Molecular phylogenies, chromosomes and dispersion in Brazilian akodontines (Rodentia, Sigmodontinae)

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ABSTRACT. A new molecular phylogeny for akodontine rodents from Brazil was proposed. The phylogenetic tree was enriched with the area of occurrence and with information on the karyotype of the samples. Based on this enriched tree, and with a described methodology, hypotheses were proposed on the karyotype and area of occurrence of the ancestors of each Clade. Thus it was possible to discuss hypotheses on chromosome evolution of the group, and on dispersion events from the "area of original differentiation" of akodontines in the Andes. Chromosome evolution started with high diploid numbers (2n=52) and showed a tendency to reduction (until 2n=14 in more recent clades). Independent side-branches of the tree showed 2n reduction and in one case the 2n increased. At least four dispersion events from the Andes down to South-eastern Brazil were proposed. The results should suggest the direction of new studies on comparative karyology.

KEYWORDS. Brazilian akodontines, chromosome evolution, biogeography, molecular phylogeny.

RESUMO. Filogenia molecular, cromossomos e dispersão em akodontinos do Brasil (Rodentia, Sigmodontinae). Uma nova filogenia molecular para roedores akodontinos do Brasil é proposta. A árvore filogenética foi enriquecida com a área de ocorrência e com informações sobre o cariótipo das amostras. Baseado nisto, e com a metodologia descrita, foram propostas hipóteses sobre as características do cariótipo e sobre a área de ocorrência dos ancestrais de cada clado. Assim, foi possível discutir hipóteses sobre evolução cromossômica do grupo, e sobre eventos de dispersão a partir da área de diferenciação original dos akodontinos nos Andes. A evolução cromossômica começou com números diplóides altos (2n=52) e mostrou uma tendência a redução (até 2n=14 em clados mais recentes). Ramos independentes da árvore mostraram redução do 2n e num caso aumentou o numero diplóide. Foram propostos pelo menos quatro eventos de dispersão dos Andes até o Brasil Sul-Oriental. Os resultados indicam a direção de novos estudos em cariologia comparada.

PALAVRAS-CHAVE. Akodontinos do Brasil, evolução cromossômica, biogeografia, filogenia molecular.

Most of the South American cricetid rodents belong to an assemblage of genera usually grouped under the subfamily Sigmodontinae. According to the view originally proposed by Hershkovitz (1966, 1972), and supported by Savage (1974), Reig (1975, 1978, 1980, 1981) and Engel *et al.* (1998), the North American ancestor entered South America through its northwestern corner by over water dispersal prior to the establishment of the Panamanian bridge, most probably between 5 and 9 MYA. This is the usually accepted hypothesis although there are some alternatives (see Pardiñas *et al.*, 2002).

According to Reig (1984) the original stock ancestral to Sigmodontinae may be found within the tribe Oryzomyini from which the Akodontini differentiated probably in the central Andean region. Reig (1986) considers that the ancestral akodontine may have been a generalized *Akodon*-like form of boreal origin, which colonized the area of the Puna from the north in Late Miocene or Early Pliocene, before the Altiplano reached considerable heights, in the Middle Pliocene (Ahlfeld, 1970).

Reig (1984) assumes that the evolution of Akodontini is a history of successful dispersion, certainly promoted by a trophic and habitat versatility of the most speciose genera such as *Akodon* Meyen, 1833 which became distributed over the greater part of South America, but prevailing in the Andean area.

To better understand the geographic history of the Sigmodontinae Reig (1984) introduced the concept of Area of Original Differentiation (AOD) as a tool to infer the patterns of geographical origin and of dispersion of the different groups of high Andean rodents. The AOD is defined as "the geographical space inside of which a certain taxon suffered its main differentiation (cladogenesis) in the subordinate taxa that compose it." In that sense, an AOD is not a "center of origin", since the ancestral lineage of a taxon that has a certain AOD does not have to be originated in the same area in which the taxon suffered its main differentiation processes. At the same time, the AOD of a taxon is the area where a new derived taxon originates by cladogenesis and that thereafter invades another area in which it suffers its main cladogenesis. This new area constitutes the AOD of the derived taxon. For the determination of the AOD of Akodontini, Reig (1984) identified the area of greatest overlapping of the geographical distributions of the different species of this tribe. The data used were the records of distribution of each species, the frequency of a given taxon in a certain geographical unit, the analysis of the overlapping of the distribution of the genera in the area and also the percentage of endemic genera.

Reig (1984) concluded that the South Central Andean Unit (SCA) and the North Southern Andean unit

(NSA) are the regions of greatest occurrence of akodontine genera and subgenera (see a definition of these units in SIMPSON (1975) and REIG (1986)), suggesting that the akodontines probably differentiated in the intermediate zone between the SCA and the NSA.

REIG (1986) considers that to explain the present distribution of members of the Akodontini it may be necessary to assume that most of the taxa originated in what is now the southern Puna region (AOD), and that they migrated northwards, southwards and to the eastern lowlands and experienced there further differentiation.

We assume that akodontines of the Atlantic Forest or their ancestrals came from the AOD. The question rises: was it a single dispersion event with differentiation in the Atlantic Forest or several events? And when did it happen?

The diploid number of species of the genus *Akodon s. l.* varies from 2n=10 in a sibling species of *Akodon montensis* Thomas, 1913 (SILVA & YONENAGA-YASSUDA, 1998) to 2n=52 in *Thaptomys nigrita* (Lichtenstein, 1829) and in other akodontine species of Southern South America. This karyotypic variation originated by several mechanisms including Robertsonian rearrangements, pericentric inversions, extranumerary chromosomes (YONENAGA *et al.*, 1976; FAGUNDES *et al.*, 1998).

To understand the karyotype evolution of akodontines it is necessary to know the direction of the chromosome rearrangements, toward fusion or fission. That is, if evolution proceeded from high to low diploid numbers by centric fusion or in the other direction by centromeric dissociation (BIANCHI *et al.*, 1971; FAGUNDES *et al.*, 1997; GEISE *et al.*, 1998).

To determine this direction as well as plesiomorphic or apomorphic character states in karyotype, a reliable external phylogeny, morphological or molecular, is necessary.

SMITH & PATTON (1993, 1999), ENGEL *et al.* (1998), GEISE *et al.* (2001) and D'ELÍA (2003) addressed the question of the phylogeny of Sigmodontinae using sequences of the Cytochrome b gene of $_{MT}$DNA. Fourteen species of *Akodon* of different geographical areas appeared in these author's cladograms as a monophyletic group with high bootstrap support.

In this paper we present a phylogenetic tree based on Cytochrome B sequences using new tissue samples and sequences taken from GenBank. Starting from Reig's theory of AODs (REIG, 1986), and with help of a molecular phylogeny, our aim is to propose new hypothesis on historical biogeography and chromosome evolution of eastern Brazilian species of Akodontini. This is probably the first study combining data from molecular phylogenies with information on karyotype and geographic distribution to understand aspects of karyotype evolution and geographic history of Brazilian akodontine rodents.

MATERIAL AND METHODS

Total DNA was obtained from grounded tissues of 20 samples belonging to eight species of the genus *Akodon* (Tab. I). Another 28 sequences were obtained from GenBank (Tab. I). Tissues were stored at the Laboratório de Citogenética de Mamíferos of the Departamento de Sistemática e Ecologia, Universidade Federal da Paraíba, and processed for DNA extraction (SAMBROOK *et al.*, 1989) at the Laboratório de Genética e Biologia Molecular, Campus Bragança of the Universidade Federal do Pará. For each DNA sample a fragment of about 800 base pairs of the mitochondrial Cytochrome B gene was amplified and sequenced using primers described by SMITH & PATTON (1993). Further details of the technique used may be found in BARROS *et al.* (2003).

The Cytochrome b gene fragment sequenced in this study was aligned to homologous sequences obtained from the GenBank using the ClustalX program (THOMPSON *et al.*, 1997) with default parameters and the data converted to FASTA and NEXUS formats. Minor modifications in the alignment were made using the BIOEDIT sequence editor (HALL, 1999). Sequences were deposited in GenBank, accession numbers pending. Nucleotide saturation was assessed by plotting transitions and transversions against K2P distances (KIMURA, 1980) using the DAMBE program version 4.0.65 (XIA & XIE, 2001). Phylogenetic reconstruction was performed using Maximum Parsimony, Neighbor-Joining and Minimum Evolution implemented in PAUP version 4.0b10 (SWOFFORD, 1998) and Mega version 2.0 (KUMAR *et al.*, 2001). As outgroup we used *Oryzomys* (*Hylaeamys*) *megacephalus* (Fischer, 1814), a member of the sister group of the akodontines in the tree of D'ELÍA (2003).

The procedure used to reconstruct the chromosome evolution and the biogeographic history of Akodontini started from the concept of AOD, and from the need to polarize the chromosome rearrangements with help of an external molecular phylogeny. We formulated hypotheses to determine the diploid number and the geographic distribution of the most recent ancestral of the terminal units of the cladogram, repeating thereafter the procedure with the nearest ancestral toward the root of the tree. This information is than incorporated in the "enriched phylogenetic tree".

Once a phylogenetic tree was obtained we established for convention that the change of the character state, that is the anagenesis, occurred between the nodes of the tree and the nodes represent the ancestrals from which originated new taxa with different characteristics. The characters presented in table I and on the right side of figure 1, correspond to the terminal taxa, and were used as a base to infer the characteristics of ancestrals. Since the characters of the terminal taxa are known, the characters of its more recent ancestral can be inferred. If all the terminal taxa of the clade considered possesses the same character state this will be attributed to its closest common ancestral. The same procedure was followed to determine the geographic distribution of the ancestral. When one of the terminal units of a certain clade occurs in the AOD, this distribution will be attributed to the ancestral of the whole clade because the basic hypothesis states that the dispersion is unidirectional starting from AOD. In that case, a dispersion event originated in the AOD may have occurred within this clade. Even if the collecting place of the studied specimen is not the AOD but the distribution of the species includes AOD we plotted for him AOD in the tree.

The hypothetical reconstruction of the ancestral will be made starting from the top of the tree. When the enriched tree is finished, reading the tree from the root allows to follow the evolutionary history of the group.

Table I. Samples studied in this paper: identification (see Fig. 1), source of sequences (this paper or GenBank), karyotype of the species with source, and geographic origin ([1]Gardner & Patton, 1976; [2]Sbalqueiro *et al*., 1982; [3]Bianchi & Merani, 1984; [4]Bianchi *et al*., 1971; [5]Myers *et al.*, 1990; [6]Sbalqueiro & Nascimento, 1996; [7]This paper; [8]Myers & Patton, 1989b; [9]Geise *et al.*, 2001; [10]Patton, 1986; [11]Myers, 1989; [12]Lobato *et al*., 1982; [13]Margarete Mattevi pers. comm.; [14]Cibele Bonvicino pers. comm.; [15]Hsu & Benirshke, 1973; [16]Vitullo *et al*., 1986; [17]Valéria P. Firme pers. comm.; [18]Myers & Patton, 1989a; [19]Reig, 1986; [20]Esbérard *et al*., 2005; [21]Reig *et al*., 1968; [22]Reig *et al*., 1971; [23]Yonenaga & Ricci, 1969; [24]Maia & Langguth, 1981; [25]Bonvicino *et al*., 1998; [26]Freitas *et al*., 1984; [27]D'Elía & Pardiñas, 2004; [28]Kasahara & Yonenaga-Yassuda, 1984. *Probably all species of *Brucepattersonius* have the same karyotype that may be 2n=52 since Bonvicino *et al*., 1997, 1998 described the chromosomes of *B. griserufescens* Hershkovitz, 1998 as having this diploid number).

Sample identification	Source of sequence	Karyotype	Geographic origin of samples
Akodon aerosus Thomas, 1913	M35703	2n = 22-40[1]	Puno/Peru
Akodon azarae (Fischer, 1829)	U03529	2n = 38[2,3,4]	Neembacu/Paraguay
Akodon boliviensis Meyen, 1833	M35691	2n = 40[4,5]	Puno/Peru
Akodon cursor (Winge, 1887) 2	this paper	2n = 14[6]	Guaraqueçaba - PR Brazil
Akodon cursor 3	this paper	2n = 14[7]	Faz. Amazonas - RJ/ Brazil
Akodon cursor 4	this paper	2n = 14[7]	Faz. Amazonas - RJ/Brazil
Akodon aff. cursor 1	this paper	2n = 16[7]	Caruaru - PE/Brazil
Akodon aff. cursor 2	this paper	2n = 16[7]	Caeté, São Miguel - AL/Brazil
Akodon aff. cursor 3	this paper	2n = 16[7]	Faz. Aldeia, Valença - BA/Brazil
Akodon aff. cursor 4	this paper	2n = 16[7]	Sapé, PB/Brazil
Akodon aff. cursor 5	this paper	2n = 16[7]	Sapé, PB/Brazil
Akodon aff. cursor 6	this paper	2n = 16[6]	Guaraqueçaba - PR/Brazil
Akodon aff. cursor 7	this paper	2n = 16[6]	Guaraqueçaba - PR/ Brazil
Akodon juninensis Myers, Patton & Smith, 1990	M35698	2n = 40[5]	Junin/Peru
Akodon kofordi Myers & Patton, 1989	M35697	2n = 40[8]	Puno/Peru
Akodon lindberghi Hershkovitz, 1990	AF184057	2n =42[9]	Minas Gerais/Brazil
Akodon mimus (Thomas, 1901)	M35710	2n = 40[10]	Puno/Peru
Akodon molinae Contreras, 1968	AY494839	2n = 42-44[11]	Argentina
Akodon mollis Thomas, 1894	U03546	2n = 22[3,12]	Puno/Peru
Akodon montensis Thomas, 1913 1	this paper	2n = 24[7]	Itatiaia - RJ/Brazil
Akodon montensis 2	this paper	2n = 24[13]	Torres - RS/Brazil
Akodon montensis 3	this paper	2n = 24[14]	Faz. Intervales - SP/Brazil
Akodon montensis 4	this paper	2n = 24[13]	Torres - RS/Brazil
Akodon mystax Hershkovitz, 1998	this paper	2n = 42[7]	Caparaó - ES/ Brazil
Akodon orophilus Osgood, 1913	M35699	2n = 26[15]	Amazonas/Peru
Akodon puer Thomas, 1902	M35693	2n = 34[16]	Puno/Peru
Akodon reigi González, Langguth & Oliveira, 1998	this paper	2n = 44[17]	Itatiaia - RJ/Brazil
Akodon serrensis Thomas, 1902 1	this paper	2n = 46[7]	Macaé - RJ/Brazil
Akodon serrensis 2	this paper	2n = 46[7]	Caparaó - ES/ Brazil
Akodon siberiae Myers & Patton, 1989	U03548	2n = 38[18]	Cochabamba/Bolivia
Akodon subfuscus Osgood, 1944	M35695	2n = 40[5]	Arequipa/Peru
Akodon toba Thomas, 1921	U03527	2n = 40[19]	Presidente Hayes/Paraguay
Akodon torques Thomas, 1917	M35700	2n = 22-24[10]	Cusco/Peru
Blarinomys breviceps (Winge, 1887) 1	AY275112	2n = 28[20]	not given
Blarinomys breviceps 2	AF108668	2n = 28[20]	Estação Experimental Djalma Bahia - BA/Brazil
Brucepatersonius iheringi (Thomas, 1896)	AF108667	2n = 52*	not given
Brucepatersonius soricinus Hershkovitz, 1998	AY277486	2n = 52*	Estação Biológica de Boracéia - SP/Brazil
Necromys urichi (J. A. Allen & Chapman, 1897)	U03549	2n = 18[21,22]	T. F. Amazonas/Venezuela
Necromys amoenus (Thomas, 1900)	M35712	2n = 34[1]	Puno/Peru
Necromys lasiurus (Lund, 1840) 1	U03528	2n = 34[23,24]	Presidente Hayes/Paraguay
Necromys lasiurus 2	this paper	2n = 34[7]	Sapé - PB/Brazil
Oryzomys megacephalus (Fischer, 1814)	U03538	2n = 52[1]	Cusco/Peru
Oxymycterus dasytrichus (Schinz, 1821)	AF516665	2n = 54[25]	Tarituba - RJ/Brasil
Oxymycterus judex (Thomas, 1909)	AF516661	2n = 54[25]	Teresópolis - RJ/Brazil
Oxymycterus rufus (Fischer, 1814)	AF516669	2n = 54[25]	Villa del Carmen - San Luis/Argentina
Scapteromys aquaticus Thomas, 1920	AY275132	2n =32[26]	not given
Scapteromys tumidus (Waterhouse, 1837)	AY275133	2n =24[26,27]	not given
Thaptomys nigrita (Lichtenstein, 1829)	this paper	2n = 52[28]	Caparaó - ES/Brazil

RESULTS AND DISCUSSION

Phylogenetic Tree. Three phylogenetic trees were constructed by the methods of Neighbor-Joining (NJ) (Fig. 1), Minimum Evolution (ME) and Maximum Parsimony (MP). The topology of the taxa was similar in all of them in spite of the low bootstrap values for the basal clades. They differed however in the positions of two clades: *Scapteromys* Waterhouse, 1837 (clade II, Fig. 1) and *A. mystax* Hershkovitz, 1998 (clade VII, Fig. 1).

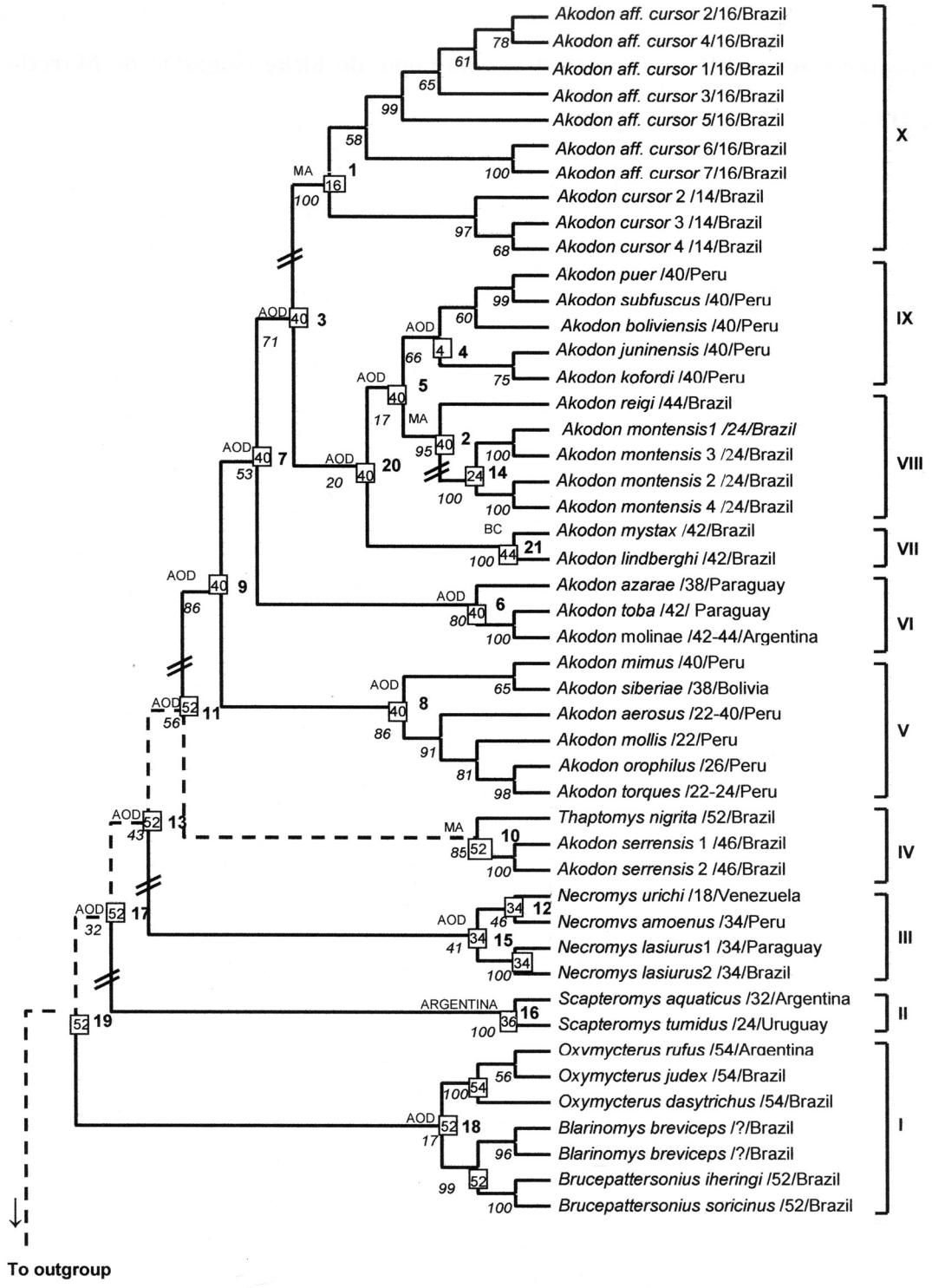

Figure 1. Phylogenetic tree obtained by the method of "Neighbor-Joining" considering genetic distances corrected by Kimura 2-parameters. The Roman numbers at right identify clades. For each sample is given: the species name followed by sample number when necessary; the diploid number between bars; the country of origin. The bootstrap values are indicated in italics below the branches. Bold numbers identify the ancestral of the clade (node). Inside the square the 2n is given. (AOD, area of original differentiation; MA, Atlantic Forest; BC, Central Brazil; //, chromosome revolution. Broken lines, lineages with ancestral karyotype).

In ME and MP the *Scapteromys* clade is the first clade at the base of the cladogram, while in NJ *Scapteromys* forms the second clade and the first is formed by *Brucepattersonius* Hershkovitz, 1998 and *Oxymycterus* Waterhouse, 1837. In ME *A. mystax* is a sister group of *A. reigi* González, Langguth & Oliveira, 1998 and *A. montensis* clade VIII. In MP *A. mystax* is a sister group of a large clade including clades VIII, IX and X (Fig. 1). This is a less parsimonious hypothesis and would imply in more dispersion events (see below).

The support for the older nodes in our topology is low, as is in the topology of Smith & Patton (1993). D'Elía (2003) grouped with high support (JK=100 and IB=22) the akodontines *Akodon*, *Deltamys* Thomas, 1917, *Necromys* Ameghino, 1889, *Thalpomys* Thomas, 1916 and *Thaptomys* Thomas, 1916 (clade E). Smith & Patton (1999) presented a clade with *Necromys*, *Thaptomys* e *Akodon* with high support (BS=97). In our topology the grouping *Akodon*, *Thaptomys* and *Necromys* had low support (BS=43; node 13 (Fig. 1)).

In D'Elía's (2003) study, *Necromys* groups with our clade IV while in our analysis *Necromys* is a sister group of all forms of *Akodon s.s.* but in both topologies support is low.

Smith & Patton (1993) found a strong support (BS=100) for the ancestral node of the genus *Akodon s.s.*, our topology shows a moderate support (BS=86; node 9) and that from D'Elía (2003) presents low support (JK; 50 and IB=3).

In our topology the basal genera, *Brucepattersonius* and *Blarinomys* Thomas, 1896 form a sister group of *Oxymycterus* (clade I). *Scapteromys* is a sister group of *Necromys* and *Akodon* with low support (BS=32, node 17). In D'Elía (2003) *Scapteromys* (clade A) occupies a more basal position being the sister group of *Brucepattersonius* and *Blarinomys* (clade B) but also with low support (JK<50, IB=1). In his cladogram the genus *Oxymycterus* (clade C) is placed closer to *Bibimys* Massoia, 1979 (clade D) and *Akodon* (clade E) and not to the genera *Brucepattersonius* and *Blarinomys* as seen in our topology. In support of our topology of these basal clades is the agreement with hypotheses of high chromosome numbers in basal forms.

Karyotype evolution. As seen above, to understand the evolution of the karyotype and establish the direction of the evolution we compared karyotype data with the molecular phylogenetic tree.

Since most rearrangements of karyotype are consequence of Robertsonian changes and inversions the 2n and NA are useful tools to trace the chromosome evolution in a certain group. Some caviomorphs, for instance, show a remarkable constancy of NA with changes in 2n. In sigmodontines both 2n and NA change. We work in our reconstruction of karyotype evolution with the diploid number alone but considering also, although not always explicitly, the size and number of arms in the whole karyotype. Primitive karyotypes in akodontines have large number of acrocentrics and derived karyotypes are formed in general by a smaller number of metacentrics usually consequence of centric fusions.

This tendency in akodontine rodents was suggested by several authors (Vitullo *et al.*, 1986; Reig, 1987; Geise *et al.*, 1998) but changes may occur in both directions and only knowledge of the phylogeny of the group can establish the direction of change. Vitullo *et al.* (1986), based on other evidence, suggested that the ancestral 2n of akodontines was 58.

In a general overview of the literature on akodontine karyotypes we observed that the differences among them may be the consequence of rearrangements. These rearrangements may be due to inversions or fusion/fission events of the whole karyotype or of large number of chromosomes ("chromosomal revolution") or to just a few Robertsonian changes in a few chromosomes ("low level chromosome diversification"). In establishing the ancestral karyotype of a clade we do not considered low level chromosome diversification. For instance 2n of 44, 42 and 40 were all considered as 2n=40. In the following paragraphs we will comment the enriched tree (Fig. 1), clade by clade, starting from the root.

Clade I is the most basal in our phylogenetic tree with ancestral in node 19. It includes three ecologically similar oxymycterine genera inhabitants of the Atlantic Forest floor. Most of them share 2n>52 in agreement with Vitullo *et al.* (1986) hypothesis that larger 2n is primitive. On the line that leads to *Blarinomys* a chromosome revolution occurred resulting in strong reduction of 2n.

Clade II includes *Kunsia tomentosus* (Lichtenstein, 1829) as a sister group of *Scapteromys aquaticus* Thomas, 1920 and *S. tumidus* (Waterhouse, 1837) according to D'Elía (2003). This clade derived from ancestral in node 17, and suffered a chromosome revolution reducing the 2n to 44 in *K. tomentosus* (Andrades-Miranda *et al.*, 1999) and, later, a further reduction to 2n=32 and 24 in *Scapteromys* by low level diversification. It includes swamp and fossorial rats inhabitants of open country. As shown by Andrades-Miranda *et al.* (1999) no autosomes are shared by *Kunsia* Hershkovitz, 1966 and *Scapteromys*. Further, *Scapteromys* differed more widely from the ancestor scapteromyine than *Kunsia*.

Clade III, derived from ancestral 2n=52 in node 13, suffered also a chromosome revolution reducing the 2n to 34, a rather stable karyotype of a genus of ample distribution in South America occupying open country from Venezuela to Argentina and Brazil. Its area of origin certainly was the AOD, because *Necromys* occurs today in this area. In D'Elía (2003) tree *Necromys* is a sister group of the genus *Thalpomys* that has a similar karyotype and is also inhabitant of open country in Brazil.

The clade IV is formed by two species of forest dwellers of the Atlantic Forest and has the ancestral number 11 with an hypothetical karyotype of 2n=52. The 2n=52 of *T. nigrita* is the old karyotype of node 19. The 2n=46 karyotype of *A. serrensis* Thomas, 1902 can be derived from the 2n=52 of *T. nigrita* by low level chromosome diversification.

The area of occurrence of ancestral 10 is the Atlantic Forest of Southeast Brazil. A similar conclusion may be reached interpreting the tree of D'Elía (2003).

Clade V derived from ancestral 9, includes a group with around 2n=40, with tendency to reduction in some members attaining a 2n=22. We suggest the 2n=40 for ancestral 8 because this number is also present in the

sister clade (ancestral 7). The area of occurrence of ancestrals 9 and 8 would be the region of Peru and Bolivia included in the AOD since most species of the clade V occur in Peru.

The clade VI derives from ancestral 7 with 2n=40. This hypothesis is confirmed by the presence of karyotype 2n=40 in the neighboring clades. Members of the clade VI occur in lowlands of Brazilian Southeast, and Northern Argentina, Paraguay and Uruguay which are the probable areas of occurrence of the ancestral. In the clade VI the 2n vary from 38 to 44 numbers derived by low level diversification from the ancestral 6.

The clade VII includes *A. mystax* with 2n=42. This karyotype may be derived by low level diversification from an ancestral 2n-40 in node 20

To check the position of *A. mystax* and *A. reigi* we prepared another tree including sequences in the GenBank used by SMITH & PATTON (1993), GEISE *et al*. (2001), D'ELÍA (2003) and specimens of *A. reigi* and *A. mystax* collected and identified by us. The result was: [*A. reigi* MNHN 3682, from Uruguay AY195865 D'ELÍA (2003). [*A. paranaensis* CIT 1131 from Venâncio Aires, RS AY195866 D'ELÍA *et al.*(2003). ["*A. mistax*" MN 48041, from Rio de Janeiro State AF184054 GEISE *et al*. (2001). [*A. reigi* from Itatiaia, RJ this paper. ["*A. mista*x" MN 48070 from Brejo da Lapa, Itatiaia, RJ AY273907 D'ELÍA (2003)]]]]].

Except for the first clade the remaining clades had BS over 90. We checked the identification of the "*mystax*" specimen MN 48070 (D'ELÍA, 2003) in the collection of the Museu Nacional do Rio de Janeiro, Brazil, it belongs to *A. reigi*. *Akodon mystax* MN 48041, from Rio de Janeiro State AF184054 GEISE *et al*. (2001) grouped with high bootstrap with our *A. reigi,* it was probably misidentified. *Akodon paranaensis* Christoff, Fagundes, Sbalqueiro, Mattevi & Yonenaga-Yassuda, 2000 is on morphological grounds e junior subjective synonym of *A. reigi*. The degree of genetic divergence sometimes considered "moderate" and others "deep" by GONÇALVES *et al*. (2007) is not a taxonomic criterion to separate species.

As a result, the true *A. mystax* form a sister group of a clade formed by *A. montensis* and *A. reigi.* The *A. lindberghi* Hershkovitz, 1990 from GEISE *et al.* (2001) groups with 100% BS with our specimen of *A. mystax.* According to GONÇALVES *et al*. (2007) *A. mystax* and *A. lindberghi* are sister species that can usually be separated morphologically.

Clade VIII includes *A. reigi* 2n=44 and *A. montensis* 2n=24. The phylogenetic line originated in node 2 suffered a karyotype revolution reducing drastically the 2n. See GEISE *et al.* (1998) for details of chromosome shuffling.

Clade IX includes a group of Peruvian species with a shared 2n=40, that is a sister group of the *A. reigi – A. montensis* assemblage of the Atlantic forest *Akodon.*

The clade X contains two parapatric sibling species *A. cursor* (Winge, 1887) 2n=14 and *A. aff. cursor* 2n=16 that form two monophyletic groups. *Akodon cursor* 2n=14 showed an extensive polymorphism (YONENAGA, 1972; FAGUNDES *et al.*, 1998) and is distributed over the Southeastern part of the Brazilian Atlantic Forest. *Akodon aff. cursor* 2n=16 show a restricted polymorphism (MAIA & LANGGUTH, 1981) and is distributed over the Northeastern part of the Brazilian Atlantic Forest with an enclave in the State of Paraná.

If the tendency was toward the reduction of the 2n, the ancestral 1 of the clade X would have 2n=16 and may have occurred in the Atlantic forest of Southeastern Brazil.

The sister group of clade IV underwent between nodes 11 and 9 another chromosomal revolution reducing the 2n to around 40 chromosomes (38-44). This group suffered subsequent chromosome revolutions reducing independently the 2n to 14 (*A. cursor*) between nodes 1 and 3 on the one side and to 24 (*A. montensis*) between nodes 2 and 14 on the other side. These sister groups are genetically close (YONENAGA *et al.*, 1975; FAGUNDES *et al.*, 1997) being sibling species but are not part of the same lineage, that is, the 2n=14 species do not derived from the 2n=24 form. Besides this, most *Akodon* clades showed low level differentiation the 2n varying from 38 to 44 and in a few cases reducing gradually to 2n=22.

In the tree shown by D'ELÍA (2003) and D'ELÍA *et al.* (2005) there is a clade below the *T. nigrita-A. serrensis* branch that includes the genus *Bibimys,* a sequence not available to us. *Bibimys* is very specialized morphologically and with an equally odd karyotype 2n=70, NF=76 or 80 (DYZENCHAUZ & MASSARINI, 1999; GONÇALVES *et al.*, 2005). This form probably derived by chromosomal revolution from an ancestral with 2n=52. The tendency of karyotype evolution was in this case not to reduction but to increase of 2n. This is the only plausible hypothesis. It will be interesting to study the mechanisms that lead to this higher chromosome number in *Bibimys* comparing his chromosome banding patterns with *Brucepattersonius* and *T. nigrita*. Certainly pericentric inversions followed by centric fissions worked out this karyotype, as is usually the case in species that increase de diploid number.

The ancestral 19 common to the group of the *Oxymycterus*-like mice and to the *Akodon*-like mice had probably a diploid number 52 in agreement with the hypothesis of VITULLO *et al.* (1986), this number (52) is present in the clade I.

Members of the clade I have a karyotype with diploid number 52-54 and its ancestral an *Oxymycterus* like form occupied the AOD.

In summary within the universe of species studied, chromosomal evolution started from high diploid numbers 2n=58 according to VITULLO *et al.* (1986) or FN=64 as found in *Oxymycterus*, followed an evolutionary line of basic 2n=52 from which derived at different times branches which reduced 2n. This 2n reduction was probably the result of drastic chromosome repatterning events (chromosomal revolution).

Derived lines lead independently to reduction of 2n in *Scapteromys*, in *Necromys*, in ancestral *Akodon*, (node 40) in *Akodon montensis*, and in *Akodon cursor.* In these cases reduction of number was not simply consequence of centric fusion but involved also pericentric inversions. The general tendency to reduction was apparently reverted in the case of *Bibimys*.

Dispersion events from the AOD. Starting from the AOD, as defined by REIG (1986), several dispersion events may be suggested. The oldest one corresponds to the

arrival of oxymycterines in Brazil (clade I). This was followed by the *Scapteromys* dispersal to eastern lowlands (clade II). The next dispersion event (clade III, *Necromys*) occurred over the lowlands of Argentina, Paraguay, Uruguay and Southern and Central Brazil as well as northwards to Venezuela. A new dispersal refers to members of clade IV (*A. serrensis* and *A. nigrita*), to the Atlantic Forest of Brazil. The next dispersion event refers to clade VI [*A. azarae* (Fischer, 1829) and *A. molinae* Contreras, 1968 etc.] over the lowlands of Argentina, Uruguay and Brazil. Ancestral of clades VII and VIII (*A. mystax, A. reigi* and *A. montensis*) dispersed to the Atlantic Forest of Brazil. The last event refers to clade X formed by *A. cursor* and *A. aff. cursor* whose ancestral dispersed independently from the AOD to the Atlantic Forest.

Dating dispersion events. We used in our calculations the age of 3,55 My for the separation of *Akodon* from *Necromys* (node 13) as suggested by SMITH & PATTON (1993) to calibrate rates of $_{MT}$DNA sequence divergence at third position transversions. The estimates we obtained show that: ancestral 15 (Fig. 1) started a dispersion event of *N. urichi* (J. A. Allen & Chapman, 1897) towards Venezuela 3,51 My ago. The ancestral 11 that gave origin to the first dispersion of *Akodon* to the Atlantic Forest has an age of 3,11 My. The ancestral 7 that originated the clade VI (*A. toba* Thomas, 1921, *A. molinae* and *A. azarae*) in Paraguay and Argentina has an age of 2,52 My. The ancestral 20 that gave origin to the second dispersion of *Akodon* to the Atlantic forest have an age of 1,82 My. The ancestral 5 that gave origin to the *A. reigi – A. montensis* group of the Atlantic forest of Brazil has an age of 1,77 My. The ancestral 3 that originated the *A. cursor* clade has 2,13 My.

These dates confirm that the events of dispersion and differentiation of the akodontines occurred mainly during the Pliocene.

As any hypothesis in science the picture of the evolution of Brazilian akodontines presented here will change with time. We hope that this paper stimulates further studies in comparative karyology, as well as sequencing of other genes or other taxa that will falsify present hypothesis.

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