An Acad Bras Cienc (2023) 95(4): e20200393 DOI 10.1590/0001-3765202320200393
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

CELLULAR AND MOLECULAR BIOLOGY

Revision and analysis of the chromosome variability in the speciose genus *Akodon* (Rodentia, Sigmodontinae), including new data from Argentina

CAROLINA A. LABARONI, VAITIARE A. PAEZ COLL MAIRHOFER, AGUSTINA A. OJEDA, AGUSTINA NOVILLO, PABLO TETA, PABLO JAYAT, RICARDO A. OJEDA, LEANDRO M. BUSCHIAZZO, EUGENIO N. CÁLCENA, ALEJANDRO D. BOLZÁN & CECILIA LANZONE

Abstract: Rodentia has a high species number and chromosomal variability. The South American genus *Akodon* is one of the most speciose muroids, with more than 40 species included in several species groups. Here, we characterize cytogenetically specimens of *Akodon* from central-western Argentina. Subsequently, we reviewed and analyzed the cytogenetic data for this genus, build a phylogeny and mapped chromosome changes to interpret its evolution. Specimens of *A. dolores* from central-western Argentina have 2n=42-44/FNa=44 (46, 48) due to a Robertsonian rearrangement. Our data expand the distribution range known for this polymorphism and confirm its geographic structure. Other specimens had 2n=40/FNa=40, representing populations of *A. oenos*, *A. polopi*, and *A. spegazzinii*. All karyotypes have a low amount of heterochromatin, concentrated in centromeres and sex chromosomes, as in other rodents. The complement with 2n=40/FNa=40 is the most frequent in *Akodon* and is shared by most species in some groups. Chromosome numbers are very diverse. The FNa shows less variability; FNa=42 was recovered as ancestral, excluding *A. mimus*, which was connected at the base of the *Akodon* tree and has FNa=44. This indicates a complex chromosome evolution in *Akodon*, and suggests that reductions and increases in the 2n and FNa evolved independently in some lineages.

Key words: Akodontini, chromosome evolution, Muroidea, phylogeny, South America.

INTRODUCTION

Each species possess a characteristic chromosome number (2n) and morphology [known as the Fundamental Number of autosomal arms (FNa) when the sex chromosomes are excluded]. Many species have a distinctive 2n and FNa, which is very useful for taxonomy. Others share with closely related species a conserved 2n and FNa, being cryptic at the chromosome level. On the other hand, some species have individuals with different chromosome complements, which can vary within and/or between populations, forming the so-called polymorphisms and polytypisms. In general, the chromosomes involved in polymorphisms and polytypisms are species-specific, which also is useful for species identification. Among mammals, rodents are a particularly variable group in chromosome numbers and morphologies. The characterization, the evolutionary dynamics, and the biological implications of this variability are

fields of intensive research (Patton & Sherwood 1983, Piálek et al. 2005, Buschiazzo et al. 2018, among others).

The subfamily Sigmodontinae constitutes a monophyletic lineage of cricetid rodents from South, Central, and North America, that radiate into major clades formalized at the rank of tribes (D'Elía 2003, Maestri et al. 2017). The tribe Akodontini encompasses a great diversity of species, being the second most diverse within Sigmodontinae in terms of genera and species (D'Elía & Pardiñas 2015). Within Akodontini, *Akodon* has a wide geographic range in Argentina, Brazil, Bolivia, Chile, Colombia, Ecuador, Paraguay, Peru, and Uruguay. This genus, with more than 40 species, is the second richest among muroid rodents (Pardiñas et al. 2015). Through morphological or/and molecular characters, several species groups were proposed to represent its evolutionary history. The better supported, with different character sets, are the *A. aerosus*, the *A. boliviensis*, the *A. cursor*, the *A. dolores,* and the *A. varius* species groups. However, the content of some of these groups varies among studies (Myers 1989, Myers et al. 1990, Braun et al. 2008, Jayat et al. 2010, Coyner et al. 2013).

The genus *Akodon* exhibits high chromosomal variability. Diploid and fundamental numbers range from 2n=44, FNa=44 in *A. paranaensis, A. reigi,* and *A. dolores* (Tiranti 1998, González et al. 1998, Christoff et al. 2000) to 2n=10, FNa=14 in *A. diauarum* (Silva & Yonenaga-Yassuda 1998). Some species share the karyotype, but others present distinctive chromosome complements (Myers 1989, Gonçalves et al. 2007, Malleret et al. 2016). Additionally, other species have polymorphisms and polytypisms, as *A. dolores* in which several species-specific Robertsonian (Rb) variants were described (Bianchi et al. 1971, 1979, Wittouck et al. 1995, Tiranti 1998). However, several of these studies were based on a few specimens and populations considering their geographic ranges, and some species were not characterized at the chromosomal level. Additionally, an extensive review and analysis of the variability of the chromosome complements in a phylogenetic context were not done for the entire genus.

In the central-western region of Argentina, in a complex landscape influenced by Andean orogeny, several nominal forms of *Akodon* were cited through the time (e.g., Myers 1989, Myers et al. 1990, Braun et al. 2008, Jayat et al. 2010, Coyner et al. 2013). Currently, the most widespread species is *A. spegazzinii*, which is extended over several types of habitats east of Los Andes, from south-central Salta province to central La Rioja province (Jayat et al. 2020). On the other hand, *A. dolores* is distributed on lowland areas of the central region, being mostly parapatric (toward the west) with *A. spegazzinii*. *Akodon oenos* replaces *A. spegazzinii* towards the south, occupying environments dominated by grasslands in San Juan and Mendoza provinces. This species has been registered in sympatry with *A. dolores* on a few localities on the western limit of the *A. dolores* distribution range, in Mendoza province (see Jayat et al. 2020). Finally, populations of *A. polopi* inhabit high altitude grasslands (between 1300 m and 2250 m elevation) on a geographically isolated context, in the mountain chain systems of Córdoba and San Luis provinces, in central Argentina (Jayat et al. 2010, 2020, Pardiñas et al. 2015).

In this work, we studied representative specimens of the genus *Akodon* from central-western Argentina at the cytogenetic level. Also, we reviewed cytogenetic data available in the literature for other species of *Akodon* to evaluate our results within the context of the chromosomal variability of the genus. Finally, we analyzed the chromosome changes

in a phylogenetic approach to investigate the chromosome evolution of this speciose taxon.

MATERIALS AND METHODS

We studied 22 specimens of *Akodon* from 9 localities coming from four different provinces of central-western Argentina. Specimens of *A. dolores* (N=8, five males and three females) and *A. oenos* (N=5, three males and two females) were collected in Mendoza province. Specimens of *A. spegazzinii* (N=6, five males and one female) come from Catamarca province, and those of *A. polopi* from Córdoba (N=2 males) and San Luis provinces (N=1 male). The collecting localities for these specimens are indicated in Figure 1. Vouchers were deposited in the mammal collection of IADIZA (Supplementary Material - Appendix S1). The specimens were determined to species level by morphological (skin and skull characters) examination and/or DNA sequencing combined with comparative analysis (Appendix S1). Additionally, some exemplars were determined by biogeographic criteria (known geographic provenance and environmental affinities for species with no overlapping distributions) considering current revisions (Myers 1989, Myers et al. 1990, Braun et al. 2008, Coyner et al. 2013, Jayat et al. 2010, 2019) and our field experience.

Chromosome preparations were obtained from bone marrow (Ford & Hamerton 1956). At least ten metaphase spreads were counted for each specimen. Fundamental Numbers (FNa) refer only to autosomes (Patton 1967). For conventional staining, the preparations were stained with a Giemsa solution (10%). To determine the chromosomal homologies of the pairs in each karyotype, the metaphases were stained with the fluorescent dye DAPI (4',

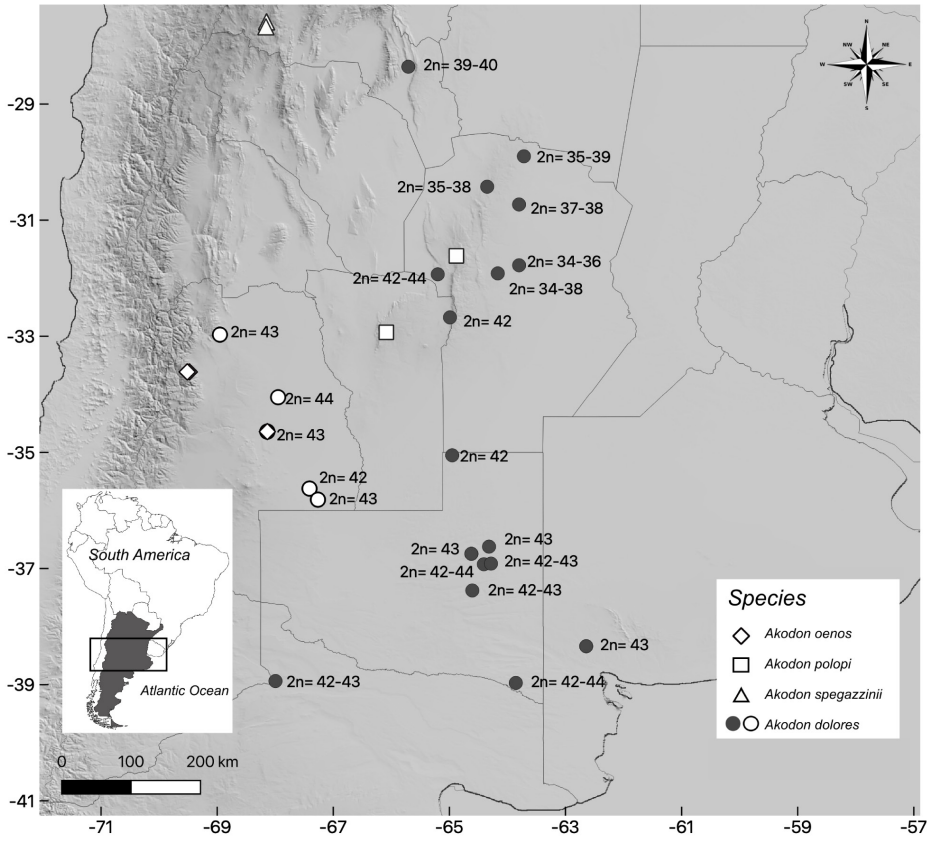

Figure 1. Map showing the different diploid numbers (2n) found in *Akodon dolores* (circles) in samples analyzed here (white), and in previously published works (grey): Bianchi et al. (1969, 1971, 1979), Wittouck et al. (1995), Tiranti (1998). The localities of *A. spegazzinii* (2n=40) from Catamarca province in samples analyzed here are indicated with white triangles; localities of *A. oenos* (2n=40) from Mendoza province with white diamonds, and localities of *A. polopi* from Córdoba and San Luis provinces are indicated with white squares.

6-diamino-2-phenylidol) following Schweizer et al. (1978). The constitutive heterochromatin (CH) was evidenced with C banding (Sumner 1972). Ag-NORs technique (Howell & Black 1980) was used to detect nucleolus organizing regions. Fluorescent *in situ* hybridization (FISH) was performed with a Cy3-conjugated PNA pan-telomeric probe [Cy3-(CCCTAA)3] obtained from PNABio Inc. (California, USA), according to the protocol provided by the supplier, as described in Lanzone et al. (2015). Slides were mounted on an antifade reagent containing DAPI (4,6-diamidino-2-phenylindole) as counterstain. Fluorescence microscopy was performed on a Nikon Eclipse 50i epifluorescence microscope equipped with an HBO 100 mercury lamp, a Nikon high-resolution digital color camera (DS-Ri-U3), and filters for DAPI and Cy3 (Chroma Technology Corp., Rockingham, VT, USA).

To investigate the chromosome variability and evolution of the genus, we combined our data with a revision of the available cytogenetic information for other *Akodon* species. The list of species and its chromosome characteristics were obtained principally from the account of Pardiñas et al. (2015), considering the species group to which they were assigned (Table SI). For some species, we procured the missing data and updated the species list to include those recently described (Table SI).

Finally, we included and analyzed molecular data, constructed a phylogeny, and mapped the evolution of the chromosome number (2n) and the fundamental number of autosomal arms (FNa) in the genus. In the genetic and phylogenetic analyses, we incorporated all species of *Akodon* for which there are available sequences of the mitochondrial cytochrome b (Cyt-b) marker in GenBank (Table SI). This is the most widely used DNA region in mammals and from which we were able to obtain the densest taxonomic sampling to species level. Also, this is the only molecular marker for which an extensive comparative analysis to investigate the levels of intra and interspecific variability was done (Baker & Bradley 2006). Also, we included sequences and chromosome data of other closely related akodontines (Salazar-Bravo et al. 2016) as outgroups: *Deltamys*, *Thalpomys, Thaptomys,* and *Castoria*.

To investigate the degree of molecular differentiation, we calculated genetic distances for all pairwise comparisons of the Cyt-b with the MEGA 6.0 software (Tamura et al. 2013). The selected model was K2P since this DNA region displayed Tv/Ts bias and showed relatively low genetic variability (Table SII); besides that, it is the most used model in molecular analyses. Then, we compared the values of genetic distances with that expected for intra and interspecific comparisons (Baker & Bradley 2006).

To build the trees, we used a combined data set that includes 48 terminals (Table SI), 801 nucleotides of Cyt-b as molecular characters (with 324 variable sites, of which 286 were parsimony informative), and two chromosome characters (2n and FNa, both parsimony informative). Modifications of 2n and FNa due to B-chromosomes and short heterochromatic arms were not included in the analysis. The chromosome characteristics used in this work were coded in accordance with the 2n and FNa observed in the group as follow, 2n: 0=10, 1=14, 2=16, 3=22, 4=24, 5=26, 6=34, 7=36, 8=38, 9=40, A=42, B=44, C=46, D=50, E=52; FNa: 0=14, 1=16, 2=18, 3=20, 4=22, 5=24, 6=26, 7=34, 8=36, 9=38, A=40, B=42, C=44, D=46, E=48, F=52. In order not to unnecessarily increase the number of different states for the chromosomal characters, in the polymorphisms, only even diploid numbers were considered. Thus, a rearrangement involving only a pair of chromosomes was coded with two numbers instead of three. See Table SI for codes in each particular species. The phylogenetic

analysis was performed by maximum parsimony (MP) using the TNT program (Goloboff et al. 2008). We performed MP heuristic searches consisting of 1000 random addition sequences with the TBR branch swapping algorithm (saving 10 trees per replication). A strict consensus tree was constructed with the most parsimonious trees obtained. To assess the robustness of the nodes of the resulting phylogeny, we performed 1000 standard bootstrap pseudoreplicates (Felsenstein 1985) consisting of 100 random addition sequences followed by TBR (retaining 10 trees in each pseudoreplicate). Optimizations of 2n and FNa by MP were done with the TNT program, considering the character states of diploid and fundamental numbers as unordered transformations.

RESULTS

New cytogenetic data in *Akodon* from central-western Argentina

Eight specimens of *A. dolores* from different localities of Mendoza Province presented chromosome complements with a 2n that varied from 42 to 44 (Appendix S1, Figs. 1-2). The 2n=42 had FNa=44 and a larger metacentric pair, double in size compared with pair 2. In the 2n=43 and 44 karyotypes, the FNa is difficult to define because the two first acrocentric autosomes (similar in size to the other large chromosomes) had short arms. If both short arms are considered, the FNa varies from 44 to 48 (Fig. 2a-b). Banding with DAPI allowed determining the chromosomal homologies of pairs, corroborating the homologous chromosomes involved in the rearrangement. The first two acrocentric pairs in the 2n=44 are part of a Robertsonian rearrangement (Rb) that involves the large metacentrics observed in the complements with 2n=43 and 42 (Fig. 2a).

Constitutive heterochromatin in the pericentromeric region of some autosomes and on the acrocentric X chromosomes was observed. Additionally, one of the subtelocentric pairs involved in the rearrangement has short heterochromatic arms, and the acrocentric Y chromosome was completely heterochromatic (Fig. 2b-c). However, the Rb metacentric does not show visible constitutive heterochromatin (Fig. 2d). With the Ag-NOR banding, positive marks in the terminal region of chromosome pairs 3 and 5 were detected (Fig. 2e). In metaphases with 2n=43 and 44, telomeric FISH signals were observed at both terminal regions of the chromosomes only. Some variation in the intensity of fluorescent signals was detected at both intra- and inter-chromosomal level (Fig. 2f-g). In the 2n=43 complements, the Rb metacentric also presented terminal telomeric signals only (Fig. 2g).

On the other hand, specimens of *A. oenos* from two localities of Mendoza (Tunuyán and San Rafael), of *A. spegazzinii* from Catamarca Province, and of *A. polopi* form Córdoba and San Luis Provinces had 2n=40, FNa=40 (Appendix S1, Figs. 1, 3, 4). The smallest autosomes (pair 19) were biarmed, but their morphology was difficult to distinguish because they were almost microchromosomes (Fig. 3). DAPI bands allowed the identification of chromosome pairs and showed a high homology among the karyotypes of the three species (Fig. 4). This complement has a low amount of heterochromatin, concentrated in the short arms of the large submetacentric X and the Y chromosomes (Fig. 3c). The Y was small and submetacentric, and in most individuals, its size was similar to that of pair 18 (Fig. 3a). However, in specimens from Córdoba and San Luis, the X chromosome had smaller short arms, and the Y chromosome presents a smaller size, similar to pair 19 (Fig. 3b). One male from Cortaderas, Catamarca province, has one

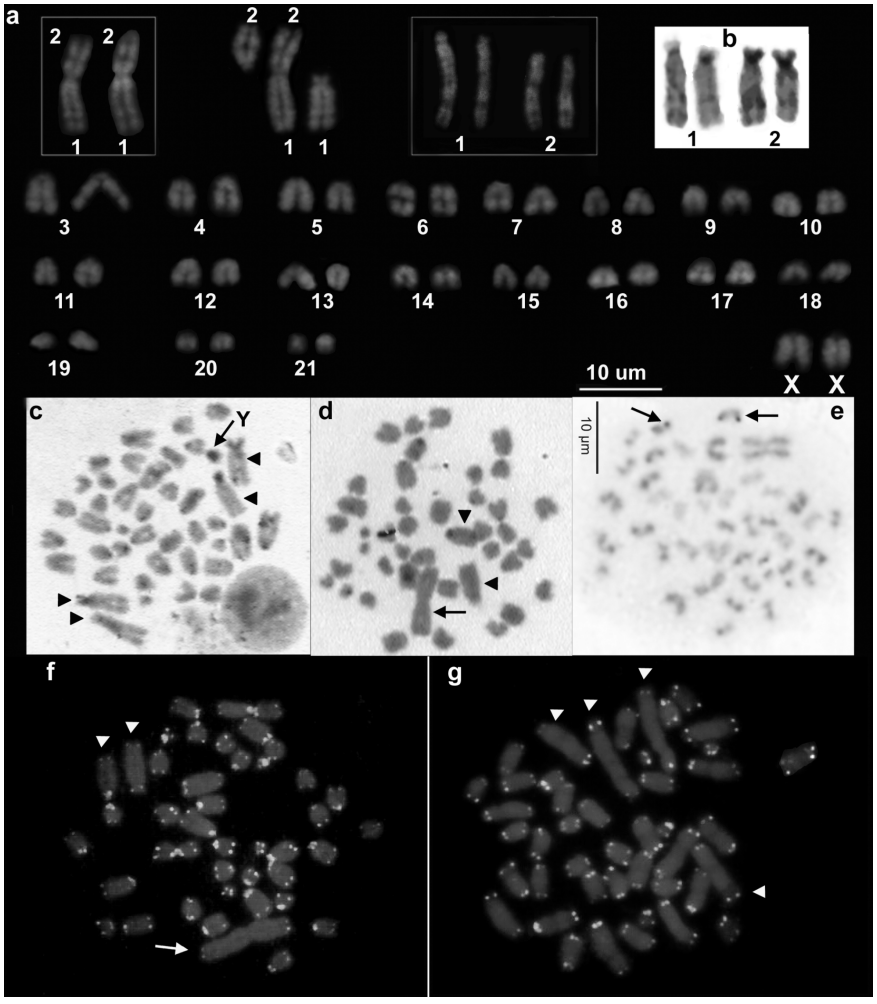

Figure 2. Chromosomes obtained from specimens of *A. dolores* with 2n=42, 2n=43, and 2n=44. a) The complement of a female with 2n=43 staining with DAPI. In boxes are chromosomes involved in the Robertsonian rearrangement (Rb) from specimens with other constitution, to the left with 2n=42 and to the right with 2n=44. b) C bands in the two first autosome pairs in the 2n=44 complement; note the short arms in both pairs, which are heterochromatic only in the second. c) C-bands in a male with 2n=44, note the heterochromatic Y chromosome; the arrowheads indicate the acrocentrics involved in the rearrangement. d) C-bands in a female with 2n=43, note the absence of heterochromatin in the Rb metacentric (arrow), the arrowheads indicate the acrocentrics homologous. e) NORs. f-g) FISH in *Akodon dolores* with the pantelomeric probe with 2n=43 (f) and 2n=44 (g). The arrow indicates the Rb metacentric and the arrowheads the homologous acrocentrics.

homologous of pair 9 with a more prominent pericentromeric positive C region (Fig. 3c). Ag-NOR banding produced positive marks in the terminal region of pairs 3 and 4, and in the pericentromeric region of pairs 7 and 8 (Fig. 3d).

DAPI and C banding comparisons indicate similar patterns among the four chromosome complements analyzed here. This is evidenced mainly in the larger chromosome pairs, which have a more marked differential banding pattern, and can be considered as conserved chromosomes (Figs. 2-4).

Review of the cytogenetic data from *Akodon* species

The compiled chromosome complements for *Akodon* species showed a wide amplitude in the 2n and FNa, but the 2n showed more dispersion. The most frequent 2n is 40, and there is a denser cluster of 2n between 33 and 44 (Table SI, Figure S1). Most species have FNa=40, and the highest frequencies are between 40 and 44 (Table SI). Considering the species groups, in the *A. aerosus* group, 2n varies from 22 to 40 and predominates the FNa=40, with the only exception of *A. mollis*, which presents a polymorphic FNa=43-44. In the *A. boliviensis* species group, most species have 2n=40, FNa=40, with some exceptions. In *A. caenosus* a reduced 2n=34 karyotype was

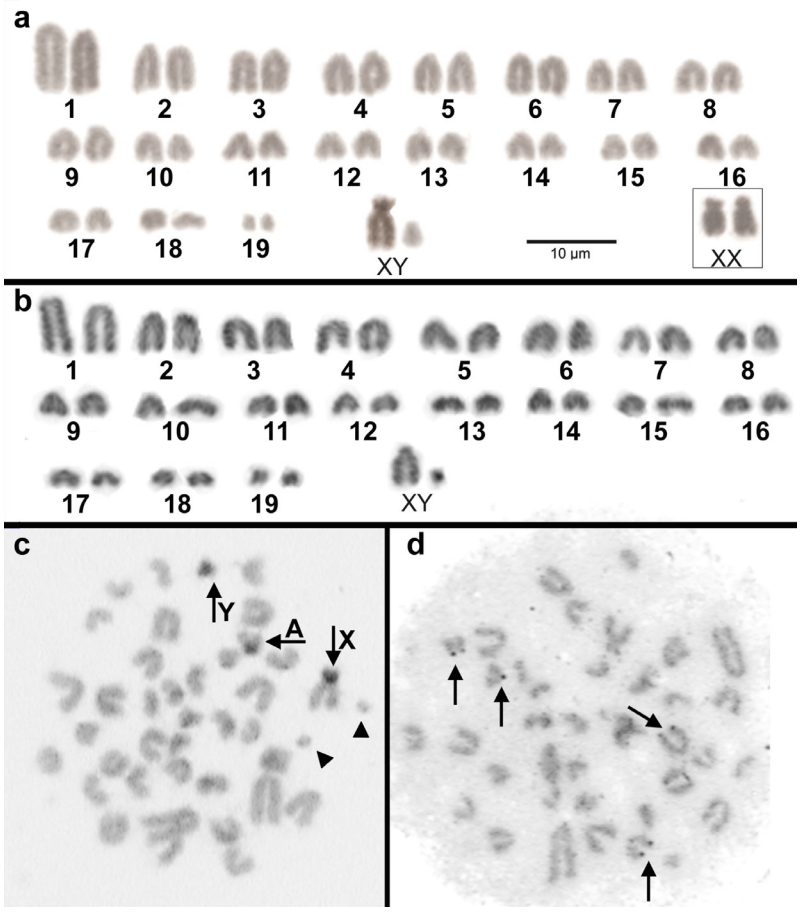

Figure 3. Chromosome complements of specimens with 2n=40. a) Conventional staining of a male from Manzano Histórico, Mendoza province, representing populations of the nominal form *A. oenos*; the sex chromosomes of a female are in a box. b) Conventional staining of a male of *A. polopi* from Pampa de Achala, Córdoba province. Note the differences in the sex chromosomes between the complements of A and B. c) C-bands in *A. spegazzinii* from Cortaderas, Catamarca province; note the heterochromatin in the short arm of the X, in the Y chromosomes and the pericentromeric region of one autosome (arrows). d) Ag-NOR staining in *A. oenos* from Manzano Histórico, Mendoza province; the arrows indicated positive marks.

described, and in *A. boliviensis* and *A. spegazzinii* increases in the FNa to 42 and 41, respectively, was registered. The most variable is the *A. cursor* species group, which ranges from 2n=10 to 2n=44 and from FNa=14 to FNa=44; the species of this group *A. diauarum* (Brandão 2022) has the most reduced chromosome complement in the genus. In the *A. dolores* group, all species with chromosome data are polymorphic due to Rb rearrangements, and the FNa varies from 42 in *A. iniscatus* to 48 considering the heterochromatic arms in *A. dolores*. The only species with chromosome data in the *A. varius* species group is *A. simulator* and also presents an Rb polymorphism with a constant FNa=42. Several species were not assigned to any of the groups, or their assignment is doubtful. Among these, 2n varied between 36 and 44 and FNa between 40 and 44 (Table SI).

Genetic and Phylogenetic analyses

In the phylogenetic analysis, molecular (801bp of cytochrome b sequences) and chromosome characters (2n and FNa) were included. Genetic distances of Cyt-b among all samples are shown in Table SII. Despite there are few intraspecific comparisons, a continuous range of genetic distances between 0.26 and 19.39 % was obtained. The major distances generally involve species designed as outgroups. At the species level, most comparisons diverge around 8-10%. The most remarkable exception corresponds to samples of *A. orophilus* and *A. josemariarguedasi* (0.03%). Also, other sequence pairs involving interspecific comparisons presented low divergences: *A. spegazzinii-A. oenos* (1.7%), *A. mystax-A. lindberghi* (2%), *A. kofordi-A. fumeus*

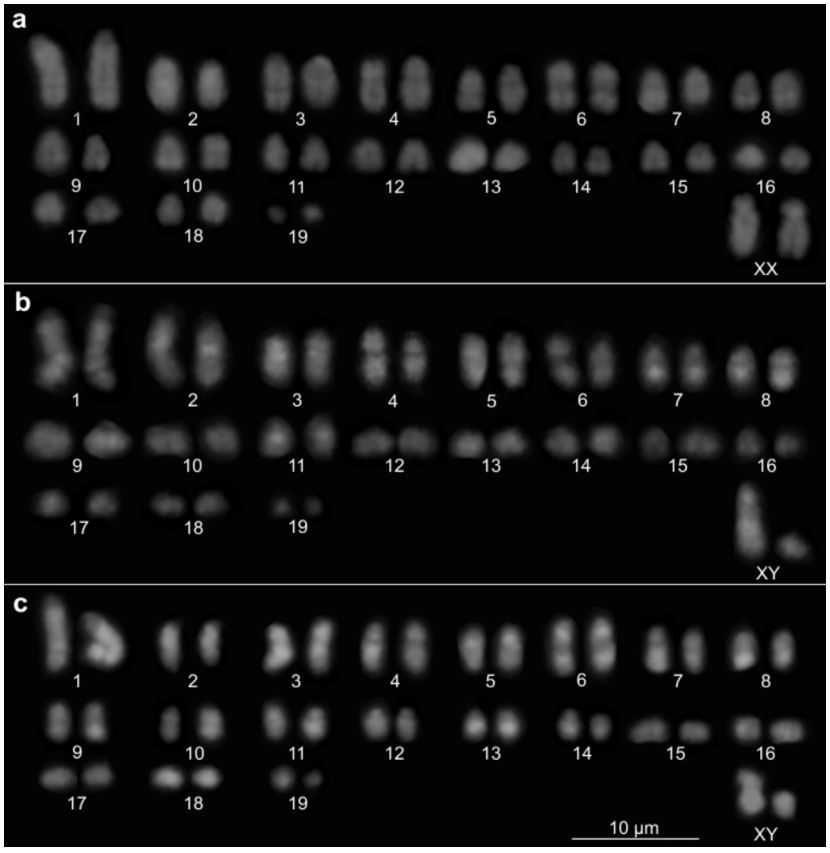

Figure 4. Karyotypes with DAPI staining of a) a female of *A. oenos* from Manzano Histórico, Mendoza province; b) a male of *A. polopi* from Pampa de Achala, Córdoba province; and c) a male of *A. spegazzinii* from Cortaderas, Catamarca province.

(2.1%), *A. spegazzinii-A. boliviensis* (2.4%), and *A. dolores-A. toba* (2.5%).

The MP analyses of combined DNA and chromosome data recovered four trees with 1561 steps (see Fig. 5 for strict consensus tree). All species groups were recovered. However, only the *A. varius* and the *A. dolores* species groups showed high bootstrap support (>80%), the rest of the groups were weakly supported (<50%; Fig. 5). Most species in the *A. boliviensis* and *A. aerosus* species groups have FNa=40, and in the trees, both species groups appeared as two independent clades. The optimization of FNa indicated that 42 was the fundamental ancestral number for *Akodon* species, excluding *A. mimus* with FNa=44, which was connected at the base of the *Akodon* phylogenetic tree (Fig. 5). In some internal branches of the *A. cursor*, *A. dolores,* and *A. aerosus* species groups, an increase from FNa=42 to FNa=44 was detected. Also, a decrease from FNa=42 to FNa=40 in the *A. boliviensis* and *A. aerosus* species groups was recovered. Additionally, in the *cursor* group, there were marked reductions in the FNa, some very extremes, as in *A. diauarum* with FNa=14.

The diploid numbers show great variation. Only in the *boliviensis* group, several species share 2n=40. In the other groups, several species are polymorphic, and then, the character state was ambiguous (Fig. 5). The optimization of 2n indicates 38 as the possible ancestral number for the genus. Nevertheless, 2n=38 is found in a few species, very separated in the tree (Fig. 5).

DISCUSSION

New cytogenetic data in *Akodon* from central-western Argentina

The chromosome polymorphism found in this work for specimens identified as *A. dolores*

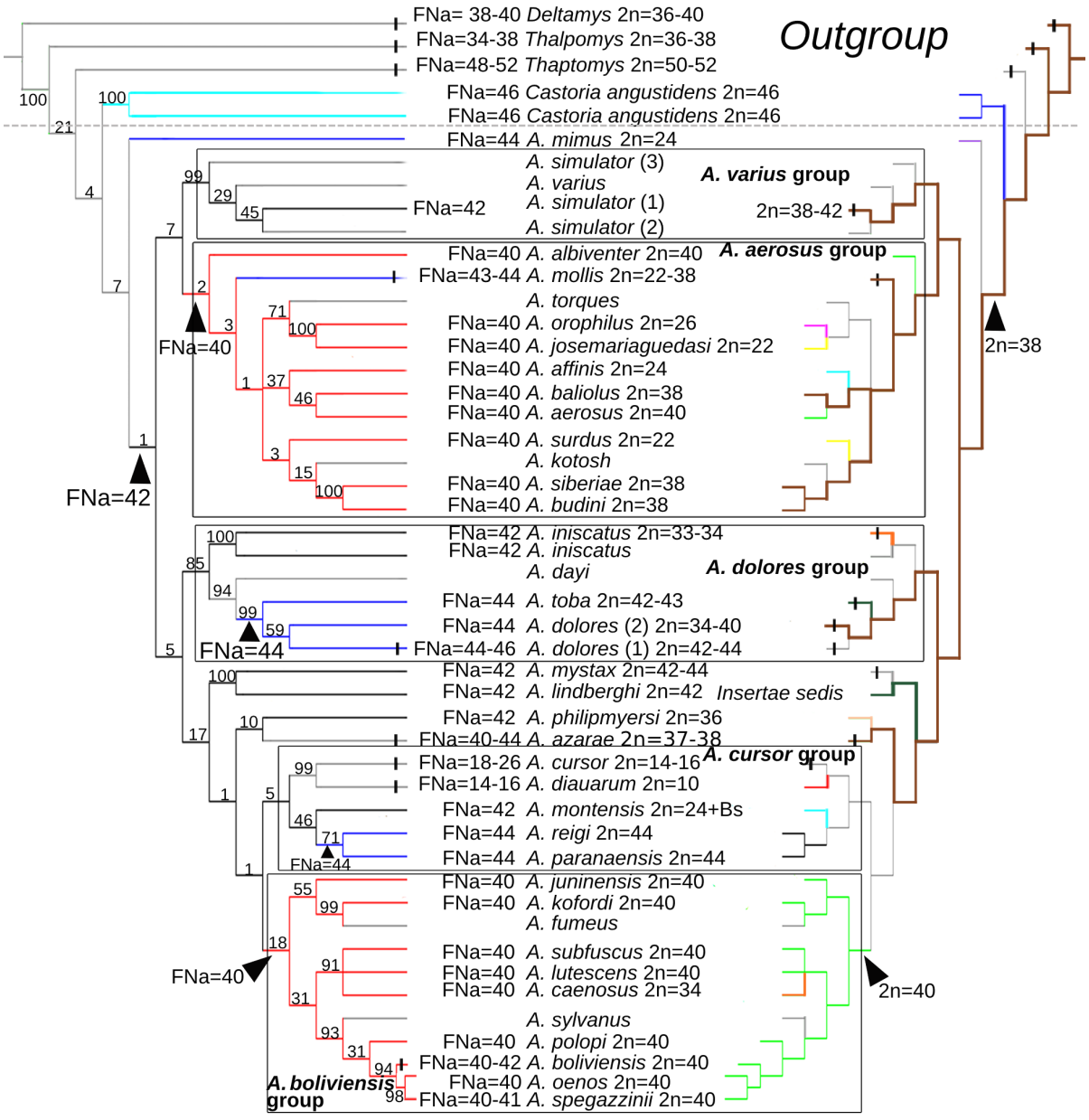

Figure 5. Strict consensus of the four most parsimonious trees obtained for the genus *Akodon* using molecular (Cyt-b) and chromosome characters, with the optimization through parsimony of FNa (left) and 2n (right). The color indicated the character states. To the left, light blue: FNa=46, blue: FNa=44, black: FNa=42, red: FNa=40. To the right, blue: 2n=46, black: 2n=44, dark green: 2n=42, light green: 2n=40, brown: 2n=38, orange: 2n=34, pink: 2n=26, light blue 2n=24, yellow 2n=22, red: 2n=10. The branch of terminals with unknown or ambiguous 2n and/or FNa are in grey. Polymorphic and polytypic taxa are indicated with small perpendicular lines in the terminals. Modifications of 2n and FNa due to B-chromosomes and short heterochromatic arms were not included in the analysis. Numbers above branches indicate bootstrap support. Numbers in parentheses identify different sequences in Table SI. In boxes are marked the species groups. The *A. aerosus* species group included *A. siberiae* and *A. budini*, not previously incorporated in this species group. In samples of *A. simulator* several chromosome numbers were registered, but as no specific number can be associated with any particular sequence, we paint only one terminal indicating its polymorphic state in the 2n.

produces a variation from 2n=42 to 44, due to one Rb translocation, and corresponds to that previously described for this species (Wittouck et al. 1995). This nominal form, with type locality in Yacanto, western Córdoba province, includes *A. molinae* (2n=34-40) and *A. neocenus* in its synonymy (Braun et al. 2008, Pardiñas et al. 2015). Chromosome arms in all complements of both *dolores* and *molinae* have an exact correspondence, and the differences are principally due to Rb rearrangements (Bianchi et al. 1979). Polymorphisms for Rb translocations were reported in several rodents, and usually, their presence does not generate reproductive isolation (Lanzone et al. 2007, 2016). The synonymy of both nominal forms is also sustained by cross-breeding experiments and morphological comparisons (Merani et al. 1978, Wittouck et al. 1995, Braun et al. 2008).

In *A. dolores*, early works showed depressed viability in individuals with 2n=43 and 44, although the studied specimens came from a few populations and laboratory strains (Merani et al. 1980, Redi et al. 1982, Bianchi & Merani 1984). The mechanism proposed to explain the reduction in fertility involves progressive degeneration of acrocentric chromosomes (Fernández-Donoso et al. 2001), but the broad distribution of this polymorphism suggest that this effect cannot be extrapolated to all individuals and populations. In these chromosome complements, in addition to the Rb rearrangement, a pericentric inversion in pair one was proposed (Bianchi et al. 1969, 1971). In the present work, we did not detect this inversion, and no inversion loop was observed in the analysis of synaptonemal complex from heterozygotes (Fernández-Donoso et al. 2001), suggesting the absence of inversions, at least in some individuals and populations. In specimens from Mendoza province with 2n=43-44, we detected short heterochromatic arms in one of the largest subtelocentric chromosomes, a finding previously unreported. Also, we observed telomeric FISH signals at the terminal regions of the short arms of the acrocentrics, but not in the homologous region of the Rb metacentric chromosome. Both telomeric regions have not structural homology with the pericentromeric region of the Rb metacentric. This suggests that a process of addition/deletion of heterochromatin and telomeric sequences, or structural changes of heterochromatin without sequences modification, was involved in the formation of the Rb metacentric chromosome. Also, in contrast with previous findings in this species (Fernández-Donoso 2001 and references cited there), the Y chromosome in the specimens analyzed in the present study was heterochromatic. This discrepancy supports the hypothesis that the population differentiation is related to the amount of constitutive heterochromatin, a relatively common phenomenon in rodents (Patton & Sherwood 1983, Buschiazzo et al. 2018).

In *A. dolores*, specimens with different diploid numbers were found in different geographic regions by several authors. Thus, in Córdoba province a variation of 2n=34 to 39 was found, in Catamarca 2n=39-40 were registered, whereas in San Luis, La Pampa, Buenos Aires, and Rio Negro a polymorphism with 2n=42, 43 and 44 was reported (Bianchi et al. 1971, 1979, Wittouck et al. 1995, Tiranti 1998). In this work, only *A. dolores* individuals with 2n=42, 43, and 44 were found, expanding the range of known distribution for these chromosome complements to Mendoza province. Thus, the 2n=42-44 polymorphism was registered to the southern and western portions of the range of *A. dolores*; in turn, the reduced chromosome complements with 2n=34-40 embraces the northeastern distribution of this species (Bianchi et al. 1979, Wittouck et al. 1995, Tiranti 1998, this work). The data presented here sustain the geographical structuring of these

chromosomal variants. New records through the Chacoan region are needed to elucidate the extension and structure of this complex chromosome polymorphism.

On the other hand, specimens of *A. spegazzinii*, *A. oenos*, and *A. polopi* analyzed here had very similar karyotypes with 2n=40, FNa=40. Specimens from Mendoza here referred to *A. oenos* were previously included in the synonymy of *A. spegazzinii* (see Pardiñas et al. 2011), but more recently, and mostly based in its morphological distinctiveness, were considered as part of a separate species by Jayat et al. (2020). The chromosome complement with 2n=40 from Mendoza characterized here is similar to those previously reported, which included specimens from the type locality of *A. oenos* (Bianchi et al. 1971, Bianchi & Merani 1984).

The specimens of *A. spegazzinii* from Catamarca had no variant in the chromosome complements, but for this species, a variation of FNa=40-41 was previously described (Barquez et al. 1980). However, the chromosome modification involved in this variation in the FNa was not elucidated. In one specimen from Catamarca, we found a heterochromatic variant not described previously, showing that in this species, variations in the amount of constitutive heterochromatin are also present. Moreover, in *A. spegazzinii,* other variations in the karyotype were observed, which were interpreted through conventional staining as a polymorphism in the X chromosome due to a pericentric inversion (Barquez et al. 1980). However, this interpretation must be taken with caution because this type of rearrangement in heterozygosis produces a considerable proportion of unbalanced gametes, in the absence of mechanisms that prevent recombination in the female meiosis. Chromosome banding and a larger number of analyzed specimens are needed to elucidate the significance of these chromosome variants.

Akodon spegazzinii is recovered as the sister species of *A. oenos*. Both nominal forms share the 2n=40, FNa=40, and are separated by low genetic distances in the Cyt-b comparisons (Table SII). However, some quantitative morphologic variations sustain their differentiation (Jayat et al. 2020).

Finally, in the specimens of *A. polopi* from San Luis and Córdoba, small variations in the morphology of the sex chromosomes were observed, which were related to different amounts of heterochromatin. Thus, the chromosome data indicate a close relationship between these three nominal forms, which are differentiated by molecular and quantitative morphological traits (in the case of *A. polopi* and *A. spegazzinii*, see Jayat et al. 2010) and by quantitative morphological traits (in the case of *A. oenos* and *A. spegazzinii*, Jayat et al. 2020).

In both complements analyzed here (2n=42-44, FNa=44-48, and 2n=40, FNa=40), most autosomes have a low amount of pericentromeric heterochromatin. In general, the submetacentric X chromosomes of the specimens with 2n=40 have heterochromatic short arms. However, the X chromosome of *A. dolores* has a similar amount of constitutive heterochromatin than the autosomes. The Y chromosome was different in morphology in both lineages but was always wholly heterochromatic. The accumulation of heterochromatin in sex chromosomes is usual in rodents, probably due to genetic degenerations (Labaroni et al. 2014, Malleret et al. 2016, Buschiazzo et al. 2018). In both chromosome complements, the NORs were evidenced in several autosomes, indicating multiple active NORs, as in other *Akodon* species (Malleret et al. 2016).

Phylogenetic analyses of chromosome variability

The MP analyses of combined DNA and chromosome data recovered all previously defined species groups, but the only ones well supported were the *A. varius* and *A. dolores* species groups (Fig. 5). Phylogenetic analyses showed that *A. dolores* (which includes into its synonymy the nominal forms *A. molinae* and *A. neocenus*) is closely related to *A. toba*, a species found in the Chaco, a biogeographic area northerly placed to the geographic range of *A. dolores* (Braun et al. 2008, Jayat et al. 2010, Coyner et al. 2013). In fact, Myers (1989) documented a 2n=42-43, FNa=44 for *A. toba*, a chromosome complement that is similar to that found in *A. dolores*, including the same Rb polymorphism. These shared chromosome characteristics, plus the low genetic distances that separate both taxa (2.55% Table SII), suggest that they could be conspecific. More sampling, in intermediate localities between the ranges of both nominal forms, is needed to corroborate this hypothesis.

Akodon dolores and *A. toba* are part of a clade (the *A. dolores* species group, Jayat et al. 2010) that also includes *A. dayi*, a poorly known species of unknown karyotype, endemic from Bolivia, and separated from the previous two species by intermediate genetic distances (7%), and *A. iniscatus*, which is distributed to the south in northern and central Patagonia (Braun et al. 2008, Jayat et al. 2010). This last species has a divergent chromosome complement, but is also polymorphic due to a Rb rearrangement (Barros et al. 1990), and presents high values of genetic distances considering all other species within this group (Table SII, Braun et al. 2008). Cytogenetic data sustained the close relationships among these lineages, and suggest that the propensity to generate and/or maintain Rb rearrangements in the polymorphic state is ancestral for the *A. dolores* species group. Morphologically, *A. dayi, A. dolores* and *A. toba* are partially symmorphic, having large and robust skulls with ridged supraorbital borders, while *A. iniscatus* is much smaller, with a delicate cranium and smooth and rounded interorbital sides (Myers 1989). This case showed a strong concordance of chromosomes with molecular and morphological data in this clade.

The species in the group of *A. dolores* (i.e., *A. dayi, A. dolores, A. toba,* and *A. iniscatus*) were firstly included within the *A. varius* species group (Myers 1989); this arrangement and the contents of the *varius* group were later redefined (Braun et al. 2008, Jayat et al. 2010, Coyner et al. 2013). In the *A. varius* group, two species were grouped with high support: *A. simulator* (including specimens representing the nominal forms *simulator*, *glaucinus* and *tartareus*), and *A. varius*. But this arrangement was polyphyletic, and the genetic distances between them were low. Only for the nominotypical form of *A. simulator* there are chromosome data which indicate a polymorphic condition involving Rb translocation, as in the *A. dolores* species group. However, the absence of cytogenetic studies in *A. varius*, and in the other two nominal forms included in *A. simulator*, precludes a more extensive analysis of the chromosome variability and evolution in this group.

The *A. boliviensis* species group, *sensu* Jayat et al. (2010) and Coyner et al. (2013) included *A. boliviensis, A. caenosus*, *A. fumeus*, *A. juninensis*, *A. kofordi*, *A. lutescens, A. oenos, A. polopi*, *A. spegazzinii*, *A. subfuscus*, and *A. sylvanus*. In this clade, most species have 2n=40, FNa=40. Sequence divergence of *A. fumeus* and *A. kofordi* samples are low, but the absence of chromosome data in *A. fumeus* precludes a more profound analysis in this chromosome context. Also, the karyotype of *A. sylvanus* is unknown. In this group, the only divergent chromosome

complement registered was that of *A. caenosus* with 2n=34, FNa=40, suggesting a reduction in the number of chromosomes in this particular species.

The *A. aerosus* species group includes *A. aerosus*, *A. affinis*, *A. albiventer*, *A. baliolus*, *A. kotosh*, *A. mollis*, *A. orophilus*, *A. surdus*, *A. torques,* and *A. josemariarguedasi*. Sequences of *A. orophilus* and *A. josemariarguedasi* presented shallow molecular divergence, but the chromosome differentiation between them seems to support their specific status. In this clade, there is high variability in the 2n, but most species have FNa=40, which is recovered as ancestral. To this clade also are joined *A. siberiae* and *A. budini*, not always recovered in this species group (Jayat et al. 2010, Coyner et al. 2013). Chromosome data sustained their inclusion in the *A. aerosus* species group, with which share a FNa=40; in addition, the karyotypes of both species resemble that of the *A. baliolus*.

The *A. cursor* species group, which comprised *A. montensis*, *A. paranaensis*, *A. reigi*, *A. cursor,* and *A. diauarum*, is the most variable at the chromosome level and includes species with extremely reduced chromosome complements. In fact, the species with one of the lowest known diploid numbers found in rodents belongs to this species group (Silva & Yonenaga-Yassuda 1998). The specimens analyzed of *A. diauarum* had an exceptional karyotype diversity due to pericentric inversions and a complex chromosome modification, which also included a fusion. These types of chromosomal mutations were shared by *A. cursor* (Fagundes et al. 1998), of which it is separates by intermediate genetic distances confirming its close relation. Polymorphism for pericentric inversion was not confirmed in other species groups of *Akodon*.

Some species were not included in the species groups. This is the case of *A. azarae*, *A. philipmyersi*, *A. mystax,* and *A. lindberghi* (and possibly also of *A. sanctipaulensis*). *Akodon mystax* and *A. lindberghi* were grouped with high support, share the chromosome complements, and are separated by low molecular divergences (this work), suggesting that they could be conspecific. On the contrary, sequences of *A. philipmyersi* and *A. azarae* are very divergent and grouped with low support, despite their chromosome similarity. However, the geographical differentiation of chromosome and molecular characters of *A. azarae* deserves additional studies (Pardiñas et al. 2005, Coyner et al. 2013).

Considering the evolutionary patterns of chromosome transformation, some species groups have very stable karyotypes, as the *A. boliviensis*, where almost all species share the 2n and FNa. Others conserve only the FNa, as the *A. aerosus* species group. Additionally, some groups are very variable, as the *A. cursor* where high levels of polymorphisms were reported (Fagundes et al. 1998, Silva & Yonenaga-Yassuda 1998). Also, in this last group, species with B-chromosomes, sex-reversed females, and trisomies were detected (Fagundes et al. 1998, Silva & Yonenaga-Yassuda 1998, Malleret et al. 2016, Labaroni et al. 2023). This indicates that these chromosomal variants have not a high negative impact on the fertility and survival of these species. Another variable group is the *A. dolores*, where Rb rearrangement predominates (Bianchi et al. 1971, 1979, Wittouck et al. 1995, Tiranti 1998, This work). This showed that different pathways of chromosomal changes occurred in the evolution of these different lineages.

The chromosomes mapping in the phylogeny revealed some general patterns for the genus. The FNa presents relatively low variability, in contrast to that observed in other sigmodontines (Lanzone et al. 2016). Both the *boliviensis* and *aerosus* species groups share

FNa=40, but in the tree, they appeared as two independent clades. This suggests convergence in the FNa, but the relationships among major clades that represent the species groups have very low supports, whereby this interpretation should be taken with caution. The optimization of FNa indicated that 42 was the ancestral number for *Akodon* species, excluding *A. mimus* that has FNa=44 and was the species with the most basal union in the phylogenetic analysis. A note of caution must be posed here, since for some previous authors, this latter species merits generic recognition (*mimus* is the type species of the genus *Microxus*). This hypothesis is not contradicted by our phylogenetic analysis and is sustained by its chromosomal and morphological distinctiveness (Gyldenstolpe 1932). In the *cursor* group, there are marked reductions in the FNa, some of which are very extremes (Silva & Yonenaga-Yassuda 1998). The diploid number is very variable. Only in the *boliviensis* group, several species share the 2n=40. In the other groups, several species are polymorphic, and then, the character state was ambiguous (Fig. 5). The optimization of 2n suggests that 38 is the possible ancestral number for *Akodon*. However, its high variability and the occurrence of 2n=38 in few species, very separated in the trees, suggest that convergence in the 2n in different evolutionary branches could have happened.

In Sigmodontinae, the ancestral chromosome complement was proposed to be possibly similar to that of *Sigmodon hispidus* (tribe Sigmodontini), which has 2n=52, FNa=52 (Swier et al. 2019). This chromosome formula also was found in other tribes, including Akodontini and Abrotrichini (Patton et al. 2015, Da Rosa et al. 2019). In the genus *Akodon*, all species have lower 2n and FNa. This suggests a general evolutionary trend towards the reduction of chromosome number and autosomal arms in *Akodon*, which in some cases appears to have occurred in independent lineages.

Acknowledgments

Our thanks to Benjamín Bender for his assistance with the material deposited in the Mammal Collection of the Instituto Argentino de Zonas Aridas (CMI), IADIZA-CONICET, Mendoza. We also appreciate the assistance in the laboratory of Genetica Evolutiva (FCEQyN-UNaM) of Alberto Taffarel. Our deep gratitude to Dr. James L. Patton for the substantial contribution to this work. Another anonymous reviewer also has suggestions that improved the manuscript. The authors thank the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Partially financed by PIP-CONICET 2015-0258 CO and 11220200103055CO (RAO, PT, CL, PJ). PIP 2012-2014 No. 0182 (ADB), PICT 2016-0537 (PT, CL).

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SUPPLEMENTARY MATERIAL

Appendix S1.
Tables SI, SII.
Figure S1.

How to cite
LABARONI CA ET AL. 2023. Revision and analysis of the chromosome variability in the speciose genus *Akodon* (Rodentia, Sigmodontinae), including new data from Argentina. An Acad Bras Cienc 95: e20200393. DOI 10.1590/0001-3765202320200393.

Manuscript received on March 25, 2020;
accepted for publication on June 12, 2020

CAROLINA A. LABARONI[1]
https://orcid.org/0000 0001 5255 0897

VAITIARE A. PAEZ COLL MAIRHOFER[1]
https://orcid.org/0009-0009-6915-6605

AGUSTINA A. OJEDA[2]
https://orcid.org/0000-0003-2007-5061

AGUSTINA NOVILLO[3]
https://orcid.org/0000-0003-1211-450X

PABLO TETA[4]
https://orcid.org/0000-0001-8694-0498

PABLO JAYAT[5]
https://orcid.org/0000-0002-6838-2987

RICARDO A. OJEDA[2]
https://orcid.org/0000-0001-5246-0496

LEANDRO M. BUSCHIAZZO[1]
https://orcid.org/0009-0001-7187-0092

EUGENIO N. CÁLCENA[6,7]
https://orcid.org/0000-0003-4535-5350

ALEJANDRO D. BOLZÁN[6,8]
https://orcid.org/0000-0002-3627-1182

CECILIA LANZONE[1]
https://orcid.org/0000-0002-3206-9809

[1]Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad Nacional de Misiones, Instituto de Biología Subtropical, Facultad de Ciencias Exactas, Químicas y Naturales, Laboratorio de Genética Evolutiva, Calle Félix de Azara 1552, 3300 Posadas, Misiones, Argentina

[2]Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto Argentino de Investigaciones de Zonas Áridas - CCT Mendoza, Laboratorio de Filogeografía, Taxonomía Integrativa y Ecología (LFTIE), Av. Ruiz Leal, s/n, Parque Gral. San Martín, Mendoza 5500, Argentina

[3]Consejo Nacional de Investigaciones Científicas y Técnicas, Tucumán, Instituto de Biodiversidad Neotropical CCT, Residencia Universitaria Horco Molle, s/n, 4017 Tucumán, Argentina

[4]Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", División Mastozoología, Av. Angel Gallardo, 470, C1405DJR Buenos Aires, Argentina

[5]Consejo Nacional de Investigaciones Científicas y Técnicas, Fundación M. Lillo, Unidad Ejecutora Lillo, Calle Miguel Lillo 251, San Miguel de Tucumán, 4000 Tucumán, Argentina

[6]Instituto Multidisciplinario de Biología Celular (IMBICE, CONICET-CICPBA-UNLP), Laboratorio de Citogenética y Mutagénesis, Calle 526 y Camino General Belgrano, La Plata, B1906APO Buenos Aires, Argentina

[7]Universidad Nacional de Quilmes, Av. Roque Sáenz Peña 352, Bernal, B1876BXD Buenos Aires, Argentina

[8]Universidad Nacional de La Plata, Facultad de Ciencias Naturales y Museo, Avenida 122 y 60, La Plata, 1900 Buenos Aires, Argentina

Correspondence to: **Cecilia Lanzone**
E-mail: cecilialanzone2016@gmail.com

Author contributions

Carolina Labaroni: Collected the data; Contributed data and analysis tools; Performed some analysis; Wrote the paper. Vaitiare A. Paez Coll Mairhofer: Collected the data; Contributed data and analysis tools; Performed some analysis. Agustina Ojeda: Collected the data; Partial writing of the manuscript. Agustina Novillo: Collected the data; Partial writing of the manuscript. Pablo Teta: Collected the data; Partial writing of the manuscript. Pablo Jayat: Collected the data; Partial writing of the manuscript. Ricardo Ojeda: Collected the data; Partial writing of the manuscript. Leandro Buschiazzo: Collected the data; Contributed data and analysis tools. Eugenio Cálcena: telomere FISH (technical support). Alejandro Bolzán: telomere FISH (image processing and analysis); Partial writing of the manuscript. Cecilia Lanzone: Conceived and designed the analysis, Performed some analysis; Wrote the paper.