Original Article

Cytogenetic and Genome Research

Cytogenet Genome Res
DOI: 10.1159/000341887

Accepted: June 12, 2012
by M. Schmid
Published online: August 18, 2012

Phylogeographic Structure and Karyotypic Diversity of the Brazilian Shrew Mouse (*Blarinomys breviceps*, Sigmodontinae) in the Atlantic Forest

K. Ventura[a] Y. Sato-Kuwabara[c] V. Fagundes[e] L. Geise[f] Y.L.R. Leite[e]
L.P. Costa[e] M.J.J. Silva[d] Y. Yonenaga-Yassuda[a] M.T. Rodrigues[b]

Departamentos de [a]Genética e Biologia Evolutiva and [b]Zoologia, Instituto de Biociências, Universidade de São Paulo, [c]Departamento de Anatomia Patológica, Fundação Antônio Prudente, Hospital A. C. Camargo, [d]Laboratório de Ecologia e Evolução, Instituto Butantan, São Paulo, [e]Departamento de Ciências Biológicas, Centro de Ciências Humanas e Naturais, Universidade Federal do Espírito Santo, Vitória, and [f]Departamento de Zoologia, Instituto de Biologia, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

Key Words
B chromosomes · Cytochrome b · Cytogenetics · Fluorescent in situ hybridization · Mitochondrial DNA · Rodentia

Abstract
Blarinomys breviceps possesses cryptic and burrowing habits with poorly documented genetics and life history traits. Due to its rarity, only a few specimens and DNA sequences have been deposited in collections worldwide. Here, we present the most comprehensive cytogenetic and molecular characterization of this rare genus. Phylogenetic analyses based on partial cytochrome b sequences were performed, attempting to establish the relationships among individuals with distinct karyotypes along the geographic distribution of the genus in the Atlantic Forest. Classical and molecular cytogenetics, using banding patterns and FISH of telomeric and whole chromosome X-specific painting probes (obtained from the Akodontini *Akodon cursor*) were used to characterize and compare the chromosomal complements. Molecular phylogenetic analyses recovered 2 main geographically structured clades, northeastern and southeastern with pairwise sequence divergences among specimens varying between 4.9 and 8.4%. Eight distinct karyomorphs are described: (A) 2n = 52 (50A, XX), (B) 2n = 52 (48A, XY+2Bs), (C) 2n = 45 (42A, XY+1B), (D) 2n = 43 (37A, XX+4Bs), (E) 2n = 37 (34A, XY+1B), (F) 2n = 34 (32A, XX), (G) 2n = 31 (27A, XX+2Bs), (H) 2n = 28 (26A, XY), all with the same number of autosomal arms (FN_A = 50). Variation of 0–4 supernumerary chromosomes (Bs) presenting heterogeneity in morphology and distribution of interstitial telomeric sequences (ITSs) is reported. ITSs are also found in some metacentric autosomes. The phylogeographic separation between 2 major lineages with high levels of genetic divergence, and the wide karyotypic diversity indicate that *B. breviceps* is a diverse group that warrants taxonomic re-evaluation.

The Brazilian Atlantic Forest, one of the top biodiversity hotspots on Earth [Myers et al., 2000; Laurance, 2009] harbors an impressive fauna of sigmodontine rodents. The radiation comprises 19 genera and 50 species,

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1424–8581/12/0000–0000$38.00/0

Accessible online at:
www.karger.com/cgr

Karen Ventura
Departamento de Genética e Biologia Evolutiva
Instituto de Biociências, Universidade de São Paulo
Rua do Matão 277, São Paulo, SP 05508-090 (Brazil)
Tel. +55 11 3091 7574, E-Mail kabiousp@yahoo.com.br

most of them arranged into 3 tribes (Oryzomyini, Thomasomyini, and Akodontini); but some genera still have uncertain phylogenetic affinities and are treated as *incertae sedis* [Wilson and Reeder, 2005; D'Elía et al., 2007]. The monotypic genus *Blarinomys* Thomas, 1896 belongs to the tribe Akodontini, an assemblage of sigmodontine rodents with adaptations to terrestrial or fossorial habits [Smith and Patton, 1999]. *Blarinomys breviceps* is endemic to the Atlantic Forest and shows a set of adaptive morphological traits, including a short conical head, very small eyes and ears, a short tail, and pronounced claws qualified for burrowing [Moojen, 1952; Matson and Abravaya, 1977].

The current distribution of the species ranges from latitude 14°S at Buerarema in the state of Bahia, northeastern Brazil, to 26°S at Eldorado in the province of Misiones, northeastern Argentina [Laemmert et al., 1946; Chebez and Massoia, 1996; Silva et al., 2003; Geise et al., 2008], extending from sea level to 1,570 meters of elevation [Geise et al., 2008].

Although the distribution of *B. breviceps* is currently restricted to the Atlantic Forest, its type locality is in the Cerrado (savanna-like) biome [Silva et al., 2003]. The species has been recorded recently from a transitional zone between the Atlantic Forest and Cerrado in the state of Minas Gerais, southeastern Brazil [Paglia et al., 2005].

Due to its cryptic and fossorial habits, *Blarinomys* is rarely collected, and it has been recorded from scattered localities in southeastern Brazil and Argentina [Silva et al., 2003; Geise et al., 2008]. There are a few specimens deposited in collections worldwide, and its life history traits are poorly known [Abravaya and Matson, 1975].

Genetic data from *Blarinomys* are scarce in the literature. DNA sequences from a couple of specimens have been used in phylogenetic analyses of sigmodontine rodents at the generic level [e.g. Smith and Patton, 1999; D'Elía et al., 2003] which turned *Blarinomys* out to be the sister-group to *Brucepattersonius* on the basis of sequences of the mitochondrial cytochrome b gene [Smith and Patton, 1999; D'Elía et al., 2003], or sister to a clade formed by *Brucepattersonius* and *Lenoxus*, when sequences of the nuclear interphotoreceptor retinoid-binding protein *(IRBP)* gene were used in the analysis [D'Elía, 2003]. Its karyotype was described only in conventional staining based on the analysis of a single specimen from the state of Rio de Janeiro, showing 2n = 28 and $FN_A = 50$ (fundamental number of autosomes) [Geise et al., 2008]. Here, on the basis of new specimens obtained at several localities we present the most comprehensive molecular and cytogenetic characterization of this rare genus, using analyses of mitochondrial DNA sequences to address its phylogeographic structure and classical and molecular cytogenetics data to address the karyotypic diversity.

Methods

The sample is composed of mtDNA sequences from 22 specimens of *B. breviceps* from 17 localities along the Brazilian Atlantic coast. Eight of them, from 7 localities, were used for karyotype analysis (table 1, fig. 1).

Taxon Sampling, DNA Extraction, Amplification, Sequencing and Phylogenetic Analysis

Phylogenetic analyses were conducted using sequences of the mitochondrial cytochrome b gene of all specimens of *B. breviceps* (table 1). We used 7 outgroups, 6 from GenBank (accession numbers in parenthesis), which were *Brucepattersonius iheringi* (AF108667), *B. soricinus* (AY277486), *Scapteromys aquaticus* (AY445550), *S. tumidus* (AY445552), *Oecomys bicolor* (AF108699), *Euryoryzomys russatus* (AF181272), and, additionally, a new sequence of *Brucepattersonius* sp. (CIT1343).

Genomic DNA was obtained from liver or muscle following the protocol described by Fetzner [1999] or Bruford et al. [1992]. Upon extraction, an 801-bp fragment was amplified using primers MVZ05 for the light-strand and MVZ16 for the heavy-strand [Smith and Patton, 1993, 1999]. Amplifications were performed in a reaction mix consisting of a final volume of 25 μl containing 2.5 μl of 10× PCR buffer (Invitrogen Inc., Grand Island, N.Y., USA), 4.0 μl of dNTPs (5 mM), 2.5 μl of $MgCl_2$ (25 mM), 2.0 μl of each primer (10 μM), 10.75 μl of milli-Q water, 0.25 μl of 5U *Taq* DNA Polymerase, and 1 μl of DNA template (5–50 ng). The thermal cycling conditions were as follows: an initial denaturation of 94°C for 5 min, 40 cycles of 94°C for 30 s, 48°C for 45 s and 72°C for 1 min, and a final extension of 72°C for 10 min. PCR products were purified with the ExoSAP-IT™ kit (USB Corporation, Cleveland, Ohio, USA) following the manufacturer's instructions. Cycle sequencing reactions were performed using ABI PRISM® Big Dye™ Terminators v 3.0 kit (Applied Biosystems, Foster City, Calif., USA). Samples were sequenced in the automatic DNA sequencers ABI PRISM® 3700 DNA Analyzer (Applied Biosystems). Light and heavy strands were edited manually, and compared using Sequence Navigator™ (Applied Biosystems). Automatic alignments were done with Clustal X. The reading frame of the obtained sequences was inferred using MacClade.

Phylogenetic analyses were carried out using maximum parsimony (MP) and maximum likelihood (ML), executed in PAUP* 4.0b10 [Swofford, 2003], and Bayesian inference (BI) was performed using MrBayes 3.1.2 [Huelsenbeck and Ronquist, 2001]. The MP analysis was performed by heuristic search with stepwise addition, TBR (tree bisection and reconnection) branch-swapping, and 10,000 replicates. ML analysis was conducted using the appropriate nucleotide substitution model selected in a jModelTest (version 0.1.1) [Guindon and Gascuel, 2003; Posada, 2008]: general time reversible [Rodríguez et al., 1990], taking into account the estimated proportion of invariable sites, and variable sites following a gamma distribution (GTR + I + G). ML heuristic searches started using TBR as branch-swapping algorithm. Confidence in the recovered nodes in the MP and ML analyses was

Table 1. Specimens of *Blarinomys*, with respective collecting localities, haplotypes from cytochrome b mtDNA sequences, karyotype description and field or museum identification number

Collecting localities				mtDNA haplotype	Karyomorphs	Specimen identification number
municipality, state	site[a]	geographic coordinates	altitude			
Jussari, BA	(1) RPPN Serra do Teimoso	15°08′S/39°31′W	300 m	H1	2n = 52 (48A, XY+2Bs); FN_A = 50	CIT1538
		15°09′S/39°31′W	250 m	H2	–	RM189
Una, BA	(2) Una	15°17′S/39°03′W	50 m	H1	–	MAS17[b]
		15°10′S/39°04′W	15 m	H3	–	RM46
Camacan, BA	(3) Serra Bonita	15°25′S/39°29′W	950 m	H4	–	MTR15858
Jequitinhonha, MG	(4) Mata Escura	16°26′S/41°00′W	230 m	H5	2n = 52 (50A, XX); FN_A = 50	MTR17328
Trancoso, BA	(5) Fazenda Nova Alegria	16°31′S/39°07′W	50 m	H8	–	MTR13511
Pinheiros, ES	(6) REBIO Córrego do Veado	18°21′S/40°09′W	715 m	H6	–	UFES969
Linhares, ES	(7) Linhares	19°39′S/40°07′W	30 m	H7	–	MTR12313
Catas Altas, MG	(8) Estrada Mariana-Catas Altas	20°11′S/43°29′W	931 m	H9	–	RM316
Ouro Branco, MG	(9) Serra do Ouro Branco	20°30′S/43°37′W	1,117 m	H9	–	59M
Alto Caparaó, MG	(10) PARNA Caparaó, Linha 2	20°25′S/41°50′W	1,350 m	H10	–	MTR10772
	(11) Córrego do Calçado	20°28′S/41°44′W	1,350 m	H11	–	MTR11577
Cariacica, ES	(12) REBIO Duas Bocas	20°17′S/40°31′W	550 m	H12	2n = 45 (42A, XY+1B); FN_A = 50	LPC1242
				H13	–	LPC1259
Valença, RJ	(13) Serra da Concórdia	22°22′S/43°47′W	815 m	H14	2n = 28 (26A, XY); FN_A = 50	MN68882
Biritiba-Mirim, SP	(14) Biritiba-Mirim	23°57′S/46°03′W	780 m	H15	–	CIT2139
Natividade da Serra, SP	(15) PE Serra Mar	23°19′S/45°05′W	720 m	H16	2n = 31 (27A, XX+2Bs); FNA = 50	ROD66
São Paulo, SP	(16) PE Serra da Cantareira	23°70′S/46°63′W	780 m	H17	2n = 43 (37A, XX+4Bs); FN_A = 50	CIT1391
São Bernardo do Campo, SP	(17) São Bernardo do Campo	23°69′S/46°56′W	760 m	H18	2n = 37 (34A, XY+1B); FN_A = 50	CIT1633
				H19	2n = 34 (32A, XX); FN_A = 50	CIT2259
				H20	–	M333

[a] Numbers 1–17 represent localities in fig. 1. [b] From GenBank (http://www.ncbi.nlm.nih.gov), accession number AF108668.

BA = Bahia; ES = Espírito Santo; MG = Minas Gerais; RJ = Rio de Janeiro; SP = São Paulo; REBIO = Reserva Biológica; RPPN = Reserva Particular do Patrimônio Natural; PARNA = Parque Nacional; PE = Parque Estadual; 2n = diploid number; FN_A = fundamental number of autosomes (excluding sex chromosomes and Bs); A = autosomes; B = supernumerary chromosomes; XX and XY = sex chromosomes.

accessed with a non-parametric bootstrap test [Felsenstein, 1985]. Bootstrap support (BS) values were obtained by data resampling with 1,000 (MP) and 1,000 (ML, using the fast stepwise-addition option in PAUP*) pseudoreplicates. Bremer support indices (BSI) [Bremer, 1994] were also calculated in MP using TreeRot.v2 [Sorenson, 1999]. BI was also performed using the GTR + I + G model and 2 simultaneous, independent runs for 200,000 generations, until the standard deviation of split frequencies dropped below 0.01 and the potential scale reduction factor was close to 1.0 for all parameters. Only groups with a Bayesian posterior probability (BPP) higher than 0.95 were considered significant. The mean pairwise genetic distances among haplotypes were calculated in PAUP* 4.0b10 [Swofford, 2003] using the Kimura 2-parameter model for comparison purposes among akodontine species.

Cytogenetics

Metaphases were obtained from in vivo bone marrow and spleen preparations, and from fibroblast cultures of ear biopsies in DMEM supplied with 20% fetal calf serum. Diploid numbers (2n) and FN_A were obtained from conventionally stained metaphases. Differential chromosomal staining for constitutive heterochromatin (C-banding), G- and R-banding were performed following standard procedures. Cells were analyzed using Axiophot and Axioskop 40 Zeiss microscopes equipped with software for image capture (Ikaros Karyotyping System, MetaSystems, Altlußheim, Germany; and Axiovision Zeiss, Oberkochen, Germany).

FISH with telomeric probes 'DAKO Telomere PNA FISH Kit/Cy3 (K 5326) or FITC (K 5325)' was employed following the manufacturer's protocol.

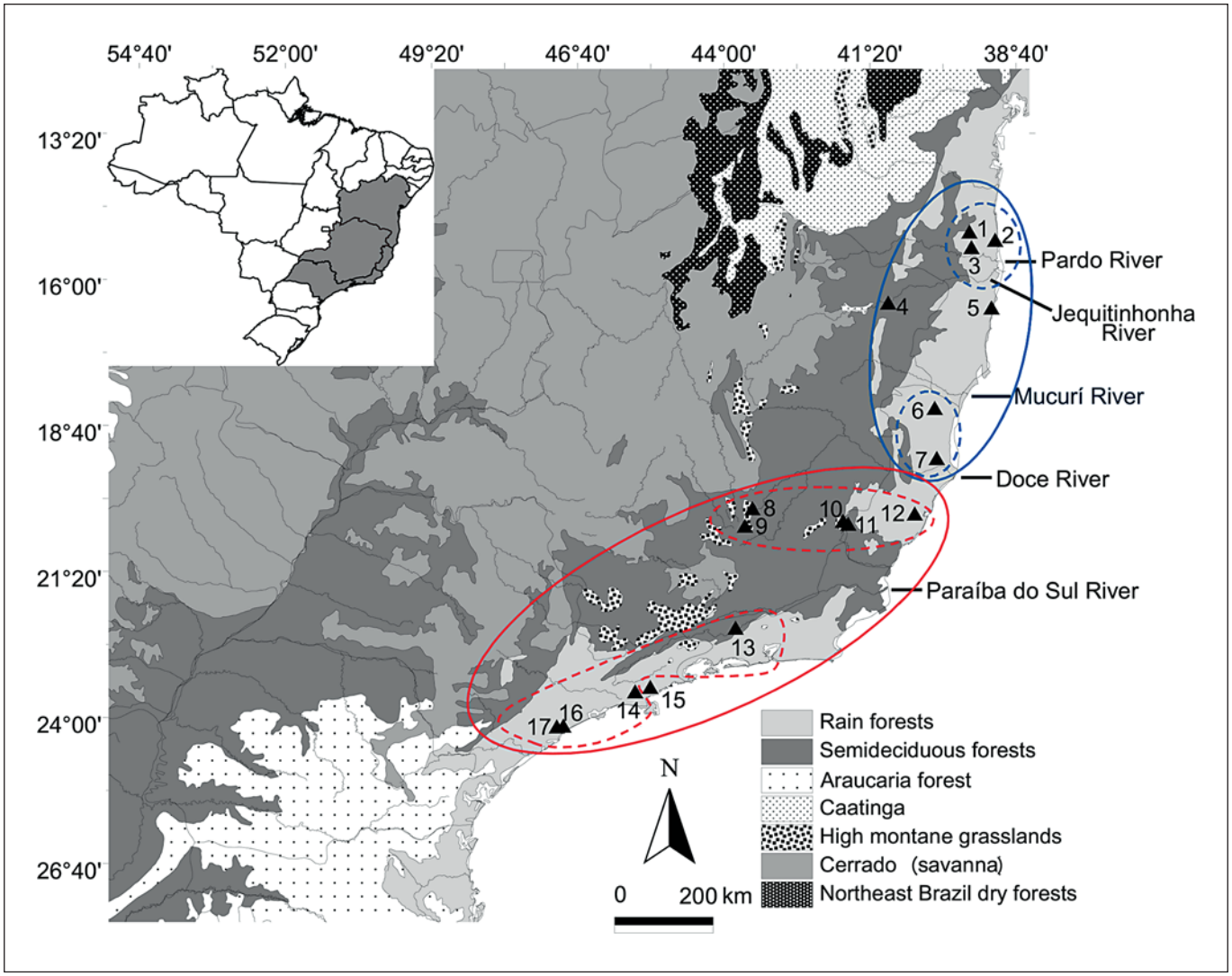

Fig. 1. Collecting localities of *Blarinomys breviceps*. (1) Jussarí, BA; (2) Una, BA; (3) Camacan, BA; (4) Jequitinhonha, MG; (5) Trancoso, BA; (6) Pinheiros, ES; (7) Linhares, ES; (8) Catas Altas, MG; (9) Ouro Branco, MG; (10) and (11) Alto Caparaó, MG; (12) Cariacica, ES; (13) Valença, RJ; (14) Biritiba-Mirim, SP; (15) Natividade da Serra, SP; (16) São Paulo, SP; (17) São Bernardo do Campo, SP. Blue lines delimit samples from the northeastern clade, and red lines correspond to samples from the southeastern clade according to fig. 2.

In order to identify the X chromosome precisely, we employed a whole chromosome X-specific painting probe obtained from the Akodontini species *Akodon cursor* (ACU) by degenerate oligonucleotide primed PCR (DOP-PCR) on flow-sorted chromosomes [Telenius et al., 1992; Yang et al., 1995; Ventura et al., 2009]. FISH of the painting probe was performed according to Yang et al. [1995]. The cross species hybridization was performed for 48–72 h at 37°C. Posthybridization washes included 2 × 5-min incubations in 50% formamide/2× SSC at 42°C followed by 2 × 5-min incubations in 2× SSC and submerged for 4 min in 4× T (100 ml 20× SSC + 400 ml H_2O + 250 μl Triton X-100 by Sigma-Aldrich, St. Louis, Mo., USA). The FITC-labeled probes were visualized with rabbit anti-FITC, followed by goat anti-rabbit antibody. The slides were counterstained with DAPI diluted with Vectashield. Images were analyzed under a Zeiss Axiophot fluorescence microscope equipped with a software for image capture system (Isis karyotyping system; MetaSystems).

Results

Molecular Phylogenetic Analyses

Twenty haplotypes were found among the 22 specimens sampled (table 1). The data matrix encompasses 756

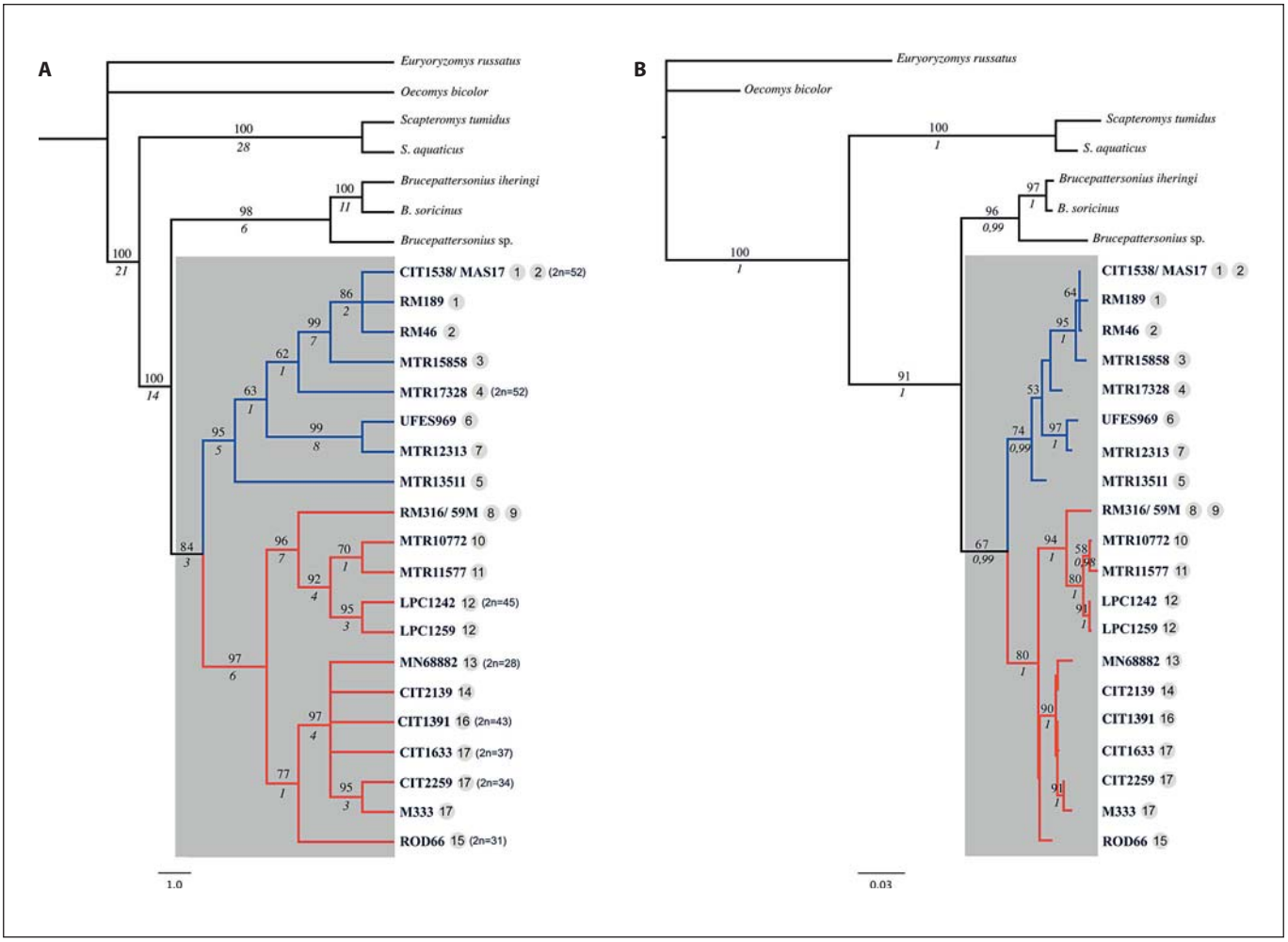

Fig. 2. Phylogenetic reconstructions for 22 specimens of *B. breviceps*, including 7 outgroups. **A** Strict consensus obtained from 2 equally most parsimonious trees (length = 488 steps, CI = 0.61 and RI = 0.77). Numbers above branches represent bootstrap values; numbers below branches are BSI values. Available diploid numbers (2n) are also provided on terminal nodes. **B** Cladogram representing the ML (-lnL = 3366.1) and Bayesian tree topologies. Numbers above branches represent likelihood bootstrap values; numbers below branches are BPPs above 0.95. Branch lengths correspond to ML estimates. The northeastern clade is highlighted in blue and the southeastern clade in red. Terminal nodes are identified by specimen identification numbers (table 1), and numbers 1–17 after them correspond to collecting localities on the map (fig. 1).

bp of cytochrome b sequence showing 511 constant sites, 49 non-informative sites and 196 parsimony informative sites.

All phylogenetic reconstructions (MP, ML and BI) indicated *Blarinomys* as monophyletic and sister to *Brucepattersonius*. The topology of the BI tree is identical to the ML tree and nearly identical to the MP trees (fig. 2).

The MP analysis resulted in 2 equally most parsimonious trees (length = 488 steps; CI = 0.61; RI = 0.77) with minor differences among a few terminal branches. The strict consensus of these 2 trees (fig. 2) clearly shows 2 main groups, the northeastern and the southeastern clades. The northeastern clade (BSI = 5 and BS = 95%) includes specimens from the states of Bahia, Espírito Santo and northern Minas Gerais, and it is geographically well-structured, with haplotypes showing a latitudinal pattern of phylogeographic affinities. The southeastern clade (BSI = 6 and BS = 97%), includes specimens from the states of Espírito Santo, Minas Gerais, Rio de Janeiro and São Paulo and is divided into 2 clades: one with specimens from Minas Gerais and western Espírito Santo, and another one with samples from eastern Espírito San-

to, Rio de Janeiro and São Paulo without a clear phylogeographic pattern on the haplotype tree (fig. 2A).

In the ML analysis, the resulting tree topology (-lnL = 3366.1; fig. 2B) is very similar to the MP analysis, with the same 2 main clades, but with lower BS in both clades: 74% in the northeastern and 80% in the southeastern (fig. 2B). The monophyly of the genus *Blarinomys* received an even lower BS of 67%. The BPPs, on the other hand, were high for both the monophyly of *Blarinomys* (0.99) and of the northeastern and southeastern clades (0.99 and 1.0, respectively).

Within the northeastern clade, localities north of the Pardo River (1–3) grouped together, as well as those haplotypes from localities between the Mucuri and Doce rivers (6, 7). In the southeastern clade, haplotypes from the Caparaó region (localities 10, 11), the southern Espinhaço Range (8, 9), and Reserva Biológica de Duas Bocas (locality 12) are also closely related. The phylogeographic pattern switches from a relatively strong, mostly latitudinal gradient in the northeastern clade, to a relatively weak gradient in the southeastern one, given the recovered polytomy.

Pairwise sequence divergences among specimens from the northeastern and southeastern clades were relatively high, varying between 4.8 and 8.4% (table 2). Genetic variation was also high within these 2 clades, ranging from 0.2 to 4.7% among specimens from the northeastern clade and from 0.1 to 5.5% within the southeastern clade. In the southeastern subclades the genetic variation ranges from 0.1 to 3.3% within the northernmost area grouping localities 8–12, and from 0.1 to 2.7% within the southernmost area grouping localities 13–17.

Karyotype Analyses

Karyomorph A (2n = 52) was observed in a female from Reserva Biológica da Mata Escura (locality 4 in fig. 1, MTR17328 in table 1) with 25 pairs of autosomes decreasing in size, all acrocentrics, and the X chromosomes were medium-sized acrocentrics (fig. 3A). FISH showed telomeric sequences present at the ends of all chromosomes.

Karyomorph B (2n = 52) was found in 1 male from Serra do Teimoso (locality 1 in fig. 1, CIT1538 in table 1) with 23 pairs of acrocentric autosomes, decreasing in size (pairs 1–3 and 5–24); 1 pair of large submetacentrics (pair 4); 1 medium-sized acrocentric X and 1 small Y, as well as the presence of 2 distinctive small metacentric B chromosomes (Bs; fig. 3B). C-banding revealed heterochromatic blocks at the pericentromeric regions of some autososomes; both Bs had heterochromatic arms and the Y was totally heterochromatic (data not shown). All telomeres presented signals after FISH, and some pericentromeric regions showed signals larger than others, reflecting colocalization of telomeric and heterochromatic regions. The Bs presented slightly larger telomeric signals on the long arms (fig. 3C).

Karyomorph C (2n = 45) was found in 1 male from Reserva Biológica de Duas Bocas (locality 12 in fig. 1, LPC1242 in table 1) that showed 4 pairs of large submetacentrics (pairs 1–4), 17 pairs of medium to small acrocentrics (pairs 5–21), 1 medium-sized acrocentric X, 1 small acrocentric Y, and 1 small metacentric B (fig. 3D). Telomeric signals were strong on all telomeres including a conspicuous signal on the long arm of the B (fig. 3E).

Karyomorph D (2n = 43) was observed in 1 female from Parque Estadual da Serra da Cantareira (locality 16 in fig. 1, CIT1391 in table 1) with 6 pairs of biarmed chromosomes (pairs 1–4, 6 and 7), 11 pairs of acrocentrics (pairs 8–18), 1 heterozygous Robertsonian rearrangement (Rb) involving 2 acrocentrics differing in size (pair 5), and the medium-sized acrocentric X. Four small metacentric Bs were detected. G-banding confirmed an Rb on pair 5 (fig. 3F) and R-banding revealed the late-replicating X and Bs (fig. 3G). C-banding revealed heterochromatin at the pericentromeric region of some acrocentrics, the X and 3 of the Bs (data not shown). All telomeres were positively hybridized after FISH and the small Bs presented a distinctive larger telomere. Interstitial telomeric sequences (ITSs) were observed at the centromere of the metacentric pair 7 (fig. 3H). The biarmed chromosome of the heteromorphic pair 5 did not present an ITS.

Karyomorph E (2n = 37) was found in 1 male from São Bernardo do Campo (locality 17 in fig. 1, CIT1633 in table 1) with 8 biarmed pairs and 9 acrocentric pairs of autosomes, decreasing in size. The X is a medium-sized acrocentric and the Y is a small acrocentric. One small metacentric B presented distal G-positive bands on both arms (fig. 3I). Pericentromeric heterochromatin was observed exclusively on the X chromosome. The B was not heterochromatic (data not shown). Telomeres were confirmed by FISH on all chromosome ends and an ITS at the centromere of the small submetacentric pair 7 was detected. The B presented a distinctive telomeric signal (fig. 3J).

Karyomorph F (2n = 34) was observed in a female from São Bernardo do Campo (locality 17 in fig. 1, CIT2259 in table 1) with 8 pairs of biarmed chromosomes (pairs 1, 2, 4, 5, 7–10), 5 pairs of acrocentrics (pairs 11–15)

Table 2. Genetic distances (Kimura 2-parameter) between the haplotypes of *Blarinomys* specimens and outgroups

		Outgroups							Northern clade								Southern clade												
		ERU	OBI	STU	SAQ	BIH	BSP	BSO	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20
Outgroups	ERU																												
	OBI	0.130																											
	STU	0.212	0.174																										
	SAQ	0.202	0.169	0.041																									
	BIH	0.203	0.171	0.159	0.148																								
	BSP	0.219	0.182	0.176	0.168	0.061																							
	BSO	0.200	0.176	0.169	0.154	0.009	0.060																						
Northern clade	H1	0.204	0.173	0.167	0.163	0.096	0.106	0.095																					
	H2	0.205	0.174	0.174	0.172	0.101	0.111	0.100	0.005																				
	H3	0.206	0.170	0.155	0.154	0.098	0.108	0.097	0.002	0.006																			
	H4	0.204	0.168	0.162	0.161	0.094	0.104	0.093	0.009	0.014	0.013																		
	H5	0.201	0.171	0.164	0.159	0.093	0.102	0.090	0.025	0.031	0.029	0.030																	
	H6	0.205	0.167	0.179	0.162	0.105	0.115	0.104	0.040	0.045	0.044	0.044	0.032																
	H7	0.198	0.164	0.174	0.161	0.105	0.117	0.104	0.038	0.043	0.044	0.043	0.032	0.011															
	H8	0.196	0.164	0.146	0.141	0.093	0.108	0.095	0.040	0.047	0.043	0.045	0.028	0.036	0.027														
Southern clade	H9	0.187	0.172	0.167	0.171	0.107	0.114	0.106	**0.078**	**0.084**	**0.075**	**0.082**	**0.062**	**0.082**	**0.079**	**0.068**													
	H10	0.200	0.180	0.171	0.168	0.110	0.115	0.114	**0.078**	**0.084**	**0.081**	**0.079**	**0.064**	**0.082**	**0.079**	**0.066**	0.031												
	H11	0.201	0.178	0.167	0.164	0.105	0.110	0.109	**0.073**	**0.079**	**0.079**	**0.075**	**0.059**	**0.078**	**0.075**	**0.061**	0.033	0.007											
	H12	0.200	0.184	0.169	0.168	0.110	0.116	0.114	**0.078**	**0.084**	**0.079**	**0.082**	**0.059**	**0.079**	**0.079**	**0.064**	0.031	0.010	0.014										
	H13	0.202	0.182	0.174	0.171	0.110	0.115	0.114	**0.075**	**0.081**	**0.077**	**0.079**	**0.056**	**0.076**	**0.076**	**0.063**	0.031	0.011	0.015	0.001									
	H14	0.185	0.173	0.178	0.164	0.098	0.107	0.099	**0.069**	**0.073**	**0.074**	**0.073**	**0.061**	**0.067**	**0.064**	**0.059**	0.051	0.050	0.046	0.052	0.053								
	H15	0.190	0.178	0.170	0.163	0.098	0.104	0.100	**0.063**	**0.067**	**0.063**	**0.070**	**0.057**	**0.061**	**0.058**	**0.051**	0.046	0.047	0.043	0.046	0.048	0.010							
	H16	0.192	0.169	0.163	0.157	0.090	0.100	0.092	**0.059**	**0.063**	**0.064**	**0.060**	**0.049**	**0.066**	**0.057**	**0.048**	0.040	0.036	0.032	0.038	0.039	0.022	0.018						
	H17	0.189	0.176	0.176	0.166	0.096	0.102	0.098	**0.061**	**0.066**	**0.062**	**0.069**	**0.056**	**0.063**	**0.060**	**0.049**	0.044	0.046	0.041	0.045	0.046	0.012	0.001	0.018					
	H18	0.191	0.178	0.174	0.166	0.096	0.106	0.098	**0.064**	**0.069**	**0.063**	**0.072**	**0.059**	**0.065**	**0.062**	**0.051**	0.046	0.048	0.043	0.046	0.048	0.014	0.003	0.019	0.001				
	H19	0.190	0.177	0.175	0.167	0.102	0.109	0.104	**0.064**	**0.068**	**0.062**	**0.071**	**0.060**	**0.065**	**0.062**	**0.052**	0.049	0.049	0.045	0.048	0.049	0.016	0.005	0.022	0.004	0.005			
	H20	0.194	0.185	0.175	0.165	0.107	0.114	0.106	**0.060**	**0.064**	**0.058**	**0.067**	**0.059**	**0.064**	**0.061**	**0.055**	0.055	0.055	0.050	0.053	0.055	0.021	0.011	0.027	0.010	0.011	0.006		

BIH = *B. iheringi*; BSO = *B. soricinus*; BSP = *Brucepattersonius* sp.; ERU = *E. russatus*; OBI = *O. bicolor*; SAQ = *S. aquaticus*; STU = *Scapteromys tumidus*.

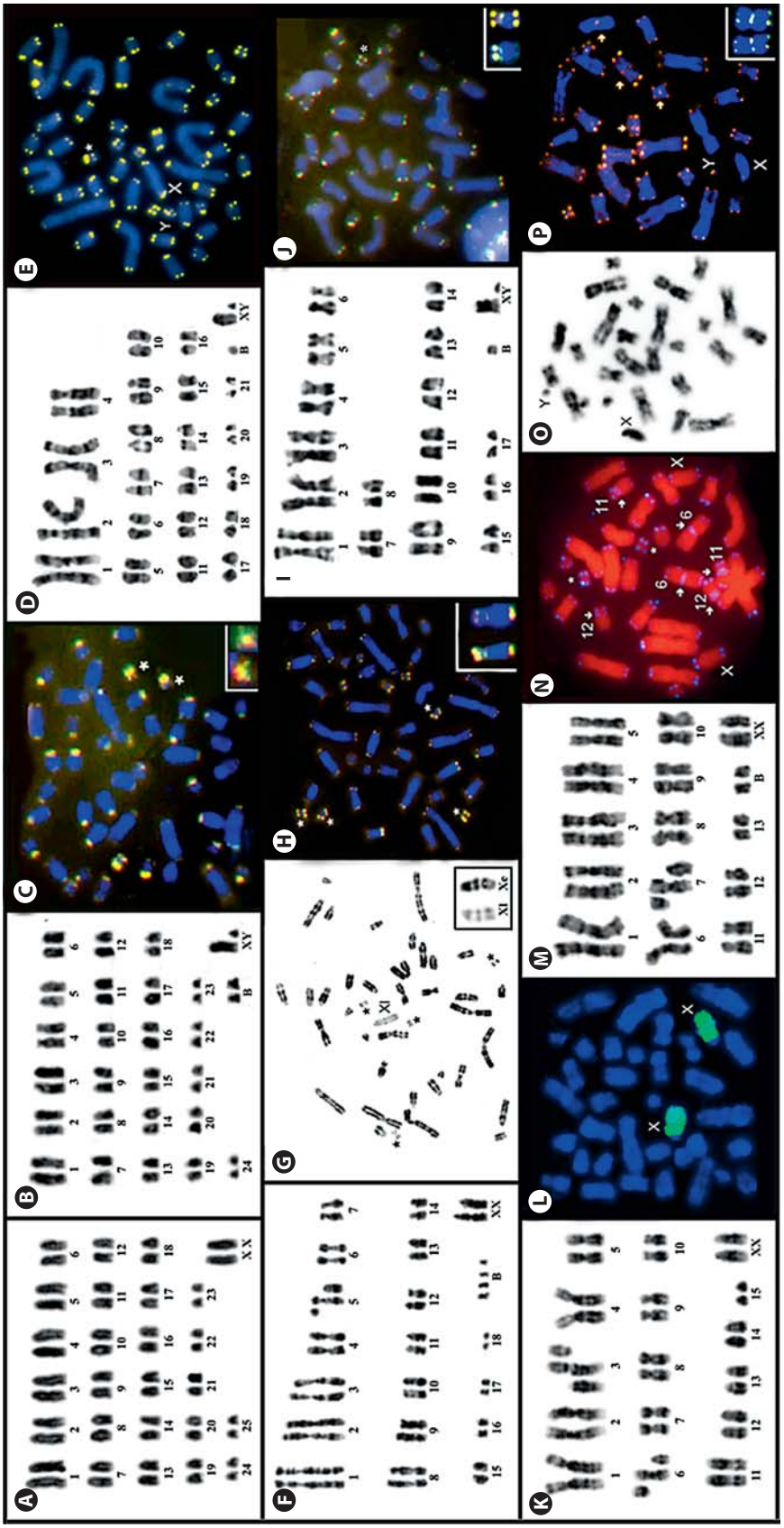

Fig. 3. Classical and molecular cytogenetics in 8 specimens of *B. breviceps*. **A** Karyomorph A of a female with 2n = 52 (50A,XX) and FN_A = 50 in conventional staining. **B**, **C** Karyomorph B of a male with 2n = 52 (48A,XY+2Bs) and FN_A = 50. **B** Karyotype in conventional staining. Note the submetacentric pair 4. **C** Metaphase after FISH with telomeric probe. Inset, small metacentrics from another metaphase with conspicuous telomeric signal. Asterisks indicate Bs. **D**, **E** Karyomorph C of a male with 2n = 45 (42A,XY+1B) and FN_A = 50. **D** Karyotype with G-banding pattern. **E** Metaphase after FISH with telomeric probe. **F–H** Karyomorph D of a female with 2n = 43 (37A,XX+4Bs) and FN_A = 50. **F** Karyotype with G-banding pattern. **G** Metaphase with R-banding pattern after BrdU incorporation. Note the 4 late-replicating small metacentric chromosomes (asterisks). **H** Metaphase after FISH with telomeric probe (asterisks: Bs). Inset: pair 7 with ITS from another metaphase. **I**, **J** Karyomorph E of a male with 2n = 37 (34A,XY+1B) and FN_A = 50. **I** Karyotype with G-banding pattern. **J** Metaphase after FISH with telomeric probe (asterisks: Bs). Inset: pair 7 with ITS from another metaphase. **K**, **L** Karyomorph F of a female with 2n = 34 (32A,XX) and FN_A = 50. **K** Metaphase with G-banding pattern. **L** Metaphase after chromosome painting using ACUX paint. Note the acrocentric chromosome X in green. **M**, **N** Karyomorph G of a female with 2n = 31 (27A,XX+2Bs) and FN_A = 50. **M** Karyotype with G-banding pattern. **N** Metaphase after FISH with telomeric probe. Arrows indicate the 6 biarmed chromosomes with ITS signal. **O**, **P** Karyomorph H of a male with 2n = 28 (26A,XY) and FN_A = 50. **O** Metaphase with G-banding pattern. **P** Metaphase after FISH with telomeric probe. Arrows indicate the 4 biarmed chromosomes with ITS signal. Inset: chromosomes of pair 5 with ITS from another metaphase.

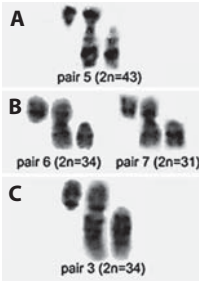

Fig. 4. Three heterozygous pairs for Rbs in *B. breviceps*. **A** Pair 5 of karyomorph D. **B** The homeologous pair 6 of karyomorph F and pair 7 of karyomorph G. **C** Pair 3 of karyomorph F which is homeologous to the homozygous pairs 4 of karyomorph C and 3 of karyomorph G.

and 6 elements involved in 2 heterozygous Rbs, represented by pairs 3 and 6. X chromosomes had the 2 G-positive bands at the long arm (fig. 3K) and cross-species chromosome painting using ACUX confirmed the medium acrocentric morphology for the X chromosome in *B. breviceps* (fig. 3L).

Karyomorph G (2n = 31) was observed in a female from Parque Estadual da Serra do Mar (locality 15 in fig. 1, ROD66 in table 1) with 11 pairs of biarmed chromosomes decreasing in size (pairs 1–6, 8–12), a heterozygous Rb (pair 7), 1 pair of small acrocentrics (pair 13), and 2 small metacentric Bs. The X chromosomes are medium-sized acrocentrics (fig. 3M). Small heterochromatic blocks were seen in the pericentromeric region of some autosomes. The X showed heteromorphic heterochromatic blocks at the pericentromeric region and the Bs were not heterochromatic (data not shown). All the telomeres were identified by FISH, as well as ITSs at the centromeres of metacentric pair 6 and submetacentric pairs 11 and 12 (fig. 3N).

Karyomorph H (2n = 28) belongs to a male obtained at Serra da Concórdia (locality 13 in fig. 1, MN68882 in table 1), and was previously reported by Geise et al. [2008]. The autosome set is composed of 12 pairs of biarmed chromosomes (pairs 1–12) and 1 small acrocentric pair 13; the X is a medium-sized acrocentric and the Y is a small acrocentric (fig. 3O). Telomeres were present at the ends of all chromosomes. ITSs were observed at the pericentromeric region of 2 metacentric pairs (fig. 3P).

Comparison of G-bands between individuals revealed homology between some autosome pairs and the sex chromosomes. The pattern of G-bands did not permit a reliable comparison of all sets of chromosomes, although we can identify the autosomes of 4 distinct heterozygous pairs for Rbs: pair 5 of the 2n = 43 karyomorph, pair 6 of the 2n = 34 karyomorph and pair 7 of the 2n = 31 karyomorph (which are homeologous), and pair 3 of the 2n = 34 karyomorph (fig. 4).

Discussion

Molecular Phylogeny

Molecular phylogenetic analyses corroborated the monophyly of the genus *Blarinomys*, despite low support in the ML analysis. Molecular analyses showed 2 geographically distinct lineages in the Atlantic Rainforest, allowing the identification of 2 broad regions in this domain, whose limit occurs approximately at a latitude of 20°S. Previous studies involving different groups of vertebrates [Costa and Leite, 2012] also recognized 2 phylogeographic regions in the Atlantic Rainforest, separating the fauna in 2 sister-group components (northeastern and southeastern), as demonstrated for species of amphibians [Carnaval et al., 2009], lizards [Vanzolini, 1988; Pellegrino et al., 2005], birds [Bates et al., 1998; Cabanne et al., 2007], and non-volant small mammals [Costa et al., 2000; Costa, 2003; Leite, 2003]. This regional pattern is attributed to the presence of the Rio Doce [Pellegrino et al., 2005].

Carnaval et al. [2009] based on simultaneous Bayesian analyses of multi-species molecular data using *Hypsiboas* compared alternative hypotheses of assemblage-scale response to late Quaternary climate change. They demonstrated that the southernmost Atlantic forest was climatically unstable during late Pleistocene and reported low genetic diversity across this area for the species sampled. However, high levels of divergence and population structure were reported for the species sampled across the central region of the Atlantic forest, which served as a large climatic refuge for neotropical species at that period, revealing a hotspot within the Brazilian Atlantic forest.

The processes uncovered by the amphibian data as postulated by Carnaval et al. [2009] may be generalized to and help to explain the patterns of diversity in the endemic Atlantic forest rodent *B. breviceps*.

Karyotypic Polymorphisms Caused by Supernumerary Chromosomes and Rbs

Different diploid numbers and the same fundamental numbers (FN_A = 50) were described for the *Blarinomys* specimens analyzed. The variation in number of chromosomes found in the karyotypes is due to the presence of 0–4 supernumerary chromosomes (Bs) and/or Rbs, leading to 8 karyomorphs: (A) 2n = 52 (50A, XX); (B) 2n = 52 (48A, XY+2Bs); (C) 2n = 45 (42A, XY+1B); (D) 2n = 43 (37A, XX+4Bs); (E) 2n = 37 (34A, XY+1B); (F) 2n = 34 (32A, XX); (G) 2n = 31 (27A, XX+2B); and (H) 2n = 28 (26A, XY).

Karyotypes herein presented characterize the genus as possessing a variable diploid number due to Rbs, con-

stancy of fundamental number ($FN_A = 50$), as well as facultative presence of Bs. Support for the supernumerary nature of these small metacentric chromosomes was determined by their late replication (fig. 3G), their variable number (0–4), the different nature of heterochromatin, and their telomeric signal variation.

Supernumerary Bs

In *B. breviceps* different features can be attributed to supernumerary chromosomes as late replication (karyomorph D), presence or absence of constitutive heterochromatin in the pericentromeric region or in 1 of the arms after applying C-banding, as well as intense signs of telomeric probe hybridizations or facultative presence of ITSs in 1 of the arms co-located with the constitutive heterochromatic region. The latter presents sequences that are similar to the telomeric repeats (karyomorph B).

According to the review by Silva and Yonenaga-Yassuda [2004], Bs have been recorded in another 8 species of 7 genera of Brazilian rodents: *A. montensis* (2n = 24+0–2Bs), *Sooretamys angouya* (2n = 58+0 or 2Bs) (referred to as *Oryzomys angouya*), *Holochilus brasiliensis* (2n = 56+0–2Bs), *Nectomys rattus* (2n = 52+0–3Bs), *N. squamipes* (2n = 56+0–3Bs), *Oligoryzomys flavescens* (2n = 64+0–2Bs), *Proechimys* sp. 2 (2n = 26+0–1B) and *Trinomys iheringi* (2n = 60+1–6Bs). In most cases, supernumerary chromosomes are heterochromatic, present late replication, are heterogeneous regarding their size, morphology, banding pattern and presence or absence of Ag-NORs or ITSs after telomeric FISH as observed in *B. breviceps*.

The Rbs

The sample presents a wide range of diploid numbers (2n = 28–52) with the number of autosomes ranging from 26, with all biarmed pairs except one, to 50, with exclusively acrocentric chromosomes and polymorphisms involving Rbs, including double heterozygotes and supernumerary chromosomes. However, considering the absence of consistent data on complete comparative analysis of banding patterns and the currently very limited sample karyotyped, it is not possible to characterize all the Rb polymorphisms (except for those presented in fig. 4) or to determine the frequency and distribution of individual Rbs and the presence/absence of monobrachial homoleogies within and between populations. Consequently, the dynamics of chromosomal evolution in *Blarinomys* is obscure given the observed diversity.

Nevertheless, the chromosomal variability found in *Blarinomys* is among the greatest found among mammals. It is comparable to the variability reported for the house mouse *Mus musculus* (2n = 22–40) [Piálek et al., 2005], the common shrew *Sorex araneus* (2n = 20–33) [Wojcik et al., 2003], the African rodent *Gerbillus nigeriae* (2n = 60–74) [Hima et al., 2011] and also for some species of *Ellobius* [Lyapunova et al., 1980, 2010; Bakloushinskaya et al., 2010].

According to Slijepcevic [1998], the mechanisms leading to Rbs are centric fusions that require the prior elimination or inactivation of telomeric sequences sometimes leading to the presence (or not) of ITSs at the sites of the fusion event. However, Ruiz-Herrera et al. [2008] reported that the telomeric sequences of ancestral chromosomes involved in centric fusions could be retained and amplified leading to the maturation of neo-telomeres/neo-centromeres, allowing a later centric fission. In the phylogenetic framework presented here for *Blarinomys*, it is not possible to evaluate whether the mechanism involves centric fusion, fission or both rearrangements.

The 2 main clades recovered in *B. breviceps* on the basis of molecular data, apparently present differences in karyotypic composition. The northeastern lineage includes 2 karyotyped specimens (CIT1538 and MTR17328) with a higher diploid number (2n = 52) composed mainly of acrocentric elements, while the southeastern lineage includes individuals with lower diploid numbers and metacentric chromosomes. We cannot speculate about the ancestral karyotype or the direction of the change (increasing or decreasing the diploid number) but we can affirm that the animals of the southeastern clade (ROD66 (MN68882, CIT2139, CIT1391, CIT1633 (CIT2259, M333))) are experiencing a visible process of genomic reorganization. This high chromosomal variability and the presence of heterozygous and double heterozygous individuals for Rbs could imply that we are dealing with individuals in a hybrid zone where different chromosomal variants segregate more or less randomly giving rise to a high number of different karyotypes. Alternatively, the chromosomal polymorphisms observed in the southeastern clade could be due to the processes of transient polymorphism (i.e. before fixation of a new rearrangement).

Conclusions

Genetic data from *B. breviceps* reveal that we are dealing with a more diverse and complex group than previously thought. Its evolutionary history is compatible with the hypothesis that the Rio Doce acts as a barrier in the Atlantic Rainforest, causing a geographic separation between the 2 main lineages. Coincidentally, other river

drainages (e.g. Pardo/Jequitinhonha, Mucuri and Paraíba do Sul rivers) in the region of the Rio Doce system appear as secondary markers of phylogeographic breaks (figs. 1 and 2). The forest lizards [Pellegrino et al., 2005; Carnaval and Moritz, 2008], amphibians [Carnaval et al., 2009] and birds [Cabanne et al., 2008] also show high diversity in the central portion of the biome relative to southern areas, and provide additional evidence for population expansion in southern regions.

Although present knowledge does not allow us to make further assumptions on the role of river systems on the evolutionary history of the small mammals occurring in the coastal rain forest, the cumulative evidence of latitudinal phylogeographic breaks in this region, as the one presented here, reinforces the urgency and importance of continuing research in this biodiversity hotspot. The investigation of the repetitive patterns so far reported may lead us to a better understanding of the historical biogeography of this region. The karyological high diversity, phylogeographic structure, and genetic divergence where Kimura 2-parameter values between haplotype pairs of both main clades in *Blarinomys*, differing by 4.8–8.4%, represent large values that fall in the range observed between pairs of akodontine sister species [e.g. Pardiñas et al., 2005; D'Elía et al., 2008; Jayat et al., 2008]. These results indicate a complex evolutionary history and call attention to the need for a taxonomic re-evaluation of this elusive taxon.

Acknowledgements

We thank Renata Cecília Amaro for some chromosomal preparations and Arlei Marcili, Agustín Camacho, Carolina Lima Neves, Caryne Braga, Dante Pavan, Felipe Curcio, Helena de Godoy Bergallo, José Cassimiro, José Mario Guellere, Laerte Bento Viola, Lucas Alves Vianna, Marco Antonio Sena, Marco Aurélio Sábato, Mauro Teixeira Jr., Patricia B. Bertola, Pedro Nunes, Raquel Moura, Renato Recoder, Sandra Favorito, Silvia Ramira Lopes Caldara, Vanessa Verdade and Victor Hugo Colombi for fieldwork support and collecting specimens. We also thank Professor Malcolm Ferguson-Smith for the English review and Craig Moritz as well as Ana Carnaval for their support. This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo a Pesquisa do Espírito Santo (FAPES), Fundação de Amparo a Pesquisa do Rio de Janeiro (FAPERJ), Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), and Fundo de Apoio à Ciência e Tecnologia do Município de Vitória do Espírito Santo (FACITEC). Field trips to Camacan and Jequitinhonha were partially funded by a National Science Foundation (DEB 0817035) grant.

References

Abravaya JP, Matson JO: Notes on a Brazilian mouse *Blarinomys breviceps* (Winge). Contr Sci Nat Hist Mus Los Angeles Co 270:1–8 (1975).

Bates JM, Hackett SJ, Cracraft J: Area-relationships in the neotropical lowlands: an hypothesis based on raw distributions of passerine birds. J Biogeogr 25:783–793 (1998).

Bakloushinskaya IY, Romanenko SA, Graphodatsky AS, Matveesky SN, Lyapunova EA, Kolomiets OL: The role of chromosome rearrangements in the evolution of mole voles of the genus *Ellobius* (Rodentia, Mammalia). Russ J Genet 46:1143–1145 (2010).

Bremer K: Branch support and tree stability. Cladistics 10:295–304 (1994).

Bruford MW, Hanotte O, Brookfield JFY, Burke T: Single-locus and multilocus DNA fingerprinting, in Hoelzel AR (ed): Molecular Genetics Analysis of Populations: a practical approach, pp 225–269 (IRL Press, Oxford 1992).

Cabanne GS, Santos FR, Miyaki CY: Phylogeography of *Xiphorhynchus fuscus* (Passeriformes, Dendrocolaptidae): vicariance and recent demographic expansion in southern Atlantic forest. Biol J Linn Soc 91:73–84 (2007).

Cabanne GS, d'Horta FM, Sari EH, Santos FR, Miyaki CY: Nuclear and mitochondrial phylogeography of the Atlantic forest endemic Xiphorhynchus fuscus (Aves: Dendrocolaptidae): biogeography and systematics implications. Mol Phylogenet Evol 49:760–773 (2008).

Carnaval AC, Hickerson MJ, Haddad CF, Rodrigues MT, Moritz C: Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. Science 323:785–789 (2009).

Carnaval AC, Moritz C: Historical climate modelling predicts patterns of current biodiversity in the Brazilian Atlantic forest. J Biogeogr 35:1187–1201 (2008).

Chebez JC, Massoia E: Mamíferos de la provincia de Misiones, in Chebez JC (ed): Fauna Misionera. Catálogo sistemático y zoogeográfico de los vertebrados de la provincia de Misiones (Argentina), pp 180–308 (Editorial L.O.L.A., Buenos Aires 1996).

Costa LP: The historical bridge between the Amazon and the Atlantic Forest of Brazil: a study of molecular phylogeography with small mammals. J Biogeogr 30:71–86 (2003).

Costa LP, Leite YL: Historical fragmentation shaping vertebrate diversification in the Atlantic forest biodiversity hotspot, in Patterson B, Costa LP (eds): Bones, Clones and Biomes: the History and Geography of Recent Neotropical Mammals (University of Chicago Press, Chicago 2012).

Costa LP, Leite YL, da Fonseca GA, Fonseca MT: Biogeography of South American forest mammals: endemism and diversity in the Atlantic Forest. Biotropica 32:872–881 (2000).

D'Elía G: Phylogenetics of Sigmodontinae (Rodentia, Muroidea, Cricetidae), with special reference to the akodont group, and with additional comments on historical biogeography. Cladistics 19:307–323 (2003).

D'Elía G, Gonzales EM, Pardiñas UF: Phylogenetic analyses of sigmodontine rodents (Muroidea), with special reference to the akodont genus *Deltamys*. Mamm Biol 68:351–364 (2003).

D'Elía G, Pardiñas UF, Teta P, Patton JL: Definition and diagnosis of a new tribe of sigmodontine rodents (Cricetidae, Sigmodontinae), and a revised classification of the subfamily. Gayana 7:187–194 (2007).

D'Elía G, Pardiñas UF, Jayat JP, Salazar-Bravo J: Systematics of *Necromys* (Rodentia, Cricetidae, Sigmodontinae): species limits and groups, with comments on historical biogeography. J Mamm 89:778–790 (2008).

Felsenstein J: Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791 (1985).

Fetzner JW: Extracting high-quality DNA from shed reptile skins: a simplified method. Bio Techn 26:1052–1054 (1999).

Geise L, Bergallo HG, Esberard CE, Rocha CF, Sluys MV: The karyotype of *Blarinomys breviceps* (Mammalia: Rodentia: Cricetidae) with comments on its morphology and some ecological notes. Zootaxa 1907:47–60 (2008).

Guindon S, Gascuel O: A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52:696–704 (2003).

Hima K, Thiam M, Catalan J, Gauthier P, Duplantier JM, et al: Extensive Robertsonian polymorphism in the African rodent *Gerbillus nigeriae*: geographic aspects and meiotic data. J Zool 284:276–285 (2011).

Huelsenbeck JP, Ronquist F: MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:754–755 (2001).

Jayat PJ, D'Elía G, Pardiñas UFJ, Miotti MD, Ortiz PE: A new species of the genus *Oxymycterus* (Mammalia: Rodentia: Cricetidae) from the vanishing Yungas of Argentina. Zootaxa 1911:31–51 (2008).

Laemmert Jr HW, Ferreira LC, Taylor RM: An epidemiological study of jungle yellow fever in an epidemic area in Brazil; epidemiology of human infections; investigations of vertebrate hosts and arthropod vectors. Am J Trop Med Hyg 26:26–69 (1946).

Laurance WF: Conserving the hottest of the hotspots. Biol Conserv 142:1137 (2009).

Leite YL: Evolution and systematics of the Atlantic tree rats, genus *Phyllomys* (Rodentia, Echimyidae), with description of two new species. Univ Calif Pub Zool 132:1–118 (2003).

Lyapunova EA, Vorontsov NN, Korobitsyna KV, Ivanitsakaya EY, Borisov YM, et al: A Robertsonian fan in *Ellobius talpinus*. Genetica 52/53:239–247 (1980).

Lyapunova EA, Bakloushinskaya IY, Saidov AS, Saidov KK: Dynamics of chromosome variation in mole voles *Ellobius tancrei* (Mammalia, Rodentia) in Pamiro-Alay in the period from 1982 to 2008 [in Russian]. Russ J Genet 46:566–571 (2010).

Matson JO, Abravaya JP: *Blarinomys breviceps*. Mamm Spec 74:1–3 (1977).

Moojen J: Os Roedores do Brasil (Instituto Nacional do Livro, Rio de Janeiro1952).

Myers, Mittermeier RA, Mittermeier CG, da Fonseca GA, Kent J: Biodiversity hotspots for conservation priorities. Nature 403:853–858 (2000).

Paglia AP, Perini FA, Lopes MO, Palmuti CF: Novo registro de *Blarinomys breviceps* (Winge, 1888) (Rodentia, Sigmodontinae) no estado de Minas Gerais, Brasil. Lundiana 6:155–157 (2005).

Pardiñas UF, D'Elía G, Cirignoli S, Suarez P: A new species of *Akodon* (Rodentia, Cricetidae) from the Northern Campos grasslands of Argentina. J Mammal 86:462–474 (2005).

Pellegrino KC, Rodrigues MT, Waite NA, Morando M, Yonenaga-Yassuda Y, Sites Jr JW: Phylogeography and species limits in the *Gymnodactylus darwinii* complex (Gekkonidae, Squamata): genetic structure coincides with river systems in the Brazilian Atlantic Forest. Biol J Linn Soc 85:13–26 (2005).

Piálek J, Hauffe HC, Searle JB: Chromosomal variation in the house mouse. Biol J Linn Soc 85:535–563 (2005).

Posada D: jModelTest: phylogenetic model averaging. Mol Biol and Evol 25:1253–1256 (2008).

Rodríguez F, Oliver JF, Marín A, Medina JR: The general stochastic model of nucleotide substitution. J Theor Biol 142:485–501 (1990).

Ruiz-Herrera A, Nergadze SG, Santagostino M, Giulotto E: Telomeric repeats far from the ends: mechanisms of origin and role in evolution. Cytogenet Genome Res 122:219–228 (2008).

Silva CR, Percequilo AR, Iack-Ximenes GE, De Vivo M: New distributional records of *Blarinomys breviceps* (Winge, 1888) (Sigmodontinae, Rodentia). Mammalia 67:147–152 (2003).

Silva MJ, Yonenaga-Yassuda Y: B chromosomes in Brazilian rodents. Cytogenet Genome Res 106:257–263 (2004).

Slijepcevic P: Telomeres and mechanisms of Robertsonian fusion. Chromosoma 107:136–140 (1998).

Smith MF, Patton JL: The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the Akodontini tribe. Biol J Linn Soc 50:149–177 (1993).

Smith MF, Patton JL: Phylogenetic relationships and the radiation of sigmodontine rodents in South America: evidence from cytochrome b. J Mamm Evol 6:89–128 (1999).

Sorenson MD: TreeRot, ver. 2 (Boston University, Boston 1999).

Swofford DL: PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.02b (Sinauer Associates, Sunderland 2003).

Telenius H, Pelmear A, Tunnacliffe A, Carter NP, Behmel A, et al: Cytogenetic analysis by chromosome painting using DOP-PCR amplified flow-sorted chromosomes. Genes Chromosomes Cancer 4:257–263 (1992).

Vanzolini PE: Distributional patterns of South American lizards, in Vanzolini PE, Heyer WR (eds): Proceedings of a workshop on neotropical distribution patterns, pp 245–274 (Academia Brasileira de Ciências, Rio de Janeiro 1988).

Ventura K, O'Brien PC, Yonenaga-Yassuda Y, Ferguson-Smith MA: Chromosome homologies of the highly rearranged karyotypes of four *Akodon* species (Rodentia, Cricetidae) resolved by reciprocal chromosome painting: the evolution of the lowest diploid number in rodents. Chromosome Res 17:1063–1078 (2009).

Wilson DE, Reeder DM: Mammal Species of the World (Johns Hopkins University Press, Baltimore 2005).

Wojcik JM, Borodin PM, Fedyk S, Fredga K, Hausser J, et al: The list of the chromosome races of the common shrew *Sorex araneus* (updated 2002). Mammalia 67:169–178 (2003).

Yang F, Carter NP, Shi L, Ferguson-Smith MA: A comparative study of karyotypes of muntjacs by chromosome painting. Chromosoma 103:642–652 (1995).