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Karyotype of *Philodryas nattereri* and *Philodryas olfersii* with a comparative analysis of the Dipsadidae family

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ABSTRACT. Cytogenetic studies of *Philodryas nattereri* and *Philodryas olfersii* revealed a diploid chromosome number 2n = 36 for both species (3 metacentrics, 4 submetacentrics, and 10 acrocentrics, with a fundamental number of 51 and 52, respectively). The results obtained are novel and similar to those previously described for species belonging to the Dipsadidae family. The conventional karyotype is also novel and divergent from other species of the Dipsadidae family, where a higher proportion of macrochromosomes predominate, revealing two distinct groups in this family. The data are reported and discussed considering the cytotaxonomy of the family. These results strongly support the current view that chromosomal alterations, such as centric fusion and Robertsonian's translocations, seems to support the distinct

importance of chromosomal rearrangements in speciation within this group.

Key words: Chromosome number; Cytotaxonomy; Dipsadidae; *Philodryas*

INTRODUCTION

The New World Dipsadidae are one of the largest radiations of colubroidean snakes, with approximately 700 species distributed throughout the Americas and the West Indies (Hedges et al., 2009; Zaher et al., 2009). Recent studies (Vidal et al., 2000, 2010; Pinou et al., 2004; He et al., 2009; Zaher et al., 2009) confirm three historically distinct lineages (Cadle, 1984).

Chromosome studies among Dipsadidae family snakes are limited to a few taxa, with chromosome numbers ranging from 2n = 24 to 56 or more (Beçak and Beçak, 1969; Ferrarezi, 1994). The increase in the number of chromosomes is accompanied by an increase in morphological derivations (Oguiura et al., 2009).

The basic chromosome number among *Philodryas* genera is considered to be 2n = 36, consisting of a karyotype with 16 macrochromosomes and 20 microchromosomes, which is relatively common in most species of this genus (Moreno et al., 1987). In this genus, the chromosomes are almost identical pair by pair in their relative lengths and centromeric indices. The only difference is in the stages of differentiation of the W chromosome: these may differ from the Z chromosome by the centromere position or by size or by both (Beçak and Beçak, 1969; Singh, 1972; Beçak et al., 1990).

Philodryas nattereri and *Philodryas olfersii* have both been identified as having a diploid chromosome number 2n = 36 (Beçak and Beçak, 1969), but there are no studies that describe other karyotypical parameters, such as a fundamental number (FN), C-banding, NOR, and karyotype differentiation, between species of the Dipsadidae family.

The aim of the present study was to describe the karyotypic parameters of the cosmopolitan species *P. nattereri* and *P. olfersii* from northeastern Brazilian. In addition, karyotype differentiation in the Dipsadidae family is discussed.

MATERIAL AND METHODS

Sampling

Six snakes of each species (*P. nattereri* and *P. olfersii*) were captured on Aroeiras Farm in the municipality of Upanema (5°38'32"S and 37°15'27"W), State of Rio Grande do Norte, and transported to NUROF-UFC. The animals were maintained in individual cages with water *ad libitum* and fed with 15 g mice every 30 days until chromosome analysis.

Mitotic stimulation and cell culture

Snake peripheral blood lymphocytes were cultured under standard conditions at 37°C for 72 h in RPMI-1640 medium, supplemented with 10% calf fetal serum. Colchicine was added at a concentration of 1 x 10^6 M. Cultures grown without an intercalator were used as

controls. Hypotonic treatment of preparations (10 mL KCl, 0.075 mM) was carried out for 40 min at 37°C. The cells were fixed in a 3:1 methanol:acetic acid mixture and dropped on wet, cooled slides (Seabright, 1972).

Chromosome, FN, and karyotype description

Cell suspensions were transferred onto a microscopy glass slide and covered with a 60°C water film, set to dry at room temperature, and then stained with phosphate-buffered Giemsa, pH 6.8, (5%) for 25 min. The metaphases resulting in ideal spreading and chromosome contraction were photomicrographed. Diploid chromosome number (2n) and FN were determined through direct counts of chromosomes and their arms, respectively. Chromosome types (metacentric, submetacentric, acrocentric, and telocentric) were identified following the method of Levan et al. (1964).

The chromosome data for *P. nattereri* and *P. olfersii* were compared with available data from other species of the Dipsadidae family (Table 1). A karyotypic parameter matrix was built, including the somatic chromosome number (2n), the FN, and the chromosomal formula. This matrix was analyzed through PC-ORD 6.0 (McCune and Mefford, 2011) to produce a UPGMA cluster based on the Bray-Curtis similarity index.

RESULTS AND DISCUSSION

Determination of the chromosome numbers of *P. nattereri* and *P. olfersii*

As shown in Figures 1 and 2, the chromosome number for both *P. nattereri* and *P. olfersii* was 2n = 36. This number agrees with those previously reported for the genus *Philodryas* (Beçak et al., 1966; Beçak and Beçak, 1969; Moreno et al., 1987).

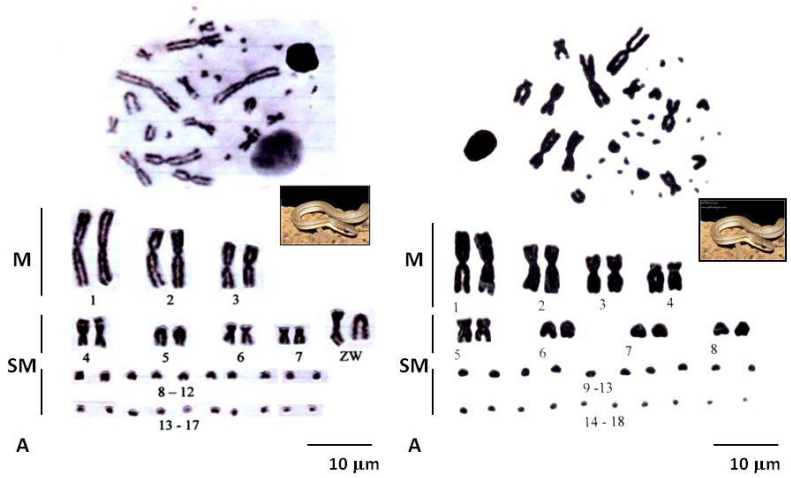

Figure 1. Metaphase of cells from female and male *Philodryas nattereri* with 2n = 36 chromosomes, respectively. Karyotype with 16 macrochromosomes and 20 microchromosomes bearing secondary constriction in the fifth pair of metacentric macrochromosomes. M = metacentric; SM = submetacentric; A = acrocentric.

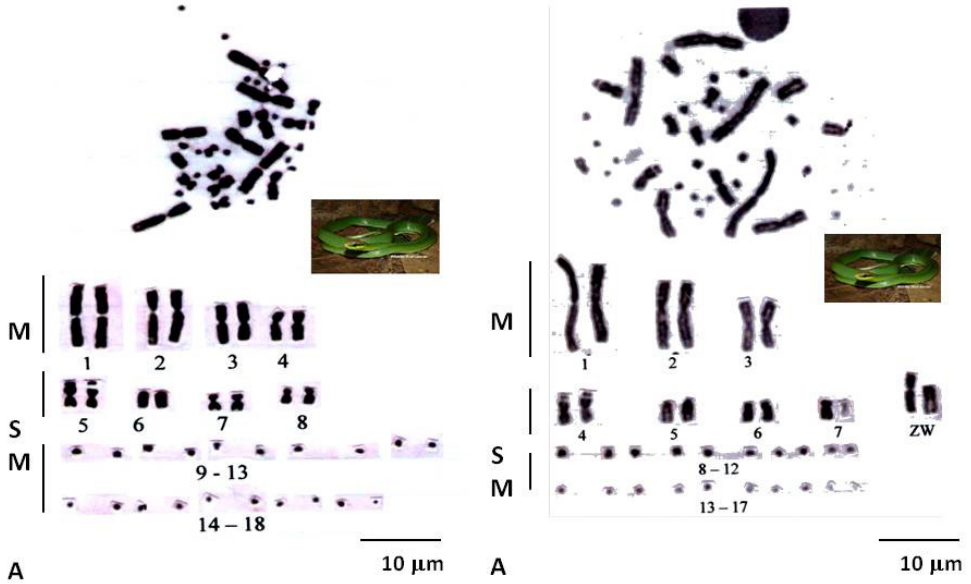

Figure 2. Metaphase of cells from female and male *Philodryas olfersii* with 2n = 36 chromosomes, respectively. Karyotype with 16 macrochromosomes and 20 microchromosomes bearing secondary constriction in the fifth pair of metacentric macrochromosomes. M = metacentric; SM = submetacentric; A = acrocentric.

No significant differences were observed between karyotypes of males and females analyzed. Thus, there was no heteromorphism related to the sex chromosome. The karyotype of female *P. nattereri* included 3 pairs of metacentric, 4 pairs of submetacentric, 10 pairs of acrocentric chromosomes, and the sexual chromosomes Z and W were submetacentric and acrocentric, respectively. The karyotype of male *P. nattereri* included 4 pairs of metacentric, 4 pairs of submetacentric, and 10 pairs of acrocentric chromosomes. The resulting FN was 51 and 52 for females and males, respectively.

In *P. olfersii*, the karyotype included 3 pairs of metacentric, 4 pair of submetacentric, and 10 pairs of acrocentric chromosomes, and the sexual chromosomes Z and W were metacentric and submetacentric, respectively. The resulting FN was 52 for females and males. The macrochromosomes (metacentric and submetacentric) showed the presence of secondary constriction in the fifth pair of metacentric chromosomes.

Some *Philodryas* species show secondary constriction in chromosome pair 5 (Beçak et al., 1971). As *P. chamissonis* exhibits NOR in the long arm of chromosome pair 2, it was proposed that translocation of insertion types may be accounted for by polymorphisms in this genus (Moreno et al., 1987).

The similarity analysis of 24 species of the family Dipsadidae based on karyotypic parameters (Figure 3) revealed two distinct groups with similarities greater than 50%. Group A, including *Hydrodynastes bicinctus*, *Hydrodynastes gigas*, *Leimadophis almadensis*, and *Xenodon severus*, presented lower FN (40 to 42) due to a higher proportion of microchromosomes (acrocentric), therefore, representing a trend towards symmetry. In group B, which includes *P. nattereri* and *P. olfersii*, a higher FN was presented in relation to group A because of the presence of a higher number of macrochromosomes (metacentric, submetacentric, telocentric) (Table 1).

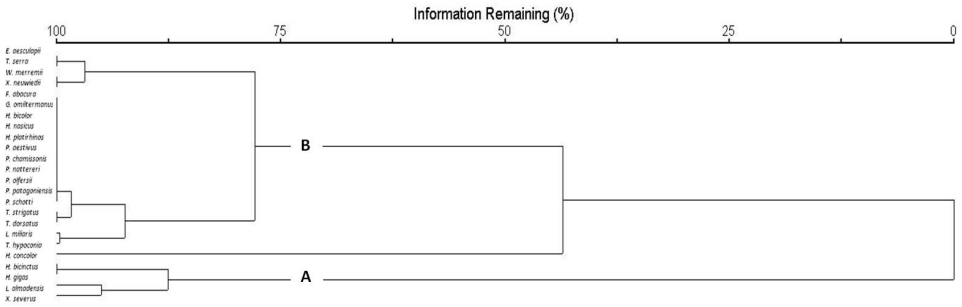

Figure 3. Bray-Curtis similarity dendrogram based on a matrix of karyotypical data. **A.** Group with predominantly symmetrical karyotypes. **B.** Group with asymmetrical karyotypes.

Table 1. Karyotypic parameters of 24 species of the Dipsadidae family.

Species	2n	FN	Karyotype	Reference
Erythrolamprus aesculapii	28	48	16M + 4SM + 8A	Beçak and Beçak, 1969
Farancia abacura	36	52	14M + 2SM + 20A	Camper and Hanks, 1995
Geophis omiltermanus	36	52	14M + 2SM + 20A	Hardy, 1976
Helicops bicolour	36	52	16M + 20A	De Smet, 1978
Heterodon nasicus	36	52	16M + 20A	Baker et al., 1972
Heterodon platirhinos	36	52	16M + 20A	Baker et al., 1972
Hydrodynastes bicinctus	24	40	16M + 8A	Beçak and Beçak, 1969
Hydrodynastes gigas	24	40	16M + 8A	Beçak and Beçak, 1969
Hydromorphus concolor	46	62	12M + 4SM + 30A/11M + 5SM + 30A/ZW	Solorzano et al., 1989
Liophis almadensis	28	42	16M + 12A	Benirschke and Hsu, 1975
Liophis miliaris	28	55	27M + 1A	Beçak and Beçak, 1969
Philodryas aestivus	36	52	12M + 18SM + 20A/ZW	Beçak and Beçak, 1969
Philodryas chamissonis	36	52	14M + 18SM + 20A/ZW	Moreno et al., 1987
Philodryas nattereri	36	51	16M+ 16SM + 20A	Present study
			12M + 18SM + 21A/ZW	
Philodryas olfersii	36	52	14M + 18SM + 20A	Present study
			13M + 3SM + 20/ZW	
Philodryas patagoniensis	36	52	12M + 4SM + 20A	Beçak and Beçak, 1969
			11M + 5SM + 20A/ZW	
Philodryas schotti	36	52		Beçak et al., 1966
Thamnodynastes hypoconia	34	54	12M + 8SM + 14A	Beçak and Beçak, 1969
			11M + 9SM + 14A/ZW	
Thamnodynastes strigatus	32	50	14M + 4SM + 14A	Beçak and Beçak, 1969
			13M + 5SM + 14A/ZW	
Tropidodryas serra	28	48	16M + 4SM + 8A/ZW	Beçak and Beçak, 1969
Tomodon dorsatus	32	50	14M + 4SM + 14A/ZW	Beçak and Beçak, 1969
Waglerophis merremii	30	46	16M + 14A/ZW	Bianchi et al., 1969
Xenodon neuwiedii	30	46	14M + 2SM + 14A/ZW	Beçak and Beçak, 1969
Xenodon severus	30	44	14M + 16A	Beçak et al., 1971

2n = diploid chromosome number; FN = fundamental number.

The observation of clustering based on similarities of a karyotype parameter (FN) (Figure 3) is in strong disagreement with recent Dipsadidae molecular phylogeny (Grazziotin et al., 2012) and seems to support the distinct importance of chromosomal rearrangements during speciation within this group.

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