

SHORT COMMUNICATION

Variation in cytochrome-*b* haplotypes suggests a new species of *Zygodontomys* (Rodentia: Cricetidae) endemic to Isla Coiba, Panama

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ABSTRACT. The identity of two insular populations of rodents of the nominal species, *Zygodontomys brevicauda* (Allen & Chapman, 1893), from the Veragua Archipelago was examined. The mitochondrial cytochrome-*b* gene was sequenced from specimens collected on Isla Coiba (n = 10), Isla Cébaco (n = 3) and on the nearby Peninsula Azuero (n = 3) in Panama and compared with sequences of *Z. brevicauda* and a number of other related species from GenBank. For Panama, phylogenetic analyses identified two clades within *Zygodontomys* Allen, 1897; one representing Isla Coiba and another clade composed of rats from Isla Cébaco and from the mainland on the Peninsula Azuero, as well as a GenBank sequence of *Z. brevicauda* from Venezuela. We suggest that the population from Isla Coiba may represent a previously undescribed species of sigmodontine rodent that is endemic to this Pacific Island.

KEY WORDS. Cébaco; island; mammal; Panamá.

Insular populations or species are often divergent from their mainland counterparts. This observation has led studies of island biota to play a central role in the development of the field of biology, specifically evolutionary theory and related empirical analyses (DARWIN 1859, RICKLEFS & BERMINGHAM 2008). The fauna of many Panamanian islands, especially those of the Pacific Coast, has not been extensively studied. The discovery that a number of historically important museum specimens purportedly from Isla Coiba were likely from other sites in Central America has further obscured our knowledge of the Pacific islands of Panama (OLSON 2008).

We sequenced a mitochondrial DNA gene from specimens recently collected on Isla Cébaco and Isla Coiba, and from mainland sites in Panama (Fig. 1). We then compared these to GenBank sequences of *Zygodontomys* Allen, 1897 from Colombia, Venezuela and French Guiana. Because the phylogenetic placement of *Zygodontomys* within the Sigmodontinae has also been controversial (CARLETON & MUSSEY 2005) and the sister taxon is unknown, we included representatives of other related species based on previous studies of their phylogenetic relationships (STEPPAN *et al.* 2004, WEKSLER 2003, 2006, WEKSLER *et al.* 2006). This molecular framework provides an initial view of

diversification of rodents of the genus *Zygodontomys* on two islands of the Veraguas archipelago.

As part of a multi-year survey of mammalian-borne pathogens (e.g., hantavirus), rodents were collected from a number of localities on the mainland in southern Veraguas Province on the Peninsula Azuero, and on the islands of Cébaco and Coiba (Fig. 1). Specimens were deposited at the Museum of Southwestern Biology in Albuquerque, New Mexico. We sequenced the mitochondrial cytochrome-*b* gene for 16 specimens of *Zygodontomys* and compared these sequences to related sequences on GenBank.

Sampling effort consisted of 6300 trap-nights in October 2006 on Isla Coiba and 4800 trap-nights in May and July 2008 on Isla Cébaco. At each site, we used 100 Sherman traps placed 10 meters apart in square grids (ca. 1 ha.) located in a variety of habitats, including crop-fields, pastures, and various stages of secondary forest. Tissue samples from previous sampling efforts on the Peninsula Azuero were also used.

The mitochondrial cytochrome-*b* gene was sequenced for 16 specimens (Isla Coiba n = 10, Isla Cébaco n = 3, Peninsula Azuero n = 3) that were tentatively identified as *Zygodontomys brevicauda* (Allen & Chapman, 1893). DNA was extracted from

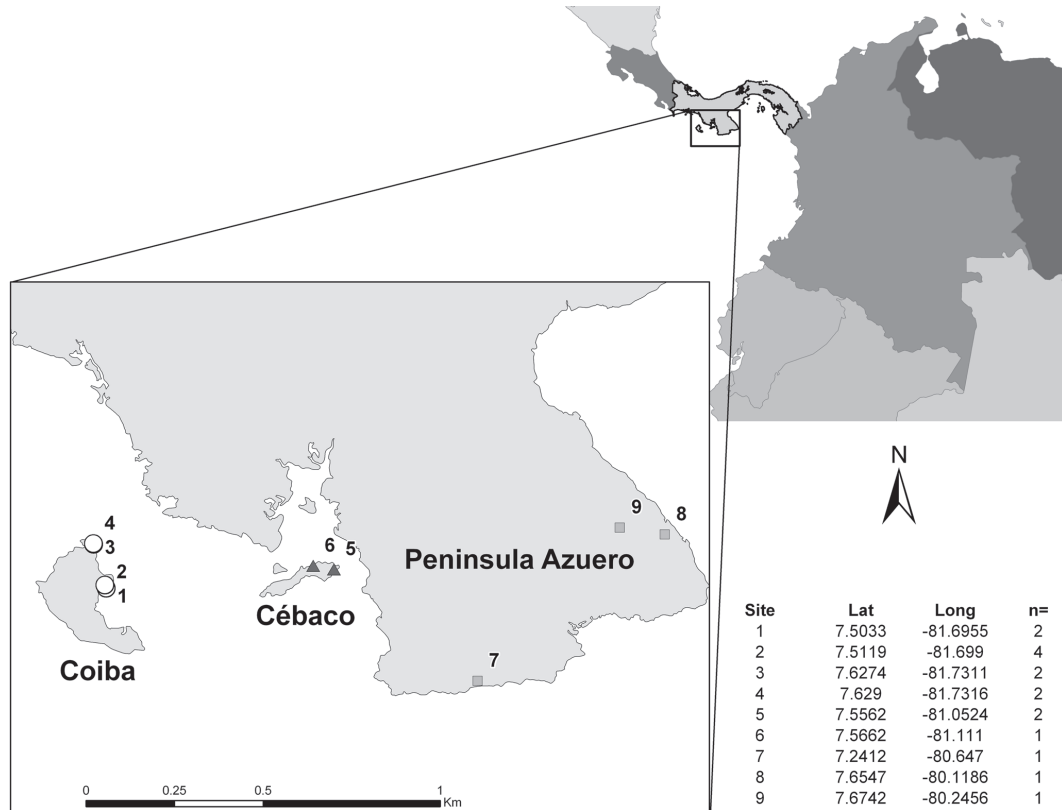


Figure 1. Specimen localities with presented corresponding latitude and longitude and number of individuals sequenced. Circles indicate the four localities on Isla Coiba, triangles for the 2 localities on Isla Cébaco, and squares for the three localities on Peninsula Azuero.

frozen heart or liver tissues using a modified salt extraction (FLEMING & COOK 2002). The polymerase chain reaction (PCR) used the primers L14734 (IRWIN *et al.* 1991) and CytB Rev (ANDERSON & YATES 2000) to obtain the complete mtDNA cytochrome-*b* sequence (1140bp). PCR conditions included 33 cycles with denaturation at 94°C for 30 seconds, annealing at 51.7° for 25 seconds, and extension at 72° for 1 minute. PCR products were sequenced using the original primers and BigDye Terminator Cycle Sequencing Ready Reaction mix. V3.1 (Applied Biosystems). All sequences were deposited in GenBank (Accession numbers: GU397410-GU397425). Additionally, because *Zygodontomys* at various times was hypothesized to be affiliated with several different tribes within the Sigmodontinae (HERSHKOVITZ 1962, HOOPER & MUSSER 1964, GARDNER & PATTON 1976), we obtained sequences from GenBank from 28 species representing 10 other genera to further assess the phylogenetic placement of *Zygodontomys* among sigmodontine rodents. *Irenomys* Thomas, 1919 was designated the outgroup.

A maximum likelihood tree was created using GARLI (D.J. ZWICKL, University of Texas at Austin, unpublished data) with 1000 replicates and a Bayes tree was created using MrBayes 3.1 (HUELSENBECK & RONQUIST 2005) with codon position partition-

ing, and 4 Monte Carlo Markov Chains that proceeded for five million generations, with each sampled every 100 generations. Using ModelTest (POSADA & CRANDALL 1998), we choose the K81uf+I+G model with gamma = 0.8938. This model was used for the GARLI and a modified version (nst = 2 and rates = invgamma) for MrBayes. Nucleotide composition and genetic distance values were computed in MEGA (TAMURA *et al.* 2007).

The Isla Coiba and Isla Cébaco sequences exhibited a base and amino acid composition expected for genuine mammalian cytochrome-*b* sequences (IRWIN *et al.* 1991). Nucleotide composition of the Isla Coiba sequences was 27.3% adenine, 31.9% thymine, 28.9% cytosine, and had an overall deficit of guanine (12.3%). The average genetic distance value within the Isla Coiba haplotypes (4 locations) was minimal (0.7%) with similar low levels of variation found within Panamanian mainland samples (0.9%) and within Isla Cébaco (0.7%). Distance values between Isla Coiba specimens and mainland representatives of *Z. brevicauda* and other species of *Zygodontomys* ranged from 7.5% to 9.2% (Tab. I), while Isla Cébaco and the nearby mainland (0.3%) was much lower (Tab. I). The higher values separating Isla Coiba from the others are similar to species-level differences for this gene for sister-species comparisons of

Table I. Distance matrix based on K2P values. The lower matrix represents the net between group average distances. The upper matrix represents the associated p-value. *Zygodontomys* sp. 1 and *Zygodontomys* sp. 2 correspond to two new species suggested by BONVICINO *et al.* (2008).

	Coiba	Cebaco	mainland	Venezuela	<i>Z. microtinus</i>	<i>Z. cherriei</i>	<i>Z. sp. 1</i>	<i>Z. sp. 2</i>	<i>Scolomys</i>
Coiba	–	0.013	0.010	0.011	0.011	0.010	0.011	0.011	0.032
Cebaco	7.5%	–	0.003	0.008	0.014	0.012	0.014	0.013	0.032
Mainland	7.6%	0.3%	–	0.006	0.011	0.010	0.011	0.011	0.033
Venezuela	9.0%	3.5%	3.8%	–	0.011	0.011	0.012	0.011	0.033
<i>Z. microtinus</i>	8.5%	8.8%	8.7%	8.7%	–	0.007	0.007	0.007	0.028
<i>Z. cherriei</i>	8.2%	7.0%	7.6%	8.2%	3.9%	–	0.006	0.005	0.029
<i>Z. sp. 1</i>	9.1%	8.4%	8.7%	9.9%	4.9%	3.0%	–	0.004	0.031
<i>Z. sp. 2</i>	9.2%	7.7%	8.2%	8.6%	4.2%	2.4%	2.2%	–	0.027
<i>Scolomys</i>	31.5%	26.5%	32.5%	31.9%	27.6%	29.7%	30.8%	28.4%	–

mammals (BAKER & BRADLEY 2006). *Scolomys* Anthony, 1924 fell out as the sister genus to *Zygodontomys*, with an approximate genetic divergence of 30%.

ML and Bayes trees showed nearly identical topologies (Fig. 2). The 10 Isla Coiba specimens formed a monophyletic clade that was supported in all analyses (Bayes = 1, ML = 66). The Isla Coiba clade associated most closely with the *Z. breviceauda* clade, while Isla Cébaco haplotypes formed a clade with mainland *Z. breviceauda* from Panama.

Mammals found on oceanic islands are often genetically isolated from other populations due to reduced migration. Among insular mammals, rodents are notorious for their devastating introductions worldwide. Rodents, however, also have a relatively high degree of endemism in insular faunas (AMORI *et al.* 2009). Worldwide, islands support an enormous and largely irreplaceable heritage of rodent biological diversity and much of it is poorly studied (CEBALLOS & BROWN 1995, CARLETON & OLSON 1999, SALVADOR *et al.* 2008).

For Panama, a number of rodent species need careful taxonomic and phylogeographic study based on expanded geographic sampling (MENDEZ 1993). Isla Coiba, which is about 18 km from the mainland, is 53,528 ha in size. This molecular perspective on the Coiba population of *Zygodontomys* indicates that these rats likely represent a distinct species based on the degree of sequence divergence relative to other distinct species of *Zygodontomys*. Careful morphological comparisons are now needed to explore the taxonomic status of this population. Another endemic rodent from this island (REID 2009) is the Coiba Island agouti, *Dasyprocta coibae* Thomas, 1902, an island form that is currently listed as Endangered by the IUCN. That taxon, however, should be reviewed through a wider study of geographic variation among various populations of Neotropical agoutis (CARLETON & MUSSEY 2005). A number of endemic birds have also been associated with Isla Coiba (WETMORE 1957, OLSON 2007). In contrast, Isla Cébaco is much smaller (8500 ha) and only about 8 km from the mainland. This island popu-

lation is less divergent from other *Zygodontomys* populations with haplotypes more closely related to those on the nearby mainland. Geologic history of the region indicates that Isla Coiba and Isla Cébaco were united with the mainland on multiple occasions in the Late Cenozoic, most recently when sea levels dropped during the Last Glacial Maximum that ended about 10,000 years ago (CASTROVIEJO *et al.* 1997). Because the nearby lowlands of the Peninsula Azuero have been highly populated by humans and deforested since the pre-Columbian period (COATES 2003), the islands of the Veragua Archipelago are important components of efforts to maintain the native fauna and flora of Panama.

The number of extant species of *Zygodontomys* has been the subject of debate (CARLETON & MUSSEY 2005) and our phylogeny suggests that this issue is far from resolved. VOSS (1991) assessed morphological variation across a large number of specimens representing populations from Costa Rica, Panama, Colombia, and Venezuela and concluded that the genus consisted of two extant species. He recognized *Z. breviceauda* as a widely distributed species that occurs from central Costa Rica south and east through northern South America to northern Brazil. The second species is *Zygodontomys brunneus* Thomas, 1898, a rat that is limited to the intermontane valleys of northern Colombia. CARLETON & MUSSEY (2005) followed that taxonomic arrangement, but BOVICINO *et al.* (2008) used chromosomal and cytochrome-*b* sequence variation to suggest the possibility of two new species in Brazil and Guiana. Those populations (Fig. 2, Sp 1 and Sp 2) show limited divergence in our analysis, while specimens from Isla Coiba are among the most distinctive identified with this assessment of cytochrome-*b* sequence variation.

Specimens from Isla Cébaco were included in the monograph of Voss (1991). His morphological analysis (Fig. 26 of Voss 1991) clustered these specimens with other island (e.g., San Miguel Island, Panama) or distant mainland (e.g., French Guiana) populations, but distinct from nearby mainland populations. In contrast, the mitochondrial data suggest a close af-

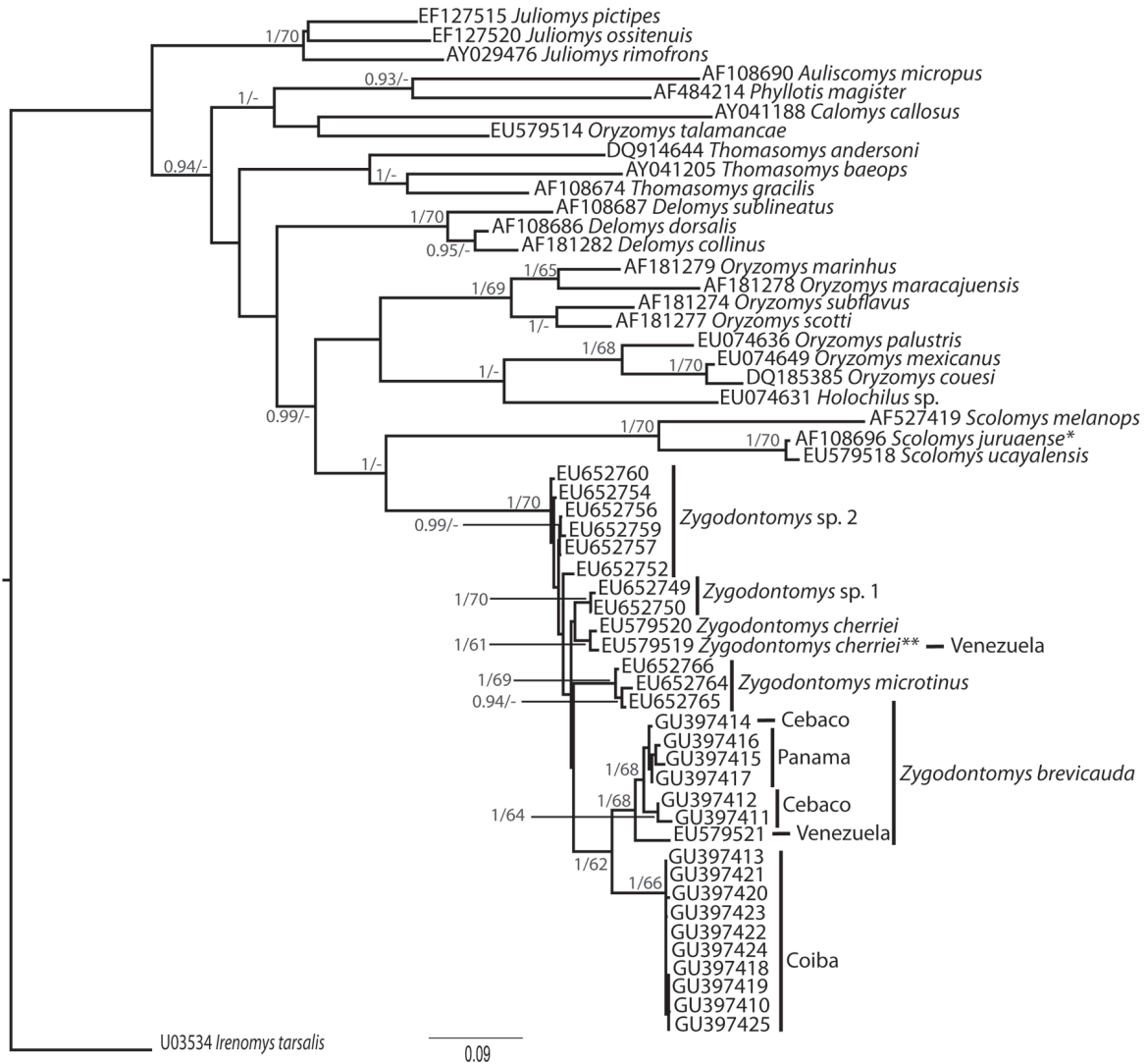


Figure 2. Phylogenetic relationship (maximum likelihood) for populations of *Zygodontomys* and from 28 other species representing 10 other sigmodontines rodent genera. Support values are the numbers on major branches reported as Bayesian posterior probability values/maximum likelihood bootstrap support values. * *Scolomys juruaense* is now considered *S. ucayalensis*. ** Source of sequence lists it as *Z. brevicauda*.

finity between populations of *Zygodontomys* on Isla Cébaco and the nearby Panamanian mainland. Further exploration of these findings will be required to interpret the taxonomic status of the Isla Cébaco population. At higher taxonomic levels, WEKSLER'S (2006) morphological and molecular assessment indicated that *Zygodontomys* was related to the oryzomine clade (WEKSLER 2006). Furthermore, *Scolomys* is considered to be sister taxon to *Zygodontomys* (WEKSLER 2006) and our expanded mitochondrial analysis is consistent with both of those views.

In summary, cytochrome-*b* haplotypes found in rodents of the genus *Zygodontomys* on Isla Coiba are not shared with

other populations and form a distinctive clade that is divergent at a level comparable to other sister-species comparisons in sigmodontine rodents (Tab. I). Further assessment of molecular and morphological variation is required to understand the taxonomic placement of these endemic rodents which could be useful indicators of overall island conservation status (AMORI *et al.* 2009). Because Isla Coiba was a penal colony for more than 70 years beginning in 1919, the island has been relatively undisturbed by humans. In 1991, Isla Coiba was declared a National Park (CASTROVIEJO *et al.* 1997). Protective status should help ensure the persistence of endemic forms so far identified

from this Pacific island, but substantial inventory and systematic work remains to document and fully understand the extent of endemism on islands in Central America.

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