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Standard karyotype and nucleolus organizer region of Neotropical blindsnake *Typhlops brongersmianus* (Serpentes: Typhlopidae)

José Augusto Ruiz García, Alejandra Hernando

Laboratorio de Herpetología, Fac. de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste, Av. Libertad 5450 W 3404 AAS, (3400) Corrientes, Argentina. Corresponding author. E-mail: ahernando@infovia.com.ar

Abstract. The karyotype of *Typhlops brongersmianus* is reported on the basis of specimens from north-eastern Argentina. The conventional Giemsa staining showed that the species has 2n = 34 chromosomes, including 8 pairs of macrochromosomes and 9 pairs of microchromosomes. Ag-NOR staining revealed the NORs location on a pair of macrochromosomes. The chromosome number and karyotypic morphology are similar to those of Neotropical typhlopid previously karyotyped.

Keywords. Cytogenetics, Scolecophidia, *Typhlops brongersmianus*, northeastern Argentina.

Among Serpentes, two major lineages are recognized, Alethinophidia and Scolecophidia. Alethinophidia include most of the extant snakes while Scolecophidia is considered a basal clade that comprises 305 species of small burrowing snakes with shiny scales, reduced eyes and traces of the pelvis girdle in most taxa (Pough et al., 2005). This group, commonly known as blindsnakes, is found on all continents except Antarctica and comprises three families: Typhlopidae, Anomalepididae and Leptotyphlopidae.

Despite their diversity, karyological knowledge of Scolecophidia lineage is still very limited. According to Olmo and Signorino (2005) chromosome data are based on non-differentially stained karyotypes for seven species that constitutes about 2% of the total blindsnakes species currently recognized (Olmo, 2005).

In this study, we describe the karyotype and the location of Ag-NORs of Neotropical *Typhlops brongersmianus* based on specimens collected from northeastern Argentina.

Six specimens of *T. brongersmianus* (four adult males and two females) were analyzed. Voucher specimens are deposited in the Herpetological Collection of the Universidad Nacional del Nordeste (UNNEC) under the following catalog numbers: UNNEC 08648 (F) from Pampa del Indio (26°03'S, 59°55'W); UNNEC 08178 (M) from Napenay (26°44'S, 60°37'W); UNNEC 08099, UNNEC 08848 (MM) from Corrientes (27°28'S, 58°51'W); UNNEC 08512 (F) from San Cosme (27°22'S, 58°31'W); UNNEC 08812 (M) from Paso de la Patria (27°19'S, 58°35'W).

Animals were injected with 0.1- 0.5 ml of 0.1% of colchicine solution 4 h before dissection. The chromosomes were obtained from intestinal epithelium and testes squash as described by Kezer and Sessions (1979). The preparations were stained with Giemsa solution at pH 7.0. The nucleolar organizer regions (NORs) were detected by silver nitrate staining (Howell and Black, 1980). The measurements of chromosomes arms were made on ten metaphase plates. Macrochromosomes were classified according to Sessions (1996).

The diploid chromosome number of *Typhlops brongersmianus* was 2n = 34 (16 M + 18 m) with clear demarcation between micro- and macrochromosomes (Fig. 1). The macrochromosomes pairs could be divided into two groups by size. The first group contained large chromosomes (pair 1-3) which were metacentric. Pairs 4-8 composed the second group with metacentric and submetacentric chromosomes (Table 1). Several microchromosomes showed metacentric morphology. In diplotene cells of males were observed 8 macrobivalents and 9 microbivalents (Fig. 1b). Heteromorphic sex chromosomes were not distinguished. The Ag-NORs were always located at the distal region of the long arm of the third macrochromosome pair (Fig. 1c).

To date about 10% of snakes have been karyotyped mostly using conventional staining methods (Olmo, 2005). The Aletinophidia lineage exhibits high chromosome variability, especially Colubroidea clade. Diploid number range from 2n = 24 to 2n = 52 and 52 different karyotypes were described (Olmo, 2005; Olmo and Signorino, 2005). Among Scolecophidia lineage, no Anomalepididae was analyzed chromosomically while one Leptotyphlopidae and five bisexual and one unisexual Typhlopidae species were studied (Wynn et al., 1987; Das and Ota, 1998; Olmo and Signorino, 2005). Three diploid chromosome number (2n = 32, 34 and 36) and five different karyotypes are known (Table 2). The highest diploid number is found in *Leptotyphlops phillipsi* (2n = 36) whereas two typhloid species of *Rhinotyphlops* analyzed share the same chromosome number (2n = 32) differing *R. schlegelii* from *R. simonii* by the macrochromosome morphologies. Only three species of the large pantropical blindsnake genus *Typhlops* have been investigated chromosomally (Olmo and Signorino, 2005). There are intrageneric differences in diploid, macrochromosome and microchromosomes numbers and macrochromosome morphologies. *T. jamaicensis* and *T. richardi* from Central America were both reported to have 2n = 34 (16 M + 18 m) (Wynn et al., 1987) and the karyotypes are comparable to our sample of *T. brongersmianus*. The karyotype of the Old World species *T. punctatus* (2n = 32) has

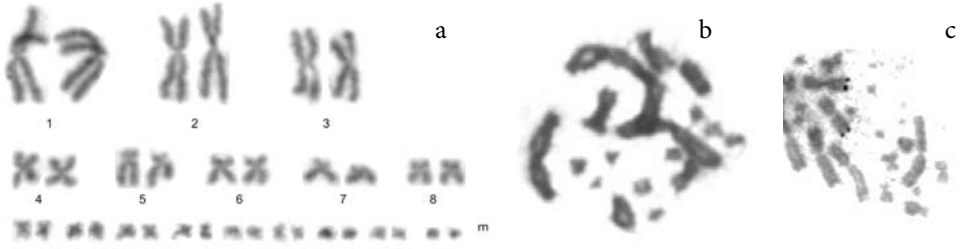

Fig. 1. a) Giemsa-stained karyotype of *Typhlops brongersmianus* (2n = 34, 16 M + 18 m) (UNNEC 08648, F). b) Male meiosis in *T. brongersmianus* showing 17 bivalents. c) Stained metaphase of *T. brongersmianus* showing the two macrochromosome bearing NORs.

Table 1. Quantitative characteristics of *Typhlops brongersmianus* chromosomes. L.R. (%): percentage length of chromosome pair over total genome length. C.I.: centromeric index

	Chromosome																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
L.R.	21.3	18.3	13.8	7.0	6.2	5.5	4.7	4.2	2.6	2.5	2.3	2.2	2.1	2	1.9	1.8	1.7
C.I.	0.42	0.42	0.43	0.39	0.31	0.32	0.33	0.30	--	--	--	--	--	--	--	--	--

Table 2. Diploid number (2n) and karyotype description (I, II, III) of species of Scolecophidia cytogenetically studied. Karyotype description = I: total number of biarmed macrochromosomes, II: total number of uniarmed macrochromosomes, III: total number of microchromosomes.

Families	Species	2n: karyotype description	References
Leptotyphlopidae	*Leptotyphlops phillipsi*	36: 10, 6, 20	Werner (1959)*
Typhlopidae	*Rhinotyphlops schlegelii*	32: 14, 2, 16	Fischman et al. (1972)*
	Rhinotyphlops simonii	32: 10, 6, 16	Werner (1959)*
	Typhlops jamaicensis	34: 16, 0, 18	Wynn et al. (1987)
	Typhlops richardi	34: 16, 0, 18	Wynn et al. (1987)
	Typhlops brongersmianus	34: 16, 0, 18	This study
	Typhlops punctatus	32: 20, 0, 12	De Smet (1978)*
	Ramphotyphlops braminus	3n: 42: 21, 0	Wynn et al. (1987); Das and Ota (1998)

*References were extracted from Olmo and Signorino (2005)

distinctive macrochromosome and microchromosome numbers and differ from the one shared by the Neotropical species.

The unisexual *Ramphotyphlops braminus* has been proposed to be triploid (2n = 3X = 42) (Wynn et al., 1987; Das and Ota, 1998).

In snakes only few banding studies exist. Among Alethinophidia lineage NORs location for about 93 species has been studied (Olmo and Signorino, 2005). Except in Natricinae and several species of Colubrinae (Colubridae subfamilies), snakes belonging to Boidae, Colubridae and Viperidae families exhibit a single pair of NOR-bearing microchromosomes (Moreno et al., 1987; Porter et al., 1991, 1994; Camper and Hanks, 1995; Aprea et al., 2006). It has been assumed that these microchromosomes are homologous representing microchromosomal NORs a primitive condition among snakes (Camper and Hanks, 1995). In Scolecophidia we document NORs location by Ag-staining method. *T. brongersmianus* presents Ag-NORs at the peritelomeric region on the long arm of chromosome 3. The same position of NORs was observed in *T. vermicularis* (16 M + 16 m) (G. Odierna, pers. comm.).

Further analyses using various banding techniques for karyotype characterization of Scolecophia blindsnakes are necessary to establish a pattern on chromosome evolution in this lineage.

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REFERENCES

Aprea, G., Gentilli, A., Zuffi, M.A.L., Odierna, G. (2006): The karyology of *Vipera aspis, V. atra, V. hugyi*, and *Cerastes vipera*. Amphibia-Reptilia **27**: 113-119.

Camper, J.D., Hanks, B. (1995): Variation in the nucleolus organizer region among New World snakes. J. Herpetol. **29**: 468-471.

Das, I., Ota, H. (1998): A checklist of chromosome numbers of South Asian reptiles. Hamadryad **23**: 179-193.

Howell, W.M., Black, D.A. (1980): Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1- step method. Experientia **36**: 1014-1015.

Kezer, J., Sessions, S.K. (1979): Chromosome variation in the plethodontid salamander, *Aneides ferreus*. Chromosoma **71**: 65-80.

Moreno, R., Navarro, J., Iturra, P., Veloso, A. (1987): The karyotype of *Philodryas chamissonis* (Colubridae). Identification of nucleolar organizer regions (NOR) and sex chromosomes by banding methods. Brazil. J. Genetics **10**: 497-506.

Olmo, E. (2005): Rate of chromosome changes and speciation in reptiles. Genetica **125**: 185-203.

Olmo, E., Signorino, G. (2005): Chromorep: a reptile chromosomes database. Internet references: http://193.206.118. 100/professori/chromorep.pdf, 15.09.06.

Porter, C.A., Haiduk, M.W., De Queiroz, K. (1994): Evolution and phylogenetic significance of ribosomal gene location in chromosomes of squamate reptiles: systematic and evolutionary implications. Copeia **1994**: 302-313.

Porter, C.A., Hamilton, M.J., Sites, Jr., J.W., Baker, R.J. (1991): Location of ribosomal DNA in chromosomes of squamate reptiles: systematic and evolutionary implications. Herpetologica **47**: 271 -280.

Pough, F.H., Janis, C.M., Heiser, J.B. (2005): Vertebrate life. Pearson Prentice Hall, New Jersey, EEUU.

Sessions, S.K. (1996): Chromosomes: Molecular. In Molecular Systematics, p. 121-168. Hillis, D., Moritz, C., Mable, B., Eds, Sinauer Associates, Sunderland, MA.

Wynn, A.H., Cole, Ch.J., Gardner, A.L. (1987): Apparent triploidy in the unisexual Brahminy Blind Snake, *Ramphotyphlops braminus*. Am. Mus. Novitates **2868**: 1-7.