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THE KARYOTYPE OF A RARE SOUTH AMERICAN MARSUPIAL, THE BUSHY-TAILED OPOSSUM GENUS *Glironia* (DIDELPHIMORPHIA: DIDELPHIDAE)

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ABSTRACT: We describe for the first time the karyotype of *Glironia venusta* (Didelphidae, Didelphimorphia), a rare South American marsupial. *G. venusta* has a diploid number 2n=18 and a number of autosomal arms NA=22. The diploid number of 18 chromosomes is somewhat unexpected, considering prior assignment of *Glironia* to the subfamily Caluromyinae (2n=14 in other species of the subfamily). The only other didelphid genus known to have 2n=18 chromosomes is *Monodelphis*. Chromosome banding, however, revealed a pattern closer to that observed for the 2n=14 species. Considering recent studies in didelphid phylogeny, wherein *Glironia* is sister to the remaining members of the family, the diploid number observed for *Glironia* may represent the ancestral state of the family, or may result from convergent evolution.

RESUMEN: Cariotipo de un marsupial suramericano raro, zarigüeya de cola peluda, del género *Glironia* (Didelphidae, Didelphimorphia). Se describe aquí por primera vez el cariotipo de *Glironia venusta*. El número diploide de *G. venusta* es 2n=18 y el número de brazos de autosómicos NA=22. El número diploide de 18 es un tanto inesperado, considerando la inclusión anterior de *Glironia* a la subfamilia Caluromyinae (2n=14 para las otras especies conocidas). El único otro género conocido de Didelphidae presentando 2n=18 es *Monodelphis*. Sin embargo, estudios de bandas de cromosomas muestran un patrón mas parecido al observado para las especies 2n=14. Considerando estudios recientes en la filogenia de los Didelphidae, donde se sugiere la hipótesis de que *Glironia* sea el grupo hermano de los restantes miembros existentes de esta familia, el número diploide observado puede representar el estado ancestral de la familia, o puede haber resultado de evolución convergente.

Key words: Ag-NOR. Amazonia. C-banding. Conventional staining.

Palabras clave: Ag-NOR, Amazonia. Bandas C. Coloración convencional.

The karyotypes of didelphid marsupials are characterized by a relatively low number of chromosomes, with diploid numbers of 2n = 22 in the larger species of opossums (*Didelphis, Philander, Chironectes* and *Lutreolina*) and one species of "mouse-opossum" *Tlacuatzin canescens* (Engstrom and Gardner, 1988; Zarza et al., 2003), 2n = 18 in the short-tailed opossums of the genus *Monodelphis* and 2n = 14 (by far the most common diploid number) in *Metachirus*, *Caluromys* and five genera of small-bodied "mouse opos-

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sums" (*Gracilinanus*, *Marmosa*, *Marmosops*, *Micoureus* and *Thylamys;* Carvalho et al., 2002, and references cited therein).

The bushy-tailed opossum *Glironia venusta* (Didelphidae, Didelphimorphia) is considered one of the rarest South American marsupials and is listed as "Vulnerable" in the 2006 IUCN Red List of Threatened Species (IUCN, 2006). Very little is known about its habits and natural history. It is considered to be arboreal and has a highly developed hallux (Marshall, 1978; Emmons and Feer, 1997). In addition to its abilities for arboreal locomotion, the postcranial morphology of *Glironia venusta* also indicates some capacities for terrestrial habits (Flores and Díaz, 2009). Indeed, *G. venusta* has also been observed to use the lower strata of the forest (MNF da Silva, pers. obs.), has been captured with live traps in the forest understory (Díaz and Willig, 2004; Santos-Filho et al., 2007) and has also been captured in pitfall traps on the ground (Bernarde and Rocha, 2003).

Known originally from Bolivia, Ecuador and Peru (Marshall, 1978; Díaz and Willig, 2004), its occurrence in Brazil has now been documented at nine widely separated localities in the Amazon and Paraguay basins (da Silva and Langguth, 1989; Patton et al., 1996; Nogueira et al., 1999; Bernarde and Rocha, 2003; Santos-Filho et al., 2007; Calzada et al., 2008, Rossi et al., 2010). Voucher specimens from Brazil are deposited in the mammal collection at the National Institute for Amazonian Research (INPA 699, 2570, 4577, 5237), in the Museu de Zoologia da Universidade de São Paulo (MZUSP 34664), and in the mammal collection of the Universidade Federal de Mato Grosso (UFMT 696) (Santos-Filho et al., 2007; Rossi et al., 2010). The sites in Rondônia (Bernarde and Rocha, 2003), Manaus (Calzada et al., 2008) and in southeastern Pará (Rossi et al., 2010) do not have voucher specimen. According to Barkley (2007), the total number of known specimens of *G. venusta* is under 25.

In this report, the description of the karyotype of the bushy-tailed opossum *Glironia venusta* is presented for the first time.

The single specimen available for cytogenetic analyses (INPA 4577; skull and body in fluid) represents a young female from Teotônio, on the right bank of the Rio Madeira, 19 km SW of Porto Velho, Rondônia, Brazil (8°52'27"S 64°0'28" W).

A suspension of mitotic metaphase bone-marrow cells was obtained in the field from this single available specimen employing a slightly modified version of Patton's (1967) colchicine-hypotonic salt and Carnoy's fixative protocol using a hand-operated centrifuge. Air-dried slides were subsequently prepared and stained with Wright's stain; a total of 30 cells were examined and five of those were photographed under oil immersion using bright-field optics. Chromosomes were sorted by decreasing size and classified according to Levan et al. (1964). C-banding (in a total of 10 cells) and silver staining of the nucleolar organizer regions (Ag-NORs - in 20 cells) were performed following protocols described by Sumner (1972) and Howell and Black (1980), respectively.

The diploid number of chromosomes in the female bushy-tailed opossum *Glironia venusta* is 18. The karyotype (**Fig. 1**) consists of 2 large pairs of metacentric/submetacentric (pairs 1 and 2), 1 pair of subtelocentric (pair 5) and 5 pairs of acrocentric autosomes (pairs 3, 4 and 6 to 9). We presume that the smallest pair of acrocentrics represents the X chromosomes, as this pattern has been found for many didelphid species (Palma and Yates, 1996; Svartman and Vianna-Morgante, 1999, 2003; Carvalho et al., 2002). The number of autosomal chromosomal arms (FN) is 22.

Silver-staining analysis revealed the activity of nucleolar organizer regions (NORs) on the short arms of both acrocentric chromosomes of autosomal pair number 6; the presumptive sex chromosomes did not show NOR activity (**Fig. 2A** and **B**).

Constitutive heterochromatin was not observed in the autosomes of *G. venusta*. C-bands are restricted to sex chromosomes where they are revealed as diffusely stained blocks in the pericentromeric region of both presumptive X chromosomes (**Fig. 2C**; **Table 1**).

G-banding and treatment with CMA3 were performed but we did not yield reliable results. These trials were probably hampered by the

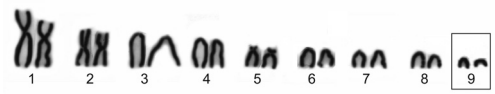

Fig. 1. *Glironia venusta* karyotype (2n = 18, FN = 22; INPA 4577); the presumptive sex chromosomes are indicated.

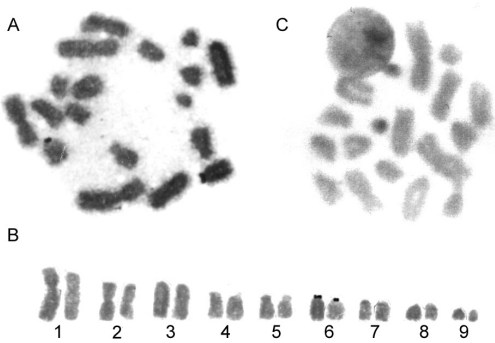

Fig. 2. (**A**) Metaphase and (**B**) karyotype showing the NOR-bearing chromosomes and (**C**) metaphase showing the C-band pattern of *Glironia venusta*.

high condensation of the chromosomes and by limited suitable material in the cell suspensions. When additional materials become available, a new trial of both sets of analyses should be performed.

In general, the distribution and expression of NORs in marsupials has been reported on one or more autosomal pairs, alone or in association with the sex chromosomes; in the latter case, NORs are either restricted to the X chromosome or they are found on both X and Y chromosomes (Svartman and Vianna-Morgante, 2003). According to Svartman and Vianna-Morgante (1999, 2003), pair 7 in species with 2n = 22, corresponds to pair 5 in species with 2n = 18 and pair 6 in species with 2n = 14 and these homeologous pairs are NOR-bearing chromosomes. Although pairs 5 and 6 are of similar size in the *Glironia* karyotype, pair 5 is approximately 9.0% larger then the NOR-bearing pair 6. This pattern contrasts to the pattern found in the 2n = 18 karyotype but fits with that of the 2n = 14 species, including its presence on the short arms, reinforcing the idea that the presence of NORs on this chromosome is a conserved condition in Didelphidae.

The only other didelphid genus previously reported to have a diploid number of 18 is *Monodelphis*. The fundamental number among species of *Monodelphis* varies considerably from 22 to 32 (Carvalho et al., 2002). Among these karyotypes (e.g., by Palma and Yates, 1996; Carvalho et al., 2002; Svartman and Vianna-Morgante, 2003), that of *M. glirina* from Bolivia (reported as *M. brevicaudata*; see Voss et al., 2001) by Palma and Yates (1996) is very similar to that of *Glironia*, despite differences between our respective classifications of chromosomes (compare op. cit.: Figure 4 and Table 1 with our **Fig. 1** and the karyotypic description above). Additional work such as chromosome banding and in-situ hybridization may determine the degree of similarity between these two karyotypes.

The similarity in diploid number and karyotype between these two genera is somewhat surprising since *Glironia* is often classified with *Caluromys* (2n=14) and *Caluromysiops* (diploid number unknown) in a distinct subfamily or family (Hershkovitz, 1992; Kirsch et al., 1995; Kirsch and Palma, 1995; McKenna and Bell, 1997; Gardner, 2005). However, the phylogenetic work of Voss and Jansa (2009), based on cladistic analyses of both molecular sequences and morphological characters, supports an alternative hypothesis, namely that *Glironia* is sister to all other opossums and is not the sister group of a *Caluromys+Caluromysiops* clade. The separation of *Glironia* from *Caluromys* and *Caluromysiops* is also reinforced by the distinctiveness of the skeletal morphology of *Glironia* when compared to these two genera (Flores and Díaz, 2009). If *Glironia* is sister to all other opossums, then the 2n=18 karyotype could either be ancestral for didelphimorphs, or the similarity in diploid number of *Glironia* and *Monodelphis* resulted from convergent evolution. The validity of these hypotheses can be examined in future analyses of cy-

Table 1

Karyotype and C-banding patterns of didelphid marsupials.

Species	2n	FN	C-banding positive			Reference
			Autossomes	X	Y	
Caluromys lanatus	14	22	Pericentromeric in all	Pericentromeric		Rofe and Hayman, 1985
Caluromys philander	14	20	Pericentromeric in all	Pericentro-meric and distal regions in the long arms	Total	Souza et al., 1990.
Didelphis albiventris	22	20	Centromeric in all	Long arms	Total	Casartelli et al., 1986
Didelphis marsupialis	22	20	Distal portion of long arms of some chromo-somes	Pericentromeric	Total	Svartman and Vianna-Morgante, 1999
Glironia venusta	18	22		Pericentromeric		This article
Gracilinanus microtarsus	14	24	Pericentromeric in all	Pericentromeric		Carvalho et al., 2002
Gracilinanus emiliae	14	24	Pericentromeric in all	Pericentromeric		Carvalho et al., 2002
Marmosa cinerea	14	20	Centromeric in all	Long arms	Total	Casartelli et al., 1986
Marmosa murina	14	20	Pericentromeric in all	Pericentromeric		Souza et al., 1990.
Marmosops incanus	14	24	Pericentromeric in all	Pericentromeric and distal blocks in the arms		Svartman and Vianna-Morgante, 1999
Metachirus nudicaudatus	14	20	Pericentromeric in pairs 5 and 6	Pericentromeric	Total	Svartman and Vianna-Morgante, 1999
Micoureus demerarae	14	20	Large pericen-tromeric blocks pairs1-4; blocks in distal long arms of 5 and 6	Pericentro-meric and distal regions of long arms	Total	Svartman and Vianna-Morgante, 1999
Monodelphis americana	18	22		Pericentromeric	Total	Pagnozzi et al., 2002
Monodelphis brevicaudata	18	30	Pericentromeric in all	Pericentromeric	Total	Carvalho et al., 2002
Monodelphis dimidiata	18	32	Pericentromeric in all	Pericentromeric	Total	Carvalho et al., 2002
Monodelphis domestica	18	20	Pericentromeric in all	Pericentromeric	Total	Merry et al, 1983; Svartman and Vianna-Morgante, 1999

(Table 1 cont.)

Monodelphis kunsi	18	30	Pericentromeric in all	Pericentromeric	Total	Carvalho et al., 2002
Philander opossum	22	20	Pericentromeric in all	Pericentromeric and lag blocks in long arm		Svartman and Vianna-Morgante, 1999
Thylamys elegans	14	20	Pericentromeric in all	Pericentromeric		Spotorno et al., 1997

togenetic and molecular markers once such are available.

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