

## Description of Karyotype of *Kerodon acrobata*, an endemic rodent in Brazilian Cerrado

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### Abstract

*Kerodon acrobata* is a caviidae rodent endemic from Brazilian Cerrado. It was described only in 1997 and the data about it is very scarce. The aim of this work was to characterize the karyotype of *K. acrobata*. Giemsa staining, nucleolar organizer region (NOR) banding, C-positive heterochromatin banding and DAPI fluorescence were used in N metaphases of a specimen collected in Asa Branca Farm, in Aurora do Tocantins municipality, Tocantins state, Brazil. *K. acrobata* showed the same diploid number, fundamental number and chromosome morphology as *Kerodon rupestris*. But its NOR location and heterochromatin distribution patterns indicated a unique cytogenetic profile when compared to its sister species, emphasizing the evolutionary uniqueness of this relatively new and unknown species. This record also extends the distribution of this species northward.

**Keywords:** cytogenetics, heterochromatin, mocó, NOR.

### Primeira descrição do cariótipo de *Kerodon acrobata*, um roedor endêmico do semiárido brasileiro

### Resumo

*Kerodon acrobata* é um roedor caviídeo endêmico do Cerrado brasileiro. A espécie foi descrita apenas em 1997, e as informações sobre ela são muito escassas. O objetivo deste trabalho foi caracterizar o cariótipo *K. acrobata*. Coloração em Giemsa, bandeamento da região organizadora do nucléolo, bandeamento da heterocromatina C-positiva e fluorescência DAPI foram utilizados em N metáfases de um espécime coletado na fazenda Asa Branca, na cidade de Aurora do Tocantins, estado do Tocantins, Brasil. *K. acrobata* apresentou o mesmo número diplóide, número fundamental e morfologia dos cromossomos de *Kerodon rupestris*. Mas a localização de sua NOR e os padrões de distribuição de heterocromatina indicam um perfil citogenético único quando comparado com sua espécie irmã, enfatizando a singularidade evolutiva desta espécie pouco conhecida. Este registro também estende a distribuição desta espécie em direção ao norte.

**Palavras-chave:** citogenética, heterocromatina, mocó, NOR.

### 1. Introduction

The suborder Hystricognathi, Caviidae (Gray, 1821) is one of the most vastly distributed families. There are three subfamilies, including Dolichotinae (Pocock, 1922) with one genus, *Dolichotis* (Desmarest, 1820); Caviinae (Gray 1821) with three genus: *Galea* (Meyen, 1831), *Microcavia* (Gervais and Ameghino, 1880) and *Cavia* (Pallas, 1766); and Hydrochoerinae (Gray, 1825) with two genus, *Hydrochoerus* (Brisson, 1762) and *Kerodon* (Cuvier, 1825). *Kerodon* was traditionally included within the subfamily Caviinae with guinea pigs and its relatives, but now it is placed as sister genus from *Hydrochoerus* within the *Hydrochoerinae* subfamily, according to new molecular studies by Rowe and Honeycutt (2002). This

subfamily occupies a wide array of habitats. *Hydrochoerus* lives from forested riversides to open savannas and flooded areas of South America (González-Jiménez, 1995; Mones and Ojasti, 1986). *Kerodon* occupies dry forests of South America (Mares and Ojeda, 1982), but its phylogeny and occupation in central portion of this continent are uncertain. One of the few phylogenetic studies with caviideans (Reis et al., 1988) shows closer relation between *Galea* and *Kerodon*, according to their ontogeny. Genus *Kerodon* has two species: *Kerodon rupestris* (Wied, 1820) and *Kerodon acrobata* (Moojen et al., 1997).

Prince Wied visited Brazil between 1815 and 1817. Two books were published by him, in German and French.

In 1940 an edition in Portuguese was edited as “Viagem ao Brasil – Maximiliano Príncipe de Wied Neuwied”. There, Prince Wied recorded notes since his disembark in Rio de Janeiro until Bahia. In Barra-da-Vereda, Minas Gerais state, he spent some days studying the natural history of the region. In his notes he talked about an undescribed mammal similar to *Cavia*, known as “mocó”. It was described as a small animal, like a rabbit, that lived under rocks from Pardo River, and with a tasty meat. The given name for it was *Cavia rupestris* at that time.

*Kerodon rupestris* lives in rock outcrops in Brazilian semiarid, the caatinga. It is found from northern Minas Gerais to Piauí state (Lacher, 1981; Mares and Oreja, 1982; Alho, 1982). Its meat is considered tasty by local communities, so the mocós are being hunted by humans with the use of “arremedo” (imitation of vocalization) and fire arms in rural areas (Alves et al., 2009), which is causing the declining of some populations.

But only in 1997, Moojen and collaborators described a new species of mocó, *Kerodon acrobata*, based on qualitative and morphometric analyses. At that time, the species was found only in Goiás state, at its type locality in São Mateus River (Moojen et al., 1997). This species was studied just from type series of individuals collected in the 1960’s in the states of Goiás and “probably” Tocantins, in dry habitats west of the Serra Geral de Goiás (Moojen et al., 1997). More recently nine individuals became available from.... (Bezerra et al., 2010).

*K. acrobata* can be identified by some characters, like a very long muzzle, reduced size of ears, long mystacial vibrissae, short and blunt nails covered by hair, large digital pads, lack of tail, and gray-light brown agouti coarse pelage. The specific name, *acrobata*, is due to its ability to jump from one branch to another. It feeds on cacti and leaves (Moojen et al., 1997). Bezerra et al. (2010) studied nine new specimens of this species and indicated that the presence of this rodent is associated to the cerrado *sensu stricto* and seasonally dry tropical forest patches in the northeastern Cerrado domain.

Through cranial analysis, a variation among *K. rupestris* and *K. acrobata* populations was showed by Lessa et al. (2005), corroborating their separation into two species. There was a complete discrimination in cranial characters multivariate space between them, which confirms the morphologic distinction of both species.

Until now no study about *K. acrobata* chromosome composition was published. The aim of this study was to describe for the first time the karyological features of a *K. acrobata* female, including chromosome composition, C-banding and NOR regions. Besides, we compared the results with information previously described for *K. rupestris*.

## 2. Material and Methods

It is extremely difficult to capture *K. acrobata* specimens alive, but a new method proposed by Portella and Vieira (unpublished data) seems to be efficient enough to catch them. They used wire traps and flues (“tarrafas”) hung in trees, and used leaves and vegetables as bait.

One female was collected using Portella and Vieira method (unpublished data), at the Asa Branca Farm, in Aurora do Tocantins municipality, Tocantins state, Brazil (12°39’35.4” S/46°28’04.4” W). It was collected in August 20, 2012. The specimen was transported to the Laboratório de Ecologia dos Vertebrados, Universidade de Brasília, Distrito Federal, Brazil, where it was dissected and then deposited in the mammal collection of the same institution, with the identification number 3276. After that, mitotic chromosomes were obtained from cell suspensions of the bone marrow of the femur, using the Ford and Hamerton protocol (1956). Metaphases were analyzed with Giemsa method. Chromosomes were analyzed with silver nitrate staining (Howell and Black, 1980) in order to see the nucleolar organizer regions (Ag-NOR). In addition, we used C-banding (Sumner, 1972) and DAPI fluorescence (Kapuscinski, 1995) to check C-positive heterochromatin with adenine-thymine richness. The chromosomes were measured using Image Pro Plus® and classified following Levan et al. (1964).

## 3. Results

The female specimen showed diploid number ( $2n$ ) = 52 chromosomes and fundamental number (FN) = 92. Its karyotypic formula was  $44m+8t$  (see Figure 1), so it didn’t have submetacentric or subtelo-centric chromosomes. At least 50 Giemsa-stained, 20 Ag-NOR, 10 C-banded and 10 DAPI fluorescence metaphases were analyzed for this animal. Sexual chromosome was the largest of the complement. Sites marked with Ag-NOR were telomeric and identified in two pairs (see Figure 2). These pairs were identified as numbers 10 and 21. In both pairs, NOR regions were distributed in the long arms. Telomeric heterochromatin was evident only in sexual chromosome pair, in the short arms, while the autosomes didn’t show this pattern (see Figure 3). DAPI fluorescence also showed this pattern for heterochromatin, with adenine-thymine richness in sexual pair of chromosomes (see Figure 4).

## 4. Discussion

We assumed that the sexual pair was the biggest one because the X chromosome of most placental mammals comprises around five percent of the genome, and it is rarely small and often biarmed in hystricognaths. In general X chromosomes of mammals have a highly conserved genetic content and a similar relative size of about 5% of a haploid set, as was estimated by Ohno (1969). That seems to be true for the genus *Kerodon*.

The karyotype analyzed was characteristic for the genus. The observed  $2n = 52$ , FN = 92 and the number of metacentric and telocentric chromosomes are the same for *K. acrobata* and *K. rupestris* (Maia, 1984). This seems to be an apomorphy for the genus *Kerodon*, once all other studied Caviidae show  $2n=64$ : *Cavia* (Gava et al., 1998), *Galea* (Maia, 1984), *Dolichotis* (Wurstler et al., 1971) and *Hydrochoerus* (Saez et al., 1971; Caldera, 2005). So, the karyotype for *K. acrobata* is extremely similar to

Karyotype of *Kerodon acrobata*

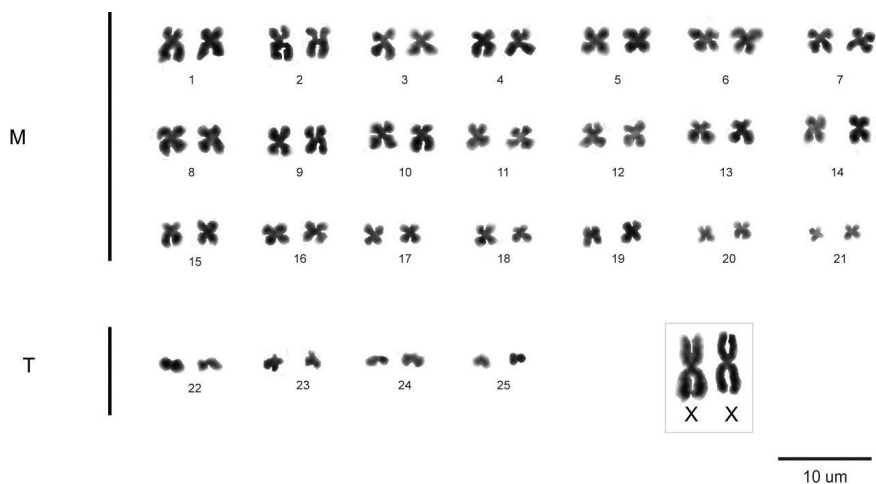


Figure 1. The karyotype of *Kerodon rupestris* from Aurora, Tocantins. Conventional staining (Giemsa).

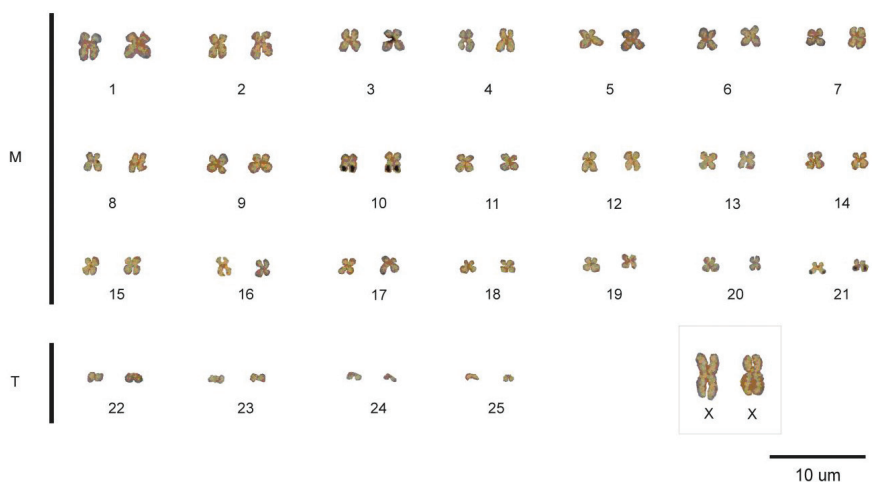


Figure 2. Ag-NOR banding marks in chromosome pairs 10 and 21 of *Kerodon acrobata*.

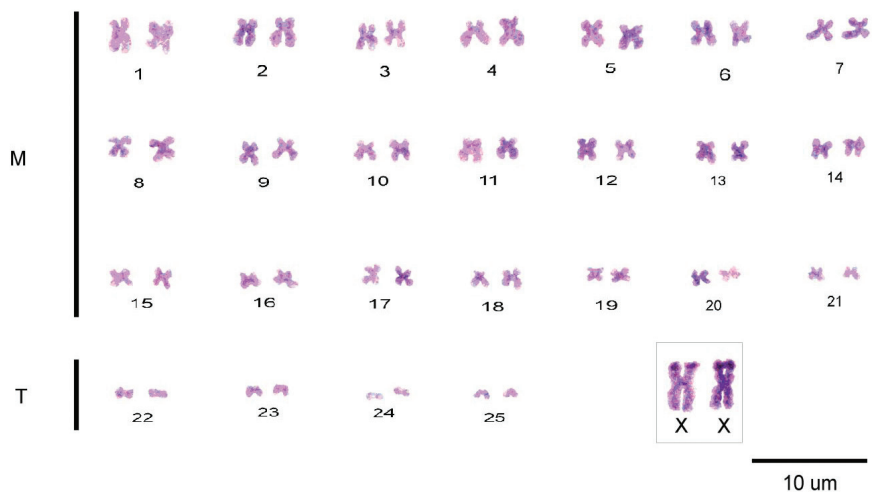
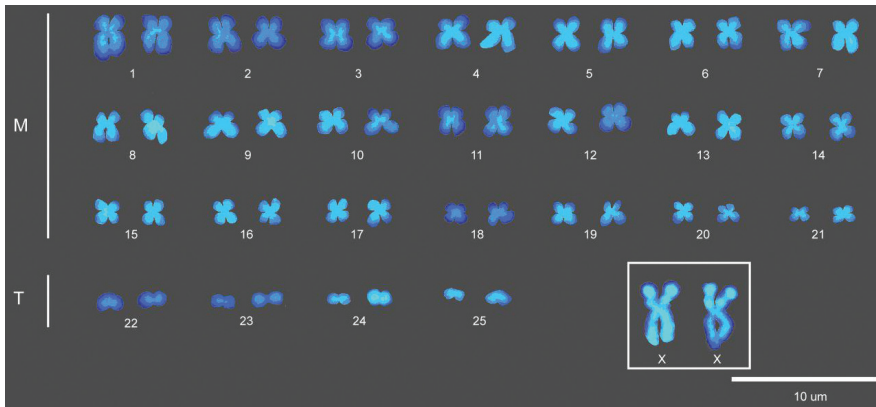


Figure 3. Heterochromatic marks obtained by C-banding protocol in sexual chromosome pair of *Kerodon acrobata*.



**Figure 4.** Heterochromatic marks obtained by DAPI fluorescence in sexual chromosome pair of *K. acrobata*.

*K. rupestris*. This karyotypical structure may be used to prove the monophyly for the genus. On the other hand, the presence of a metacentric sexual X chromosome pair as the largest of the complement in *Kerodon* seems to be characteristic of the entire group hystricomorpha (Wurstler et al., 1971).

Lessa et al. (2013) studied *K. rupestris* cytogenetics. NOR regions were checked, being present pairs of chromosomes 10 and 11. *K. acrobata* shows the same diploid number ( $2n=52$ ), but the other patterns were distinct from *K. rupestris*. In *K. acrobata* NOR regions are present in pairs 10 and 21, and only the telomeric region of the short arms of X chromosome is heterochromatic. Thus it has great relevance for understanding the karyotypical evolution of this species.

NOR regions are usually present in two pairs of autosomes for the family Caviidae, as in *Cavia* (Gava et al., 2012) and *K. rupestris* (Lessa et al., 2013). But all show at least one different pair. Despite of being close related species, some minor karyotype differences show distinct evolutionary histories among them. Such divergences manifest themselves in structural terms, by differential expression of the NORs.

Karyological variation was studied in *Cavia*, *Galea* and *Kerodon* (Maia, 1984). It concluded that 80% of their chromosomes is composed of biarmed ones. The diploid and fundamental numbers for *K. rupestris* was  $2n=52$  and  $FN=92$ . C-band results showed that all constitutive heterochromatin was in X chromosome, differing from the pattern observed in *Cavia* and *Galea* (Maia, 1984). *K. acrobata* is even more different, showing a small and telomeric region in the sexual pair. Equilocal heterochromatic markings are considered to be plesiomorphic within taxa. The different pattern observed in *K. acrobata* suggests the occurrence of paracentromeric inversions in this species. Chromosomal inversions are crucial for the reproductive isolation of populations (Stefansson et al., 2005) and contribute to the speciation process (Hoffmann and Rieseberg, 2008). In general, chromosomal rearrangements are involved in the processes of speciation (King, 1993). It is hypothesized that chromosomal rearrangements need to be fixed in a short period of time (Aniskin et al., 2006), so they may

create reproductive isolation between populations (Ayala and Coluzzi, 2005), because heterochromatic regions are regarded as the main contenders for the role of stabilizing or destabilizing evolution (Graphodatsky, 1989; Hennig, 1999).

South American rodents derive from two colonizations: the hystricognath, from the end of Eocene to the present (Huchon and Douzery, 2001; Adkins et al., 2003); and the sciurognath which entered the continent during the transition Miocene/Pliocene (Eisenberg and Redford, 1999). Reig (1986) showed that the Caviidae originated in northeastern Brazil, followed by two diversifications, one in the south, and the other associated with the uplift of the Andes. *Dolichotis* and *Kerodon* probably derived first, while *Cavia* and *Galea* remained as sister groups later, during the Late Miocene, around 10 million years ago (Dunnun and Salazar-Bravo, 2010). Climate changing at the end of the Pleistocene introduced modifications in the dominant ecosystems in the inter-tropical Brazil, with the expansion of dry regions (Ab'saber, 2005). Rain forests that once dominated Brazilian northeast scenario probably retracted from east to west within the continent, because of isolated islands of caatinga and cerrado, which exposed the rocky outcrops. This fact can be corroborated by studies with primate fossils that could only have lived in humid forests in the region, around 11000 years ago (Vivo, 1997). These cerrado islands which occurred in the states of Goiás and Tocantins, Brazil, may have isolated *Kerodon* populations, and that could have caused this differentiation in *K. acrobata*, making it endemic of that region of cerrado, through genetic drift or founding effect, according to Thorpe (1983).

The unique cytogenetic features of *K. acrobata*, when compared to *K. rupestris* or other Caviidae from close related biogeographical units suggests that this species is a separate phylogenetic unit that faces extinction risks, also due to its small range area.

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