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Research Article

Comparative cytogenetic analysis in the species *Uroderma magnirostrum* and *U. bilobatum* (cytotype 2n = 42) (Phyllostomidae, Stenodermatinae) in the Brazilian Amazon

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Abstract

The genus *Uroderma* includes two species: *U. magnirostrum* and *U. bilobatum.* These species are characterized by their high degree of karyotypic evolution, diverging from most other species of the subfamily Stenodermatinae, which have a lower degree of chromosomic evolution. The present study reports the first banding patterns of *U. magnirostrum* (G-, C-banding and Ag-NOR) and *U. bilobatum* (C-banding and Ag-NOR). The chromosomic data in conventional staining of *U. magnirostrum* (2n = 36, NF = 62) and *U. bilobatum* (cytotype 2n = 42, NF = 50) are equivalent to that described in the literature. When compared, chromosomal homeologies are found in both karyotypes, as well as differences, confirming that karyotypic evolution in the *Uroderma* genus is intense. Fission, fusion, inversion or translocation events are required to explain the karyotypic evolution of this genus. The comparison of karyotype, described here, to one of the species of the genus *Artibeus* (2n = 30/31), suggests that some chromosomic forms are apomorphic and shared between the two species of *Uroderma*. This confirms the monophyly of the genus, and that *U. magnirostrum* presents a more primitive karyotype when compared to *U. bilobatum.*

Key words: cytogenetics, chromosomal evolution, Phyllostomidae, *Uroderma.*

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Introduction

The *Uroderma* genus is a group of Stenodermatines bats primarily recognized as frugivores with slightly round faces, small snout and lancet nose. They also possesses four facial stripes and a very evident dorsal stripe (Nowak, 1996). *Uroderma* comprises two species, *U. bilobatum* (common) and *U. magnirostrum* (uncommon), and their geographic range encompasses southern Mexico to south-eastern Brazil (Hill and Smith, 1984; Nowak, 1996).

The Stenodermatinae subfamily comprises 18 genera (Nowak, 1996) and the relationships of the *Uroderma* genus with other members of the subfamily are the subject of discussion. The phylogenetic proposals using data from different characters show different associations of the genus *Uroderma* with other genera of Stenodermatinae. Using karyotypic information, Gardner (1977) placed this genus as a different lineage, occupying a basal position in the subfamily. Using morphological information, Owen (1987) related *Uroderma* with *Ectophylla* and *Artibeus* genera. Using molecular data, Van Den Bussche (1992) suggested that *Uroderma* is more closely related to *Vampyrops* and *Vampyrodes* than with the other genera. With the help of morphological and karyotypic information, Lim (1993) related this genus strictly to *Artibeus*, in spite of the great chromosomal divergence among them.

This genus is an exception to the karyotypic conservation found in most species of the Stenodermatinae subfamily, because it presents both intraspecific and interspecific variability (Baker, 1967, 1973, 1979; Varella-Garcia *et al.*, 1989). The species *U. magnirostrum* had its karyotype described by Baker and Lopez (1970), without banding information. *U. bilobatum* presents intraspecific variability with, at least, three chromosomal cytotypes: 2n = 38, 42 and 44 (Baker and Lopez, 1970;

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Baker *et al.*, 1972, 1975, 1982). The cytotypes with 2n = 38 and 2n = 44 are derived from three mutational events; two translocation and one centric fusion (Baker, 1979, 1981). The cytotypes with 2n = 42 and 2n = 44 are distinguished by one centric fusion and one pericentric inversion (Baker *et al.*, 1982).

The Amazonian area is, without a doubt, one of the largest centers of biodiversity on the planet, but little is known about the cytogenetics of the species that are part of this diversity. The objective of this work is to study the karyotype of *U. magnirostrum* and *U. bilobatum* (cytotype 2n = 42) species, collected in the Brazilian Amazon using G-, C-banding and Ag-NOR staining, and to compare the data to the karyotypes already described for both species. They will also be compared to the karyotype of *Artibeus* (2n = 30/31), which is supposed to be the primitive karyotype of the subfamily. This information will be used to define the pattern of chromosomal evolution within the genus *Uroderma*.

Material and Methods

The sample consists of four *U. magnirostrum* (3M and 1F) and two *U. bilobatum* (1M and 1F) collected in Belém, Pará, Brazil and were deposited in the Mammal collection at the Museu Paraense Emílio Goeldi (MPEG 27271, 27272, 27476, 27557, 27568, 27572). The animals were collected according to Bredt *et al.* (1996), with the use of mist nets.

The metaphase chromosomes were obtained using the technique of direct extraction of bone marrow, according to Ford and Hamerton (1956). The animals were previously submitted to mitotic induction by yeast stimulation, according to Lee and Elder (1980). The G-bands were obtained by incubating slides in a solution of 2XSSC (60 °C) from 2 to 10 min and staining in a Wright Giemsa solution (0.25% added to 3 mL of phosphate buffer, pH 6.8) for 2 min and 30 s. The C-banding was performed according to Sumner (1972). The Nucleolar Organizer Regions (NORs) staining was performed according to Howell and Black (1980).

The chromosomes were grouped according to morphology and in decreasing size, following Baker and Lopez (1970).

Results

The *U. magnirostrum* specimens presented 2n = 36, NF = 62 (Figure 1a). The autosome set is composed of 4 metacentric, 10 submetacentric and 3 acrocentric pairs. The X chromosome is a submetacentric of medium size and the Y is a small submetacentric. In one specimen the submetacentric pair 5 showed a heteromorphism in the short arm. The C-banding pattern (Figure 1b) shows that the constitutive heterochromatin is located in the pericentromeric region of all the autosomes and in the distal region of the short arm of all the submetacentrics. The few exceptions are the heteromorphic pair 5 whose homologues have a interstitial band in the short arm and the pair 6 that has proximal band in the long arm. The X chromosome has centromeric and terminal heterochromatin in the short arm. The Y has a heterochromatic block in the long arm. The number of Ag-NORs ranges from five to eight per metaphase, and they are located in the distal region of the short arm of submetacentric chromosomes (Figure 3a).

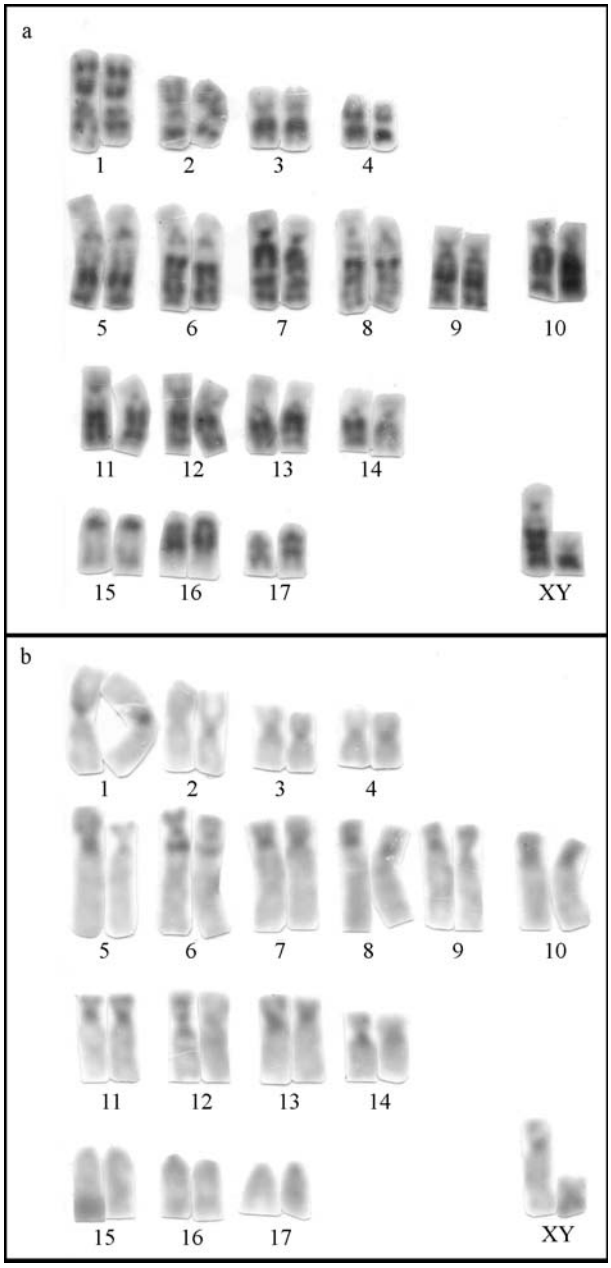

Figure 1 - G- (a) and C-banded (b) karyotypes of *U. magnirostrum.* Note the heteromorphism of pair 5.

The *U. bilobatum* specimens had 2n = 42, NF = 50 (Figure 2a). The autosome set is composed of 5 submeta-

centric and 15 acrocentric pairs. The X chromosome is a medium sized submetacentric and the Y, a small submetacentric. The C-banding pattern (Figure 2b) shows the constitutive heterochromatin in the pericentromeric region of all the autosomes and in the distal region of the short arm of two submetacentric chromosomes (pairs 1 and 2). The X chromosome shows centromeric and terminal heterochromatin in the short arm. The Y has a block of heterochromatin in the long arm. The Ag-NORs were located in the distal region of the short arm of submetacentric/acrocentric chromosomes (Figure 3b), ranging from five to eight per metaphase.

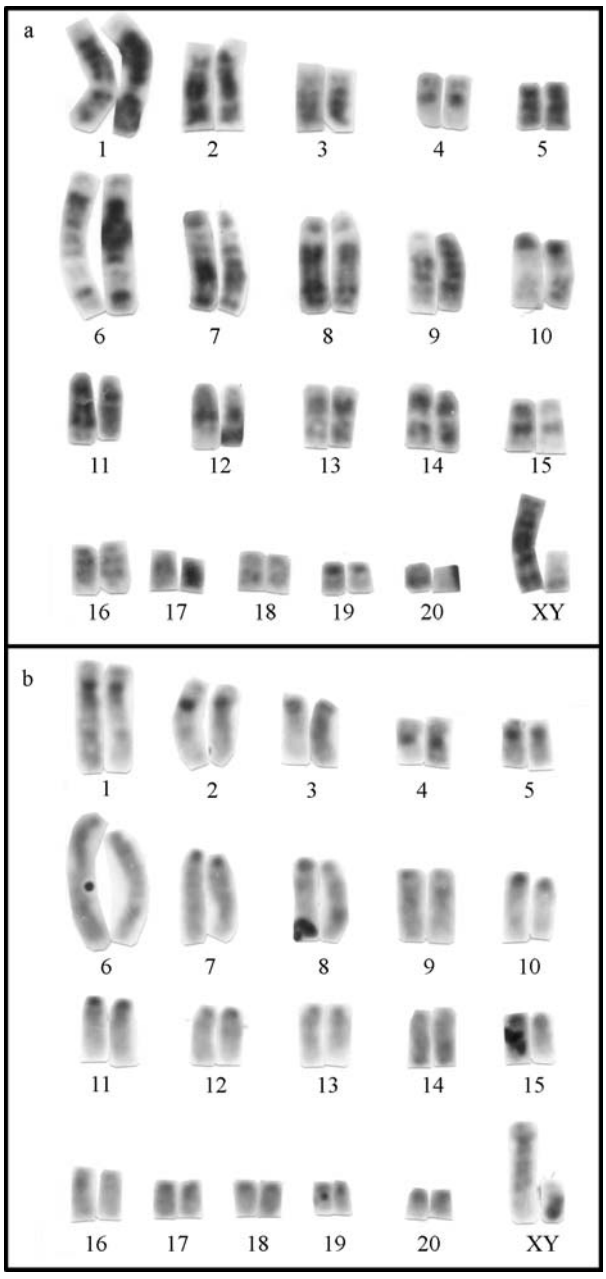

Figure 2 - G- (a) and C-banded (b) karyotypes of *Uroderma bilobatum.*

The G-banding comparison of the karyotypes of the two species of *Uroderma* (Figure 4) shows homeologies of whole chromosomes (Figure 4a), segments or arms of chromosomes (Figures 4b, 4c and 4d) and chromosomes that have no correspondence (Figures 4e and 4f). Both species present a chromosomal system of sexual determination of the type Neo-XY, but the G-banding obtained on the sex chromosomes, mainly the X, demonstrates that these chromosomes have no similar banding patterns (Figure 4g). The C-banded karyotype of *U. magnirostrum* (Figure 1b) shows a larger amount of heterochromatin than *U. bilobatum* (Figure 2b). The 5-8 multiple Ag-NORs were located in different chromosomes as can be seen in Figure 3.

Discussion

The *U. magnirostrum* species, collected in the Brazilian Amazon, has a similar karyotype (2n = 36, NF = 62) to that published by Baker and Lopez (1970) from animals collected in Central America, whose chromosomes were analyzed under giemsa staining. The authors also found two males with 2n = 35, NF = 62, due to the presence of a large metacentric chromosome (possibly originating from a fusion of two acrocentrics), not seen in the present work.

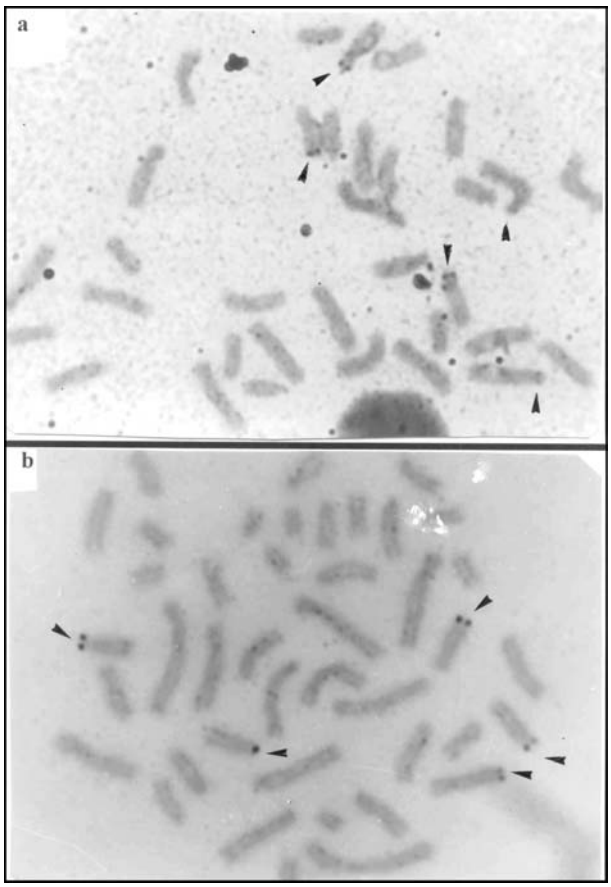

Figure 3 - Metaphases of *U. magnirostrum* (a) and *U. bilobatum* (b) showing chromosomes with Ag-NORs (arrow).

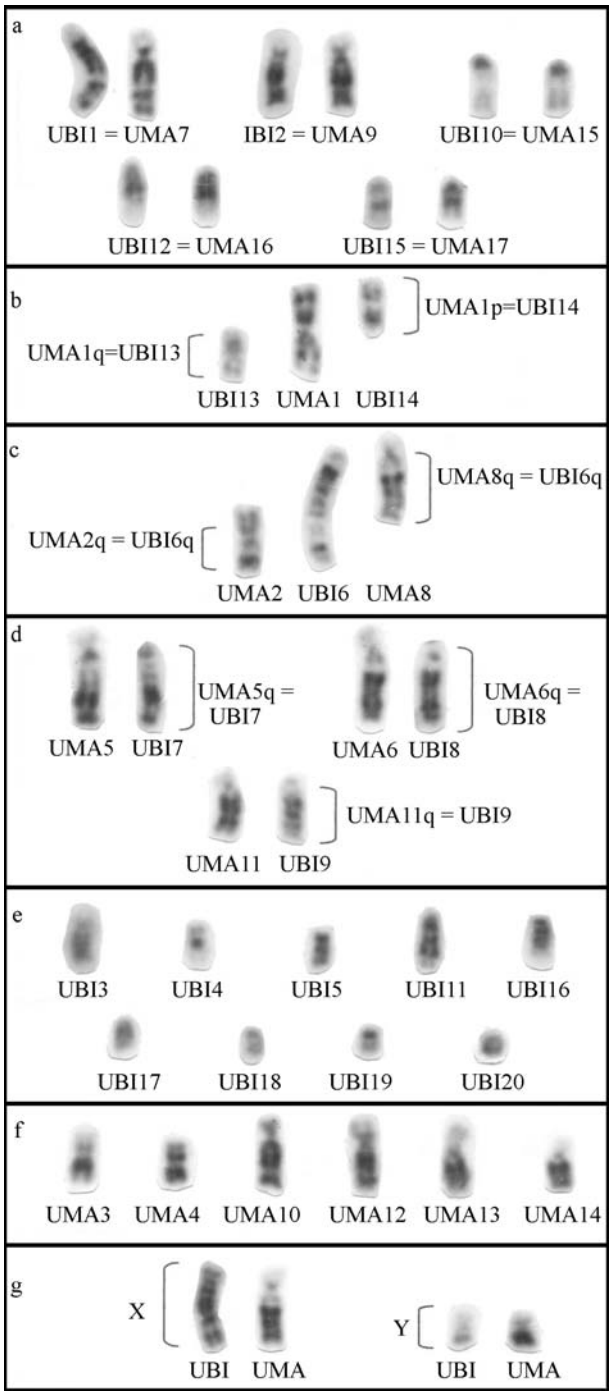

Figure 4 - Karyotypic comparison between *Uroderma bilobatum* (UBI) and *U. magnirostrum* (UMA). a) Homeologies of whole chromosomes: 1, 2, 10, 12 and 15 of UBI, and 7, 9, 15, 16 and 17 of UMA; b) Homeologies of the short arms (p) of the UMA1 with the acrocentric pair UBI14, while the long arms (q) correspond to the acrocentric pair UBI13, with a paracentric inversion; c) The long arm of pair UMA8 is similar to proximal region of long arm of the pair UBI6, while terminal region of long arm of pair UBI6 is similar to the long arm of pair UMA2; d) Homeologies of the long arm of the pairs 5, 6 and 11 of UMA with the acrocentric pairs 7, 8 and 9 of UBI, respectively; e) Pairs of UBI that have no correspondence in the karyotype of UMA; f) Pairs of UMA that have no correspondence in the karyotype of UBI; g) Comparison of the sex chromosomes: both have chromosomal system of sexual determination of the type Neo-XY.

The unique variation observed was a specimen that showed a heteromorphism in the short arm of the largest submetacentric (chromosome 5). At least three hypotheses could be advanced to explain this heteromorphism: 1) a heterochromatinization in only one of the homologues (see C-banding); 2) amplification of the rDNA cistron (see Ag-NOR banding); 3) a deletion in the short arm of only one of the homologues.

The *U. bilobatum* species (cytotype 2n = 42), has a similar karyotype to that published by Baker and Lopez (1970) and Baker *et al.* (1982). From a chromosomal evolution viewpoint, this species is very important, because it possesses karyotypes ranging from 2n = 38 to 44 (Baker and Lopez, 1970; Baker 1979, 1981; Baker *et al.*, 1982). According to Baker *et al.* (1982), two evolutionary histories for these karyotypes can be inferred. In the first, there is a trichotomy, because the three karyotypes appeared at the same time, and the cytotype 2n = 44 would represent the primitive condition. In the second, a fusion and an inversion separate a group with 2n = 42 and other with 2n = 44/38 and two translocations/fusion separate the 2n = 38 from the 2n = 44. However, even using the data analyzed here and those from the literature, the chromosomal phylogeny of the cytotypes of the *U. bilobatum* species remains uncertain, and more chromosomic information is necessary to solve this puzzle.

The high degree of karyotypic homeologies found between *Uroderma* species reinforces its relationship. They possess exclusive features such as pair 15 of *U. magnirostrum* or pair 10 of *U. bilobatum* not found in any other species of Stenodermatinae bats with G-banded karyotypes described so far. These species also shared two pairs of chromosome (16 and 17 of *U. magnirostrum;* 12 and 15 of *U. bilobatum*) that arose by fission of a metacentric chromosome. These chromosomes have some similarities to the chromosome 4 of *A. lituratus* (Silva, 2000) and 5 of *Phyllostomus hastatus* (Rodrigues, 1998). According to Baker *et al.* (1979), the karyotypes of the members of the Stenodermatinae subfamily have two chromosomes that are not found in any other species of the family Phyllostomidae, and are considered a plesiomorphic character in the *Uroderma* species.

Taking into account that the karyotype 2n = 30/31 of *Artibeus* represents the most primitive for the subfamily (Baker *et al.*, 1979; Silva, 2000) and that *U. magnirostrum* might present the most primitive karyotype for the genus, because of the highest similarity to *Artibeus*, we can infer karyoevolutionary pathways for the genus *Uroderma*: 1) one paracentric inversion in the long arm and subsequent fission of the pair 1 of *U. magnirostrum* originated the pairs 13 and 14 of *U. bilobatum*; 2) at least, two fission events and one fusion *in tandem* is necessary to explain the homeologies of the chromosomic segments among pair 6 of *U. bilobatum* and pairs 2 and 8 of *U. magnirostrum*; 3) fission events might have occurred also, to give rise to the

acrocentric chromosomes 7, 8 and 9 of *U. bilobatum* from the submetacentric chromosomes 5, 6 and 11 of *U. magnirostrum*, respectively. For the other chromosomes, the resolution of G-bands was too low to allow good comparison in small euchromatic areas that suffered chromosomal rearrangements. However, new techniques of molecular cytogenetics (such as FISH) can help explain doubts that still remain on the chromosomal evolution of this genus.

Regarding the chromosomal systems of sex determination of these two species (neo-XY/neo-XX), Tucker (1986) and Tucker and Bickham (1986) proposed that two chromosomal rearrangements possibly gave rise to the sex chromosomes observed in the karyotypes of the species of Phyllostomidae. First, a translocation X-autosome gave rise to a chromosomal pattern XY_1Y_2, as observed in most of the species of the *Artibeus* genus. Second, a translocation Y-autosome originated the Neo-XY pattern, as observed in the species of the present work. Thus, the chromosomal system of sex determination neo-XY is probably derived from system XY_1Y_2, as corroborated by the similarity in the size and in the C-banding pattern of the acrocentric chromosomes of the system XY_1Y_2 (genus *Artibeus*) and metacentric chromosomes of the system neo-XY (genus *Uroderma*).

Our comparative analysis of the two species of *Uroderma* allowed us to conclude that they are monophyletic. The species *U. bilobatum* presents an apomorphic karyotype, while whole *U. magnirostrum* presents a pleisiomorphic karyotype. This last species showed larger number of chromosomal homeologies with the species of the *Artibeus* genus (2n = 30/31), that possess the presumed ancestral karyotype for the subfamily Stenodermatinae (Baker, 1979; Baker *et al.*, 1979; Lim, 1993). The direction of the karyotypic evolution of the genus *Uroderma* trended toward an increase in the chromosome number by events of fission followed by events of fusion, inversion and/or translocation.

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