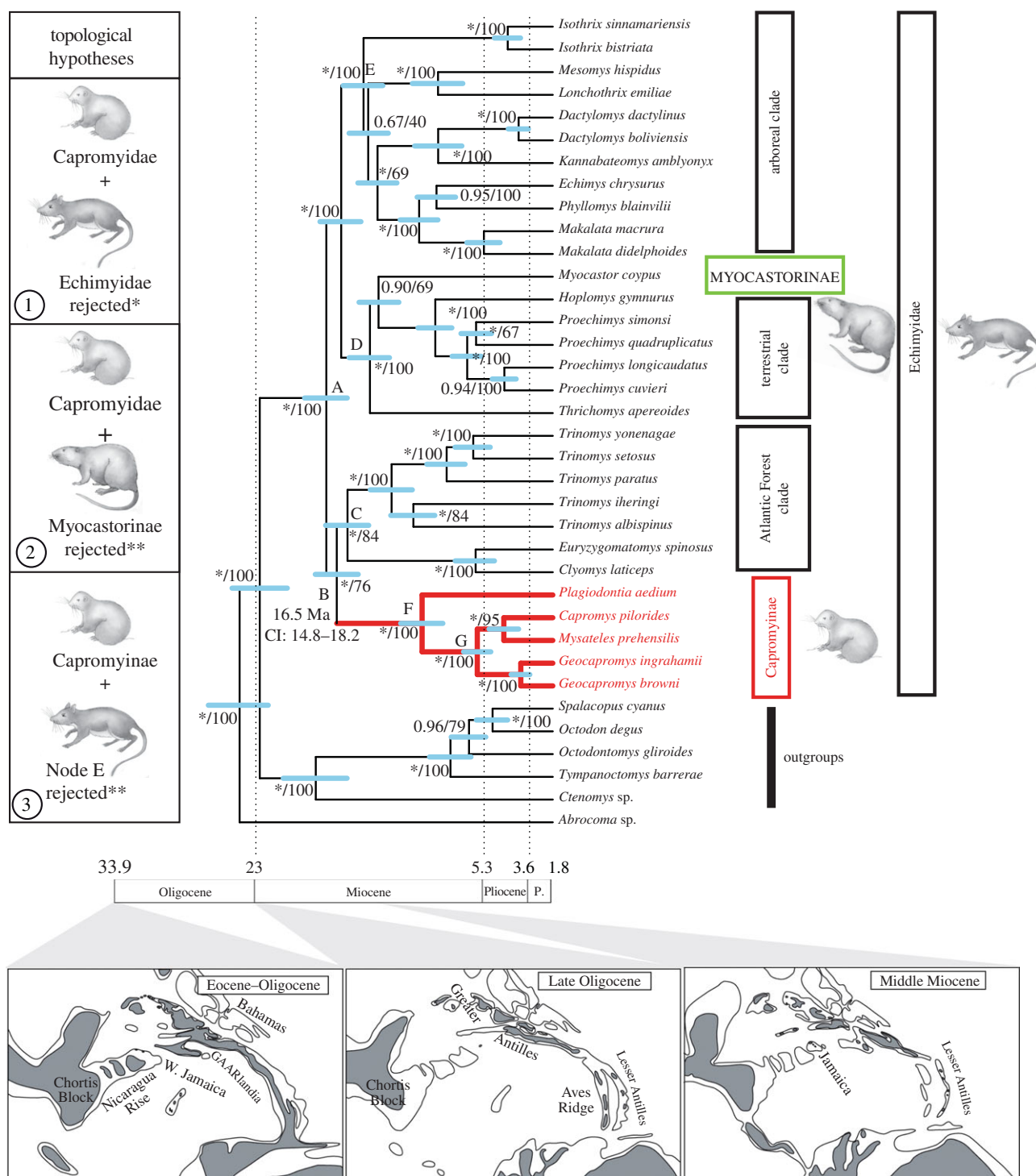
Figure 2. Phylogenetic and molecular dating results for hutias and close-relative lineages inferred from eight mitochondrial and nuclear genes. The timescale is expressed in million years. For clarity, non-octodontoid taxa have been pruned from the chronogram. Bayesian posterior probabilities and maximum-likelihood bootstrap support values are indicated at the nodes (asterisk (*) denotes maximum support). The maps display the transition zone between North America and Central America (after [5]) and show the distribution of land and sea from the Oligocene to the present at $14-16$ Ma intervals: grey, land; white, sea; black lines surrounding islands separate deep sea from the shallow sea nearer the land (which may at times have been above sea-level). P., Pleistocene. Left insert: Summary of the topological test analyses. 'Rejected' indicates strong statistical evidence against tested hypothesis (*$p < 0.05$, **$p < 0.01$). Original artwork by Laurence Meslin, ©Laurence Meslin. (Online version in colour.)

2. Material and methods

(a) Taxon sampling, amplification and sequencing

We constructed a 50-taxon mitochondrial and nuclear multigene dataset comprising five hutias and 25 echimyids sampled across major clades (figure 1), and several South American caviomorph outgroups. DNA extraction, primers, amplification and NGS procedures are detailed in the electronic supplementary material.

(b) Phylogenetic analyses

Probabilistic inferences were implemented to assess phylogenetic relationships within Capromyidae and Echimyidae from the eight-marker supermatrix. Models of nucleotide substitution were selected for each gene individually by applying the Akaike Information Criterion. As the nucleotide frequencies and Γ distribution differed among genes, we applied a partitioned analysis of the combined dataset. We then used a relaxed molecular clock to

estimate the divergence dates. To calibrate the clock, we selected four fossil constraints already considered in previous studies on rodents [10,11]. We tested the robustness of our molecular dating results using a leave-one-out approach. These analyses are described in greater detail in the electronic supplementary material, with explicit reference to methods and software.

3. Results and discussion

Analyses of the 9000-site alignment as well as topological tests demonstrate that hutias, here represented by genera *Capromys*, *Geocapromys*, *Mysateles* and *Plagiodontia*, constitute a monophyletic assemblage (figure 2). Moreover, these capromyids appear nested within echimyids (node A), a family of South American spiny rats whose radiation represents more than 95 extant species. More specifically, hutias are related to Atlantic Forest echimyids (node B) which comprise both fossorial (*Clyomys*, *Euryzygomatomys*) and terrestrial (*Trinomys*) taxa (node C). Hutias and their echimyid relatives are the sister-group of a clade subdivided into terrestrial (node D) and arboreal (node E) echimyids. This suggests that hutias should be considered a subfamily of Echimyidae (figure 2, Capromyinae) rather than a separate family (Capromyidae, Hypothesis 1). Our data also significantly reject a Myocastorinae + Capromyinae relationship (figure 2, Hypothesis 2).

Using our set of fossil constraints (see the electronic supplementary material, S2), we estimated the divergence between hutias from the West Indies and South American echimyids at 16.5 Ma (95% credibility interval (CI): 14.8–18.2), and dated the first divergence among extant species of hutias to the Middle–Late Miocene 9.4 Ma (95% CI: 7.8–11.1). Our results have major implications about the biogeographic origin of terrestrial fauna of the West Indies. These divergence ages clearly postdate those involved by the GAARlandia vicariance hypothesis [17], which assumes an Eocene/Oligocene (figure 2) connection between South America and the West Indies, in relation to both a continental landbridge and a sea-level drop [14–16]. Our results suggest instead that an echimyid-like ancestor colonized the Greater Antilles during the Early Miocene when the land bridge was fully submerged. This implies a Southern American origin of capromyids, possibly via rafting. Therefore, hutias constitute a new example among non-flying vertebrates of a late, post-GAARlandia colonization of the Greater Antilles.

The Cuban *Zazamys* fossil [12] is the oldest known capromyid representative. Its Early Miocene age (16 Ma) is in accordance with our 16.5 Ma estimate for the split of hutias from their South American relatives. Moreover, the oldest continental South American octodontoid relative (*Deseadomys*) dates back to the Deseadan epoch (29–24.5 Ma). This delimits a time-interval for the arrival of a Southern American ancestor into the West Indies, a time frame compatible with our molecular dating

estimate. Late Miocene divergence among *Geocapromys* and Cuban hutias suggests the extinction of *Zazamys* close relatives on Cuba.

The major biogeographic outcome of our study relates to the Hispaniola Island as a potential source for subsequent Greater Antilles and Bahamas colonizations. A hutia origin in the Eastern Antilles was postulated [16] and linked to the GAARlandia event, with hutia ancestors crossing the Caribbean Sea via the Lesser Antilles and first reaching Puerto Rico and Hispaniola. Our analysis clearly rejects the GAARlandia hypothesis but supports a basal split between *Plagiodontia* from Hispaniola and other hutias (figure 2), as well as a sister relationship between *Geocapromys* (Bahamas and Jamaican islands) and the Cuban hutias. In agreement with myological [9] and cytochrome *b* data [16], we therefore confirm that *Plagiodontia* hutias, which are endemic to Hispaniola, can be considered as a distinct tribe (Plagiodontini). This suggests either (i) a vicariant event between eastern (Hispaniola) and western (Bahamas, Cuba, Jamaica) hutias or (ii) stepping stone colonizations from east to west. Divergence dates between hutias and echimyids (node B: 16.5 Ma) as well as among hutias (node F: 9.4 Ma) is compatible with a westward vicariance hypothesis as already suggested for Caribbean sloths [14,18]. This vicariant pattern was likely due to reduced connections among Carribean islands during the Mid and Late Miocene and the Windward Passage opening between Cuba and Hispaniola [14,15,19]. The recent divergence between *Geocapromys* and Cuban hutias (node G: 5.8 Ma) indicates that several dispersal events might have taken place from the Late Miocene. Multiple sweepstake colonizations could have been the consequence of island connections or rafting events during Plio-Pleistocene glacial cycles [14].

Apart from very old lineages like solenodons, it seems that most of the Caribbean terrestrial fauna is restricted to few diversified vertebrate subfamilies (e.g. anolines, eleutherodactylines, capromyines), which arrived from South America during the Mid Miocene [3,17,20]. While the GAARlandia landbridge might explain few terrestrial vertebrate colonizations [19], hutias constitute a new Miocene over-water dispersal example from South America to the West Indies. Further sequencing of extinct mammal subfossils (rodents, sloths) will be required to confirm the pivotal role of Hispaniola, as well as the recent dispersal scenarios at the origin of western Caribbean terrestrial biotas.

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