# Sequence and structure of the mitochondrial control region of the Cuban rodent Capromys pilorides (Rodentia: Capromyidae)

∠ Alejandro Silva¹, Adriana Artiles<sup>2,3</sup>, William Suárez<sup>4</sup>, Gilberto Silva<sup>4</sup>

 <sup>1</sup>Grupo de Tecnología, Empresa de Gestión del Conocimiento y la Tecnología, GECYT Calle 20 e/ 41 y 47 #4110, Playa, La Habana, Cuba
<sup>2</sup>Laboratorio de Genética Molecular, Hospital Hermanos Ameijeiras San Lázaro 701 esq. Belascoaín, Centro Habana, CP 10 300, La Habana, Cuba
<sup>3</sup>Laboratorio de Sanidad Acuícola, Centro de Investigaciones Pesqueras, CIP 5ta. Avenida y 246, Barlovento, Santa Fe, Playa, CP 19100, La Habana, Cuba
<sup>4</sup>Departamento de Paleogeografía y Paleobiología, Museo Nacional de Historia Natural de Cuba, MNHNCu
Obispo 61, Plaza de Armas, Habana Vieja, CP 10100, La Habana, Cuba E-mail: alejo@gecyt.cu

# ABSTRACT

The complete mitochondrial DNA (mtDNA) control region from *Capromys pilorides*, an autochthon Cuban rodent, was sequenced and compared to two other species of hystricognath caviomorph rodents, in order to know patterns of variation and to explore the existence of previously described domains and other elements in rodents. The results revealed that the complete D-loop region of this species is 1336 base pairs long. Our data were compatible with the proposal of three domains [extended terminal associated sequences (ETAS), central (CD), and conserved sequence blocks (CSB)] within the control region, as well as the subsequences ETAS1, ETAS2, CSB1, CSB2, and CSB3. Likewise, a repetitive DNA region between the subsequences CSB1 and CSB2 was observed. The most conserved domain in the mitochondrial control region was the CD domain followed by ETAS and CSB domains in that order. The comparative analysis on base composition and genetic distance support the rationale of using the mitochondrial control region as well of useful markers for population genetic studies with application to the conservation of this and other related Cuban rodent species, some of them under severe extinction risk.

Keywords: Capromys pilorides, D-loop structure, rodents

Biotecnología Aplicada 2011;28:136-141

## RESUMEN

Secuencia y estructura de la región control mitocondrial del roedor cubano Capromys pilorides (Rodentia: Capromyidae). Con el objetivo de explorar los patrones de variación y la presencia de los dominios y subsecuencias se secuenció la región control mitocondrial (D-loop) completa de Capromys pilorides, un roedor autóctono cubano, y se comparó con la descripción de otros dos roedores hystricognathos caviomorfos. Los resultados mostraron que la región control mitocondrial completa de esta especie tiene con 1336 pares de bases, y se verificó la presencia de los dominios y las secuencias extendidas asociadas a la terminación (ETAS), central (CD), y bloque de secuencias conservadas (CSB) y las subsecuencias ETAS1, ETAS2, CSB1, CSB2, y CSB3. A su vez, se observó una región de ADN repetitivo entre las subsecuencias CSB1 y CSB2. La región más conservada resultó ser la correspondiente al dominio CD, a la que siguen los dominios ETAS y CSB. El análisis comparativo de la composición de bases entre dominios y de la distancia genética, apoya el propósito de utilizar estas secuencias como fuentes de marcadores útiles para los estudios de genética poblacional, con aplicación a la conservación de esta y otras especies de roedores cubanos afines, algunas de ellas en severo riesgo de extinción.

Palabras clave: Capromys pilorides, estructura D-loop, roedores

# **I**ntroduction

The maternal inheritance pattern of vertebrate mitochondrial DNA, together with the presence of orthologous genes in single copies, an extremely low recombination rate [1], high mutation rates [2] and number of copies that facilitates its amplification, have made this biomolecule an essential tool for studies in genetics, taxonomy, systematics and evolution, as well as the ideal target for genetic research on biodiversity conservation. Mitochondrial DNA has been the most recurrent source of molecular markers during the last three decades [3].

Mammalian mitochondrial genomes are closed double-stranded circular molecules containing 13

protein-coding genes, 2 ribosomal RNA genes and 22 tRNA genes. Non-coding regions are circumscribed to two areas, called the control region or D-loop, involved in the replication and transcription of these molecules, and the OL region, involved in replication initiation [4]. Studies have revealed that the most rapidly evolving part of the mitochondrial genome is the control region or D-loop [5]. Research on the mammalian D-loop [6] show that can be divided into 3 domains: Extended Termination-Associated Sequences (ETAS; from the proline tRNA to the central domain), the central domain (CD), and Conserved Sequence Blocks (CSB) (from the CD to the phenylalanine

1. Hurst GDD, Jiggins FM. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. Proc R Soc Lond B Biol Sci. 2005;272:1525-34.

 Nabholz B, Glémin S, Galtier N. Strong variations of mitochondrial mutation rate across mammals the longevity hypothesis. Mol Biol Evol. 2008;25(1):120-30.

3. Galtier N, Nabholz B, Glémin S, Hurst GD. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. Mol Ecol. 2009;18(22):4541-50. tRNA). Comparative studies of the mitochondrial control region (MCR) of mammals have demonstrated that each domain has a different pattern of variation, as ETAS and CSB evolve rapidly, whereas CD is strongly conserved between species [6, 7].

The analysis of 25 full-length MCR sequences from 23 species of the *Sciurognathi* and *Hystricognathi* suborders of the *Rodentia* order, plus one of *Lagomorph* order [8], suggested that the only sequence elements of this region that is conserved across all rodent species is the central domain (CD), a conserved region of the ETAS domain adjacent to CD called ETAS1, and the conserved sequence block 1 (CSB1) from domain CSB. The sample used in this study, however, included only 4 species of the *Hystricognathi* rodent suborder.

Efforts to map world biodiversity have uncovered around 2000 species of rodents; of which, more than 40 species and 12 genera have been discovered in neotropical zones alone since 1992 [9]. This mammalian group is increasingly vulnerable, as illustrated by the extinction of 50 to 51% of its species in the last 500 years [10]. There are 388 living species of island rodents, classified into 127 genera and 10 families. The *Capromyidae* family, endemic to the Antilles, belongs to the hystricognath caviomorph rodents of the New World, and represents the only endemic family exclusively composed of island dwellers [11].

*Capromyinae*, one of the subfamilies grouped into the *Capromyidae* family, contains all living and extinct species of hutia. Five genera with 26 species are currently recognized in this subfamily; of them, 17 (66%) are extinct. There are 7 living species in Cuba [12], five of which currently face the risk of extinction to certain degrees [13]. In addition, the living species of hutia represent the only examples of Cuban indigenous land mammals still observable in the wild, as the rest are either extinct or have not been sighted recently, as in the case of *Solenodon cubanus* [12].

*Capromys pilorides* (CP) is the most abundant and widely distributed capromid species in the Cuban archipelago, occupying widely dissimilar habitats and exhibiting an extensive phenotypic variability [14-17]. It therefore represents a prime candidate for studies of the sequence and structure of the D-loop region that may contribute to genetic research for conservation efforts targeted at these species.

To fulfill this objective, we have sequenced and determined the structure of the D-loop region of CP, which was then compared to those of two other hystricognath caviomorph rodents: *Cavia porcellus* (CV) and *Octodon degus* (OD).

#### **M**aterials and methods

#### Species included in the study

Table 1 contains relevant data on the species of this study, including their taxonomic classification at the family and suborder levels within the Rodentia order, as well as the GenBank accession number for the sequences used in the comparisons.

#### Extraction and amplification of DNA

Total DNA from two CP specimens belonging to the collection of frozen biological materials of the Nation-

#### Table 1. Rodent species included in this study

Species	Specimen	Family	Suborder	Accession Number <sup>a</sup>		
Capromys pilorides	MNHNCCu-25.0066	Capromyidae	Hystricognathi	FR686471		
Capromys pilorides	MNHNCCu-25.0067	Capromyidae	Hystricognathi	NE <sup>ь</sup>		
Octodon degus	ND	Octodontidae	Hystricognathi	AY007362		
Cavia porcellus	ND	Caviidae	Hystricognathi	AJ222767		
Mus musculus	ND	Muridae	Sciurognathi	AJ512208		
Rattus norvegicus	ND	Muridae	Sciurognathi	NM181627		

°GenBank/EMBL

<sup>b</sup>NE: Sequence does not exist in the database.

ND: Not determined.

al Museum of Natural History of Cuba was obtained from liver samples, using the *DNeasy Tissue* system (Qiagen, USA) and the protocol recommended by the manufacturer. This material was used to amplify a mitochondrial DNA fragment of approximately 2.3 kb long, containing the sequences for the 3' end of the cytochrome b gene, threonine and proline tRNA, the MCR, phenylalanine tRNA, and a portion of the 12s gene, using primers O-009 (5'-GCCTATGCCATC-CTACGCTC-3') and O-012 (5'-GGTGTGCTTGA-TACCCGCTC-3') (Figure 1). Both primers were designed based on published sequences of mitochondrial cytochrome b and 12s genes from CP [18, 19], using the FastPCR software application [20] (Figure 1).

The amplification reactions (PCR) were set up in a volume of 50  $\mu$ L, using the components of the *GoTAQ Core* system and 2.5 U of Taq polymerase, both obtained from Promega (USA). The amplification used an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of a denaturation step at 94 °C for 45 s, an annealing step at 58 °C for 45 s, and an extension step at 72 °C for 2.5 min, followed by a final single extension step at 72 °C for 10 min.

Amplification products were examined in 8% agarose gel in TBE buffer (Tris base 54 g/L, boric acid 27.5 g/L, 20 mL of 0.5 M EDTA pH 0.8), and the 2.3 kb product was purified with the *Wizard SV Gel and PCR Clean-Up System* from Promega (USA).

### Cloning and sequencing

The purified fragments were ligated into pGEMT-easy, using the conditions and components of the pGEM-T and pGEM-T Easy Vector Systems (Promega, USA). XL-1 Blue competent cells [21], obtained from the Center for Genetic Engineering and Biotechnology of Cuba, were transformed with the ligation mixture and the positive clones were selected on LBA plates (tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L, pH 7.2, agar 15 g/L, ampicillin 100 µg/mL) to which 40 µL of both 100 mM IPTG and X-gal at 20 mg/mL 4. Shadel GS, Clayton DA. Mitochondrial DNA maintenance in vertebrates. Annu Rev Biochem. 1997;66:409-35.

5. Saccone C, Lavane C, Pesole G, Sbisa E. Peculiar features and evolution of mitochondrial genomes in mammals. In: DiMauro S, Wallace DC, editors. Mitochondrial DNA in human pathology. New York: Raven Press;1993. p. 27-37.

6. Sbisà E, Tanzariello F, Reyes A, Pesole G, Saccone C. Mammalian mitochondrial D-loop region structural analysis: identification of new conserved sequences and their functional and evolutionary implications. Gene. 1997;205(1-2):125-40.

7. Pesole G, Gissi C, De Chirico A, Saccone C. Nucleotide substitution rate of mammalian mitochondrial genomes. J Mol Evol. 1999;48(4):427-34.

 Larizza A, Pesole G, Reyes A, Sbisà E, Saccone C. Lineage specificity of the evolutionary dynamics of the mtDNA Dloop region in rodents. J Mol Evol. 2002; 54(2):145-55.

9. Amori G, Gippoliti S. A higher-taxon approach to rodent conservation priorities for the 21st century. Anim Biodivers Conserv. 2003;26(2):1-18.

10. Macphee RDE, Flemming C. Requiem Aeternam. The last five hundred years of mammalian species extinctions. In: MacPhee RDE, editor. Extinctions in near time. New York: Kluwer Academic / Plenum Publisher. 1999; p. 333-71.

 Amori G, Gippoliti S, Helgen KM. Diversity, distribution, and conservation of endemic island rodents. Quat Int. 2008; 182:6-15.

 Silva T G, Duque S W, Diaz-Franco S. Mamíferos terrestres autóctonos de Cuba. Ciudad de la Habana: Ediciones Boloña: 2007.

 International Union for the Conservation of Nature (IUCN). Red list of threatened species [CD-ROM]. Cambridge: The IUCN Species Survival Commission, 2008.



Figure 1. Sketch of the amplified region of the mitochondrial genome of Capromys pilorides, displaying the approximate position of primers O-009 and O-012, used for initial amplification and later sequencing, and primers O-048 and O-049, used only during sequencing.

were added to facilitate the identification of recombinant clones.

Four white colonies and one blue colony obtained from the amplification of DNA from each CP specimen were submitted to colony PCR [22] to corroborate the presence of the 2.3 kb insert. Positive plasmids were purified with the *Wizard Plus SV Minipreps DNA Purification System* (Promega, USA), following the manufacturer's instructions.

Plasmid DNA samples were shipped to Macrogen (South Korea) for sequencing both strands with universal primers, and also primers O-048 (5'-TCTG-GTTCTTTCTTCAGG-3'), and O-049 (5'-GAGAT-GTCTTATTTAAGGG-3'), binding to a subsequence of the central domain (Figure 1). They were designed based on the MCR from CV, using the FastPCR software application [20].

#### Sequence analysis

MCR sequences from both CP specimens were aligned to their corresponding orthologs in CV and OD using Clustal X 2.0.10 [23], analyzing nucleotide composition with DAMBE v5.0.48 [24] and PAUP 4.10 beta [25]. Genetic distance values used to estimate sequence homology between the three species were calculated with MEGA 4.0 [26], using Kimura's 2-parameter evolution model (K2P) [27].

The presence or absence of the main subsequences (ETAS, CD and CSB) reported for mammalian [5, 6] and, specifically, rodent MCR [8], was ascertained by visual inspection, since they exhibited an acceptable level of homology. The absence or presence of the ETA2 subsequence was corroborated with a separate alignment that included rodent species *Mus musculus* and *Rattus norvegicus* which, unlike CV and OD, do have this subsequence previously identified.

#### **R**esults and discussion

# Sequence and characterization of the MCR from C. *pilorides*

Both CP specimens had an MCR that was 1336 bp long. As shown in previous studies of this region using mammals, and specifically rodents [5, 6, 8], it was also divided into a highly conserved central domain flanked by ETAS and CSB domains. There was also a repetitive DNA segment within the CSB domain (Figure 2). Given the high sequence identity (98%) of the two CP specimens included in this study, the results of their analysis will not be reported individually, but to the species in general.

#### **ETAS** domain

The ETAS domain is 350 bp long in CP. Two conserved subsequences have been described within this region; they are named ETAS1 and ETAS2. While ETAS2 is conserved across different mammalian species [5, 6, 28, 29], it is reportedly absent in certain rodents [8]. Using comparisons with MCR sequences from CV, OD, *M. musculus*, and *R. norvegicus*, it was possible to corroborate the presence of both subsequences in CP (Figure 1, supplementary material). Likewise, an ongoing phylogenetic study (Silva A, unpublished observations), using ETAS sequences from 20 species of hystricognath rodents, has also confirmed the

presence of ETA2 subsequences. Although a previous study reported a repetitive region within this domain in rodents [8], we did not find it in CP.

#### **Central Domain**

This domain is 309 bp long in CP. Subsequences A, B and C (Figure 2), involved in the binding of cytoskeletal elements associated to the mitochondria [30], were confirmed.

#### CSB domain

The CSB domain was 676 bp long in CP, structured into the three canonical sequence blocks of this region (CSB1, CSB2 and CSB3). Additionally, CSB from CP has a 300 bp-long repetitive DNA region between CSB1 and CSB2 (Figure 2), in agreement with previously published data for other mammals and, especially, rodents [5, 6, 8, 29, 31, 32]. In CP the repetitive DNA region is composed of 50 hexamers, not all of which are identical (Table 2).

#### Comparison to CV and OD

The fundamental goal of this study was to determine the sequence and structure of the mitochondrial control region of a representative species of Cuban rodents from the *Capromyinae* subfamily to apply molecular genetic tools to future efforts for their conservation. It was therefore necessary to compare the MCR sequence from CP to that from phylogenetically close rodents to evaluate the feasibility of using our results as the basis of future population, inter-species and supra-species studies.

The species chosen for the comparison, CV and OD, are also New World hystricognath rodents. OD is evolutionarily closer to CP than CV [33-36]; it is therefore expected to cluster with CP and away from CV on the basis of sequence similarity alone. Results shown on Table 3 confirm these expectations regarding both domain length and genetic distance (homology).

When comparing domain length (Table 3), however, there is an important disparity in the case of ETAS in OD. This is not a contradictory finding in itself, however, as the length of this domain is known to vary in rodents [8], although this is clearly not a conclusive structural and functional explanation. Apart from this exception, the remaining domains have similar lengths across all three species compared.

An examination of the calculated genetic distance values (Table 3) indicates that the homologies of domains ETAS and CD (Table 3) are similar to those described for other mammalian families [37, 38]. In the specific case of domain CD in the CP/OD pair, the computed genetic distances are even close to the average for genera within the same rodent family [39], although both species belong to different families (CP to *Capromyidae* and OD to *Octodontidae*). This confirms the close phylogenetic relationship of these families, which, not coincidentally, are grouped together in superfamily *Octodontoidea*.

CV, on the other hand, belongs to family *Caviidae* belonging to the *Cavioidea* superfamily. Consequently, its genetic divergence (inverse of homology) is larger when compared to the other two species, because they are not so closely related from an evolutionary viewpoint [37].

 Berovides AV, Alfonso SMA, Camacho PA. Variabilidad de la jutía conga Capromys pilorides (Rodentia, Capromyidae) de Cuba. Doñana Acta Vertebr. 1990;17(1):122-7.

 Berovides AV, Camacho PA, Comas GA, Borroto PR. Variación ecológica en poblaciones de la jutía conga Capromys pilorides (Rodentia, Capromyidae). Cienc Biol. 1990;23:44-58.

 Berovides AV, Gutiérrez AA. Grado de heterocigocidad y peso corporal de la jutía conga Capromys pilorides (Rodentia, Capromyidae). Rev. Biol .1999; 13(1):59-60.

17. Berovides AV.Variaciones morfofisiológicas en poblaciones de jutía conga Capromys pilorides (Rodentia, Capromyidae) en hábitats de bosques y manigua costera. Cubazoo 2006;13:11-5.

 Nedbal MA, Allard MW, Honeycutt RL. Molecular systematics of hystricognath rodents: evidence from the mitochondrial 12S rRNA gene. Mol Phylogenet Evol. 1994;3(3):206-20.

19. Leite YL, Patton JL. Evolution of South American spiny rats (Rodentia, Echimyidae): the star-phylogeny hypothesis revisited. Mol Phylogenet Evol. 2002; 25(3):455-64.

20. Kalendar R, Lee D, Schulman AH. FastPCR Software for PCR Primer and Probe Design and Repeat Search. In: Mansour A, editor. Focus on Bioinformatics. Genes, Genomes and Genomics. 2009;3(Special Issue 1):1-14.

21. Tu Z, He G, Li KX, Chen MJ, Chang J, Chen L, et al. An improved system for competent cell preparation and high efficiency plasmid transformation using different Escherichia coli strains. Electron J Biotechnol. 2005;8(1):113-20.

22. Zon Ll, Dorfman DM, Orkin SH. The polymerase chain reaction colony miniprep. Biotechniques, 1989;7(7):696-8.

23. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al. Clustal W and Clustal X version 2.0. Bioinformatics. 2007;23(21):2947-8.

24. Xia X. Data analysis in molecular biology and evolution. Boston/Dordrecht/ London: Kluwer Academic Publishers; 2000.

25. Swofford DL. PAUP: Phylogenetic analysis using parsimony (and other methods). Version 4. Sunderland: Sinauer Associates; 2000.

26. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol. 2007;24(8):1596-9.

27. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 1980;16(2):111-20.

 Reyes A, Nevo E, Saccone C. DNA sequence variation in the mitochondrial control region of subterranean mole rats, Spalax ehrenbergi superspecies, in Israel. Mol Biol Evol. 2003;20(4):622-32.

29. Matson CW, Baker RJ. DNA sequence variation in the mitochondrial control region of red-backed voles (*Clethrionomys*).

10	20	30	40	50	60	70	80	90
CCCCATATATTTA	TATAATGTAT	ATAATACACT	TTACTATGTA	CGTCGTGCAT	TAATGCATGT	CCCCATACAA	TATATGCAAG	AGTACAT
ETAS DOMAIN								
100	110	120	130	140	150	160	170	180
ATTATGTATAATA	GTACATAGAC	CATACTATGT	TTAATCAACA	TTAAACCTTT	GCCCCATGCA	TATAAGCATG	TTACCATTTA	ACTAAGC
	ETAS 1							
	200	21.0	220	220	240	250	260	270
CGTGCATAATACA					∠₄∪ ۵ͲႺ۵ͲͲ۵ͲϹϹ		∠00 ͲϪͲͲϹϪͲͲͲͲ	
ETAS 2								
ETAS DOMAIN								
280	290	300	310	320	330	340	350	360
ACATACAATGTGT	TATTATACAT	TAGTACATGT.	AATTAAATTA	TCCTTGTCAA	CACGTCTATT	ACTAACCATT.	AGAAATCTAT	TAATAAC
ETAS DOMAIN								
370	380	390	400	410	420	430	440	450
			GIGICCCCCT	CETEGETEEG	GGCCCATTAA	ATGTGGGGGT.	AGCTAGAGTG	AAACTTT
CENTRAL DOMAIN								
460	470	480	490	500	510	520	530	540
AACAGACATCTGG	TTCTTTCTTC	AGGGCCATAA	AATTCAAATT	GCTCATTCGT	TCCCTATAAA	TAAGACATCT	CGATGAAATT	GGGTCTA
SEQUENCE B					SEQUE	NCE C		
CENTRAL DOMAIN								
550	560	570	580	590	600	610	620	630
CTGGAAAGAAACC	CAGCAACAACC	TTACTAAATA	CATTTGGTAA	CTATTTAATT	TTAGGGATGC	TGTGACTCAG	CATAGCCGTC.	AAGGCAT
	CERT							
640	650	660	670	690	690	700	710	720
CAACCCTTCCAAC	000 'TTTAACTCTAC'	ਗ਼ਗ਼ਗ਼ ਗ਼			090 Сатаатаааа		╯⊥∪ C⊉⊉ͲͲϹͲͲͲͲ	
0121000011001210								
							CSB 1	
CSB DOMAIN							CSB 1	
CSB DOMAIN 730	740	750	760	770	780	790	CSB 1 800	810
CSB DOMAIN 730 GGAGGACATAAGA		750 ACATACACGC	760 ATACACACAC	770 ACATACACAC	780 ACGCATACAT	790 ACACACACAT	CSB 1 800 ACACACACAT.	810 ACGCATA
CSB DOMAIN 730 GGAGGACATAAGA CSB 1	740 AAAATTATACA	750 ACATACACGC. PETITIVE DNA	760 ATACACACAC	770 ACATACACAC	780 ACGCATACAT	790 ACACACACAT	CSB 1 800 ACACACACAT.	810 ACGCATA
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN	740	750 ACATACACGC	760 ATACACACAC	770 ACATACACAC	780 ACGCATACAT	790 ACACACACAT	CSB 1 800 ACACACACAT	810 ACGCATA
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820		750 ACATACACGC. PETITIVE DNA 840	760 ATACACACAC 850	770 ACATACACAC 860	780 ACGCATACAT 870	790 ACACACACAT. 880	CSB 1 800 ACACACACAT. 890	810 ACGCATA 900
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820 CGCACACACATAC	740 AAAATTATAC RE 830 CACATACACAT	750 ACATACACGC PETITIVE DNA 840 ACACATACAC	760 ATACACACAC 850 ATACACATAC	770 ACATACACAC 860 ACATACGCAT	780 ACGCATACAT 870 ACACATACAC	790 ACACACACAT 880 ATACACATAC	CSB 1 800 ACACACACAT. 890 ACATACACAT.	810 ACGCATA 900 ACACATA
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820 CGCACACACATAC REPETITIVE DNA CSB DOMAIN	740	750 ACATACACGC. PETITIVE DNA 840 ACACATACAC	760 ATACACACAC 850 ATACACATAC	770 ACATACACAC 860 ACATACGCAT	780 ACGCATACAT 870 ACACATACAC	790 ACACACACAT 880 ATACACATAC	CSB 1 800 ACACACACAT. 890 ACATACACAT.	810 ACGCATA 900 ACACATA
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820 CGCACACACATAC REPETITIVE DNA CSB DOMAIN 910	740 AAAATTATACA 830 CACATACACATA 920	750 ACATACACGC. PETITIVE DNA 840 ACACATACAC. 930	760 ATACACACAC 850 ATACACATAC 940	770 ACATACACAC 860 ACATACGCAT 950	780 ACGCATACAT 870 ACACATACAC 960	790 ACACACACAT. 880 ATACACATAC. 970	CSB 1 800 ACACACACAT. 890 ACATACACAT. 980	810 ACGCATA 900 ACACATA 990
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820 CGCACACACATAC REPETITIVE DNA CSB DOMAIN 910 CGCATACGCATAC	740 AAAATTATAC 830 CACATACACAT 920 CGCATACACAT	750 ACATACACGC. PETITIVE DNA 840 ACACATACAC. 930 ACGCATACAC.	760 ATACACACAC 850 ATACACATAC 940 ATACACATAC	770 ACATACACAC 860 ACATACGCAT 950 ACATACACAT	780 ACGCATACAT 870 ACACATACAC 960 ACACACACAT	790 ACACACACAT. 880 ATACACATAC. 970 ACGCATACAC.	CSB 1 800 ACACACACAT. 890 ACATACACAT. 980 ATACACATAC.	810 ACGCATA 900 ACACATA 990 ACATACA
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820 CGCACACACATAC REPETITIVE DNA CSB DOMAIN 910 CGCATACGCATAC REPETITIVE DNA	740 AAAATTATAC 830 ACATACACAT 920 CGCATACACAT	750 ACATACACGC. PETITIVE DNA 840 ACACATACAC. 930 ACGCATACAC.	760 ATACACACAC 850 ATACACATAC 940 ATACACATAC	770 ACATACACAC 860 ACATACGCAT 950 ACATACACAT	780 ACGCATACAT 870 ACACATACAC 960 ACACACACAT	790 ACACACACAT. 880 ATACACATAC. 970 ACGCATACAC.	CSB 1 800 ACACACACAT. 890 ACATACACAT. 980 ATACACATAC.	810 ACGCATA 900 ACACATA 990 ACATACA
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820 CGCACACACATAC REPETITIVE DNA CSB DOMAIN 910 CGCATACGCATAC REPETITIVE DNA CSB DOMAIN	740 AAAATTATAC 830 CACATACACATA 920 CGCATACACATA	750 ACATACACGC. PETITIVE DNA 840 ACACATACAC. 930 ACGCATACAC.	760 ATACACACAC 850 ATACACATAC 940 ATACACATAC	770 ACATACACAC 860 ACATACGCAT 950 ACATACACAT	780 ACGCATACAT 870 ACACATACAC 960 ACACACACAT	790 ACACACACATA 880 ATACACATAC 970 ACGCATACAC	CSB 1 800 ACACACACAT. 890 ACATACACAT. 980 ATACACATAC.	810 ACGCATA 900 ACACATA 990 ACATACA
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820 CGCACACACATAC REPETITIVE DNA CSB DOMAIN 910 CGCATACGCATAC REPETITIVE DNA CSB DOMAIN 1000	740 AAAATTATAC: 830 ACATACACAT: 920 GCATACACAT: 1010	750 ACATACACGC PETITIVE DNA 840 ACACATACAC 930 ACGCATACAC 1020	760 ATACACACAC 850 ATACACATAC 940 ATACACATAC 1030	770 ACATACACAC 860 ACATACGCAT 950 ACATACACAT 1040	780 ACGCATACAT 870 ACACATACAC 960 ACACACACAT 1050	790 ACACACACATA 880 ATACACATAC 970 ACGCATACAC 1060	CSB 1 800 ACACACACAT. 890 ACATACACAT. 980 ATACACATAC. 1070	810 ACGCATA 900 ACACATA 990 ACATACA 1080
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820 CGCACACACATAC REPETITIVE DNA CSB DOMAIN 910 CGCATACGCATAC REPETITIVE DNA CSB DOMAIN 1000 CATACGCATACGC	740 AAAATTATAC 830 ACATACACAT 920 CGCATACACAT 1010 ATACGCATAC	750 ACATACACGC PETITIVE DNA 840 ACACATACAC 930 ACGCATACAC 1020 GCATACGCAT	760 ATACACACAC 850 ATACACATAC 940 ATACACATAC 1030 ACGCATACGT	770 ACATACACAC 860 ACATACGCAT 950 ACATACACAT 1040 ACGTACGTAT	780 ACGCATACAT 870 ACACATACAC 960 ACACACACAT 1050 TAATTACCAA	790 ACACACACATA 880 ATACACATAC 970 ACGCATACAC 1060 TAATTATCTT	CSB 1 800 ACACACACATA 890 ACATACACATA 980 ATACACATAC 1070 TTAACAAACC	810 ACGCATA 900 ACACATA 990 ACATACA 1080 CCCCTTA
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820 CGCACACACATAC REPETITIVE DNA CSB DOMAIN 910 CGCATACGCATAC REPETITIVE DNA CSB DOMAIN 1000 CATACGCATACGC REPETITIVE DNA	740 AAAATTATAC: RE 830 ACATACACAT: 920 GCATACACAT: 1010 ATACGCATACO	750 ACATACACGC PETITIVE DNA 840 ACACATACAC 930 ACGCATACAC 1020 GCATACGCAT	760 ATACACACAC 850 ATACACATAC 940 ATACACATAC 1030 ACGCATACGT	770 ACATACACAC 860 ACATACGCAT 950 ACATACACAT 1040 ACGTACGTAT	780 ACGCATACAT 870 ACACATACAC 960 ACACACACAT 1050 TAATTACCAA	790 ACACACACATA 880 ATACACATAC 970 ACGCATACAC 1060 TAATTATCTT	CSB     1       800     800       ACACACACAT.     890       ACATACACAT.     980       ATACACATAC.     1070       TTAACAAAACC     CSB	810 ACGCATA 900 ACACATA 990 ACATACA 1080 CCCCTTA
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820 CGCACACACATAC REPETITIVE DNA CSB DOMAIN 910 CGCATACGCATAC REPETITIVE DNA CSB DOMAIN 1000 CATACGCATACGC REPETITIVE DNA CSB DOMAIN	740 AAAATTATACA 830 CACATACACATA 920 CGCATACACATA 1010 CATACGCATACO	750 ACATACACGC. PETITIVE DNA 840 ACACATACAC. 930 ACGCATACAC. 1020 GCATACGCAT.	760 ATACACACAC 850 ATACACATAC 940 ATACACATAC 1030 ACGCATACGT	770 ACATACACAC 860 ACATACGCAT 950 ACATACACAT 1040 ACGTACGTAT	780 ACGCATACAT 870 ACACATACAC 960 ACACACACAT 1050 TAATTACCAA	790 ACACACACATA 880 ATACACATAC 970 ACGCATACAC 1060 TAATTATCTT	CSB 1 800 ACACACACAT. 890 ACATACACAT. 980 ATACACATAC. 1070 TTAACAAACC CSB 2 1160	810 ACGCATA 900 ACACATA 990 ACATACA 1080 CCCCTTA
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820 CGCACACACATAC REPETITIVE DNA CSB DOMAIN 910 CGCATACGCATAC REPETITIVE DNA CSB DOMAIN 1000 CATACGCATACGC REPETITIVE DNA CSB DOMAIN 1090	740 AAAATTATACA 830 CACATACACATA 920 CGCATACACATA 1010 CATACGCATACO 1100	750 ACATACACGC. PETITIVE DNA 840 ACACATACAC. 930 ACGCATACAC. 1020 GCATACGCAT. 1110	760 ATACACACAC 850 ATACACATAC 940 ATACACATAC 1030 ACGCATACGT 1120	770 ACATACACAC 860 ACATACGCAT 950 ACATACACAT 1040 ACGTACGTAT	780 ACGCATACAT 870 ACACATACAC 960 ACACACACAT 1050 TAATTACCAA 1140	790 ACACACACAT. 880 ATACACATAC. 970 ACGCATACAC. 1060 TAATTATCTT 1150	CSB 1     800     ACACACACAT.     890     ACATACACAT.     980     ATACACATAC.     1070     TTAACAAACC     CSB 2     1160     CCADAAACAACAACAACAACAAACAAACAAACAAAACA	810 ACGCATA 900 ACACATA 990 ACATACA 1080 CCCCTTA
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820 CGCACACACATAC REPETITIVE DNA CSB DOMAIN 910 CGCATACGCATAC REPETITIVE DNA CSB DOMAIN 1000 CATACGCATACGC REPETITIVE DNA CSB DOMAIN 1090 CCCCCCCCATAAAA	740 AAAATTATACA 830 CACATACACATA 920 CGCATACACATA 1010 CATACGCATACA 1100 ATTACAAATTTA	750 ACATACACGC. PETITIVE DNA 840 ACACATACAC. 930 ACGCATACAC. 1020 GCATACGCAT. 1110 AATACATAGG	760 ATACACACAC 850 ATACACATAC 940 ATACACATAC 1030 ACGCATACGT 1120 CATTTAATCC	770 ACATACACAC 860 ACATACGCAT 950 ACATACACAT 1040 ACGTACGTAT 1130 CATGTACCGT	780 ACGCATACAT 870 ACACATACAC 960 ACACACACAT 1050 TAATTACCAA 1140 ACTTGATATC	790 ACACACACAT. 880 ATACACATAC. 970 ACGCATACAC. 1060 TAATTATCTT 1150 CTGCCAAACC CSE 3	CSB 1     800     ACACACACAT.     890     ACATACACAT.     980     ATACACATAC.     1070     TTAACAAACC     CSB 2     1160     CCAAAAACAA	810 ACGCATA 900 ACACATA 990 ACATACA 1080 CCCCTTA 1170 GAGGGAC
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820 CGCACACACATAC REPETITIVE DNA CSB DOMAIN 910 CGCATACGCATAC REPETITIVE DNA CSB DOMAIN 1000 CATACGCATACGC REPETITIVE DNA CSB DOMAIN 1090 CCCCCCCCATAAAA CSB 2 CSB DOMAIN	740 AAAATTATAC: RE 830 CACATACACAT: 920 CGCATACACAT: 1010 CATACGCATACO 1100 ATTACAAATTT:	750 ACATACACGC. PETITIVE DNA 840 ACACATACAC. 930 ACGCATACAC. 1020 GCATACGCAT. 1110 AATACATAGG	760 ATACACACAC 850 ATACACATAC 940 ATACACATAC 1030 ACGCATACGT 1120 CATTTAATCC	770 ACATACACAC 860 ACATACGCAT 950 ACATACACAT 1040 ACGTACGTAT 1130 CATGTACCGT	780 ACGCATACAT 870 ACACATACAC 960 ACACACACAT 1050 TAATTACCAA 1140 ACTTGATATC	790 ACACACACATA 880 ATACACATAC 970 ACGCATACAC 1060 TAATTATCTT 1150 CTGCCAAACC CSE 3	CSB 1     800     ACACACACAT.     890     ACATACACAT.     980     ATACACATAC.     1070     TTAACAAACC     CSB 2     1160     CCAAAAACAA	810 ACGCATA 900 ACACATA 990 ACATACA 1080 CCCCTTA 1170 GAGGGAC
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820 CGCACACACATAC REPETITIVE DNA CSB DOMAIN 910 CGCATACGCATAC REPETITIVE DNA CSB DOMAIN 1000 CATACGCATACGC REPETITIVE DNA CSB DOMAIN 1090 CCCCCCCATAAAA CSB 2 CSB DOMAIN 1180	740 AAAATTATAC: RE 830 ACATACACAT: 920 GCATACACAT: 1010 CATACGCATACC 1100 ATTACAAATTT: 1190	750 ACATACACGC. PETITIVE DNA 840 ACACATACAC. 930 ACGCATACAC. 1020 GCATACGCAT. 1110 AATACATAGG 1200	760 ATACACACAC 850 ATACACATAC 940 ATACACATAC 1030 ACGCATACGT 1120 CATTTAATCC 1210	770 ACATACACAC 860 ACATACGCAT 950 ACATACACAT 1040 ACGTACGTAT 1130 CATGTACCGT	780 ACGCATACAT 870 ACACATACAC 960 ACACACACAT 1050 TAATTACCAA 1140 ACTTGATATC	790 ACACACACATA 880 ATACACATAC 970 ACGCATACAC 1060 TAATTATCTT 1150 CTGCCAAACC CSB 3 1240	CSB 1     800     ACACACACAT.     890     ACATACACAT.     980     ATACACATAC.     1070     TTAACAAAACC     CSB 2     1160     CCAAAAAACAA     1250	810 ACGCATA 900 ACACATA 990 ACATACA 1080 CCCCTTA 1170 GAGGGAC
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820 CGCACACACATAC REPETITIVE DNA CSB DOMAIN 910 CGCATACGCATAC REPETITIVE DNA CSB DOMAIN 1000 CATACGCATACGC REPETITIVE DNA CSB DOMAIN 1090 CCCCCCCATAAAA CSB 2 CSB DOMAIN 1180 AAGCACAAAAATC	740 AAAATTATAC: RE 830 ACATACACAT: 920 GCATACACAT: 1010 ATACGCATACO 1100 ATACAAATTT: 1190 TAATATTTTA	750 ACATACACGC. PETITIVE DNA 840 ACACATACAC. 930 ACGCATACAC. 1020 GCATACGCAT. 1110 AATACATAGG 1200 CGATCTTCCT	760 ATACACACAC 850 ATACACATAC 940 ATACACATAC 1030 ACGCATACGT 1120 CATTTAATCC 1210 GATACTGTAT	770 ACATACACAC 860 ACATACGCAT 950 ACATACACAT 1040 ACGTACGTAT 1130 CATGTACCGT 1220 CATAGAGTGC	780 ACGCATACAT 870 ACACATACAC 960 ACACACACAT 1050 TAATTACCAA 1140 ACTTGATATC 1230 AAAAAATAAA	790 ACACACACATA 880 ATACACATAC 970 ACGCATACAC 1060 TAATTATCTT 1150 CTGCCAAACC CSB 3 1240 ATTTAACCCT	CSB 1 800 ACACACACAT. 890 ACATACACAT. 980 ATACACATAC. 1070 TTAACAAACCA CSB 2 1160 CCAAAAACAA 1250 CATGTCAGTA	810 ACGCATA 900 ACACATA 990 ACATACA 1080 CCCCTTA 1170 GAGGGAC 1260 CAATATG
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820 CGCACACACATAC REPETITIVE DNA CSB DOMAIN 910 CGCATACGCATAC REPETITIVE DNA CSB DOMAIN 1000 CATACGCATACGC REPETITIVE DNA CSB DOMAIN 1090 CCCCCCCATAAAA CSB 2 CSB DOMAIN 1180 AAGCACAAAAATC	740 AAAATTATAC: 830 ACATACACAT 920 CGCATACACAT 1010 ATACGCATACC 1100 ATACGCATACC 1100 ATACAAATTT	750 ACATACACGC. PETITIVE DNA 840 ACACATACAC 930 ACGCATACAC. 1020 GCATACGCAT. 1110 AATACATAGG 1200 CGATCTTCCT	760 ATACACACAC 850 ATACACATAC 940 ATACACATAC 1030 ACGCATACGT 1120 CATTTAATCC 1210 GATACTGTAT	770 ACATACACAC 860 ACATACGCAT 950 ACATACACAT 1040 ACGTACGTAT 1130 CATGTACCGT 1220 CATAGAGTGC	780 ACGCATACAT 870 ACACATACAC 960 ACACACACAT 1050 TAATTACCAA 1140 ACTTGATATC 1230 AAAAAATAAA	790 ACACACACATA 880 ATACACATAC 970 ACGCATACAC 1060 TAATTATCTT 1150 CTGCCAAACC CSE 3 1240 ATTTAACCCT	CSB 1 800 ACACACACAT. 890 ACATACACAT. 980 ATACACATAC. 1070 TTAACAAACCA CSB 2 1160 CCAAAAACAA 1250 CATGTCAGTA	810 ACGCATA 900 ACACATA 990 ACATACA 1080 CCCCTTA 1170 GAGGGAC 1260 CAATATG
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820 CGCACACACATAC REPETITIVE DNA CSB DOMAIN 910 CGCATACGCATAC REPETITIVE DNA CSB DOMAIN 1000 CATACGCATACGC REPETITIVE DNA CSB DOMAIN 1090 CCCCCCCCATAAAA CSB 2 CSB DOMAIN 1180 AAGCACAAAAATC	740 AAAATTATAC: 830 ACATACACAT: 920 GCATACACAT: 1010 ATACGCATACO 1100 ATTACAAATTT: 1190 TAATATTTTAC	750 ACATACACGC. PETITIVE DNA 840 ACACATACAC. 930 ACGCATACAC. 1020 GCATACGCAT. 1110 AATACATAGG 1200 CGATCTTCCT	760 ATACACACAC 850 ATACACATAC 940 ATACACATAC 1030 ACGCATACGT 1120 CATTTAATCC 1210 GATACTGTAT	770 ACATACACAC 860 ACATACGCAT 950 ACATACACAT 1040 ACGTACGTAT 1130 CATGTACCGT 1220 CATAGAGTGC	780 ACGCATACAT 870 ACACATACAC 960 ACACACACAT 1050 TAATTACCAA 1140 ACTTGATATC 1230 AAAAAATAAA	790 ACACACACATA 880 ATACACATAC 970 ACGCATACACA 1060 TAATTATCTT 1150 CTGCCAAACC CSE 3 1240 ATTTAACCCT	CSB 1 800 ACACACACATA 890 ACATACACATA 980 ATACACATACA 1070 TTAACAAACC CSB 2 1160 CCAAAAACAA 1250 CATGTCAGTA	810 ACGCATA 900 ACACATA 990 ACATACA 1080 CCCCTTA 1170 GAGGGAC 1260 CAATATG
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820 CGCACACACATAC REPETITIVE DNA CSB DOMAIN 910 CGCATACGCATAC REPETITIVE DNA CSB DOMAIN 1000 CATACGCATACGC REPETITIVE DNA CSB DOMAIN 1090 CCCCCCCATAAAA CSB DOMAIN 1090 CCCCCCCATAAAA CSB DOMAIN 1180 AAGCACAAAAAATC	740 AAAATTATAC: RE 830 ACATACACAT 920 CGCATACACAT 1010 ATTACGCATACCATAC 1100 ATTACAAATTT 1190 TAATATTTTAC	750 ACATACACGC PETITIVE DNA 840 ACACATACAC 930 ACGCATACACA 1020 GCATACGCAT 1110 AATACATAGG 1200 CGATCTTCCT 1290	760 ATACACACAC 850 ATACACATAC 940 ATACACATAC 1030 ACGCATACATAC 1120 CATTTAATCC 1210 GATACTGTAT	770 ACATACACAC 860 ACATACGCAT 950 ACATACACAT 1040 ACGTACGTAT 1130 CATGTACCGT 1220 CATAGAGTGC	780 ACGCATACAT 870 ACACATACAC 960 ACACACACACAT 1050 TAATTACCAA 1140 ACTTGATATC 1230 AAAAAATAAA 1320	790 ACACACACATA 880 ATACACATAC 970 ACGCATACACA 1060 TAATTATCTT 1150 CTGCCAAACC CSB 3 1240 ATTTAACCCT	CSB 1     800     ACACACACATA     890     ACATACACATA     980     ATACACATACATAC     1070     TTAACAAACC     CSB 2     1160     CCAAAAACAA     1250     CATGTCAGTA     1336	810 ACGCATA 900 ACACATA 990 ACATACA 1080 CCCCTTA 1170 GAGGGAC 1260 CAATATG
CSB DOMAIN 730 GGAGGACATAAGA CSB 1 CSB DOMAIN 820 CGCACACACATAC REPETITIVE DNA CSB DOMAIN 910 CGCATACGCATAC REPETITIVE DNA CSB DOMAIN 1000 CATACGCATACGC REPETITIVE DNA CSB DOMAIN 1090 CCCCCCCCATAAAA CSB 2 CSB DOMAIN 1180 AAGCACAAAAATC CSB DOMAIN 1180 AAGCACAAAAAATC	740 AAAATTATAC: 830 ACATACACAT: 920 GCATACACAT: 1010 ATACGCATACC 1100 ATACGCATACC 1190 TAATATTTAC 1280 ACAGAGTGCA:	750 ACATACACGC. PETITIVE DNA 840 ACACATACAC. 930 ACGCATACAC. 1020 GCATACGCAT. 1110 AATACATAGG 1200 CGATCTTCCT 1290 TTTATTTGCA	760 ATACACACAC 850 ATACACATAC 940 ATACACATAC 1030 ACGCATACGT 1120 CATTTAATCC 1210 GATACTGTAT 1300 CTTGCCTATG	770 ACATACACAC 860 ACATACGCAT 950 ACATACACAT 1040 ACGTACGTAT 1130 CATGTACCGT 1220 CATAGAGTGC 1310 TAATATTTT	780 ACGCATACAT 870 ACACATACAC 960 ACACACACACAT 1050 TAATTACCAA 1140 ACTTGATATC 1230 AAAAAATAAA 1320 CACGTCTAAT	790 ACACACACATA 880 ATACACATAC 970 ACGCATACACA 1060 TAATTATCTT 1150 CTGCCAAACC CSE 3 1240 ATTTAACCCT 1330 ACAGCCCTCT	CSB 1     800     ACACACACAT.     890     ACATACACAT.     980     ATACACATAC     1070     TTAACAAACC     CSB 2     1160     CCAAAAACAA     1250     CATGTCAGTA     1336     TTC	810 ACGCATA 900 ACACATA 990 ACATACA 1080 CCCCTTA 1170 GAGGGAC 1260 CAATATG

Figure 2. Complete sequence of the mitochondrial control region of Capromys pilorides, showing the ETAS, Central and CSB domains and subsequences ETAS1, ETAS2, A, B, C, CSB1, CSB2, CSB3 and repetitive DNA.

The above results are confirmed on examining the alignments for domains ETAS (Figure 1, supplementary material), CD (Figure 2, supplementary material) and CSB (Figure 3, supplementary material; excluding the repetitive DNA region from each species) as well as Table 3.

The three alignments demonstrate a greater similarity between domain sequences from CP and OD, evidencing that the incidence of insertions and deletions between these two species is much lower to that of these two and CV.

The largest genetic distance, largest numbers of insertions and deletions, and highest proportion of insertions and deletions larger than 1 bp (parentheses in Table 3, insertions/deletions) are observed in the specific case of domain CSB, confirming the greater variability of this domain compared to ETAS and CD. This is even more evident in CV in respect to the other two species, underscoring once again the degree of evolutionary divergence between these species.

A repetitive DNA region was also observed in domain CSB for the three species, located between subsequences CSB1 and CSB2. This region, however, had inter-species differences for the number of repeats and their composition (Table 2). For instance, CV had several copies of a single repeat, whereas CP and OD were heterogeneous in repeat sequence and numbers.

The presence of repetitive DNA in mammalian CSB domains has been well documented. This repetitive region is highly variable, and may even be entirely absent [5, 6, 8]. In any case, its role within the context of the mitochondrial control region is still unknown.

The alignments for domain CSB (Figure 3, supplementary material) demonstrate the presence of sequence blocks CSB1, CSB2, and CSB3, with a high degree of sequence conservation except for small variations in CSB1 (Figure 3). These three blocks are not a conserved, general feature in rodents or in mammals, in general, since out of the 7 hystricognath with published full-length sequences of the mitochondrial control region, only those examined here have all three blocks present.

In summary, despite the availability of previous sequences from CP and other capromids published in the literature in studies of intra- and supra-species phylogenetic relationships within the *Capromyidae* family [18, 19, 40], this is the first published full-length D-loop sequence for a member of this taxon, and does not only enhance the knowledge on the ge-



Figure 3. Alignment of sequence blocks CSB1, CSB2 and CSB3 from domain CSB in Capromys pilorides (CP), Octodon degus (OD) and Cavia porcellus (CV). CSB2 and CSB3 are highly conserved, whereas CSB1 differentiates between the three species. Each subsequence was colored so as to highlight differences compared to the consensus (Cons.).

Table 2 Composition of	the renetitive	DNA region in	the three studied	snecies
Tuble 2. Composition of	me repennie	DIALICGIONI	The fiffee stouled	species

		5	
Species	Repeat Unit	Bases/Unit	Copies
Cavia porcellus	GTACGCACAGACGTGT	16	19
Octodon degus	TACACACGTA	12	11
	TACACACGTA	10	8
	TACGCACACGTA	12	7
	CGTACACACGTA	12	1
	TACACACA	8	1
			(28)°
Capromys pilorides	CATACA	6	21
	CATACG	6	7
	TACGTA	6	7
	TACACA	6	6
	CACACA	6	4
	TACGTA	6	2
	CACATA	6	1
	CACACG	6	1
	TACACG	6	1
			(50)°

<sup>a</sup>Total number of copies.

Table 3. Comparison of Capromys pilorides (CP), Octodon degus (OD), and Cavia porcellus (CV) in domain length, sequence homology and deletions

				-												
Domain length (bp)					Homology (%)ª					Insertions/deletions						
	Species	<b>ETAS</b> <sup>ь</sup>	CD	CSBd	ET.	AS	С	Ď	ĊCS	B*	ET,	AS	С	D	CS	B*
					1	2	1	2	1	2	1	2	1	2	1	2
	CP	350	309	677	-	-	-	-	-	-	-	-	-	-	-	-
	OD	266	310	679	73	-	84	-	69	-	2	-	0	-	8(2	2) -
	CV	351	315	684	49	50	60	68	54	60	5	7	7	7	16	15
															(12)	(11)

<sup>©</sup>K2P homology. Numbers in parenthesis refer to the number of insertions and deletions longer than 1 bp. <sup>b</sup>ETAS: Extended termination-associated sequences.

<sup>c</sup>CD: Central domain.

<sup>d</sup>CSB: Conserved sequence block

\*Excluding the repetitive DNA region.

netic resources of our country, but it is a starting point for exploring this region in mitochondrial DNA of other Cuban capromid species.

Results indicate that the sequence and structure of the MCR in CP correspond to those published for other rodents, in complete agreement with already established phylogenetic relationships within the Hystricognathi suborder.

The strong homology between the two full-length MCR CP specimens sequences (98%), and the coherence of the values obtained from comparisons of ETAS and CD domains between species, regarding their length, genetic distance and number of insertions and deletions with those obtained for these domains in other rodents [41-43] in population genetics studies, lead to the conclusion that these sequences may be useful for population studies of Cuban capromids focused on their conservation.

Mol Biol Evol. 2001;18(8):1494-501.

30. Jackson DA, Bartlett J, Cook PR. Sequences attaching loops of nuclear and mitochondrial DNA to underlying structures in human cells: the role of transcription units. Nucleic Acids Res. 1996;24(7):1212-9.

31. Gemmell NJ, Western PS, Watson JM, Graves JA. Evolution of the mammalian mitochondrial control region-comparisons of control region sequences between monotreme and therian mammals. Mol Biol Evol. 1996;13(6):798-808.

32. Stewart DT, Baker AJ. Patterns of sequence variation in the mitochondrial D-loop region of shrews. Mol Biol Evol. 1994;11(1):9-21.

33. Huchon D, Douzery EJ. From the Old World to the New World: a molecular chronicle of the phylogeny and biogeogra-

# **A**cknowledgements

The authors wish to thank the direction of the Molecular Genetics Laboratory of Hermanos Ameijeiras Hospital, its specialists and the hospital management for the use of their facilities and their constant support during the experimental stage of this study. This was a project funded by the World Wildlife Fund (WWF) of Canada.

phy of hystricognath rodents. Mol Phylogenet Evol. 2001;20(2):238-51.

34. Honeycutt RL, Rowe DL, Gallardo MH. Molecular systematics of the South American caviomorph rodents: relationships among species and genera in the family Octodontidae. Mol Phylogenet Evol. 2003;26(3):476-89.

35. Opazo JC. A molecular timescale for caviomorph rodents (Mammalia, Hystricognathi). Mol Phylogenet Evol. 2005;37(3):932-7.

36. Galewski T, Mauffrey JF, Leite YL, Patton JL, Douzery EJ. Ecomorphological diversification among South American spiny rats (Rodentia; Echimyidae): a phylogenetic and chronological approach. Mol Phylogenet Evol. 2005; 34(3):601-15.

Received in August, 2010. Accepted for publication in June, 2011.

 Johns GC, Avise JC. A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome b gene. Mol Biol Evol. 1998;15(11):1481-90.

38. Castresana J. Cytochrome b phylogeny and the taxonomy of great apes and mammals. Mol Biol Evol. 2001;18(4):465-71.

39. Bradley RD, Baker RJ. A test of the genetic species concept: Cytocrhome b sequences and mammals. J Mammal. 2001;82(4):960-73.

40. Woods CA, Borroto R, Kilpatrick CW. Insular patterns and radiations of West Indian rodents. In: Woods CA, Sergile FE, editors. Biogeography of the West Indies: patterns and perspectives. 2nd ed. Boca Raton: CRC Press, 2002. p. 335-53.

41. Ojeda AA. Phylogeography and genetic variation in the South American rodent Tympanoctomys barrerae (Rodentia: Octodonti-dae). J Mammal. 2010;91(2):302-13.

42. Meshchersky IG, Feoktistova NY. Intraspecific organization of dwarf hamsters Phodopus campbelli and Phodopus sungorus (Rodentia: Cricetinae) based on mtDNA analysis. Dokl Biol Sci. 2009;424:35-8.

43. Trucchi E, Gentile G, Sbordoni V. Development of primers to amplify mitochondrial DNA control region of Old World porcupines (subgenus Hystrix). Mol Ecol Resour. 2008;8(5):1139-41.

# Suplementary material



Figure 1. Alignment of the ETAS domain sequences of Capromys pilorides (CP), Octodon degus (OD), Cavia porcellus (CV), Mus musculus (MM) and Rattus norvegicus (RN), showing the ETAS1 and ETAS2 sequence blocks.

Consen	btettaa-täaecateeteögagaaaccateaaecegeexggeaggtgteeeeteeteeteegggeeeataaaiiegte
1. CV	TTC <b>A</b> TAA <b>BCCAG</b> CATCCTCCGTGAAACCAGCAACCCGCTAG <b>A</b> CAGGG <b>A</b> TCCCTCTGCTCGCCCCGGGCCCATAGACCGTG
2.00	NTCTTAA-TAACCATCCTC <mark>GLGGAAACCATCAACCC</mark> GCCAGGCAGGTGTC <b>M</b> CCCTCCTCGCTCCGGGCCCATAAAMCGTG
3. CP	TTAA-TAACCATCCTC
Consen	SCGGTAGETÄDANTGAAAE <sup>10</sup> TTTAACAGAC <sup>10</sup> TETGGTTET <sup>10</sup> TETTCAGGG <sup>10</sup> CATAGAATT <sup>10</sup> ANCTBGETC <sup>20</sup> TTEGTTEC <sup>10</sup>
1. CV	GGGGT <b>TA</b> CTA <b>RAACTGCCT</b> TTTAA <mark>G</mark> ACACCTGGTTCTTTCTTCAGGGCCATAGEATTAAECTEGCTCATTCGTTCCCC
2.00	3GGGTAGCTA <b>mam</b> tgaaactttaaca <mark>cacatctggttctttcttcagggccat</mark> agaattaa <mark>g</mark> ct <mark>g</mark> gctcattcgttcc <b>m</b> c
3. CP	3GGGTAGCTA <mark>G</mark> AGTGAAACTTTAACA <mark>CATCTGGTTCTTTCTTCAGGGCCAT</mark> A <b>M</b> AATT <b>U</b> AMAT <b>U</b> GCTCATTCGTTCCC <b>U</b>
	SUBSECCENCIAC
Consen	PTAAATAAGÃCATOTEGATĜWAATDAGGTÊTACTGGEŴAGAAACCAAĈAACACTWTAÃTTCAATACAÊTTGGTAACTÃ
Consen 1. GV	P <mark>TAAATAAGÄCATOTOGATČMAATDAGGTÖT</mark> – <mark>AGTGGOŴAGAAACCAA</mark> ÄAAC <mark>ACTWTAÄTTCAATACAÄTTGGT</mark> AACTÄ ITAAATAAGACATCTCGATG <mark>G</mark> AETAA <b>TTA</b> CT <mark>NG</mark> ACTGGC <b>ECATG</b> ACCAACA <b>TA</b> ACT <mark>G</mark> GAATTCCATGCATTGGT – ATTT
Consen 1. GV 2. OD	ITAAATAAC <mark>XATTTCGATCWAATDAGGTÖT – ACTGGCWAGAAATCAAXAACATWTAXTTCAATACAXTTGGTAACTX</mark> ITAAATAAGACATCTCGATGGA <b>TTACTNG</b> ACTGGCCAATGACCAACATAACTGGAATTCGAATTCGGT-ATTT ITAAATAAGACATCTCGATG <b>CU</b> AGGAGGTCT – ACTGGC <mark>G</mark> AGAAACCAACAACACATATAATACAAGACATTTGGTAACTG
Consen 1. GV 2. OD 3. GP	ITAAATAAC <sup>XX</sup> CATCTCGATC <sup>X</sup> WAATDAGGT <sup>X</sup> T - ACTGGC <sup>X</sup> AGAAACCA <sup>X</sup> AACACTWTA <sup>X</sup> TTCGATACA <sup>X</sup> TTGGTAACT <sup>X</sup> TTAAATAAGACATCTCGATGGAETAATTACTNGACTGGCCCATGACCAACATAACTGCAATTCCAATGCATTGGTAACT <sup>X</sup> TTAAATAAGACATCTCGATG <sup>CT</sup> AGGAGGTCT - ACTGGC <mark>G</mark> AGAACCAACAACACTATAATTCCAAGACATTTGGTAACGA TTAAATAAGACATCTCGATG <sup>CT</sup> AGGAGGTCT - ACTGGC <mark>G</mark> AGAACCAACAACACTATAATTCCAAGACATTTGGTAACGA TTAACATCTCGATG <sup>CT</sup> AGTGGGGTCT - ACTGGCAAGAACCAACAACATTACATTCCAATTTGGTAACTA
Consen 1. GV 2. OD 3. GP Consen	PTAATAACĂCATOTOGATĞWAATDAGGTÖT ACTGGWAGAAACCAA <sup>P</sup> AAGACTWTAÄTTCAATACA <sup>P</sup> TTGGTAACTÄ TTAAATAAGACATCTCGATGGATTACTAGACTGGCCATGACCAACATAACTGGAATTCCATGCATTGGTAACTĂ TTAAATAAGACATCTCGATGGAGAGGGCCT - ACTGGCGAGAAACCAACACTTAATACAAGACATTGGTAACGA NTAAATAAGACATCTCGATGAATTGGGTCT - ACTGGCAAGAAACCAACACTTAATACAAGACATTGGTAACGA NTAAATAAGACATCTCGGTGAATTGGGTCT - ACTGGCAAGAAACCAACACTTAATACAAGACATTGGTAACGA NTAAATAAGACATCTCGGTGAATTGGGTCT - ACTGGCAAGAAACCAACCACAACTTAATACAAGACATTGGTAACGA NTAAATAAGACATCTCGGTGAATTGGGTCT - ACTGGCAAGAAACCAACAACTTAATACAAGACATTGGTAACTA NTAAATAAGACATCTCGGTGAATTGGGTCT - ACTGGCAAGAAACCAACCAACCTTAATACAAGACATTGGTAACTA NTAAATAAGACATCTCGGTGAACTGGCGTCA - ACTGGCAACCAACCAACCTTAATACAACTACAATTGGTAACTA
Consen 1. CV 2. OD 3. GP Conson 1. CV	PTAAATAAGĂCATOTOGATĞMAATDAGGTÖT ACTGG MAGAAACCAAÜAACACTMTAÄTTCAATACAÜTTGGTAACTĂ TTAAATAAGACATCTCGATGGATAATTACTAGACTGGCCATGACCAACATAACTGGAATTCCATGCATTGGT-ATTT MANDATATAAGACATCTCGATGCTAGGAGGTCT - ACTGGCGAGAAACCAACACATATAATACAAGACATTTGGTAACGA TTAAATTAAGACATCTCGATGGTAATTGGGTCT - ACTGGAMAGGAAACCAACAACTTATAATACAAGACATTTGGTAACGA TTAAATTATAGACATCTCGATGGTATTGGGATCT - ACTGGAMAGGAAACCAGCAACATTATAATACAAGACATTTGGTAACGA TTAATTTTTÄGGGATGGTCTGGACATGGGATGGCATGGACCAGCAACTATACTAGTCAATTGTACĞ TTAATTTTTÄGGGATGGTCTGGCATGGCATGGCGCAAGGGGCTTGGTACCA TTAATTTTTGGGGTTGGCATGGCA
Consen. 1. GV 2. OD 3. GP Conson 1. GV 2. OD	ITAAATAAGCATCTCGATCWAATDAGGTÜT – ACTGGCWAGAAACCAACATWTACTTCAATACAA <sup>TT</sup> TGGTAACT TTAAATAAGACATCTCGATGGATTAATTACTNGACTGGCCATGACCAACATAACTGCAATTCGAATTCGATTGGTAACTA TTAAATAAGACATCTCGATGCAGGGGCCT – ACTGGCGGAAACCAACAACATATACAATGCATTTGGTAACTA TTAAATAAGACATCTCGATGCAGGGGCCT – ACTGGCAGGAAACCAACAACATTAATACAATGCATTTGGTAACTA TTAAATTTTCGGCATGCATGCAGGGGCCT – ACTGGCAAGAAACCAGCAACAACTTTAATTCAATGCATTTGGTAACTA TTAAATTTTCGGGCATGCTCGCATGGCATCGCATGC – MCCAACCAATTCTACTTCGCA TTTAATTTTCGGGTTGCCTGGCATCACCATGCCGCCACACGGCCTGGTATCCTAATTCAATTCTACTTGGTAGC TTTAATTTTTGGGGTTGCCTGGGATCACCATGCCGCACAGGGGCCTGGTATCCTAATTCTAGTGAAGGTGGGAC TTTAATTTTTGGGGTTGCTGGGATCCACCATGCCGCTCA – MCCAATCCAATTCTAGTGAAGTGGGAC TTTAATTTTTGGGGTTGCTGGGATCCACCATGCCGTCA – AGGCCTGGT TCCTAATCCATTGTAGCTTGGGAC

Figure 2. Alignment of Central Domain sequences of Capromys pilorides (CP), Octodon degus (OD), and Cavia porcellus (CV), also showing subsequences A, B and C

Consen.	TATAAGTO	AGTACCOT	AGCCOGCATZ	30	ATA	AAAATCDDO				90			ATTOTTT
1. CV	ATATAAGGC	AGTATCCT	GCCCCACAT?	GAGTGGAC	CACCACATA	AAGATTGG	GETCATTAT	ACATCAAGT	ATCCTCCC	TOCOATAC	GATTAAG	TGAGTA	PATTCATA
2. OD	TTACAAATC	-AGTTCCCT	TAGCCCGCATA	4	ATA	AAAATCAAG	;					AGGTG	ATACTITI
3. GP	TTATTAGTC	AGTACCCT	TAGCCCGCATA	1	ATA	AAATCCTT						TAATG	ATTCTTT
Consen.	AATGOTAGA	AGGACATAN	NAMAAAT TATT	DATTTAC-			TCTTTTAAC	AAACCCCCC	TTACCCCCC	GINT AAAAT	TATAHAT	TANTINC	ATWGGHAHT
1. CV	AATGCTTGT	AGGACAT	AATCCTT	ATATGCG	CAAGTGTGA	CAACAAGTA	TCTATTAAC	AAACCCCCC	TTACCCC	GTTAAAAT	ACTACTT-		ATOGGAGOT
2. OD	AATGCTAGA	AGGACATA <mark>G</mark> I	ATAAATTAT	GTTTTAC-	(	CAA-AAGTA	TCTTTTAAC	AAACCCCCC	TTACCCC	CG-TAAAAT	TATATAT	TATTGC	ATGGGTAAA
3. CP	AATGCTAGA	AGGACATA <mark>A</mark> G	GAAAAATTAA	TAATTAC-	(	CAATAATTA	TCTTTTAAC		TTACCCC	CATAAAAT	TACAAAT	TAATAC.	ATAGGCATT
Consen.	TTA-CCCAT	GCACOGTAT		TGCCAAAC	CCCAAAAAACI	AAGAANNNN	AAMMMAA	MAINNTANNA	TTTTACGIN		CCATATA-		<b>TGTAATAA</b>
1. CV	OTA CGCTO	GCAAAATGT	AGGCGAGCCC	TGCCAAAC	CCCAAAAAC)	AAGAA				CCAAC	CCACATA	CATA0	<b>STATAATAA</b>
2. OD	TTA CCCAT	GCACCGTAT'	TTATTAGCCC	TGCCAAAC	CCCAAAAAC/	A <mark>g</mark> gaa <b>aat</b> a	AATACAAAA	TAACTACAA	TTTTACG	TACCCTAC	CCATAT	CATAG	<b>GTGTAAT</b> A
а ср	TAATCCCAT	GTACCGTAC	TTGATATCC-	TGCCAAAC	CCCAAAAAC	AAGAGGGA	AAGCACAAAA	AATGTAATA	TTTTACCA	ICTTCCTCA	TACTCTA	CATAGA	GTGCAAAAA
Consen.	MANANIT'TIN	NACCOTCAT	GTOAGTACAAT	370	ATTTECCON	390 NO07	TTCATAGAA	410		C-TTTGAA		ICWT AAT	ATHITCCAC
1. CV	0	CCTACATO	GTGATCACAC		-TTTOCCCA-	CP	ATTCATAG <b>G</b> A	TGTAAAAAC	CAAATCAT	C-TATGAA	CCAAGCT	CATAAT	GATATCCGC
2. OD	TAATATTA	ACCCTCAT	GTCAGTACAA	ATTACATT	ATTTECCCA	CACCOTCP	TTCATAGAA	TG	ACCAC	C CTTAAA	CAAACCO	COTTO	AAACCCTAA
3. CP	ATAAAATT	ACCCTCAT	GTCAGTACAA		ATGTGCCCA		TTCACAGAG	TG	CAT	TATTTGCA	CTTGCCT	TGTAAT	ATTTCAC
Consen	GCCTAAN	470	472										
1. CV	ACCCCC												
2.00	GCCTAAG												
3 CP	GTCTAATAC	ACCOUTTY	rc										

Figure 3. Alignment of CSB domain sequences of Capromys pilorides (CP), Octodon degus (OD) and Cavia porcellus (CV), excluding the repetitive DNA regions. Notice the presence of the CSB1, CSB2 and CSB3 sequence blocks, and the frequent deletions and insertions larger than single-base in CV as compared to the other species.