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SHORT COMMUNICATION

A new karyotypic formula for the genus *Amphisbaena* (Squamata: Amphisbaenidae)

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Palabras claves: Squamata, Amphisbaenidae, *Amphisbaena bolivica*, cromosomas, Ag-NOR, Argentina.

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Based on the phylogenetic hypothesis of Mott and Vieites (2009), the "worm lizards" of the family Amphisbaenidae are represented by *Amphisbaena* and *Mesobaena* in the New World. The genus *Amphisbaena* Linné, 1758 includes about 94 morphologically diverse species (Gans 2005, Thomas and Hedges 2006, Mott *et al.* 2008, 2009, Ribeiro *et al.* 2008, Strüssmann and Mott 2009). Chromosome information is available for 19 species of *Amphisbaena* (Olmo and Signorino 2005, Laguna *et al.* 2010).

Although karyotype data are scarce, they indicate that species of *Amphisbaena* are highly variable in the number, as well as in morphology of macro- and microchromosomes. Diploid numbers range from 25 (*A. dubia;* Beçak *et al.* 1972) to 50 (*A. innocens* [Huang and Gans 1971]; *A. leberi* [Cole and Gans 1987]). The macrochromosomes and arms number vary from 12–24 and from 24–36, respectively (Huang *et al.* 1967, Huang and Gans 1971, Beçak *et al.* 1972, Cole and Gans 1987, Hernando 2005, Laguna *et al.* 2010). The numbers of microchromosomes range from 10–28, but the morphology is difficult to resolve because their size is in the order of 1 μm (Cole and Gans 1987). Nucleolus Organizer Region (NOR) locations also were found to be variable in *Amphisbaena,* differing among the five species previously studied (Hernando 2005, Laguna *et al.* 2010).

To contribute to the knowledge of chromosome evolution in *Amphisbaena,* we describe the karyotype constitution and the NOR location of *Amphisbaena bolivica* Mertens, 1929. This species occurs in central and northern Argentina, east of Bolivia and southwest of Paraguay (Gans 2005).

Specimens examined were deposited in Herpetological Collection of Universidad Nacional del Nordeste (UNNEC). The catalogue number and collecting localities are as follow: UNNEC 09314, female, from Paraje Las

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Figure 1. Localities where *Amphisbaena bolivica* was obtained for this study. 1 = Paraje Las Malvinas; 2 = Paraje Los Pichis (Provincia de Chaco, Argentina).

Malvinas (25˚16'05" S, 62˚16'14" W) and UNNEC 10470, male, from Paraje Los Pichis (25˚39'25" S, 61˚8'46" W), Provincia de Chaco, Argentina (Figure 1).

Chromosome preparations were obtained from the intestinal epithelium by squash techniques (Kezer and Sessions 1979). Chromosome number and standard morphology were determined by conventional Giemsa staining at pH 6.8. We describe the karyotype following the formula 2n (I; II; III) of Peccinini-Seale (1981) in which Group I consists of all metacentric and submetacentric macrochromosomes, Group II comprises subtelocentric and telocentric macrochromosomes, and Group III, the microchromosomes. Nucleolar organizer regions were detected by the silver nitrate staining method (Ag-NOR) (Howell and Black 1980).

Based on the analysis of 37 cells, the specimens of *Amphisbaena bolivica* have a karyotype 2n = 44 (0; 24; 20). Twelve pairs are uniarmed macrochromosomes that gradually decrease in size and 10 pairs are microchromosomes (Figure 2). No secondary constrictions were observed.

Figure 2. Karyotype of *Amphisbaena bolivica* (2n = 44) using conventional procedure.

The Ag-NORs are located in the telomeric region of a medium-sized macrochromosome pair that we considered to be pair 6 (Figure 3).

Chromosomal variation in this genus is as great as that within the family Amphisbaenidae (Gans 1978). To date, 20 karyotypes of *Amphisbaena*, including *A. bolivica* have been described, and 13 karyotypic formulae and one polymorphic species (*A. dubia*) are known (Beçak *et al*. 1972, Olmo and Signorino 2005, Laguna *et al*. 2010) (Table 1).

Ten species of *Amphisbaena* have karyotypes with 12 metacentric or submetacentric macrochromosomes and variable numbers of microchromosomes: 14 (*A. kingii*), 18 (*A. angustifrons, A. darwini, A. hiata, A. trachura*), 22 (*A. microcephalum*) and 24 (*A. xera, A. manni, A. caeca, A. fenestrata*) (Huang *et al*. 1967, Huang and Gans 1971, Hernando 2005).

The karyotypes of the remaining nine species of *Amphisbaena* previously analyzed have variable numbers of uni- and bi-armed macrochromosomes that exceed a minimum of six pairs. There are nine pairs in *A. mertensi* and *A. ridleyi*, and eleven pairs in *A. alba*, *A. vermicularis*, *A. fuliginosa*, *A. innocens,* and *A. gonavensis*. There are 12 pairs in *A. camura* and *A. bolivica* (Huang *et al.* 1967, Huang and Gans 1971, Beçak *et al*. 1972, Beçak *et al.* 1973 in Olmo and Signorino 2005, Hernando 2005, Laguna *et al.* 2010).

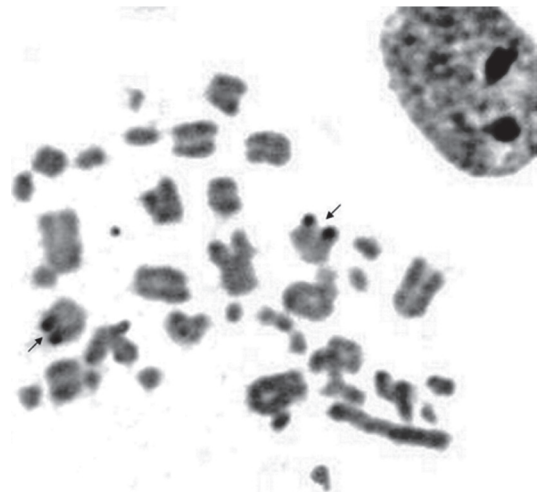

Figure 3. Metaphase of *Amphisbaena bolivica* after Ag-NOR technique.

The karyotype of *Amphisbaena bolivica* is distinctive among its congeners because all the macrochromosomes are telocentric (2n = 44 = 0; 24; 20); however, the same diploid number was reported for *A. vermicularis* (2n = 44 = 2; 20;

Table 1. Chromosome information for species of *Amphisbaena*. In the formula, I = number of bi-armed macrochromosomes; II = number of uni-armed macrochromosomes; and III = number of microchromosomes (Peccinini-Seale 1981).

Species of *Amphisbaena*	2n	Formula	Authority
A. alba	38	14; 8; 16	Huang and Gans (1971), Beçak *et al.* (1972)
A. angustifrons	30	12; 0; 18	Huang *et al.* (1967)
A. bolivica	44	0; 24; 20	This study
A. caeca	36	12; 0; 24	Huang *et al.* (1967)
A. camura	44	4; 20; 20	Huang *et al.* (1967)
A. darwini	30	12; 0; 18	Huang *et al.* (1967)
A. dubia	25	12; 3; 10	Beçak *et al.* (1972)
—	26	12; 2; 12	Beçak *et al.* (1972)
—	27	12; 1; 14	Beçak *et al.* (1972)
—	28	12; 0; 16	Beçak *et al.* (1972)
A. fenestrata	36	12; 0; 24	Huang and Gans (1971)
A. fuliginosa	48	6; 16; 26	Huang and Gans (1971)
A. gonavensis	50	8; 14; 28	Cole and Gans (1987)
A. hiata	30	12; 0; 18	Hernando (2005)
A. innocens	50	8; 14; 28	Huang and Gans (1971)
A. kingii	26	12; 0; 14	Huang and Gans (1971)
A. manni	36	12; 0; 24	Huang and Gans (1971)
A. mertensi	40	6; 12; 22	Hernando (2005)
A. microcephalum	32	12; 0; 20	Huang *et al.* (1967)
—	34	12; 0; 22	Huang *et al.* (1967)
—	34	12; 0; 22	Beçak *et al.* (1972), Hernando (2005)
A. ridleyi	46	14; 4; 28	Laguna *et al.* (2010)
A. trachura	30	12; 0; 18	Huang *et al.* (1967)
A. vermicularis	44	2; 20; 22	Beçak *et al.* (1973) in Olmo and Signorino 2005
A. xera	36	12; 0; 24	Huang *et al.* (1967)

22) and *A. camura* (2n = 44 = 4; 20; 20) (Beçak *et al.* 1973 in Olmo and Signorino 2005, Huang *et al*. 1967). This chromosome number also is known for the rhineurid *Rhineura floridana* (2n = 44 = 16; 8; 20) (Huang *et al.* 1967)

Huang and Gans (1971) and Cole and Gans (1987) proposed that the primitive karyotype is the most frequently observed in the extant worm lizards. It consists of 12 bi-armed macrochromosomes and 22 or 24 microchromosomes and occurs in representatives of Trogonophidae and Amphisbaenidae, including five species of *Amphisbaena* (*A. microcephalum, A. xera, A. manni, A. caeca,* and *A. fenestrata*). According to this hypothesis, reduction in number of microchromosomes in *A. angustifrons, A. darwini, A. hiata, A. trachura, A. kingii,* and the blanid, *Cynisca leucura,* may be a derived condition. Fission of macrochromosomes may explain the karytotypes with uni-armed macrochromosomes, as seen in 11 amphisbaenid species (*Amphisbaena alba, A. mertensi, A. bolivica, A. vermicularis, A. camura, A. ridleyi, A. fuliginosa, A. innocens, A. gonavensis, Mesobaena huebneri,* and *Geocalamus acutus*), Bipedidae, and Rhineuridae (Cole and Gans 1987).

The analyses by silver staining in six species of *Amphisbaena*, including this study, revealed that the Ag-NORs vary in number and location. *Amphisbaena darwini* has multiple NORs on macrochromosome pairs 1, 3, and 4, whereas a single pair bears the NORs in *A. hiata* (pair 4) and *A. ridleyi* (pair 2). In *A. bolivica,* the NOR is located on a medium-sized macrochromosome, as it is in *A. mertensi* (Hernando 2005, Laguna *et al*. 2010). These differences suggest that Ag-NOR may be a valuable cytogenetic marker in *Amphisbaena* species (Laguna *et al*. 2010).

Descriptive chromosomal studies on additional species of amphisbaenians are necessary before we can speculate on the pattern of karyotype evolution in Amphisbaenia.

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