

# The karyotype of *Cabassous unicinctus* (Dasypodidae, Xenarthra)

PEREIRA JUNIOR HÉLIO RUBENS JACINTHO<sup>1,2,\*</sup>, VALQUIRIA SANTILONI<sup>1</sup>, PATRICIA SANMARCO ROSA<sup>3</sup>, LIGIA SOUZA LIMA SILVEIRA DA MOTA<sup>1</sup>, WILHAM JORGE<sup>1,4</sup>.

<sup>1</sup>Departamento de Genética, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, SP, Brazil.

<sup>2</sup>Faculdade de Tecnologia, Centro Universitário Nossa Senhora do Patrocínio, Salto, SP, Brazil.

<sup>3</sup>Instituto Lauro de Sousa Lima, Bauru, SP, Brazil.

<sup>4</sup>Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

**Abstract** — Armadillos was belonged to the Xenarthra Order, Dasypodidae family. This family has been comprising the largest number of genera and species among the Xenarthrans; eight (8) and 21, respectively. Two adult males of the species *Cabassous unicinctus* were analyzed in this study. Lymphocyte cultures from whole blood were used and the cells were then submitted to conventional staining by C- and Ag-NOR banding. Data regarding the number of chromosomes showed discrepancies among the species described in the literature. The 46 chromosomes observed were distributed in six large metacentric pairs, five medium submetacentric pairs, five medium and small metacentric pairs and six acrocentric pairs. The Y chromosome was classified such as the smallest acrocentric of the group. The X chromosome was classified as medium submetacentric, it considered atypical for mammals, because X was generally large size metacentric when it compared to the remaining karyotype. The diploid number reduction from 62 to 46 chromosomes may be explained by Robertsonian fusion and the inversion of acrocentric, or even the fission of centromeric regions of metacentric, given that the species *Cabassous centralis* had 23 acrocentric pairs and that this number was reduced to 14 pairs in the *Cabassous tautouay* and six pairs in the species described in the present work.

**Key words:** *Cabassous*, chromosome evolution, *Cingulata*, Dasypodidae, karyotype, *Xenarthra*.

## INTRODUCTION

Armadillos was classified as placental mammals belonging to the Xenarthra Group, Cingulata Order, Dasypodidae family (WETZEL 1985; NOWAK 1999). The Order Cingulata has been comprising three families: Pampatheridae (†), Glyptodontidae (†) and Dasypodidae of living and extinct specimens (ENGELMANN 1985; WETZEL 1985; NOWAK 1999). The Dasypodidae family was been currently composed of 21 species, distributed in four subfamilies and eight genera (WETZEL 1985; VIZCAINO 1995; NOWAK 1999).

What differentiates armadillos from other living Xenarthrans (vermilinguas and sloths) was been the presence of a protective shield or carapace (WETZEL 1985), formed by bands composed of small superposed osseous plates of dermal origin

(BENIRSCHKE 1968). This osteoderm was been a specialized anti-predatory structure (BENIRSCHKE 1968; NOWAK 1999; PEREIRA JR. *et al.* 2003).

Present species of the Dasypodidae family was exclusive to neotropical fauna, located in parts of Central and South America, except for the nine-banded armadillo (*Dasypus novemcinctus*), which was introduced to North America between the XIX and XX centuries. Today its geographic distribution ranges from the Strait of Magellan (Southern Patagonia) to Central Southern United States (WETZEL 1985; NOWAK 1999). Armadillos present crepuscular/nocturnal fossorial-terrestrial habits and they was considered asocial. They spend their lives building burrows, usually underground, and digging soil looking for their foods.

The genus *Cabassous* was considered the second largest in number of species, surpassed only by the *Dasypus* genus. It has been having four species: *Cabassous tautouay*, *Cabassous centralis*, *Cabassous unicinctus* and *Cabassous chacoensis* (WETZEL 1980). They were known as naked-tail armadillos, because of the minimal dispersion of

\* Corresponding author: e-mail: hejaper@yahoo.com.br

osteoderm plates on their tails. They belonged to the Tolypeutinae tribe and were considered a sister group to the *Tolypeutes* genus (DELSUC *et al.* 2003; 2004). This group of animals was considered myrmecophilous, i.e., they feed only on termites and ants (REDFORD 1985). They were present from Southeastern Mexico and Belize to Uruguay and Argentina. The largest size species was the *Cabassous tautoauay*, approximately 50 centimeters long (WETZEL 1980). *Cabassous unicinctus* had the widest geographic distribution ranging from the Andes, close to Venezuela, to Southeastern Brazil (WETZEL 1980). *Cabassous centralis* was the only species of this genus that has never been sighted in Brazilian territory (WETZEL 1985; 1980). According to the IUCN (2008), the species *Cabassous chacoensis* needed more attention concerning preservation, because its geographic distribution was restricted to swampy areas formed by the Great Chaco.

The first Xenarthran to have its karyotype described was the *Dasybus novemcinctus* species, by BEATH *et al.* in 1962. The Dasypodidae family presents the largest karyotypic variation among

Xenarthrans, the *Tolypeutes matacus* species with  $2n=38$  chromosomes and the species of the *Dasybus* genus with  $2n=64$  chromosomes. Up to now, 16 armadillo species have had their karyotype described and/or reported (Table 1) (JORGE and PEREIRA JR 2008, REDI *et al.* 2005). The *Cabassous* genus presents the largest intragenus chromosome variation. The *Cabassous centralis* had the highest diploid number ( $2n=62$  chromosomes) (BERNIRSCHKE *et al.* 1969) and *Cabassous tautoauay* has  $2n=50$  (BARROSO and SEUÁNEZ 1991). The present work aimed to describe the karyotype of the *Cabassous unicinctus* species.

## MATERIAL AND METHODS

Two males of the *Cabassous unicinctus* species were studied. The animals were maintained in the scientific collection of the Lauro de Souza Lima Institute, located in Bauru, São Paulo State, Brazil.

Both animals came from the forest reservation bordering the Institute's land. The armadillos were anesthetized according to the standardized

Table 1 — Chromosome data regarding all armadillo species: diploid number ( $2n$ ), morphology of sex chromosomes (X and Y), banding pattern and bibliography. The acronyms A, SM, M, C, G and NOR respectively signify, submetacentric acrocentric chromosomes, metacentric chromosomes, C banding, G banding and Ag-NOR banding.

Tribe	Species	$2n$	X	Y	Banding	References
Chlamyphorinae	<i>Chl. truncatus</i>	58	–	–	C e G	JORGE <i>et al.</i> , 1985
	<i>Chl. retusus</i>	–	–	–	–	No date
Dasypodinae	<i>D. hybridus</i>	64	SM	A	G	SAEZ <i>et al.</i> 1964
	<i>D. kappleri</i>	64	–	–	–	REDI <i>et al.</i> , 2005
	<i>D. novemcinctus</i>	64/65	SM	A	G, NOR	BEATH <i>et al.</i> 1962, BERNIRSCHKE <i>et al.</i> 1969, JORGE <i>et al.</i> 1977
	<i>D. pilosus</i>	64	–	–	–	REDI <i>et al.</i> , 2005
	<i>D. sabanicola</i>	64	–	–	–	REDI <i>et al.</i> , 2005
	<i>D. septemcinctus</i>	63/64	SM	A	G, NOR	BARROSO and SEUÁNEZ 1991
	<i>D. yepesi</i>	–	–	–	–	No date
Euphractinae	<i>Cha. nationi</i>	62	SM–	A	–	COOK <i>et al.</i> 1991
	<i>Cha. vellerosus</i>	62	M	A	–	LIZARRALDE <i>et al.</i> 2005
	<i>Cha. villosus</i>	60	A	A	C e G	JORGE <i>et al.</i> 1977
	<i>E. sexcinctus</i>	58	SM/A	SM/A	C, G e NOR	JORGE <i>et al.</i> 1977, BARROSO and SEUÁNEZ 1991
	<i>Z. pichiy</i>	62	A	A	G	MERITT and BERNIRSCHKE 1973, JORGE <i>et al.</i> 1977
Tolypeutinae	<i>C. centralis</i>	62	SM	M	–	BERNIRSCHKE <i>et al.</i> 1969
	<i>C. chacoensis</i>	–	–	–	–	No date
	<i>C. tatouay</i>	50	M	A	–	BARROSO and SEUÁNEZ 1991
	<i>C. unicinctus</i>	46	M	A	C e NOR	This paper
	<i>P. maximus</i>	50	SM	M	–	BERNIRSCHKE and WURSTER 1969
	<i>T. matacus</i>	38	M	A	–	JORGE <i>et al.</i> 1977
	<i>T. tricinctus</i>	–	–	–	–	No date

protocol of the Institution: subcutaneously pre-medicated with atropine sulphate (0.04 ml/kg) and intramuscularly anesthetized with Zoletil® (tiletamine/zolazepan 0.2 ml/kg). Three milliliters of blood were collected from the subclavian vein with a heparinized syringe.

A series of procedures were tested to adequate the cultures and chromosome preparation conditions (PEREIRA JR *et al.* 2004). The best metaphases were obtained with C and Ag-NOR banding, described for the first time in this work.

## RESULTS AND DISCUSSION

The first study that described chromosomes in the genus *Cabassous* was realized by BENIRSCHKE *et al.* (1969), who described the karyotype of the species *Cabassous centralis* with  $2n=62$  chromosomes.

BARROSO and SEUÁNEZ (1991) described another species, *Cabassous unicinctus*. Later on, after the karyotype had already been described, the authors verified a problem in the identification of the species. They initially described the species as *Ca-*

*bassous unicinctus* throughout their initial study; however, the species was renamed *Cabassous tautoauay*. In the PhD thesis of BARROSO (1982), the karyotype of the species *Cabassous tautoauay* was described with  $2n=50$  chromosomes.

BARROSO (1982), described the karyotype of the species *Cabassous tautoauay* with  $2n=50$  chromosomes. They initially (BARROSO and SEUÁNEZ 1991) described the species as *Cabassous unicinctus*, however the species was renamed *Cabassous tautoauay*.

In the present work, two specimens of *Cabassous unicinctus* had their karyotype analyzed, revealing  $2n=46$  chromosomes. Twelve of the chromosomes were acrocentric, ten submetacentric and 22 metacentric. They were divided in four groups: 1) six pairs of large metacentric; 2) five pairs of median and small submetacentric; 3) five pairs of small metacentric; and 4) six pairs of acrocentric. The X chromosome was classified as medium submetacentric equivalent to the seventh pair. The Y chromosome was classified as the smallest of the karyotypic group and is considered a punctiform chromosome (Fig. 1).



Fig. 1 — Karyotype of the species *Cabassous unicinctus* with  $2n=46$  chromosomes. The X sex chromosome is a medium sized metacentric and the Y a small acrocentric chromosome, the smallest in the group.

Table 2 — Chromosome characteristics of the genus *Cabassous*.

Species	2n	Chromosome Morphology					Reference
		Metacentric	Submetacentric	Acrocentric	X	Y	
<i>C. centralis</i>	62	10	4	46	SM	M	BERNIRSCHKE <i>et al.</i> , 1969
<i>C. tautoauy</i>	50	8	12	28	SM	A	BARROSO and SEUANEZ, 1991
<i>C. unicinctus</i>	46	24	10	12	SM	A	This paper
<i>C. chacoensis</i>	-	-	-	-	-	-	No date

A, SM and M are signifying respectively, Acrocentric, Submetacentric and Metacentric.

In contrast to the other two species described, the number of metacentric chromosomes was larger and the acrocentric was small-

est (Table 2). This suggests that a metacentric chromosome formation event occurred (group 1) by fusion of large and medium acrocentric



Fig. 2 — Ag-NOR banding of *Cabassous unicinctus*, arrows indicate the nucleolus organizing regions.

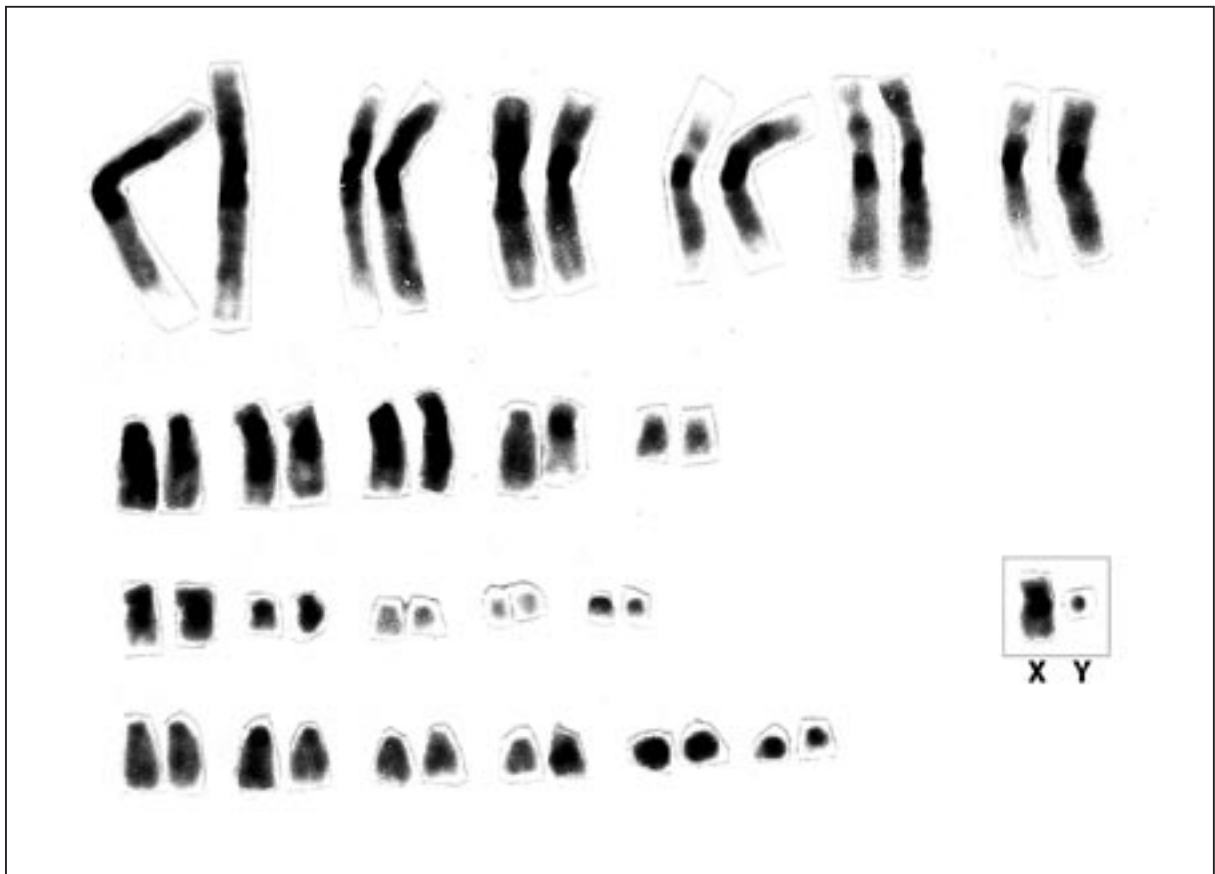


Fig. 3 — C banding showing centromeric markings on the chromosomes. The small size chromosomes barely present markings for this banding pattern.

chromosomes, or metacentric fission (group 1) originating from acrocentric chromosomes. The morphology of the X chromosome remained stable in this genus while the Y chromosome lost or gained material, such that it was transformed from a punctiform chromosome (acrocentric) in the *Cabassous tautouay* and *Cabassous unicinctus* species to a small metacentric chromosome in *Cabassous centralis*.

Two banding techniques were used: C (JORGE *et al.* 1977) and Ag-NOR (HOWELL and BLACK 1980). In order to observe Ag-NOR regions, twenty metaphases were counted, marked on two different chromosomes (Fig. 2), a strongly stained region on a medium metacentric (open arrow) and a region on one third of the long arm of a medium sized submetacentric chromosome (bold arrow). In the C-band staining (Figure 3), the large and medium chromosomes stained well, while the small chromosomes presented faint staining. The first two

pairs of large metacentric presented centromeric and highly evident pericentromeric markings, which indicate a chromosomal fusion process.

In the present work, the karyotype of the *Cabassous unicinctus* species was described with  $2n=46$  chromosomes, including C and Ag-NOR banding. In order to make inferences regarding the chromosomal evolutionary process in the *Cabassous* genus, it was necessary to obtain chromosomal data on the *Cabassous chacoensis* species (not yet described) and it improved chromosome preparations of the species *Cabassous tautouay* and *Cabassous centralis*, besides acquiring further information regarding phylogenetic relationships among the genus *Cabassous*.

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## REFERENCE

- BARROSO C.M.L., 1982 — Estudos cromossômicos nos gêneros *Dasybus*, *Euphractus* e *Cabassous* (Edentata - Dasypodidae) Rio de Janeiro, UFRJ, (Doctoral Thesis).
- BARROSO C.M.L. AND SEUNÁNEZ H., 1991 — Chromosome studies on *Dasybus*, *Euphractus* and *Cabassous* genera (Edentata: Dasypodidae). *Cytobios*, 68: 179-196.
- BEATH M.M., BENIRSCHKE K., AND BROWNHILL L.E., 1962 — The chromosomes of nine-banded armadillo, *Dasybus novemcinctus*. *Chromosoma*, 13:27-38.
- BENIRSCHKE K., 1968 — Why armadillos? *Animal Models for Biomedical Research*, 4: 45-54.
- BENIRSCHKE K. AND WURSTER D.H., 1969 — The chromosomes of the giant armadillo, *Prionotus giganteus* Geoffroy. *Acta Zoologica et Pathologica Antverpensis*, 49: 125-30.
- BENIRSCHKE K., LOW R.J., AND FERM V.H., 1969 — Cytogenetic studies of some armadillos. In: "Comparative mammalian cytogenetics" Springer-Verlag, New York.
- COOK J.A., VEGA, F.C. AND MOREIRA C.M., 1991 — Cariotipo del quirquincho (*Chaetophractus nationi*). *Ecología em Bolivia*, 18: 21-27.
- DELSUC F., VIZCAÍNO S.F. AND DOUZERY E.J.P., 2004 — Influence of Tertiary paleoenvironmental changes on the diversification of South American mammals: a relaxed molecular clock study within xenarthrans. *Evolutionary Biology* 4(11).
- DELSUC F., STANHOPE M.J. AND DOUZERY E.J.P., 2003 — Molecular systematics of armadillos (*Xenarthra*, *Dasypodidae*): contribution of maximum likelihood and Bayesian analyses of mitochondrial and nuclear genes. *Molecular Phylogeny and Evolution*, 28: 261-75.
- ENGELMANN G.F., 1985 — The phylogeny of *Xenarthra*, In: MONTGOMERY, G.G. (Ed). The evolution and ecology of armadillos, sloths and vermilinguas, p. 51-64, Smithsonian Institution Press, Washington and London.
- HOWELL W.M. AND BLACK D.A., 1980 — Controlled silver-staining of nucleolus organizer regions with protective colloidal developer: a 1-step method. *Experiencia*, 36: 1014.
- IUCN, 2008 — Red List of Threatened Species 2006. <http://www.iucnredlist.org/> (extract in June 2008)
- JORGE W. AND PEREIRA JR H.R.J., 2008 — Chromosomal studies in the *Xenarthra*, In: VIZCAÍNO S.F. AND LOUGHRY W.J. (eds.) *The Biology of the Xenarthra*. p 217- 238. University Press of Florida, Gainesville.
- JORGE W., ORSI-SOUZA A.T. AND BEST R.C., 1985 — The somatic chromosomes of *Xenarthra*. In: MONTGOMERY, G.G. (Ed). The evolution and ecology of armadillos, sloths and vermilinguas, p. 12-129, Smithsonian Institution Press, Washington and London.
- JORGE W., MERITT D.A. AND BENIRSCHKE K., 1977 — Chromosomes studies in Edentata. *Cytobios*, 18: 157-172.
- LIZARRALDE M.S., BOLZAN A.D., POLJAK S., PIGOZZI M.I., BUSTOS J. AND MERANI M.S., 2005 — Chromosomal localization of the telomeric (TTAGGG) n sequence in four species of Armadillo (*Dasypodidae*) from Argentina: an approach to explaining karyotype evolution in the *Xenarthra*. *Chromosome Research*, 13: 777-784.
- MERITT D. AND BENIRSCHKE K., 1973 — The chromosomes of *Zaedyus pichiy* Ameghino, 1889. *Mammalian Chromosomes Newsletter*, 14(03): 108-109.
- NOWAK R.M., 1999 — Walker's mammals of the world Sixth edition, The Johns Hopkins University Press, Baltimore and London.
- PEREIRA JR H.R.J., BAGAGLI E. AND JORGE W., 2003 — Por que tatu? *Ciência Hoje*, 34(199): 70-73.
- REDFORD, K.H., 1985 — Food habitats of armadillo (*Xenarthra:Dasypodidae*), In: MONTGOMERY, G.G. (Ed). The evolution and ecology of armadillos, sloths and vermilinguas, p. 429-437, Smithsonian Institution Press, Washington and London.
- REDI C.A., ZACHARIAS H., MERANI S., OLIVEIRA-MIRANDA M., AGUILERA M., ZUCCOTTI M., GARAGNA S. AND CAPANNA E., 2005 — Genome Sizes in Afrotheria, *Xenarthra*, Euarchontoglires, and Laurasiatheria. *Journal of Heredity*, 96(5): 485-490.
- SAEZ F.A., DRETS M.E. AND BRUM N. 1964 — Chromosomes of the mulita (*Dasybus hybridus* Desmarest): a mammalian edentate of South America. In: *Mammalian cytogenetics and related problems in radiobiology*. p. 163-170, Oxford, London, New York, Paris: Pergamon Press.
- VIZCAÍNO S.F., 1995 — Identificación específica de las "mulitas", género *Dasybus* L. (*Mammalia*, *Dasypodidae*), del noroeste argentino, descripción de una nueva especie. *Mastozoología Neotropical*, 2(1): 5-13.
- WETZEL R.M., 1980 - Revision of the naked-tailed armadillos, genus *Cabassous* McMurtrie. *Annals of Carnegie Museum*, 49(20): 323-357.
- WETZEL R.M., 1985 — Taxonomy and distribution of armadillos, *Dasypodidae*, In: MONTGOMERY, G.G. (Ed). The evolution and ecology of armadillos, sloths and vermilinguas, p. 23-46, Smithsonian Institution Press, Washington and London.

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