Karyotype characterization and nucleolar organizer regions of marsupial species (Didelphidae) from areas of Cerrado and Atlantic Forest in Brazil

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Abstract

The karyotypes of 23 specimens belonging to 16 species from nine genera of Brazilian marsupials (family Didelphidae) were studied. The animals were collected in eight localities of Cerrado or Atlantic Forest biomes in the states of Goiás, Tocantins and São Paulo. The karyotypes were analyzed after conventional Giemsa staining and silver staining of the nucleolus organizer regions (Ag-NORs). New karyotypic data were obtained for Gracilinanus microtarsus (2n = 14, FN = 24), Marmosops paulensis (2n = 14, FN = 24), Micoureus paraguayanus (2n = 14, FN = 20) and Monodelphis rubida (2n = 18, FN = 32) and are discussed in detail. The karyotypes of G. microtarsus, M. paulensis and M. paraguayanus include three large pairs of submetacentrics (pairs 1, 2 and 3) and a medium-sized metacentric or submetacentric pair 4. Pairs 5 and 6 are small submetacentrics in G. microtarsus and M. paulensis and acrocentrics in M. paraguayanus. M. paulensis presented a single Ag-NOR in pair 6 (6p6p), while M. paraguayanus exhibited multiple Ag-NORs in pairs 5 and 6 (5pq5pq6p6p). There was variation in size and morphology of the sex chromosomes among these species. Monodelphis rubida presented a karyotype with 2n = 18 and FN = 32 composed of a large submetacentric pair 1, a medium-sized metacentric pair 2 and six pairs of submetacentrics (pairs 3 through 8). The X was a small acrocentric and the Y was dot-like. A single Ag-NOR bearing pair (5p5p) characterized M. rubida. Relevant karyotypic information was obtained for 19 specimens belonging to 12 species collected in areas sampled for the first time [Caluromys lanatus and C. philander (2n = 14, FN = 20), Gracilinanus emiliae (2n = 14, FN = 24), Marmosa murina, Metachirus nudicaudatus and Micoureus demerarae (2n = 14, FN = 20), Monodelphis americana (2n = 18, FN = 32) and M. domestica (2n = 18, FN = 20), and Didelphis marsupialis, Philander frenata, P. opossum and P. sp (2n = 22, FN = 20)]. Although the karyotypes were relatively conserved with respect to the morphology of the autosomes among species with the same diploid number, some differences regarding FN, sex chromosomes morphology and Ag-NORs patterns were detected.

Key words: marsupials, karyotypes, cytogenetics, Didelphidae, NORs.

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There are currently 331 recognized species of living marsupials assembled into seven orders, three of which occur in the American continent (Didelphimorphia, Paucituberculata and Microbiotheria). They represent an extreme example of karyotype conservation, which is observed in most living species from the Australian and American continents. Some marsupials present a 2n = 14 karyotype, while others exhibit a karyotype with 2n = 22 (Pagnozzi et al., 2000; 2002; Svartman and Vianna-Morgante, 2003). The diploid number of 2n = 18 also occurs, but so far it has only been observed in four species of the genus Monodelphis found in Brazil and Bolivia (Pagnozzi et al., 2002; Carvalho et al., 2002).

The most frequent 2n = 14 karyotype has been traditionally considered ancestral to both American and Australian species (Rofe and Hayman, 1985; Hayman, 1990) and was suggested to have given rise to the highest diploid numbers through a series of centric fissions (Rofe and Hayman, 1985; Metcalfe et al., 2004). An alternative hypothesis claimed that the ancestral karyotype was similar to that with 2n = 22 and that centric fusions played a role on the origin of the derived karyotypes with lower diploid numbers (Svartman and Vianna-Morgante, 1998; Carvalho and Mattesi, 2000).
Didelphidae belongs to the order Didelphimorphia and is the most diverse American family of marsupials, with 19 genera and 103 species (Wilson and Reeder, 2005). About 25 Brazilian species of marsupials have already had their karyotypes reported (Yonenaga-Yassuda et al., 1982; Casartelli et al., 1986; Souza et al., 1990; Pagnozzi et al., 2000, 2002; Carvalho et al., 2002; Svartman and Vianna-Morgante, 1999; 2003; Paresque et al., 2004).

Karyological studies have demonstrated that the nucleolus organizer regions (NORs) and C-banding patterns represent important cytogenetic markers in conserved karyotypes because they reveal patterns that may characterize different species. According to Svartman and Vianna-Morgante (1999), marsupial karyotypes with the same diploid numbers differed in the amount of pericentromeric constitutive heterochromatin and in the number and distribution of Ag-NORs. These differences among species may be useful in the taxonomy of Didelphidae.

We analyzed 23 specimens belonging to 16 species from nine genera of Didelphidae collected in eight different localities of Cerrado and Atlantic Forest in the states of São Paulo, Goiás and Tocantins (Brazil). The karyotypes were analyzed after conventional staining and after silver staining of the nucleolus organizer regions (Ag-NORs) (Table 1). We present data on 19 specimens belonging to 12 species collected in areas not previously surveyed (Table 1). We discuss in detail the new karyotypic data obtained for specimens of *Gracilinanus microtarsus*,

### Table 1 - Chromosome data of Didelphidae from Brazil.

| Species                     | Specimen/filed numbers | Collection (locality and coordinates)         | 2n/FN | Sex X Y | Number of cells analyzed | NORs position | Number of NORs |
|-----------------------------|------------------------|----------------------------------------------|-------|---------|--------------------------|---------------|----------------|
| *Caluromys lanatus*         | MM 11 MM12             | UHE Corumbá IV (Luziânia, GO) 16° 15'09" S, 47° 57'01" W | 14/20 | M SM D  | 28 10                   | 6p6p           | 2              |
| *Caluromys philander*       | UNIBAN 2589 UNIBAN 2622| Biritiba-Mirim (SP) 23° 34’21” S, 46° 02’19” W | 14/20 | F A -   | 34 12                   | 6p6p           | 2              |
| *Gracilinanus emiliae*      | MM 10                  | UHE Corumbá IV (Luziânia, GO)                | 14/24 | M SM A  | 30 6                    | 6p6p           | 2              |
| *Gracilinanus microtarsus*  | UNIBAN 2602            | Biritiba-Mirim (SP) 23° 34’21” S, 46° 02’19” W | 14/24 | F M -   | 24 -                    | -              | -              |
| *Marmosa murina*            | MM 13 MM14             | UHE Corumbá IV (Luziânia, GO)                | 14/20 | M SM A  | 30 22                   | 6p6p           | 2              |
| *Marmosops paulensis*       | UNIBAN 2307            | Biritiba-Mirim (SP) 23° 34’21” S, 46° 02’19” W | 14/24 | M M A  | 31 28                   | 6p6p           | 2              |
| *Metachirus nudicaudatus*   | APC 1436               | PESM (Caraguatatuba, SP) 23° 37’13” S, 45° 24’47” W | 14/20 | F A -   | 33 5                    | 6p6p           | 2              |
| *Micocebus dumerarae*       | UNIBAN 2074 UNIBAN 2134| Biritiba-Mirim (SP)                         | 14/20 | F A -   | 62 2                    | 6p6p           | 2              |
| *Micocebus paraguayanus*    | APC 1469               | PESM (Caraguatatuba, SP)                     | 14/20 | F A -   | 32 4                    | 6p6p           | 2              |
| *Monodelphis americana*     | UNIBAN 2133            | Biritiba-Mirim (SP)                         | 18/32 | F A -   | 14 -                    | -              | -              |
| *Monodelphis domestica*     | MM 15 MM16             | UHE Corumbá IV (Luziânia, GO)                | 18/20 | M A A  | 15 -                    | -              | -              |
| *Monodelphis rubida*        | UNIBAN 2311            | Biritiba-Mirim (SP) 23° 32’51” S, 45° 24’47” W | 18/20 | F A -   | 30 -                    | -              | -              |
| *Didelphis marsupialis*     | MM 17                  | UHE Peixe Angelical (TO) 12° 01’30” S, 48° 32’21” W | 18/20 | F A -   | 25 -                    | -              | -              |
| *Phylad er frenata*         | UNIBAN 3645            | Serra da Cantareira (SP)                    | 22/20 | M A A  | 31 17                   | 5p5p7q 7q     | 4              |
| *Phylad er opossum*         | MM 18                  | PEAMP (GO) 16° 30’38” S, 49° 01’26” W       | 22/20 | M A A  | 34 44                   | 5p5p7q 7q     | 4              |

* New karyotypic data described in the present study.

2n – diploid number; FN – fundamental (FN) numbers; M-metacentric; SM-submetacentric; A-acrocentric; D-dot-like; CS – conventional staining; AgS – silver nitrate staining, p- short arm; q- long arm.

UHE: Usina Hidrelétrica, PESM: Parque Estadual da Serra do Mar; PEAMP: Parque Ecológico Altamiro de Moura Pacheco (GO);
GO – Goiás, SP – São Paulo, TO – Tocantins.

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Marmosops paulensis, Micoureus paraguayanus and Monodelphis rubida.

Mitotic preparations were obtained from bone marrow and spleen following routine protocols. After Giemsa staining, at least 15 metaphases/individual were analyzed to establish the diploid (2n) and fundamental numbers (FN = number of autosome arms) and the chromosomes morphology. Metaphases were photographed with a 100X objective under a Leica CW 4000 or Zeiss Axiophot photomicroscope equipped with image capture systems and softwares for chromosome analyses (Karyo, Leica and Ikaros Karyotyping System, MetaSystems). After silver staining (Howell and Black, 1980), the Ag-NORs were analyzed in the maximum number of cells possible.

Three diploid numbers were found in the studied Didelphidae: (I) 2n = 14 in Caluromys lanatus, C. philander, Gracilinanus emiliae, G. microtarsus, Marmosa murina, Marmosops paulensis, Metachirus nudicaudatus, Micoureus demerarae, M. paraguayanus; (II) 2n = 18 in Monodelphis americana, M. domestica and M. rubida; and (III) 2n = 22 in Didelphis marsupialis, Philander frenata, P. opossum and P. sp. A summary of the karyotypic data is presented in Table 1.

I) Karyotypes with 2n = 14

Gracilinanus microtarsus and Marmosops paulensis presented karyotypes with 2n = 14/FN = 24 composed of three pairs of large submetacentrics (pairs 1, 2 and 3), a medium-sized submetacentric pair 4 and two pairs of small submetacentrics (pairs 5 and 6); the X chromosome was a small metacentric (Figure 1A and B). The Y chromosome was acrocentric in Marmosops paulensis (Figure 1B). The karyotypes reported here for G. microtarsus and M. paulensis are very similar, despite the difference in the size of the X chromosome, larger in G. microtarsus than in M. paulensis. (Figure 1A and B).

The karyotype of a female Gracilinanus microtarsus from Biritiba-Mirim, São Paulo (Table 1), differed from specimens previously collected in the state of Rio Grande do Sul and also from individuals of G. agilis and G. emiliae trapped in Goiás and Minas Gerais (Carvalho et al. 2002; this work). While pair 4 was submetacentric and the X chromosomes were metacentric in our specimen of G. agilis and G. emiliae, the X chromosome was a small metacentric (Figure 1A and B). The Y chromosome was metacentric in Marmosops paulensis (Figure 1B). The karyotypes reported here for G. microtarsus and M. paulensis are very similar, despite the difference in the size of the X chromosome, larger in G. microtarsus than in M. paulensis. (Figure 1A and B).

The karyotype of Micoureus paraguayanus exhibited 2n = 14 and FN = 20 and a karyotype with three pairs of large submeta-
centrics (pairs 1 through 3), one pair of medium meta-
centrics (pair 4) and two small acrocentric pairs (pairs 5 and
6; Figure 1C). The X chromosome was a small acrocentric.
This karyotype is identical to that previously described for
specimens of M. demerarae collected in the Amazon and in
the states of Pernambuco, Mato Grosso, Bahia and São
Paulo (Casartelli et al., 1986; Souza et al., 1990; Pagnozzi
et al., 2000; Svartman and Vianna-Morgante, 1999). A
karyotype with 2n = 14 and FN = 24 was reported in five in-
dividuals identified as M. demerarae trapped in localities of
Goiás and Rio Grande do Sul (Carvalho et al. 2002). We
believe that the difference in the reported FNs is due to dis-
tinct degrees of chromatin condensation of the short arms
of pairs 5 and 6, which were considered as biarmed by
Carvalho et al. (2002).

According to Voss and Jansa (2003), the specimens
identified as Micoureus demerarae collected in Rio Grande
do Sul and analyzed by Carvalho et al. (2002) were mis-
identified and were actually M. paraguayanus. We cannot
discard the possibility that other previously karyotyped
specimens of M. demerarae were also misidentified. Nev-
evertheless, the present paper is the first to describe the
karyotype and the Ag-NORs distribution in a specimen
identified as M. paraguayanus.

Micoureus paraguayanus was the only species from
our sample with more than two Ag-NORs. Besides the
Ag-NORs at pair 6, this species also had Ag-NORs at the
telomeres of both chromosome arms of pair 5 and there was
variation in the number of Ag-NORs per cell. Out of ten
cells analyzed, two presented five Ag-NORs: on the telo-
meres of the long arms of one homologue of pair 5, on both
telomeres (long and short arms) of the other element of pair
5 and on the telomeres of the short arms of pair 6
(5pq5pq6p6p). The remaining eight cells presented six
Ag-NORs (5pq5pq6p6q) (Figure 2B; Table 1). This
5pq5pq6q6q Ag-NORs pattern was also reported in speci-
mens of M. demerarae from Rio Grande do Sul (Carvalho
et al., 2002) and by Svartman and Vianna-Morgante
(2003), who described six positive signals after FISH with a
ribosomal probe and after silver staining in a single speci-
m of M. demerarae from an unknown Brazilian locality.
It is important to point out that the two specimens of M.
demerarae herein studied were collected in a locality not
previously sampled (Biritiba-Mirim) and exhibited a single
pair with an Ag-NOR (pair 6; Table 1), differing from the
multiple Ag-NORs (four to six sites) reported in the litera-
ture for specimens collected in the Amazon (Casartelli et
al., 1986) and in the states of Pernambuco (Souza et al.,
1990), Goiás and Rio Grande do Sul (Carvalho et al., 2002).
Nevertheless, we analyzed the Ag-NORs in only six
cells (Table 1) and more cells have to be analyzed in order
to confirm this result.

The specimens of Marmosa murina from areas of
Cerrado in Goiás and Tocantins presented 2n = 14 and
FN = 20 (Table 1), a karyotype identical to the one found in
specimens from Pernambuco (Souza et al., 1990) and
Espírito Santo (Paresque et al., 2004). However, karyo-
types with 2n = 14 but presenting FN = 22 and 24 were de-
scribed for specimens from Tocantins [Porto Nacional:
FN = 22 (Lima, 2004) and FN = 24 (Carvalho et al., 2002)],
Amãpã (FN = 24, Carvalho et al., 2002) and Goiás (Serra
da Mesa: FN = 24, Carvalho et al., 2002). The difference in
FNs among different specimens of M. murina reported in the
literature reflects distinct classifications of pairs 5 and
6, which were considered biarmed by Carvalho et al.
(2002) and Lima (2004). Moreover, while the X chromo-
some was a submetacentric in the specimens of M. murina
studied herein (Table 1), only acrocentric X chromosomes
were reported previously (Souza et al., 1990; Carvalho
et al., 2002, Paresque et al., 2004).

II) Karyotype with 2n = 18

The karyotype of Monodelphis rubida had 2n = 18
and FN = 32, and the eight pairs of autosomes included one
large pair of submetacentrics (pair 1), one medium-sized
metacentric pair (pair 2) and six pairs of submetacentrics
(pairs 3 through 8). The X chromosome was a small acro-
centric and the Y was dot-like (Figure 1D).

Although all the species of Monodelphis already de-
scribed presented karyotypes with 2n = 18, there was varia-
tion in the FNs among and within species. For instance,

![Figure 2 - Ag-NORs in the karyotypes of three species of Didelphidae: (A) Marmosops paulensis male (Ag-NORs in 6p6p); (B) Micoureus paraguayanus female (Ag-NORs in 5pq5pq6p6p); (C) Monodelphis rubida male (Ag-NORs in 5p5p).](image-url)
karyotypes with FN = 20 and FN = 28 (sampled in localities of Goiás) were both described for specimens of *M. domestica* (Swartman and Vianna-Morgante, 1999; Carvalho et al., 2002, respectively). The lack of information on the collection sites for the specimens analyzed by Swartman and Vianna-Morgante (1999) prevents further considerations of a possible geographical variation within this species. A fundamental number as high as 30 was described in specimens of *M. domestica* from Espírito Santo (Paresque et al., 2004), *M. kunsi* collected in localities of Goiás and *M. breviceudata* from Roraima and Pará (Carvalho et al., 2002). A karyotype similar to that found in *M. rubida* (present work), with exclusively biarmed chromosomes and FN = 32, also characterizes *M. dimidiata* from Rio Grande do Sul (Carvalho et al., 2002) and specimens of *M. americana* from São Paulo (Biritiba Mirim; Table 1) and from Espírito Santo (Paresque et al., 2004).

*M. rubida* exhibited a single Ag-NOR on the telomeres of the short arms of pair 5 (Figure 2C; Table 1). An identical pattern was found in two species of *Monodelphis* (*M. kunsi* and *M. dimidiata*) from Goiás and Rio Grande do Sul, but a different pattern was reported in *M. breviceudata* (from the states of Roraima and Pará) and *M. domestica* (Goiás), in which Ag-NORs were observed on the telomeres of the short arm of the X chromosome (Carvalho et al., 2002). Moreover, in specimens of *M. domestica* analyzed by Swartman and Vianna-Morgante (2003) four NORs (5p5p, XpXp) were reported in females and three (5p5p, Xp) in males.

A significant variation is observed in the FNs reported for specimens of *Monodelphis domestica* from different localities in Brazil. Karyotypes with 2n = 18 and FN = 20 occurred in specimens from two localities of Cerrado in Goiás and Tocantins (present study, Table 1) and in specimens analyzed by Swartman and Vianna-Morgante (1999, 2003; without information on locality). Karyotypes with FN = 28 and FN = 30 were described for specimens from Cerrado of Goiás (Serra da Mesa and Ipameri; Carvalho et al., 2002) and areas of Atlantic Forest in Espírito Santo (Paresque et al., 2004), respectively. The reports of FNs higher than 20 is due to subtelocentric condensation. The standardization of chromosome nomenclature in marsupial species would prevent the artificial differences that make up for the confusing literature, especially for species in which there is no clear evidence of geographical variation.

In this study we described the karyotypes of three species of Didelphidae from Brazil (*Marmosops paulensis*, *Monodelphis rubida* and *Micoureus paraguayanus*) and a new karyotype for *Gracilinanus microtarsus*. Our work also increased the number of localities surveyed for 12 marsupial species. Although there was conservation of the autosomes morphology among karyotypes of species with the same diploid number, some differences regarding FN, morphology of the sex chromosomes and Ag-NORs distribution were detected, indicating that marsupial karyotypes are less uniform than believed.

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