KARYOTYPES AND NUCLEOLUS ORGANIZER REGIONS IN FOUR SPECIES OF THE GENUS *PHYSALAEMUS* (ANURA, LEPTODACTYLIDAE)

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ABSTRACT

Cytogenetic studies were performed in *Physalaemus biligonigerus* (Cope, 1861), *P. crombiei* (Heyer & Wolf, 1989), *P. olfersii* (Lichtenstein & Martens, 1856) and *P. spiniger* (Miranda-Ribeiro, 1926) collected at Southern and South Brazil. The four species have similar 2n=22 karyotypes. The Ag-NORs are located on Chromosome 8 in *P. biligonigerus* and *P. crombiei*, on Chromosome 11 in *P. spiniger*, and on Chromosome 3 and 4 in *P. orfersii*. The C-banding pattern for *P. biligonigerus* is also described.

KEYWORDS. Ag-NORs, Anura, *Physalaemus*, chromosome, cytogenetics.

INTRODUCTION

The family Leptodactylidae is one of the most diversified groups of Anura, comprising 54 genera and a great number of species which are distributed throughout the world (Frost, 1985; Duellman, 1993). The genus *Physalaemus* (Fitzinger, 1826) is one of the most abundant, comprising 39 species, found in Mexico and South America (Frost, 1985; Duellman, 1993; Pombal & Madureira, 1997; Feio **et al.**, 1999). Until now, 17 species of *Physalaemus* have been karyotyped (Beçak, 1968; Brum-Zorrila & Saez, 1968; Beçak **et al.**, 1970; Denaro, 1972; De Lucca **et al.**, 1974; Morescalchi & Gargiulo, 1968 and León, 1970 **apud** Kuramoto, 1990; Lourenço **et al.**, 1998), the majority of which only under conventional staining. Recently, cytogenetic data based on Ag-NOR and FISH techniques were presented for one *Physalaemus* species (Lourenço **et al.**, 1998). The available karyograms (Beçak, 1968; Beçak **et al.**, 1970; Denaro, 1972; De Lucca **et al.**, 1974; Lourenço **et al.**, 1998) show that the species of *Physalaemus* share very similar 2n=22 karyotypes, but some differentiation in the morphology and size of

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chromosome pairs as well as in the number and position of secondary constriction or Ag-NOR seems to occur.

The karyotypes and the Ag-NOR patterns of *Physalaemus biligonigerus* (Cope, 1861), *P. crombiei* (Heyer & Wolf, 1989), *P. olfersii* (Lichtenstein & Martens, 1856) and *P. spiniger* (Miranda-Ribeiro, 1926) are described; also the C-banding pattern in *P. biligonigerus*. The later species belongs to the *P. biligonigerus* group whereas the other three are included in the *P. signifer* group.

MATERIAL AND METHODS

We performed cytogenetics analysis in four species of *Physalaemus*: 2♂ *P. biligonigerus*, from Santa Maria, RS (29°41'S, 53°48'W, 150m altitude); ♂ *P. crombiei*, from Aracruz, ES (19°49'16"W, 60m altitude); 2♂, 4♀ *P. olfersii*, from Ribeirão Branco, SP (24°13'S, 48°46'W, 875m altitude) and juvenile *P. spiniger*, from Ilha do Cardoso, Cananéia, SP (27°00'S, 47°44'W, 8m altitude). The animals were deposited in the amphibian collection of Departamento de Zoologia, Instituto de Biociências, UNESP, Rio Claro, São Paulo, Brazil.

Direct chromosome preparations of bone marrow, liver, and testis were made by cellular suspension (BALDISSERA **et al.**, 1993). In order to improve the mitotic index, it was injected Phytohemagglutinin-P (Difco) in some specimens, before colchicine treatment, in the proportion of 0.1 ml/10g animal weight, between 48 to 72 hours before sacrifice, according to the procedure of BAKER **et al.** (1971) and WILEY (1982). Conventional staining was performed with Giemsa diluted in phosphate buffer, pH 6.8. Ag-NOR staining followed the procedures described by HOWELL & BLACK (1980). C-banding pattern, obtained only for *P. biligonigerus*, followed SUMNER (1972).

RESULTS

The description of the 2n=22 karyotype in *Physalaemus biligonigerus* is based on C-banded metaphases (fig. 1). The 22 chromosomes can be divided into three large metacentric (Chromosome 1) and submetacentric (Chromosomes 2 and 3) pairs, four intermediate metacentric (Chromosomes 5 and 6) and submetacentric (Chromosomes 4 and 7) pairs, and four smaller metacentric or submetacentric (Chromosomes 8, 9, 10 *and* 11) pairs. No heteromorphic sex chromosome pair was identified in the male karyotype.

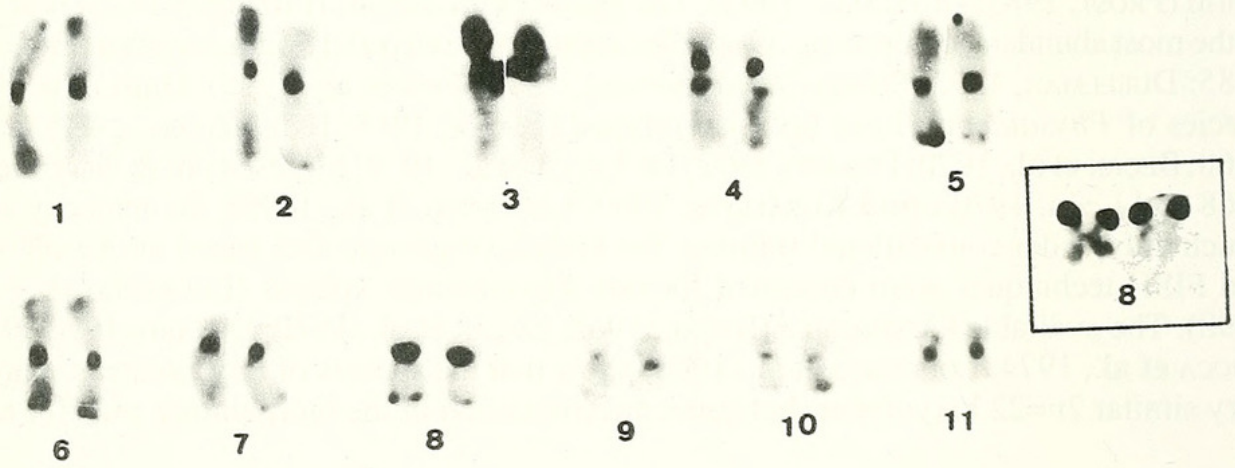
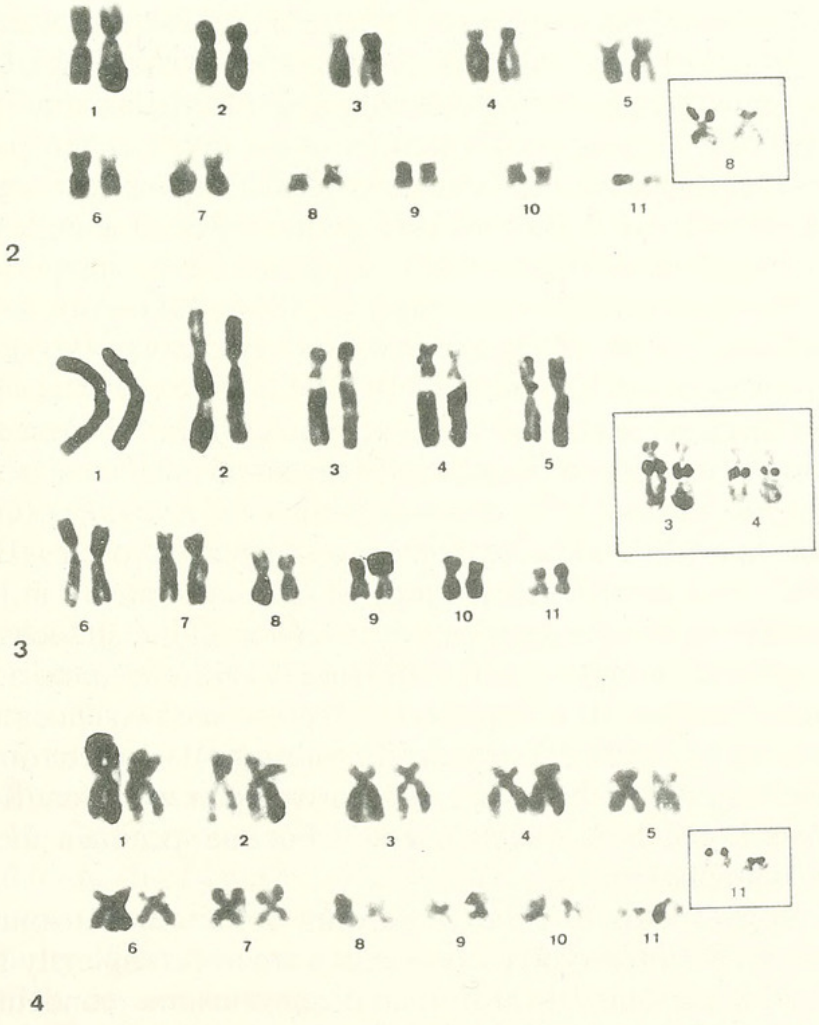


Fig. 1. C-banded karyotype (2n=22) and Ag-NOR stained chromosomes (inset) of a male specimen of *Physalaemus biligonigerus*.

C-bands were located mainly on the centromeric region. Chromosome 4 also presents an interstitial C-band on the proximal region of the long arms, which usually appears more heavily stained in one of the homologues. The telomeric regions of some chromosomes appear positively stained in some metaphases. Chromosome 8 has C-banding pattern that distinguishes it from the other smaller chromosomes. The chromosomes of this pair exhibit C positive staining along the short arms, except at the distal end, which corresponds to the Ag-NOR site in the karyotype of *P. biligonigerus* (fig. 1). Both Ag-NORs are equal in size and in some metaphases appear associated to one another.

The 22 chromosomes of *P. crombiei* can be divided into one large metacentric pair (Chromosome 1), six intermediate submetacentric pairs (Chromosomes 2 to 7), and four smaller pairs of which Chromosomes 8, 9 and 10 are metacentric or submetacentric and Chromosome 11 is telocentric (fig. 2). No heteromorphic sex chromosome pair was identified in the male karyotype. Chromosomes 8 and 11 are distinguished from the other smaller chromosomes. Chromosome 8 by the presence of a secondary constriction in the interstitial region of the short arms, and Chromosome 11 due to its telocentric morphology. In this specimen, only one Ag-NOR per metaphase was visualized (fig. 2), in the site of the secondary constriction.

The 22 chromosomes of *P. olfersii* can be divided into two large metacentric



Figs. 2-4. Giemsa-stained karyotype (2n=22) and Ag-NOR stained chromosomes (inset): 2, male of *Physalaemus crombiei*; 3, male of *P. olfersii*; 4, juvenile of *P. spiniger.*

(Chromosome 1) and submetacentric (Chromosome 2) pairs, five intermediate metacentric (Chromosomes 5 and 6) and submetacentric (Chromosomes 3, 4 and 7) pairs, and four smaller metacentric or submetacentric (Chromosomes 8, 9, 10 and 11) pairs (fig. 3). No heteromorphic sex chromosome pair was identified in male or female karyotypes. Chromosomes 3 and 4 have secondary constriction on the proximal region of the long arms. Chromosome 4 is slightly more submetacentric. These chromosome pairs are both Ag-NOR bearers (fig. 3).

The 22 chromosomes of *P. spiniger* can be divided into three large metacentric (Chromosome 1) and submetacentric (Chromosomes 2 and 3) pairs, four intermediate metacentric (Chromosome 7) and submetacentric (Chromosomes 4, 5 and 6) pairs, and four smaller pairs of which Chromosomes 8, 9, and 10 are metacentric or submetacentric and Chromosome 11 is telocentric (fig. 4). Chromosome 11 is distinguished from the other smaller chromosomes due to its telocentric morphology and by the presence of a proximal secondary constriction. The secondary constriction is Ag-NOR bearer (fig. 4).

DISCUSSION

Physalaemus biligonigerus, P. crombiei, P. olfersii and *P. spiniger* have 2n= 22 chromosomes as do other species of the genus (KING, 1990; KURAMOTO, 1990). The karyograms of the species of *Physalaemus* hitherto studied exhibit great similarity between chromosome pairs, most of which are metacentrics or submetacentrics. Chromosome 11 has a variable morphology, generating two karyotypic formulae among the species of *Physalaemus*. The first karyotypic formula is made up of seven pairs of large or intermediate biarmed chromosomes and four pairs of small biarmed chromosomes. Besides *P. biligonigerus* and *P. olfersii*, it is found in *P. aguirrei* (Bokermann, 1966), *P. albifrons* (Spix, 1824), *P. centralis* (Bokermann, 1962), *P. cicada* (Bokermann, 1966), *P. cuvieri* (Fitzinger, 1826), *P. fuscomaculatus* (Steindachner, 1864), *P. gracilis* (Boulenger, 1883), *P. kroyeri* (Reinhardt & Lutken, 1862), and *P. soaresi* (Izecksohn, 1965), as described by BEÇAK (1968); BEÇAK **et al.** (1970); DENARO (1972); DE LUCCA **et al.** (1974). The second karyotypic formula includes seven pairs of large or intermediate biarmed chromosomes, three pairs of small biarmed chromosomes and one pair of small uniarmed chromosome, being found in *P. crombiei* and *P. spiniger* as well as in *P. signifer* (Girard, 1853), *P. nattereri* (Steindachner, 1863) and in two other non-identified species (BEÇAK, 1968; DE LUCCA **et al.**, 1974). The distinct morphology of Chromosome 11 in both karyotypic formulae might be consequence of a pericentric inversion, although such a rearrangement cannot be confirmed since longitudinally differentiated chromosomes are not available. *Physalaemus petersi* (Jiménez de la Espada, 1872) presented two uncommon karyotypic patterns (LOURENÇO **et al.**, 1998), in which Chromosome 11 is heteromorphic in male specimens. For the majority of them, this chromosome pair was identified as XY, the X being a submetacentric and the Y, a subtelocentric. For one specimen, the heteromorphic chromosomes are submetacentrics.

Full homeology seems to occur regarding some chromosome pairs among *Physalaemus* species. Slight karyotypic variations are not completely ruled out and to some extent, may be attributed to differential chromosome condensation or small rearrangements mainly those involving constitutive heterochromatin. Such idea is based on the fact that species of amphibians belonging to the same genus, exhibiting great

uniformity in chromosome number and morphology, can show extensive differences in the amount of constitutive heterochromatin (SCHMID **et al.**, 1990). Unfortunately, C-banding data for species of *Physalaemus* are still very scanty to clarify this question.

C-banding was obtained only for *P. biligonigerus*. If the pattern of interstitial and telomeric C-bands is species-specific, this technique might be another important tool to better characterize the karyotypes of *Physalaemus*, as occurred for other amphibian groups. For example, C-band comparisons have been useful to differentiate taxonomically distinct forms within the same genera (MATSUI **et al.**, 1985; ANDERSON, 1991; MIURA, 1995), mainly among those including species which show similar karyotypes under conventional staining.

The chief interspecific difference among the karyotypes of *Physalaemus* is related to the number and position of Ag-NORs or secondary constrictions. *Physalaemus biligonigerus, P. crombiei* and *P. spiniger* have one pair of small-sized chromosomes bearing Ag-NORs (Chromosome 8 in *P. biligonigerus* and *P. crombiei*, and Chromosome 11 in *P. spiniger*), whereas *P. olfersii* has two large chromosome pairs bearing Ag-NORs (Chromosome 3 and 4). It is interesting to note that, unlike our data, DE LUCCA **et al.** (1974) described secondary constriction in a single chromosome pair, Chromosome 3, in *P. olfersii*. Although the possibility that Chromosome 4 also carries Ag-NORs cannot be ruled out, the data on *P. olfersii* could represent a case of geographical karyotypic variation.

The cytogenetic data on *Physalaemus* indicate that the majority of the species have a single pair of chromosomes bearing nucleolus organizer regions, as it is usually observed in anuran species (SCHMID **et al.**, 1990). The secondary constriction (*P. nattereri,* BEÇAK, 1968; *P. cuvieri* and *P. fuscomaculatus,* BEÇAK **et al.**, 1970; *P. centralis,* DENARO, 1972; *P. signifer* and *P. soaresi,* DE LUCCA **et al.**, 1974) or Ag-NOR is frequently found among the smallest in the karyotype (Chromosome 8, 9, or especially 11), although in some species it can also be one of the large or intermediate chromosomes. Less frequently, there are reports on species of *Physalaemus* with more than one chromosome bearing secondary constriction or Ag-NOR (*P. albifrons,* DENARO, 1972; *P. petersi,* LOURENÇO **et al.**, 1998). Evidently, more data about the Ag-NORs in species of *Physalaemus* are still necessary to establish an evolutionary pattern for the distribution of this chromosome marker.

The species *P. crombiei, P. olfersii* and *P. spiniger* are included in the *P. signifer* morphological group. Other eight representatives of this group are *P. bokermanni* (Cardoso & Haddad, 1985), *P. caete* (Pombal & Madureira, 1997), *P. maculiventris* (A. Lutz, 1925), *P. maximus* (Feio, Pombal & Madureira, 1999), *P. moreirae* (Miranda-Ribeiro, 1937), *P. nanus* (Boulenger, 1888), *P. obtectus* (Bokermann, 1966), and *P. signifer* (FROST, 1985; HADDAD & POMBAL, 1998; FEIO **et al.**, 1999), of which only the karyotype of *P. signifer* has been described (DE LUCCA **et al.**, 1974). The present study show that *P. olfersii* has divergent cytogenetic characteristic from the remaining karyotyped species of *P. signifer* group. While *P. crombiei, P. spiniger* and *P. signifer* share a telocentric chromosome 11 in their karyotypes, *P. olfersii* presents a distinct karyotypic formula with biarmed chromosome 11. This fact may reinforce the previous statement that *P. olfersii*, due to its morphological traits, should not be allocated in *P. signifer* group, like the newly described species *P. maximus* (see FEIO **et al.**, 1999). According to these authors, although the external similarity of the two species as well as of *P. aguirrei* and *P. soaresi*, additional evidence is still necessary to suggest a new species group for them. For the moment, a common known cytogenetic characteristic for *P. aguirrei, P. olfersii* and *P. soaresi* is the karyotypic formula.

Acknowledgments. To Rogilene Aparecida Prado (UNESP) for technical assistance. Anne d'Heursel (UNESP) for critically reading earlier versions of the manuscript. This work was supported by CNPq and FAPESP.

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Recebido em 30.06.1999; aceito em 23.11.1999.