CARYOLQGIA Vol. 53, no. 2: 283-288,2000

Cytogenetic analysis of three *Physalaemus* species (Amphibia, Anura)

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Abstract — *Physalaemus biligonigerus, P. fuscomaculatus* and *P.* sp. have *2n=2* chromosomes, consistent with the number of chromosomal pairs found in the genus. There were no substantial differences in the karyotypes of the three species, except for chromosome 3 which was submetacentric in *P. fuscomaculatus* and metacentric in *P. biligonigerus* and *P.* sp. All chromosomes were characterised by the presence of a C-band in the pericentromeric region. Some interstitial bands were also detected at the same position in all karyotypes, but the band located on the short arm of chromosome 3 was larger in *P. biligonigerus* and *P.* sp. than in *P. fuscomaculatus.* Also, only *P.* sp. had a large block of pericentromeric heterochro-matin in the short arm of chromosome 9 and another one in the telomeric band on the long arm. The NOR was located on the long arm of chromosome 9 in the three species. These findings confirmed that the three species are indeed closely related cytogenetically.

Key words: Ag-NOR, C-band, cytogenetics, cytotaxonomy, karyotypes, *Physalaemus biligonigerus, Physalaemus fuscomaculatus, Physalaemus* sp.

INTRODUCTION

The genus[1] *Physalaemus* shows extensive polymorphism and an abundance of cryptic species (BARRIO 1965; FROST 1985). LYNCH (1970) recognized four groups of species in the genus *Physalaemus: pustulosus, signifer, cuvieri* and *biligonigerus,* mainly for its morphologic similarities. However, some recently described species could not be included in any of these groups (CARAMASCHI *et al.* 1991). According to LYNCH (1970) and FROST (1985), the following species *P. fuscomaculatus, P. nattereri* and *P. biligonigerus* was included in the *biligonigerus* group.

Between these species, the recognition of *P. fuscomaculatus* and *P. biligonigerus* is complex and this taxonomic confusion surrounding the recognition of *P. fuscomaculatus* and *P. biligonigems* was mentioned in PARKER'S revision (1927 — apud BARRIO 1965). According to CEI (1990), the inappropriate use of the specific name *fuscomaculatus* was very common up to the publication of MILSTEAD'S critical note (1963). This included several populations of P. *biligonigerus,* extending into southern South America, from southern Brazil, Uruguay and the Paraguayan Chaco to Central Argentina. Based on taxonomic priority and on the morphological analysis done by MILSTEAD (1963), it was determined that species without vomerine teeth would be recognized as *P. biligonigerus* and species with vomerine teeth, as *P. fuscomaculatus.* In addition, the small species from southern Brazil would be recognized as *P. biligonigerus* and the large one, as *P. fuscomaculatus* (MILSTEAD 1963). According to BARRIO (1965), this last criterion does not justify the separation of P. *biligonigerus* and *P. fuscomaculatus.* This author argued that the specimens of *P. fuscomaculatus* collected by Milstead in Mato Grosso (Brazil) did not differ significantly from *P. biligonigerus* collected in Argentina.

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Considering the controversy involving the identification of these species, we analyzed both of them cytogenetically, including *P. fuscomaculatus* from Caceres in the midwestern region of Brazil (type locality) and *P. biligonigerus* from Santo Antonio da Patrulha, southern Brazil, next to Buenos Aires (type locality). We also analyzed a population from Cuiaba, also in the midwestern region, which had mostly *P. biligonigerus* morphological characteristics. This species is referred to as *P.* sp. in the present paper.

MATERIAL AND METHODS

Animals

Twenty specimens of *P. fuscomaculatus* from Caceres and twenty one of *P.* sp. from Cuiaba (both sites in the state of Mato Grosso (midwestern region), and thirteen *P. biligonigerus* from Santo Antonio da Patrulha, a municipality in the state of Rio Grande do Sul (southern Brazil), were studied. All the specimens were deposited in the "Professor Adao Jose Cardoso" Natural History Museum of the State University of Campinas, Campinas, Brazil.

Chromosome Preparation

Mitotic metaphases were obtained from a suspension of intestinal and testis cells from animals treated with colchicine for at least 4 h, as described by SCHMID *(1978a)* and SCHMID *et al.* (1979).

Chromosome Morphology

For morphological studies, the slides were stained with 10% Giemsa solution. Mean descriptive values of the karyotype were calculated from information obtained from a minimum of five well-spread mitotic metaphase plates from at least five individuals. The nomenclature of GREEN and SESSIONS (1991) was used to describe the chromosome morphology.

C-banding and Ag-NOR

Chromosomes were C-banded according to SUMNER (1972), with slight modifications. The Ag-NOR method was that described by HOWELL and BLACK (1980).

RESULTS

All three species had *2n=22* chromosomes consisting of seven large and four small chromosome pairs. Morphometric analysis of mitotic chromosomes showed that in the three species chromosome pairs 1, 2, 4, 6 and 8-11 were metacentric whereas pairs 5 and 7 were submetacentric. The only difference occurred in pair 3 which was metacentric in *P. biligonigerus* and *P.* sp. and submetacentric in *P. fuscomaculatus* (Figs. 1 and 4; Table 1). A large secondary constriction was located close to the telomere on the long arm of chromosome 9, in the three species (Fig. 1).

TABLE 1 — Analysis of the somatic complements *of P. biligonigerus, P.* sp. and *P. fuscomaculatus.*

Taxon		Chromosomes										
		1	2	3	4	5	6	7	8	9	10	11
Physalaemus biligonigerus (Santo Antônio da Patrulha - RS)	RL	0.139	0.128	0.122	0.106	0.103	0.083	0.078	0.061	0.058	0.054	0.050
	AR	1.19	1.45	1.55	1.22	2.46	1.30	2.86	1.46	1.38	1.21	1.17
	CP	M	M	M	M	SM	M	SM	M	M	M	M
Physalaemus fuscomaculatus (Cáceres - MT)	RL	0.125	0.123	0.113	0.101	0.091	0.089	0.076	0.067	0.056	0.048	0.040
	AR	1.32	1.21	2.29	1.16	2.35	1.24	2.0	1.65	1.38	1.31	1.41
	CP	M	M	SM	M	SM	M	SM	M	M	M	M
Physalaemus sp. (Cuiabá - MT)	RL	0.136	0.127	0.122	0.103	0.093	0.089	0.080	0.065	0.059	0.058	0.049
	AR	1.32	1.25	1.53	1.12	2.47	1.16	2.00	1.42	1.25	1.14	1.08
	CP	M	M	M	M	SM	M	SM	M	M	M	M

RL = relative length, AR = arm ratio, CP = centromere position, M = metacentric, SM = submetacentric.

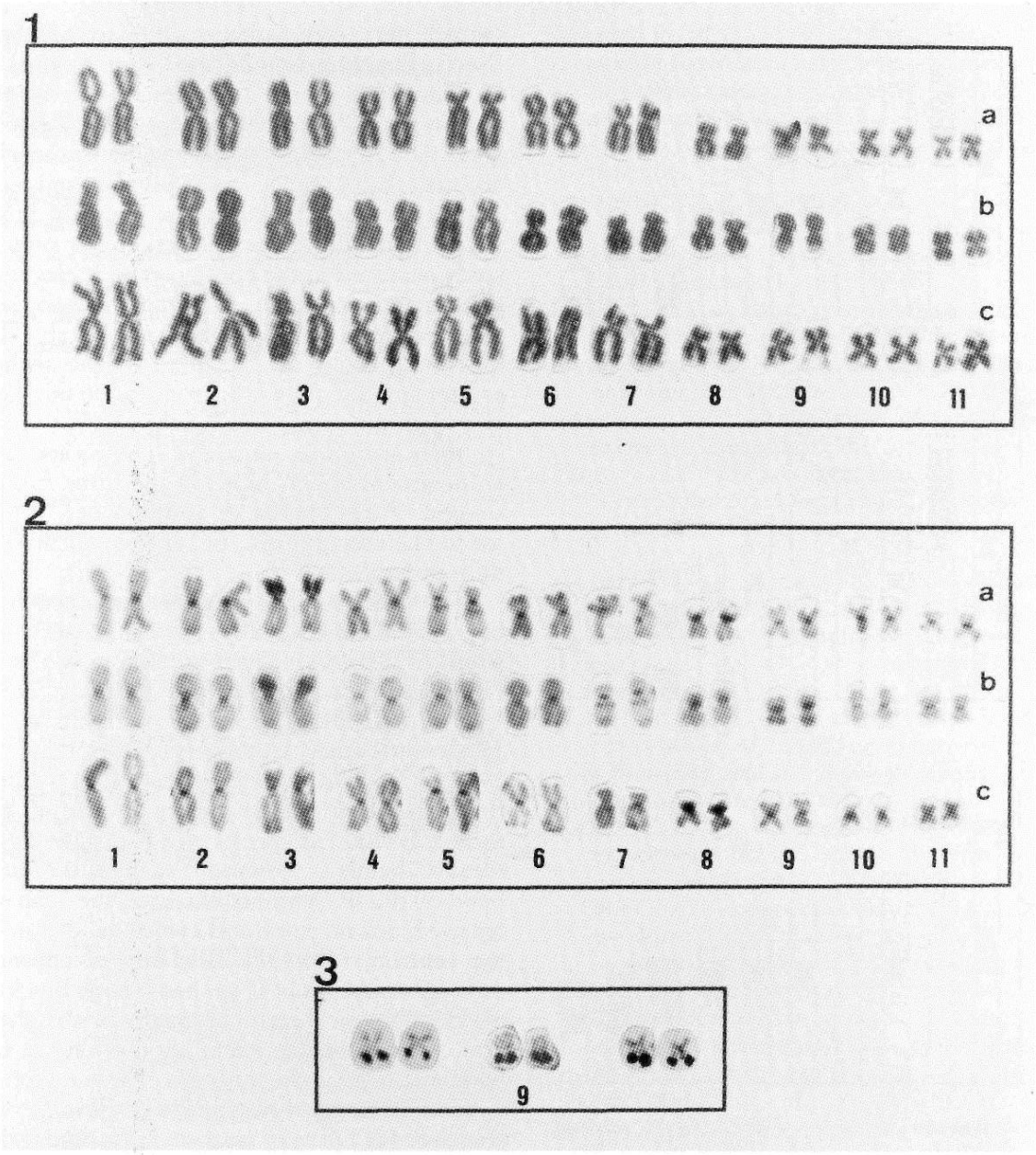

Fig. 1 — Karyotypes of *Physalaemus biligonigerus* (a), P. sp. (b) and *P. fuscomaculatus* (c). 2500x.
Fig. 2 — C-banded metaphases *of Physalaemus biligonigerus* (a), P. sp. (b) and *P. fuscomaculatus* (c). 2500x.
Fig. 3 — Silver-stained NOR *of Physalaemus biligonigerus* (a), P. sp. (b) and *P. fuscomaculatus* (c). 2500x.

The C-banded karyotypes of the three species showed heterochromatin bands at the centromeres of all chromosomes, a big block of interstitial heterochromatin close to the centromere on the short arms of pairs 3 and 8 and a small interstitial band on the long arm of pairs 5 and 7. In addition, only *P.* sp. had a large block of pericentromeric heterochromatin in the short arm of chromosome 9 and another one in the telomeric band of the long arm (Figs. 2 and 4).

The NOR was located on the long arm of chromosome pair 9 in all three species and coincided with a secondary constriction (Figs. 3 and 4).

DISCUSSION

All species of the genus *Physalaemus,* as well as many other species of Leptodactylidae have

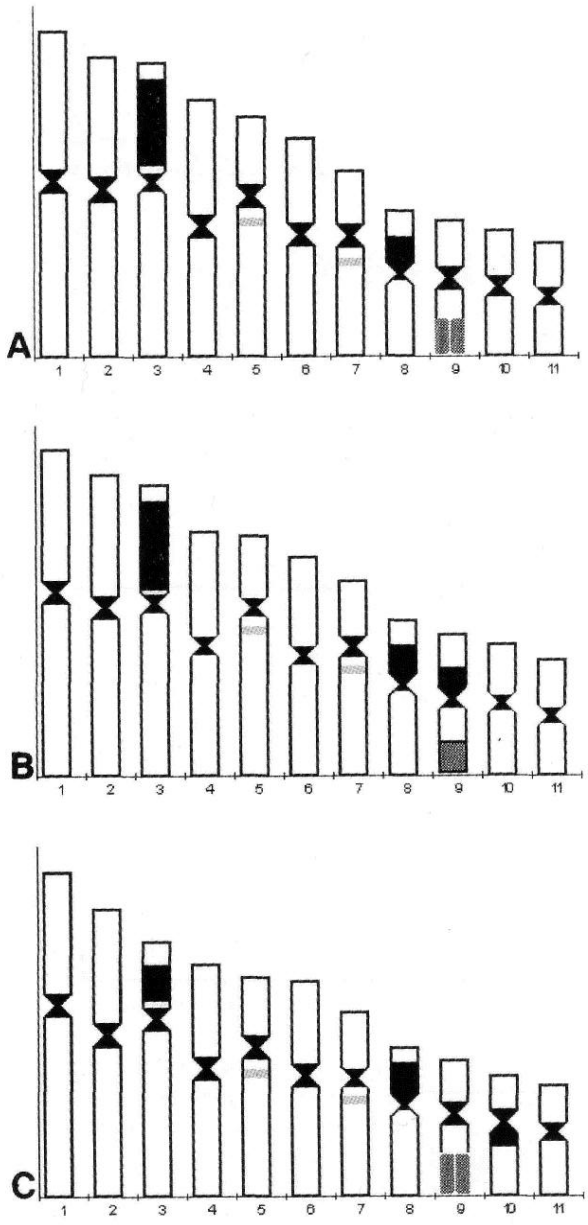

Fig. 4 — Idiograms of *Physalaemus* karyotypes. A: *Physataemus biligonigems.* B: P. sp. C: *P. fuscomaculatus.* Black areas denote dark C-bands, grey areas denote faint C-bands and shaded areas denote the positions of NOR.

2n=22 chromosomes (BRUM-ZoRRiLA and SAEZ 1968; BECAK *et al.* 1970; BOGART 1970; KURAMOTO 1990; LOURENCO *et al.* 1998, 1999). The karyotype of *P. fuscomaculatus* was first described by BECAK *et al.* (1970). Nevertheless, the same metaphase was used in another paper by DENARO (1972), to show the karyotype of *P. albifrons.* The kayotype reported in the above two papers was much more similar to *P. albifrons* (in analysis in our laboratory- Recco-Pimentel, pers. com.), than to *P. fuscomaculatus* (Recco-Pimentel, unpublished observations).

Thus, this is the first description of the karyotypes of *P. fuscomaculatus* and *P. biligonigerus.* The karyotypes of all *Physalaemus* species are remarkably similar in the number and morphology of their chromosomes. The only difference among the three species in the present study occurred in the chromosome 3, classified as submetacentric in *P. fuscomaculatus* and metacentric in *P biligonigerus* and P. sp. This difference could be explained by the slightly greater amount of heterochromatin on the short arm of chromosome 3 in *P. biligonigerus* and *P.* sp. than in P. *fuscomaculatus.* The presence of an heterochromatin block in the three species suggests a mechanism of addition or deletion for the heterochomatin, rather than euchromatin transformation.

The species of *Physalaemus* analyzed here had a small amount of heterochromatin compared to *Physalaemus petersi* (LOURENCO *et al.* 1999), *Leptodactylus ocelatus* (BIANCHI *et al.* 1973), *Mixophyes fasciolatus* (SCHMID *et al.* 1990) and other leptodactylids (Ruiz *et al.* 1981; SCHMID *et al.* 1985; KING 1991). Apart from the centromeric heterochromatin, the three *Physalaemus* species also had some interstitial C-bands in common, such as the large band on the short arm of chromosome 3, an extensive block on pair 8 and a small band close to the centromere on the long arm of chromosomes 5 and 7. Only P. sp. had a large block of pericentromeric heterochromatin in the short arm of chromosome 9 and another one in the telomeric band in the long arm. The variation in C-banding in the three species of *Physalaemus* analized was not very marked, with similar differences occurring among anuran populations, such as *Hyla prasina* (ANANIAS 1996; BALDIS-SERA *et al.,* 1993) and *Rana. ishikawae, R. ornativentris, R. japonica* and *R. chensinensis* (SETO *et al.* 1984, MIURA *et al.* 1995).

All three species had only one active ribosomal cistron in the long arm of chromosome 9, coincident with the secondary constriction. In the karyotypes of species that belong to the same or closely related species groups, the Ag-stained NORs always lie in comparable chromosomal regions (SCHMID *I978a,b;* 1982; SCHMID *et al.,* 1990). However, a distinct location for NORs was observed in two geographically distinct populations of *Hyla ebraccata.*

Such chromosomal rearrangements may be indicative of incipient speciation (KAISER *et al.* 1996).

The cytogenetic data obtained for the three species of *Physalaemus* showed more similarities (homologies in chromosomal morphology, C-banding and NOR localization) than differences. The small chromosomal differences among these three species may reflect the dynamic process of response of each species to its environment and may indicate incipient speciation, as postulated for *Hyla ebraccata* populations. The species studied here are closely related chromosomally. However, additional data at the molecular level are needed for a more detailed assessment of speciation among *Physalaemus* species.

Acknowledgements — The authors thank Giovani Vinciprova and Christine Strussmann for providing the *P. biligonigerus* and *P.* sp. specimens, respectively, Klelia A. Carvalho for technical assistance and Heidi Bolder for reviewing the English. This work was supported by FAPESP (grant n° 1997/12675-8). M.J.E.V.A. was the recipient of a Ph.D. scholarship from CAPES-PICDT. This paper is dedicated to memory of Adao Jose Cardoso who died before this work was completed.

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Received 16 August 2000; accepted 9 November 2000

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