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The Karyotype of the Stream Dwelling Frog *Megaelosia massarti* (Anura, Leptodactylidae, Hylodinae)

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Leptodactylids are a large and diversified family of Neotropical anurans with about sixty genera and 710 species (Frost 1985, Duellman and Trueb 1986). The subfamily Hylodinae is mainly related to rushing, clean, cold water rivulents of the Atlantic Forest in Southeast Brazil; *Megaelosia* and *Hylodes* are endemic genera of this vegetal formation. The subfamily is composed of three genera, nominally: *Hylodes* (14 spp.), *Crossodactylus* (5 spp.) (Frost 1985) and *Megaelosia* (4 spp.) (Giaretta *et al.* 1993). The hylodine frogs form a monophyletic group in which *Hylodes* and *Crossodactylus* are more related to one another than with *Megaelosia* (Heyer 1975). *Megaelosia* species are very different by their large size and aquatic frog-eating habits. They are also particularly rare in collections, probably due to their restricted distribution and cryptic behavior (Giaretta *et al.* 1993).

Karyological information is currently available for five species of the genus *Hylodes* and three of the genus *Crossodactylus* (Beçak 1968, Denaro 1972, De Lucca and Jim 1974, Bogart 1970, 1991). The most widespread diploid number among the subfamily, as well as the family, is 2n=26 (Kuramoto 1990, Bogart 1991). Here, we describe the karyotype of *Megaelosia massarti* (De Witte 1930), a first karyological report on the genus.

Materials and methods

The karyological features of *Megaelosia massarti* were based in the analysis of 3 adult males caught in Paranapiacaba, São Paulo, Brazil. Voucher specimens are the same cited in Giretta *et al.* (1993). The individuals were injected intraperitoneally with 1% colchicine solution (0.01 ml/g of body weight) then killed 3–4 hr later. The mitotic chromosomes were obtained from cell suspensions of bone marrow (Schmid 1978), gut epithelium (King and Rofer 1976), and from testis squash preparations (Ohno *et al.* 1964). Conventional staining was performed with Giemsa 10% in 0.1 M phosphate buffer pH 6.8, for 2 min. Ten complete and wellspread metaphases were chosen and photographed for the chromosomes measurements. The percentage relative length, arms ratio (length of long arm/length of short arm) and centromeric index (length of short arm/total chromosome length) were determined according to Levan *et al.* (1964). The nomenclature for centromeric position on mitotic chromosomes types was based on Green and Sessions (1991). Ag-NOR staining was obtained by the technique of Howell and Black (1980).

Results

A diploid number of 2n=28 chromosomes was found in *M. massarti* (Fig. 1). The karyotypes are formed by 07 metacentric, 06 submetacentric and 01 subtelocentric pairs (Table 1). There is no heteromorphic chromosome pair. A conspicuous secondary constriction is

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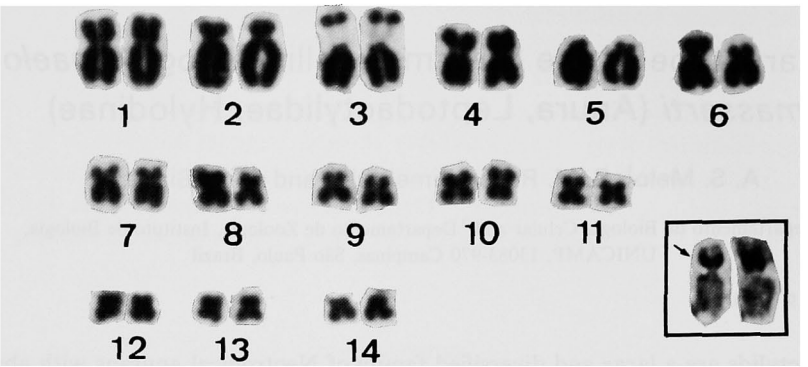

Fig. 1. Karyotype of a *Megaelosia massarti* male. In the inset, the respective NOR-bearing 3th chromosome pair stained by the Ag-NOR technique. (2750× and 2250×, respectively).

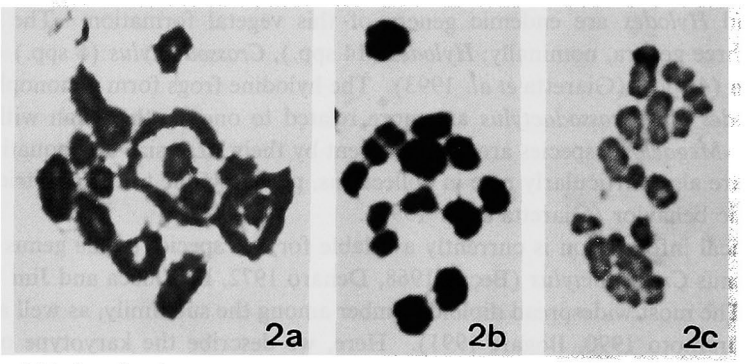

Fig. 2. Meiotic cells of males of *Megaelosia massarti*. (a) diplotene and (b) metaphase I with 14 bivalents; (c) metaphase II with 14 chromosomes. (a) and (c): AgNOR technique (2210×); (b): Giemsa conventional staining (2300×).

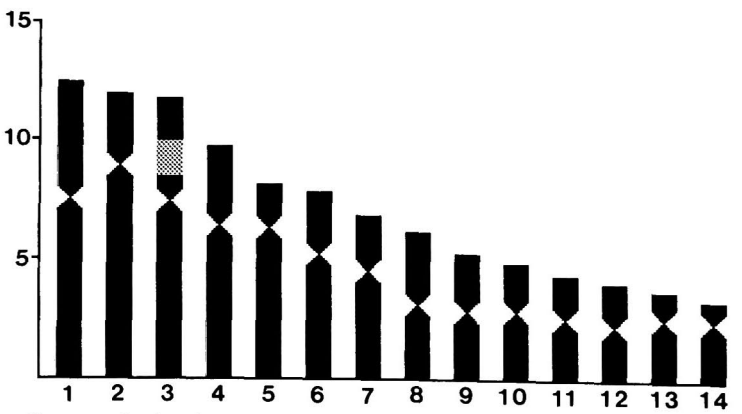

Fig. 3. Idiogram of *Megaelosia massarti* species derived from data in Table 1. The scale represents the percentage of the total genome length. Position of secondary constriction is indicated by the dotted area in the third chromosome.

present in the short arms of chromosome pair 03 (Figs. 1, 3). The karyotype show no major differences among the relative lengths of chromosomes and therefore they also do not form distinct size groups of chromosomes (Table 1, Fig. 3). NORs appears at the secondary constriction of the short arms of the chromosome pair 3 (Fig. 1). Meiotic analysis of the males

Table 1. Percentual relative length (RL), arm ratio (AR), centromere index (CI) and centromere position (CP) of *M. massarti* chromosomes (m = metacentric; sm = submetacentric; st = subtelocentric)

Chromosome pair no	RL	AR	CI	CP
1	12.42	1.55	0.39	m
2	11.96	2.97	0.25	sm
3	11.69	1.74	0.36	sm
4	9.74	1.60	0.38	m
5	8.10	3.50	0.22	st
6	7.77	2.00	0.33	sm
7	6.82	1.96	0.34	sm
8	6.09	1.02	0.49	m
9	5.16	1.15	0.46	m
10	4.84	1.34	0.43	m
11	4.32	1.32	0.43	m
12	4.05	1.19	0.46	m
13	3.72	2.02	0.33	sm
14	3.32	2.57	0.28	sm

revealed 14 bivalents in profase I and metaphase I cells (Fig. 2a, b) and 14 chromosomes in metaphase II cells (Fig. 2c). The majority of the bivalents were ring shaped, with two terminal chiasmata. One bivalent showed an open configuration with only one terminal chiasma (Fig. 2a).

Discussion

M. massarti differs from others hylodine frogs in some karyotypic aspects. Until now this is the only species in the subfamily with 28 chromosomes, and a secondary constriction on pair 3. The majority of *Hylodes* and *Crossodactylus* species agree in karyotype features, with 26 chromosomes (with exception of *H. nasus*, 2n = 24) and a very large first pair of chromosomes (with exception of *C. dispar*) containing a secondary constriction and frequently one more constriction in another chromosome pair (Beçak 1968, Bogart 1970, 1991, Denaro 1972, De Lucca and Jim 1974). Like all hylodine there are no distinctive size group of chromosome in *M. massarti*. Comparing all available hylodine karyotypes, excluding the differences pointed out above, *M. massarti* karyotype is similar to the others hylodinae. There is no Ag-NOR analysis made in any other hylodinae species. *M. massarti* showed NOR only in one chromosome pair and in the same position as the secondary constriction. Ag-NOR have been analyzed in a large number of anuran species and it was shown that most of them present only one pair of NORs (Schmid *et al.* 1990) and just as we found, it was located on the secondary constriction.

Morphological similarities and phylogenetic relationship between the hylodine and dendrobatid frogs was first advocated by Lynch (1971). Bogart (1991) reinforces this arguments with karyological data. However, *M. massarti* karyotypes do not have some of the characteristics, found in both Dendrobatidae and *Hylodes* which allowed Bogart to conclude that they have similar karyotypes: presence of a relatively large No 1 chromosome, presence of telocentric pairs, as well as, the number of chromosomes. It seems that *Megaelosia* is karyologically more distant from Dendrobatidae than *Hylodes* species.

Heyer (1975) in his analysis of the intergeneric relationship of members of the Leptodactylidae Family considered all hylodine frogs as having 26 chromosomes, with no citation of authors or species. If the diploid number of 28 chromosomes of *M. massarti* is a representative feature of the genus, this number may be regarded as an autapomorphie of the genus.

Summary

The karyotype of the hylodine frog *Maegaelosia massarti* is reported. The species has 2n= 28 chromosomes, of which 07 pairs are metacentric, 06 submetacentric and 01 subtelocentric. NOR is located at the secondary constriction on the short arms of the submetacentric third pair. The chromosome number of *M. massarti* differs from the modal number (2n=26) of hylodine.

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