

# C- and R-banding patterns and nucleolus organizer regions in the karyotype of *Hemidactylus mabouia* (Sauria, Gekkonidae)

Katia Cristina Machado Pellegrino<sup>1</sup>, Maria Cecília Araripe Sucupira<sup>2</sup>, Sanae Kasahara<sup>2</sup>, Miguel Trefaut Rodrigues<sup>3</sup> and Yatiyo Yonenaga-Yassuda<sup>1</sup>

## ABSTRACT

Chromosomes of *Hemidactylus mabouia* ( $2n=42$ ) from the states of Paraíba and São Paulo, Brazil, were studied by conventional and differential staining (C- and R-banding and Ag-NORs). Most of the chromosomes are subtelocentrics or acrocentrics, with gradation in size. Banding patterns and Ag-NORs location in *H. mabouia* are described here for the first time.

## INTRODUCTION

The Gekkonidae is the most diverse and ancient group of reptiles and has a worldwide distribution (King, 1990). It constitutes the largest family of lizards, comprising 100 genera and 943 species (Kluge, 1991). In spite of the great number of species in the family, only a few karyotypes have been reported, except for the Australian species, that are fairly well studied and exhibit a high level of chromosomal variation.

The diploid number among gekkonid lizards ranges from  $2n=16$  to  $2n=46$  (Gorman, 1973; Schmid *et al.*, 1994). The typical karyotype consists of a gradual series of acrocentric chromosomes, and there is no distinction between macro and microchromosomes. When banded chromosomes are present, the centromere is often subterminal (Gorman, 1973).

According to the most recent checklist, the genus *Hemidactylus* includes 74 species and several subspecies (Kluge, 1991). Taxonomically, it is the most diverse group of gekkonid lizards (Darevsky *et al.*, 1984). Karyological analyses of the genus have been performed on some diploid and triploid species. Among the diploid species studied are included: *H. frenatus*, *H. brookii* and *H. flaviridis* (all with  $2n=40$ ), *H. turcicus* ( $2n=44$ ) and *H. bowrigii* with  $2n=46$  (Singh *et al.*, 1970; Gorman, 1973; King, 1978; De Smet, 1981; Kupriyanova and Darevsky, 1989). The triploid species, which are all parthenogenetic, include: *H. vietnamensis* ( $2n=3x=60$ ), *H. garnotii* ( $2n=3x=70$ ) and *H. stejnegeri* with  $2n=3x=56$  (Kluge and Eckardt, 1969; Darevsky *et al.*, 1984; Ota and Hikida, 1989a, b; Ota *et al.*, 1989). In all these papers only conventionally stained karyotypes were described.

It has been postulated that most of the relatively few endemic New World gekkonine genera evolved from Old World ancestors. According to Kluge (1969), *Hemidactylus mabouia* is derived from west African coast stock, that reached the New World by natural trans-Atlantic dispersal. This hypothesis was criticized by Vanzolini (1978), who argued that the

<sup>1</sup> Departamento de Biologia, Instituto de Biociências, USP, Caixa Postal 11461, 05422-970 São Paulo, SP, Brasil. Send correspondence to Y.Y.-Y.

<sup>2</sup> Departamento de Biologia, Instituto de Biociências, UNESP, Caixa Postal 199, 13506-900 Rio Claro, SP, Brasil.

<sup>3</sup> Departamento de Zoologia, Instituto de Biociências, USP, São Paulo, SP, Brasil.

species probably arrived in the New World on several different occasions, both by natural and human means of transportation. In the eastern coast of South America, *H. mabouia* is known to occur from Uruguay to Guyana; along most of the length of the Amazon river in Brazil; from its headwaters in Ecuador and Peru; and from the Antilles islands (Kluge, 1969). It was also reported that the species was not restricted to a narrow margin along the eastern coast, being found far inland in the upper Amazon, the upper Madeira and the edges of the Pantanal in the State of Mato Grosso (Vanzolini, 1978), indicating that this expansion has a clear association with human settlements.

Until now, the data on the karyotype of *H. mabouia* are restricted to Giemsa stained preparations from populations of São Paulo, Brazil, and Trinidad (Beçak *et al.*, 1972; MacBee *et al.*, 1987).

## MATERIAL AND METHODS

Cytogenetic studies were carried out on ten specimens of *H. mabouia* (five males and five females) collected in: Fazenda Bravo, Cabaceiras, State of Paraíba (7°28'S, 36°15'W); and Itatiba (23°00'S, 46°51'W), Rio Claro (22°24'S, 47°30'W) and São Paulo (23°32'S, 46°21'W) all in the State of São Paulo. All the specimens were deposited in the Museum of Zoology, University of São Paulo (MZUSP).

Chromosomal preparations were obtained from bone marrow, liver and fibroblast cultures (according to Yonenaga-Yassuda *et al.*, 1988). Meiotic studies were performed on male specimens, following the technique described by Eicher (1966). Chromosomes were analyzed after conventional and differential staining, which included C-banding (Sumner, 1972), and R-banding after *in vitro* incorporation of 5-bromodeoxyuridine (Dutrillaux and Couturier, 1981) and Ag-NORs (Howell and Black, 1980).

## RESULTS

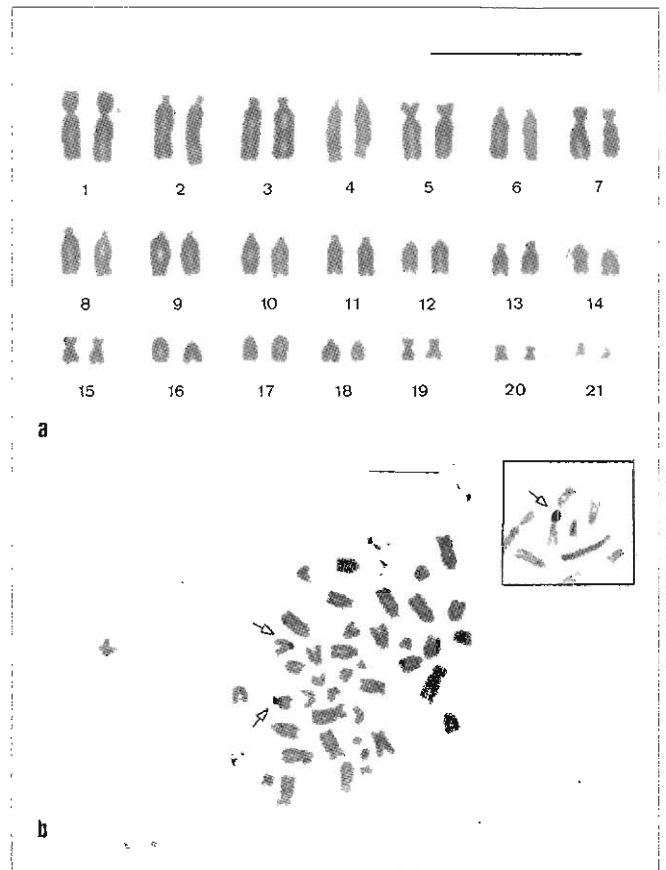
*Hemidactylus mabouia* presented a diploid number of  $2n=42$  and a karyotype consisting of: eight submetacentric chromosomes (pairs 1, 5, 7 and 15), 28 subtelocentrics or acrocentrics (pairs 2-4, 6, 8-14, 16-18) and six small metacentrics (pairs 19-21). Macro and microchromosomes were indistinguishable and no sex chromosome heteromorphism could be observed (Figure 1a). The silver stained metaphases showed that

the Ag-NORs occur in the short arms of one medium sized subtelocentric or acrocentric pair which is not morphologically distinctive. The Ag-NOR-bearing pair was frequently observed in association (Figure 1b). The C-banding pattern revealed that only lightly stained heterochromatic blocks are present in the telomeric region of most chromosomes. Some smaller chromosomes were almost devoid of positive C-bands (Figure 2a). The R-banding pattern allowed the identification of most chromosomal pairs (Figure 2b). Meiotic studies revealed 21 bivalents in diplotene cells (Figure 3a) and 21 chromosomes in metaphase II (Figure 3b). No heteromorphic bivalent was detected.

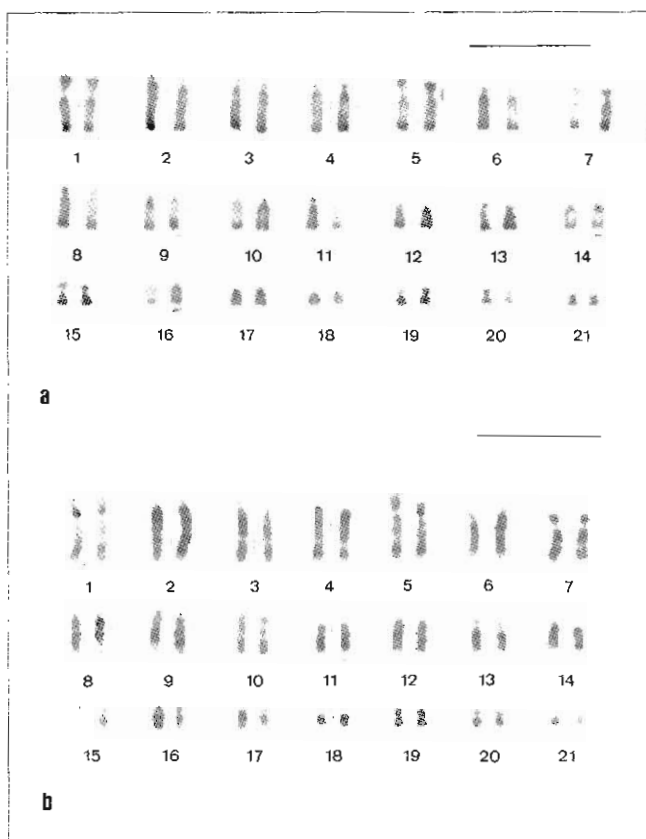
## DISCUSSION

The diploid number of  $2n=42$  found in *H. mabouia* is situated within the range of the family.

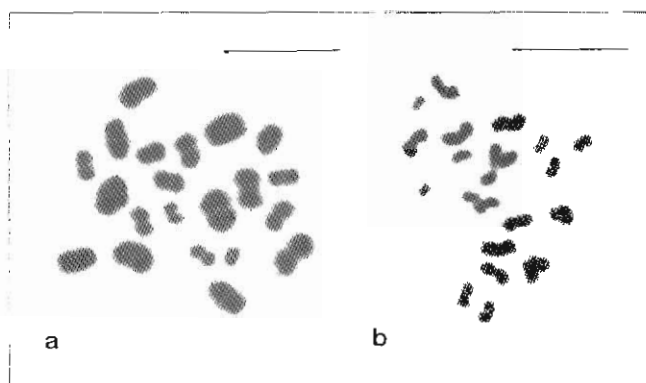
Karyotypes with no distinction between macro and microchromosomes, as the one we found in *H. mabouia*, are also typical of the Gekkonidae.



**Figure 1** - (a) Karyotype of *Hemidactylus mabouia* female ( $2n=42$ ) after conventional staining. (b) Metaphase with the Ag-NORs stained in a medium size subtelocentric/acrocentric pair (arrows). Inset: the Ag-NOR-bearing pair in association (arrow). Bar = 10 µm.



**Figure 2** - Banding patterns in *Hemidactylus mabouia* female ( $2n=42$ ). (a) C-banding. (b) R-banding after 5-BrdU incorporation *in vitro*. Bar = 10  $\mu\text{m}$ .



**Figure 3** - Meiotic cells of *Hemidactylus mabouia* male ( $2n=42$ ). (a) Diplotene with twenty-one bivalents. (b) Metaphase II with twenty-one chromosomes. Bar = 10  $\mu\text{m}$ .

An essentially identical karyotype was previously reported by Beçak *et al.* (1972) and MacBee *et al.* (1987) for the same species. There are some slight differences between the karyotype described by the first authors and the one presented here: the pairs 4 and 5, 6 and 7 in our study correspond to pairs 5, 4, 7 and 6, respectively, in their karyotypes, and pair 15 differs in morphology. We consider the latter pair a submetacentric while it was described as a metacentric by them. These discrepancies are probably due to

differences in the quality of the cytological preparations and to differential condensation of the chromosomes.

Heteromorphic sex chromosomes were not observed in *H. mabouia*. This was corroborated by the absence of a distinctive heteromorphic bivalent in male cells. This result is in agreement with that previously found by Beçak *et al.* (1972). The presence of heteromorphic sex chromosomes in Gekkonidae is known only from 10 species belonging to five genera (Ota *et al.*, 1992) and as far as we know, there is no reference to sexual chromosomes in *Hemidactylus*. Most gekkonids have a chromosomal mechanism of sex determination of the ZZ:ZW type, in which the heterogametic sex is the female, such as in *Heteronotia binoei* (Moritz, 1984a; 1990), *Gehyra australis* (King, 1983), *Gehyra purpuracens* (Moritz, 1984b) and *Cyrtodactylus pubisulcus* (Ota *et al.*, 1992). However, in a few species, the mechanism is of the XX:XY type, such as in *Gekko gekko* (Solleder and Schmid, 1984), *Gekko japonicus* (Yoshida and Masahiro, 1974 *apud* Moritz, 1990) and *Gonatodes ceciliae* (MacBee *et al.*, 1987).

Chromosomal studies based on differential staining techniques were mainly performed in Australian genera, such as *Gehyra* (King, 1984; Moritz, 1984b; 1986), *Phyllodactylus* (King and Rofe, 1976) and *Heteronotia* (Moritz, 1984a; 1990). In these cases, banding techniques helped to clarify aspects of the chromosomal variability of these lizards.

C- and R-banding patterns, as well as Ag-NORs location are presented here for the first time for the genus *Hemidactylus*.

There are only a few reports of R-banding patterns of gekkonid lizards. For instance, in *Lepidodactylus lugubris* (Volobouev and Pasteur, 1988; Volobouev *et al.*, 1993) and *Ailuronyx seychellensis* (Volobouev and Ineich, 1994), R-banding patterns were also established with preparations obtained from fibroblast cultures.

Although most karyological studies on lizards have been performed with conventionally stained chromosomes, the application of banding patterns represents an essential tool to precisely characterize the complements, as well as to elucidate the evolutionary karyotypic trends of the group.

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## RESUMO

*Hemidactylus mabouia* (2n=42) proveniente dos estados da Paraíba e São Paulo, Brasil foi estudado citogeneticamente através de coloração convencional e diferencial (bandas C-, R- e Ag-RONs). A maioria dos cromossomos é de subtlococêntricos ou acrocêntricos com variação gradativa de tamanho. Os padrões de bandamento e a localização das Ag-RONs (em um par subtlococêntrico ou acrocêntrico de tamanho médio) são descritos aqui pela primeira vez.

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