Karyotypic analyses and morphological comments on the endemic and endangered Brazilian painted tree rat *Callistomys pictus* (Rodentia, Echimyidae)

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Abstract

The genus *Callistomys* belongs to the rodent family Echimyidae, subfamily Echimyinae, and its only living representative is *Callistomys pictus*, a rare and vulnerable endemic species of the state of Bahia, Brazil. *Callistomys* has been previously classified as *Nelomys*, *Loncheres*, *Isothrix* and *Echimys*. In this paper we present the karyotype of *Callistomys pictus*, including CBG and GTG-banding patterns and silver staining of the nucleolus organizer regions (Ag-NORs). Comments on *Callistomys pictus* morphological traits and a compilation of Echimyinae chromosomal data are also included. Our analyses revealed that *Callistomys* can be recognized both by its distinctive morphology and by its karyotype.

Key words: *Callistomys*, karyotype, banding patterns, Echimyinae.

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Table 1 - Karyotypes of Echimyinae taxa (Suborder Hystricognathi).

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Referred as</th>
<th>2n</th>
<th>FN</th>
<th>Locality</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echimys pictus</td>
<td></td>
<td>42</td>
<td>76</td>
<td>Ilhéus, BA, Brazil</td>
<td>Present paper</td>
</tr>
<tr>
<td>Echimys semivillosus</td>
<td>Aff. Lasiuromys villosus</td>
<td>94</td>
<td>114</td>
<td>Venezuela</td>
<td>Aguilera et al., 1998; Emmons, 2005</td>
</tr>
<tr>
<td>Lasiuromys villosus</td>
<td></td>
<td>60</td>
<td>116</td>
<td>Rio Juruá, AC and UHE</td>
<td>Patton et al., 2000; Leal-Mesquita, 1991*</td>
</tr>
<tr>
<td>Isothrix bistriata</td>
<td></td>
<td>70</td>
<td>120</td>
<td>UHE Samuel, RO, Brazil</td>
<td>Leal-Mesquita, 1991*</td>
</tr>
<tr>
<td>Isothrix negrensis</td>
<td></td>
<td>60</td>
<td>112</td>
<td>Rio Negro, AM, Brazil</td>
<td>Bonvicino et al., 2003</td>
</tr>
<tr>
<td>Isothrix pagurus</td>
<td></td>
<td>22</td>
<td>38</td>
<td>Manaus, AM, Brazil</td>
<td>Patton and Emmons, 1985</td>
</tr>
<tr>
<td>Isothrix sinnamariensis</td>
<td></td>
<td>28</td>
<td>42</td>
<td>French Guiana</td>
<td>Vié et al., 1996</td>
</tr>
<tr>
<td>Makalata didelphoides</td>
<td></td>
<td>66</td>
<td>106</td>
<td>Balbina Hydroelectric Dam on the Uatumá River, AM, Brazil</td>
<td>Lima et al., 1998</td>
</tr>
<tr>
<td>Phyllomys blainvilli</td>
<td></td>
<td>72</td>
<td>ND</td>
<td>RS, Brazil</td>
<td>Leite, 2003</td>
</tr>
<tr>
<td>Echimys sp.</td>
<td></td>
<td>90/92</td>
<td>ND</td>
<td>SP and RS, Brazil</td>
<td>Yonenaga, 1975; Leite, 2003</td>
</tr>
<tr>
<td>Echimys dasythrix</td>
<td></td>
<td>96</td>
<td>ND</td>
<td>SC, Brazil</td>
<td>Sbalqueiro, 1998 apud Leite, 2003</td>
</tr>
<tr>
<td>Echimys sp. / E. thomasi</td>
<td>Echimys pictus</td>
<td>52</td>
<td>ND</td>
<td>RJ, Brazil</td>
<td>Leite, 2003</td>
</tr>
<tr>
<td>Echimys sp. / E. thomasi</td>
<td>P. aff. dasythix</td>
<td>80/72</td>
<td>112/114</td>
<td>ES and RJ, Brazil</td>
<td>Zanchin, 1988* Leite, 2003</td>
</tr>
</tbody>
</table>


Nelomys (Pictet, 1843; Goldman, 1916; Thomas, 1916), Echimys (Tate, 1935; Moojen, 1952) and Isothrix (Waterhouse, 1848; Ellerman, 1940; Cabrera, 1961; Honacki et al., 1982; Patton and Emmons, 1985) and it has only recently been placed in its own genus *Callistomys* (Emmons and Vucetich, 1998).

In a review of the genus *Isothrix*, Patton and Emmons (1985) followed Cabrera (1961) and Honacki et al. (1982) and kept *Nelomys pictus* within *Isothrix*. The same *I. pictus* was later classified as *Nelomys* (Emmons and Feer, 1990) and then included in *Echimys* (Woods, 1993; Emmons and Feer, 1997). Emmons and Vucetich (1998) examined the fossil mandible of one specimen identified as *Lasiuromys villosus* (a synonym of *Isothrix bistriata*) by Winge (1888) and several specimens of *Nelomys pictus* (including the holotype) and concluded that they belonged to the same genus. A comparison of these specimens with the three genera in which *Nelomys pictus* had been previously included led Emmons and Vucetich (1998) to conclude that *N. pictus* did not belong to any of them. Considering its distinct morphology, these authors suggested a new genus, *Callistomys*, to contain *N. pictus* and *Callistomys* sp. (formerly identified as *Lasiuromys villosus*).

Here we report new cytogenetic data on the endemic, rare and endangered Echimyinae *Callistomys pictus*, including Ag-NOR staining, CBG- and GTG-banding, as well as comments on the external morphology, cranial anatomy and geographical distribution of the species.

The specimen reported herein was incidentally captured in April 2002, preyed and injured by a domestic dog in a cacao plantation at Fazenda Santo Antônio (14°41'46" S, 39°15'22" W), Ilhéus, state of Bahia, Brazil. Ilhéus is the type-locality of *Nelomys pictus* (Pictet, 1843). The animal was sacrificed according to Ethical Issues in the Use of Animals (Colégio Brasileiro de Experimentação Animal, COBEA, 1991), tissues were deposited in the collection of the Instituto de Biociências, Universidade de São Paulo (IBUSP) and the voucher specimen was deposited at the Museu de Zootogia, Universidade de São Paulo (MZUSP), São Paulo, Brazil, under the number MZUSP 31404.

Morphology - We employed Wahlert (1974, 1983, 1985) and Woods and Howlands (1979) for the nomenclature of cranial foramina. Dental nomenclature followed Lack-Ximenes et al. (2005) which was modified from Lavocat (1976) with further considerations from Butler (1985), Jaeger et al. (1985), Flynn et al. (1986), Jaeger...
(1989), Bryant and McKenna (1995), and Candela (1999a; 1999b; 2002). Besides the specimen from Fazenda Santo Antônio, Ilhéus, state of Bahia, eight other specimens of *Callistomys pictus* were examined, as follows: Brazil: Bahia: South America: NHM: 52.1.5.22 (skull, mounted skin) and 80.9.15.1 (skull, mounted skin); Brésil: MHNN: 94.2464A (mounted skin) and 94.2463 (mounted skin); Fazenda 7 Voltas, Ilhéus, Bahia: MZUSP: 31404 (skull, skin); Lavapés de Dentro, Rio do Braço, Bahia: MN: 11207 (skull, skin); Ilhéus, Bahia: MN15453 (skull, skull) and 31546 (skin, skull); no locality: MNK: 4809 (skull, skin) (Abbreviations: NHM: Natural History Museum, London, England; MHNN: Museum d’Histoire Naturelle du Neuchâtel, Neuchatel, Switzerland; MZUSP: Museu de Zoolo- logia, Universidade de São Paulo, São Paulo, Brazil; MNK - Museum für Naturkunde, Berlin, Germany, MN: Museu Nacional do Rio de Janeiro, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil).

**External morphology:** fur includes only soft hairs; hairs brown at base with tip white or black. Pelage is dense and long. *Callistomys* has a unique color pattern among echimyid rats: body white with black coat, extending from the top of head, nape, and back to the basis of tail. Tail densely haired, black at base and white distally. Limbs broad and stout, hands and feet relatively short but broad. Cranial anatomy: Skull is large and strong, rostrum and nasals medium sized and broad. Lateral wings of frontal well-developed forming a roof over orbital region; postorbital process of zygoma rounded and formed by squamosal. Petrosal bone covered by squamosal posteriorly. Incisive foramina long and fusiform, mostly formed by premaxillar. Septum of incisive foramina wide and long and formed by premaxillar. Palatal region rectangular, long and slender; palate extending up to M1. Sphenopalatine foramen double. Mesopterygoyd fossae with slit-shaped lateral openings. Alisphenoid region wide; alisphenoid channel not differentiated; buccinator and masticator foramina confluent; foramen ovale medium sized; maxillary vein passes through foramina; transverse canal foramen well-developed. Bullae round, inflated, with tiny stiliform process, tegmen timpani short and wide; external auditory meatus opening in a short and strong tube. Upper molariforms tetralophodonts; anteroloph and the fourth loph (metalo- loph+protoloph) connected lingually as well as mesoloph and posteroloph. In young adults and younger specimens hypoflexus and mesoflexus deep, isolates anteroloph proto- loph U-shaped from mesoloph posteroloph; but in older ones, the M1-M3 with narrow mure connecting protocone to hypocone. Lower molar trilophodonts and dP4 tetra- lophodont with metafossetid between first loph (antero- lophid+metallocodont) and mesolophid; mesolophid connected by mure to hipolophid; hipolophid and posterolophid connected labially by hypoconid.

**Distribution -** *Callistomys pictus* occurs in Bahia State, Brazil, and most records are from the municipality of Ilhés and nearby areas (Moojen, 1952; Emmons and Vucetich, 1998; Vaz, 2002, 2005). *C. pictus* was recently recorded in Serra da Jibóia, Eliso Medrado, Bahia State, about 150 km North of Ilhés (Encarnação et al., 2000). Two specimens were collected by Auguste de Meuron, a tobacco dealer, and the locality was labeled as “Brésil”. De Meuron lived in the city of Salvador, formerly known as Bahia, a name now used for the state. In view of the specimens from other species (*P. pattoni, T. setosus*) sent by de Meuron to the MHNN, it is possible that the specimens of *Callistomys pictus* came from Salvador (MHNN: 94.2464A and 94.2463) or from somewhere in the neighbourhood. *Callistomys pictus* is endemic to the Atlantic forest of the state of Bahia, Brazil, where it is also found in cacao plantations shaded by native trees. *C. pictus* is either locally rare or difficult to capture with traditional live trapping methods, since ecological studies with a high sampling effort carried out in different habitats in South Bahia did not record the species (Pardini, 2004; Moura RT, 1999, MSc Dissertation, Universidade Federal de Minas Gerais, BH, Brazil). Nevertheless, *Callistomys pictus* seems to be more common in cacao plantations in Ilhés, where local people reported frequent sightings of the animal.

**Cytogenetics -** Metaphases from the male of *Callistomys pictus* were obtained from bone marrow and spleen 40 min after an *in vivo* subcutaneous injection of 0.1% colchicine. Conventional staining with Giemsa, Ag-NORs staining (Howell and Black, 1980), GTG- (Sea- bright, 1971) and CBG-banding (Sumner, 1972) were car-
ed out following standard cytogenetic procedures.

The karyotype of *Callistomys pictus* revealed 2n = 42 and FN (number of autosomal arms) = 76 (Figure 1A) and consisted of 18 pairs of meta or submetacentric autosomes decreasing in size (pair 1 to 16, 18 and 20) and two pairs of small acrocentrics (17 and 19). The X and Y chromosomes were, respectively, medium and small acrocentrics; the X being perfectly distinguishable as a medium sized acrocentric. CBG-banding allowed the recognition of all auto-
some pairs and the sex chromosomes (Figure 1B). CBG-
banding evidenced small heterochromatic blocks in the pericentromeric regions of some autosomes and in the sex chromosomes (Figure 2A). The single Ag-NOR was de-
tected in a secondary constriction at the long arm of pair 13 (Figure 2B).

*Callistomys pictus*, the only living species of the gen-
us, differs from other extant Echimyidae in many major cranial characters and could represent the last survivor of an old clade of Echimyidae. Other species of this genus represented by a single fossil from the Upper Pleisto-
cene-Recent collected at Lapa do Capão Seco, Lagoa Santa, Minas Gerais State, Brazil, was recognized (Emmons and Vucetich, 1998). A cladistic analysis based on morphological data of Echimyidae placed *Callistomys* in the basal position of the Dactylomyinae/Echimyinae clade (Carvalho and Salles, 2004). The authors found a
Figure 1 - Karyotype of a male *Callistomys pictus* with 2n = 42 and FN = 76 after: (A) Conventional staining and (B) GTG-banding.

Figure 2 - Metaphases of *Callistomys pictus* after: (A) CBG-banding, arrows indicate the X and Y chromosomes and (B) Ag-NORs, arrows indicate the NOR-bearing pair 13.
slightly distinct topology when fossil taxa were included: *Callistomys, Maruchito trilocodonte* Vucetich *et al.*, 1993 (a fossil echimyid genus from Middle Miocene), and the Dactylomyine/Echimyinae formed a basal politomy. These results suggest that *Maruchito* and *Callistomys* can be related, as previously proposed by Emmons and Vucetich (1998), suggesting a recent reduction in its geographic distribution (Figure 3). Records of *Callistomys pictus* from Lagoa Santa are restricted to Pleistocene fossil specimens.

The taxonomy of Echimyinae is still very complex and the diploid numbers can be helpful in species identification since karyotypes seem to be important diagnostic markers (Table 1), except for *Isothrix bistriata, I. negrensis* and *Echimys* sp. with 2n = 90-92. Diploid numbers within Echimyinae are usually high, with the exception of *Isothrix pagurus* and *I. sinnamariensis*, with 2n = 22 and 2n = 28, respectively (Patton and Emmons, 1985; Vié *et al.*, 1996), and of the odd 2n = 42 and FN = 76 described herein for *Callistomys pictus*.

Our results show that the karyotype associated to the restricted geographical range and the unique set of morphological traits are useful in identifying *Callistomys pictus*. Our data bring important new information thus reducing the knowledge gap of Brazilian biodiversity and, most importantly, contributing to improve conservation and management initiatives. This is specially important for species like *Callistomys pictus*, which was included as vulnerable in the red list of endangered species published by the Brazilian Institute for Environment (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis, IBAMA) in 2003. In fact, as pointed out by Costa *et al.* (2005), the major threat to endangered small mammals is the scarcity of scientific knowledge about their distribution, systematics, taxonomy and natural history, since most of them are rare and poorly known and very few sites of Brazil have been adequately surveyed.

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