doi: 10.5123/S2176-6223201100100007

Identification of a long-standing colony of *Proechimys* at the Instituto Evandro Chagas, Pará, Brazil, based on cytogenetic information

Identificação de uma colônia de longo prazo de *Proechimys* no Instituto Evandro Chagas, Pará, Brasil, com base em informações citogenéticas

Identificación de una colonia de largo plazo de *Proechimys* en el Instituto Evandro Chagas, Pará, Brasil, con base en informaciones citogenéticas

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ABSTRACT

The taxonomic classification of the genus *Proechimys* is complex because many of its species are morphologically similar but chromosomally different, with diploid (2n) values ranging from 14 to 62. The "Seção de Criação e Produção de Animais de Laboratório do Instituto Evandro Chagas" (The Division for Breeding and Production of Laboratory Animals, Instituto Evandro Chagas, Brazil) maintains a *Proechimys* colony for biomedical research. The colony members have been classified as *P. guyannensis*, which reportedly has 2n=40 and a fundamental number (FN)=54. However, using karyotype analysis to aid in their taxonomic classification, we instead observed that a sample of the animals in this colony have 2n=30 and FN=56, with a medium-sized submetacentric X chromosome and a small acrocentric Y chromosome. Constitutive heterochromatin was distributed as follows: in the pericentromeric regions of chromosomes 6, 7, 9, 10, 11, 12, 13, 14 and X; on the distal short arms of chromosomes 3, 6, 10 and X; on the distal long arm of chromosome 12; on the long arm of the Y chromosome; and distally on both arms of chromosomes 7, 9 and 11. The nucleolar organizer regions (NORs) are located on the long arm of chromosome 9. This karyotype is consistent with that described previously for *P. roberti*, but not *P. guyannensis*, thus demonstrating the importance of using karyotyping for the taxonomic identification of *Proechimys*.

Keywords: Chromosomes; *Proechimys*; Rodentia; Karyotyping.

INTRODUCTION

The taxonomic classification of representatives of the family Echimyidae (Hystricognathi – Rodentia) is controversial, with the number of recognized genera ranging from 14[1] to 16[2]. Patton and Rogers[3] stated that *Proechimys* is one of the less well-known and more taxonomically complex genera among the Neotropical rodents. There is debate regarding the number of species in this genus because many of these species vary little in their morphological traits. Furthermore, many of the traits that are traditionally used in the systematic classification of mammals, such as fur color, dental enamel pattern, and the number and position of plantar tubercles[4,5,6,7], differ in these species based on geography and the age of the animal, further complicating the clear definition of populations and taxa (Figure 1).

The number of *Proechimys* species identified in the literature ranges across different authors and papers: Tate[8] recognized 46 species, Gardner and Emmons[1] identified 32, Ellerman[9] reported 21, Moojen[5] found 15, and Cabrera[10] recognized only 12. Patton[11] used bacular and skull data to define 59 species of *Proechimys* organized into nine groups:

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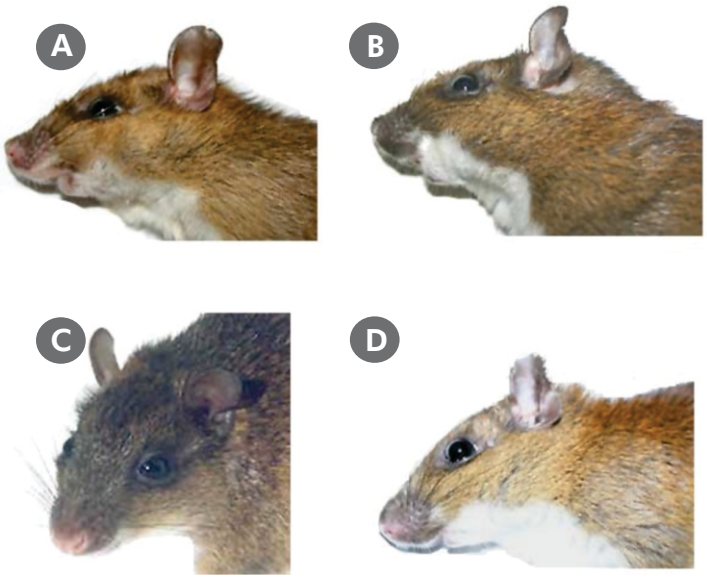

Figure 1– Proechimys belonging to the species *P. simonsi*: (**A**), *P. steerei* (**B** and **D**, with different fur color), and *P. roberti* (**C**)

three monotypic groups (*decumanus*, *canicollis*, and *simonsi*) and six polytypic groups (*semispinosus, longicaudatus, goeldii, cuvieri, trinitatus* and *guyannensis*). According to this classification, the guyannensis group was composed of the species *P. guyannensis*, *P. cherriei*, *P. roberti*, *P. vacilator*, *P. oris*, *P. warreni*, *P. boimensis*, *P. arescens*, *P. riparum*, and *P. arabupu*.

These species range from the coastal lands of the Guyanas to the Rio Negro basin and the eastern half of the Amazon basin. Some populations are also found in Goiás and Minas Gerais in Brazil. From this study, the geographic distribution of many species of this genus was defined, such as *P. guyannensis*, which was found in the left banks of the Amazon River, and *P. roberti*, which was found in the right banks. This was confirmed by other authors[2,12,15,16]. However, despite this extensive classification, the number of species in each group remains unclear. More recently, Emmons and Feer[12] reported that there are probably no more than 20 or 30 species of *Proechimys*, whereas Nowak[2] found 32 species. Clearly, additional lines of evidence, such as skull structure and karyotype, must also be analyzed concurrently to devise a more precise strategy for identifying the species within *Proechimys*[11].

Based on studies of the sequence of the mitochondrial gene Cytochrome b, Lara et al[13] suggested elevating *Trinomys*, a former subgenus of *Proechimys*, to the genus level. Leite and Patton[14] analyzed the sequences of Cytochrome b, as well as the 12S and 16S genes, which provided support for the proposal of Lara et al[13]. These data show the potential of molecular studies to elucidate the taxonomy and phylogenetic relationships of this group of rodents.

As *Proechimys* has huge karyotypic variability, with a diploid (2n) number ranging from 14[17] to 62[18], cytogenetic analysis appears to be particularly well-suited for resolving the taxonomic problems of this genus. Weksler et al[19] used cytogenetic data and other information to evaluate the taxonomic status of the species *P. roberti* Thomas, 1901 and *P. oris* Thomas, 1904, and concluded that *P. oris* is actually a junior synonym of *P. roberti*. The authors combined their data with that of previous reports to compile a table of the 48 karyotypes described for *Proechimys* (Table 1), organized according to the species groups proposed by Patton[11]. The karyotypic characterization of *P. roberti* was later confirmed by Machado et al[20], using samples obtained from Piauí, Tocantins, and Mato Grosso States in Brazil.

Table 1– Karyotypic formulas on *Proechimys* species modified from Weksler et al[19]. Species grouped according to Patton[11]

Species groups	2n	FN	Locality	Reference
guyannensis				
P. oris	30	56	Curuá-Uma, Pará, Brazil	1, 21
P. roberti	30	54-55	Goiás, Tocantins, Maranhão, Brazil	19
P. cherriei	40	54	Cairara del Orinoco, Venezuela	18
P. guyannensis	40	54	Cayenne and Saül, French Guiana	22
P. cf. pattoni (guyanensis)	40	56	Balta, Loreto, Peru	7
P. patonni	40	56	Juruá River, Amazonas, Brazil	23
P. gardneri	40	56	Juruá River, Amazonas, Brazil	23
goeldii				
P. steerei	24	42	Pucallpa, Peru; Loreto, Peru; Southern Peru	1, 7, 18
P. cf. steerei	24	44	Ucayali, Peru	24

continuation

Table 1– Karyotypic formulas on *Proechimys* species modified from Weksler et al[19]. Species grouped according to Patton[11] (continuation)

P. amphichoricus	26	44	Amazonas Federal Territory, Venezuela	18
P. quadruplicatus	28	44	Limoncocha, Napo, Ecuador	1
P. quadruplicatus longicaudatus	28	42	La Poza, Santiago, Peru	1
P. longicaudatus	28	48	Jamari River, Roraima, Brazil	21
P. brevicauda	28-30	48-50	Southern Peru	1
P. gularis	30	48	Limoncocha, Ecuador	1
Proechimys sp. 1	28	51-52	Ucayali, Peru	24
Proechimys sp. 2	30	50	Ucayali and Loreto, Peru	24
Proechimys sp. 3	28	51-52	Loreto, Peru	24
Proechimys sp. 4 simonsi	34	56	Loreto, Peru	24
P. simonsi (hendeei)	32	58	Balta, Peru; Putumayo, Colombia	7,18
P. simonsi	32	58	Ecuador and Southern Peru	1
P. cf. simonsi cuvieri	32	57-58	Ucayali and Loreto, Peru	24
P. cuvieri	28	46	Uatumã River, Amazonas, Brazil	25
P. cuvieri trinitatis	28	50	Cayenne, French Guiana	22
P. poliopus	42	72	Táchira, Zulia and Merida, Venezuela	18
P. poliopus	42	76	Kasmera, Los Angeles del Tucuco, Venezuela	26
P. guairae	44-50	72	Aragua, Venezuela	27
P. guairae	46-52	72-74	El Limon, Turiamo, Palmero, Turén, Cueva de Agua and San Juan de Areo, Venezuela	26
P. guairae	46	68	Aragua, Carabobo and Falcon, Venezuela	18
P. guairae	46	70	Ocumare, Aragua, Venezuela	28
P. mincae	48	68	Minca (topotypes), Magdalena, Colombia	1
P. guairae spp.	50	66	Cojedes and Portuguesa, Venezuela	18
P. trinitatis	62	80	Cueva del Guacharo, Venezuela	26
P.trinitatis (P. urichi)	62	76	Monagas, Venezuela	18
P. urachi (Proechimys sp.)	62	66	Barinas, Venezuela	18
Proechimys sp. semispinosus	62	74	Guaquitas, Tierra Buena las Matas, and la Nilita, Venezuela	26
P. semispinosus	30	50	Isla Gorgona, Colombia; Choco, Colombia	29,30
P. semispinosus rosa	30	52	Santa Rosa, Ecuador	1
P. semispinosus	30	50-54	Limón, Costa Rica; Canal Zone, Panama; Valle, Colombia; Esmeraldas and El Oro, Ecuador	1,7
P. oconnelli Canicollis	32	52	Meta, Colombia	1
P. canicollis decumanus	24	44	Bonda (topotypes), Magdalena, Colombia; Rio Cachiri, Venezuela	1,26
P. decumanus	30	54	Aguas Verdes (topotypes), Tumbes, Peru and Guayas and El Oro, Ecuador	1
Unknown				
P. echinothrix	32	60	Juruá River, Amazonas, Brazil	23
P. kulinae	34	52	Juruá River, Amazonas, Brazil	23
Proechimys sp. 5	14-16	18	Amazonas, Brazil	17
Proechimys sp. 6	30	52	Juruá River, Amazonas, Brazil	21
Proechimys sp. 7	32	54	Boyacá, Colombia	31
Proechimys sp. 8	44	52	Manaus, Amazonas, Brazil	21
Proechimys sp. 9	36	58	Plácido de Castro, Acre, Brazil	32

Patton and Gardner[7], Gardner and Emmons[11], George and Weir[12], Barros[17], Reig and Useche[18], Weksler et al[19], Leal-Mesquita [21], Reig et al[22], Silva[23], Aniskin[24], Maia and Langguth[25], Aguilera and Corti[26], Reig[27], Bueno and Gomez-Laverde[29], Gomez-Laverde et al[30], Bueno et al[31], Ribeiro[32].
2n= diploid number FN= fundamental number

MATERIAL AND METHODS

The karyotypes of four animals (two males and two females) of *Proechimys* belonging to the SACPA-IEC colony were studied. The chromosomes were obtained by direct extraction from bone marrow[33] and analyzed by conventional staining using Giemsa, G-banding[34], C-banding[35] and Ag-NOR staining[36]. The metaphase chromosomes were photographed under Carl Zeiss III and Axiophot Zeiss microscopes, and karyotypes were mounted based on chromosome morphology, in order of decreasing size. This work was performed in accordance with the Brazilian national ethic rules (Law no. 11.794/08; Arouca Law).

RESULTS

The four animals were found to have 2n=30 , fundamental number (FN)=56. The autosomes comprised 13 pairs of biarmed chromosomes, ranging in size from large to small, and one pair of subtelocentrics. The X chromosome was found to be a medium-sized submetacentric chromosome, whereas the Y was a small acrocentric chromosome (Figure 2A). The determination of the chromosome pairs was made by G-banding pattern analysis (Figure 2B).

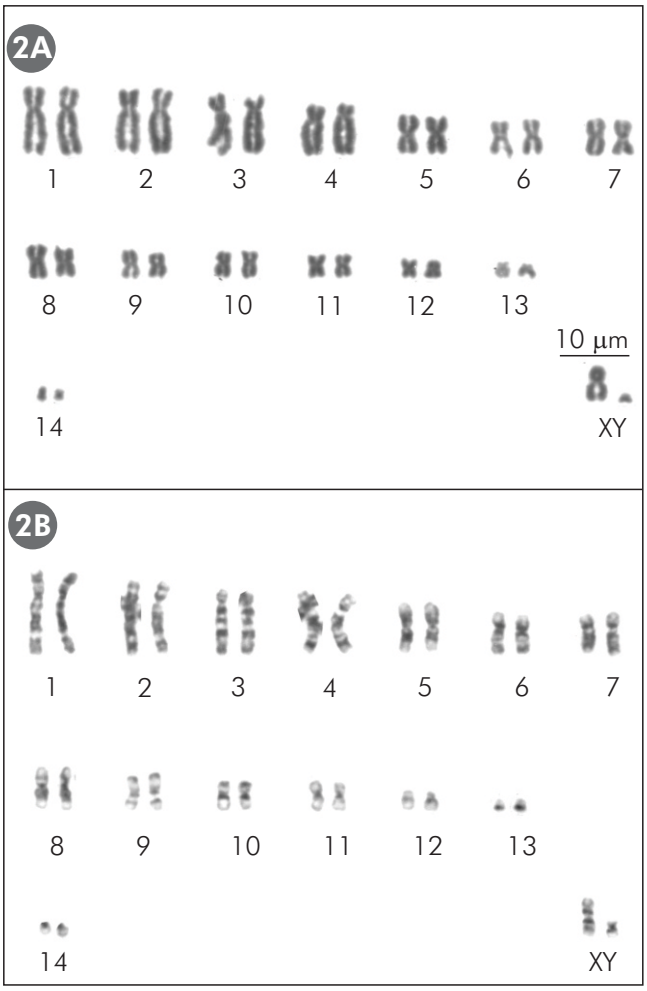

Figure 2 – Karyotype of *Proechimys* from the Instituto Evandro Chagas colony: **2A** conventional staining; **2B** G-banding.

C-banding (Figure 3A) revealed the presence of small blocks of pericentromeric constitutive heterochromatin (CH) in nine autosome pairs (6, 7, 9, 10, 11, 12, 13, 14, and X). CH was also found on the distal short arm of chromosomes 3, 6, 10, and X, on the distal long arm of chromosome 12, on the long arm of the Y chromosome, and distally on both arms of chromosomes 7, 9, and 11.

The nucleolar organizer regions (NORs) were located in the interstitial region of the long arm of chromosome 9 (Figure 3B).

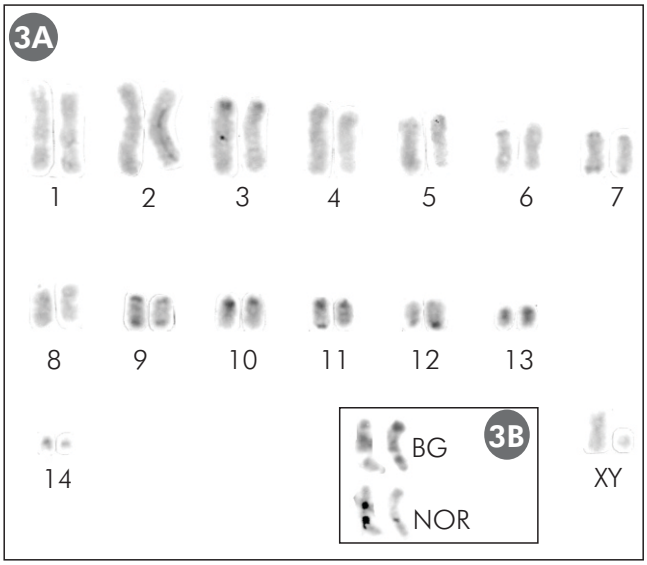

Figure 3 – Karyotype of *Proechimys* from the Instituto Evandro Chagas colony: **3A** C-banding; **3B** Ag-NOR staining after G-banding

DISCUSSION

When the rodent colonies were initially established at the Instituto Evandro Chagas, the *Proechimys* were classified as *P. guyannensis*. The published cytogenetic data on this species show karyotypic formulas of 2n=40, FN=56[7] for a sample from Balta (Peru), and 2n=40, FN=54[22] for samples from Cayenne and Saül (French Guyana). In sharp contrast, we found that the sample from the SACPA-IEC colony had 2n=30, FN=56. Comparison of our data (karyotypic formula, G- and C-banding, and Ag-NOR staining patterns) with those found in the literature for *Proechimys* shows that our cytogenetic results match those described by Weksler et al[19] and Machado et al[20] for *P. roberti*. Additionally, these animals were originally collected in the southwestern region of the Para state. This further suggests that the tested samples must belong to the *P. roberti* species because previous studies[2,12,15,16] demonstrated that *P. roberti* is found only at the right banks of the Amazon River, where there are no records of *P. guyannensis*.

Based on these results and those of other studies[19,20], the karyotype of the species *P. roberti* does not have numerical variation, with all tested samples having 2n = 30. However, there are morphological differences involving chromosome 14, the last autosome pair. Populations can be found with a

homomorphic, acrocentric pair (FN = 54), a heteromorphic pair, with one acrocentric chromosome, and a bi-armed chromosome (FN = 54-56), and a homomorphic pair, with two bi-armed chromosomes (FN = 56). As previously defined[19,20], such chromosomal differences are typical in populations with a defined geographic distribution. This makes them useful to identify populations with biogeographical and phylogenetic similarities.

Weksler et al[19] analyzed samples from Goiás and from Pará, Maranhão and Tocantins in the Amazonian region. In the north of Tocantins and Maranhão, they found karyotypes with FN = 54, in which both copies of chromosome 14 were acrocentric. In the other localities, the entire sample had FN = 56, with both copies of chromosome 14 being bi-armed. This is in agreement with the data of Gardner and Emmons[1] and Leal-Mesquita[21] for samples collected in Curuá-Una, Pará State, and with the data of Barros[17] for samples of the Transamazonia Road in Pará. The *Proechimys* colony from SACPA – IEC originated from the Barros[17] collection. Thus, our data for this colony are in agreement with Barros. Apart from the homomorphic states of the chromosome 14 pair (acrocentric or bi-armed), Weksler et al[19] identified some karyotypes with FN = 56 and FN = 54-56 in the same locality, meaning the chromosome 14 pair is heteromorphic. According to the authors, this is a consequence of a pericentric inversion in one of the homologues. These karyotypic patterns (NF = 56 and NF =54-56) were found in populations of the Cavalcante Farm, northeastern Goiás, and Primavera farms, northeastern Pará. Machado et al[20] also analyzed karyotypes of *P. roberti* from six regions of three Brazilian states where they found karyotypes with FN = 56 and bi-armed chromosome 14 homologues: the Ecological Station at Uruçuí-Uma, Piauí; Paranã and Peixe, Tocantins; Cláudia, Gaúcha do Norte and Vila Rica, Mato Grosso.

From the geographic and karyotypic analyses of *P. roberti*, the existence of two populations can be noted: a western population, in Pará, north of Tocantins, Goiás and Mato Grosso, with FN = 56 and a homomorphic, bi-armed chromosome 14 homolog pair; and an eastern population, located in Maranhão, south of Tocantins, with FN = 54 and an acrocentric chromosome 14 homolog pair. However, Weksler et al[19] found karyotypes with FN = 54-56 (with heteromorphic copies of chromosome 14) in populations from the Cavalcante farm, northeastern Goiás, and Primavera farm, northeastern Pará. However, it is possible that these are hybrid populations, resulting from the mix of the homomorphic groups (one-armed in the east and bi-armed in the west), as the heteromorphic populations are observed in the contact region of both the homomorphic types (northeast of Para and Goiás). This region may thus constitute a potential hybridization zone.

CONCLUSION

This mistake in classification is very understandable given all the problems related to classifying members of genus *Proechimys*, such as the relative lack of morphological variability among species and the intrapopulational variations in some traits typically used for mammal systematics. At the time of the colony's initial classification four decades ago, taxonomic identification was made exclusively using morphology.

Since many biomedical studies deal with the species-specific relationships among vertebrate reservoirs and pathogenic species (viruses, bacteria, fungi, protozoa, helminthes, etc.) that have been established over thousands of years of co-evolution, with different vertebrate species having different reactions to pathogens, it is very important that model animals be correctly characterized. Due to the morphological similarities among species of *Proechimys*, these rodents should be accurately identified using techniques beyond morphological examination. Here, we show that cytogenetic studies, in conjunction with other data, are very useful tools for the precise definition of members of *Proechimys*.

By comparing the cytogenetic data of the sample of *P. roberti* from SACPA-IEC with published samples, we concluded that the SACPA-IEC sample belongs to the largest populational group of this species, with FN = 56 and a geographical distribution ranging from Pará to Mato Grosso. This suggests that the sample shares a similar evolutionary origin with that group and therefore must have the same genetic and biological properties that allow its distribution and survival in a forest environment characterized by a particular complement of pathogenic species. The SACPA-IEC *P. roberti* samples may thus represent an ideal model system in which to study species-specific relationships with pathogens.

ACKNOWLEDGMENTS

We thank Dr. Regina Barros (UFPa) for her expertise in the field of cytogenetics, Doctor Reinaldo Carvalho (chairman of the SACPA-IEC at the time of this study) for permission to collect samples, the Instituto Evandro Chagas and Universidade Federal do Pará for the use of laboratory facilities, and CNPq and CAPES for financial support.

FINANCIAL SUPPORT

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Conselho Nacional de Desenvolvimento Científico e Tecnológico, Universidade Federal do Pará, Instituto Evandro Chagas.

Identificação de uma colônia de longo prazo de *Proechimys* no Instituto Evandro Chagas, Pará, Brasil, com base em informações citogenéticas

RESUMO

A classificação taxonômica do gênero *Proechimys* é complexa porque muitas de suas espécies são morfologicamente semelhantes, porém diferentes cromossomicamente, com números de diploide (2n) que variam entre 14 e 62. A Seção de Criação e Produção de Animais de Laboratório do Instituto Evandro Chagas mantém uma colônia de *Proechimys* para pesquisa biomédica. Os membros da colônia foram classificados como *P. guyannensis*, que possui 2n = 40 e um número fundamental (NF) = 54. No entanto, ao utilizar a análise do cariótipo para auxiliar em sua classificação taxonômica, observamos que uma amostra dos animais desta colônia possuem 2n = 30 e NF = 56, com um cromossomo X submetacêntrico de tamanho médio e um cromossomo Y acrocêntrico pequeno. A heterocromatina constitutiva foi distribuída da seguinte forma: nas regiões pericentromérica dos cromossomos 6, 7, 9, 10, 11, 12, 13, 14 e X; na porção distal dos braços curtos dos cromossomos 3, 6, 10 e X; na porção distal do braço longo do cromossomo 12; no braço longo do cromossomo Y; e nas porções distais de ambos os braços dos cromossomos 7, 9 e 11. As regiões organizadoras nucleolares (NORs) localizam-se no braço longo do cromossomo 9. Este cariótipo é consistente com o descrito anteriormente para *P. roberti*, não para *P. guyannensis*, o que demonstra a importância do uso de cariotipagem para a identificação taxonômica de *Proechimys*.

Palavras chave: Cromossomos; *Proechimys*; Roedores; Cariotipagem.

Identificación de una colonia de largo plazo de *Proechimys* en el Instituto Evandro Chagas, Pará, Brasil, con base en informaciones citogenéticas

RESUMEN

La clasificación taxonómica del género *Proechimys* es compleja porque muchas de sus especies son morfológicamente semejantes, aunque diferentes cromosómicamente, con números de diploide (2n) que varían entre 14 y 62. La Sección de Cría y Producción de Animales de Laboratorio del Instituto Evandro Chagas mantiene una colonia de *Proechimys* para investigación biomédica. Los miembros de la colonia fueron clasificados como *P. guyannensis*, que posee 2n = 40 y un número fundamental (NF) = 54. Sin embargo, al utilizar el análisis del cariotipo para auxiliar en su clasificación taxonómica, observamos que una muestra de los animales de esta colonia tenía 2n = 30 y NF = 56, con un cromosoma X submetacéntrico de tamaño mediano y un cromosoma Y acrocéntrico pequeño. La heterocromatina constitutiva fue distribuida de la siguiente forma: en las regiones pericentromérica de los cromosomas 6, 7, 9, 10, 11, 12, 13, 14 y X; en la porción distal de los brazos cortos de los cromosomas 3, 6, 10 y X; en la porción distal del brazo largo del cromosoma 12; en el brazo largo del cromosoma Y; y en las porciones distales de ambos brazos de los cromosomas 7, 9 y 11. Las regiones organizadoras nucleolares (NORs)se localizan en el brazo largo del cromosoma 9. Este cariotipo es consistente con lo descrito anteriormente para *P. roberti*, no para *P. guyannensis*, lo que demuestra la importancia del uso de cariotipado para la identificación taxonómica de *Proechimys*.

Palabras clave: Cromossomos; *Proechimys*; Roedores; Cariotipificación.

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Recebido em: 9/9/2010
Aprovado em: 4/5/2011