# Diversity of tuco-tucos (*Ctenomys*, Rodentia) in the Northeastern wetlands from Argentina: mitochondrial phylogeny and chromosomal evolution

**Diego A. Caraballo · Giselle A. Abruzzese · María Susana Rossi**

**Abstract** Tuco-tucos (small subterranean rodents of the genus *Ctenomys*) that inhabit sandy soils of the area under the influence of the second largest wetland of South America, in Northeastern Argentina (Corrientes province), are a complex of species and forms whose taxonomic status were not defined, nor are the evolutionary relationships among them. The tuco-tuco populations of this area exhibit one of the most ample grades of chromosomal variability within the genus. In order to analyze evolutionary relationships within the Corrientes group and its chromosomal variability, we completed the missing karyotypic information and performed a phylogenetic analysis. We obtained partial sequences of three mitochondrial markers: D-loop, cytochrome b and cytochrome oxidase I. The Corrientes group was monophyletic and split into three main clades that grouped related karyomorphs. The phylogeny suggested an ancestral condition of the karyomorph with diploid number (2n) 70 and fundamental number (FN) 84 that has evolved mainly via reductions of the FN although amplifications occurred in certain lineages. We discuss the relationship between patterns of chromosomal variability and species and groups boundaries. From the three main clades the one named *iberá* exhibited a remarkable karyotypic homogeneity, and could be considered as an independent and cohesive evolutionary lineage. On the contrary, the former recognized species *C. dorbignyi* is a polyphyletic lineage and hence its systematic classification should be reviewed.

D. A. Caraballo · G. A. Abruzzese · M. S. Rossi (✉)
IFIBYNE-CONICET. Laboratorio de Fisiología y Biología
Molecular, Dep. Fisiología, Biología Molecular y Celular,
Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II,
2do piso, EHA1428 Buenos Aires, Argentina
e-mail: srossi@fbmc.fcen.uba.ar

## Introduction

*Ctenomys* subterranean rodents (named popularly as tuco-tucos) inhabit the austral cone of South America, from Perú and Brazil to Tierra del Fuego, Argentina (Reig et al. 1990). The origin of the genus *Ctenomys* dates back 10 mya in the early upper Miocene based on fossil evidence (Quintana 1994), but the profuse cladogenesis is placed at the late Pliocene-early Pleistocene, as revealed by fossil record (Reig 1989; Verzi et al. 1991; Reig and Quintana 1992; Quintana 1994) as well as cyt-b-based clocks (Parada et al. 2011; Slamovits 2002).

The genus is characterized by one of the highest number of living species for mammals: about 60 (Woods and Kilpatrick 2005), and the broadest range of chromosomal number, from 2n = 10 to 2n = 70, known for a mammalian genus (Cook et al. 1990; Ortells et al. 1990). This extremely high karyotypic variability led to postulate early that *Ctenomys* was an example of chromosomal speciation (Reig and Kiblisky 1969). In most of the 60 living species each one has a distinctive 2n, with the exception of the high-Andean puna species *C. opimus*, *C. fulvus* and *C. robustus* sharing 2n = 26 and very similar C-banding patterns (Gallardo 1979), as well as the species *C. mendocinus*, *C. porteousi*, *C. australis*, *C. azarae*, *C. flamarioni* share the same 2n = 48, which conform the *mendocinus* group that includes *C. rionegrensis*, although its diploid number is 50 (Massarini et al. 1991; Freitas 1994; Massarini and Freitas 2005). Within the limits of a species, chromosomal polytypisms or polymorphisms

can also be found as is the case of *Ctenomys pearsoni*, with karyotypes ranging between 2n = 56 and 2n = 70 (Tomasco and Lessa 2007).

The cyt-b-based phylogenies obtained define main species groups within the genus, although the relationships among them remained unresolved and fall into a polytomy (Lessa and Cook 1998; D'Elía et al. 1999; Mascheretti et al. 2000; Slamovits et al. 2001; Parada et al. 2011). The star-like pattern of the phylogeny of the genus produced concern whether it was a soft (character dependent) or a hard (taxon dependent) polytomy. But the strong phylogenetic signal observed above and below the polytomy (Lessa and Cook 1998), as well as a coincident star-like phylogeny using intron sequences (Castillo et al. 2005) suggest that the radiation of tuco-tucos was explosive.

Based on allozymes and chromosomal characters, Ortells and Barrantes (1994) studied a *Ctenomys* group of species and forms that inhabit an area under the wide influence of the Iberá marsh and its natural channels and lagoons, in the Corrientes Argentinean province, between the Paraná and the Uruguay rivers. The sandy soils predominant in this area are favourable habitats for tuco-tucos digging activity; however the proximity to wetlands makes these habitats temporary and spatially fragmented.

The *Ctenomys* Corrientes group is currently comprised of 25 known populations some of which are alleged to belong to three species: *C. roigi*, *C. dorbignyi* and *C. perrensi*, but most of them have an undefined taxonomic status. The Corrientes group is characterized by a high chromosomal variability with diploid numbers ranging from 2n = 41 to 2n = 70 (Ortells et al. 1990; García et al. 2000b; Argüelles et al. 2001; Giménez et al. 2002; Lanzone et al. 2007). This conspicuous chromosomal variability, together with the patchy distribution of the demes, product of discontinuities of suitable habitats in the wetland, suggest that chromosomal evolution is an ongoing active and recurrent process in this group. The phylogeny of the Corrientes species and forms has not been resolved because of incomplete taxa representation (Parada et al. 2011). On the other hand, the work that included more species and forms from Corrientes did not yield a sufficiently resolved topology to clarify the phylogeny (Giménez et al. 2002).

The study of the *Ctenomys* Corrientes group implies several challenges: to assign populations to the described species (or to new species or forms), to describe a comprehensive scenario of chromosomal evolution and to evaluate the influence of ancestral variability versus ongoing processes in the patterns of genetic and chromosomal diversity. These three aspects overlap each other, so different approaches and data sets are necessary. The difficulty of delimiting species (and even populations) boundaries resides in the low genetic differentiation among populations when compared with the intrapopulation ones

(Ortells and Barrantes 1994; Mirol et al. 2010). However, it is difficult to distinguish whether this relatively low genetic differentiation among populations is due to the absence of fixation of ancestral variants (incomplete lineage sorting) or to genetic flux among them (Mirol et al. 2010). Defining the limits among species is not only relevant from a theoretical point of view, but also for biodiversity conservation and management programs in the Iberá Reserve as well as in other natural environments of the Corrientes province.

In order to bring a phylogenetic framework to the main questions that this complex group of species offers, we re-examined the phylogeny of the group, based on three mitochondrial markers: cyt-b, D-loop and COI. The objectives of our study are: (1) to delimitate the species and forms which comprise the Corrientes group, (2) to establish the main clades within it, and (3) to analyze chromosomal variability in this phylogenetic context.

## Materials and methods

### Chromosome preparations

Metaphase preparations were obtained from bone marrow following Ford and Hamerton (1956) and stained with Giemsa. Chromosomes were classified according to Levan et al. (1964) and fundamental numbers (FNs) were computed considering autosomes and sexual chromosomes, being 1 for telocentric chromosomes and 2 for biarmed chromosomes. For each specimen a minimum of 20 metaphases was analyzed.

### *Ctenomys* tissue samples

A total of 42 specimens collected between October 2007 and October 2010 correspond to the ingroup. The captures were performed using live traps at 23 populations located in Corrientes: Estancia la Tacuarita (27°58′42.7″S; 56°33′40.6″W), Pago Alegre (28°8′53″S; 58°21′44.8″W), Saladas (28°14′20.3″S; 58°37′40.4″W), Saladas Km-Sur (28°17′37.5″S; 58°41′19.2″W), San Alonso (28°17′76″S; 57°24′45″W), Rincón de Ambrosio (28°15′5.2″S; 58°53′40.9″W), Estancia San Luis (28°6′43.7″S; 58°51′48.1″W), Colonia 3 de Abril (28°23′26.9″S; 58°53′37.5″W), Goya (29°11′17.2″S; 59°12′36.7″W), San Roque (28°41′S; 58°42′S), Santa Rosa (28°10′47.4″S; 58°8′11.5″W), Chavarría (28°58′S; 58°35′W), Curuzú Laurel (27°55′24.4″S; 57°29′23.5″W), Loma Alta (28°5′2.5″S; 58°19′29.9″W), Loreto (27°44′43.7″S; 57°14′35.2″W), Contreras Cué (28°5′28.6″S; 56°33′53.7″W), Mbarigüí (27°33′S; 57°31′W), Paraje Angostura (27°33′S; 57°31′W), Mburucuyá (28°5′51.4″S; 58°16′38.6″W), San Miguel (28°0′58.6″S; 57°36′19.2″W), Paraje Caimán (28°3′3.1″S;

57°40′38.4″W), Manantiales (27°57′54.5″S; 58°7′20.9″W) and Paraje Sarandicito (30°14′43.1″S; 59°33′46.1″W). Ingroup sample numbers were assigned in the field. As outgroup candidates we included in the analyses 10 specimens corresponding to 7 *Ctenomys* species: *C. rionegrensis*, *C. conoveri*, *C. torquatus*, *C. minutus*, *C. argentinus* and *C. pearsoni*. The samples of *C. pearsoni* are from the type locality, Colonia (Uruguay) and also from Médanos, Entre Ríos, Argentina (33°25′37.1″S; 59°5′35.5″W) that were described to this species (Reig and Kiblisky 1969; Ortells et al. 1990; García et al. 2000a).The specimens from Médanos were sampled by us in October 2010, while the other samples correspond to a part of the set in Slamovits et al. (2001) and references therein, and Parada et al. (2011) (sample EV1454). Tissue samples collected by us were obtained from liver and/or phalanx preserved in ethanol 100 % for further DNA extraction.

## DNA extraction, PCR amplification, 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 760 bp fragment involving the 3′ end of the cyt-b coding sequence, and a 775 bp fragment concerning a 53 bp non-coding flanking sequence and 722 bp of the 5′ end of the COI coding sequence, were amplified by PCR. Primer information is shown in Table 1. PCR amplifications were performed in reaction volumes of 50 μl containing 2.5 U of *Taq* polymerase (Life Technologies, Brazil), 25–100 ng of genomic DNA, 0.4 μM of each primer, 0.2 mM deoxynucleoside triphosphate (dNTP), 1× *Taq* buffer, 2.5 mM MgCl$_2$. The D-loop amplification PCR protocol consisted of an initial 1 min denaturation step at 94 °C, 30 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, extension at 72 °C for 30 s, and a final extension of 5 min at 72 °C. In cyt-b amplification, thermal cycling consisted of an initial denaturation step at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 1 min, annealing at 49 °C for 1 min, extension at 72 °C for 1 min, and a final extension step of 5 min at 72 °C. The thermal cycling protocol for the amplification of the 5′ end of the COI coding sequence was identical to the one used to amplify cyt-b but annealing temperature was 53 °C. PCR products were purified and directly sequenced. For each amplicon 5–10 samples were sequenced for both strands, revealing no differences in the obtained contigs. Contigs and single stranded sequences were analysed using Contig Express (Vector NTI 10.3.0. Invitrogen). The sequences generated in this study were deposited in Gen-Bank (accession numbers JX275502-JX275655). Ambiguous alignments were not evident in any of the employed markers. All 52 specimens yielded the expected PCR

**Table 1** Primer sequences, final alignment length (expressed in base pairs), number (and percentage %) of variable as well as potentially informative sites, selected substitution models (for BE), and distance comparisons between members of ingroup, outgroup and ingroup versus outgroup, are summarized for the three markers used in this study

| Genomic region | D-loop | Cyt-b | COI |
|---|---|---|---|
| Primer sequences | TUCO-Dloop-F 5′ TTCTAATTAAACTATTTCTTG 3′ | TUTU-F 5′ CCTTCATAGGCTACGTAC 3′ | COI CT F 5′ CTGTNYTTAGATTTACAGTCTAA 3′ |
| | TDKD 5′ CCTGAAGTAGGAACCAGATG 3′* | TUTU-R 5′ CTTCATTTGAGTAGTTTAT 3′ | COI CT R 5′ GGGTGNCCRAARAATCARAA 3′ |
| Alignment length (bp) | 409 | 688 | 693 |
| Number of variable bases (%) | 68 (17 %) | 145 (21 %) | 137 (20 %) |
| Number of parsimony informative bases (%) | 30 (7 %) | 70 (10 %) | 55 (8 %) |
| Selected substitution model (for Bayesian estimation) | GTR + I + Γ | HKY + Γ | TVM + I + Γ |
| | Selected model for the concatenated data set: GTR + I + Γ (pinvar = 0.5765; α = 0.8579) | | |
| Mean divergence | | | |
| Ingroup | 1.24 ± 0.55 % | 1.14 ± 0.53 % | 0.94 ± 0.43 % |
| Outgroup | 4.37 ± 1.98 % | 6.00 ± 3.07 % | 4.71 ± 3.94 % |
| Ingroup-Outgroup | 3.59 ± 1.58 % | 4.46 ± 2.57 % | 3.85 ± 2.65 % |

* Primer TDKD was designed by Kocher; see reference in Slade et al. (1994)

products of amplification, except *C. argentinus*, that did not yield any product neither for COI nor for cyt-b, probably because of the partial degradation observed in total genomic DNA agarose electrophoresis.

Sequence alignment and analyses

DNA sequence alignments were performed using CLUSTAL X2 program (Larkin et al. 2007). Statistics on nucleotide composition were compiled using DAMBE (Xia and Xie 2001), performing a test introduced by Xia et al. (2003) to measure substitution saturation in a set of aligned sequences to evaluate whether these sequences retain phylogenetic signal.

The three partitions were analyzed with maximum parsimony (MP), distance methods (DM), and Bayesian inference analysis (BI) in two combinations: (1) individual alignments for each marker, and (2) all 3 sequences concatenated together.

For BI analyses the Akaike Information Criterion (AIC) approach was used to find the simplest substitution model that explained the data using the program ModelTest 3.7 (Posada and Crandall 1998). Maximum parsimony and DM searches were carried out with PAUP 4.0 (Swofford 2003), and BI was performed with MrBayes 3.1 (Ronquist and Huelsenbeck 2003).

Distance methods inferences were carried out using the Neighbor-Joining method (NJ; Saitou and Nei 1987) and clade support was evaluated performing 10,000 bootstrap replicates.

Maximum parsimony searches were performed using equal transformation costs and weights, as well as using the weighting scheme 2, 5 and 1 for first, second and third codon positions (cyt-b and COI), respectively, and a stepmatrix contemplating a transition/transversion (Ts/Tv) ratio of 6. Since D-loop is a non genic sequence, we assayed two weighting schemes 1 and 2 i.e. the same weight of the third or the first codon positions of coding sequences, respectively. Gaps were either treated as missing data or as a fifth character. Heuristic searches were performed with the same search parameters for all analyses: 1,000 Random Addition Sequences (RAS) hold 10 and TBR swapping algorithm. To assess clade support we ran 1,000 bootstrap replicates for each partition and for the concatenated data set, and we further calculated a majority rule consensus of the replicates.

Bayesian inference was conducted with random unconstrained starting trees; four simultaneous Markov chains were run for 300,000 generations, and trees were sampled every 100 generations. The standard deviation of split frequencies was analyzed to determine when the Markov chain reaches stationarity, and samples prior to "burn-in" were discarded. We stopped the analyses when the standard deviation of split frequencies was smaller or equal to 0.01

and when the "burn-in" phase corresponded at most to one-third of total generations. 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. With the concatenated sequences we performed two runs for 1,000,000 generations: (a) using independent substitution models for each partition and (b) using a single substitution model inferred by treating the whole concatenated matrix as a unique partition.

## Results

D-loop, cytochrome b and cytochrome oxidase I sequence variability

We employed three mitochondrial sequences: D-loop, 3′ fragment of cyt-b and the 5′ fragment of COI.

No deletions or insertions or stop codons were observed in the alignment of cyt-b sequences for the 51 specimens, which preclude the inclusion of nuclear pseudogenes that were found in *Ctenomys* (Mirol et al. 2000). No saturation was revealed by the substitution saturation test implemented for 1st and 2nd codon positions (Iss = 0.0620 < Iss.c = 0.6974 DF = 116 and $p < 0.0001$) neither for 3rd codon position (Iss = 0.1752 < Iss.c = 0.6848 DF = 136 and $p < 0.0001$).

The total sequence length of the D-loop fragment varied from 406 to 409 bp, consequently the final alignment of this marker contained gaps reflecting possible indels among the 52 total sequences. However, ambiguous alignments were not evident in this case or in the other two markers. No saturation was revealed by the substitution saturation test implemented (Iss = 0.115 < Iss.c = 0.691 DF = 119 and $p < 0.0001$), thus validating the use of this marker for phylogenetic inference.

No variation in length was detected in the 693 bp alignment of the COI gene fragment obtained for 51 individuals. The substitution saturation test showed no or little saturation for 1st and 2nd codon positions (Iss = 0.026 < Iss.c = 0.698 DF = 111 and $p < 0.0001$), either for 3rd codon positions and the 5′ non-coding region taken as a whole (Iss = 0.152 < Iss.c = 0.685 DF = 151 and $p < 0.0001$).

The mean divergence (average of the uncorrected pairwise distances) among the members of the ingroup, outgroup and the ingroup/outgroup, as well as the number of variable and potentially informative sites, for each marker are shown in Table 1. The amplified sequences of cyt-b and COI contain more variable and also more potentially informative characters than those of D-loop (Table 1).

We found an even number of unique haplotypes for the three markers, being 32 for cyt-b, 36 for D-loop and 34 in the case of COI. The concatenated matrix (D-loop + cyt-
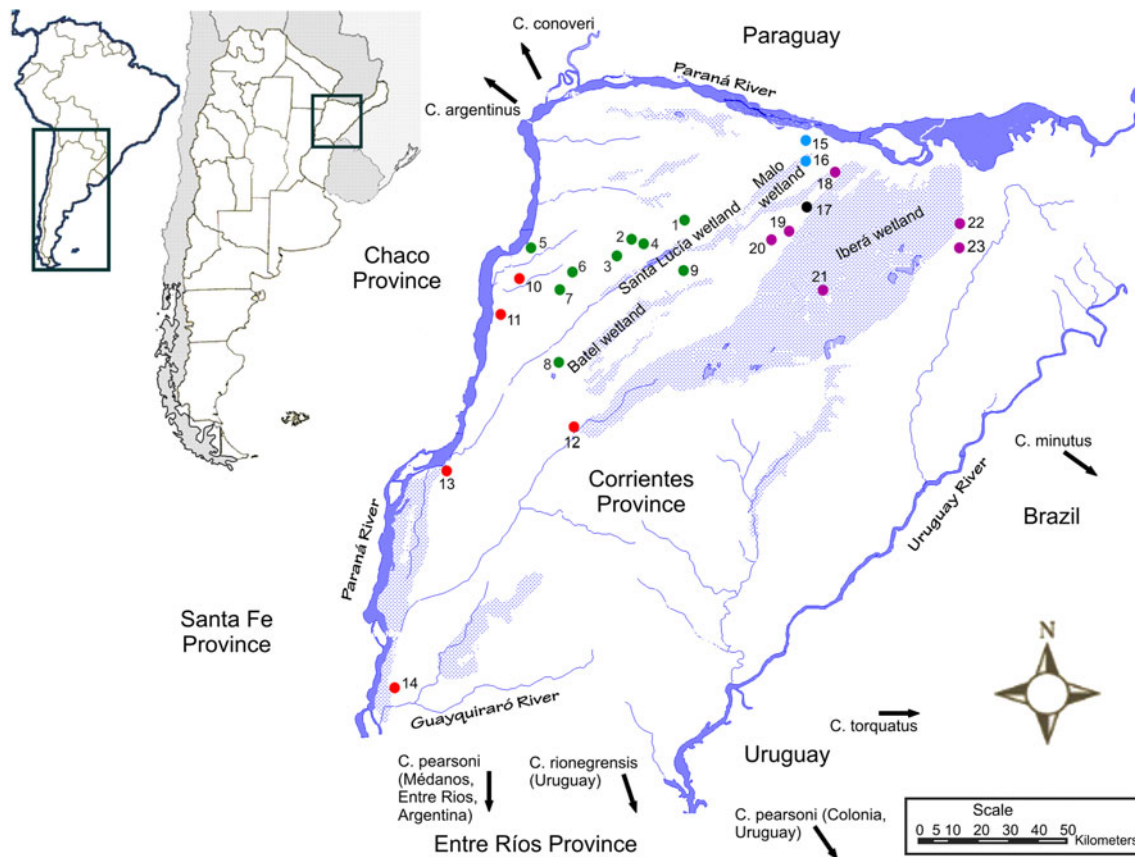
**Fig. 1** Map showing sampling localities. Each locality has a color key according to the main clades within Corrientes group. The *numbers* correspond to: (*1*) Manantiales, (*2*) Loma Alta, (*3*) Pago Alegre, (*4*) Mburucuyá, (*5*) Estancia San Luis, (*6*) Saladas, (*7*) Saladas Km-Sur, (*8*) San Roque, (*9*) Santa Rosa, (*10*) Rincón de Ambrosio, (*11*) Colonia 3 de abril, (*12*) Chavarría, (*13*) Goya, (*14*) Paraje Sarandicito, (*15*) Mbarigüí, (*16*) Paraje Angostura, (*17*) Curuzú Laurel, (*18*) Loreto, (*19*) San Miguel, (*20*) Paraje Caimán, (*21*) San Alonso, (*22*) Estancia La Tacuarita and (*23*) Contreras Cué. The direction towards the geographic distribution of each outgroup is pointed with black arrows. The Corrientes province as well as neighboring provinces or countries is indicated. Water bodies, such as main rivers and wetlands are shown in blue. (Color figure online)

b + COI) contains 1,790 characters from which 350 are variable, defining 44 haplotypes for the 42 individuals from the Corrientes group plus 10 individuals considered potential members of the outgroup.

Phylogenetic analysis

The trees obtained with the three markers separately did not completely resolve the relationship among the Corrientes lineages but were congruent among them (not shown). The concatenated data set analyzed under MP, NJ and Bayesian methods were also congruent and yielded a resolved topology (Fig. 2). Congruent topologies were obtained using any of the weighting schemes and gap treatment. Differences among the three methods resided on the statistical support of certain groups but not, as mentioned, on the topologies (Fig. 2). Since D-loop, cyt-b and COI fit to different evolutionary models (Table 1), these were considered in Bayesian phylogenetic analysis.

However, we also obtained a congruent topology using a unique evolutionary model (see Table 1) for the concatenated data set.

In general, bootstrap values for MP and NJ were lower than Bayesian posterior probabilities (Fig. 2). However, discrepancy among characters was not the cause of this difference, but instead the low proportion of synapomorphic characters. Indeed, the strict consensus and majority rule trees for MP and NJ recovered the same topology that resulted from the posterior distribution of the Bayesian probabilities.

As putative outgroups we include species distributed beyond both margins of the Paraná and Uruguay Rivers, the two plausible physical barriers for the Corrientes group (see Fig. 1). We constrained the root in *C. rionegrensis*, since this species belongs to a sister group of a clade that included members of the Corrientes group (Parada et al. 2011). However, the topology did not change when other species are set as outgroup. *Ctenomys pearsoni*, distributed
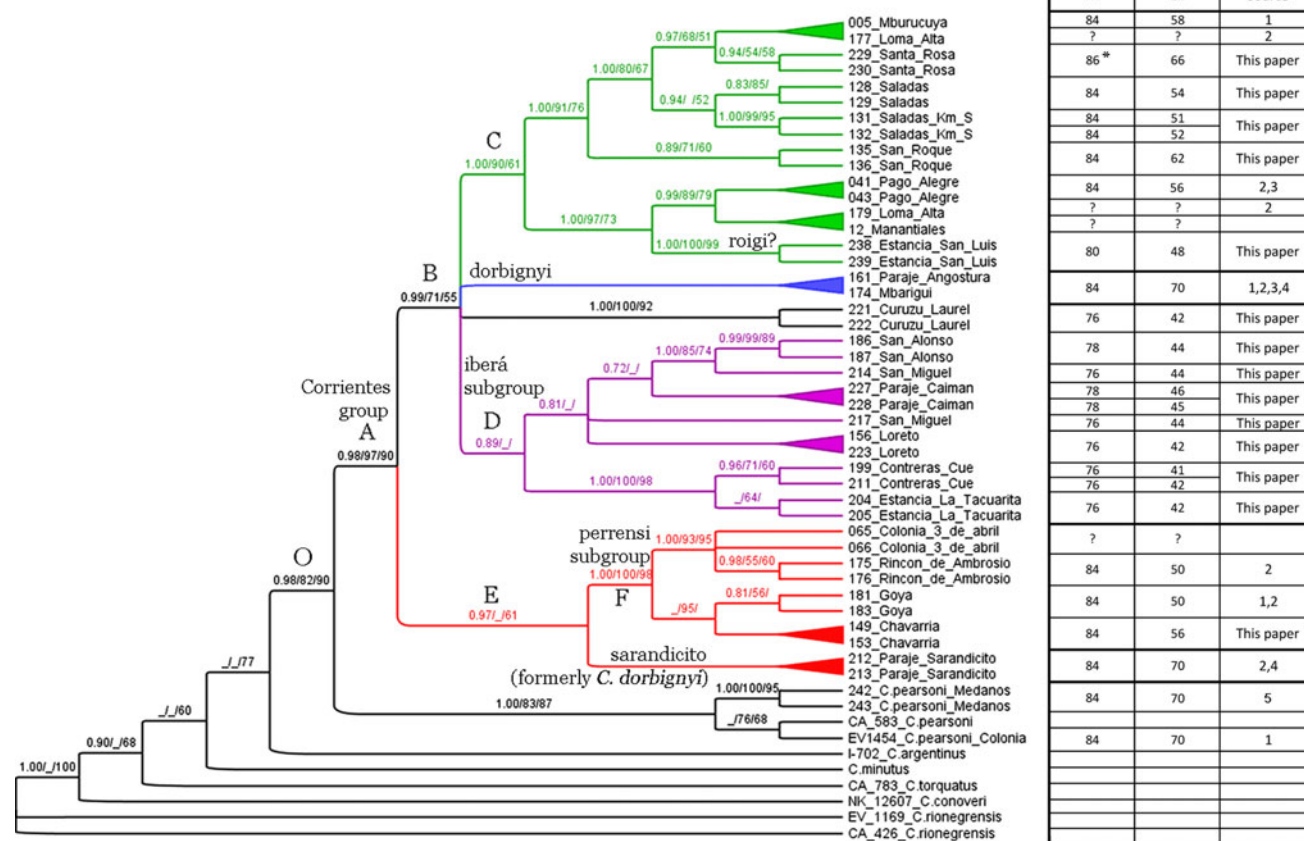
| | FN | 2n | Source |
|---|---|---|---|
| 005_Mburucuya | 84 | 58 | 1 |
| 177_Loma_Alta | ? | ? | 2 |
| 229_Santa_Rosa | 86 * | 66 | This paper |
| 230_Santa_Rosa | | | |
| 128_Saladas | 84 | 54 | This paper |
| 129_Saladas | | | |
| 131_Saladas_Km_S | 84 | 51 | This paper |
| 132_Saladas_Km_S | 84 | 52 | |
| 135_San_Roque | 84 | 62 | This paper |
| 136_San_Roque | | | |
| 041_Pago_Alegre | 84 | 56 | 2,3 |
| 043_Pago_Alegre | | | |
| 179_Loma_Alta | ? | ? | 2 |
| 12_Manantiales | ? | ? | |
| 238_Estancia_San_Luis | 80 | 48 | This paper |
| 239_Estancia_San_Luis | | | |
| 161_Paraje_Angostura | 84 | 70 | 1,2,3,4 |
| 174_Mbarigui | | | |
| 221_Curuzu_Laurel | 76 | 42 | This paper |
| 222_Curuzu_Laurel | | | |
| 186_San_Alonso | 78 | 44 | This paper |
| 187_San_Alonso | | | |
| 214_San_Miguel | 76 | 44 | This paper |
| 227_Paraje_Caiman | 78 | 46 | This paper |
| 228_Paraje_Caiman | 78 | 45 | |
| 217_San_Miguel | 76 | 44 | This paper |
| 156_Loreto | 76 | 42 | This paper |
| 223_Loreto | | | |
| 199_Contreras_Cue | 76 | 41 | This paper |
| 211_Contreras_Cue | 76 | 42 | |
| 204_Estancia_La_Tacuarita | 76 | 42 | This paper |
| 205_Estancia_La_Tacuarita | | | |
| 065_Colonia_3_de_abril | ? | ? | |
| 066_Colonia_3_de_abril | | | |
| 175_Rincon_de_Ambrosio | 84 | 50 | 2 |
| 176_Rincon_de_Ambrosio | | | |
| 181_Goya | 84 | 50 | 1,2 |
| 183_Goya | | | |
| 149_Chavarria | 84 | 56 | This paper |
| 153_Chavarria | | | |
| 212_Paraje_Sarandicito | 84 | 70 | 2,4 |
| 213_Paraje_Sarandicito | | | |
| 242_C.pearsoni_Medanos | 84 | 70 | 5 |
| 243_C.pearsoni_Medanos | | | |
| CA_583_C.pearsoni | | | |
| EV1454_C.pearsoni_Colonia | 84 | 70 | 1 |
| I-702_C.argentinus | | | |
| C.minutus | | | |
| CA_783_C.torquatus | | | |
| NK_12607_C.conoveri | | | |
| EV_1169_C.rionegrensis | | | |
| CA_426_C.rionegrensis | | | |

**Fig. 2** Phylogenetic tree using a combined data set of 3 mitochondrial markers: cytochrome-b, cytochrome oxidase I, and D-loop. Specimen catalogue number/field code and population procedence are shown in the terminal nodes. *Numbers* above internal nodes indicate support for posterior probability, parsimony bootstrap (%) and distance bootstrap (%) (BE/MP/NJ). Only values higher than 50 % of MP and NJ and/or higher than 0.7 of *p* are shown, while nodes with lower values were collapsed. Identical haplotypes are schematized with *triangles*, and main clades in the Corrientes group are shown with *different colours* and/or *capital letters* (*A–F*). Tentative names for well supported clades that share chromosomal affinities are displayed above the nodes. The *O* shows the hypothetical 2n = 70 FN = 84 common ancestral node, that involves the Corrientes group as well as the lineage leading to *C. pearsoni*. At the right of the tree a table shows the FN and 2n of a population's karyotype (reference numbers) or a specimen's karyomorph (this paper). Reference numbers stand for 1: Ortells et al. (1990), 2: Giménez et al. (2002), 3: Lanzone et al. (2007), 4: Argüelles et al. (2001), 5: García et al. (2000a). In this last citation the authors describe the karyomorph found in Médanos as indistinguishable in number and morphology from the one found in *C. dorbignyi*. The karyotype of *C. dorbignyi* has been extensively described as 2n 70 and FN 84, so we decided to consider this as the morph for both *C. dorbignyi* and the karyomorph found in Médanos. However we shall mention that these authors mention a FN 88 for *C. dorbignyi* and Médanos, although chromosomal morphology is not clear in their karyograms. *See reference in text. (Color figure online)

in both margins of the Uruguay River (Fig. 1), is the closest species to the Corrientes group (Fig. 2). The phylogenetic analysis supported the monophyletic condition of the Corrientes group that split apart from *C. pearsoni* with a high support for the three methods (Fig. 2, see node O).

Within the Corrientes group, the haplotypes from any population were identical or constituted a monophyletic group, except in the cases of Loma Alta and San Miguel (Fig. 2, see below). We would focus the attention in five main and well-supported nodes (B, C, D, E and F) (Fig. 2). The node C grouped the population of Estancia San Luis, 9.87 km apart from Costa Mansión, the type locality of *C. roigi* (Contreras 1989), with several populations whose

taxonomic status were undetermined: Manantiales, Loma Alta, Pago Alegre, San Roque, Saladas, Saladas Km-Sur, Santa Rosa and Mburucuyá. All these last populations are distributed in the area under the influence of the Santa Lucía wetland, a small basin without discharge, located at the West of the Iberá marsh whose hydrologic flux is mainly conditioned by rain regimes (Bohn and Campo 2010). All nodes derived from node C are quite well supported by the three methods. As mentioned, the only case in this group in which haplotypes from the same population fell into different groups is Loma Alta. One of the haplotypes from Loma Alta is identical to one from Manantiales, while the other is identical to one of Mburucuyá.

Node D showed a strong statistical support in the Bayesian analysis, but not under MP or NJ. However, as mentioned above, the cause of the low bootstrap values were not homoplasies. This node persisted when the strict consensus of the most parsimonious trees was computed. If more characters were included in the analysis, bootstrap values would be probably higher.

Node D included several populations whose taxonomic status was undefined (Ortells et al. 1990). This group clustered populations distributed in the Eastern (Contreras Cué and Tacuarita) and Western (Loreto, San Miguel and Paraje Caimán) margins of the Iberá marsh, as well as the population of San Alonso, a recently discovered population located in an island within the marsh (Fig. 1). All the populations of this group are under the direct influence of the water level of the big Iberá marsh. Curuzú Laurel is the only population located in the margin of the Iberá marsh whose ownership to this group did not receive enough support (Fig. 2).

The other well-supported node (F) included the population from Goya, the type locality of one of the three nominal species described in the Corrientes group, *C. perrensi*, as well as the populations of Colonia 3 de Abril and Rincón de Ambrosio, classified by other authors as belonging to this species (Giménez et al. 2002). This group also included the two identical haplotypes from Chavarría population that joined those from Goya population. However, this relationship was only held by MP analysis, but not by the other two methodologies (Fig. 2), suggesting some degree of conflict among characters.

Previous studies described the third nominal species, *C. dorbignyi*, as represented by the Northern populations of Paraje Angostura and Mbarigüí and the Southern population of Paraje Sarandicito (Fig. 1), since all these populations shared the same karyomorph (2n = 70, FN = 84) (Ortells et al. 1990; Argüelles et al. 2001). However, the phylogenetic tree showed *C. dorbignyi* as polyphyletic: Northern and Southern populations split apart into two strongly supported nodes, B and E, respectively (Fig. 2).

Chromosomes

The same fundamental number 84 is shared between the karyomorphs found in both Northern and Southern populations previously alleged to *C. dorbignyi*, as well as groups C and F, with the exception of Estancia San Luis and Santa Rosa (Fig. 2). Estancia San Luis exhibited a FN of 80, unique in this group, and 2n = 48, the same karyomorph found in Costa Mansión, the type locality of *C. roigi* (Contreras 1989).

In the case of Santa Rosa the 4 analyzed individuals (2 of them included in this study) showed a complex karyomorph. To begin with, they presented the highest FN in

the Corrientes group, being 86. However, we cannot avoid mentioning the existence of a variable number of extra short "arms" in (otherwise monobrachial) chromosomal pairs that could not be ignored because they appeared in the 4 studied specimens from Santa Rosa. Since the number of these tiny arms varied between metaphases in the same individual, we are performing chromosomal banding techniques to try and distinguish whether this is a case of mosaicism or a technical artifact. The smallest FN of the Corrientes group was found in Curuzú Laurel and in the specimens of the group of node C, ranging from 76 to 78 (Fig. 2).

Regarding group C, chromosomal numbers ranged from 48 of Estancia San Luis to 66 of Santa Rosa (Fig. 2). In addition to the FN variability observed in Santa Rosa, we found a minor but detectable mosaicism in the 2n for all individuals of this population, counting 65 chromosomes, where the lacking chromosome was always one of the smallest telocentric pairs. Group D exhibited a narrower range of chromosomal numbers, from 41 in one individual from Contreras Cué, to 46 in one of the individuals from Paraje Caimán. Curuzú Laurel showed an undistinguishable karyotype (FN = 76 2n = 42) from those found in Loreto, Contreras Cué and Estancia La Tacuarita, all populations belonging to node D (unpublished result). Chromosomal numbers of group F were 50 or 56. As mentioned, the populations of Paraje Sarandicito, Paraje Angostura and Mbarigüí presented a 2n = 70, the highest chromosomal number for all *Ctenomys* species.

Discussion

The phylogeny of the Corrientes group we presented here has included the highest number of populations considered up to now. The inclusion of a large number of lineages, as well as a broad spectrum of outgroups, enabled us to delimitate the monophyletic group. As happened with other analysis based on cyt-b including 4 representatives of the Corrientes group (Parada et al. 2011), in our preliminary cyt-b based analysis, some specimens of *C. pearsoni* appeared intermixed with individuals of the Corrientes group but in our case split apart with high support when the 3 markers and more individuals from *C. pearsoni* were included (Fig. 2, node O). The Corrientes group was strongly sustained by node A (Fig. 2). The present phylogenetic analysis showed that *C. pearsoni*, is the sister lineage to the Corrientes group.

Regarding the Corrientes group, two basic ranges of FNs were found (Fig. 2): 76–78 and 80–84–86. Fundamental numbers fit with well-supported nodes: C (FN = 80–84–86), E (FN = 84), and node D (FN = 76–78). The occurrence of the karyomorph FN = 84 2n = 70 in the Corrientes group

(*sarandicito* and *dorbignyi* lineages) as well as in its sister clade, *C. pearsoni* (Kiblisky et al. 1977), suggests the ancestral condition of this karyomorph, as postulated by Ortells et al. (1990). In addition, the karyomorphs of these two lineages exhibit very similar chromosomal banding patterns (Argüelles et al. 2001; García et al. 2000a). Taking into account the topology of the tree, it is reasonable to hypothesize that the FN = 84 of the ancestral karyotype suffered a moderate reduction in the lineage leading to the one found in Estancia San Luis and a more extreme diminution in that leading to node D. However, moderate increases in FN would also have occurred within nodes C and node D (see below). Assuming the ancestry of the FN = 84 2n = 70, the overall variability in chromosomal numbers could be explained as mainly due to recurrent Robertsonian rearrangements, in which there is a global trend for reduction, since there are no populations with 2n > 70.

As regards the potential effects of centric fusions in heterozygous fertility, previous studies in 17 specimens of the Corrientes group showed that meiosis of heterozygous individuals for one or two Robertsonian translocations occurs with normal disjunctions (Lanzone et al. 2007). It is worth noting that no more than two Robertsonian changes were found in heterozygosis in this study, suggesting that this could be the threshold for this type of rearrangement beyond which meiosis could be anomalous. In fact the model of speciation by monobrachial centric fusions proposed by Baker and Bickham (1986) holds that if there is hybridization between two isolated stocks with several different fixated Robertsonian rearrangements the resulting hybrid would be sterile. The cause of sterility is claimed to be the simultaneous occurrence of several rearranged chromosomes (which would individually cause little or no loss of fertility when heterozygous) which in meiosis form quadrivalents or more complex multivalents that usually do not segregate normally. In the Corrientes group there are two cases where similar karyomorphs differ in Robertsonian rearrangements. The sister karyomorphs of Saladas (2n = 54) and Saladas Km-Sur (2n = 52) differ in a single fusion/fission event that would not be sufficient to constitute a barrier to gene flow according to the findings of Lanzone et al. (2007). However, the three fusions of 6 acrocentric chromosomal pairs (not shown; a detailed analysis of chromosomal morphology and banding patterns will be published separately) that distinguish the karyomorphs of Goya (2n = 50) and Chavarría (2n = 56) might constitute a case of speciation by centric fusions.

The phylogeny showed several strongly held clades within the Corrientes group. However, we propose to name those subgroups that not only received phylogenetic support, but also exhibited homogeneous chromosomal characteristics: *perrensi* and *iberá*. The *perrensi* subgroup was strongly supported in the phylogeny based on the

concatenated sequences and also by the phylogenies that resulted from the three markers separately (not shown). This subgroup shared the same FN = 84 and, as mentioned above, closely related karyomorphs. Since patchy favorable habitats (sandy soils) occur almost all over the Corrientes province, and taking into account that the Southeastern part of the province has not been completely surveyed, it is probable that intermediate karyomorphs occur or have occurred in populations of this area.

As mentioned above, we considered subgroup *iberá* as a robust clade since Bayesian posterior probability is high (0.89) and, although the bootstrap value for MP (nor NJ) did not reach 50 %, the strict consensus of the most parsimonious trees held it, indicating the lack of substantial conflict between characters in defining this group. The chromosomal homogeneity of the *iberá* subgroup is remarkable. The difference in FNs 76 and 78 could have involved the non-centric fission of a chromosomal segment and the formation of a neocentromere with the subsequent increase of both FN and 2n. We propose that FN evolution in this group was mainly towards amplification since the most parsimonious reconstruction of the FN in the basal node of this clade is 76.

Ortells et al. (1990) originally suggested that Northern and Southern Correntinean populations of *C. dorbignyi*, as well as the populations of *C. pearsoni* from Conchillas, Uruguay, may be the relics of a widespread paleodistribution of the karyomorph 2n = 70 FN = 84. Our phylogeny showed that the Southern population of Paraje Sarandicito previously classified as *C. dorbignyi* is related to the subgroup *perrensi* and not to the Northern populations of Paraje Angostura and Mbarigüí. The polyphyletic state of *C. dorbignyi*, together with its basal position in the two main clades of the Corrientes group (nodes B and E, Fig. 2) is congruent with the scenario proposed by Ortells et al. (1990). In addition, microsatellite variability showed that these two groups of populations belong to different clusters (Mirol et al. 2010) also indicating the polyphyletic condition of this taxa. The designation of *C. dorbignyi* was hence restricted to the specimens of the Northern populations since Mbarigüí is the type locality of this species (Contreras and Contreras 1984).

Node C is a very robust clade, and the relationships among the lineages that it comprises are particularly resolved with our data set (Fig. 2). Contrary to what was observed in subgroups *iberá* and *perrensi*, chromosomal variability within this node is high, and it is not possible to hypothesize clear sequences of chromosomal evolution. For this reason we did not name this clade. The absence of hypothetical intermediate karyomorphs may be due to incomplete sampling and/or extinction (the area is under extensive anthropic influence via agricultural activity and the presence of several small towns).

Strikingly, Loma Alta had two different and distantly related haplotypes, one identical to that of Mburucuyá and the other identical to that of Manantiales (Fig. 2). The distribution of both Loma Alta's haplotypes between the two major clades diverging from node C was also well supported by the phylogenies obtained when the three markers were run separately. The presence of these two different haplotypes in Loma Alta may be reflecting the persistence of mitochondrial variants of an ancestral pool (incomplete lineage sorting), or the occurrence of genetic flux between individuals from Mburucuyá and Manantiales.

The haplotypes from the populations of Paraje Angostura and Mbariguí fall into a polytomy involving the clade diverging from node C, subgroup iberá and the haplotypes from Curuzú Laurel. As discussed in other *Ctenomys* phylogenies, the polytomy observed in the divergence of these groups of karyomorphs might reflect a virtually simultaneous process of cladogenesis producing a star phylogeny (hard polytomy) or may be due to the lack of informative characters and/or high levels of conflict between them (soft polytomy). The argument in favor of a hard polytomy in this node is that the polytomy is preceded and followed by nodes with high support values as proposed by Lara et al. (1996). However there is extra phylogenetic data that support the hypothesis of a soft polytomy in node B. The karyomorph of Curuzú Laurel is undistinguishable from those of the *iberá* subgroup with FN = 76, 2n = 42, although its membership to this subgroup is not resolved by this data set. If the positional uncertainty of Curuzú Laurel is due to a soft polytomy, the inclusion of additional markers will incorporate this taxon into the *iberá* subgroup.

The intragroup distances between pairs of sequences in the nodes C, D and F were very similar (not shown). Consequently, branch lengths within these three nodes were comparable in NJ analysis. Instead, chromosomal variability exhibited different grades among them, being higher within node C when compared with subgroups *iberá* and *perrensi*, suggesting that karyotypic evolution may have occurred at higher intensity within the first group.

Regarding the relationship between chromosomal variability and groups boundaries, we found three main patterns:

1. A set of *similar* karyomorphs exclusively found in a monophyletic group of populations, in contrast to species-specific karyotypes frequently found in the rest of *Ctenomys*. The example of this pattern is the *iberá* subgroup. Karyomorphs of the members of clade F would reflect this pattern with a lower range of chromosomal variability and if the 2n = 56 NF = 84 from Pago Alegre is actually a different karyomorph from the one found in Chavarría.

2. A differentiated karyomorph exclusively found in a unique lineage. This is the case of Estancia San Luis, quite possibly *C. roigi*, as argued above.

3. The presence of the same karyomorph shared by polyphyletic lineages, this is the case of *C. dorbignyi* and the lineage from Paraje Sarandicito both sharing the 2n = 70 FN = 84. This case is radically different from the monophyletic groups of species-complexes as are the *mendocinus* and the *opimus* groups which share similar karyotypes.

   Except Estancia San Luis, with a remarkably differentiated karyomorph, the remaining members of clade C exhibit a complex chromosomal variability and, in the light of the basic karyotypic information depicted in this work, could not be included in any of the former patterns.

Most of the populations surveyed in the present study, were also sampled during 1994–2003 and subjected to population genetics analyses based on microsatellite nuclear loci (Mirol et al. 2010). Five out of eight microsatellite-based clusters did not disagree with our results. For instance, in both studies, Sarandicito, on the one hand and Mbariguí and Paraje Angostura, on the other, resulted in different groups. In addition, populations alleged to *C. roigi* also constituted a separate cluster. The populations depicted in the *iberá* subgroup fall into two separate clusters but were not intermixed with other populations different to those included in node D (subgroup *iberá*). The only exception is Curuzú Laurel, which in our analysis, as mentioned, did not join the *iberá* subgroup, but falls in the same polytomy (Fig. 2). However, as stated above, the undefined position of Curuzú Laurel is not incongruent with its hypothetical membership to the *iberá* subgroup, as suggested by nuclear and chromosomal evidence. In addition its geographical location at the margins of Iberá wetland coincides with the one of the members of the *iberá* subgroup (Fig. 1). Finally, Pago Alegre, Mburucuyá and Manantiales populations that belong to the same cluster pertained all to node C in our analysis.

However, two microsatellite-based clusters are to some extent discordant with our phylogeny. The populations of Goya, Chavarría, Santa Rosa and San Roque, resulted in a unique cluster, while Colonia 3 de Abril, Rincón de Ambrosio and Saladas populations resulted grouped into a separate one. This clusterings bring together populations that belong to different deeply splitting branches (nodes C and F, see Fig. 2). Phylogenetic clades, based on mitochondrial markers, and microsatellite-based population clustering may be incongruent because of the different inheritance modes and differing influence of historical processes that both markers reflect (Avise 2004).

We can interpret the microsatellite-based clusterings as the trace of a certain degree of gene flow between populations of the *perrensi* subgroup and populations grouped in node C. It is worth noting that the karyotypes of these nodes are potentially compatible (FN = 84 for *perrensi* subgroup and FN = 80–84–86 for node C). Reinforcing the gene flow hypothesis, the populations from both clusters had mixed ancestry: the membership to a certain cluster of the populations was not absolute; instead the populations had a major probability of belonging to a specific cluster, but also a second most probable membership to another one. The populations from the first cluster (Goya, Chavarría, Santa Rosa and San Roque) have a second highest probability of being members of the second cluster (Colonia 3 de Abril, Rincón de Ambrosio and Saladas) and vice versa.

Incongruence between mtDNA and nuclear microsatellites as a product of male-mediated dispersal has been well documented in rodents (Bryja et al. 2010), in elephants (Nyakaana and Arctander 1999), as well as in lizards (Godinho et al. 2008). Polygyny has been described as the regular mating system in non social tuco-tucos species, such as *C. pearsoni*, *C. talarum*, *C. australis* and *C. flamarioni* (Zenuto and Busch 1998; Zenuto et al. 1999a, b, 2002; Mora et al. 2010; Fernández-Stolz et al. 2007; Francescoli 2010), and associated to a higher home range in males than in females (Cutrera et al. 2006, 2010). This predominant mating system may lead to territorial struggles with the concomitant expulsion of males, favoring occasional male migrations, and therefore producing the observed lack of congruence between mtDNA and nuclear groupings. However, habitat fragmentation should be taken into consideration in the case of the Corrientes group, since habitat discontinuities may hinder long distance dispersals. Although discordances between mitochondrial and nuclear markers may be due to a differential vagility between sexes, as either argued by Mirol et al. (2010), we should also take into consideration that mtDNA is more sensitive to genetic drift than nuclear markers are (Larmuseau et al. 2010).

The present work shows the monophyletic condition of the Corrientes group, and in addition brings a phylogenetic framework to interpret chromosomal evolution, as well as future morphological studies in this group. Regarding the delimitation of species boundaries, our results show that subgroup *iberá* could be considered an independent evolutionary lineage. Morphological as well as ecological data should be added in order to assess if *iberá* is a new species in the Corrientes group. The subgroup *perrensi* and populations from node C, may not be completely isolated one from the other. In order to consider *C. dorbignyi* as a natural group, it should be restricted to the, monophyletic, Northern populations. Species boundaries in these cases should take into account

the intensity of these contacts in order to distinguish whether it is an occasional introgression or a wide spread hybridization.

## References

Argüelles CF, Suárez P, Giménez MD, Bidau CJ (2001) Intraspecific chromosome variation between different populations of *Ctenomys dorbignyi* (Rodentia, Ctenomyidae) from Argentina. Acta Theriol 46(4):363–373

Avise JC (2004) Molecular markers, natural history and evolution. Sinauer Associates, Sunderland

Baker RJ, Bickham JW (1986) Speciation by monobrachial centric fusions. Proc Natl Acad Sci USA 83:8245–8248

Bohn VY, Campo AM (2010) Estimación de escurrimientos superficiales para cuencas no aforadas en Corrientes, Argentina. Investigaciones Geográficas 71:31–42

Bryja J, Granjon L, Dobigny G et al (2010) Plio-Pleistocene history of West African Sudanian savanna and the phylogeography of the *Praomys daltoni* complex (Rodentia): the environment/geography/genetic interplay. Mol Ecol 19:4783–4799

Castillo AH, Cortinas MN, Lessa EP (2005) Rapid diversification of South American tuco-tucos (*Ctenomys*; Rodentia, Ctenomyidae): contrasting mitochondrial and nuclear intron sequences. J Mammal 86:170–179

Contreras JR (1989) *Ctenomys roigi*, una nueva especie de 'anguya tutu' de la Provincia de Corrientes, Argentina (Rodentia: Ctenomyidae). Bol Inst Estud Almerienses 1988 (extra)(1989): 51–67

Contreras JR, Contreras ANCH (1984) Diagnosis preliminar de una nueva especie de 'anguyá tutú' (género *Ctenomys*) para la provincia de Corrientes, Argentina (Mammalia, Rodentia). Hist Nat 4:131–132

Cook JA, Anderson S, Yates TL (1990) Notes on Bolivian mammals. 6, The genus *Ctenomys* (Rodentia, Ctenomyidae) in the highlands. Am Mus Novit 2980:1–27

Cutrera AP, Antinuchi CD, Mora MS, Vassallo AI (2006) Home-range and activity patterns of the South American subterranean rodent *Ctenomys talarum*. J Mammal 87:1183–1191

Cutrera AP, Mora MS, Antenucci CD, Vassallo AI (2010) Intra- and interspecific variation in home-range size in sympatric tuco-tucos, *Ctenomys australis* and *C. talarum*. J Mammal 91:1425–1434

D'Elía G, Lessa EP, Cook JA (1999) Molecular phylogeny of tuco-tucos, genus *Ctenomys* (Rodentia: Octodontidae): evaluation of the *mendocinus* species group and the evolution of asymmetric sperm. J Mamm Evol 6:19–38

Fernández-Stolz GP, Stolz JFB, De Freitas TRO (2007) Bottlenecks and dispersal in the tuco-tuco das Dunas, *Ctenomys flamarioni* (Rodentia: Ctenomyidae), in Southern Brazil. J Mammal 88: 935–945

Ford CE, Hamerton JL (1956) A colchicine, hypotonic citrate, squash sequence for mammalian chromosomes. Stain Technol 31: 247–251

Francescoli G (2010) Tuco-tucos' vocalization output varies seasonally (*Ctenomys pearsoni*; Rodentia, Ctenomyidae): implications for reproductive signaling. Acta Ethol 14:1–6

Freitas TRO (1994) Geographical variation of heterochromatin in *Ctenomys flamarioni* (Rodentia-Octodontidae) and its cytogenetic relationships with other species of the genus. Cytogenet Cell Genet 67:193–198

Gallardo M (1979) Las especies Chilenas de *Ctenomys* (Rodentia, Octodontidae). I. Estabilidad Cariotípica. Archivos de Biología y Medicina experimental 12:71–82

García L, Ponsa M, Egozcue J, García M (2000a) Comparative chromosomal analysis and phylogeny in four *Ctenomys* species (Rodentia, Octodontidae). Biol J Linn Soc 69:103–120

García L, Ponsa M, Egozcue J, García M (2000b) Cytogenetic variation in *Ctenomys perrensi* (Rodentia, Octodontidae). Biol J Linn Soc 71:615–624

Giménez MD, Mirol PM, Bidau CJ, Searle JB (2002) Molecular analysis of populations of *Ctenomys* (Caviomorpha, Rodentia) with high karyotypic variability. Cytogenet Genome Res 96:130–136

Godinho R, Crespo EG, Ferrand N (2008) The limits of mtDNA phylogeography: complex patterns of population history in a highly structured Iberian lizard are only revealed by the use of nuclear markers. Mol Ecol 17:4670–4683

Kiblisky P, Brum-Zorrilla N, Perez G, Saez F (1977) Variabilidad cromosómica entre diversas poblaciones uruguayas del roedor cavador del genero *Ctenomys* (Rodentia-Octodontidae). Mendeliana 2:85–93

Lanzone C, Giménez MD, Santos JL, Bidau CJ (2007) Meiotic effects of Robertsonian translocations in tuco-tucos of the *Ctenomys perrensi* superspecies (Rodentia: Ctenomyidae). Caryologia 60:233–244

Lara MC, Patton JL, Da Silva MNF (1996) The simultaneous diversification of South American echimyid rodents (Hystricognathi) based on complete cytochrome b sequences. Mol Phylogenet Evol 5:403–413

Larkin MA et al (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948

Larmuseau MHD, Raeymaekers JAM, Hellemans B et al (2010) Mito-nuclear discordance in the degree of population differentiation in a marine goby. Heredity 105:532–542

Lessa EP, Cook JA (1998) The molecular phylogenetics of tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae) suggests an early burst of speciation. Mol Phylogenet Evol 9:88–99

Levan A, Fredga K, Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. Hereditas 52:1–22

Mascheretti S, Mirol PM, Giménez MD, Bidau CJ, Contreras JR, Searle JB (2000) Phylogenetics of the speciose and chromosomally variable rodent genus *Ctenomys* (Ctenomyidae, Octodontoidea), based on mitochondrial cytochrome b sequences. Biol J Linn Soc 70:361–376

Massarini A, Freitas TRO (2005) Morphological and cytogenetics comparison in species of the *mendocinus*-group (genus *Ctenomys*) with emphasis in *C. australis* and *C. flamarioni* (Rodentia-Ctenomyidae). Caryologia 58:21–27

Massarini A, Barros MA, Ortells MO, Reig OA (1991) Evolutionary biology of fossorial Ctenomyine rodents (Caviomorph: Octodontidae). I. Chromosomal polymorphism and small karyotypic differentiation in Central Argentinian populations of tuco-tucos. Genetica 83:131–144

Mirol PM, Mascheretti S, Searle JB (2000) Multiple nuclear pseudogenes of mitochondrial cytochrome b in *Ctenomys* (Caviomorpha, rodentia) with either great similarity to or high divergence from the true mitochondrial sequence. Heredity 84(Pt 5):538–547

Mirol P, Giménez MD, Searle JB, Bidau CJ, Faulkes CG (2010) Population and species boundaries in the South American subterranean rodent *Ctenomys* in a dynamic environment. Biol J Linn Soc 100:368–383

Mora MS, Mapelli FJ, Gaggiotti OE et al (2010) Dispersal and population structure at different spatial scales in the subterranean rodent *Ctenomys australis*. BMC Genet 11:9

Nyakaana S, Arctander P (1999) Population genetic structure of the African elephant in Uganda based on variation at mitochondrial and nuclear loci: evidence for male-biased gene flow. Mol Ecol 8:1105–1115

Ortells MO, Barrantes GE (1994) Genetic distances and variability study in several species of the genus *Ctenomys* (Rodentia: Octodontidae), with special reference to a probable causal role of chromosomes in speciation. Biol J Linn Soc 53:189–208

Ortells MO, Contreras JR, Reig OA (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

Parada A, D'Elía G, Bidau CJ, Lessa EP (2011) Species groups and the evolutionary diversification of tuco-tucos, genus *Ctenomys* (Rodentia: Ctenomyidae). J Mammal 92:671–682

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14(9):817–818

Quintana CA (1994) Ctenominos primitivos (Rodentia, Octodontidae) del Mioceno de la Provincia de Buenos Aires, Argentina. Bol R Soc Esp Hist Nat (Sec Geol) 89:19–23

Reig OA (1989) Karyotypic repatterning as one triggering factor in cases of explosive speciation. In: Fontdevila A (ed) Evolutionary biology of transient unstable populations. Springer, Berlin, pp 246–289

Reig OA, Kiblisky P (1969) Chromosome multiformity in the genus *Ctenomys* (Rodentia, Octodontidae), a progress report. Chromosoma 28:211–244

Reig OA, Quintana CA (1992) Fossil ctenomyine rodents of the genus *Eucelophorus* (Caviomorpha: Octodontidae) from the Pliocene and early Pleistocene of Argentina. Ameghiniana 29:363–380

Reig OA, Busch C, Ortells MO, Contreras JR (1990) An overview of evolution, systematics, population biology, cytogenetics, molecular biology and speciation in *Ctenomys*. Prog Clin Biol Res 335:71–96

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Slade RW, Moritz C, Heideman A (1994) Multiple nuclear-gene phylogenies: application to pinnipeds and comparison with a mitochondrial DNA gene phylogeny. Mol Biol Evol 11:341–356

Slamovits CH (2002) Filogenia molecular y dinámica del ADN satélite: su relación con la evolución de los roedores tuco-tucos (*Ctenomys*, Octodontidae) PhD thesis. Universidad de Buenos Aires, Buenos Aires, Argentina

Slamovits CH, Cook JA, Lessa EP, Rossi MS (2001) Recurrent amplifications and deletions of satellite DNA accompanied chromosomal diversification in South American tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae): a phylogenetic approach. Mol Biol Evol 18:1708–1719

Swofford DL (2003) PAUP: phylogenetic analysis using parsimony (and other methods), version 4. Sinauer Associates, Sunderland, MA

Tomasco IH, Lessa EP (2007) Phylogeography of the tuco-tuco: mtDNA variation and its implication for chromosomal differentiation. In: Kelt DA, Lessa EP, Salazar-Bravo JA, Patton JL (eds) The quintessential naturalist: honoring the life and legacy of

Oliver P. Pearson. University of California Publications in Zoology, San Diego, pp 859–882

Verzi DH, Montalvo CI, Vucetich MG (1991) Nuevos restos de *Xenodontomys simpsoni* Kraglievich y la sistemática de los más antiguos Ctenomyinae (Rodentia, Octodontidae). Ameghiniana 28:325–331

Woods C, Kilpatrick C (2005) Infraorder Hystricognathi Brandt, 1855. In: Wilson DE, Reeder DM (eds) Mammal species of the world: a taxonomic and geographic reference, 3rd edn. John Hopkins University Press, Baltimore, MD, pp 1538–1600

Xia X, Xie Z (2001) DAMBE: data analysis in molecular biology and evolution. J Hered 92:371–373

Xia X, Xie Z, Salemi M, Chen L, Wang Y (2003) An index of substitution saturation and its application. Mol Phylogenet Evol 26:1–7

Zenuto RR, Busch C (1998) Population biology of the subterranean rodent *Ctenomys australis* (tuco-tuco) in a coastal dune-field in Argentina. Zeitschrift für Säugetierkunde 63:357–367

Zenuto RR, Lacey EA, Busch C (1999a) DNA fingerprinting reveals polygyny in the subterranean rodent *Ctenomys talarum*. Mol Ecol 8:1529–1532

Zenuto RR, Malizia AI, Busch C (1999b) Sexual size dimorphism, testes size and mating system in two populations of *Ctenomys talarum* (Rodentia: Octodontidae). J Nat History 33:305–314

Zenuto RR, Vasallo AI, Busch C (2002) Comportamiento social y reproductivo del roedor subterráneo solitario *Ctenomys talarum* (Rodentia: Ctenomyidae) en condiciones de semicautiverio. Rev Chil Hist Nat 75:165–177