Mastozoología Neotropical, 23(1):39-49, Mendoza, 2016

Versión impresa ISSN 0327-9383
Versión on-line ISSN 1666-0536

Copyright ©SAREM, 2016
http://www.sarem.org.ar
http://www.sbmz.com.br

Artículo

PHYLOGENETIC RELATIONSHIPS BETWEEN TUCO-TUCOS (*Ctenomys*, RODENTIA) OF THE CORRIENTES GROUP AND THE *C. pearsoni* COMPLEX

Diego A. Caraballo[1], Ivanna H. Tomasco[2], Denise H. Campo[1], and María Susana Rossi[1]

[1] IFIBYNE-CONICET. Laboratorio de Fisiología y Biología Molecular, Dep. Fisiología, Biología Molecular y Celular. Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II, 2do piso, EHA1428, Buenos Aires, Argentina. [Correspondence: María Susana Rossi <srossi@fbmc.fcen.uba.ar>], Tel. +54 11 4576 3386/68

[2] Laboratorio de Evolución. Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay. Iguá 4225 esq. Mataojo. Montevideo, 11.400

ABSTRACT. Lineage delimitation is of extreme importance in evolutionary biology and constitutes an essential tool in basic and applied fields including conservation. We addressed the delimitation of two closely related species complexes of South American rodents of the genus *Ctenomys* (tuco-tucos), namely the *C. pearsoni* and Corrientes groups, whose relationships have been unclear or conflicting in previous phylogenetic studies. We performed a molecular phylogenetic analysis, increasing the number of representatives of each group and including sequences of three mitochondrial loci (the complete cytochrome b coding sequence, and partial regions of the cytochrome oxydase I gene and the D-loop of the control region). The trees obtained using Bayesian Inference and Maximum Parsimony methods show the Corrientes group and the *C. pearsoni* complex as reciprocally monophyletic sister clades, with higher values of intergroup compared to intragroup genetic distances. These results indicate that the Corrientes group and the *C. pearsoni* complex are differentiated lineages. The resulting tree topology is in agreement with a scenario of independent chromosomal rearrangements from the ancestral karyomorph (2n = 70 FN = 84), leading to the considerable chromosomal diversity that characterizes both groups.

RESUMEN. Relaciones filogenéticas entre los tuco-tucos (*Ctenomys*, Rodentia) del grupo Corrientes y del complejo *C. pearsoni*. La delimitación de linajes tiene una gran importancia en biología evolutiva y constituye una herramienta fundamental en disciplinas básicas y aplicadas incluyendo la conservación. Abordamos la delimitación de dos complejos de especies de roedores sudamericanos del género *Ctenomys* (tuco-tucos), denominados grupos *C. pearsoni* y Corrientes, cuyas relaciones resultaron poco claras o conflictivas en estudios filogenéticos previos. Realizamos una filogenia molecular, aumentando el número de representantes de cada grupo e incluyendo secuencias de tres marcadores mitocondriales (la secuencia completa de la región codificante del gen del citocromo b y secuencias parciales del gen de la enzima citocromo oxidasa I, y del D-loop de la región control). Los árboles obtenidos mediante métodos de inferencia bayesiana y máxima parsimonia muestran que el grupo Corrientes y el complejo *C. pearsoni* son grupos hermanos recíprocamente monofiléticos con mayores valores de distancias genéticas entre grupos que dentro de los grupos. Estos resultados indican que el grupo

Recibido 28 septiembre 2015. Aceptado 17 marzo 2016. Editor asociado: E Palma

Corrientes y el complejo *C. pearsoni* son linajes diferenciados. La topología resultante es consistente con un escenario de fijación de reordenamientos cromosómicos independientes a partir del cariomorfo ancestral (2n = 70 NF = 84), dando origen a la considerable diversidad cromosómica que caracteriza a ambos grupos.

Key words: Corrientes group. *Ctenomys pearsoni*. Mitochondrial phylogeny.

Palabras clave: *Ctenomys pearsoni*. Filogenia mitocondrial. Grupo Corrientes.

INTRODUCTION

Subterranean rodents of the genus *Ctenomys* that inhabit the southern cone of South America have one of the highest numbers of living species among mammals; at least 60 have been recognized (Woods and Kilpatrick, 2005). Although the relationship among different groups of species is not fully resolved, chromosomal and molecular phylogenetic studies clustered related species into several species groups (Gallardo, 1979; Massarini et al., 1991; Ortells and Barrantes, 1994; Cook and Yates, 1994; Parada et al., 2011). One of these groups, named *torquatus* by Parada et al. (2011) includes the species that inhabit the eastern part of the distribution of the genus: *C. torquatus*, *C. lami*, *C. minutus*, *C. pearsoni*, and three species belonging to the Corrientes group, *C. roigi*, *C. perrensi* and *C. dorbignyi*. Recently a new member was described as a distinct species in the *torquatus* group: *C. ibicuiensis* (Freitas et al., 2012). However, the specific status or relationships among the species of tuco-tucos of the *torquatus* group are not fully resolved. In a phylogeny based on complete cytochrome b (cyt-b) sequences that includes representatives from all the species of the group, four reciprocally monophyletic clades could be distinguished: *C. ibicuiensis*, *C. torquatus*, *C. lami* + C. *minutus*, and *C. pearsoni* + Corrientes group (Freitas et al., 2012). The relationship between *C. pearsoni* and the Corrientes group is poorly resolved in the published phylogenies, but these groups were never subjected to an exhaustive survey of molecular characters and taxon representation. Parada et al. (2011), using complete sequences of cyt-b, recovered a paraphyletic Corrientes group that included *C. pearsoni*, a result reaffirmed by Freitas et al. (2012). In contrast, other phylogenies based on partial sequences of the same mitochondrial gene split *C. pearsoni* from the Corrientes group (Fernandes et al., 2009; Caraballo et al., 2012). In all these phylogenies, no more than four representatives of *C. pearsoni* were included.

The tuco-tucos of the Corrientes group inhabit a large area under the direct or indirect influence of the Iberá marsh and its lagoons and channels in the Province of Corrientes, Argentina (**Fig. 1**). Based on allozymes and chromosome banding, this group was delimited by the pioneering work of Ortells and Barrantes (1994). *Ctenomys pearsoni* inhabits the coastal plains of southern Uruguay (Lessa and Langguth, 1983; Tomasco and Lessa, 2007) and the western part of the Province of Entre Ríos, Argentina (García et al., 2000) (**Fig. 1**).

The Corrientes group and the *C. pearsoni* complex share extreme chromosomal variability, intraspecific in the case of *C. pearsoni* with chromosomal numbers (2n) ranging between 56-58, 64-66 and 70 and fundamental numbers (FN) 80, 82 and 84 (Tomasco and Lessa, 2007, and references therein), and intra and interspecific in the case of the Corrientes group with chromosomal numbers that range between 41-44, 44-46, 48 and 50-70 and with FN 76, 78, 80 and 84, respectively (Caraballo et al., 2015, and references therein). The karyomorph 2n = 70 FN = 84 is shared by the *C. pearsoni* complex and the Corrientes group and was proposed by Ortells et al. (1990) as the ancestral morph for both groups. These authors stated that the extant 2n = 70 FN = 84 karyomorphs found in Argentina and Uruguay are relicts of a more widespread distribution, from which lower chromosomal numbers

Fig. 1. Map showing sampling localities. The numbers correspond to the following localities: (1) Mbarigüí, (2) Paraje Angostura, (3) Manantiales, (4) Mburucuyá, (5) Loma Alta, (6) Pago Alegre, (7) Saladas Centro, (8) Estancia San Luis, (9) Rincón de Ambrosio, (10) Saladas Sur, (11) Colonia 3 de abril, (12) San Roque, (13) Santa Rosa, (14) Paraje Caimán, (15) San Miguel, (16) Curuzú Laurel, (17) Loreto, (18) Estancia La Tacuarita, (19) Contreras Cué, (20) San Alonso, (21) Chavarría, (22) Goya, (23) Paraje Sarandicito, (24) Médanos, (25) Limetas, (26) Arazatí, (27) Roosevelt, (28) Chihuahua, (29) José Ignacio, (30) Laguna de Rocha and (31) Valizas.

could have originated by means of Robertsonian rearrangements.

Lineage and species delimitation are non-trivial issues in evolutionary biology. Underestimation or inflation of the number of species in certain groups of organisms could adversely affect biodiversity conservation and management efforts (Zachos et al., 2013, and references therein). In addition, a reliable phylogeny is a crucial tool to understand the evolution of behavioural (Strier et al., 2014), morphological (Morgan and Álvarez, 2013) or genomic characters (Slamovits et al., 2001). In the case of the tuco-tucos of the Corrientes group and the *C. pearsoni* complex, tracking the conspicuous chromosomal evolution that characterizes both groups is not possible in the absence of clear delimitation of independent evolutionary lineages and knowledge of their historical relationships.

The aim of the present work is to examine the relationship between *C. pearsoni* and the

tuco-tucos that inhabit the Corrientes Province with an expanded taxon and character sampling. We included complete cyt-b sequences of 42 representatives of the Corrientes group, 15 of *C. pearsoni* and 15 additional specimens, including representatives of the *torquatus* group and other *Ctenomys* species. We also added to the analysis partial sequences from two additional mitochondrial loci, the control region (D-loop) and the cytochrome oxidase I coding gene (COI).

MATERIALS AND METHODS

Specimens

Animals were captured with modified Oneida Victor Number 0 snap traps. Guidelines of the American Society of Mammalogists (Gannon and Sikes, 2007) were followed. Well-preserved skulls were submitted to the Colección de Mastozoología of the MuseoArgentino de Ciencias Naturales "Bernardino Rivadavia" for Argentinean specimens, and to the collection of the Laboratorio de Evolución in the Facultad de Ciencias of the Universidad de la República, Montevideo, for the Uruguayan specimens. Trapping localities with their geographical location (**Fig. 1**) as well as field / catalog numbers (when available) were recorded (**Table 1**).

This study included a total of 73 samples; 42 individuals were from 23 localities of the Corrientes Province (Argentina), two samples of *C. pearsoni* are from Médanos, Entre Ríos Province (Argentina), while 13 were sampled at 7 Uruguayan localities (**Table 1**). The rationale of the sampling strategy was to cover the overall haplotypic diversity observed among these two groups along their known geographic distributions, based on previous mtDNA studies (Tomasco and Lessa, 2007; Caraballo et al., 2012). To test the Corrientes group + *C. pearsoni* complex monophyly, we also included sequences from other members of the *torquatus* group: 4 specimens of *C. torquatus* (Fernandes et al., 2009), 3 specimens of *C. minutus* (Lopes et al., 2013), 1 *C. lami* (Lopes and Freitas, 2012) and 1 *C. ibicuiensis* (Freitas et al., 2012). As outgroup for the *torquatus* group, we included sequences of the species: *C. conoveri* (1), *C. rionegrensis* (3), *C. sociabilis* (1), *C. leucodon* (1) and *Octodon degus* (1) (Tomasco and Lessa, 2011; Caraballo et al., 2012).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega). A 482 bp partial fragment encompassing the 5' end of the mitochondrial D-loop, plus a 775 bp fragment covering a 53 bp non-coding flanking sequence and 722 bp of the 5' end of the COI coding sequence were amplifyied using primers and protocols detailed in Caraballo et al. (2012). Complete cyt-b sequences were obtained by PCR amplification using the following primer pairs: MVZ05TUCO and tuco06, tuco07 and tuco14a, TUTU-F and TUTU-R, MVZ06 and tuco16. Primers tuco06, tuco07, tuco14a and tuco16 were designed by Wlasiuk et al. (2003) while MVZ06, by Smith and Patton (1999). MVZ05TUCO is a modification of MVZ05 (Smith and Patton, 1999) designed by us: 5'CAAGACTAATGATATGAAAAACCATTGTT 3'. Primers TUTU-F and TUTU-R were designed by Caraballo et al. (2012).

PCR products were amplified and directly sequenced on one strand, since previous contigs of both strands did not reveal differences in these loci (Caraballo et al., 2012). Cytochrome oxydase I, D-loop and the 3' end of cyt-b sequences from the Corrientes group were previously obtained and deposited in GenBank under the accession numbers JX275502 to JX275655 (Caraballo et al., 2012). The remaining cyt-b partial sequences of the Corrientes group, as well as the complete dataset of *C. pearsoni* and the samples CA 426 (*C. rionegrensis*), EV 1169 (*C. rionegrensis*), CA 783 (*C. torquatus*) and NK 12607 (*C. conoveri*) were generated in this study and deposited in GenBank (accession numbers KT818638 - KT818684 and KT900 – KT900168). The sequences corresponding to an additional *C. rionegrensis*, *C. ibicuiensis*, *C. lami*, *C. minutus*, 3 additional *C. torquatus*, *C. sociabilis*, *C. leucodon* and *Octodon degus* specimens were retrieved from GenBank (see **Table 2**, **Supplementary Material**).

Sequence alignment and analyses

Sequence electropherograms were visually inspected using Bioedit (Hall, 1999) and aligned with CLUSTAL X2 (Larkin et al., 2007). Ambiguous alignments were not evident in any of the sequenced loci and four indels were postulated only for D-loop. Nucleotide variability indexes (number of variable sites, parsimony informative sites, nucleotide and haplotype diversity indexes) were computed for each locus using DNASP 5.10.01 (Librado and Rozas, 2009). Substitution saturation was assessed with DAMBE (Xia and Xie, 2001), performing a test

Table 1

Sampling localities, specimen field codes, voucher numbers (when available), geographical location and GenBank accession numbers of the samples of the Corrientes group and the *C. pearsoni* complex included in this study. Acronym MACN-Ma corresponds to vouchers deposited at the Colección de Mastozoología of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", while acronyms CA and EV correspond to the collection of the Laboratorio de Evolución in the Facultad de Ciencias of the Universidad de la República, Montevideo, Uruguay.

Species group/ complex	Country	Sampling Locality	Field codes / voucher numbers	Lat (S)	Long (W)	GenBank Accession Numbers
Corrientes group	Argentina	Estancia La Tacuarita	204, 205	27° 58' 42.7"	56° 33' 40.6"	
		Saladas Centro	128, 129	28° 14' 20.3"	58° 37' 40.4"	
		Saladas Sur	131, 132	28° 17' 37.5"	58° 41' 19.2"	
		San Alonso	186, 187	28° 17' 76"	57° 24' 45"	
		Rincón de Ambrosio	175, 176	28° 15' 5.2"	58° 53' 40.9"	
		Estancia San Luis	238/MACN-Ma 26486, 239/ MACN-Ma 26487	28° 6' 43.7"	58° 51' 48.1"	
		Colonia 3 de Abril	65, 66	28° 23' 26.9"	58° 53' 37.5"	
		Goya	181, 183	29° 11' 17.2"	59° 12' 36.7"	
		San Roque	135, 136	28° 41'	58° 42'	
		Santa Rosa	229, 230	28° 10' 47.4"	58° 8' 11.5"	
		Chavarría	149, 153	28° 58'	58° 35'	
		Curuzú Laurel	221, 222	27° 55' 24.4"	57° 29' 23.5"	JX275502 to JX275655
		Loma Alta	177, 179	28° 5' 2.5"	58° 19' 29.9"	
		Loreto	156, 223/MACN-Ma 26479	27° 44' 43.7"	57° 14' 35.2"	
		Contreras Cué	199, 211	28° 5' 28.6"	56° 33' 53.7"	KT818638 to KT818684
		Mbarigüí	174	27° 33'	57° 31'	
		Pago Alegre	41, 43	28° 8' 53"	58° 21' 44.8"	
		Paraje Angostura	161	27° 33'	57° 31'	
		Mburucuyá	05	28° 5' 51.4"	58° 16' 38.6"	
		San Miguel	214/MACN-Ma 26478, 217	28° 0' 58.6"	57° 36' 19.2"	
		Paraje Caimán	227/MACN-Ma 26481, 228/ MACN-Ma 26482	28° 3' 3.1"	57° 40' 38.4"	
		Manantiales	12	27° 57' 54.5"	58° 7' 20.9"	
		Paraje Sarandicito	212/ MACN-Ma 26476, 213/ MACN-Ma 26477	30° 14' 43.1"	59° 33' 46.1"	
		Médanos	242, 243	33° 25' 37.1"	59° 5' 35.5"	
C. pearsoni complex	Uruguay	Limetas	EV 1439, EV 1454	34° 09' 00"	58° 05' 30"	AY755438 to AY755458*
		Arazatí	CA 381, EV 1471	34° 33' 00"	57° 00' 00"	
		Roosevelt	CA 493	34° 51' 18"	56° 02' 38"	
		Chihuahua	CA 489, CA 581, CA 583, CA 586	34° 56' 44"	54° 56' 47"	JX275650
		Jose Ignacio	CA 475, CA 455	34° 50' 22"	54° 38' 52"	KT900149 to KT900168
		Laguna de Rocha	CA 466	34° 37' 21"	54° 15' 27"	
		Valizas	CA 498	34° 21' 06"	53° 50' 30"	

*Serial numbers ending in 40, 43, 45, 46, 48 to 50, 53, 55, 57 and 59 were not included.

introduced by Xia et al. (2003, 2009) in 5 different partitions: 1st and 2nd cyt-b codon positions, 3rd cyt-b codon position, 1st and 2nd COI codon position, 3rd COI codon position, D-loop. P-distances and net divergence among pairs of species and complexes were calculated with MEGA (Tamura et al., 2013).

Phylogenetic inference

Phylogenetic analyses were performed with the concatenated data set since all 3 loci are linked in the mitochondrial genome. Trees were rooted on the branch leading to *Octodon degus*. Different schemes and models were assayed by Bayesian Inference and Maximum Parsimony.

Maximum parsimony (MP) searches were performed in PAUP 4.0 (Swofford, 2003) using equal transformation costs and weights while internal gaps were treated as a fifth character. Heuristic searches were performed running 1000 independent Random Addition Sequences (RAS) with hold 10 and using TBR swapping. Clade support was assessed running 10 000 bootstrap replicates with the same search parameters.

Bayesian inference (BI) analyses were carried out using MrBayes 3.2.5 (Ronquist et al., 2012) under 3 different data treatments: (1) fixing a substitution model for each of the three mitochondrial loci, (2) estimating a substitution model for each locus and (3) estimating a substitution model discriminating codon positions and non-coding sequences. For (1), the corrected Akaike Information Criterion (AICc) was used to find the simplest substitution model that explained the data, using the program MrModeltest v2 (Nylander, 2004). In the first two treatments, the dataset was partitioned into three subsets, D-loop, cyt-b and COI, while in the third treatment the dataset was partitioned into 5 subsets: D-loop, 1st and 2nd codon positions for cyt-b, 3rd codon position for cyt-b, 1st and 2nd codon positions for COI and 3rd codon position for COI.

For each data treatment, two simultaneous runs with four Markov chains each were run for 2 000 000 generations, and trees were sampled every 500 generations. The standard deviation of split frequencies was analyzed to determine when the Markov chain reached stationarity and to determine the number of samples to be discarded as "burn-in". Additionally, the effective sample sizes (ESS) of parameters sampled from Markov chain Monte Carlo were analyzed using TRACER 1.6 (Rambaut and Drummond, 2013). We stopped the analyses when we verified that the standard deviation of split frequencies was smaller or equal to 0.01, ESS for all estimated parameters were higher than 200 and the "burn-in" phase corresponded at most to one third of total generations. These conditions were accomplished with the initial settings for data treatments 1 and 2, while we needed to run 12 000 000 additional generations for data treatment 3. A 50% majority rule consensus tree was constructed with the post "burn-in" distribution and the percentage of samples recovering each clade was used to estimate its posterior probability. These consensus trees were visualized in FigTree 1.4.2 (Rambaut, 2014).

RESULTS

Sequence variability

Different measures of polymorphism and nucleotide diversity were computed for the Corrientes group and the *C. pearsoni* complex. The 697 bp sequence alignment obtained for COI yielded 70 polymorphic sites (48 of which are parsimony informative sites) defining 37 different haplotypes for 55 of the 57 individuals (samples EV1471 and CA 455 did not yield PCR products). Uncorrected nucleotide diversity (Pi) and haplotype diversity (HD) indexes were 0.015 (SD 0.001) and 0.980 (SD 0.008), respectively.

The 1140 bp sequence alignment obtained for cyt-b yielded 102 polymorphic sites (77 of which are parsimony informative) defining 35 different haplotypes for the 57 studied specimens. Uncorrected nucleotide diversity (Pi) and haplotype diversity (HD) indexes were 0.014 (SD 0.002) and 0.982 (SD 0.006), respectively.

The 438 bp sequence alignment obtained for D-loop yielded 39 polymorphic sites (26 of which were parsimony informative) defining 38 different haplotypes for the 57 studied specimens. Uncorrected nucleotide diversity (Pi) and haplotype diversity (HD) indexes were 0.019 (SD 0.001) and 0.986 (SD 0.006), respectively.

Table 3 shows net distances within species and groups of species of the *torquatus* group. As expected, distances between any member of the *torquatus* group and outgroups were higher than between any of its members. Within the *torquatus* group, with the exception of the low distance between *C. minutus* and *C. lami* (0.002) that reflects the close relationship between these two hybridizing species (Gava

Table 3

Net divergence among pairs of species and complexes within the *torquatus* group and between them and outgroups.

	Corrientes group	*C. pearsoni*	*C. torquatus*	*C. minutus*	*C. lami*	*C. ibicuiensis*	*C. rionegrensis*	*C. leucodon*	*C. sociabilis*	*C. conoveri*
Corrientes group	-									
C. pearsoni	0.012	-								
C. torquatus	0.019	0.025	-							
C. minutus	0.022	0.027	0.011	-						
C. lami	0.027	0.031	0.016	0.002	-					
C. ibicuiensis	0.029	0.033	0.027	0.026	0.037	-				
C. rionegrensis	0.043	0.048	0.044	0.040	0.049	0.049	-			
C. leucodon	0.086	0.088	0.088	0.092	0.089	0.100	0.084	-		
C. sociabilis	0.116	0.119	0.106	0.106	0.107	0.114	0.101	0.129	-	
C. conoveri	0.108	0.110	0.111	0.115	0.110	0.116	0.106	0.128	0.120	-
Octodon degus	0.259	0.264	0.262	0.267	0.318	0.273	0.266	0.291	0.289	0.266

and Freitas, 2003), net distance values are comparable. However, the net distances between *C. torquatus* and *C. lami* or *C. minutus* are lower (0.011 and 0.016, respectively) than the distances between *C. torquatus* and *C. pearsoni* or the Corrientes group (0.025 and 0.019, respectively), confirming again that *C. torquatus* is most closely related to these two Brazilian coastal species (Freitas, 2012). The *Ctenomys pearsoni* complex and the Corrientes group showed a net distance value (0.012), comparable with the *C. torquatus-C. lami* or *C. torquatus-C. minutus* distances, verifying also their close relatedness. However, the intragroup distances are lower (0.014 for *C. pearsoni* complex and 0.011 for the Corrientes group) than the intergroup distance (0.024), which indicates these two groups are genetically distinctive.

No saturation was revealed (Iss<Iss.c, $p<0.0001$) for any of the five partitions (COI 1st and 2nd codon positions, COI 3rd codon position, cyt-b 1st and 2nd codon position, cyt-b 3rd codon position, and D-loop), indicating they are usable for phylogenetic analyses.

Phylogenetic relationships

The concatenated matrix (D-loop + COI + complete cyt-b) is 2282 bp long; 733 characters are variable, 401 of which are parsimony informative. For the Corrientes group + *C. pearsoni* complex, 141 characters are variable, 103 of which are parsimony informative, defining a total of 40 haplotypes.

The trees obtained by BI under the three conditions and by MP were congruent and the main clades (CP, C and P, see below) received high bootstrap support in all cases (see **Table 2**, **Supplementary Material**). **Fig. 2** shows the BI consensus tree resulting from the estimation of independent substitution models distinguishing between different codon positions as well as non-coding sequences (see **Materials and Methods**; model parameters estimated by MrBayes, as well as fixed model parameters obtained by MrModeltest are shown in **Table 2**, **Supplementary Material**). As expected, the *torquatus* group divergence from the remaining *Ctenomys* species is represented by a well-supported basal node (node O). The *torquatus*

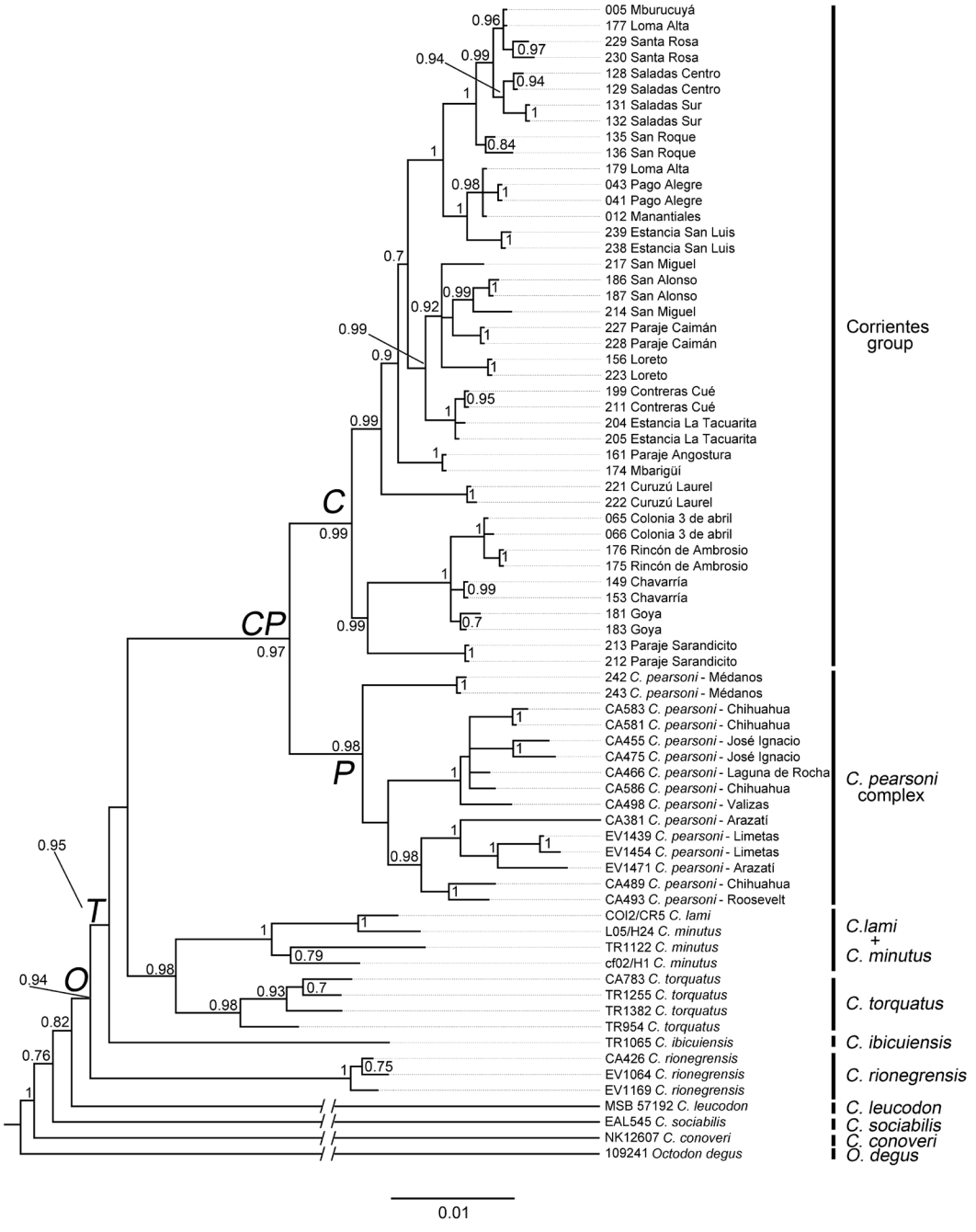

Fig. 2. Bayesian Inference phylogenetic tree based on concatenated sequences of cyt-b, COI and D-loop, estimating different model parameters for codon positions (1st + 2nd, and 3rd position separately) and non-coding sequences (see **Material and Methods**). The numbers in the nodes indicate posterior probability. T: *torquatus* group, C + P: Corrientes group + *C. pearsoni* complex group, C: Corrientes group and P: *C. pearsoni* complex group. Branch lengths are proportional to the number of changes.

group (node T) splits into three well supported lineages: the recently described *C. ibicuiensis* (Freitas et al., 2012), *C. minutus* + *C. lami* + *C. torquatus* and the Corrientes group + *C. pearsoni* (node CP). This basal splitting within the *torquatus* group coincides with the phylogeny previously published by Freitas et al. (2012). However, while the individual of *C. pearsoni* grouped to a representative from Paraje Sarandicito (a member of the Corrientes group, which turned out paraphyletic) in Freitas et al. (2012), in our analysis all the 15 representatives of *C. pearsoni*, including two individuals from the locality of Médanos in the Province of Entre Ríos, Argentina, clustered above a well supported node (node P). On the other hand, the 42 individuals from 23 Correntinean tuco-tucos populations, including those from Paraje Sarandicito, formed a well-supported clade (C). Thus, *C. pearsoni* and the Corrientes group are monophyletic sister clades that belong to the *torquatus* group.

DISCUSSION

We reanalyzed the evolutionary relationship between the *C. pearsoni* complex and the Corrientes group of tuco-tucos that was controversial in the literature. We constructed a molecular phylogeny by enlarging the number of representatives of each group and including sequences of three mitochondrial loci, resulting in the largest data matrix for these groups to date: 2275 characters and 57 taxa within the Corrientes group + *C. pearsoni* complex. Concordant with previous analyses (Freitas et al., 2012), the tree recovered three main basal lineages of the *torquatus* group: *C. ibicuiensis*, *C. torquatus* + *C. lami* + *C. minutus* and *C. pearsoni* complex + Corrientes group. The addition of a higher number of representatives and characters clearly split the *C. pearsoni* complex and the Corrientes group into two sister clades. These two divergent groups of tuco-tucos should be recognized by biodiversity management programs, as they are independent lineages. However, these mitochondrial data should be supplemented with nuclear markers.

Within the Corrientes group, most populations are monophyletic or (as in the cases of Loma Alta and San Miguel) are included in clades with closely related karyomorphs (Caraballo et al., 2015). This may be the case in the *C. pearsoni* complex, although there is evidence of maintenance of ancestral polymorphisms, as shown by Tomasco and Lessa (2007).

These two groups of tuco-tucos have undergone high rates of chromosomal evolution. The 2n = 70 FN = 84 karyomorph is the only form common to both clades, and hence it is likely to be ancestral. The ample chromosomal variability observed in both groups would be the result of the fixation of independent rearrangements across the lineages. Although most chromosomal rearrangements postulated are Robertsonian fusions/fissions, which involve changes in chromosomal number without altering the fundamental number, inversions and/or translocations have been postulated as responsible of the changes in the fundamental number (Ortells and Barrantes, 1994; Caraballo, 2013), and hence are likely to constitute reproductive barriers.

Finally, we would note that although the population of Médanos falls into the *C. pearsoni* clade (node P) and the 2n = 70 FN = 84 karyomorph of specimens of this population was indistinguishable from others of *C. pearsoni* (García et al., 2000), this is the only population of *C. pearsoni* of Entre Ríos included in phylogenetic analyses to date. An expanded survey in the Province of Entre Ríos should be performed to further document the phylogenetic position of these tuco-tucos. *Ctenomys pearsoni* is not the only tuco-tuco species on both sides of the Uruguay River: populations of *C. rionegrensis* are distributed in both Uruguay and Argentina at the 31° 44' S - 34° 56' S latitude (Bidau et al., 2008 a,b). The association of these species with the history of the Uruguay River remains to be explored.

ACKNOWLEDGMENTS

This work was supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 11220110100910CO) and the Linnean Society (Systematics Research Fund) from Argentina and the United Kingdom, respectively. D.A.C. is supported by a postdoctoral fellowship awarded by CONICET. M. S. R. is career investigator of CONICET. We are grateful to Thales de Freitas for

assistance in matching individual sequences of Brazilian tuco-tucos. Finally, we would like to thank J.A.C., an anonymous reviewer and the editor whose comments improved the manuscript.

LITERATURE CITED

BIDAU C, E LESSA, and R OJEDA. 2008a. *Ctenomys rionegrensis*. The IUCN Red List of Threatened Species 2008: e.T136635A4320816.http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T136635A4320816.en. Downloaded on 28 January 2016.

BIDAU C, E LESSA, and R OJEDA. 2008b. *Ctenomys pearsoni*. The IUCN Red List of Threatened Species 2008: e.T5819A11735567.http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T5819A11735567.en. Downloaded on 28 January 2016.

CARABALLO DA, GA ABRUZZESE, and MS ROSSI. 2012. Diversity of tuco-tucos (*Ctenomys*, Rodentia) in the Northeastern wetlands from Argentina: Mitochondrial phylogeny and chromosomal evolution. Genetica 140:125-136.

CARABALLO DA. 2013. Evolución de un complejo de especies de *Ctenomys* (Octodontidae, Rodentia) del noreste argentino: filogenia, variabilidad cromosómica y dinámica del ADN satélite. Tesis de doctorado. Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales.

CARABALLO DA, PC JABLONSKI, PJ REBAGLIATI, and MS ROSSI. 2015. Chromosomal variability in tuco-tucos (*Ctenomys*, Rodentia) from the Argentinean northeastern wetlands. Mastozoología Neotropical 22:289-301.

COOK JA and TL YATES. 1994. Systematic relationships of the Bolivian tuco-tucos, genus *Ctenomys* (Rodentia: Ctenomyidae). Journal of Mammalogy 75:583-599.

FERNANDES FA, GL GONÇALVES, SS XIMENES, and TR FREITAS. 2009. Karyotypic and molecular polymorphisms in *Ctenomys torquatus* (Rodentia: Ctenomyidae): Taxonomic considerations. Genetica 136:449-459.

FREITAS TR, FA FERNANDES, R FORNEL, and PA RORATTO. 2012. An endemic new species of tuco-tuco, genus *Ctenomys* (Rodentia: Ctenomyidae), with a restricted geographic distribution in southern Brazil. Journal of Mammalogy 93:1355-1367.

GALLARDO MH. 1979. Las especies chilenas de *Ctenomys* (Rodentia, Octodontidae). I. Estabilidad cariotípica. Archivos de Biología y Medicina experimental 12:71-82.

GANNON WL and RS SIKES. 2007. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 88:809-823.

GARCÍA L, M PONSÀ, J EGOZCUE, and M GARCÍA. 2000.Comparative chromosomal analysis and phylogeny in four *Ctenomys* species (Rodentia, Octodontidae). Biological Journal of the Linnean Society 69:103-120.

GAVA A and TRO FREITAS. 2003. Inter- and intra-specific hybridization in tuco-tucos (*Ctenomys*) from Brazilian coastal plains (Rodentia: Ctenomyidae). Genetica 119:11-17.

HALL TA. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symposium series 41:95-98.

LARKIN MA, G BLACKSHIELDS, NP BROWN, R CHENNA, PA McGETTIGAN, H McWILLIAM, et al. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23:2947-2948.

LESSA EP and A LANGGUTH. 1983. *Ctenomys pearsoni* n. sp (Rodentia: Octodontidae) del Uruguay. Resúmenes, Comunicaciones Jornadas de Ciencias Naturales del Uruguay 3:86-88.

LIBRADO P and J ROZAS. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451-1452.

LOPES CM and TRO FREITAS. 2012. Human impact in naturally patched small populations: Genetic structure and conservation of the burrowing rodent, tuco-tuco (*Ctenomys lami*). Journal of Heredity 103:672-681.

LOPES CM, SSF XIMENES, A GAVA, and TRO FREITAS 2013. The role of chromosomal rearrangements and geographical barriers in the divergence of lineages in a South American subterranean rodent (Rodentia: Ctenomyidae: *Ctenomys minutus*). Heredity 111:293-305.

MASSARINI AI, MA BARROS, MO ORTELLS, and OA REIG. 1991. Chromosomal polymorphism and small karyotypic differentiation in a group of *Ctenomys* species from Central Argentina (Rodentia: Octodontidae). Genetica 83:131-144.

MORGAN CC and A ÁLVAREZ. 2013. The humerus of South American caviomorph rodents: Shape, function and size in a phylogenetic context. Journal of Zoology 290:107-116.

NYLANDER JAA. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

ORTELLS MO, JR CONTRERAS, and OA REIG. 1990. New *Ctenomys* karyotypes (Rodentia, Octodontidae) from north-eastern Argentina and from Paraguay confirm the extreme chromosomal multiformity of the genus. Genetica 82:189-201.

ORTELLS MO and GE BARRANTES. 1994. A study of genetic distances and variability in several species of the genus *Ctenomys* (Rodentia: Octodontidae) with special references to a probable causal role of chromosomes in speciation. Biological Journal of the Linnean Society 53:189-208.

PARADA A, G D'ELÍA, CJ BIDAU, and EP LESSA. 2011. Species groups and the evolutionary diversification of tuco-tucos, genus *Ctenomys* (Rodentia: Ctenomyidae). Journal of Mammalogy 92:671-682.

RAMBAUT A and AJ DRUMMOND. 2013. Tracer v1.5 Available from http://beast.bio.ed.ac.uk/Tracer.

RAMBAUT A. 2014. FigTree: Tree figure drawing tool, version 1.4. 0.

RONQUIST F, M TESLENKO, P VAN DER MARK, DL AYRES, A DARLING, S HÖHNA, et al. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and

model choice across a large model space. Systematic Biology 61:539-542.

SLAMOVITS CH, JA COOK, EP LESSA, and MS ROSSI. 2001. Recurrent amplifications and deletions of satellite DNA accompanied chromosomal diversification in South American tuco-tucos (genus *Ctenomys*, Rodentia, Octodontidae): A phylogenetic approach. Molecular Biology and Evolution 18:1708-1719.

SMITH MF and JL PATTON. 1999. Phylogenetic relationships and the radiation of sigmodontine rodents in South America: Evidence from cytochrome b. Journal of Mammalian Evolution 6:89-128.

STRIER KB, PC LEE, and AR IVES. 2014. Behavioral flexibility and the evolution of primate social states. Plos One.DOI: 10.1371/journal.pone.0114099

SWOFFORD DL. 2003. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates Inc Publishers, Sunderland Massachusetts.

TAMURA K, G STECHER, D PETERSON, A FILIPSKI, and S KUMAR. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30:2725-2729.

TOMASCO I and EP LESSA. 2007. Phylogeography of the tuco-tuco *Ctenomys pearsoni*: mtDNA variation and its implication for chromosomal differentiation. Pp. 859-882, in: The quintessential naturalist: honoring the life and legacy of Oliver P. Pearson (D Kelt, EP Lessa, J Salazar-Bravo, and JL Patton). University of California Publications in Zoology.

TOMASCO IH and EP LESSA. 2011. The evolution of mitochondrial genomes in subterranean caviomorph rodents: Adaptation against a background of purifying selection. Molecular Phylogenetics and Evolution 61(1):64-70.

WLASIUK G, J CARLOS GARZA, EP LESSA, and TB SMITH. 2003. Genetic and geographic differentiation in the Rio Negro tuco-tuco (*Ctenomys rionegrensis*): Inferring the roles of migration and drift from multiple genetic markers. Evolution 57:913-926.

WOODS *CA* and CW KILPATRICK. 2005. Infraorder Hystricognathi. Mammal species of the world: A taxonomic and geographic reference, Johns Hopkins University Press. Baltimore, Maryland.

XIA X and Z XIE. 2001. DAMBE: software package for data analysis in molecular biology and evolution. Journal of heredity, 92(4), 371-373.

XIA X, Z XIE, M SALEMI, L CHEN, and Y WANG. 2003. An index of substitution saturation and its application. Molecular Phylogenetics and Evolution 26:1-7.

XIA X and P LEMEY. 2009. Assessing substitution saturation with DAMBE. Pp. 615-630 in: The Phylogenetic Handbook: A Practical Approach to DNA and Protein Phylogeny (P Lemey, M Salemi, and A-M Vandamme, eds.). 2nd edition. Cambridge University Press.

ZACHOS FE, M APOLLONIO, EV BÄRMANN, M FESTA-BIANCHET, U GÖHLINCH, JC HABEL, E HARING, L KRUCKENHAUSER, S LOVARI, AD McDEVITT, C PERTOLDI, GE RÖSSNER, MR SANCHEZ-VILLAGRA, MA SCANDURA, and F SUCHENTRUCK. 2013. Species inflation and taxonomic artefacts-A critical comment on recent trends in mammalian classification. Mammalian Biology 78:1-6.

SUPLEMENTARY MATERIAL ON-LINE

Fig 2. Maximum Parsimony consensus tree computed for 10 000 bootstrap replicates (node values indicate bootstrap support) of heuristic searches consisting in 1000 RAS with hold 10.
https://www.sarem.org.ar/wp-content/uploads/2016/06/SAREM_MastNeotrop_23-1_Caraballo-sup1.docx