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Karyotypic analysis in species of the genus *Dasyprocta* (Rodentia: Dasyproctidae) found in Brazilian Amazon

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ABSTRACT

A total of 30 animals of the genus *Dasyprocta* were cytogenetically studied. They belong to the following species: *D. prymnolopha* (N=20), *D. leporina* (N=6), *D. fuliginosa* (N=1) and *Dasyprocta* sp. (N=3) (Dasyproctidae, Hystricognathi). Cell suspensions were obtained by peripheral blood culture, besides bone marrow and spleen cells, from *D. prymnolopha* and *D. leporina*. The diploid number was 64/65 for all samples. The karyotypes showed similarity, and chromosomal polymorphism was not detected by Giemsa conventional **staining** and G banding. The constitutive heterochromatin distribution at the pericentromeric region of all the chromosomes was similar in all species. *D. prymnolopha, D. leporina* and *Dasyprocta* sp. presented variation in the heterochromatical block size at one of the homologues of the A18 pair. *D. fuliginosa* presented the heterochromatin uniformly distributed in all chromosomes. There was not variation in the NORs pattern in the species studied.

Key words: Cytogenetics, Hystricognathi, *Dasyprocta*, Karyotype.

INTRODUCTION

The *Dasyprocta* (Hystricognathi) genus includes about 11 species and 33 subspecies, and occurs from the south Mexico as far as high Amazon, including the Antilles, Colombia and Peru (Eisenberg 1989, Emmons 1990). Some forms are not easily recognizable due to taxonomic problems, and absence of any modern taxonomic revision (Emmons 1990).

Cytogenetic data in this genus are scanty. From eleven accepted species, only three (*D. leporina, D. fuliginosa* and *D. variegata*) have their karyotypes studied, presenting 2n=64 chromosomes with conventional staining (Hungerford and Snyder 1964, Fredga 1966, Hsu and Benirschke 1968, Kasahara and Yonenaga-Yassuda 1984). Lima (1993) studied *D. leporina* and *D. fuliginosa* species through G and C banding standards and through Nucleolus Organizer Regions (NORs). Both species present well-defined constitutive heterochromatin located in the pericentromeric region of all the autosome chromosomes, with variable intensity. The X chromosome presents pericentromeric C banding and the Y chromosome of *D. leporina* is almost totally heterochromatic. In both species, the Ag-NOR is located in the telomeric region of only one acrocentric pair.

Therefore, the presented research has the proposal of characterize karyotypes of *D. leporina, D. prymnolopha, D. fuliginosa* and *Dasyprocta* sp. in specimens from the Brazilian Amazon, using G (GTG) and C (CBG) bandings and NOR techniques

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TABLE I

Species and number of animals specified by sex, using preparations obtained with different tissues.

Species	Peripheral blood	Bone Marrow	Spleen
D. prymnolopha	20 (9F and 11M)	6 (1F and 5M)	3M
D. leporina	6 (2F and 4M)	2 (1F and 1M)	2 (1F and 1M)
D. fuliginosa	1F		
Dasyprocta sp.	3 (1F and 2M)		
Total	30	8	5

F = female; M = male

to verify chromosomal polymorphism.

MATERIALS AND METHODS

Twenty specimens of *D. prymnolopha*, six of *D. leporina*, one of *D. fuliginosa* and three of *Dasyprocta* sp. were cytogenetically analyzed. These specimens were maintained at the vivarium of the Federal University of Pará (UFPA) and Museu Paraense Emílio Goeldi Zoobotanic Park. The progeny from couples kept in the vivarium of the UFPA are included in this sample. Most of these animals are from unknown origin, including those from MPEG. All the animals were subjected to peripheral blood collection: eight to bone marrow and five to spleen.

Table I shows species, number and genus of animals analyzed by peripheral blood, bone marrow and spleen. To obtain metaphasic chromosomes from lymphocytes culture, the modified methodology described by Moorhead et al. (1960) was used, where the mitogenic agent utilized was the Pokeweed. To obtain chromosomes from bone marrow, the methodology described by Baker et al. (1982) with modifications was used.

Live Animals

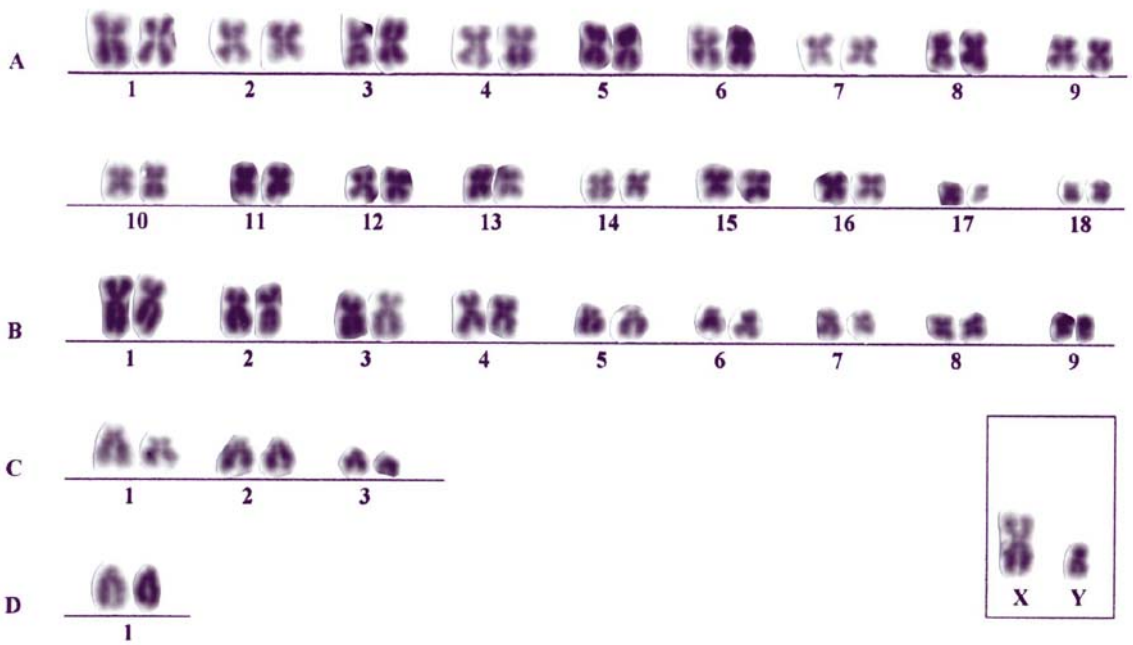
The animals were sedated with Ketalar (Parke-Davis & Co., Aché Laboratórios Farmacêuticos S.A. Brasil) and Rompum (Bayer do Brasil S.A. Veterinária, Brasil) by intramuscular way in proportions of 0.3 and 0.1 ml/kg of body weight, respectively. The bone marrow cells collection was done in the region of femur-tíbio-patelar joint with syringe and BD (Luerlok) 30 × 12 special needle with mandril. The collected material was transferred to the centrifuge's tube that contained 5ml of medium HAM-F10, added of 20% of foetal serum and 0,025 ml of colchicine at 0.02% being incubated at 37°C during 40-60 minutes.

Sacrificed Animals

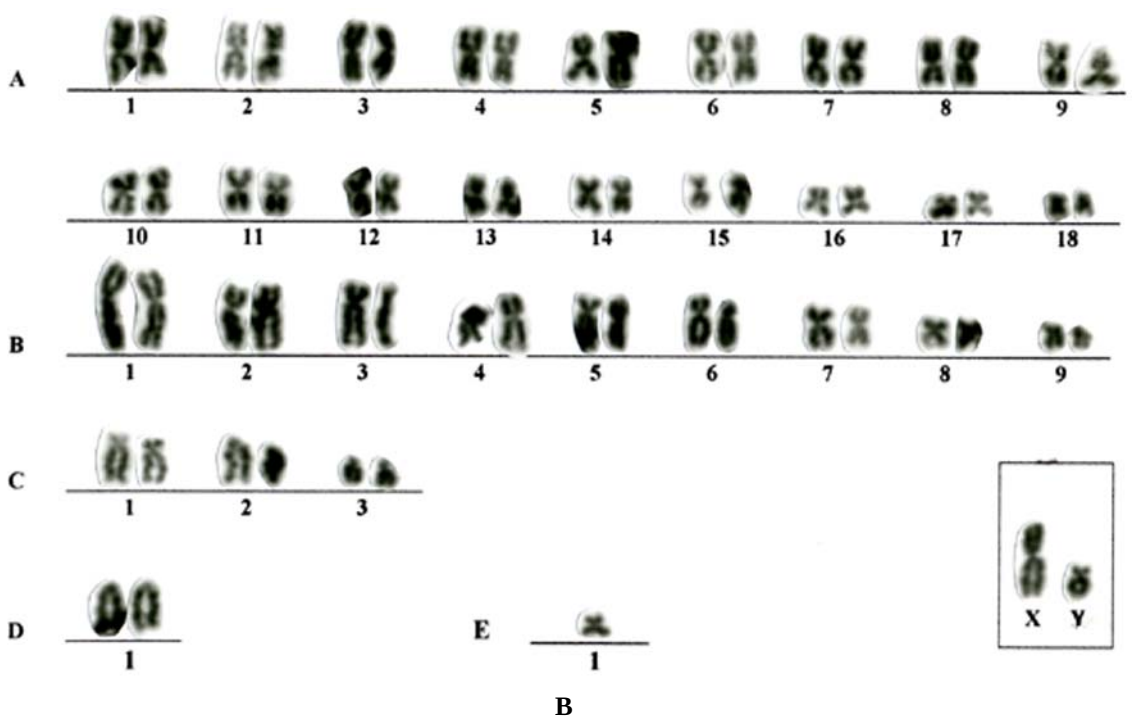
The animals were sacrificed with an excess dosis of Ketalar and Rompum associated with ether or chloroform inhalation. Four hours before the sacrifice, a proportion of 1ml to 1000g of the body weight of colchicine at 0.02% was injected by intramuscular way. In addition to the bone marrow cells collected, the spleen was removed. To obtain metaphasic cells from spleen, the technique described by Ford and Hamerton (1956) was used. To obtain the G and C banding and NOR the methodologies described by Seabright (1971), Sumner (1972) and Howell and Black (1980) were used. The karyotypes were set up in decreasing size order in according to Hungerford and Snyder (1964) model.

RESULTS

All analyzed specimens presented a diploid number of 64/65 chromosomes (Figures 1A to 3B). This variation was observed with a frequency of about



A



B

Fig. 1 – Karyotypes of the lineages 2n=64 and 2n=65 (lymphocytes culture) from the male sample (N=277) of *D. prymnolopha* in conventional Giemsa stain. A) lineage 2n=64; B) lineage 2n=65.

70% in cells with $2n = 64$ and 30% in cells with $2n = 65$, in the three different tissues: peripheral blood in all species; bone marrow and spleen in *D. prymnolopha* and *D. leporina* (Tables II, III and IV). The lineage $2n = 65$ presents a supernumerary metacentric chromosome with size ranging between medium and small (Figures 1B, 2B and 3B) in *D. prymnolopha, D. leporina* and *Dasyprocta* sp., whereas in *D. fuliginosa* it seems to be a submetacentric (Figure 4). Through the conventional Giemsa stain, the chromosomes of the lot A ($2n = 64$) showed the same morphology in all studied species. The karyotypes were characterized by the following model:

Group A – 18 pairs of metacentric chromosomes being the A1 pair the largest metacentric and the A18 pair, the smallest, with other pairs varying in size from large to small. The A18 pair presents, in the short arm, a morphology similar to a lower degree of spiralization looking like a secondary constriction, in conventional stain (Figure 2A).

Group B – nine pairs of submetacentric chromosomes, ranging from large to small.

Group C – three pairs of subtelocentric ones, pairs C1 and C2 of medium size, and C3 pair with small size.

Group D – only one acrocentric pair with large size.

X Chromosome – submetacentric with large size.

Y Chromosome – small submetacentric, smaller than the B9 pair. This chromosome was observed in *D. prymnolopha, D. leporina* and *Dasyprocta* sp.; however only one female of *D. fuliginosa* was karyotyped.

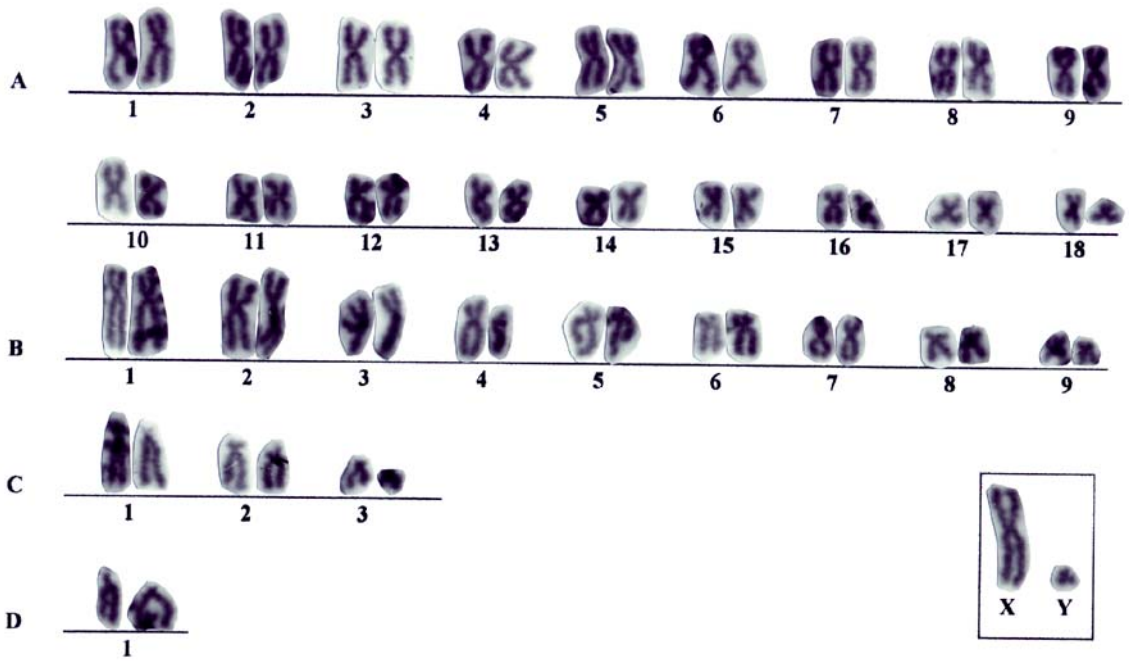
G band pattern was similar in all analyzed samples (Figure 4). C banding technique allows the identification in all species, in the different analyzed tissues, of constitutive heterochromatin in the pericentromeric region of the most of the autosomes, including the supernumerary chromosomes (Figure 5A).

In the X chromosome, the constitutive heterochromatin was restricted to pericentromeric region, whereas in the Y chromosome, was observed variation in the distribution of this chromatin. In the *D. prymnolopha* the constitutive heterochromatin was limited to pericentromeric region (Figure 5). In *D. leporina* and *Dasyprocta* sp., the Y chromosome was almost totally heterochromatic (Figures 5B and C). The A6 pair in *D. prymnolopha, D. leporina* and *Dasyprocta* sp. presented polymorphism in the amount of constitutive heterochromatin. One of the chromosomes showed the heterochromatic block twice the size of the other homologous of the pair (Figures 5A, B and C). In *D. fuliginosa* karyotype no polymorphism was observed (Figure 6). The A18 pair in *D. prymnolopha* and *Dasyprocta* sp. karyotypes showed constitutive heterochromatin in only one of the homologues (Figures 5A and C). This pair, in the *D. leporina* and *D. fuliginosa* karyotypes did not show variation (Figures 5B and 6). *D. fuliginosa* karyotype showed uniformity in the standard distribution of constitutive heterochromatin in all of chromosomes (Figure 6).

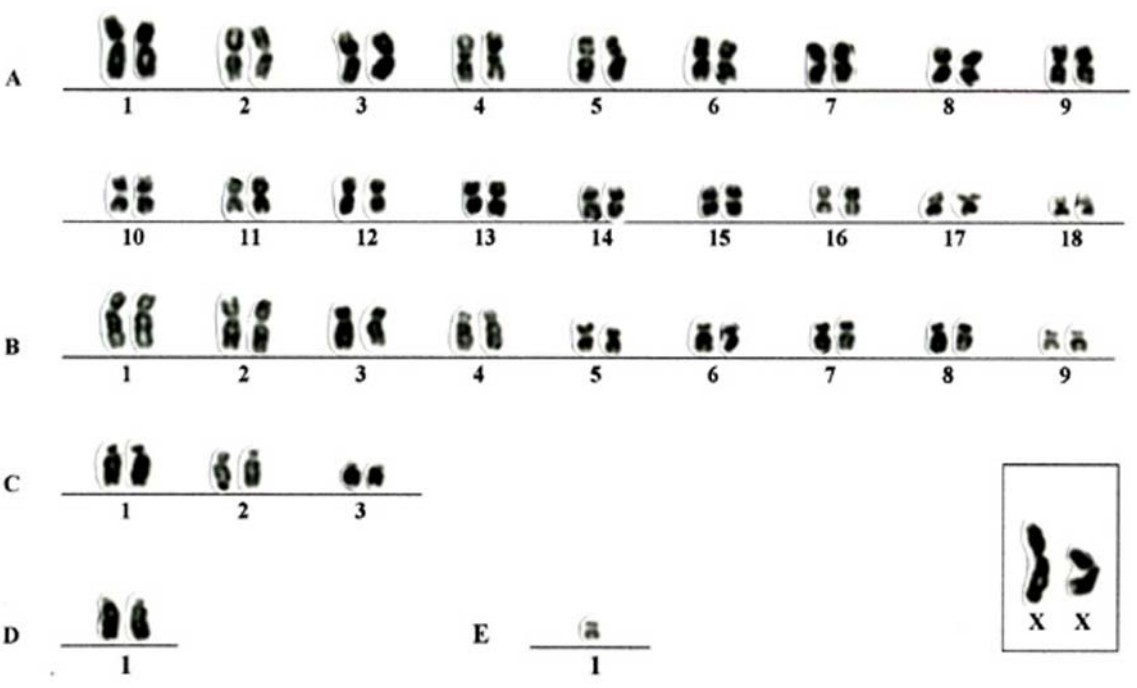
The NORs were observed in the telomeric region of D1 acrocentric pair short arm, in the karyotypes obtained using different tissues of all specimens studied (Figure 7).

DISCUSSION

All specimens of *D. prymnolopha, D. leporina, Dasyprocta* sp. and *D. fuliginosa* presented two cellular lineages, with $2n = 64$ and $2n = 65$ chromosomes. No animal showed only the basic karyotype of $2n = 64$, as described in the literature (Kasahara and Yonenaga-Yassuda 1984, Lima 1993, Lima and Langguth 1995, 1998). This variation is because of the presence of one supernumerary chromosome metacentric in the *D. prymnolopha, D. leporina* and *Dasyprocta* sp. and submetacentric in *D. fuliginosa*. Supernumerary chromosomes are considered common among the Hystricognathi, mainly in the Chinchillidae, Octodontidae, Caviidae, Ctenomyidae, Hydrochaeridae and Echimyidae (George and Weir 1974, Kasahara and Yonenaga-Yassuda 1984, Leal-Mesquita 1991a, b,

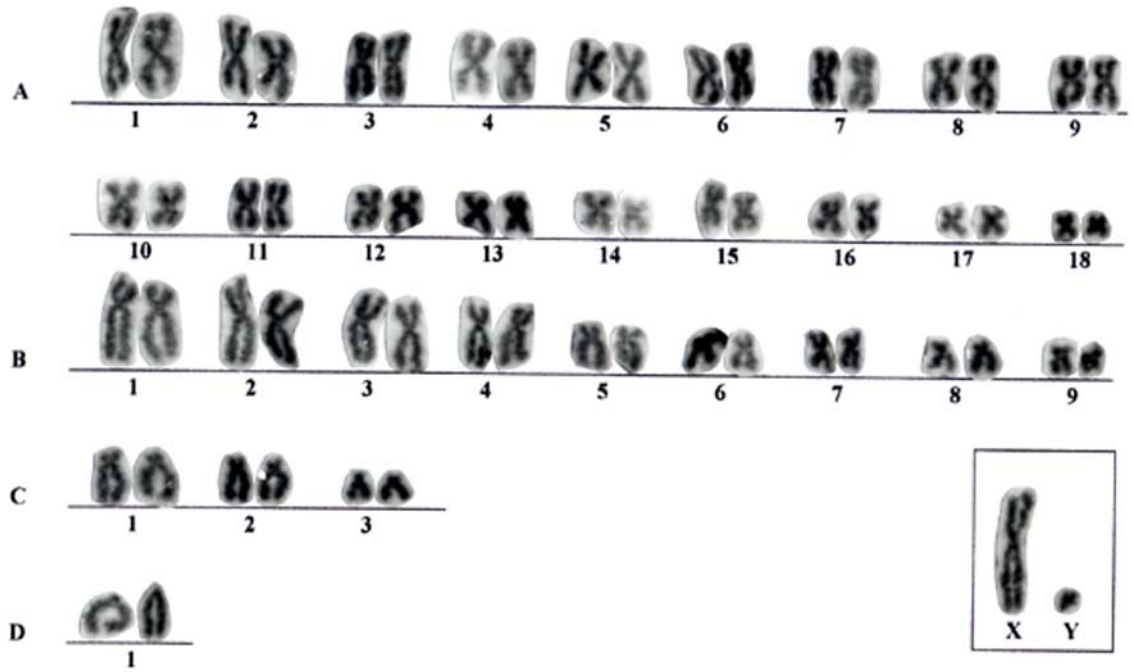


A

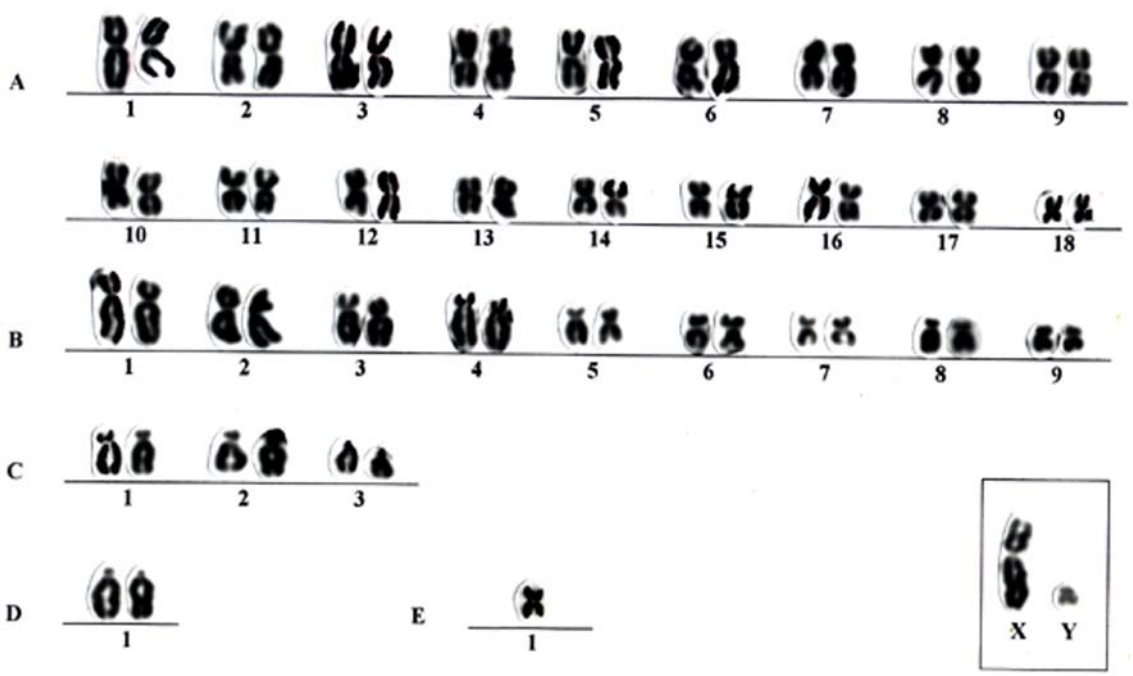


B

Fig. 2 – Karyotype of the lineage 2n=64 (marrow bone) and 2n=65 (lymphocytes culture) of *D. leporina* in conventional Giemsa stain. A) lineage 2n=64 from a male; B) lineage 2n=65 from a female.



A



B

Fig. 3 – Karyotypes of the lineages 2n=64 and 2n=65 (lymphocytes culture) from a male sample (N=283) of *Dasyprocta* sp. in conventional Giemsa stain. A) lineage 2n=64; B) lineage 2n=65.

TABLE II

Number of 2n=64 and 2n=65 cells, collected from peripheral blood, bone marrow and spleen, in *D. prymnolopha*.

Ident.	Sex	Peripheral blood				Bone Marrow				Spleen				Total
		Conven-tional 64/65	GTG 64/65	CBG 64/65	NOR 64/65	Conven-tional 64/65	GTG 64/65	CBG 64/65	NOR 64/65	Conven-tional 64/65	GTG 64/65	CBG 64/65	NOR 64/65	
F03	F	53 / 34	17 / 08	07 / 05	13 / 04	–	–	–	–	–	–	–	–	90/ 51
101	F	63 / 27	16 / 04	09 / 06	09 / 04	–	–	–	–	–	–	–	–	97/ 41
132	F	65 / 12	08 / 05	16 / 07	06 / 03	–	–	–	–	–	–	–	–	95/27
217	F	53 / 25	17 / 03	12 / 04	08 / 04	75 / 19	16 / 04	14 / 07	08 / 03	–	–	–	–	203/ 69
218	M	87 / 17	09 / 06	13 / 07	16 / 07	65 / 15	12 / 03	16 / 06	09 / 03	–	–	–	–	227/ 64
226	F	133 / 42	44 / 19	29 / 07	43 / 18	–	–	–	–	–	–	–	–	249/86
260	F	57 / 33	28 / 17	10 / 05	24 / 08	–	–	–	–	–	–	–	–	119/ 63
263	F	54 / 21	08 / 07	12 / 03	08 / 02	–	–	–	–	–	–	–	–	82 / 33
267	M	74 / 26	20 / 10	17 / 03	09 / 06	41 / 34	16 / 04	09 / 08	08 / 04	44 / 22	12 / 07	08 / 07	08 / 05	266/136
270	M	55 / 25	23 / 12	09 / 04	07 / 02	–	–	–	–	–	–	–	–	94/ 43
272	M	47 / 30	07 / 03	08 / 02	10 / 05	36 / 24	06 / 02	08 / 04	07 / 03	30 / 25	17 / 03	14 / 08	09 / 08	199/117
273	M	75 / 2 5	08 / 06	09 / 03	05 / 04	54 / 18	07 / 04	07 / 03	09 / 02	–	–	–	–	174 / 65
274	F	67 / 22	12 / 03	13 / 05	07 / 02	–	–	–	–	–	–	–	–	99 / 32
277	M	83 / 16	27 / 04	08 / 04	09 / 03	56 / 34	10 / 05	17 / 08	27 / 18	45 / 30	09 / 04	09 / 06	07 / 03	307/135
278	M	66 / 12	09 / 06	17 / 06	10 / 07	–	–	–	–	–	–	–	–	102/ 31
281	M	65 / 20	08 / 02	07 / 03	09 / 09	–	–	–	–	–	–	–	–	89/ 34
282	M	71 / 16	20 / 08	13 / 07	07 / 03	–	–	–	–	–	–	–	–	111/ 34
285	F	81 / 09	06 / 04	10 / 07	06 / 06	–	–	–	–	–	–	–	–	103/ 26
294	M	95 / 15	08 / 06	37 / 15	35 / 22	–	–	–	–	–	–	–	–	175/ 58
295	M	106 / 14	08 / 04	18 / 04	26 / 09	–	–	–	–	–	–	–	–	158/ 31
Total	–	1450 / 441	303/ 137	274/ 107	267/ 128	327/ 144	67 / 22	71 / 36	68 / 33	119 / 77	38 / 14	31 / 21	24 / 16	4215

Ident. = Identification.

TABLE III

Number of 2n=64 and 2n=65 cells, collected from peripheral blood, bone marrow and spleen, in *D. leporina*.

Ident.	Sex	Peripheral blood				Bone Marrow				Spleen				Total
		Conventional 64/65	GTG 64/65	CBG 64/65	NOR 64/65	Conventional 64/65	GTG 64/65	CBG 64/65	NOR 64/65	Conventional 64/65	GTG 64/65	CBG 64/65	NOR 64/65	
M01	M	58 / 29	31 / 27	06 / 04	17 / 09	–	–	–	–	–	–	–	–	112 / 69
M02	M	59 / 29	08 / 04	14 / 06	09 / 01	–	–	–	–	–	–	–	–	90 / 40
190	F	47 / 38	09 / 06	18 / 07	08 / 05	–	–	–	–	–	–	–	–	82 / 56
216	M	34 / 06	09 / 03	05 / 03	03 / 01	10 / 05	09 / 05	06 / 03	08 / 05	08 / 02	07 / 04	06 / 02	04 / 04	109 / 43
220	M	22 / 11	10 / 05	15 / 08	16 / 04	27 / 17	09 / 06	07 / 03	05 / 04	14 / 07	05 / 02	03 / 01	04 / 03	137 / 71
261	M	47 / 23	15 / 05	09 / 03	11 / 05	–	–	–	–	–	–	–	–	82 / 36
Total	–	267 / 136	82 / 50	67 / 31	64 / 25	37 / 22	18 / 11	13 / 06	13 / 09	22 / 09	12 / 06	09 / 03	08 / 07	927

Ident. = Identification.

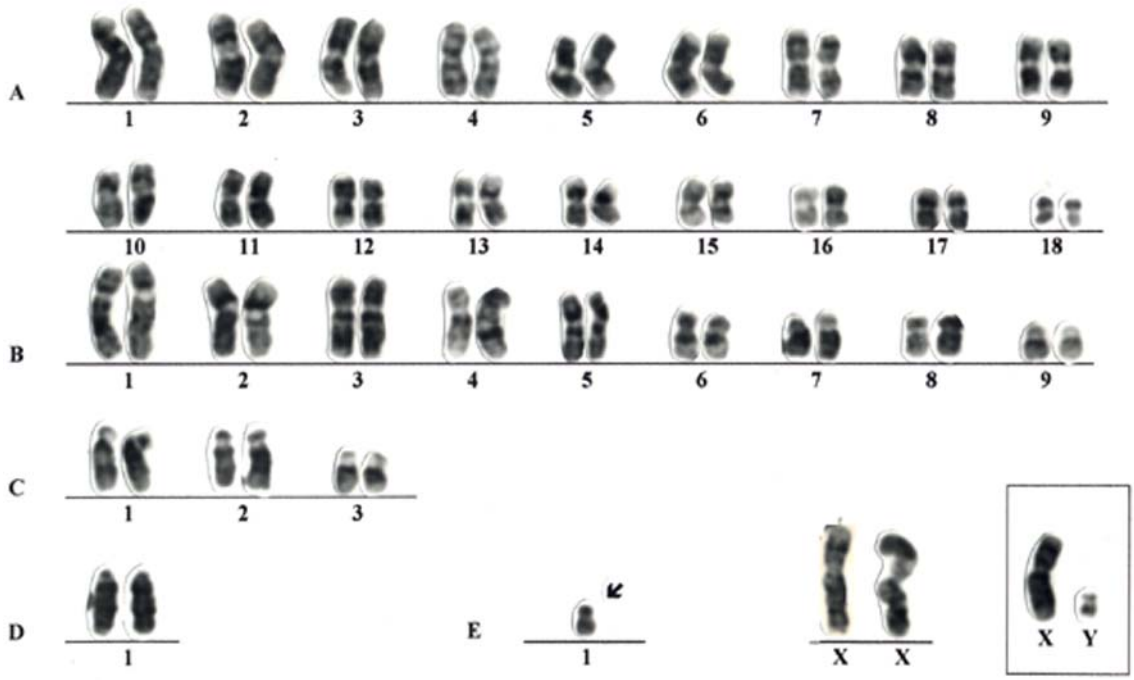


Fig. 4 – Banding G (GTG) standard of the lineage 2n=65 from a female sample of *D. fuliginosa*. The arrow points to the banded supernumerary chromosome. Standing out, it appears the sexual pair: X and Y chromosomes, from a male of *D. prymnolopha*.

TABLE IV

Number of 2n=64 and 2n=65 cells, collected from peripheral blood, bone marrow and spleen, in *Dasyprocta* sp. and peripheral blood, in *D. fuliginosa*.

Species		Staining and Banding					
		Sex	Conven-tional 64/65	GTG 64/65	CBG 64/65	NOR 64/65	Total
*D.*sp.	01	F	63 / 17	10 / 08	13 / 10	12 / 09	98 / 44
	283	M	84 / 08	32 / 13	09 / 06	08 / 04	133 / 31
	284	M	79 / 16	32 / 27	20 / 10	15 / 05	146 / 58
Total			226 / 41	74 / 48	42 / 26	35 / 18	510
D.fulig.	53	F	74 / 21	43 / 17	15 / 06	17 / 07	149 / 51

D. sp. = *Dasyprocta* sp.; *D. fulig.* = *D. fuliginosa.*

Gallardo 1991, 1992, Fagundes and Yassuda 1995, Moreira et al. 1995). The presence of lineage $2n = 65$, with one supernumerary chromosome in our sample, can characterize a geographic variation, since all constants species of literature were derived from different South America regions other than the Northern region.

In *D. prymnolopha*, *D. leporina* and *D. fuliginosa* we detected a small variation in the A18 pair in relation to *Dasyprocta* sp. due to the fact that 100%

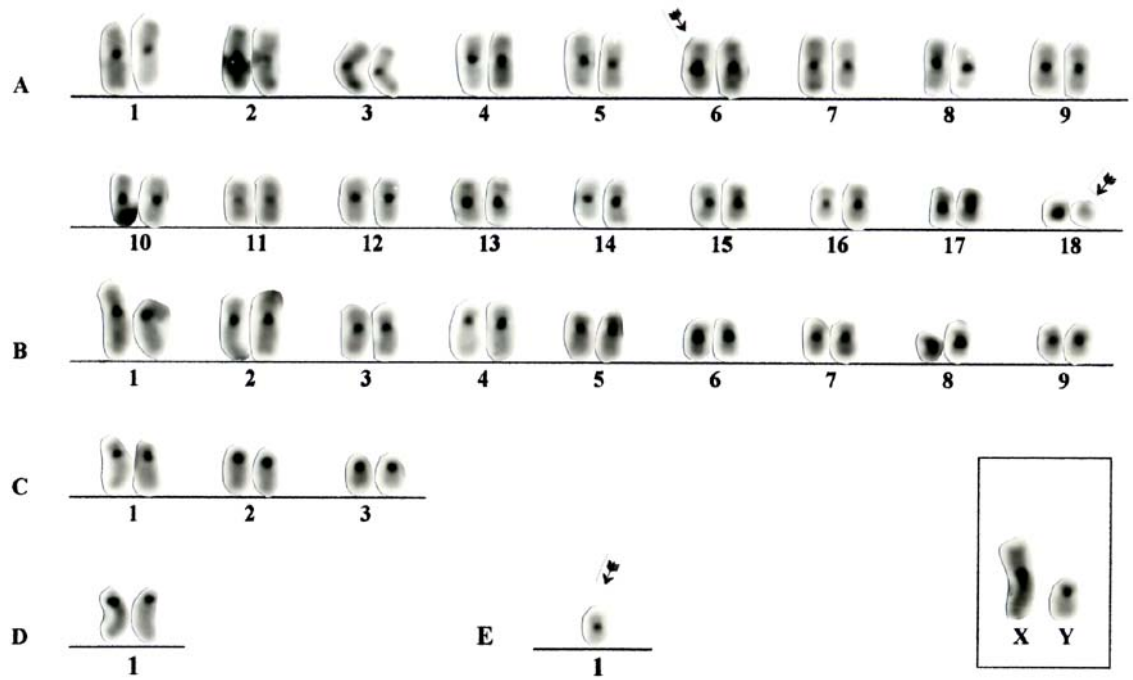

A
1 2 3 4 5 6 7 8 9
10 11 12 13 14 15 16 17 18
B
1 2 3 4 5 6 7 8 9
C
1 2 3
D
1
E
1
X Y

Fig. 5A

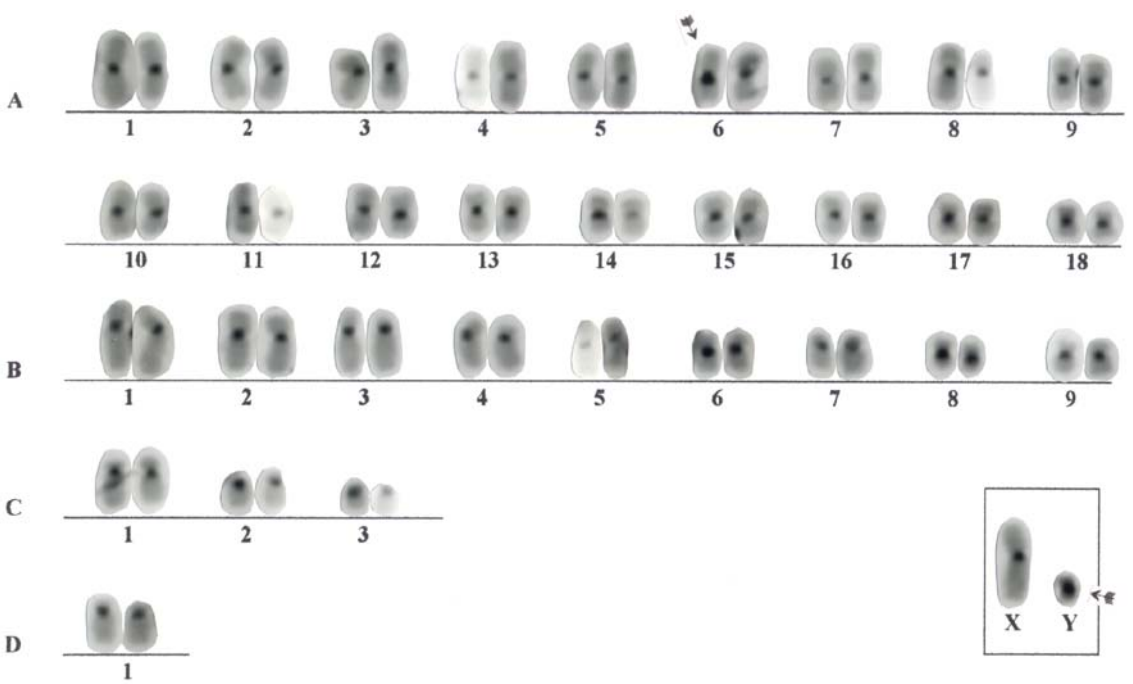

A
1 2 3 4 5 6 7 8 9
10 11 12 13 14 15 16 17 18
B
1 2 3 4 5 6 7 8 9
C
1 2 3
D
1
X Y

Fig. 5B

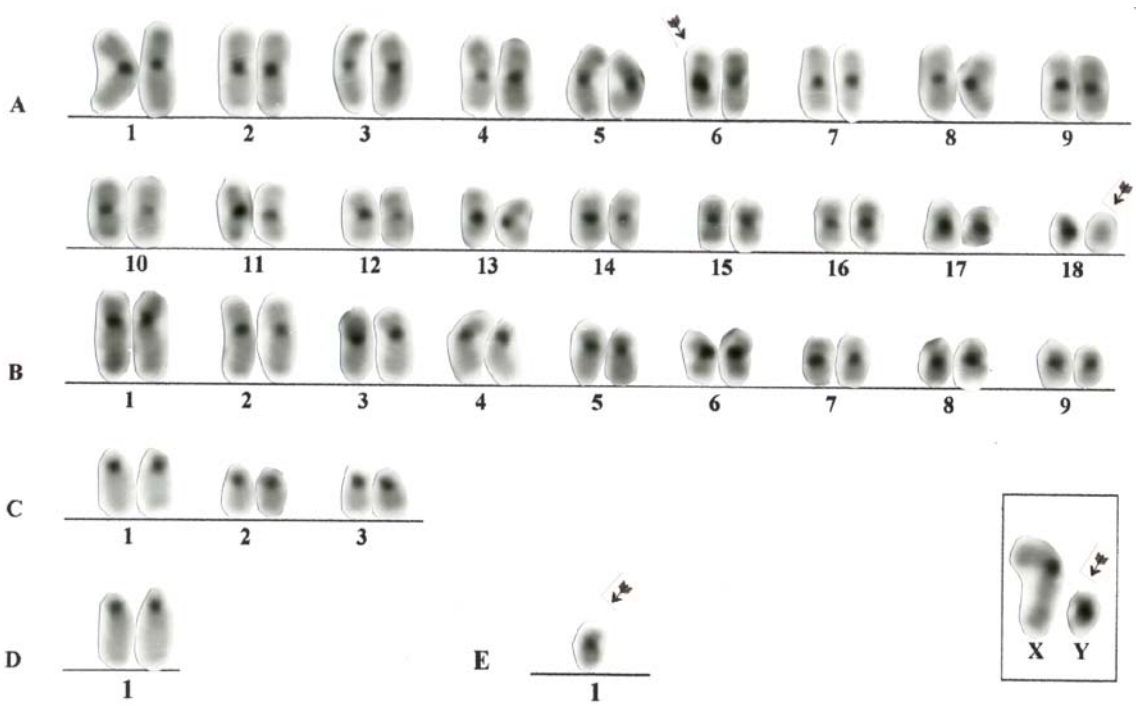


Fig. 5C

Fig. 5 – Banding C (CBG) standard in the lineages 2n=64 and 2n=65. A) and C) the arrows point to one of the homologues from the pair A18, lacking the heterochromatical block (*D. prymnolopha* and *Dasyprocta* sp., respectively.); A), B) and C) one of the homologues from the pair A6 had an increasing of the heterochromatical block (*D. prymnolopha*, *D. leporina* and *Dasyprocta* sp., respectively); A) and C) the supernumerary chromosome with a constitutive heterochromatin in the pericentromeric region (*D. prymnolopha* and *Dasyprocta* sp., respectively). B) and C) the arrows point to the Y chromosome, almost totally heterochromatical (*D. leporina* and *Dasyprocta* sp., respectively).

of the analyzed cells showed one of the homologues with a secondary constriction and about 40% from the cells of both lineages present this morphology in two homologues. However, there is a necessity of a more detailed research to confirm this characteristic. The marking character of A18 chromosome of our samples was first described here. The remaining chromosome complement agrees with Lima (1993), Lima and Langguth (1995, 1998) researches in *D. leporina* and *D. fuliginosa*.

The X chromosome of the four species here studied is submetacentric, different from the one found in *D. leporina* and *D. fuliginosa* described as large metacentric (Kasahara and Yonenaga-Yassuda 1984, Lima and Langguth 1995, 1998). The Y chromosome of *D. prymnolopha*, *D. leporina* and *Dasyprocta* sp. is a small submetacentric, similar to the one described by *D. variegata* (Hungerford and Snyder 1964), but different from the described small metacentric chromosome of *D. leporina* (Kasahara and Yonenaga-Yassuda 1984, Lima 1993, Lima and Langguth 1995, 1998).

G banding comparison between species showed several chromosomes similarities (Figure 8), suggesting that these species are conserved at the chromosome level. This finding is similar to that found in four species of *Ctenomys* (*C. flamarioni*, *C. mendocinus*, *C. porteousi* and *C. australis*), in relation to 1, 2, 4-8, 11-14, 16, 18, 21 and 23 pairs (Freitas 1994), and in two species of *Cavia* (*C. aperea* and

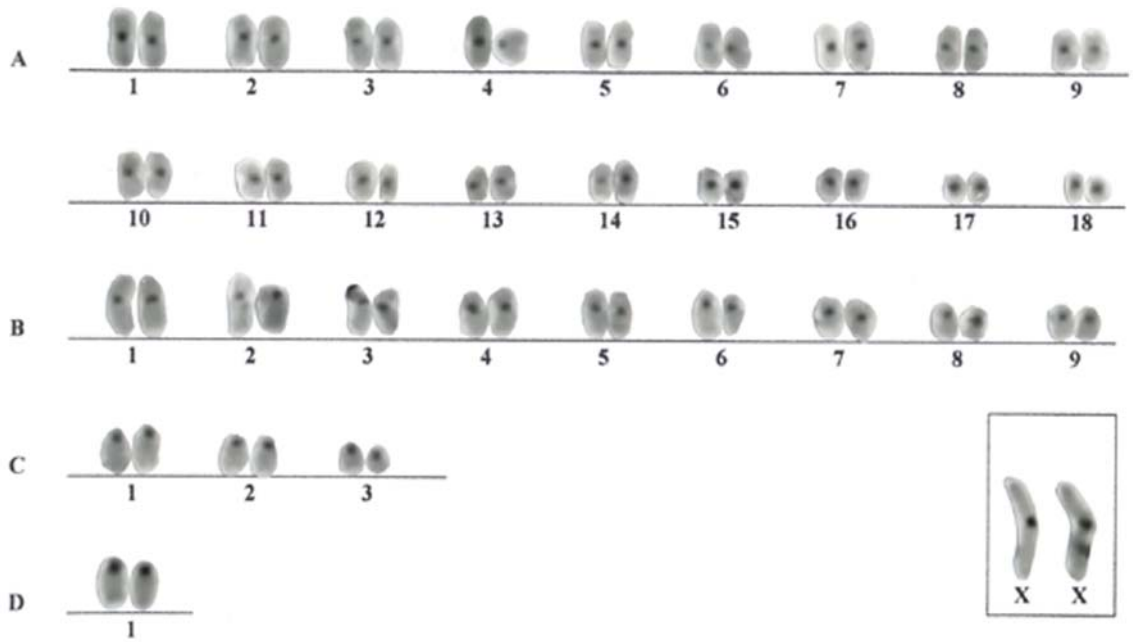


Fig. 6 – Banding C (CBG) standard of the lineage 2n=64 of *D. fuliginosa*.

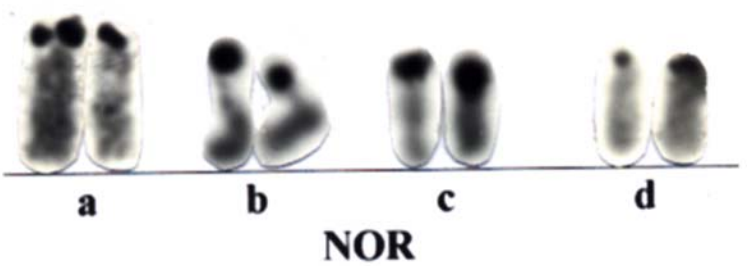


Fig. 7 – Localization of the Nucleolous Organizer Regions in the pair D1 in: A) *D. prymnolopha*; B) *D. leporina*; C) *D. fuliginosa*; D) *Dasyprocta* sp.

C. aperea pamparum) that present similarity in the G banding standard in most of the autosomes (Maia 1984).

In spite of the difficulties in obtaining banded trypsin-Giemsa karyotypes of *D. leporina* and *D. fuliginosa*, we presented an idiogram (Figure 9) where we suggest the interpretation of positive (dark) and negative (clear) bands distribution like an essay of G banding standard in *Dasyprocta*.

In C banding analysis, it was evident the pericentromeric constitutive heterochromatin is all chromosomes, including the supernumerary chromosome and X and Y sexual pair. This pattern is similar to the one found in other Hystricognathi as *Cavia porcellus* (Natarajan and Raposa 1974); *Clyomys laticeps laticeps* (Souza and Yonenaga-Yassuda 1982) and *P. longicaudatus* (Maia et al. 1988). *D. leporina* and *Dasyprocta* sp. presented the Y chromosome almost totally heterochromatic, as found in *D. leporina* (Lima 1993, Lima and Langguth 1995, 1998) and in some species from *Ctenomys* that presented Y chromosome varying from totally hete-

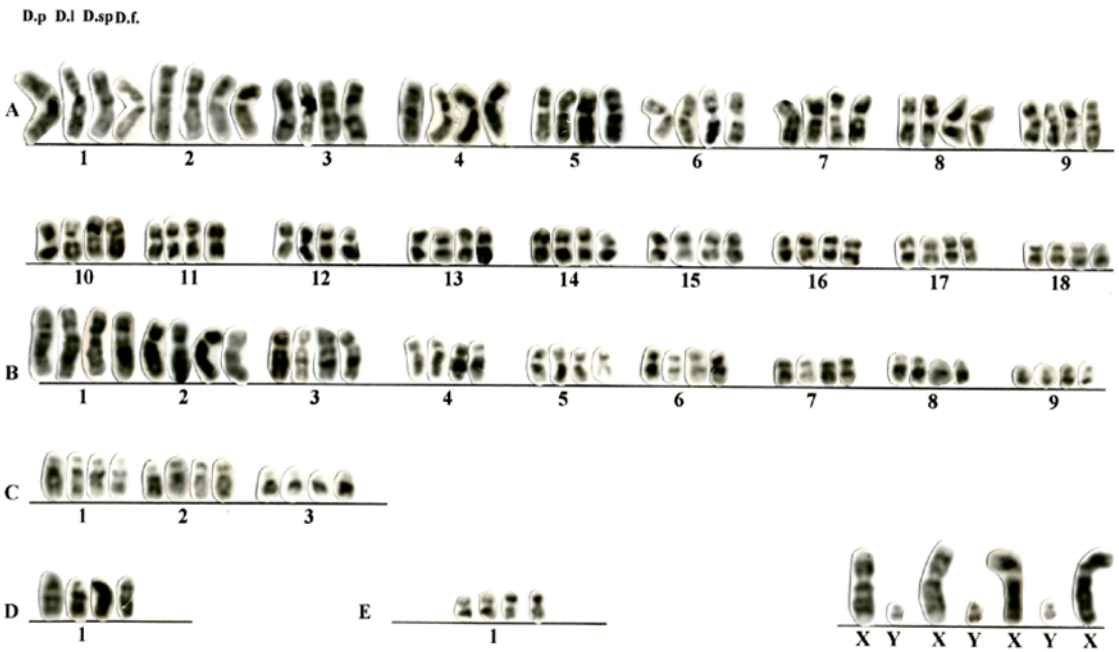


Fig. 8 – Comparisons of the banding standard G (GTG) in the four analized species. D.p – *D. prymnolopha*; D.l – *D. leporina*; D.sp – *Dasyprocta* sp.; D.f – *D. fuliginosa.*

rochromatic to C banding negative standard (Freitas 1994). *D. prymnolopha, D. leporina* and *Dasyprocta* sp. presented variation in the amount of heterochromatin in the A6 pair, similar to the observed by Reig et al. (1990) in the Ctenomyídeos (Hystricognathi). *D. fuliginosa* did not show variation in C banding and probably, this karyotype represents the basic standard of distribution and amount of constitutive heterochromatin in *Dasyprocta*.

In the four species the NORs were located in telomeric region of the short arm of both homologues of D1 pair, without size variation or NORs absence in any analyzed cell. This results are similar to the ones described in *D. leporina* and *D. fuliginosa* (Lima 1993).

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RESUMO

Foram estudados citogeneticamente um total de 30 animais das espécies *D. prymnolopha* (N=20), *D. leporina* (N=6), *D. fuliginosa* (N=1) e *Dasyprocta* sp. (N=3) (Dasyproctidae, Histricognathi). As preparações cromossômicas foram obtidas do cultivo de sangue periférico, além de medula óssea e baço em *D. prymnolopha* e *D. leporina*. O número diplóide foi de 64/65 em todos os exemplares. O cariótipo mostrou similaridade, não sendo detectado, através de coloração convencional de giemsa e de banda G, polimorfismo cromossômico em qualquer uma das espécies estudadas. A distribuição da heterocromatina constitutiva na região pericentromérica de todos os cromossomos foi similar nas quatro espécies. *D. prymnolopha*, *D. leporina* e *Dasyprocta* sp. apresentaram variação no tamanho do bloco heterocromático em um dos homólogos do par A18. *D. fuliginosa* apresentou a heterocromatina uniformemente distribuída em todos os

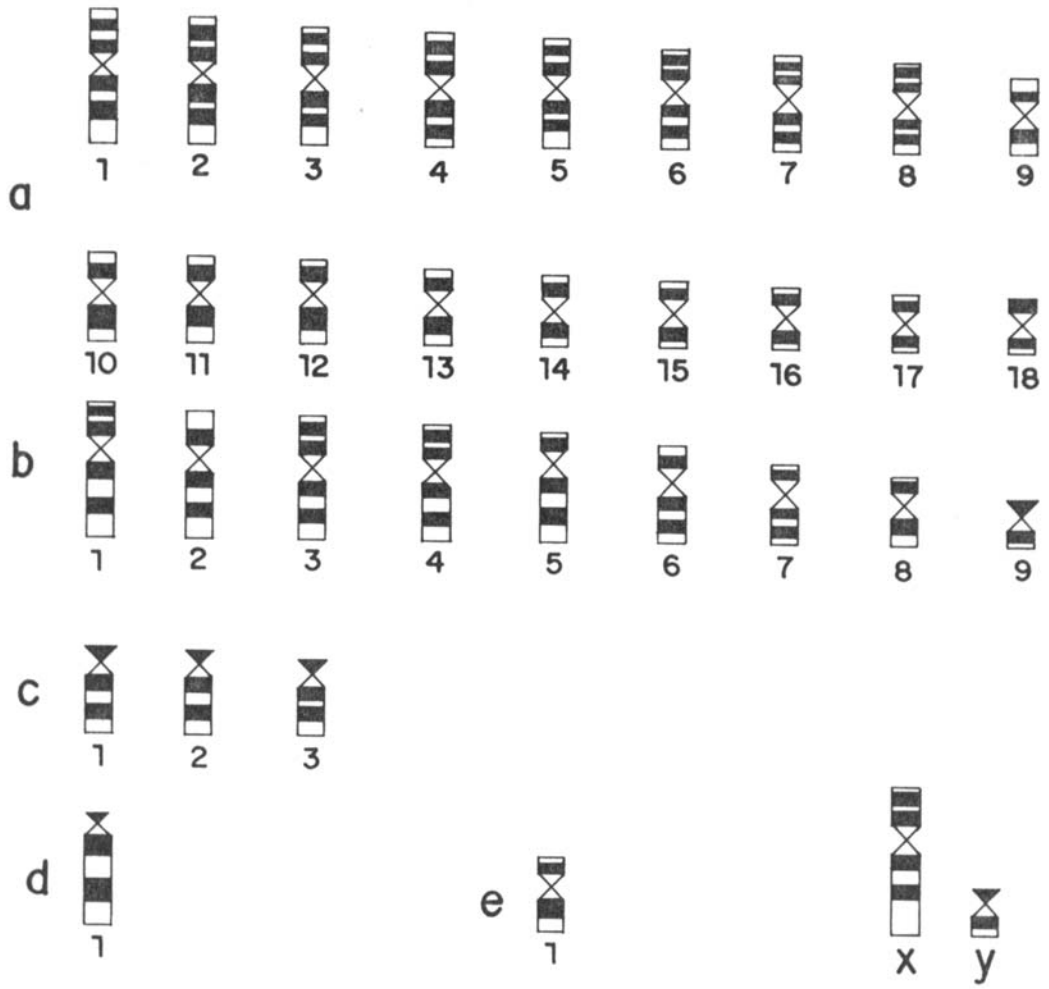

Fig. 9 – Idiogram presenting the banding G standards in *D. prymnolopha, D. leporina, Dasyprocta* sp. and *D. fuliginosa*, including the supernumerary chromosome (e1) of the last species.

cromossomos. Não houve variação no padrão das RONs entre as espécies estudadas.

Palavras-chave: Citogenética, Hystricognathi, *Dasyprocta*, Cariótipo.

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