

## CHROMOSOME BANDING STUDIES OF *Chrotopterus auritus* (CHIROPTERA: PHYLLOSTOMIDAE)

Eliana Morielle-Versute<sup>1</sup>, Valdir Antonio Taddei<sup>1</sup> and Marileila Varella-Garcia<sup>2</sup>

### ABSTRACT

Chromosomal characterization of *Chrotopterus auritus* by GTG, CBG and Ag-NOR techniques are presented in order to clarify the chromosomal changes occurred in the evolutionary process in phyllostomid bats.

### INTRODUCTION

Peter's woolly false vampire bat *Chrotopterus auritus* ranges from southern Mexico to southern Brazil, Paraguay and northern Argentina (Jones and Carter, 1976). This species has been traditionally included in the subfamily Phyllostominae (Miller, 1907; Smith, 1976; Koopman, 1984). However, considering that this classification does not reflect the evolutionary relationships defined by synapomorphies, Baker *et al.* (1989) proposed a revised classification of the higher taxonomic levels within the Phyllostomidae, based on a synthesis of classical morphological, chromosomal, immunological, and biochemical data. Three subfamilies were recognized, and the genera *Chrotopterus*, *Vampyrum* and *Trachops* were removed from Phyllostominae to constitute the subfamily Vampyrinae.

The monophyletic origin for the subfamily Vampyrinae has been supported by immunological data (Honeycutt and Sarich, 1987). Although Baker *et al.* (1989) postulated the lack of elements contradicting such an alignment, only the standard karyotype has been published for each of the three species, so additional data are needed.

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<sup>1</sup> Departamento de Zoologia and <sup>2</sup> Departamento de Biologia, Instituto de Biociências, Letras e Ciências Exatas - UNESP, Caixa Postal 136, 15055 São José do Rio Preto, SP, Brasil. Send correspondence to E.M.-V.

This paper presents the chromosomal characterization of *C. auritus* by GTG and CBG bands, and by Ag-NOR staining. It also points out the presumed chromosomal homologies with the karyotype of *Macrotus waterhousii* (Patton and Baker, 1978; Baker, 1979; Baker *et al.*, 1982) which is taken as the most primitive for the family.

## MATERIAL AND METHODS

A male specimen of *C. auritus* was captured in Sales, State of São Paulo (ca. 21°23'S; 49°30'W) and is deposited in the Chiroptera collection of the Department of Zoology, Universidade Estadual Paulista - UNESP, at São José do Rio Preto, State of São Paulo, Brazil. Mitotic chromosome spreads were prepared after the usual colchicine arresting and hypotonic (1% sodium citrate) and fixation (methanol - acetic 3:1) treatments of fibroblast-like cells obtained from ear biopsies. Cultures were grown in Ham F-10 medium supplemented with 20% fetal calf serum, L-glutamine, penicillin, and streptomycin. GTG and CBG banding and Ag-NOR staining were performed according to De Grouchy and Turleau (1977), Sumner (1972) and Howell and Black (1980) with the modifications referred to by Varella-Garcia and Taddei (1989).

## RESULTS AND DISCUSSION

The karyotype of *C. auritus* has  $2n=28$  and  $FN=52$ . It is composed of thirteen pairs of metacentric or submetacentric autosomes, ranging from large to medium size, and one pair of sexual chromosomes, the X chromosome is a medium sized metacentric element and the Y chromosome is a minute acrocentric element. Each chromosome pair is identifiable by a unique GTG-banding pattern (Figure 1A). Heterochromatin blocks identified by the CBG-banding technique appear only at the pericentromeric regions in every chromosome (Figure 1B). Silver staining showed a single pair of nucleolus organizer regions (NOR) located at the secondary constriction on the proximal region of the long arm of the third largest chromosome pair (Figure 1C).

An extensive similarity can be detected by comparing the G-banding pattern of *C. auritus* with the karyotype of *M. waterhousii* presented in Patton and Baker (1978) and Baker *et al.* (1982). The autosomes 5, 7, 8, 9, 11, 12, and 13 of *C. auritus* are identifiable in the karyotype of *M. waterhousii*, corresponding respectively to the banded chromosomes 1/2, 4/5, 6/7, 10/11, 15/16, 19/20, and 23/24. Chromosomes 1, 2 and 3 of *C. auritus* seem to be Robertsonian fusion products of chromosome arms of *Macrotus*, respectively 8/9, 18/3 and 17/12, which are shared also by *Tonatia minuta* (= *T. brasiliense*), *Mimon crenulatum*, *Phyllostomus hastatus* and *P. discolor*. Additionally,

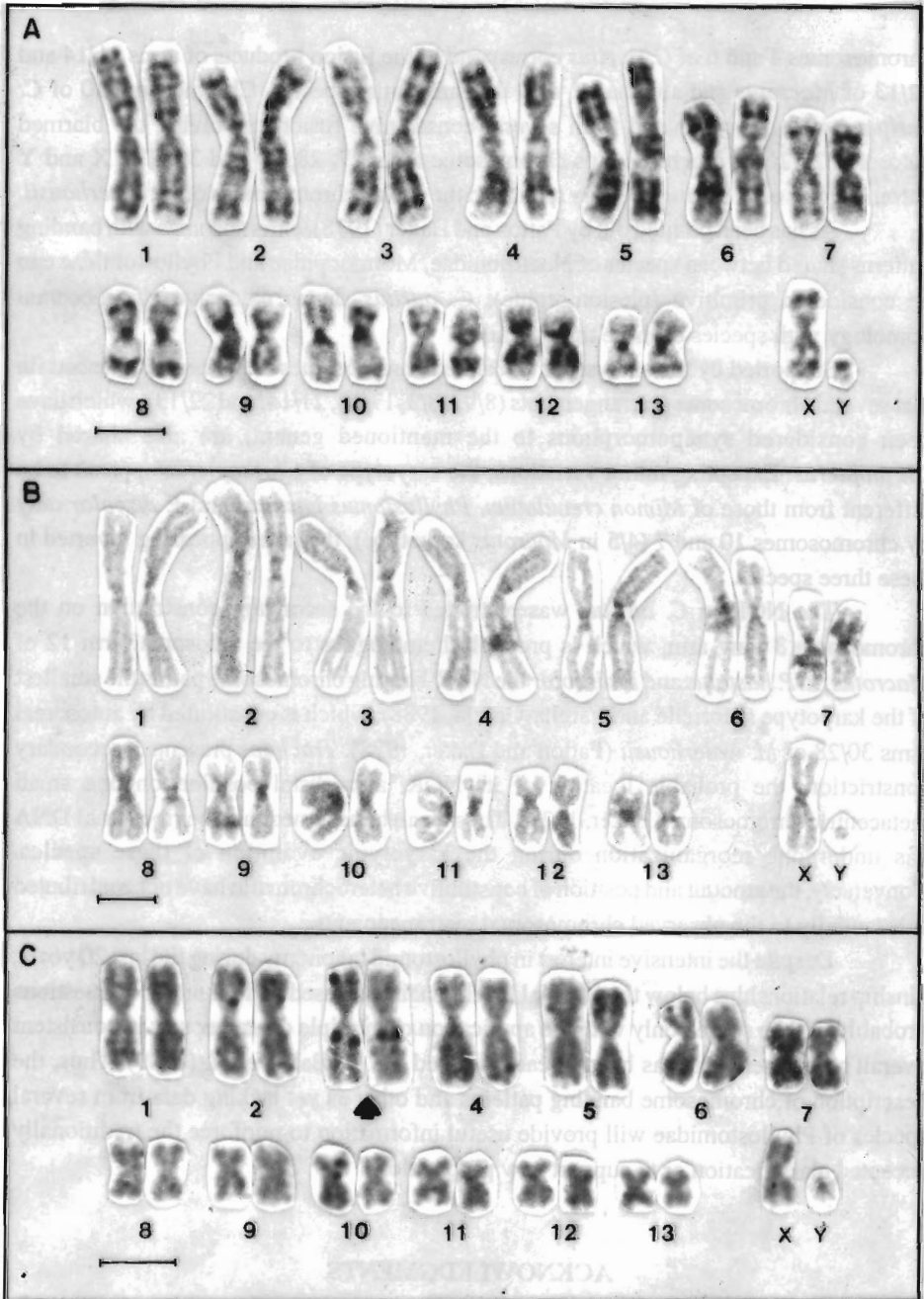


Figure 1 - Karyotype of a male *Chrotopterus auritus*. The bar represents 5 µm. A: GTG-banded chromosomes.

B: CBG-banded chromosomes. C: Ag-NOR staining.

chromosomes 4 and 6 of *C. auritus* correspond to the fusion products of arms 21/14 and 22/13 of *Macrotus* and are shared with the three latter species. Chromosome 10 of *C. auritus* may have originated from several consecutive fusions involving the banded autosome 25/26 of *Macrotus* plus chromosome arms 27, 28, 29 and 30. The X and Y chromosomes of *C. auritus* are very similar to the sexual chromosomes of *M. waterhousii*.

As has been pointed out by Patton and Baker (1978), chromosomes with banding patterns shared between species of Noctilionidae, Mormoopidae and Phyllostomidae can be considered primitive (plesiomorphic). *C. auritus* shares extensive chromosomal homology with species of these three families.

Supported by the analysis of G-banded chromosomes it is possible to ascertain that several chromosome rearrangements (8/9, 18/3, 17/12, 21/14, and 22/13), which have been considered synapomorphous to the mentioned genera, are also shared by *Chrotopterus*. Excepting minor variations, the karyotype of *Chrotopterus* appears to be different from those of *Mimon crenulatum*, *Phyllostomus hastatus* and *P. discolor* only by chromosomes 10 and 7 (4/5 in *Macrotus* karyotype), the latter appearing inverted in these three species.

The NOR in *C. auritus* was restricted to the secondary constriction on the chromosome 3 long arm, which is probably homologous to the autosomal arm 12 of *Macrotus*. In *P. hastatus* and *P. discolor* the NOR-bearing chromosome pair is the smallest of the karyotype (Morielle and Varella-Garcia, 1988), which is constituted by autosomal arms 30/28 of *M. waterhousii* (Patton and Baker, 1978). *Trachops* presents a secondary constriction, the probable location of its NOR, at a distal position on one small metacentric chromosome (Baker, 1979). Thus it can be assumed that the ribosomal DNA has undergone reorganization during the karyotypic evolution of these species. Conversely, the amount and position of constitutive heterochromatin have not contributed substantially to the observed chromosomal rearrangements.

Despite the intensive interest in phyllostomid taxonomy during the last 20 years, kinship relationships below the familial level remain confused and a number of questions probably will be solved only with the application of multiple character sets in consistent overall assessments, as has been already pointed out by Baker *et al.* (1989). Thus, the description of chromosome banding patterns and other as yet lacking data from several species of Phyllostomidae will provide useful information to reinforce the traditionally accepted classification or to support new propositions.

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## RESUMO

Apresenta-se a caracterização cromossômica de *Chrotopterus auritus* pelas técnicas de bandamentos GTG, CBG e Ag-NOR, como uma contribuição para o esclarecimento das mudanças cromossômicas ocorridas no processo evolutivo dos morcegos fillostomídeos.

## REFERENCES

- Baker, R.J. (1979). Karyology. Pp. 107-155, in *Biology of bats of the New World family Phyllostomatidae, Part III* (R.J. Baker, J.K. Jones, Jr., and D.C. Carter, eds.). *Spec. Publ. Mus., Texas Tech Univ.*, 16: 1-441.
- Baker, R.J., Haiduk, M.W., Robbins, L.W., Cadena, A. and Koop, B.F. (1982). Chromosomal studies on South American bats and their systematic implications, in *Mammalian biology in South America* (Mares, M.A. and H.H. Genoways, eds.). *Spec. Publ. Ser. Pymatuning Lab. Ecol. IV*: 303-327.
- Baker, R.J., Hood, C.S. and Honeycutt, R.L. (1989). Phylogenetic relationships and classification of the higher categories of the New World bat family Phyllostomidae. *Syst. Zool.* 38: 228-238.
- De Grouchy, J. and Turleau, C. (1977). *Atlas des maladies chromosomiques*. Paris, Expansion Scientifique Française.
- Honeycutt, R.L. and Sarich, V.M. (1987). Albumin evolution and subfamilial relationships among New World leaf-nosed bats (family Phyllostomidae). *J. Mamm.* 68: 508-517.
- Howell, W.M. and Black, A.D. (1980). Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia* 36: 1014-1015.
- Jones, J.K. Jr. and Carter, D.C. (1976). Annotated checklist, with keys to subfamilies and genera. Pp. 7-38, in *Biology of bats of the New World family Phyllostomatidae, Part I* (R.J. Baker, J.K. Jones Jr. and D.C. Carter, eds.). *Spec. Publ. Mus. Texas Tech Univ.* 10: 1-218.
- Koopman, K.F. (1984). A synopsis of the families of bats. *Bat. Res. News* 25: 25-29.
- Miller, G.S. Jr. (1907). The families and genera of bats. *Bull. U.S. Natl. Mus.* 57: i-xvii + 1-282.
- Morielle, E. and Varella-Garcia, M. (1988). Variability of nucleolus organizer regions in phyllostomid bats. *Rev. Bras. Genet.* 11: 853-871.
- Patton, J.C. and Baker, R.J. (1978). Chromosomal homology and evolution of phyllostomatoid bats. *Syst. Zool.* 27: 447-462.
- Smith, J.D. (1976). Chiropteran evolution. Pp. 49-79, in *Biology of bats of the New World family Phyllostomatidae, Part I* (R.J. Baker, J.K. Jones, Jr. and D.C. Carter, eds.). *Spec. Publ. Mus. Texas Tech. Univ.* 10: 1-218.
- Sumner, A.T. (1972). A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell. Res.* 75: 304-306.
- Varella-Garcia, M. and Taddei, V.A. (1989). Citogenética de quirópteros: métodos e aplicações. *Rev. Bras. Zool.* 6: 297-323.