*Genetics and Molecular Biology, 23, 3, 595-599 (2000)*

# Chromosome comparison between two species of *Phyllostomus* (Chiroptera - Phyllostomidae) from Eastern Amazonia, with some phylogenetic insights

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*Abstract*

The karyotypes of *Phyllostomus discolor* and *P. hastatus* from Eastern Amazonia were studied by G-, C-, G/C sequential and Ag-NOR techniques. Both species presented 2n = 32, with the autosome complement composed of 30 bi-armed in *P. discolor* and 28 bi-armed plus 1 acrocentric in *P. hastatus.* In both species, the X chromosome is medium submetacentric while the Y is minute acrocentric. The present study found only one difference between the karyotypes of *P. discolor* and *P. hastatus*: the smallest autosome (pair 15) is bi-armed in *discolor* and acrocentric in *hastatus*, a result best explained by pericentric inversion. The C-banding revealed constitutive heterochromatin only at the centromeric regions of all chromosomes, with the NOR site located at the distal region of short arm of pair 15, in both species. The taxon *P. discolor* is considered primitive for genus *Phyllostomus* and the bi-armed form of pair 15 is the assumed primitive condition which, rearranged by a pericentric inversion originated the acrocentric form found in *P. hastatus.*

## INTRODUCTION

The neotropical bat genus *Phyllostomus* (Chiroptera - Phyllostomidae) traditionally includes four species: *P. hastatus*, *P. discolor*, *P. elongatus* and *P. latifolius* (Valdez, 1970). The genera *Phyllostomus* and *Phylloderma*, solely comprising the species *Phylloderma stenops*, are closely related by morphology (Williams and Genoways, 1980), karyotypes (Baker, 1973, 1979) and albumin data (Honeycutt and Sarich, 1987). Baker *et al.* (1988) suggested the inclusion of *Phylloderma stenops* in *Phyllostomus.* Evidence of strong affinity between *Phyllostomus* and *Phylloderma* was reinforced by cytochrome-b sequence analysis (Van den Bussche and Baker, 1993). In this paper, by adding *Phyllostomus stenops* (Baker *et al.*, 1988), we increased to five the number of species in the genus *Phyllostomus*.

Widely distributed throughout in the Central and South America, *P. hastatus* occurs from Honduras to Southeastern Brazil; *P. discolor* occurs from Mexico to Argentina while *P. elongatus* is restricted to South America in Colombia, Venezuela, the Guianas, Brazil, Ecuador, Peru and Bolivia; *P. latifolius* was registered in the Kanuko Mountains, of Guyana and in Suriname whereas *P. stenops* is found from Mexico to Northern Bolivia and Southeastern Brazil (Valdez, 1970; Jones and Carter, 1976; Honeycutt *et al.*, 1980; Wilson and Reeder, 1993).

Karyotypic studies in *Phyllostomus* have demonstrated that all species possess 2n = 32; *P. discolor* has FN = 60 while *P. hastatus*, *P. elongatus*, *P. stenops* and *P. latifolius* have FN = 58 (Baker, 1973, 1979; Honeycutt *et al.*, 1980). Chromosome comparisons by G-banding between *P. discolor* and *P. hastatus* from Central America (Patton and Baker, 1978) have demonstrated that only the smallest autosome (pair 15), a chromosome metacentric in *P. discolor* and acrocentric in *P. hastatus*, underwent rearrangement after divergence of the two species. A similar result was found in specimens from Southeastern Brazil (Varella-Garcia *et al.*, 1989). C-banding studies revealed constitutive heterochromatin only at centromeric regions in *P. hastatus*, *P. discolor* and *P. elongatus* (Baker, 1979; Varella-Garcia *et al.*, 1989). Ag-NOR staining identified NOR site, located only on chromosome 15 in *P. hastatus* and *P. discolor* (Morielle and Varella-Garcia, 1988).

According to Patton and Baker (1978), the karyotype of *Macrotus waterhousii* (2n = 46, FN = 60) is considered primitive to the family Phyllostomidae. *Phyllostomus hastatus*, *P. discolor*, *Mimon crenulatum* and *Tonatia minuta* are associated by five synapomorphic chromosome rearrangements: four Robertsonian fusions (18/3, 8/9, 17/12 and 29/27) and one inversion (4/5 inv.). *Phyllostomus* and *Mimon* share three additional synapomorphic fusions (22/13, 14a/21 and 28/30). The monophyly of *Phyllostomus* was established but the interspecific relationships remain unresolved (Honeycutt and Sarich, 1987; Baker *et al.*, 1988; Van Den Bussche and Baker, 1993).

The Amazonian Region bat fauna is the world's richest (Findley, 1993). Despite this, few studies have been made of bats from that region, at least from Brazilian Amazonia. This study presents a chromosome comparison between *Phyllostomus discolor* and *P. hastatus*, through G-band-

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ing, C-banding, sequential G/C-banding and NOR staining patterns, in specimens exclusively from Brazilian Amazonia and discusses the phylogeny of genus *Phyllostomus* from the chromosomal standpoint.

## MATERIAL AND METHODS

The sample included 3 specimens of *Phyllostomus discolor* (1M, 2F) from Belém (1°11' S, 48°29' W), and 4 *P. hastatus* (1M, 3F) from Peixe-Boi (1°11' S, 47°19' W), both in Pará State, Brazil. All specimens were taken from natural populations and collected with mist nets; voucher specimens are found in the mammal collections of the Emílio Goeldi Museum in Pará. Access numbers are: 26328, 26329 and 26330 for *P. discolor* and 26331, 26332, 26333 and 26334 for *P. hastatus*. Metaphase spreads were got with fibroblast cultures from ear biopsies and bone marrow preparations (Baker and Qumsiyeh, 1988). Slides were prepared using air-drying method and G-bands obtained by trypsin treatment (Scheres, 1972), C-bands were performed according to Sumner (1972), sequential G/C-banding followed both techniques (some metaphases were G-banded, photographed and later submitted to a C-banding process), and Ag-NOR staining followed Howell and Black (1980).

## RESULTS

The karyotype of *Phyllostomus discolor* possesses 2n = 32 and FN = 60; the autosome complement is composed of 15 bi-armed pairs, while that *P. hastatus* has 2n = 32, FN = 58, with 14 bi-armed plus one acrocentric pair. In both species, the X chromosome is medium submetacentric and the Y is minute acrocentric. Figures 1 and 2 show, respectively, the G- and C-bands representative of *P. hastatus* and similar those of *P. discolor.* C-banding revealed constitutive heterochromatin restricted to the pericentromeric region of all chromosomes.

Comparative analysis of G-banding pattern between *P. discolor* and *P. hastatus* shows that both species conserved all chromosomes without rearrangements, except for chromosome 15, altered by pericentric inversion (Figure 3). Both species presented Ag-NOR labelling at a distal region of pair 15 (Figure 3d).

## DISCUSSION

Karyotypes of *P. discolor* and *P. hastatus* from Eastern Amazonia are identical to those described for Central American specimens (Patton and Baker, 1978), and Southeastern Brazil (Morielle and Varella-Garcia, 1988; Varella-Garcia *et al*., 1989). Our results corroborate the hypothesis that *Phyllostomus* has a conservative rate of chromosomal evolution, in contrast to other widespread bat genera, e.g., *Tonatia* and *Micronycteris* that present a high rate of chromosomal evolution (Baker and Bickham, 1980).

*P. discolor* and *P. hastatus* differ cytogenetically by a single pericentric inversion in chromosome 15, probably derived by fusion of acrocentrics 28 and 30 from *Macrotus waterhousii* (Patton and Baker, 1978). This chromosome, a small metacentric in *P. discolor*, is acrocentric in *P. hastatus*, *P. elongatus*, *P. stenops* and *P. latifolius* (Baker, 1973, 1979; Honeycutt *et al*., 1980). Considering that *P. discolor* is primitive to genus *Phyllostomus* (Valdez, 1970; Honeycutt and Sarich, 1987; Baker *et al*., 1988), and shares

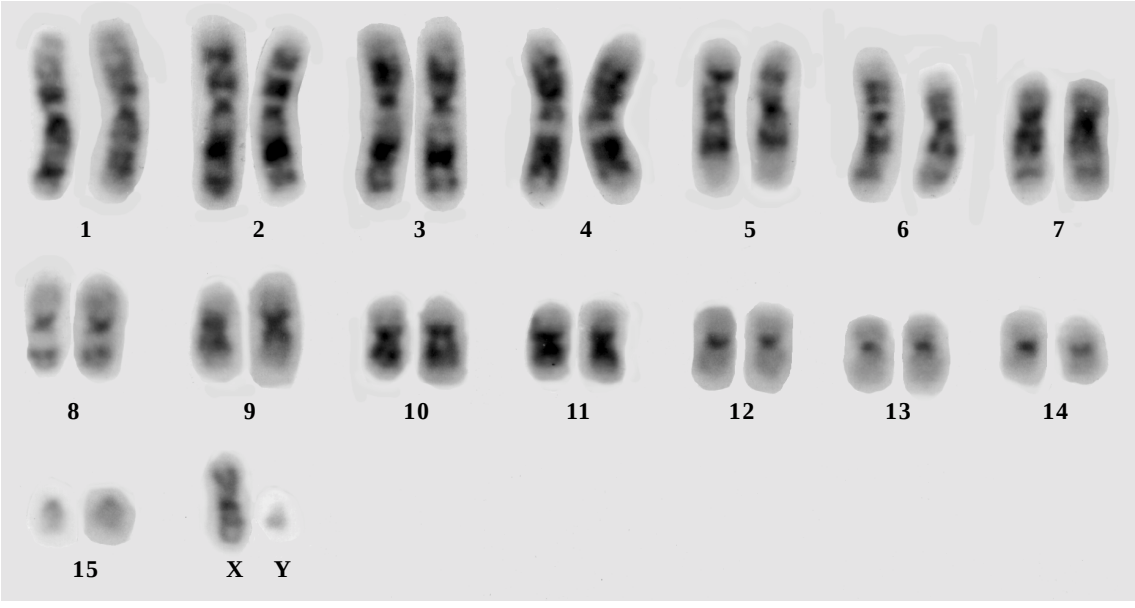


**Figure 1** - G-banding pattern of *Phyllostomus hastatus*.

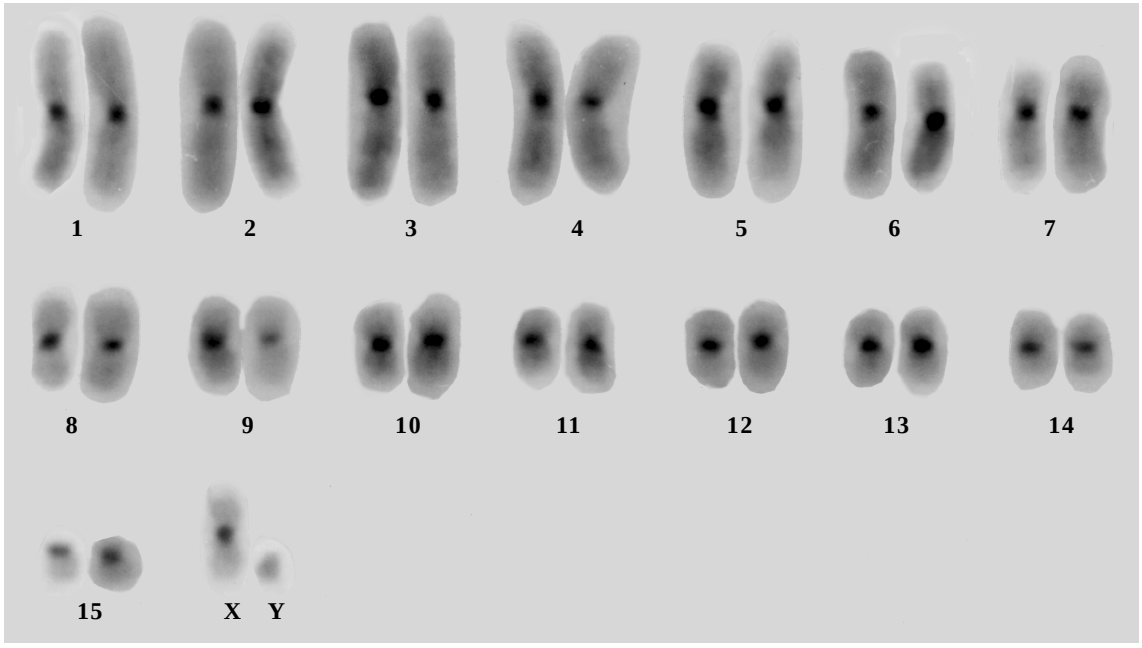


**Figure 2** - C-banding pattern of *Phyllostomus hastatus*.

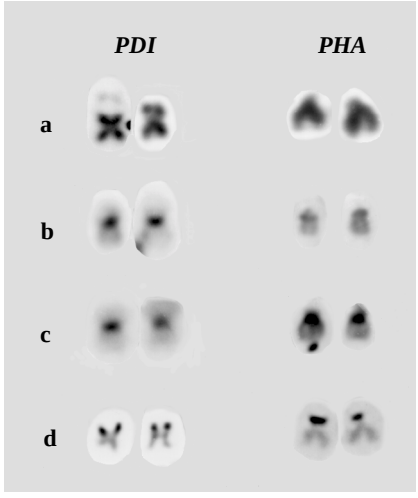


**Figure 3** - Pair 15 of *Phyllostomus discolor* (*PDI*) and *P. hastatus* (*PHA*). a) Giemsa stained, b) G-banded, c) C-banded and d) Ag-NOR stained.

with *Mimon crenulatum* the metacentric chromosome 15 (Patton and Baker, 1978), the apparently derived acrocentric chromosome 15 is shared by *P. hastatus*, *P. elongatus*, *P. stenops* and *P. latifolius*.

Chromosome 15 of *P. discolor* presents the NOR on the distal region of one arm, while it appears on the distal region of a short arm in the 15 of *P. hastatus*. Therefore, the chromosome portion involved in pericentric inversion seems unlikely to contain DNAr sequences, because of the distal position of the NOR site. In general, few species of the family Phyllostomidae have been studied by Ag-NOR staining (Baker, 1979). In the specific case of *Phyllostomus*, NOR studies are needed to verify if: 1) *P. elongatus* and *P. stenops* present NORs at distal regions of the short arm of chromosome 15 as in *P. hastatus*, 2) *Mimon crenulatum* has NOR sites on chromosome 15 as in *P. discolor*, and 3) *Macrotus waterhousii* presents NORs in the acrocentric 28 or 30. This information will increase understanding of chromosomal evolution in the genus *Phyllostomus*.

Interspecific relationships in the genus *Phyllostomus* have been difficult to clarify. Using morphological characters, Valdez (1970) proposed a close relationship between *P. elongatus* and *P. latifolius*. In contrast, albumin distance analysis (Honeycutt and Sarich, 1987) indicated a closer connection between *P. hastatus* and *P. elongatus*, joined by *P. stenops* in one group while *P. discolor* and *P. latifolius* emerged at the cladogram base. The allozymic data from Baker *et al.* (1988), while not adequately resolving interspecific relationships in *Phyllostomus*, suggested that the five species diverged at approximately the same time. Phylogenetic analysis with cytochrome-B sequence (Van Den Bussche and Baker, 1993) confirmed the *hastatus-elongatus* association suggested by Honeycutt and Sarich (1987), and suggested that *P. discolor* is sister taxa to *hastatus-elongatus*, with *P. latifolius* joining this group and *P. stenops* at the root of the tree.

We suggest a chromosomal phylogeny for genus *Phyllostomus* (Figure 4), based on the data (see references: Patton and Baker, 1978; Baker, 1979; Honeycutt *et al*., 1980; Honeycutt and Sarich, 1987; Baker *et al*., 1988; Van Den Bussche and Baker, 1993) and assuming that: a) *P. dis-*

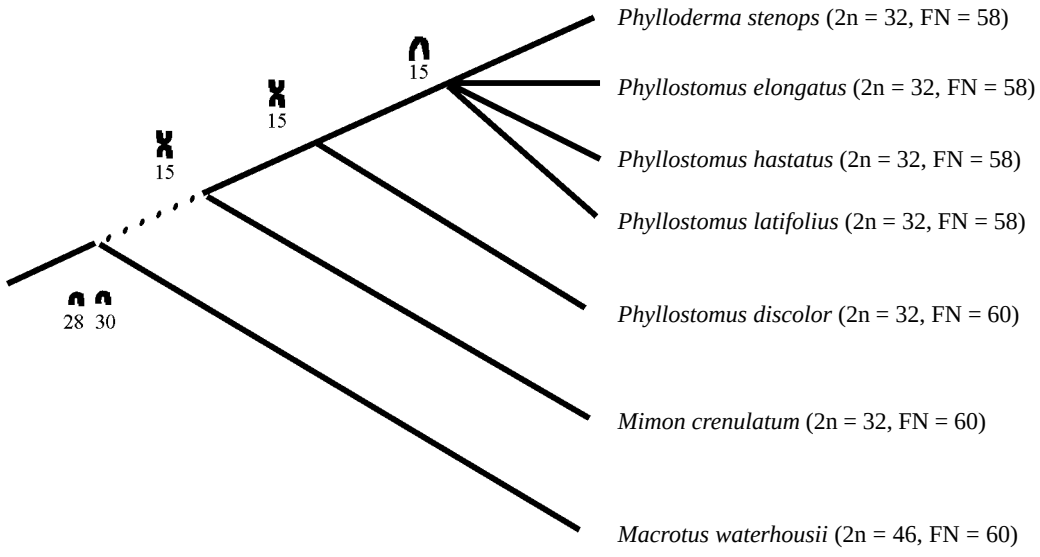


**Figure 4** - Chromosomal phylogeny of the genus *Phyllostomus* based on literature data.

*color* is primitive to *Phyllostomus*, b) the bi-armed chromosome 15 shared with *Mimon crenulatum* arose by fusion between two acrocentrics (28 and 30) from *Macrotus waterhousii*, followed by pericentric inversion in the ancestral lineage of *P. hastatus*, *P. elongatus*, *P. latifolius* and *P. stenops*.

Cytogenetic data are compatible with the phylogeny based on albumin data, proposed by Honeycutt and Sarich (1987). On the other hand, from the cytogenetic standpoint, the hypothesis based on cytochrome-B (Van Den Bussche and Baker, 1993) is less parsimonious because if the bi-armed form of chromosome 15 is primitive, then the derived form (acrocentric) would have twice evolved: once at lineage *P. stenops*, again at lineage *P. latifolius* and the finally at lineage *hastatus-elongatus*.

Additional studies are need for clarifying the interspecific relationships in the genus *Phyllostomus*. Special attention should be given to Ag-NOR staining or DNAr hybridization studies in *Mimon crenulatum*, *Phyllostomus stenops* and *P. elongatus* to ascertain the supposed homeology of chromosome 15.

## ACKNOWLEDGMENTS

We thank CAPES, CNPq, FINEP and PROPESP-UFPA, Brazil, for financial support. We also thank the students and technicians from Laboratório de Citogenética-UFPA and Emílio Goledi Museum who contributed to this research, especially to the Chiroptera Team (Carol, Renata, Nelson, Adailton and Ciane).

## RESUMO

Os cariótipos de *Phyllostomus discolor* e *P. hastatus* da Amazônia oriental são estudados por bandeamentos G, C, G/C sequencial e coloração Ag-NOR. Ambas as espécies apresentaram 2n = 32, sendo o complemento autossômico composto por 15 pares bi-armed em *P. discolor* e 14 bi-armed mais 1 par acrocêntrico em *P. hastatus*. O cromossomo X é um submetacêntrico médio e o Y é um pequeno acrocêntrico em ambas as espécies. O presente estudo encontrou apenas uma diferença entre os cariótipos de *P. discolor* e *P. hastatus*: o menor autossomo (par 15) é metacêntrico em *discolor* e acrocêntrico em *hastatus*. Este resultado é melhor explicado por uma inversão pericêntrica. O bandeamento C revelou heterocromatina constitutiva na região centromérica de todos os cromossomos, e os sítios NOR foram localizados na região distal do par 15, em ambas as espécies. O táxon *P. discolor* é considerado primitivo para o gênero *Phyllostomus* e supõe-se que a forma metacêntrica do par 15 seja a condição primitiva, que foi rearranjada por uma inversão pericêntrica, originando a forma acrocêntrica encontrada em *P. hastatus*.

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*(Received August 20, 1999)*