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The role of cytogenetic variation in *Akodon cursor* species complex speciation (Rodentia: Sigmodontinae)

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ABSTRACT: Chromosome polymorphism in populations of *Akodon cursor* complex (134 specimens) and the karyotypes of other *Akodon* species (94 specimens) from Brazil were analyzed. Five taxa were considered: *Akodon cursor* with 2n = 14, *Akodon* aff. *cursor* with 2n = 16, *Akodon montensis* with 2n = 24, *Akodon paranaensis* with 2n = 44 and *Akodon serrensis* with 2n = 46. Chromosome polymorphism was observed in *A. cursor* and *A.* aff. *cursor* due to pericentric inversions, and also in *A. paranaensis* and *A. montensis* by the presence of supernumerary chromosomes. Pericentric inversion polymorphism affecting two autosome pairs in *A. cursor* karyotype was found in Hardy-Weinberg equilibrium. *Akodon* aff. *cursor* with 2n = 16 occurred from Bahia to Rio Grande do Norte states and *A. cursor* with 2n = 14 from Bahia to Paraná states. Our results as well as recent literature suggest that the karyotype with 2n = 16 is fixed in the northern part of the distribution of *Akodon cursor* complex. Captive hybrid males between *Akodon* [2n = 14] x [2n = 16] are apparently sterile, strengthen the proposition these populations may represent two full species.
Key words: Akodontini, chromosome polymorphism, karyotype, geographic distribution.

RESUMO (O papel da variação citogenética na especiação do complexo de espécies *Akodon cursor* (Rodentia: Sigmodontinae)): O polimorfismo cromossômico foi analisado em populações do complexo *Akodon cursor* (134 amostras), assim como os cariótipos de outras espécies de *Akodon* (94 amostras) do Brasil. Foram considerados cinco táxons: *Akodon cursor* com 2n = 14, *Akodon* aff. *cursor* com 2n = 16, *Akodon montensis* com 2n = 24, *Akodon paranaensis* com 2n = 44, e *Akodon serrensis* com 2n = 46. O polimorfismo cromossômico observado em *A. cursor* e *A.* aff. *cursor* foi devido a inversões pericêntricas, e no caso de *A. paranaensis* e *A. montensis* a causa foi a presença de cromossomos supranumerários. O polimorfismo de inversão pericêntrica afetando dois pares de autossomos no cariótipo de *A. cursor* foi encontrado em equilíbrio de Hardy-Weinberg. *Akodon* aff. *cursor* com 2n = 16 ocorreu do estado da Bahia ao do Rio Grande do Norte, e *A. cursor* com 2n = 14 do estado da Bahia ao do Paraná. Nossos resultados e o da literatura recente sugerem que o cariótipo com 2n = 16 esteja fixado na parte norte da distribuição do complexo *Akodon cursor*. Os machos híbridos gerados em cativeiro entre *Akodon* [2n = 14] x [2n = 16] são aparentemente estéreis, reforçando a proposição de que essas populações possam representar, de fato, duas espécies.
Palavras-chave: Akodontini, polimorfismo cromossômico, cariótipo, distribuição geográfica.

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INTRODUCTION

Karyologic data are most useful for understanding the diversity of *Akodon* Meyen, 1833, a genus whose striking karyologic differentiation accounts for differences in diploid number, which may vary from 9–10 to 46 (Bianchi *et al.* 1983, Yonenaga-Yassuda *et al.* 1987, Silva & Yonenaga-Yassuda 1998, Bonvicino 2011). Recent phylogenetic and phylogeographic studies, using Cytochrome-*b* mtDNA sequence data, have also shown that this genus is highly diverse (Gonçalves *et al.* 2007).

Akodon presents a widespread distribution in South America except for the north of the Amazonas river (Oliveira & Bonvicino, 2006), and, in Brazil, this genus occurs mainly in the Atlantic Forest and the Cerrado biomes. Four *Akodon* species can be easily captured in the Atlantic Forest: *Akodon cursor* (Winge, 1887), *Akodon montensis* Thomas, 1913, *Akodon serrensis* Thomas,1902, and *Akodon paranaensis* Christoff *et al*., 2000. Whereas *A. paranaensis*, *A. montensis,* and *A. serrensis*, are karyologically stable, *Akodon cursor*, shows a remarkable karyological polymorphism (Fagundes *et al.* 1998), and the geographic distribution of karyomorphs indicates a trend to fixation in southern and northern populations. This is one of the most studied species of the genus, and its remarkable karyotype variation includes diploid numbers from 14 to 16, and numbers of autosomal arms from 18 to 26 due to complex rearrangements involving pericentric inversions and fusion/fission centric (Yonenaga *et al*. 1975, Geise *et al*. 1998, Bonvicino 2011, Massariol 2016). This variation in diploid number is caused by the Robertsonian process that includes either fission or centric fusions, without compromising the genome size or its genic significance. The result is an unaffected phenotype for individuals bearing such chromosomal changes, however, may cause reproductive disadvantage due to fertility reduction by gametic aneuploidy of heterozygotes (Capanna *et al.* 1977). The effects of chromosomal rearrangements within populations are variable, with fusions and inversions with little or no impact on gametogenesis, that may be maintained as variants within populations. Conversely, under fairly stringent conditions, fusions may be associated with significant deleterious outcomes in other taxa (Dobigny *et al.* 2015). Such pericentric inversions and Robertsonian translocations play an important role in the karyotypic evolution of mammals (Qumsiyeh 1994). Most records of *Akodon cursor* with 2n = 16 are restricted to the northeast region, through the states of Bahia and Paraíba, where there are no records of animals with 2n = 14 or 15 (Maia & Langguth 1981). However, despite being at a low frequency, one specimen with 2n = 16 has already been registered in the state of Paraná (Sbalqueiro & Nascimento 1996).

In this work, we have analyzed the chromosome complement of *Akodon cursor*, *A. montensis, A. paranaensis,* and *A. serrensis* from the Atlantic Forest biome emphasizing the role of chromosome polymorphisms in speciation process in the *A. cursor* species complex.

MATERIAL AND METHODS

Two hundred and twenty-eight *Akodon* specimens, including the *A. cursor* species complex (134), *A. montensis* (66), *A. serrensis* (6) and *A. paranaensis* (22) were karyotyped from different Brazilian localities and distinct elevations to detect possible altitudinal variation (Appendix 1, Figure 1). Chromosome preparations were obtained from bone marrow cultures following a 2-hour

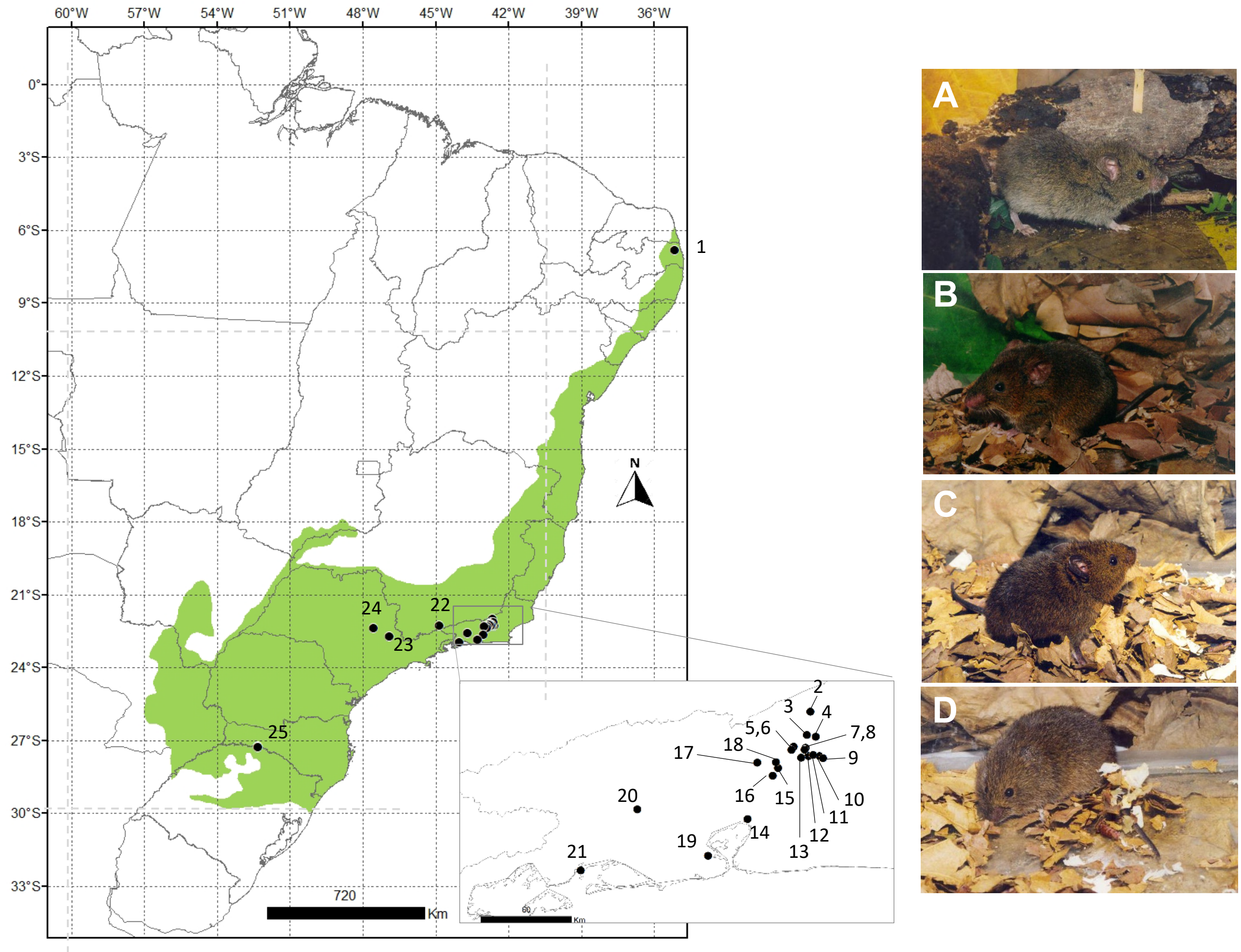

Figure 1. Sampling localities and the four *Akodon* species considered in this study. Left and center: Map showing the remaining Atlantic Forest domain in green. Localities numbered, Brazil: Paraíba state: (1) Mamanguape; Rio de Janeiro state: Sumidour o municipality: (2) vale da Bela Joana, (3) Campinas, (4) Dona Mariana, (7) Santo André; Teresópolis municipality: (6) Teresópolis, (15) Bonsucesso, (16) Canoas, (17) Fazenda Alpina, (18) Venda Nova, (5) Vieira; Nova Friburgo municipality: (8) Conquista, (9) Mottas, (10) Campo do Coelho, (11) Rio Grande and Salinas, (12) Córrego Grande, (13) Cardinot; (14) Magé municipality; (19) Rio de Janeiro municipality; (20) Paracambi municipality; (21) Mangaratiba municipality; Minas Gerais state: (22) Itamonte municipality; São Paulo state: (23) Pedreira municipality; (24) Rio Claro municipality; Santa Catarina state: (25) Itá municipality. See Gazetteer on Appendix 1 for more details. On the right side: specimens of *Akodon.* A. *A. cursor.* B. *A. montensis*. C. *A. serrensis*. D. *A. paranaensis.*

incubation, at 37°C, in RPMI 1600 medium with fetal bovine serum (20%), colchicine (10^{-6}M) and ethidium bromide (5µg/ml) (Andrade & Bonvicino 2003). G-banding was performed as described by Seabright (1971). Fundamental Numbers (FN) refers to the number of autosomal arms. Specimens were deposited in the mammal collection of the Museu Nacional - UFRJ, Rio de Janeiro, Brazil (tagged as MN), and in the collection of the Laboratório de Biologia e Parasitologia de Mamíferos Reservatórios Silvestres, Instituto Oswaldo Cruz (tagged as LBCE), Rio de Janeiro, Brazil. CRB refers to Cibele R. Bonvicino field number.

Selected samples of *A. cursor* from Rio de Janeiro state, Sumidouro and Teresópolis municipalities where the number of karyotyped specimens was greater than 40, were submitted to Hardy-Weinberg Equilibrium (HWE) test, for each chromosome pair that presented an inversion polymorphism (pairs 2 and 4). A similar procedure was carried on published data for a Paraná state Guaraqueçaba municipality population which sample was also over 40 specimens (Sbalqueiro & Nascimento, 1996).

In order to assess the degree of reproductive isolation between karyologically different populations, cross-breeding experiments were carried out in captivity considering three karyotypes, for two valid species in two arrangements. The first arrangement was set between an *A. cursor* female with 2n = 14 from Mangaratiba (Rio de Janeiro state) and one *A.* aff. *cursor* male with 2n = 16 from Mamanguape (Paraíba state). The Second arrangement was drawn with an *A. cursor* male with 2n = 14 from Sumidouro (Rio de Janeiro state) and an *A. montensis* female with 2n = 24 from Itamonte (Minas Gerais state). Hybrids' fertility was evaluated by the detection of spermatozoids within its testis. This procedure was carried on a sample of spermatic secretion collected from the epididymis, buffered in saline solution, and set on a petri dish examined under an optical microscope.

RESULTS

Akodon cursor was the most common species in all sampled localities from seven municipalities of Rio de Janeiro state, Magé, Mangaratiba, Nova Friburgo, Paracambi, Rio de Janeiro, Sumidouro, and Teresópolis, with sympatry with *A. montensis* in several localities, and syntopy in Sumidouro at Dona Mariana locality, Nova Friburgo at Córrego Grande locality, and Teresópolis at Mottas and Vieira localities (Appendix 1). *Akodon montensis* was found in Nova Friburgo, Teresópolis and Sumidouro (Rio de Janeiro state); Pedreira and Rio Claro (São Paulo state); and Itá (Santa Catarina state). *A. serrensis* occurred in Nova Friburgo (Rio de Janeiro state). *Akodon paranaensis* was capured in Itamonte (Minas Gerais state), Nova Friburgo (Rio de Janeiro state) and Itá (Santa Catarina state). *Akodon montensis* also occurred in syntopy with *A. serrensis* in São Lourenço locality in Nova Friburgo (Rio de Janeiro state).

Karyologic analyses of 66 specimens of *A. montensis* showed 2n = 24 and FN = 42 (Figure 2A, Appendix 1). The autosome complement is composed of 10 biarmed chromosome pairs varying in size from large to small and one small acrocentric pair; the X chromosome is a medium-small acrocentric and the Y chromosome is a very small acrocentric (Figure 2A.). One specimen (MN50264) showed 2n = 25 and FN = 44 due to the presence of a biarmed supernumerary chromosome. Karyologic analyses of 20 specimens of *A. paranaensis* showed 2n = 44 and FN = 44 (Figure 2B, Appendix 1). The autosome complement was composed of 20 acrocentric pairs varying in size from large to small, and one very small biarmed chromosome pair; the X chromosome is a small acrocentric and the Y chromosome is a very small acrocentric. Two specimens showed 2n = 45 and FN = 45 due to the presence of one supernumerary acrocentric chromosome. Karyologic analyses of six specimens of *A. serrensis* showed 2n = 46 and FN = 46 (Figure 2C, Appendix 1). The autosome complement was composed of 21 acrocentric pairs varying in size from large to small, and one very small biarmed chromosome pair; the X chromosome is a small acrocentric and the Y chromosome is a very small acrocentric.

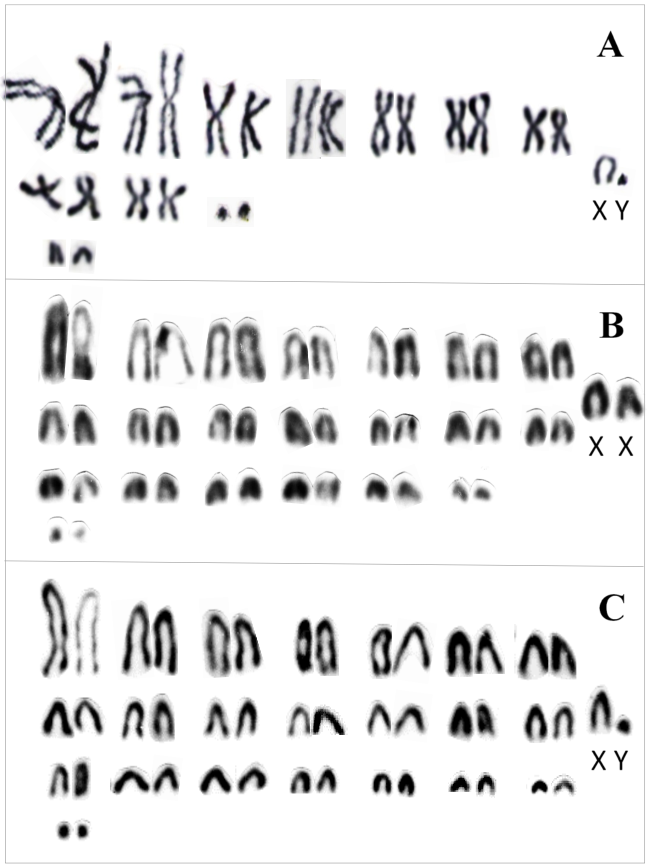

Figure 2. Conventional staining karyotypes of *Akodon* species. A. *Akodon montensis* LBCE1602 with 2n = 24. B. *Akodon paranaensis* CRB1327 with 2n = 44. C. *Akodon serrensis* LBCE2052 with 2n = 46.

Karyologic analyses of 134 specimens of *A. cursor* showed the same 2n = 14, but with fundamental number variation, 18, 19, 20, 21, and 22. The X chromosome was a small acrocentric and the Y chromosome a very small acrocentric. Three autosome pairs were morphologically identical in all karyotypes: the largest biarmed chromosome pair no. 1+3, the medium-sized biarmed chromosome pair no. 5, and the smallest biarmed chromosome pair number 7. The remaining three autosome pairs (2, 4, and 6) showed pericentric inversions. Altogether, these rearrangements accounted for 10 different karyotypes identified by letters from 'A' to 'J' (Figure 3; Tables 1 and 2). Most natural populations were polymorphic for pairs number 2 and 4, and the variation in chromosome types and pair combinations found in samples from distinct localities herein studied are shown in Table 1. There was no clear pattern in geographic variation based on the frequency of acrocentric and biarmed chromosomes 2 and 4. Moreover, these inversion polymorphisms were in HWE (Table 2). The pair number 6 consisted, in most cases, of two acrocentric chromosomes and, more rarely, of an acrocentric chromosome and a biarmed counterpart.

Cross-breeding experiments between *A. cursor* specimens with 2n = 14 from Rio de Janeiro and *A.* aff. *cursor* specimens with 2n = 16 from Paraíba produced first-generation hybrids (F1) with 2n = 15 and FN = 22 (Figure 4A). Crosses between *A. cursor* specimens with 2n = 14 from Rio de Janeiro and specimens with *A. montensis* 2n = 24 from Minas Gerais produced F1 hybrids with 2n = 19 and FN = 32 (Figure 4B). Both karyotypes showed a small acrocentric X chromosome and a very small acrocentric Y chromosome (Figure 4A,B). Male F1 hybrids herein analyzed were sterile due to azoospermia.

DISCUSSION

The syntopy of *A. montensis* and *A. cursor* was herein confirmed in elevations around 750m in three municipalities of Rio de Janeiro state: Sumidouro (Dona Mariana locality), Teresópolis (localities of Mottas and Vieira) and Nova Friburgo (Córrego Grande locality). These findings would give support to the occurrence of natural hybrids (with 2n = 19) between *A. montensis* and *A. cursor* reported for São Paulo state by Yonenaga *et al.* (1975), but put on debate the hypothesis that *A. cursor* and *A. montensis* were allopatrically segregated by altitude. Such assumptions came from the fact that *A. cursor* would be limited to altitudes below 600 m and *A. montensis* could be only found at higher elevations in Rio de Janeiro (Geise 1995). In this study *A. cursor* was the most abundant species, and the only one occurring in all sampled localities, confirming previous suggestions that this species is a habitat generalist compared to *A. serrensis* and *A. paranaensis* that were almost restricted to high elevations. *A. montensis* was also found in syntopy with *A. paranaensis* and *A. serrensis*, whereas *A.* aff. *cursor* is the distinctive *Akodon* species occurring in northeastern Brazil.

Table 1. Karyotypes of *Akodon* with 2n = 14, 2n = 16, and captive hybrids, specifying the morphology of chromosome pairs. 'Karyo' indicates the 2n + Morphotype (see Figure 3). FN = autosome fundamental number, AA = acrocentric homozygotes, AB = heterozygotes, BB = biarmed homozygotes, non = without variation. Sample size from this study (ts) or literature (lit), see Fagundes *et al*. (1998) for a revision of *Akodon* with 2n = 14, Teixeira (1993), and Maia & Langguth (1981) for a revision of *Akodon* with 2n = 16.

Karyo	FN	Chromosome pair										Sample size		
		1+3	1	2	3	4	5	6	7	X	Y	ts	lit	Total
14A	18		non	AA	non	AA		AA		A	A	18	35	53
14B	19		non	AB	non	AA		AA		A	A	18	22	40
14C	19		non	AA	non	AB		AA		A	A	43	64	107
14D	20		non	AB	non	AB		AA		A	A	15	31	46
14E	20		non	AA	non	BB		AA		A	A	17	19	36
14F	20	BB	non	BB	non	AA	BB	AA	BB	A	-	1	3	4
14G	20		non	AA	non	AB		AB		A	A	3	1	4
14H	21		non	AB	non	BB		AA		A	A	16	13	29
14I	21		non	BB	non	AB		AA		A	A	2	2	4
14J	22		non	BB	non	BB		AA		A	A	1	0	1
Total												**134**	**190**	**324**
16A		non	BB	BB	BB	BB	BB	AA	BB	A	A	1	6	
16B		non	BB	BB	BB	BA	BB	AA	BB	A	A		4	
Total													**10**	
Hybrid		B	B	AB	B	AB	BB	AA		A	A	2		

Akodon montensis and *A. paranaensis* showed diploid and fundamental number variation due to the presence of supernumerary B chromosomes but diploid and fundamental number in *A. serrensis* were apparently invariable. The observed variation in fundamental autosome number in *A. cursor* with 2n = 14 was herein confirmed in 10 different karyotypes, nine of which previously described (Fagundes *et al.* 1998), and another one, designated as 'J' karyotype with FN = 22, herein reported for the first time (Figure 3, Table 1). This remarkable variation in autosome fundamental number, ranging from 18 to 22, resulted from pericentric inversions affecting three autosome pairs (2, 4, and 6), accounting for chromosome polymorphisms in this species. In *A. cursor* with 2n = 14, the distribution of chromosome pairs no. 2 and 4 over the large sampled populations (N>40) is in Hardy-Weinberg Equilibrium (Table 2), indicating that these chromosome rearrangements did not result in a selective disadvantage in heterozygous carriers and that, to present, have not accounted for any apparent evolutionary diversification of *A. cursor.* Conversely, the pericentric inversion occurring in pair number 4 in *Akodon* with 2n = 16 from the northeast of Brazil accounts for only two fundamental autosome numbers (Teixeira 1993), which is probably resulted by the very small number of karyotyped specimens.

Differences in *A. cursor* diploid chromosome number, 2n = 14 and 15, have been reported for few specimens from Rio de Janeiro state (Fagundes *et al.* 1998) and from 2n = 14 to 16 for São Paulo and Paraná states specimens (Sbalqueiro & Nascimento 1996, Fagundes *et al.* 1998), as a result from similar fusion/fission events. Events of diploid number variation are quite rare. Even with 134 *A. cursor* specimens analyzed from Rio de Janeiro state, in the present study we were not able to detect such variation. Contrary to this possible polymorphic variation in diploid number in the southern part of the distribution of *A. cursor*, the northern population shows a constant diploid number (2n = 16; Maia & Langguth 1981). This stability suggests the 2n = 16 karyotype is fixed in the northeastern population, in contrast to the most common 2n = 14 karyotype in the southern population.

Cross-breeding between specimens with 2n = 14 from Rio de Janeiro state and 2n = 16 from Paraíba state successfully produced hybrids. Despite azoospermia was detected in the sole male specimen analyzed, it is noteworthy that *Akodon*

Figure 3. Giemsa conventional stained karyotypes of *Akodon cursor* with 2n = 14. A. FN = 18, LBCE1404-F1. B. FN = 19, LBCE1811. C. FN = 19, LBCE2080. D. FN = 20, LBCE652. E. FN = 20, LBCE2158. F. FN = 20, LBCE1142. G. FN = 20, LBCE1428. H. FN = 21, LBCE2167. I. FN = 21, LBCE2269. J. FN = 22, LBCE2131. Numbers on the bottom of the figure refers to chromosome pairs as shown in Tables 1 and 2. X and Y indicate sexual chromosomes. The purple polygon emphasizes the most variable chromosome pairs for this species.

Table 2. Polymorphism for chromosome pairs 2 and 4* in *Akodon cursor*, specifying the locality of specimens karyotyped, sample size (N), morphology of pairs 2 and 4* [acrocentric (A) or biarmed (B)], values of qui-square test for Hardy-Weiberg Equilibrium (χ^2 HW; df=2; p=0.05) for samples with N>40, and source. *pair 3 in some cited literature.

Locality		**2**				**4**				**Source**
	N	**AA**	**AB**	**BB**	**χ^2 HW**	**AA**	**AB**	**BB**	**χ^2 HW**	
RJ, Teresópolis	42	30	12	0	1.167	11	22	9	0.105	this study
RJ, Sumidouro	77	40	34	3	1.692	24	31	22	2.906	this study
PR, Guaraqueçaba	97	51	42	4	1.682	17	38	42	2.510	Sbalqueiro & Nascimento, 1996
BA Una	2	0	2	0		0	2	0		Fagundes *et al*., 1998
RJ, Campus UFRRJ	2	0	2	0		1	1	0		Yonenaga-Yassuda, 1979
RJ, Rio de Janeiro	4	3	0	1		1	2	1		this study
RJ, Magé	1	0	1	0		1	0	0		this study
RJ, Paracambi	6	5	1	0		1	3	2		this study
RJ, Nova Friburgo	4	3	1	0		0	4	0		this study
RJ, Piraí	2	1	1	0		1	1	0		Yonenaga-Yassuda, 1979
SP, Casa Grande	1	1	0	0		1	0	0		Yonenaga-Yassuda ,1979
SP, Iguape	6	3	2	1		0	5	1		Fagundes *et al*., 1998
SP, Sete Barras	6	4	2	0		2	3	1		Fagundes *et al*., 1998
SP, Juquitiba	3	3	0	0		1	1	1		Fagundes *et al*., 1998
SP, Salesópolis	3	2	1	0		2	0	1		Fagundes *et al*., 1998
SP, Picinguaba	4	3	1	0		1	1	2		Fagundes *et al*., 1998
SP, Ariri	1	1	0	0		0	1	0		Fagundes *et al*., 1998
SP, Iporanga	1	0	1	0		1	0	0		Fagundes *et al*., 1998
SP, Ilha do Cardoso	2	1	1	0		0	2	0		Fagundes *et al*., 1998

cursor stands out among sigmodontine rodents for its high spermatogenic and reproductive efficiency (Balarini 2013). This result is similar to other findings in crossbreeding experiments carried by Massariol (2016). This author pointed out that crossing between 2n=14–15 females and 2n = 16 males showed a low reproductive success, with a strong fertility reduction in 2n = 15 individuals, especially in females. It was also detected a reproductive success in 2n = 14 males, but not with 2n = 16 males. Chromosomal rearrangements usually cause species reproductive issues caused by gametes due to genetic unbalancing (White 1978). This was true for *Akodon* karyomorphotype with 2n = 15 with pericentric inversions of pairs no. 2 and 4, while other variants of the karyotype 2n = 15 showed a greater potential to generate descendants after crossing with the cytotype 2n = 14 than with the 2n = 16, revealing the role of the karyotype in the process of differentiation of populations in *A. cursor*, featuring a rare case of speciation in real-time (Massariol 2016). Furthermore, although individuals with 2n = 15 are fertile, they have reduced reproductive success, mainly when the female presents this heterozygous condition. Crossings between 2n = 15 and 2n = 16 did not generate descendants, but between 2n = 15 and 2n = 14 individuals did (Massariol 2016). Furthermost, molecular studies, based on cytochrome *b* sequence data, showed the northeastern and southern populations as two divergent evolutionary lineages (Geise *et al*. 2001) in disagreement with Fagundes *et al.* (1998) who postulated that the northern and southern populations comprised a single evolutionary unit. These data did not support the postulation that this species is monotypic (Patton *et al*. 2015).

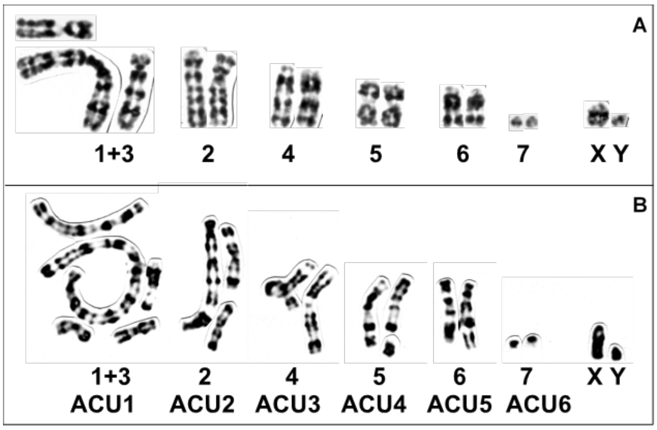

Figure 4. G band karyotype of hybrids. A. *Akodon cursor* with 2n = 14 from Rio de Janeiro state and *Akodon* aff. *cursor* with 2n = 16 from Paraíba state. B. F1 hybrid between *Akodon cursor* with 2n = 14 from the state of Rio de Janeiro and *Akodon montensis* with 2n = 24 from Minas Gerais state. Chromosome number follows the criterion of Teixeira (1993) for *Akodon* aff. *cursor* with 2n = 16 and the criteria of Geise *et al*. (1998) for *Akodon cursor* 2n = 14 (ACU).

The type locality of *A*. *cursor* (Lagoa Santa, in the state of Minas Gerais) is located within the geographic range of specimens with 2n = 14. We thus consider this species as the one showing a basic karyotype with 2n = 14, and *A*. aff. *cursor* as the species with 2n = 16. There is no available name for this latter species that is morphologically very similar to *A. cursor*. Their high similarity concerning external and cranial characteristics makes their morphological distinction very difficult for a precise diagnosis of this new species, which will require accurate morphological analyses. Cryptic sigmodontine rodent species are very common, and identification at species level is a central question not only to taxonomy but is vital to address the occurrence of zoonoses. This is dissociation is unsuitable since many sigmodontine species present high specificity in their parasitic relationships with etiological agents, as is the case of hantaviruses (Romano-Lieber *et al*. 2001, Oliveira *et al*. 2014). Nonetheless, the pursuit of unequivocal identifications will strengthen biodiversity surveys uplifting our knowledge on the group taxonomy and thus resulting in suitable conservation and epidemiological policies.

CONCLUSION

Akodon cursor and *A.* aff. *cursor* karyotypes are characterized by chromosome polymorphism due to pericentric inversions, the latter with 2n = 16 occurring from Brazilian states of Bahia to Rio Grande do Norte and *A. cursor* with 2n = 14-16 from Bahia to Paraná states. The karyotype with 2n = 16 is fixed in the northern part of the distribution of *Akodon cursor* complex. Captive hybrid males between *Akodon* [2n = 14] x [2n = 16] are apparently sterile, strengthen the proposition these populations may represent two full species, *A. cursor* as the one showing a basic karyotype with 2n = 14, and *A.* aff. *cursor* as the species with 2n = 16.

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APPENDIX 1. Supplementary material

Supplementary data associated with this article can be found at 10.6084/m9.figshare.12685463.

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