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Chromosomal phylogeny of four Akodontini species (Rodentia, Cricetidae) from Southern Brazil established by Zoo-FISH using *Mus musculus* (Muridae) painting probes

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Abstract

We established chromosome homology maps between *Mus musculus* (MMU) and five species of the Akodontini tribe, *Akodon cursor* (2n = 14, 15 and 16), *A. montensis* (2n = 24), *A. paranaensis* (2n = 44), *A. serrensis* (2n = 46) and *Oligoryzomys flavescens* (2n = 66) by Zoo-FISH (fluorescence *in situ* hybridization) using mouse chromosome-specific probes. The aims of this study were (1) to detect the chromosomal rearrangements responsible for the karyotype variation in this tribe and (2) to reconstruct the phylogenetic relationships among these species. We observed four common syntenic associations of homologous chromosome segments, of which the MMU 7/19 has been described previously in other rodents from Africa, Asia and Europe, and might represent a phylogenetic link between the Old World and Neotropical rodents. The remaining three associations (3/18, 6/12 and 8/13) have been observed exclusively in Neotropical rodents so far, which at present can be considered synapomorphic traits of this group. Five further mouse chromosomes (MMU 4, 9, 14, 18 and 19) were each found evolutionarily conserved as a separate syntenic unit. Our phylogenetic analysis using parsimony and heuristic search detected one consistent group, separating the Akodontini from other rodents.

Abbreviations

ACU	*Akodon cursor*
AMO	*Akodon montensis*
APA	*Akodon paranaensis*
ASE	*Akodon serrensis*
ASY	*Apodemus sylvaticus*
CGR	*Cricetulus griseus*
FISH	fluorescence *in situ* hybridization
FNa	number of autosomal chromosome arms
MMU	*Mus musculus*
MPL	*Mus platythix*
OFL	*Oligoryzomys flavescens*
OIR	*Otomys irroratus*
PAUP	Phylogenetic Analysis Using Parsimony
RNO	*Rattus norvegicus*
RPU	*Rhabdomys pumilio*
TOS	*Tokudaia osimensis*
TTO	*Tokudaia tokunoshimensis*

Electronic supplementary material

The online version of this article (doi:10.1007/s10577-007-1211-5) contains supplementary material, which is available to authorized users.

Introduction

South America is inhabited by 450 rodent species, corresponding to 43% of the mammals of the continent (Reig 1984). Within the Sigmodontinae, the Akodontini stand out as one of the most diversified tribes with approximately 35% of the total variety of this subfamily. The Akodontini tribe is composed of 9 genera and 81 species (Musser & Carleton 2005), with similar appearance to rats (Reig 1981, 1984). The genus *Akodon* comprises 41 species (Musser & Carleton 1993 and 2005) spreading throughout South America, mainly in Brazil, Argentina, Paraguay, Chile, Venezuela and Uruguay. This genus presents a very complex and little understood taxon, including several species with a high degree of morphological similarity. The phenotypic uniformity stays in contrast to the high karyotypic divergence observed in these rodents, ranging from 2n = 10 to 2n = 46 (Sbalqueiro *et al*. 1986, Christoff *et al*. 2000). The number of autosomal chromosome arms (FNa = 14–16 to 46) (Sbalqueiro & Nascimento 1996, Silva *et al*. 2006), makes this karyotype divergence even more intricate.

Several reports have attempted to address the questions concerning the phylogenetic relationships of this group using chromosomal (Bianchi *et al*. 1971, Liascovich & Reig 1989) or molecular markers. Smith & Patton (1991, 1993) and Geise *et al*. (2001) performed comparative analyses of the mitochondrial cytochrome *b* sequences in *Akodon* species from various regions in South America in order to establish the phylogenetic relationships of this genus. Montes *et al*. (2008) analysed the partial sequence of the nuclear gene *interphotoreceptor retinoid binding protein* from 29 *Akodon* species and proposed a more detailed phylogeny. According to this phylogeny, two principal clusters are separated into four clear groups that correlate with their geographic distribution in the Northern Andes, Southern Andes, Pampas and the Atlantic Forrest. Comparative G-banding analysis has revealed numerous chromosome rearrangements, leading to proposals of phylogenetic relationships in Akodontini (Sbalqueiro & Nascimento 1996, Geise *et al*. 1998). The chromosomal variability within this group was believed to be due to the occurrence of various centric fusions, tandem fusions and inversions. However, there are many gaps in our knowledge and controversies that still exist could be resolved using a molecular cytogenetic approach.

During the last decade, several rodent species have been investigated by Zoo-FISH using mouse (*Mus musculus*) chromosome painting probes. Among these are *Rattus norvegicus* (Guilly *et al*. 1999, Stanyon *et al*. 1999, Cavagna *et al*. 2002) and *Apodemus sylvaticus* (ASY; Matsubara *et al*. 2004, Stanyon *et al*. 2004) from Europe; *Mus platythix* (MPL; Matsubara *et al*. 2003), *Cricetulus griseus* (CGR; Yang *et al*. 2000), *Tokudaia osimensis* (TOS) and *Tokudaia tokunoshimensis* (TTO) (both Nakamura *et al*. 2007) from Asia; and *Rhabdomys pumilio* (RPU; Rambau & Robinson 2003) an *Otomys irroratus* (OIR, Engelbrecht *et al*. 2006) from Africa. Including another 18 hamster species from 10 genera (Cricetidae), Sitnikova *et al*. (2007) and Romanenko *et al*. (2006, 2007b) proposed an ancestral karyotype for the Muroidea that comprises 2n = 48 chromosomes with homology to the *M. musculus* syntenic units 7/19, 16/11b, 2a, 3, 4, 6, 9, 5a/11a, 1a/17a, 14, 12a, 18, 1b, 13b/15a, 15b, 2b/13a, 8a, 8b, 5b, 10c/17c, 17b/10b, 12b/17d, 10a, X and Y. According to this proposal, the *M. musculus* karyotype would be relatively derived even in Muridae, which themselves present extensively reshuffled karyotypes among rodents, when compared, for example, with those of squirrels (Stanyon *et al*. 2003, Li *et al*. 2004).

Concerning South American rodents, the comparative molecular cytogenetic data available so far are limited to the delineation of homology between chromosome 1b of *A. cursor* and chromosomes 7, 8 and 9q of *A. montensis* by Zoo-FISH, using a microdissection probe (Fagundes *et al*. 1997). Here we analysed four species of genus *Akodon,* together with *Oligoryzomys flavescens* as a phylogenetic outgroup, by cross-species chromosome painting using *Mus musculus* chromosome-specific probes. Our aim was to establish reliable comparative chromosome maps between these species and to utilize this dataset for the reconstruction of chromosomal phylogenies within this taxonomically controversial group of Cricetidae species.

Materials and methods

Sample characterization

The specimens were collected in several localities of Paraná, Southern Brazil (Table 1) and catalogued, and their skulls and skins were deposited at the Departmentos de Genética and Zoologia (Coleção Científica de Mastozoologia) of the Universidade

Table 1. Species analysed in this study by FISH with *Mus musculus* chromosome painting probes

No.[a]	Species	Sex	2n	Locality and coordinates
P702	*Akodon cursor*	F	14	I. Laranjeiras; 25°19′S, 48°18′W
P722	(ACU)	M	16	I. Rasa; 25°21′S, 48°25′W
P732	(ACU)	F	15	I. das Gamelas; 25°20′S, 48°24′W
P827	*Akodon montensis*	M	24	Quatro Barras; 25°21′S, 49°04′W
P871	(AMO)	F	24	Três Barras; 25°26′S, 48°53′W
P875	*Akodon paranaensis*	F	44	Três Barras; 25°26′S, 48°53′W
P890	(APA)	M	44	Curitiba; 25°22′S, 49°13′W
P851	*Akodon serrensis*	F	46	Quatro Barras; 25°21′S, 49°04′W
P856	(ASE)	M	46	Piraquara; 25°28′S, 49°21′W
P931	*Oligoryzomys flavescens*	F	66	S. J. dos Pinhais; 25°37′S, 49°05′W
P952	(OFL)	M	66	S. J. dos Pinhais; 25°37′S, 49°05′W

[a]No. = original sample number.

Federal do Paraná. We have studied 11 individuals from five species of the Akodontini tribe: *Akodon cursor* (ACU), *A. montensis* (AMO), *A. paranaensis* (APA), *A. serrensis* (ASE) and *Oligoryzomys flavescens* (OFL) (IBAMA, Brazil reference number 026/2004). Metaphases were prepared according to Ford & Hamerton (1956), with modifications from Sbalqueiro & Nascimento (1996). G-banding was performed as described by Seabright (1971), with modifications.

Preparation and labelling of mouse chromosome-specific painting probes

Painting probes specific for *Mus musculus* (MMU) chromosomes 1–20 and X, previously established by flow sorting, were obtained from the Resource Centre for Comparative Genomics, Department of Veterinary Medicine, University of Cambridge, UK. All painting probes were amplified and labelled with biotin-dUTP, dinitrophenol-dUTP or Tamra-dUTP by degenerate oligonucleotide-primed PCR, as previously described by de Oliveira *et al.* (2002). 500 ng to 2 μg per mouse paint probe was used in single-, dual- or triple-colour cross-species FISH experiments.

In situ hybridization and probe detection

In situ hybridization of *M. musculus* chromosome-specific painting probes with Akodontini and *Oligoryzomys flavescens* chromosomes was performed as previously described by de Oliveira *et al.* (2002), with the following modifications. The hybridization was performed for 48–72 h at 37°C. Post-hybridization washes included 2 × 5 min incubations in 50% formamide/2× SSC at 37°C, followed by 2 × 5 min incubations in 2× SSC at 37°C. Biotin-labelled probes were visualized with Cy3-avidin or by Cy5-avidin, whereas dinitrophenol-labelled probes were visualized with rabbit anti-dinitrophenol, followed by goat α-rabbit–Alexa 488 antibodies. All slides were counterstained with actinomycin D (0.5 mg/ml, 50% EtOH/50%, 1× PBS) at 1:50 dilution for 20 min and 4′,6′-diamino-2-phenylindole (DAPI, 2 μg/ml in 2× SSC) for 5 min.

Microscopy and image analysis

Metaphases were analysed with a cooled Photometrics C250/A CCD camera equipped with a KAF1400 chip, or with a cooled VDS-CCD camera, in either case coupled to a Zeiss Axiophot microscope. Images were captured using SmartCapture 2 or BandView/FISHView software. The FISH images were processed using Adobe Photoshop version 7.0. Chromosomes were identified by computer-enhanced DAPI– actinomycin D banding patterns.

Maximum parsimony analysis (PAUP)

The characters observed in the five species that were analysed in this study by chromosome painting using mouse probes, are summarized in Supplementary Table S1. The characters correspond to the conserved chromosomal homologies and syntenic associations verified in the outgroup *O. flavescens*. A data matrix was established (Supplementary Table S2) based on the presence or absence of these discrete characters. This data set was subjected to maximum parsimony analysis (PAUP* v.4.0b10 software; Phylogenetic

Analysis Using Parsimony, Swofford 2001), using the exhaustive search option. All characters had the same weight, based on the premise that chromosome rearrangements occur by equal chance. The relative stability of nodes was assessed by bootstrap estimates based on 1000 iterations. Each bootstrap replicate involved a heuristic parsimony search with 10 random taxon additions and tree-bisection-reconnection branch swapping.

Results

The hybridization with the 20 chromosome-specific painting probes from *Mus musculus* (MMU 1–19 and X) to *Akodon* and *O. flavescens* metaphase spreads showed reproducible results, except for the MMU chromosome 10 and 17 probes in *O. flavescens* and MMU 10 probe in *A. serrensis*. Centromeric and heterochromatic regions were not hybridized by any probe. In each experiment a minimum of 10 metaphases were analysed. Figure 1 shows representative metaphases after *in situ* hybridization with mouse chromosome-specific probes in *O. flavescens* and in the four *Akodon* species.

Akodon cursor (ACU)

The 19 autosomal and the X painting probes of *M. musculus* detected 32 regions of homology in the *A. cursor* genome. All mouse paint probes produced single hybridization signals, except for probes of MMU 1 and MMU 17, which showed four and three FISH signals, respectively. The chromosomal pair ACU 6 did not show homology with any painting probe of MMU. Twenty-eight syntenic associations of mouse homologous chromosomal segments were observed (MMU 1/3, 1/6, 1/9, 1/10, 1/11, 1/16, 1/17, 2/5, 2/7, 3/18, 4/5, 4/15, 4/17, 4/18, 5/6, 5/7, 5/12, 6/10, 6/12, 6/14, 7/19, 8/13, 9/10, 10/12, 10/17, 12/17, 14/17, 15/16). The majority of these associations were part of the complex translocation products MMU 2/5/7/19, MMU 6/10/14/17, MMU 12/17/10/9/1/3/1/10 and MMU 3/18/4/5/12/6/1/17/4/15/16/1/11. The associations 2/7 and 10/17 were identified considering the heteromorphic chromosome pair ACU 3 and ACU 2, respectively. Further, the *A. cursor* individual investigated was heterozygous for a rearrangement of ACU chromosome 1, resulting in chromosome forms 1 and 1a/1b, respectively. The chromosomal homology map between *Mus musculus* and *A. cursor* is shown in Figure 2a.

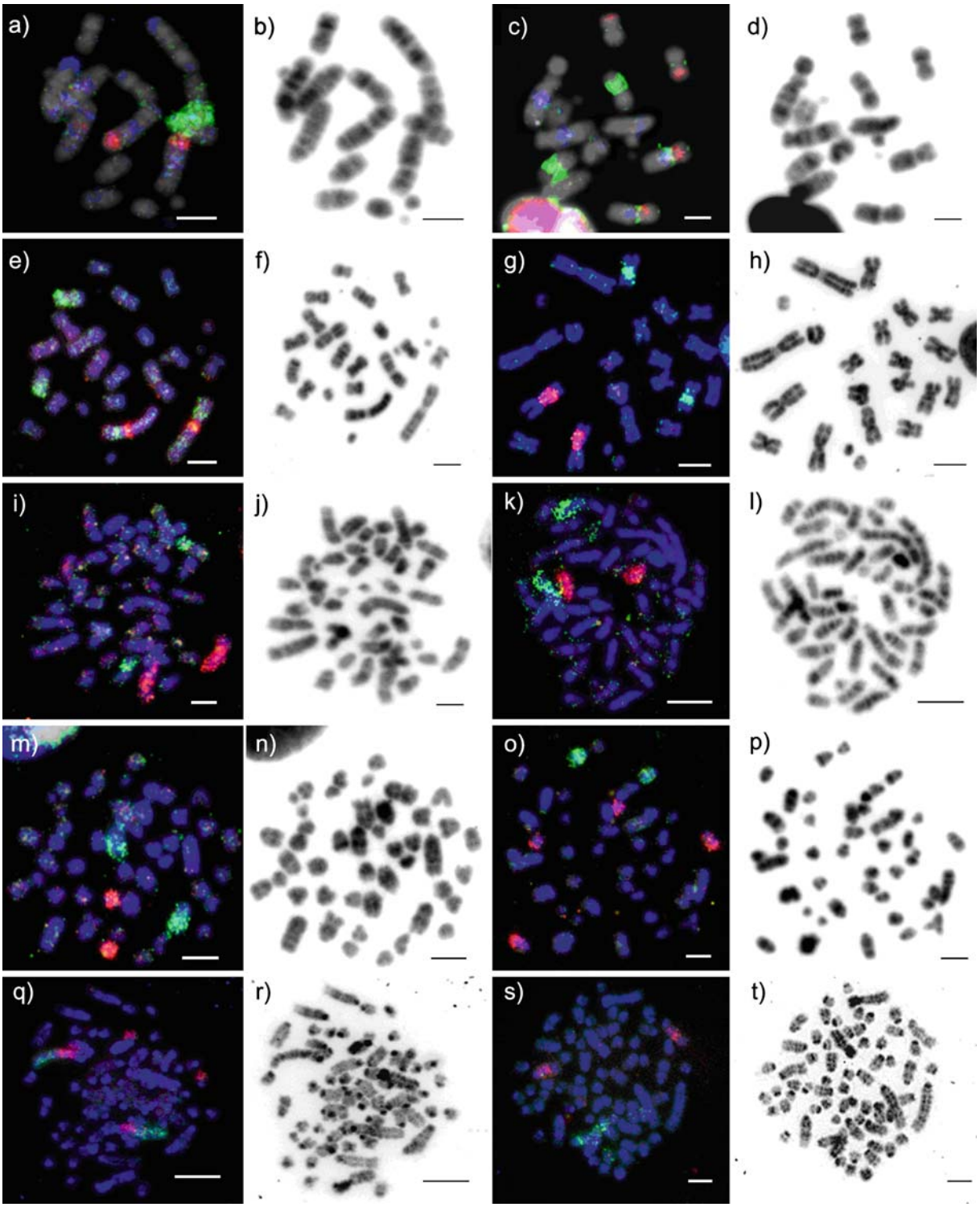
Akodon montensis (AMO)

The hybridizations with the 20 *M. musculus* painting probes revealed 27 regions of homology in the *A. montensis* genome, summarized in Figure 2b. The MMU painting probes 3, 6, 12, 13 and 16 each produced two FISH signals on *A. montensis* karyotype, while MMU 17 yielded three signals. The remaining 14 probes produced one signal each (MMU 1, 2, 4, 5, 7, 8, 9, 10, 11, 14, 15, 18, 19 and X), although in each case forming syntenic associations. The smallest chromosomal pair AMO 11 did not show homology to any *M. musculus* paint probe. We identified 16 syntenic associations (1/11, 2/7, 3/12, 3/16, 3/18, 4/15, 5/17, 5/18, 6/12, 6/14, 6/17, 7/19, 8/13, 8/17, 9/10, 13/16), some of these part of the more complex translocation events 2/7/19, 12/3/16, 13/8/17 and 12/6/17/5/18/3.

Akodon paranaensis (APA)

The 20 *M. musculus* chromosomal painting probes allowed the identification of 29 conserved segments of homology in *A. paranaensis*. MMU 1–4, 7–9, 13–16, 18, 19 and X produced single hybridization signals, of which eight hybridized one chromosome of the *A. paranaensis* complement entirely (MMU 1, 2, 4, 9, 14, 15, 16 and X). Seven syntenic associations were observed: MMU 3/10, 3/18, 6/12,

Figure 1. In situ hybridization with specific painting probes of MMU in *Akodon* species **(a–p)** and *O. flavescens* **(q–t)**. ***A. cursor* (a–d)**: (a) mouse chromosome 1 in blue, 2 in green and 3 in red, counterstain in grey; (c) mouse chromosome 4 in blue, 5 in green and 6 in red, counterstain in grey. ***A. montensis* (e–h)**: (e) mouse chromosome 5 in red and 6 in green, counterstain in blue; (g) mouse chromosome 7 in red and 8 in green, counterstain in blue. ***A. paranaensis* (i–l)**: (i) mouse chromosome 7 in red and 8 in green, counterstain in blue; (k) mouse chromosome 11 in red and 12 in green, counterstain in blue. ***A. serrensis* (m–p)**: (m) mouse chromosome 11 in red and 12 in green, counterstain in blue; (o) mouse chromosome 13 in red and 14 in green, counterstain in blue ***Oligoryzomys flavescens* (q–t)**: (q) mouse chromosome 5 in red and 6 in green, counterstain in blue; (o) mouse chromosome 7 in red and 8 in green, counterstain in blue. **(b, d, f, h, j, l, n, p, r, t)** Illustrations of the respective metaphases counterstained with actinomycin D/DAPI (inverted display). Scale bar = 5 μm.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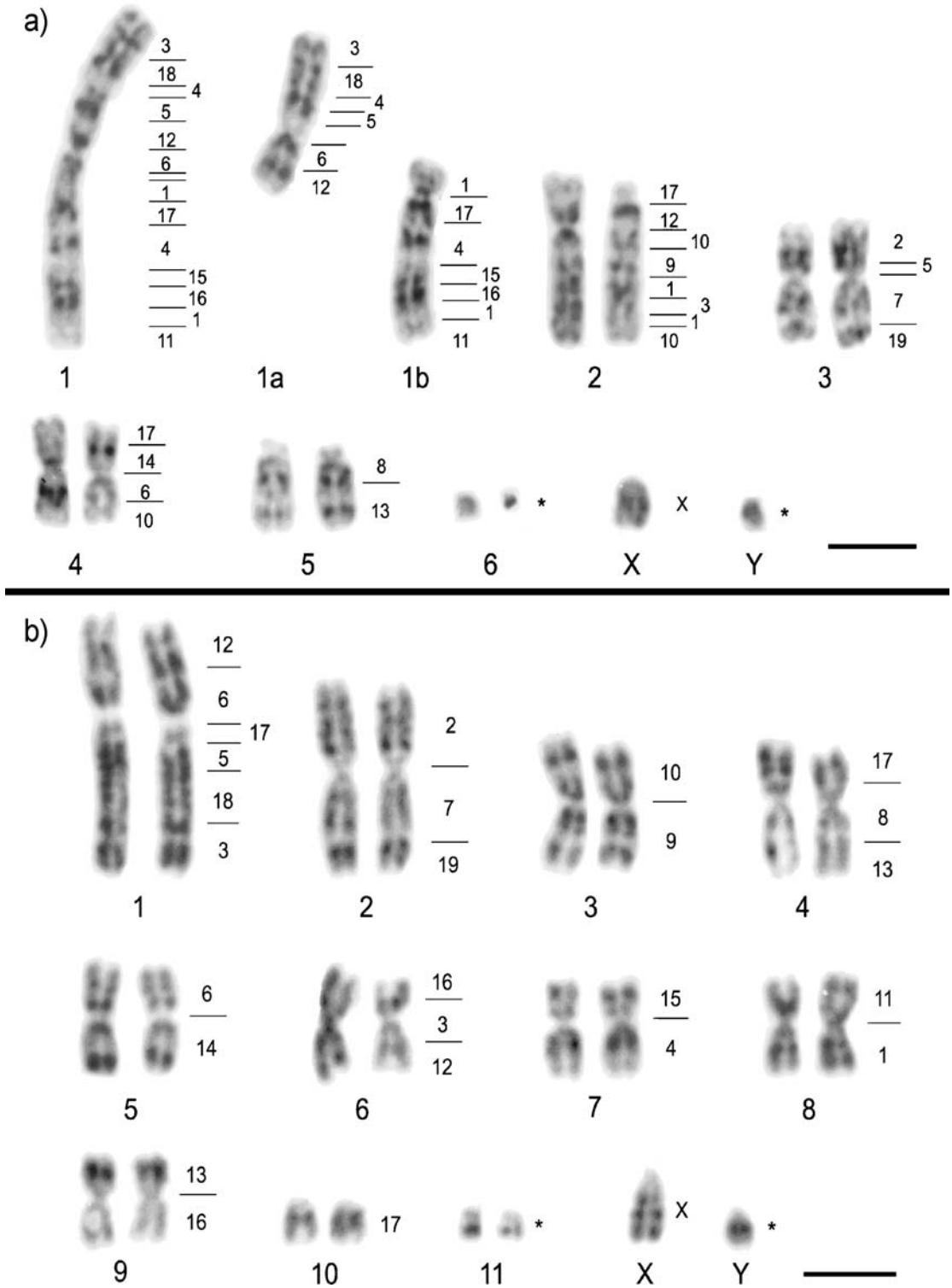

Figure 2. **(a)** G-banded karyotype of *Akodon cursor*, 2n = 15; chromosomes are numbered below. **(b)** G-banded karyotype of *Akodon montensis*, 2n = 24; chromosomes are numbered below. The hybridization signals of mouse probes are assigned to the right of each chromosome. Asterisks denote regions not hybridized by any mouse paints. Scale bar = 5 μm.

7/19, 8/13, 10/11 and 11/12. Synteny MMU 10/11 was observed in two different chromosomes of APA (15 and 17). Figure 3a shows the chromosomal homology map between *M. musculus* and *A. paranaensis*. The smallest chromosomal pair APA 21 did not show homology to any mouse paint probe.

Akodon serrensis (ASE)

In *A. serrensis*, the 20 MMU painting probes delineated 25 homologous chromosome segments, of which 13 probes produced single FISH signals (MMU 1, 2, 4, 7, 9, 11, 12, 14–16, 18, 19 and X). Four of these were present in syntenic association with other homologies (MMU 3/18, 6/12, 7/19 and 8/13). The remaining paint probes produced two signals each. The *A. serrensis* chromosome 7, the smallest chromosomal pair 22 and part of chromosome 15 were not labelled by any of the *M. musculus* painting probes. These results are summarized in Figure 3b.

Oligoryzomys flavescens (OFL)

The cross-species painting experiments between *M. musculus* and *O. flavescens* revealed 27 regions of chromosomal homology, with 10 chromosomes showing evolutionary conservation (MMU 1, 4, 6, 7, 11, 14, 15, 18, 19 and X). The *M. musculus* painting probes 6, 7 and 19, however, participated in syntenic associations. Seven other probes each produced two signals in *O. flavescens* (MMU 2, 3, 5, 8, 9, 12 and 16) and only MMU 13 labelled three different *O. flavescens* chromosome segments. This species presented the three associations MMU 5/6, 7/19 and 8/13. The two painting probes of MMU 10 and MMU 17 displayed no reproducible FISH signal in *O. flavescens* chromosomes. Further, *O. flavescens* chromosomes 18, 24–27, and 29–31, the proximal regions of OFL chromosomes 2 and 5, as well as the short heterochromatic arm of the X chromosome, were not labelled by any mouse painting probe. The chromosomal homology map between *M. musculus* and *O. flavescens* is shown in Figure 4.

The hybridization patterns of the 20 mouse probes in these five Akodontini species are summarized in Table 2. Nine mouse chromosomes (MMU 2, 4, 7, 9, 14, 15, 18, 19 and X) were found entirely conserved in four *Akodon* species. MMU 2 and 9 delineated two segments in the outgroup species, *O. flavescens*. Four syntenic associations (MMU 3/18, 6/12, 7/19 and 8/13) were shared by all four *Akodon* species investigated so far; however, only the associations of MMU 7/19 and 8/13 were observed in *O. flavescens*; and the association MMU7/19 was part of the ancestral karyotype of Muroidea. Another three associations (MMU1/17, 10/17 and 12/17) were present in *A. cursor* and the ancestral Muroidea karyotype.

Phylogenetic analysis in four Akodon species

In the PAUP analysis, we included characters based on chromosome painting experiments in five Akodontini rodents from the present report (i.e. four *Akodon* species and *Oligoryzomys flavescens*). The chromosomal homology data established by Zoo-FISH with chromosome-specific painting probes of *M. musculus* were translated into binary characters (Supplementary Table S1). For each character in a given species, the following values were attributed: 0 = absent state of the character, 1 = present state and 9 = unknown state (Supplementary Table S2). Fifty-eight characters were observed in total, comprising conserved and disrupted syntenies, and syntenic associations, verified in the outgroup and in the hypothetical ancestral Muroidea.

A single most parsimonious tree was obtained (Figure 5), 54 steps long, with a consistency index (CI) of 0.9444 and a retention index (RI) of 0.7273. It grouped three of the four *Akodon* species (*A. cursor*, *A. montensis*, and *A. paranaensis*) to the exclusion of *A. serrensis,* although with a moderate to low bootstrap value of 61. The clade that unites *A. cursor* and *A. montensis* is supported by the bootstrap value of 96. One apomorphy (MMU 15) and four synapomorphies (fission of MMU 3 into two segments, 7/19, 8/13) support the clade of Neotropical rodents. The monophyletic Akodontini clade is supported by four synapomorphies (fission of MMU 6 into two segments, 3/18, 6/12) and two plesiomorphies (MMU 2 and 9). One fusion signal (MMU 8) represents the apomorphy shared by *A. cursor*, *A. montensis* and *A. paranaensis*. The last clade within *A. cursor* and *A. montensis* shares eight synapomorphies (fission of MMU 17 into thee segments, 1/11, 2/7, 4/15, 6/14, 9/10). Each branch presented characters comparing with outgroup and ancestral Muroidea (Supplementary Table S1): *O. flavescens* (3, 7, 10, 13, 18, 33, 38, 44), *A. serrensis* (16, 17), *A. paranaensis* (4, 30, 51, 54), *A. montensis* (7, 12, 15, 17, 31, 32, 41, 42, 46,

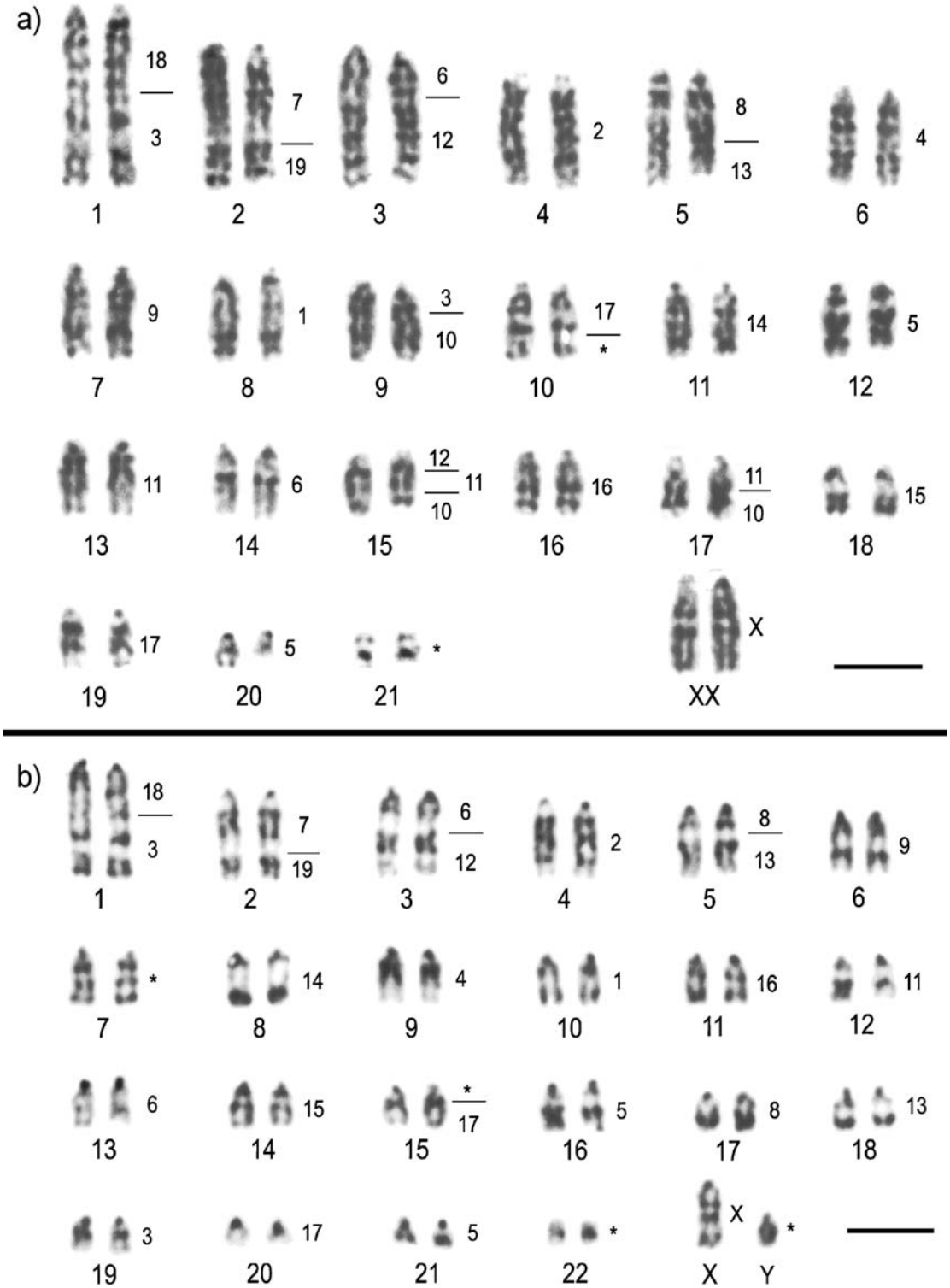

Figure 3. **(a)** G-banded karyotype of *Akodon paranaensis*, 2n = 44; chromosomes are numbered below. **(b)** G-banded karyotype of *Akodon serrensis*, 2n = 46; chromosomes are numbered below. The hybridization signals of mouse probes are assigned to the right of each chromosome. Asterisks denote regions not hybridized by any mouse paints. Scale bar = 5 μm.

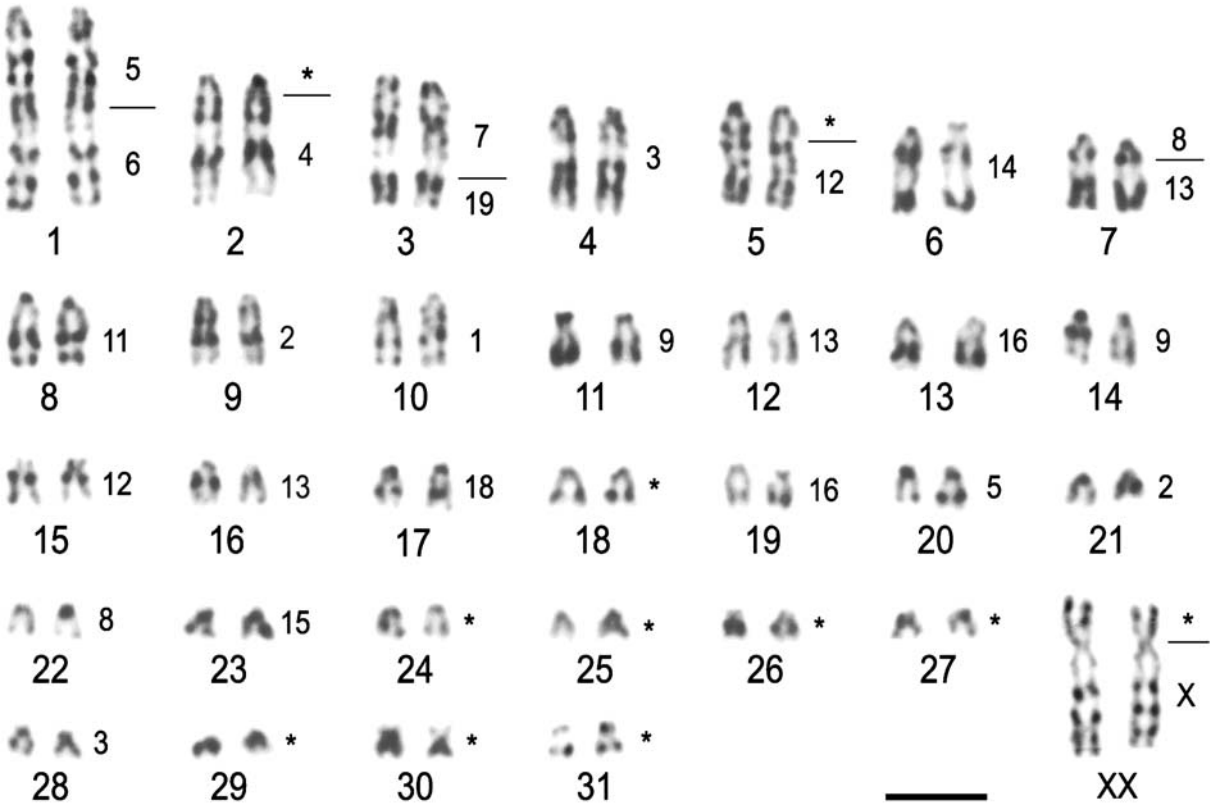

Figure 4. G-banded karyotype of *Oligoryzomys flavescens*, 2n = 64; chromosomes are numbered below. The hybridization signals of mouse probes are assigned to the right of each chromosome. Asterisks denote regions not hybridized by any mouse paints. Scale bar = 5 μm.

49, 56) and *A. cursor* (1, 2, 21–24, 26–28, 34, 36–40, 43, 52, 53, 55, 57, 58).

Discussion

Knowledge about evolutionary chromosome rearrangements in New World rodents by cross-species chromosome painting with mouse chromosome-specific probes has, until now, been limited to the analysis of *Peromyscus eremicus* (Muridae, Neotominae) from North America. Here we present the first Zoo-FISH-based chromosomal homology maps between *Mus musculus* and five Neotropical rodents from the subfamily Sigmodontinae. We focused our analysis on members of the genus *Akodon* using *Oligoryzomys flavescens* as phylogenetic outgroup, since the genus *Akodon* shows a large variability in diploid chromosome numbers. All five species included in our study were challenging to analyse with mouse chromosome painting probes. This might indicate considerable sequence divergence between the mouse and the analysed species (i.e. species from different families), a finding that is supported by comparative sequence data (Smith & Patton 1993, Geise *et al*. 2001, Steppan *et al*. 2004). Technical difficulties could be compensated to a certain degree by increasing the probe concentration and by performing low-stringency post-hybridization washes. Nevertheless, the Zoo-FISH experiments frequently yielded low signal intensity and low signal/noise ratios (Figure 1). This rendered difficult the interpretations of resulting hybridization patterns in some instances, and may explain why in certain *Akodon* and *Oligoryzomys* chromosomes no mouse homologous segments could be recorded (Figures 2–4). We excluded insufficient painting probe quality as a reason for these difficulties, since control experiments to mouse chromosomes showed intense and uniform FISH signal intensities. However, hybridization efficiency on *Akodon* chromosomes was still superior to chromosome painting with human probes (data not shown). Further, our cross-species chromo-

Table 2. The total number of signals observed by each of the 20 mouse chromosomal paints. The numbers of different chromosomes target by each probe are given in parentheses. Bold numbers highlight syntenic associations. The presence of syntenic associations is indicated by '+', their absence by '−'

Probes and associations	*Oligoryzomys flavescens:* 27	*Akodon cursor:* 32	*Akodon montensis:* 27	*Akodon paranaensis:* 29	*Akodon serrensis:* 25	Ancestral Muroidea: 33
1	1	3 (2)	1	1	1	2 (2)
2***	2 (2)	1	1	1	1	2 (2)
3**	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	1
4	1	2 (1)	1	1	1	1
5	2 (2)	2 (2)	1	2 (2)	2 (2)	2 (2)
6***	1	2 (2)	2 (2)	2 (2)	2 (2)	1
7*	1	1	1	1	1	1
8	2 (2)	1	1	1	2 (2)	2 (2)
9***	2 (2)	1	1	1	1	1
10	−	3 (2)	1	3 (3)	−	3 (3)
11	1	1	1	3 (3)	1	2 (2)
12	2 (2)	2 (2)	2 (2)	2 (2)	1	2 (2)
13	3 (3)	1	2 (2)	1	2 (2)	2 (2)
14*	1	1	1	1	1	1
15**	1	1	1	1	1	2 (2)
16	2 (2)	1	2 (2)	1	1	1
17	−	3 (3)	3 (3)	2 (2)	2 (2)	4 (4)
18*	1	1	1	1	1	1
19*	1	1	1	1	1	1
X*	1	1	1	1	1	1
7/19*	+	+	+	+	+	+
8/13**	+	+	+	+	+	−
6/12***	−	+	+	+	+	−
3/18***	−	+	+	+	+	−
1/11	−	+	+	−	−	−
1/17	−	+	−	−	−	+
2/7	−	+	+	−	−	−
4/15	−	+	+	−	−	−
5/6	+	+	−	−	−	−
6/14	−	+	+	−	−	−
9/10	−	+	+	−	−	−
10/17	−	+	−	−	−	+
12/17	−	+	−	−	−	+

*Character shared by Neotropical rodents and the Ancestral Muroidea.
**Character shared by Neotropical rodents.
***Character shared by Akodontini.

some painting data corroborate published Zoo-FISH results of Fagundes *et al.* (1997) using a microdissection-derived probe of the chromosomal segment/arm ACU 1b to delineate chromosomal homologies with AMO 7, 8 and 9q, indicating that the use of *Mus musculus* chromosomal panting probes is feasible in species from genera *Akodon* and *Oligoryzomys*.

Ancestral rodent chromosome forms conserved in Neotropical Sigmodontinae

According to a recent study, 26 rearrangements are required to derive the karyotype of *Mus musculus* from that of the Muroidea ancestor (Romanenko *et al.* 2006). Therefore, the chromosomal genome organization in mouse has to be considered as extremely derived compared with other rodents from various geographic regions analysed by chromosome painting. The karyotype of the New World rodent *Peromyscus eremicus* showed in contrast a remarkably conserved karyotype that differs from the ancestral Muroidea by only seven rearrangements (Romanenko *et al.* 2006, 2007a).

The five species of Neotropical Sigmodontinae analysed here share four of the conserved syntenies that are present in the mouse and the putative ancestral

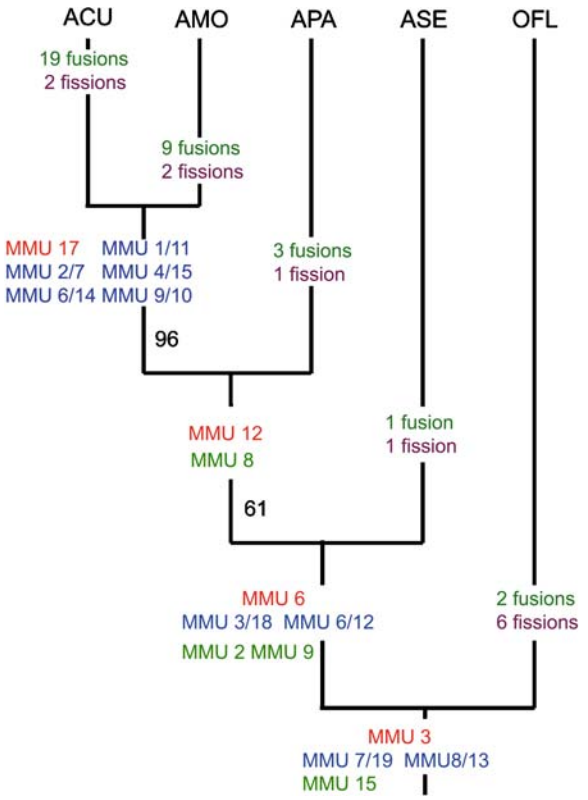

Figure 5. Phylogenetic reconstruction within four *Akodon* species according to the most parsimonious tree, using *O. flavescens* as outgroup (Supplementary Tables S1 and S2). Bold numbers indicate bootstrap values. Clades show the synapomorphies (red and blue) and in green apomorphy (MMU 8 and 15) and plesiomorphy (MMU 2 and 9), and branches the species-specific rearrangements.

rodent (MMU 4, 7, 18 and 19), thus confirming their ancestral state. In addition, the presumed ancestral rodent syntenic association of MMU 7/19 was identified in all five Neotropical Sigmodontinae. Another interesting syntenic association is MMU 5/6 observed in *O. flavescens*, which is shared with the murids from Europe (*Apodemus sylvaticus* (ASY)) and from the islands of the Nansei Shoto archipelago (*Tokudaia osimensis* (TOS) and *Tokudaia tokunoshimensis* (TTO)). This might be an ancient association, maintained in species similar to the founder of the occupation on the different continents, reinforcing the idea that *O. flavescens* is a direct descendent of one of the basal species that initially colonized South America, and suggesting that this syntenic association was lost secondarily in the genus *Akodon*. This finding may also confirm the basal position of *Oligoryzomys* species in phylogenetic reconstructions based on morphological and molecular data (Smith & Patton 1991, Steppan 1995, Montes *et al*. 2008).

Three further putatively ancestral syntenic associations (MMU 1/17, 10/17, 12/17) are present in ACU 1, ACU 2 metacentric and ACU 2 acrocentric, respectively (heteromorphic par 2 in *A. cursor*). The segments MMU 2a and 2b were observed in *O. flavescens* 9 and 21; MMU 5a and 5b in *A. cursor* 1 and 3, *A. paranaensis* 12 and 20, *A. serrensis* 16 and 21, *O. flavescens* 1 and 20; MMU 8a and 8b in *A. serrensis* 5 and 17, *O. flavescens* 7 and 22; and MMU 12a and 12b in *A. cursor* 1 and 2, *A. montensis* 1 and 6, *A. paranaensis* 3 and 15, *O. flavescens* 5 and 15. None of the other proposed ancestral rodent syntenic units have been observed in *Akodon* and in *Oligoryzomys*, indicating that their karyotypes comprise of several derived features. Notably, the four species of genus *Akodon* showed entirely conserved synteny of nine homologous chromosomes of *M. musculus* (MMU 2, 4, 7, 9, 14, 15, 18, 19 and X), with seven of these also present in the Neotropical outgroup *Oligoryzomys* (MMU 4, 7, 14, 15, 18, 19 and X). Presumably, a convergent fusion of MMU 2 and 9 occurred in the Akodontini tribe, while in *Oligoryzomys* those two *M. musculus* homologues share the ancestral split signal pattern with other rodents previously studied.

Chromosomal rearrangements in Genus Akodon

The three syntenic associations MMU 3/18, 6/12 and 8/13 and the fission of MMU 6 into two segments could be considered as shared derived chromosome forms, which define Neotropical rodents as a distinct group of Sigmodontinae, especially the Akodontini tribe. It might be speculated that these chromosome rearrangements occurred after their last common ancestor reached South America approximately 7 million years ago (Marshall 1979). In common with Akodontini, the species *O. flavescens* shows the synteny 8/13, which until now we consider an exclusive association for Neotropical rodents. We also identified nine chromosomal fissions comparing *Oligoryzomys* and the *Akodon* species with *M. musculus* chromosome painting. One fission (MMU 3′, 3″) is shared by all species studied, whereas three others are shared by all but one species: MMU 2′, 2″ except in *O. flavescens*, MMU 5′, 5″ absent in *A.*

montensis and MMU 12′, 12″ not observed in *A. serrensis*. The fission of MMU 8 represents a synapomorphy of the ACU/AMO/APA clade, while fissions of MMU 17 and associations MMU 1/11, 2/7, 4/15, 6/14 and 9/10 are shared derived traits of ACU and AMO. Those associations and chromosomal fissions may explain and/or confirm the participation of just a few groups in the colonization process of South America, in concordance with Marshall (1979).

Chromosomal phylogeny of Akodontini

Previous studies on phylogenetic relationships of the rodent species of the Akodontini tribe, and in particular those on the genus *Akodon*, have led to inconclusive results concerning the taxonomical status and the species nomenclature. Some studies attempted to resolve the phylogeny of the group, including cytogenetic and morphological traits, for example those of Bianchi *et al*. (1971), Espinosa & Reig (1991) and Geise *et al*. (1998), and others through studies of molecular data (Smith & Patton (1991) and Geise *et al*. (2001)).

The phylogeny obtained in the present study is largely in concordance with our unpublished data on comparative G-banding analysis, involving the species *A. montensis* (AMO), *A. paranaensis* (APA) and *A. serrensis* (ASE), with *Oligoryzomys flavescens* (OFL) used as an outgroup. Both phylogenies agree on the formation of one clade that groups the *Akodon* species, with ASE placed most externally in the clade. A similar constellation was proposed by Geise *et al*. (1998) for the species *A. cursor* (ACU), *A. montensis* (AMO) and *A. serrensis* (ASE), in which ASE also was encountered more externally at the *Akodon* clade. Morphological and cytogenetic data, molecular markers and gene sequencing (Geise *et al*. 1998, Christoff *et al*. 2000, Geise *et al*. 2001, Montes *et al*. 2008) as well as the Zoo-FISH data of the present work suggest the remoteness of *Akodon serrensis* from the other species of the genus. For a more precise taxonomic positioning of *Akodon* serrensis, analysis of a larger number of species would be indicated.

The genera *Mus* and *Akodon* belong to the families Muridae and Cricetidae, respectively; their last common ancestor can be traced back to 24.7 million years ago (Steppan *et al*. 2004). The use of painting probes derived from a different family, together with drastic karyotypical differences and the rapid DNA sequence divergence in certain rodents, might have contributed to the hybridization failure of some mouse probes (e.g. MMU 10 in ASE and OFL and MMU 17 in OFL). It has to be emphasized that in almost every species of rodents studied so far, these two painting probes showed multiple hybridization signals, which could be involved in unidentified fusions/fissions or might be present in small missed translocations segments in Akodontini. For future studies, which would allow for a more detailed comparative analysis of evolutionary chromosomal rearrangements in Neotropical rodents, multidirectional chromosomal painting including the use of chromosome paint probes from a member of this species group would be helpful, for example from *Oligoryzomys flavescens*. Alternatively, so-called 'overgo' probes (Thomas *et al*. 2002) could be used to screen large-insert clone libraries of *Mus musculus* for clones from genomic regions with a high degree of evolutionary sequence conservation. Such conserved clones could then by used in comparative molecular cytogenetic analyses.

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