



Karyological analysis of *Proechimys cuvieri* and *Proechimys guyannensis* (Rodentia, Echimyidae) from central Amazon

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Abstract

The aim was to characterize the karyotype of rodents of the genus *Proechimys* from three localities in the central Brazilian Amazon, in the search for new markers that might shed light on our understanding of the taxonomy and evolutionary history of this taxon. Two karyotypes were found, viz., $2n = 28$, $FN = 46$ in individuals from the NRSP (Cuieiras River) and REMAN (Manaus), and $2n = 46$, $FN = 50$ in individuals from the Balbina Hydroelectric Plant. While individuals with the karyotype with $2n = 28$ chromosomes were morphologically associated with *Proechimys cuvieri*, their karyotype shared similarities with those of the same diploid number in two other regions. Although three karyotypes are described for *Proechimys cuvieri*, no geographic distribution pattern that defined a cline could be identified. Based on the morphological examination of voucher specimens and additional results from molecular analysis, the karyotype with $2n = 46$ and $FN = 50$ could be associated with *P. guyannensis*.

Key words: spiny rats, C-banding, Amazon region, karyotypes, FISH.

Received: July 28, 2011; Accepted: November 4, 2011.

Spiny rats of the genus *Proechimys* are among the most abundant terrestrial mammals in the Amazon forests (Malcolm *et al.*, 2005), the genus consisting of 25 valid species (Wilson and Reeder, 2005), and 57 karyotype forms (da Silva, 1998; Weksler *et al.*, 2001; Bonvicino *et al.*, 2005; Machado *et al.*, 2005). Patton and Gardner (1972) had previously proposed that chromosome data could be used as efficient markers in the separation of *Proechimys* species, this having proven to be especially true for locations in which there is sympatry of as yet unknown species of the genus (see da Silva, 1998; Patton *et al.*, 2000).

The *cuvieri*-group comprises only one nominal form, *Proechimys cuvieri* Petter 1978 (Patton, 1987), with a diploid number of 28 chromosomes. Nevertheless, three karyotype forms have been found in which the fundamental number (FN) is variable (Table 1). Revelation of the existence of clades with divergent mitochondrial DNA has placed in, evidence that *P. cuvieri* sensu Patton (1987) is composite (da Silva, 1998; Patton *et al.*, 2000). The *guyannensis* group includes two valid species, viz., *P. roberti* and *P. guyannensis* (Weksler *et al.*, 2001; Wilson and Reeder, 2005), although eight karyotype forms have already been described (Table 1).

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Herein, the cytogenetic data of specimens recognized as *Proechimys cuvieri* and *Proechimys guyannensis*, from three sites in the central Brazilian Amazon, were described, thereby contributing to an understanding of the geographic variation and taxonomy of these taxa.

Eleven individuals of the genus *Proechimys* were collected for analysis from three localities in the central Brazilian Amazon (Figure 1). The specimens were prepared and deposited in the Mammal Collection of the National Institute of Amazonian Research (INPA, Manaus). Voucher specimens were identified, according to Patton (1987).

Mitotic chromosomes were prepared from femur bone marrow, in accordance with the Ford and Hamerton (1956) protocol. C-banding, G-banding and the detection of nucleolus organizer regions (NORs), were according to protocols described by Sumner (1972), Seabright (1971), and Howell and Black (1980), respectively.

18S rDNA units were amplified according to Gross *et al.* (2010) from total genomic DNA extracted from *Caluromys philander* liver tissues (heterologous probe), "all human telomeric probes" were obtained according Ijdo *et al.* (1991). Probe labeling was with biotin-14-dATP by nick translation (BioNick Labeling System, Invitrogen). *In situ* fluorescent hybridization was based on protocols described by Pinkel *et al.* (1986) and Martins and Galetti Jr (2001), with modifications.

Table 1 - Chromosome characterization of *Proechimys* of the *guyannensis* and *cuvieri* groups, collecting localities and references (2n = diploid number; FN = fundamental number).

Group/species	2n	FN	Locality	Coordinate	Reference
<i>guyannensis</i> group					
<i>P. roberti</i> (cytotype D)	30	56	Ecologic Station Uruçuí-Una, Piauí, Brazil	08°52' S, 44°58' W	1
<i>P. roberti</i> (cytotype D)	30	56	Paraná e Peixe, Tocantins, Brazil	12°36' S, 47°54' W	1
<i>P. roberti</i> (cytotype E)	30	56	Cláudia, Mato Grosso, Brazil	11°35' S, 55°08' W	1
<i>P. roberti</i> (cytotype E)	30	56	Gaúcha do Norte, Mato Grosso, Brazil	13°02' S, 53°12' W	1
<i>P. roberti</i> (cytotype F)	30	56	Vila Rica, Mato Grosso, Brazil	09°54' S, 51°12' W	1
<i>P. roberti</i> (oris)	30	54-55	Goiás, Brazil	14°04' S, 47°45' W	2
<i>P. roberti</i> (oris)	30	54-55	Tocantins, Brazil	05°17' S, 48°18' W; 9°53' S, 48°17' W	2
<i>P. roberti</i> (oris)	30	54-55	Maranhão, Brazil	04°04' S, 44°58' W	2
<i>Proechimys</i> sp. A	38	52	Barcelos, Amazonas, Brazil	00°09'89" N 63°30'49" W	3
<i>Proechimys</i> sp. A	38	52	Santa Isabel, Amazonas, Brazil	00°18'50" N 64°01'40" W	3
<i>P. guyannensis</i>	40	56	Balta, Peru	10°08' S, 71°13' W	4
<i>P. guyannensis</i>	40	54	Cayenne, French Guyanna	04°37' N, 53°22' W	7
<i>P. guyannensis</i>	46	50	São João da Baliza, Roraima, Brazil	00°57'01" N 59°54'40" W	3
<i>P. guyannensis</i>	46	50	Balbina Hydroelectric Reservoir, Uatumã River, Amazonas, Brazil	01°55' S, 59°28' W	present work
<i>P. cherriei</i>	40	54	Cairara del Orinoco, Venezuela		5
<i>cuvieri</i> group					
<i>P. cuvieri</i>	28	46	Balbina Hydroelectric Reservoir, Uatumã River, Amazonas, Brazil	02°00' S 59°30' W	6; present work
<i>P. cuvieri</i>	28	46	Macaco, Jaú River, Brazil	02°05'29" S 62°08'22" W	7
<i>P. cuvieri</i>	28	48	Altamira, Pará, Brazil	6°35' S 6°54' W	7
<i>P. cuvieri</i>	28	50	La Trinité Mountains, French Guyanna	04°37' N, 53°22' W	8
<i>P. cuvieri</i>	28	50	Acre, Brazil	8°40' S, 72°47' W	7

1-Machado *et al.* 2005 ; 2-Weksler *et al.* 2001; 3-Bonvicino *et al.* 2005; 4- Patton and Gardner 1972; 5-Reig and Useche 1976; 6- Maia and Langguth 1993; 7-Patton *et al.* 2000; 8-Reig *et al.* 1979.

Hybridized chromosomes were analyzed using an Olympus BX 51 microscope and the images were captured with a digital camera (Olympus DP70), using the Image-Pro MC 6.0 software. Mitotic metaphases were processed on the Adobe Photoshop CS4 program, and chromosomes measured by means of the Image J public domain program. Chromosomes were classified according to Levan *et al.* (1964). The fundamental number was based on the number of autosomal arms (FN), as described by Gardner and Patton (1976).

Proechimys cuvieri individuals collected from the NRSP (two males) and the REMAN (two males and one female) showed 2n = 28 chromosomes and FN = 46. The autosomes consisted of seven metacentric, two submetacentric, one subtelocentric and three acrocentric, pairs. The sexual X chromosome was a medium sized acrocentric and the Y was a puntiform chromosome. The largest chromosomes in the karyotype consisted of one metacentric pair (pair 1), one subtelocentric pair (pair 2) and one acrocentric pair (pair 3) (Figure 2a).

Constitutive heterochromatin was encountered in the centromeric region of seven autosomal pairs, viz., three small metacentric pairs, submetacentric pair 5 and all the acrocentric pairs. Nevertheless, heteromorphism was also observed in the heterochromatic blocks in pair 9. Among the sex chromosomes, only the X chromosome had a weakly stained, proximal heterochromatic block in the long arm (Figure 2b). The G-banding pattern was useful in recognizing chromosome pairs (Figure 2c). As visualized by conventional coloration in some metaphases, the nucleolus organizer region (NOR) and 18 S DNAr sequences were all located interstitially in the long arms of submetacentric pair 5, coinciding with secondary constriction (Figure 2d). Telomeric probe hybridization occurred in the telomeric regions of both arms of all the chromosomes (data not shown).

The *Proechimys guyannensis* individuals collected at the Balbina Hydroelectric Plant (four males and two females) presented a diploid number of 46 chromosomes and FN = 50. Autosomes comprised two metacentric, one submetacentric and 19 acrocentric pairs (Figure 2e). X and Y

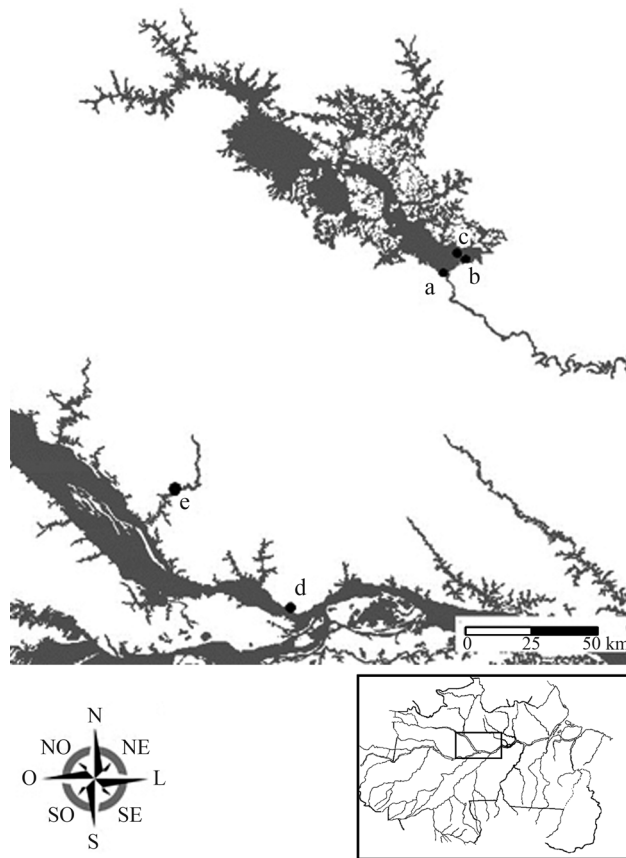


Figure 1 - Map indicating collection points of analyzed species: a) UHE Balbina location - 01°55' S, 59°28' W; Reservoir islands - b) 01°52'24.16586" S 59°24'49.09481" W, c) 01°50'59.95936" S, 59°26'24.72089" W; d) Isaac Sabbá oil refinery, REMAN - 03°08' S, 59°57' W; e) NRSP (at Cuieiras River) - 02°47' S, 60°27' W.

chromosomes were acrocentric, with the Y chromosomes approximately half the size of the X.

The positive C band, observed in the centromeric region, was distributed among 10 chromosome pairs, one metacentric and one submetacentric pair, the acrocentric pairs and the sex chromosomes. Whereas in X chromosomes, heterochromatin was located only in the centromeric region, Y chromosomes were completely heterochromatic (Figure 2f). The G-banding pattern was useful in recognizing chromosome pairs (Figure 2g). NOR and the 18S rDNA sequences, located interstitially in the long arm of a submetacentric pair (pair 11), coincided with secondary constrictions visualized in a few metaphases of conventional coloration (Figure 2h). Telomeric probe hybridization was observed in the telomeric regions of both arms of all the chromosomes (data not shown).

A diploid number of $2n = 28$ was encountered in four species of *Proechimys* belonging to three species groups (*sensu* Patton, 1987), viz., in *P. quadruplicatus* from the *goeldii* group (Gardner and Emmons, 1984; Patton *et al.*, 2000; Bonvicino *et al.*, 2005), *P. longicaudatus* (Machado *et al.*, 2005) and *P. brevicauda* from the *longicaudatus*

group (Patton and Gardner, 1972; Gardner and Emmons, 1984; Patton, 1987; Patton *et al.*, 2000), and *P. cuvieri* from the *cuvieri* group (Maia and Langguth, 1993, Patton *et al.*, 2000, and references therein). Considering only those species with $2n = 28$, 15 karyotype forms have been found, with fundamental number ranging from 44 to 50 (Weksler *et al.*, 2001).

Proechimys cuvieri is distributed throughout the Amazon basin, from southeastern Peru and the state of Acre (Brazil) in the west, to the Guyanas and the state of Pará (Brazil), in the East. There is partial overlapping of geographic distribution with the *goeldii*, *longicaudatus*, *simonsi* and *guyannensis* species groups (*sensu* Patton, 1987). Among all, the only one bearing karyotypes similar to that described here ($2n = 28$ and $FN = 46$) is the *longicaudatus* group. Nevertheless, differences in the size and position of the centromere in the sex chromosomes in *P. cuvieri* from our material and from the *longicaudatus* group (Patton *et al.*, 2000; Weksler *et al.*, 2001; Machado *et al.*, 2005) were noted. Based on sex-chromosome morphology and the distribution of constitutive heterochromatin of *P. cuvieri* specimens from REMAN and the NRSP, their karyotype could be related with those previously described for individuals from the Jaú River (Patton *et al.*, 2000), the Balbina Hydroelectric Plant in the Uatuma River (Maia and Langguth, 1993), and the Jari Valley (Eler *et al.* in press), all assigned to the *cuvieri* group.

Currently, three karyotype forms have been recorded for the *cuvieri* group, all with $2n = 28$ chromosomes, but with different fundamental numbers. $FN = 46$ prevailed in individuals collected from four localities in the central Amazon, namely the Balbina Hydroelectric Plant in the Uatuma River (Maia and Langguth, 1993), the Jaú River (Patton *et al.*, 2000), REMAN and NRSP (present study). $FN = 48$ occurred in the eastern Amazon, south of the Amazon River in Altamira, and the state of Pará (Brazil) (Patton *et al.*, 2000), and $FN = 50$ in localities as far distant as the upper Juruá River (state of Acre, Brazil) (Patton *et al.*, 2000) and Cayenne in French Guyana (Reig *et al.*, 1979).

On comparing our data with the conventional coloration of karyotypes from the Jaú River (Patton *et al.*, 2000), it is evident that these karyotypes are very similar to one another, but differ from those from the Balbina Hydroelectric Plant (Maia and Langguth, 1993), as to morphology of the sex chromosomes. In terms of C-band patterns, seven autosomal pairs and a tenuous band in the long arms of sexual chromosome X were present in individuals from REMAN and NRSP, whereas, in individuals from the Balbina Hydroelectric Plant (Maia and Langguth 1993), large heterochromatic blocks were noted in 11 autosomal pairs, and X and Y chromosomes were fully heterochromatic. C-band data for individuals from the Jaú River are inexistent.

The geographic distribution of *P. cuvieri* cytotypes does not presuppose a cline. Patton *et al.* (2000) reported

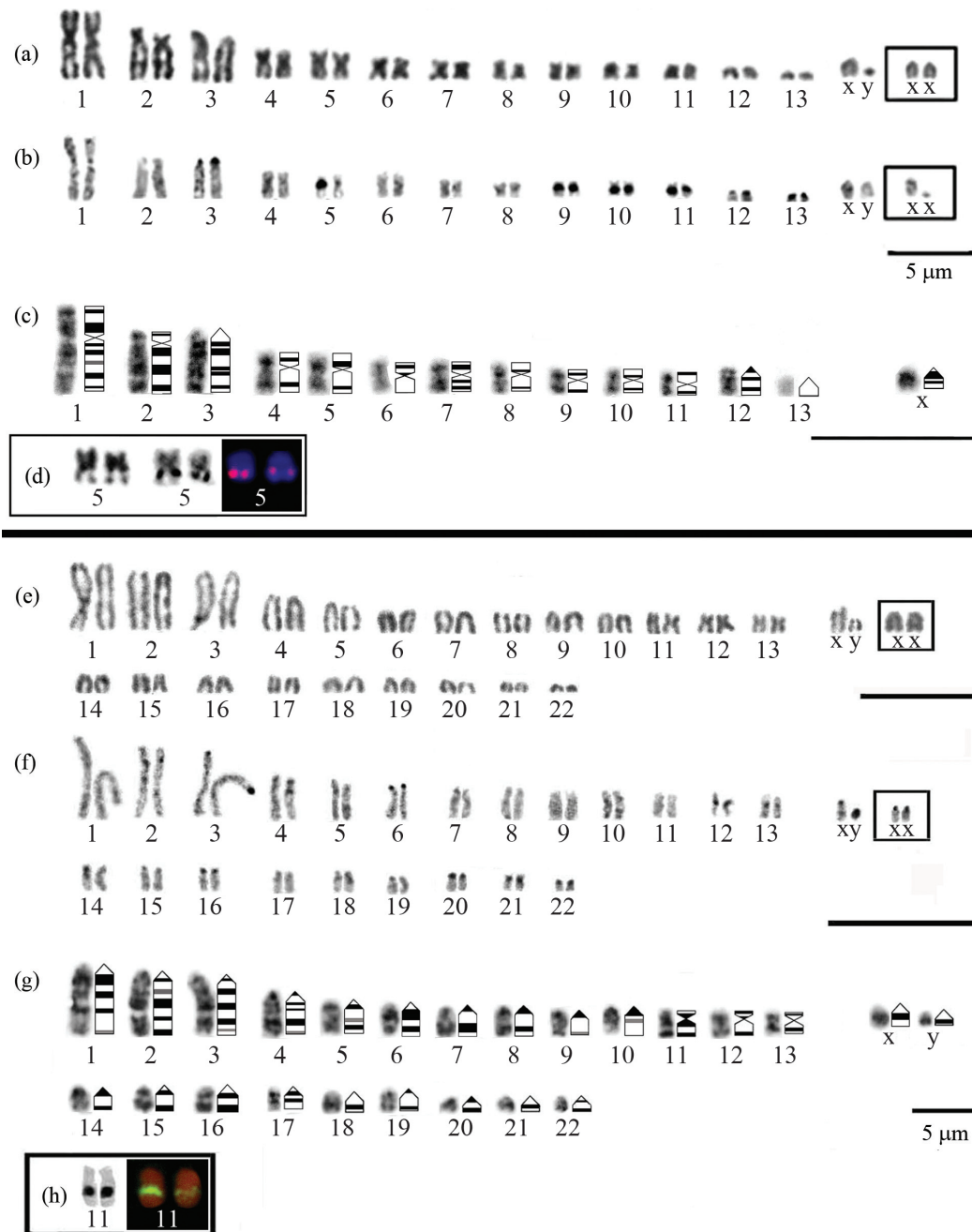


Figure 2 - Chromosome visualization for *Proechimys cuvieri* ($2n = 28$; $NF = 46$) by Giemsa staining (a), C-banding (b), G-banding (c), Ag-NOR staining (d), and for *Proechimys guyannensis* ($2n = 46$; $NF = 50$) by Giemsa staining (e), C-banding (f), G-banding (g), Ag-NOR staining (h). (m = metacentric; sm = submetacentric; st = subtelocentric; a = acrocentric).

FN = 50 in locales situated at both extremes of its distribution, in western (Juruá River, Acre, Brazil) and northeastern (Cayenne, French Guyana) Amazon, FN = 48 in the southeastern section of the Solimões-Amazonas axis, and FN = 46 in central Amazon and the northern part of the Solimões-Amazonas axis. Thus, despite the proposed uniformity in morphological characters for the *cuvieri* group (Patton, 1987), the presence of chromosome rearrangements appears to indicate that this group is composite. Molecular analysis of the cytochrome b mitochondrial gene

(da Silva, 1998; Patton *et al.*, 2000; da Silva *et al.*, unpublished data) certainly indicated differentiated regional units within the *cuvieri* group presenting degrees of divergence similar to those among other species of *Proechimys*. These results underscore the need for associating a more ample geographic sampling of *P. cuvieri* throughout its entire range, with genetic and morphological studies, in order to clarify species composition of this taxon.

In the *guyannensis* group, four diploid numbers have been described (30, 38, 40 and 46), as well as a variation in

the fundamental number from 50 to 56 (Table 1). Weksler *et al.* (2001) also associated the diploid number of 40 found in *P. pattoni* and *P. gardneri* (da Silva, 1998) with the same group, although without presenting an explanation for an association which is not supported by the limited molecular data currently available (see Figure 13 in da Silva, 1998). Weksler *et al.* (2001) also included specimens from Balta (Peru) with diploid number 40 in the *guyannensis* group, possibly since these animals, originally referred to as *P. guyannensis* by Gardner and Patton (1976), were considered as closely related to this species by Gardner and Emmons (1984). Patton (1987), on listing the specimens from Balta as *Proechimys* sp., placed them provisionally in the group *cuvieri*. Later on, da Silva (1998) included these specimens in *P. pattoni*.

The karyotype with 46 chromosomes, as described in the present study, was found in animals from the Balbina Hydroelectric Plant, situated within the areas of geographic distribution of the *goeldii*, *guyannensis* and *cuvieri* species groups (Patton, 1987). This diploid number, identified in *P. guairae* from the *trinitatis* group, presents a variation in FN = 68, 70 and 72 (George and Weir, 1973; Reig and Useche, 1976; Aguilera and Corti, 1994). As to diploid ($2n = 46$) and fundamental (FN = 50) numbers, the form found here is identical to that described by Bonvicino *et al.* (2005) for specimens from the Jatapu River in the state of Roraima (Brazil), listed as *Proechimys* sp. (B) and associated with the *guyannensis* group.

Based on the morphological examination of our voucher specimens, and due to the karyotypic similarities observed between individuals from the Balbina site and those described by Bonvicino *et al.* (2005), the karyotypic form $2n = 46$, FN = 50 from the Balbina Hydroelectric Plant was also associated with the *guyannensis* group.

Many similarities were found, on comparing this form with that described by Machado *et al.* (2005) for the region to the north of Manaus, with diploid number $2n = 44$ and FN = 52. The apparent differences between the two are the presence of a pair of large submetacentric chromosomes in $2n = 44$, and the morphology of the sex chromosomes. In the $2n = 46$ karyotype, the X and Y chromosomes are acrocentric, whereas in the $2n = 44$, the X chromosome is subtelocentric. Despite the similarities between the two, and the relatively short distance separating the two sampling areas, individuals with $2n = 44$ were not assigned by the to any of the *Proechimys* species groups. We also refrain from doing so here, due to the apparent inexistence of voucher specimens associated to this karyotype. However, when considering the statement (Machado *et al.*, 2005) that the karyotype $2n = 44$, FN = 52 belongs to a different taxonomic entity other than *P. cuvieri* and *P. guyannensis*, both currently the only two taxa of *Proechimys* recognized for central Amazon, it is not clear why they make a claim which certainly lacks support from Malcolm (1994). Thus, it remains unclear whether the diploid number with 44

chromosomes represents a case of chromosome polymorphism in *P. guyannensis* or, as suggested, a third taxon, as yet unknown in the region. In fact, molecular evidence implies that *P. cuvieri* and *P. guyannensis* may be composite.

The distribution of constitutive heterochromatin also differs between the two karyotypes. In the karyotype with $2n = 44$, constitutive heterochromatin is found in the centromeric region of all the autosomes and in the Y chromosome, except in pair 1. The X chromosome presented only a pale mark in the proximal region of the short arm. As to $2n = 46$, its presence was noted only in autosomal pairs (11, 12, 4, 5, 6, 16, 20, 21, 22), whereas in the X chromosome, centromeric labeling, as well as more tenuous labeling in the interstitial region, were perceptible.

Regarding the C-band pattern, the $2n = 46$ karyotype is very similar to the $2n = 38$ (*guyannensis* group) even as regards morphology of the sex chromosomes.

Comparative analysis between the G-band patterns of *P. cuvieri* and *P. guyannensis* was inconclusive, as to the identification of homeologies, possibly due to the high number of chromosomal rearrangements within the two. Indeed, molecular evidence presupposes high levels of genetic divergence among *Proechimys* species (da Silva, 1998).

As *Proechimys* sp. A ($2n = 38$), *Proechimys* sp. B ($2n = 46$) and *Proechimys guyannensis* ($2n = 40$) form a well supported monophyletic clade (Bonvicino *et al.*, 2005), and without discarding inversion re-arrangements, plausibly centric fission/fusion events were involved in differentiation of the diploid number groupwise.

As regards nucleolus organizer regions, Yonenaga-Yassuda *et al.* (1985) proposed that the nucleolar pair is homeologous in all the species of the Echimyidae family. However, there is considerable variation in the position of this pair in the karyotypes of several *Proechimys* species, due to non-Robertsonian re-arrangements in other autosomes. The two species under analysis are no exception. This region was located in the 5th pair in *P. cuvieri*, and in the 11th in *P. guyannensis* ($2n = 46$, FN = 50).

Interstitial telomeric signals (ITS) were not detected in *P. cuvieri* and *P. guyannensis*, when using telomeric probes, and neither in *P. gr. goeldii*, $2n = 15$ (Machado *et al.*, 2005) and *P. guairae guairae*, $2n = 48$ (Garagna *et al.*, 1997). To date, data on repetitive DNA mapping on *Proechimys* is very scanty. The lack of chromosome-banding information leaves many gaps, and hinders a more precise analysis of homologies between karyotypes. Consequently, it is, as yet, impossible to define either evolutionary trends, or which karyotype could be considered as the most basal for the genus *Proechimys*, to thereby trace main chromosomal evolutionary trends, as has been done for other groups of Amazonian organisms, such as bats (Silva *et al.*, 2005), and various families of fish (Feldberg *et al.*, 2003; Artoni and Bertollo, 2001).

The lack of a robust phylogenetic hypothesis for this genus is a primary problem (Lara *et al.*, 1996; da Silva, 1998; Leite and Patton, 2002; Galewski *et al.*, 2005). Obviously, supplementary studies of phylogenetics, which include chromosome markers and a larger number of representative species, are crucial in furthering understanding of chromosomal evolution and genome organization in *Proechimys*.

In fact, this lack of knowledge is representative of practically all the taxa of the Amazonian mammalian fauna, and underscores the need for biodiversity surveys and associated collection-based data on most of the forest organisms throughout the region.

Acknowledgments

The authors wish to thank R.A. Andrade and M.A.A. Schetino for help with field work, and C.H. Schneider, M.C. Gross and N.D.M. Carvalho for laboratory help and tips. This work was supported by the Instituto Nacional de Pesquisas da Amazônia (INPA) through the Research Institutional Projects (PRJ.05-73), Petrobrás S/A, Instituto de Pesquisas Ecológicas (IPÊ), Programa de apoio a Núcleos de Excelência em Ciência e Tecnologia (PRONEX). C.E.F. e Silva received a grant from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). The English was revised by Glenn Sheperd.

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Associate Editor: Yatiyo Yonenaga-Yassuda

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