Original Article

Cytogenetic and Genome Research

Comparative Chromosome Maps of Neotropical Rodents *Necromys lasiurus* and *Thaptomys nigrita* (Cricetidae) Established by ZOO-FISH

I. Hass[a–c]   S. Müller[c]   R.F. Artoni[b]   I.J. Sbalqueiro[a]

[a]Departamento de Genética, Universidade Federal do Paraná, Curitiba, and [b]Programa de Pós-Graduação em Biologia Evolutiva, Universidade Estadual de Ponta Grossa, Ponta Grossa, Brasil; [c]Institute of Human Genetics, University Hospital, Ludwig-Maximilians University, Munich, Germany

Key Words

Akodontini · Chromosome homology · Cytogenetic · Phylogeny · Rodentia

Abstract

This work presents chromosome homology maps between *Mus musculus* (MMU) and 2 South American rodent species from the Cricetidae group: *Necromys lasiurus* (NLA, 2n = 34) and *Thaptomys nigrita* (TNI, 2n = 52), established by ZOO-FISH using mouse chromosome-specific painting probes. Extending previous molecular cytogenetic studies in Neotropical rodents, the purpose of this work was to delineate evolutionary chromosomal rearrangements in Cricetidae rodents and to reconstruct the phylogenetic relationships among the Akodontini species. Our phylogenetic reconstruction by maximum parsimony analysis of chromosomal characters confirmed one consistent clade of all Neotropical rodents studied so far. In both species analyzed here, we observed the syntenic association of chromosome segments homologous to MMU 8/13, suggesting that this chromosome form is a synapomorphic trait exclusive to Neotropical rodents. Further, the previously described Akodontini-specific syntenic associations MMU 3/18 and MMU 6/12 were observed in *N. lasiurus* but not in *T. nigrita*, although the latter species is considered a member of the Akodontini tribe by some authors. Finally, and in agreement with this finding, *N. lasiurus* and *Akodon serrensis* share the derived fission of MMU 13, which places them as basal sister clades within Akodontini.

The order Rodentia is the most abundant of all mammals with more than 1,700 recognized species [Musser and Carleton, 2005]. Neotropical rodents in particular show a complex taxonomic classification and are considered to present some of the most diverse karyotypes within mammals. Diploid chromosome numbers range from 9, X0/10,XX in *Akodon* sp. from Central Brazil [Silva and Yonenaga-Yassuda, 1998], to 102 in *Tympanoctomys barrerae* endemic to the arid regions of central-western Argentina [Gallardo et al., 1999; Diaz et al., 2000].

Necromys lasiurus (NLA; 2n = 34) and the *Akodon* species group are recognized as closely related genera in some publications according to morphological, chromosomal G-banding and mtDNA sequence data [Reig, 1987; Smith and Patton, 1991; Fagundes and Yonenaga-Yassuda, 1998]. Using G-banding and fluorescence in situ hy-

Dr. Iris Hass
Universidade Federal do Paraná, Laboratório de Citogenética Animal
Departamento de Genética, Setor de Ciências Biológicas, s/nº – Jardim das Américas
Curitiba, PR 81530-000 (Brasil)
Tel. +55 41 3361 1781, E-Mail irish@ufpr.br

Table 1. Total number of signals observed in each of the 20 mouse chromosomal paints

Probes and associations	N. lasiurus this study	T. nigrita this study	O. flavescens Hass et al., 2008	A. cursor Hass et al., 2008	A. montensis Hass et al., 2008	A. paranaensis Hass et al., 2008	A. serrensis Hass et al., 2008	Ancestral Eumuroida Romanenko et al., 2007
1	1	1	1	3 (2)	1	1	1	2 (2)
2	1	2 (2)	2 (2)	1	1	1	1	2 (2)
3	1	1	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	1
4	1	1	1	2 (1)	1	1	1	1
5	4 (4)	2 (2)	2 (2)	2 (2)	1	2 (2)	2 (2)	2 (2)
6	2 (2)	2 (2)	1	2 (2)	2 (2)	2 (2)	2 (2)	1
7*	1	1	1	1	1	1	1	1
8	2 (2)	2 (2)	2 (2)	1	1	1	2 (2)	2 (2)
9	1	1	2 (2)	1	1	1	1	1
10	3 (3)	3 (2)	–	3 (2)	1	3 (3)	–	3 (3)
11	1	2 (2)	1	1	1	3 (3)	1	2 (2)
12	1	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)	1	2 (2)
13	2 (2)	2 (2)	3 (3)	1	2 (2)	1	2 (2)	2 (2)
14*	1	1	1	1	1	1	1	1
15**	1	1	1	1	1	1	1	2 (2)
16	1	1	2 (2)	1	2 (2)	1	1	1
17	1	2 (2)	–	3 (3)	3 (3)	2 (2)	2 (2)	4 (4)
18*	1	1	1	1	1	1	1	1
19*	1	1	1	1	1	1	1	1
X*	1	1	1	1	1	1	1	1
7/19*	+	+	+	+	+	+	+	+
8/13**	+	+	+	+	+	+	+	–
6/12***	+	–	–	+	+	+	+	–
3/18***	+	–	–	+	+	+	+	–
1/11	–	–	–	+	+	–	–	–
1/17	–	–	–	+	–	–	–	+
2/7	–	–	–	+	+	–	–	–
4/15	–	–	–	+	+	–	–	–
5/7	+	–	–	+	–	–	–	–
6/14	–	–	–	+	+	–	–	–
9/10	–	–	–	+	+	–	–	–
10/17	–	–	–	+	–	–	–	+
12/17	–	–	–	+	–	–	–	+
Total	28	31	27	32	27	29	25	33

The number of different chromosomes, targeted by each single probe, are given in parentheses. Bold numbers highlight syntenic associations.

+ = Presence; – = absence of syntenic associations. * Character shared by Neotropical rodents and Ancestral Muroidea, ** character shared by Neotropical rodents, *** character shared by Akodontini.

bridization (FISH) with telomere sequences, Fagundes and Yonenaga-Yassuda [1998] proposed the karyotypic homology between *Akodon cursor* (ACU), *A. montensis* (AMO), and NLA *(= Bolomys lasiurus).*

The second species analyzed here, *Thaptomys nigrita* (TNI; 2n = 52), is considered a member of the Akodontini group by some authors [Musser and Carleton, 2005]. Recent phylogenies based on molecular data (cytochrome b gene and the nuclear gene interphotoreceptor retinoid binding protein) indicated that TNI is a peripheral clade

of the Akodontini group, closely related to *A. serrensis* (ASE) [D'Élía, 2003; D'Élía et al., 2003].

More than 20 rodent species have so far been investigated by ZOO-FISH using *Mus musculus* (MMU) chromosome painting probes. Among these are *Apodemus sylvaticus* [Matsubara et al., 2004; Stanyon et al., 2004] from Europe, *Mus platythix* [Matsubara et al., 2003], *Cricetulus griseus* [Yang et al., 2000], *Tokudaia osimensis* and *T. tokunoshimensis* [Nakamura et al., 2007] from Asia, and *Rhabdomys pumilio* [Rambau and Robinson, 2003]

Table 2. Summary of characters derived from mouse chromosomal homologies observed in the species analyzed in our study that were used to establish a binary matrix (table 3): evolutionary conserved and syntenic associations of homologous chromosome segments

Character	Homologous segments	Character	Homologous segments	Character	Homologous segments
1	1	21	1/11	41	6/14
2	4	22	1/16	42	6/17
3	9	23	1/17	43	7/19
4	11	24	2/5	44	8/13
5	16	25	2/7	45	8/17
6	2 (2 segments)	26	3/10	46	9/10
7	3 (2 segments)	27	3/12	47	10/11
8	5 (2 segments)	28	3/16	48	10/12
9	6 (2 segments)	29	3/18	49	9/14
10	8 (2 segments)	30	4/5	50	11/12
11	10 (3 segments)	31	4/15	51	12/17
12	12 (2 segments)	32	4/17	52	13/16
13	13 (2 segments)	33	5/9	53	14/17
14	13 (3 segments)	34	5/6	54	15/16
15	17 (2 segments)	35	5/7	55	2/10
16	17 (3 segments)	36	5/12	56	8/15
17	1/3	37	5/17	57	13/18
18	1/5	38	5/18	58	16/17
19	1/9	39	6/10		
20	1/10	40	6/12		

and *Otomys irroratus* [Engelbrecht et al., 2006] from Africa. Concerning South American rodents, comparative FISH data using mouse painting probes include 4 species of the genus *Akodon* and *Oligoryzomys flavescens* (OFL) [Hass et al., 2008]. A second recent study based on chromosome painting probes obtained by chromosome flow sorting of *Akodon* sp. (ASP), 2n = 10; *A. cursor*, 2n = 15; *A. montensis*, 2n = 24; and *A. paranaensis* (APA), 2n = 44, recognized further chromosomal homologies in these species [Ventura et al., 2009].

To obtain a broader view on the karyotype evolution of Neotropical rodents, we analyzed NLA and TNI by cross-species chromosome painting using MMU probes and established comparative chromosome maps. We then integrated our results and previously published data on other rodent species from South America to reconstruct chromosomal phylogenies within this taxonomically controversial group of Cricetidae.

Materials and Methods

Sample Characterization

All specimen were collected in localities of Paraná and Santa Catarina, Southern Brazil: *N. lasiurus* (sample number – F6750; female; locality and coordinates: São Domingos – SC: 26°33′S, 52°31′W) and *T. nigrita* (sample number – F6559; male; locality and coordinates: Blumenau – SC: 26°55′S, 49°03′W and sample number – P889; female; locality and coordinates: Piraquara – PR: 25°28′S, 49°21′W). Their skulls and skins were catalogued and deposited at the Departamentos de Genética e Zoologia (Coleção Científica de Mastozoologia – DZUP) of the Universidade Federal do Paraná (UFPR) – (IBAMA – Brazil liberation number 026/2004). Metaphases were prepared according to Ford and Hamerton [1956], with modifications from Sbalqueiro and Nascimento [1996]. G-banding was performed as described by Seabright [1971], with modifications.

Preparation and Labeling of Mouse Chromosome-Specific Painting Probes

Painting probes specific for MMU chromosomes 1–20 and X, previously established by flow sorting, were kindly provided by Malcolm Ferguson-Smith, Resource Centre for Comparative Genomics, Veterinary School, University of Cambridge, UK. All painting probes were amplified and labeled with Biotin-dUTP, Dinitrophenol-dUTP or Tamra-dUTP by degenerate oligonucleotide-primed PCR (DOP-PCR), as previously described by Hass et al. [2008]. 500 ng–2 μg per mouse paint probe was used in single- or dual-color cross-species FISH experiments.

In situ Hybridization and Probe Detection

In situ hybridization of MMU chromosome-specific painting probes to NLA and TNI chromosomes was performed as previously described by Hass et al. [2008]. The hybridization was performed for 48–72 h at 37°C. Post-hybridization washes included

Table 3. Binary data matrix used in the parsimony analysis, comprising for the 58 informative characters defined in table 2

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
OFL	1	1	0	1	0	1	1	1	0	1	9	1	0	1	9	9	0	0	0	0	0
ACU	0	0	1	1	1	0	1	1	1	0	1	1	0	0	0	1	1	0	1	1	1
AMO	1	1	1	1	0	0	1	0	1	0	0	1	1	0	0	1	0	0	0	0	1
APA	1	1	1	0	1	0	1	1	1	0	1	1	0	0	1	0	0	0	0	0	0
ASE	1	1	1	1	1	0	1	1	1	1	9	0	1	0	1	0	0	0	0	0	0
NLA	1	1	1	1	1	0	0	0	1	1	1	0	1	0	0	0	0	1	0	0	0
TNI	0	1	1	0	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0

	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
OFL	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
ACU	1	1	1	1	0	0	0	1	1	1	1	0	1	1	1	0	0	1	1	1	0
AMO	0	0	0	1	0	1	1	1	0	1	0	0	0	0	0	1	1	0	1	1	1
APA	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
ASE	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
NLA	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	1	0	0
TNI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58
OFL	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ACU	1	1	0	1	0	1	0	0	1	0	1	1	0	0	0	0
AMO	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0
APA	1	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0
ASE	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NLA	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0
TNI	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1

0 = Absent; 1 = present; 9 = unknown state.

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-labeled probes were visualized by Cy3-Avidin, and Dinitrophenol-labeled probes by Rabbit-α-Dinitrophenol, followed by Goat-α-Rabbit – Alexa 488 antibodies. All slides were counterstained with Actinomycin D/DAPI as described in Hass et al. [2008].

Microscopy and Image Analysis

Metaphases were analyzed with a cooled CCD camera Photometrics C250/A, equipped with a KAF1400 chip coupled to a Zeiss Axioplan 2 microscope, or with a cooled VDS-CCD camera coupled to a Zeiss Axiophot microscope. Images were captured using SmartCapture VP 4.15 or with BandView/FISH-View software. The FISH images were processed using the software Adobe Photoshop version 7.0. Chromosomes were identified by computer-enhanced Actinomycin D/DAPI banding patterns.

Phylogenetic Analysis

The chromosomal characters of the 7 Neotropical rodent species that were analyzed by chromosome painting using mouse probes to date (5 species studied by Hass et al. [2008] and 2 species from the present study), and inferred ancestral Muroidea chro-

mosome forms [Romanenko et al., 2007], are summarized in table 1. The species *O. flavescens* was used as outgroup. The characters correspond to homologies with mouse chromosomes and syntenic associations thereof (table 2). A data matrix was established based on the presence or absence of these discrete characters (table 3). 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 2,000 iterations. Each bootstrap replicate involved a heuristic parsimony search with 10 random taxon additions and tree-bisection-reconnection branch swapping.

Results

In situ hybridization in 2 Neotropical rodents species, NLA and TNI with the 20 chromosome-specific painting probes from MMU (1–19 and X), showed re-
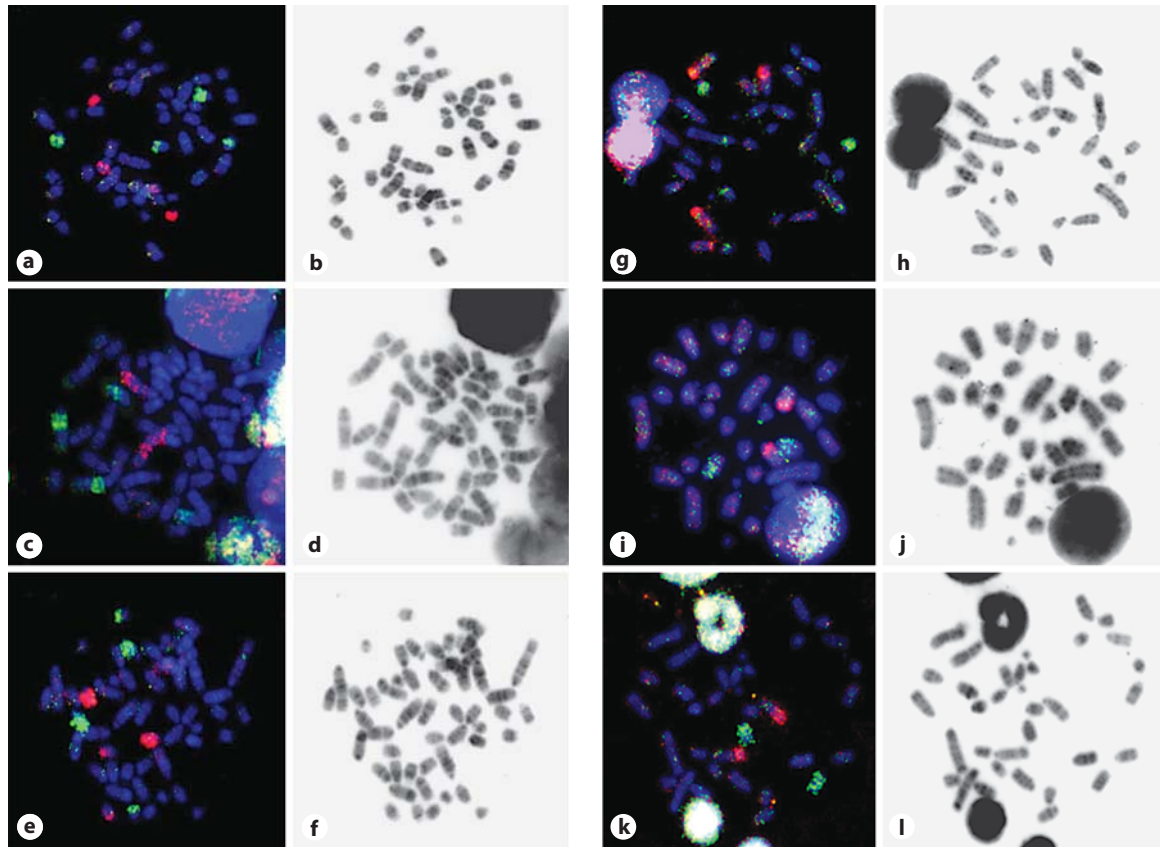
Fig. 1. In situ hybridization with mouse-specific painting probes in *T. nigrita* (**a–f**) and *N. lasiurus* (**g–l**). *T. nigrita*: **a** mouse chromosome 5 in green, 6 in red, counterstain in blue; **c** mouse chromosome 7 in red, 8 in green, counterstain in blue; **e** mouse chromosome 11 in red, 12 in green, counterstain in blue. *N. lasiurus*: **g** mouse chromosome 5 in red and 6 in green, counterstain in blue; **i** mouse chromosome 15 in red, 16 in green, counterstain in blue; **k** mouse chromosome 19 in red, X in green, counterstain in blue. **b**, **d**, **f**, **h**, **j**, and **l** illustrate the respective metaphases counterstain with Actinomycin D/DAPI (inverted display).

producible results (fig. 1). Centromeric and heterochromatic regions were not hybridized by any probe. In each experiment, a minimum of 10 metaphases were analyzed.

N. lasiurus

The 20 MMU chromosomal painting probes allowed the identification of 28 conserved segments of homology in NLA. MMU 1–4, 7, 9, 11, 12, 14–19, and X produced single hybridization signals, of which two hybridized to one chromosome of the *N. lasiurus* (MMU 2 and 4). Twelve syntenic associations were observed: MMU 1/5, 3/18, 5/7, 5/9, 5/10, 6/12, 7/19, 8/13, 8/17, 9/14, 10/16, and 11/15. Figure 2a shows the chromosomal homology map between *M. musculus* and *N. lasiurus*. The smallest chromosomal pair NLA 16 did not show hybridization of any mouse paint probe.

T. nigrita

In *T. nigrita*, the 20 MMU painting probes delineated 31 homologous chromosome segments, of which 11 probes produced single FISH signals (MMU 1, 3, 4, 7, 9, 14, 15, 16, 18, 19, and X). Four of these were present in syntenic associations (MMU 8/15, 16/17, 13/18, and 7/19). Another 2 syntenic associations between chromosome segments were identified: MMU 2/10 and MMU 8/13. The remaining probes produced 2 signals each and the probe MMU 10 produced 3 signals. The proximal regions of TNI chromosomes 12 and 18, as well as the smallest chromosomal pair TNI 25, were not labeled by any mouse painting probe. These results are summarized in figure 2b.

The hybridization patterns of the 20 mouse probes in NLA and TNI are summarized and compared with those on other 5 South American Cricetidae species [Hass et al., 2008] and with the inferred ancestral eumu-
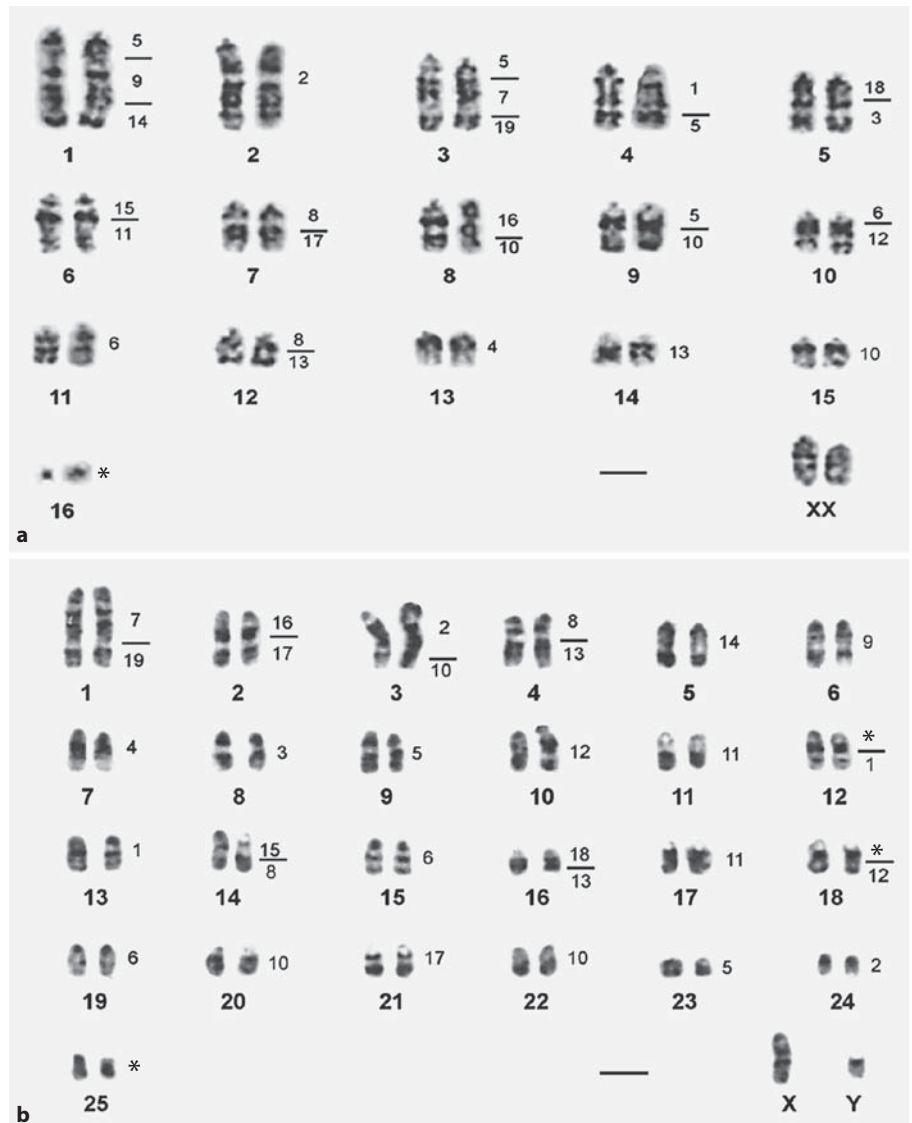
Fig. 2. a G-banded karyotype of *N. lasiurus*, 2n = 34, and **b** G-banded karyotype of *T. nigrita*, 2n = 52, chromosomes are numbered below. The hybridization signals of mouse probes were assigned to the right of each chromosome. Asterisks denote regions not hybridized by any mouse paint. Scale bar = 5 μm.

roid [Romanenko et al., 2007] in table 1. Seven mouse chromosomes (MMU 7, 9, 14, 15, 18, 19, and X) were found entirely conserved in NLA and TNI, as in 4 previously analyzed *Akodon* species. Conserved synteny of chromosomes MMU 7, 14, 18, 19, and X, together with syntenic association MMU 7/19 were shared by TNI and NLA with other Cricetidae species including the inferred ancestral eumuroid. Association of MMU 8/13 was found so far only in TNI, NLA and in other Neotropical Cricetidae. Syntenic associations MMU 6/12 and 3/18 were only observed in NLA and in genus *Akodon*. The association MMU 5/7 was present in NLA and ACU.

Phylogenetic Analysis in Cricetidae

We then performed a phylogenetic analysis using PAUP software. We included chromosomal characters from 7 Neotropical rodents (*Akodon* species: *A. cursor*, *A. montensis*, *A. paranaensis* and *A. serrensis*, and *O. flavescens*; Hass et al. [2008], *N. lasiurus* and *T. nigrita* from the present study) analyzed by ZOO-FISH. The ZOO-FISH data with chromosome-specific painting probes of *M. musculus* were translated into binary characters (table 3). For each character in a given species, the following values were attributed: 0 = absent state of the character and 1 = present state. In total, 58 characters were identified, comprised of single or multiple labeled signals and syntenic associations.
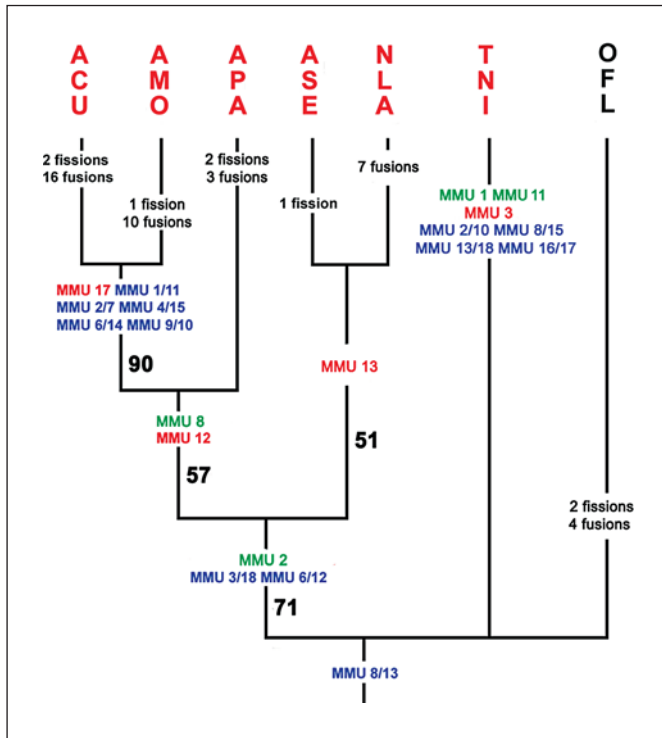
Fig. 3. Chromosomal phylogeny of the 7 Neotropical rodent species analyzed so far using mouse chromosome paint probes. *O. flavescens* was used as na outgroup. The most parsimonious tree from the PAUP analysis is depicted, together with the evolutionary changes that could be assigned to each clade (tables 1, 2 and 3). Bold numbers indicate bootstrap values. Clades show the synapomorphies (fissions in red and syntenic associations in blue), apomorphies and plesiomorphies (green), branches the species-specific rearrangements.

A single most parsimonious tree was obtained, 67 steps long, with a consistency index (CI) of 0.8358 and a retention index (RI) of 0.5417 (fig. 3). It grouped the Akodontini species, including NLA, but not TNI, in one clade with a moderate bootstrap value of 71, supported by 3 synapomorphies (fusion of MMU 2 and MMU 3/18 and 6/12). The bootstrap value 57 was observed in the closely related *Akodon* species ACU, AMO and APA, supported by 2 synapomorphies (fission of MMU 12 in 2 segments and MMU 8). Three fissions products of MMU 17 and 5 syntenic associations (MMU 1/11, 2/7, 4/15, 6/14, and 9/10) represented the synapomorphies in the clade of *A. cursor* and *A. montensis*, supported by a bootstrap value of 90. One synapomorphy (MMU 13 in 2 segments) supported the clade of ASE and NLA, with a bootstrap value of 51. In the individual branches, the following characters were present compared with the outgroup: ACU (1, 2, 17,

19, 20, 22, 23, 24, 30, 32, 34, 35, 36, 39, 48, 51, 53, and 54), AMO (5, 8, 11, 13, 27, 28, 37, 38, 42, 45, and 52), APA (4, 15, 26, 47, and 50), ASE (13), NLA (7, 8, 18, 33, 35, 45, and 49), and TNI (1, 4, 7, 55, 56, 57, and 58).

Discussion

In this paper, we established ZOO-FISH-based chromosomal homology maps between *M. musculus* and *N. lasiurus* and *T. nigrita*, extending our previous studies on the karyotype evolution in Neotropical rodents. The 2 species were challenging to analyze with mouse chromosome painting probes because the ZOO-FISH experiments frequently yielded low signal intensity and low signal/noise ratios (fig. 1). Control FISH experiments on mouse chromosomes (data not shown) excluded insufficient painting probe quality as a cause. This rendered difficult interpretations of the hybridization patterns in some instances and may explain that in certain chromosomes no mouse homologous segments could be recorded.

N. lasiurus

Our data could confirm some G-banding homologies between AMO and NLA suggested by Fagundes and Yonenaga-Yassuda [1998]. These authors proposed that chromosomes NLA 5 and 10 correspond to AMO 1, homology of NLA 3 with part of AMO 2, NLA12 with the long arm of AMO 4, partial homology of NLA1 with AMO 5, and the homology of NLA 13 with AMO 8q. Other homologies presented by Fagundes and Yonenaga-Yassuda [1998] are not in agreement with our analysis. For example, it was proposed that AMO 3 is the result of a centric fusion between NLA 6 and 7, whereas our data indicate that AMO 3 is homologous to 4 NLA chromosomes (NLA 1, 8, 9, and 15; Hass et al. [2008], this study) and that AMO 3 is the product of complex rearrangements. The cross-species chromosome painting experiments in *N. lasiurus* reveled syntenic associations MMU 7/19, 8/13, 6/12, and 3/18 which were also previously observed in *Akodon* species [Hass et al., 2008], thus indicating that both genera are closely related. The syntenic association 5/7, which was previously described in *A. cursor*, but not in other Akodontini [Hass et al., 2008], may represent a homoplasy, the result of a convergent chromosome change that occurred independently in the 2 different phylogenetic lineages. Alternatively, this rearrangement could be a hemiplastic character [Robinson et al., 2008], which emerged in the last common ancestor of

genera *Akodon* and *Necromys* as a polymorphism but became only fixed in extant *A. cursor* and *N. lasiurus*. The remaining 7 syntenies MMU 1/5, 5/9, 5/10, 8/17, 9/14, 10/16, and 11/15 have to date to be considered as species-specific traits. Smith and Patton [1999] studied mitochondrial cytochrome-*b* gene sequences and proposed that NLA *(= B. lasiurus) and* TNI are to be included in the Akodontini group but as a sister species of genus *Akodon*. This hypothesis is corroborated in our phylogeny for *Necromys*. Another study on cytochrome-*b* gene sequence combined with karyotype analysis also indicated that NLA is closely related with genus *Akodon* [Geise et al., 2001].

T. nigrita

Thaptomys was originally considered a monotypic species with 2n = 52 chromosomes, but Ventura et al. [2004] recently described a form of *Thaptomys* sp. with 2n = 50 chromosomes. This reduction in chromosomal number could be a result of a fusion involving TNI 2 and 24. This suggestion is corroborated by our FISH data, where TNI 3 (homolog of TNI 2 in this paper) and TNI 24 showed homology to MMU 2. Eight conserved syntenies that are also present in the mouse and the putative ancestral rodent (MMU 3, 4, 7, 9, 14, 16, 18, and 19) and in the segments MMU 8a and 8b, MMU 10a, 10b and 10c, and MMU 13a and 13b were observed in species NLA and TNI, thus reinforce that those characters are ancestral and represent a conserved state also in Neotropical rodents. The syntenic association MMU 8/13 confirmed cytogenetically, as expected, that this species is a Neotropical rodent, but the absence of derived associations MMU 3/18 and 6/12 discards the inclusion of this species in the *Akodon* group. Our phylogenetic inference is in agreement with comparative gene sequencing data, where

TNI was placed in a separated clade, or was regarded as a sister clade of ASE, a species which inclusion within the genus *Akodon* is questionable [Smith and Patton, 1999; D'Élía, 2003; D'Élía et al., 2003].

In summary, the comparative molecular cytogenetic analysis of 7 Neotropical Cricetidae species using mouse chromosome-specific paining probes so far revealed the following landmark rearrangements: (a) the inferred ancestral eumuroid chromosome form MMU 7/19 is retained also in Neotropical muroids, (b) the derived syntenic association MMU 8/13 can be assigned to an inferred ancestral South American Cricetidae, (c) chromosome rearrangements resulting in associations MMU 3/18 and 6/12 may have occurred in the last common ancestor of the Akodontini species complex, and (d) the syntenic association 5/7 within *N. lasiurus* and *A. cursor* which could be a hemiplasy. All together, the chromosomal data suggest that genus *Necromys* should be classified as a member of the Akodontini tribe, while no chromosomal evidence was found for an inclusion of genus *Thaptomys*. We conclude that the inferred phylogenies presented here, based on characters from chromosome rearrangements, are very informative and able to explain the evolutionary relationship within this controversially discussed group of rodents.

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