CYTOGENETIC STUDIES IN SIX SPECIES OF TROPIDURIDAE (SAURIA)

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ABSTRACT

Cytogenetic studies were performed on six tropidurid lizards: *Tropidurus mucujensis*, *T. hispidus*, *T. montanus*, *T. semitaeniatus*, *T. spinulosus* and *Uranoscodon superciliosus*. Conventional and differential (C- and R-bands and Ag-NORs) staining techniques were employed for the characterization of the karyotypes.

The analyses were carried out in order to establish the morphological characterization of macro and microchromosomes, the R- and C-banding patterns as well as the silver staining of nucleolus organizer regions (Ag-NORs). R-banding was obtained after *in vitro* incorporation of 5-bromodeoxyuridine (BrdU). For some species, the banding patterns are described for the first time.

All species presented a diploid number of 2n=36, with a basic karyotype constituted by 12 metacentric or submetacentric macrochromosomes and 24 microchromosomes (12M+24m). Although these species present a conservative karyotype, most of them are distinguished by the morphology of the macro and microchromosomes, by location of Ag-NORs, and by amount and distribution of constitutive heterochromatin. Sex determination of the XX:XY type was found in *T. hispidus*, *T. montanus* and *U. superciliosus*. The presence of species specific karyotypes in *T. spinulosus* and *U. superciliosus* is suggested.

INTRODUCTION

The former and traditional Iguanidae has recently undergone a major revision (Frost and Etheridge, 1989) and was split into eight monophyletic families. One of these, the neotropical Tropiduridae, is now composed also of three monophyletic subfamilies: Liolaminae, mainly Andean in distribution; Leiocephalinae, restricted to Central America; and Tropidurinae, occurring both in cis and transandean South America and the Galapagos islands. Furthermore, two tribes are presently recognized for Tropiduridae: Stenocercini for the genus *Stenocercus* and the Tropidurini. The systematics of Tropidurini was improved after a cladistic analysis by Frost (1992) that placed the forest genera *Plica*, *Strobilurus* and *Uracentron* and the heliophilic *Tapinurus* under the synonymy of *Tropidurus*. He also moved several species formally considered *Tropidurus* occurring west of Andes and in the Galapagos islands to *Microlophus* and *Plesiomicrolophus*. As it now stands the Tropidurini include *Uranoscodon*, *Plesiomicrolophus*, *Microlophus* and *Tropidurus*.

Although some Liolaminae have been studied karyotypically and show important variations in chromosome numbers, the karyotypes of Tropidurinae (especially the Tropidurini), with few exceptions, have remained as a classic example of conservative chromosome number in lizards. Most Tropidurini species present a diploid number of 36 chromosomes and a basic karyotype constituted by 12 metacentrics or submetacentrics macrochromosomes and 24 microchromosomes. This karyotype has been considered primitive (Gorman, 1973; Paull et al., 1976; Bickham, 1984). Nevertheless, the majority of the data have been based on nondifferentially stained chromosomal preparations.

Some Brazilian species of the genus *Tropidurus* have been investigated cytogenetically with banding techniques (Kasahara et al., 1983; 1987a,b; 1989; Yonenaga-Yassuda et al., 1988; Rodrigues et al., 1989). Characterization of 12 species of *Tropidurus* belonging to the *torquatus* group (Kasahara et al., 1989;
Kasahara et al., unpublished results) showed that some chromosome differences involving mechanisms of sex determination, variability of amount and distribution of constitutive heterochromatin and both location and number of Ag-NORs occur among these species.

MATERIAL AND METHODS

The six species of Tropiduridae, localities, sample size and sex of the specimens are listed in Table I. All the specimens were deposited in the Museum of Zoology, University of São Paulo (MZUSP).

Mitotic chromosomes were obtained from either bone marrow, intestine, liver and spleen preparations or fibroblast cultures from hind-leg and dorsum muscle cultivated in Dulbecco’s modified Eagle medium (DMEM) with 20% fetal bovine serum (Yonenaga-Yassuda et al., 1988).

The morphological analyses of the chromosomes were performed after Giemsa. The differential staining included C-banding (Sumner, 1972), Ag-NOR (Howell and Black, 1980) and R-banding (Dutrillaux and Couturier, 1981), in which the chromosomes were differentially stained with 33258 Hoechst-Giemsa (FPG staining).

RESULTS

All species presented a diploid number of 2n=36 (12M+12m) and a basic karyotype with 12 metacentric or submetacentric macrochromosomes and 24 microchromosomes (Figures 1a, 1b, 2a, 3a, 3b). Except for U. superciliosus (Figure 4a, 4b), the karyotypes of all species included the metacentric pairs 1, 3, and 4 and the submetacentric pairs 2, 5 and 6. The majority of species exhibited 12 pairs of acrocentric microchromosomes. Sex determination of the XX:XY type in three species (T.
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T. montanus, T. hispidus, and U. superciliosus) could be identified. The Y appeared as a dot-like microchromosome present only in males, while the X could not be precisely identified and is probably a middle size acrocentric microchromosome (Figures 1b, 2a, 4a, 4b).

*T. mucujensis* showed a small secondary constriction in pair 11 which was Ag-NOR positive (Figure 1a). The C-banding technique revealed a small amount of heterochromatin in the centromeric and telomeric regions of macrochromosomes. Some microchromosomes also appeared slightly stained (Figure 8d). The R-banding pattern allowed the precise identification of the macrochromosomes. The distal region of the long arm of submetacentric pair 2 was a late-replication DNA region. The precise identification of microchromosomes by R-banding was not possible (Figure 9).

Three karyotypes (A, B and C) were established in *T. hispidus* after a study of seven populations in Brazil (Kasahara et al., 1983; 1987). Karyotype A was found in specimens from São Luís (MA) and Karyotype C in a specimen from Jacobina (BA). The basic difference among these karyotypes is determined by the location of the Ag-NORs which was present either in a secondary constriction of pair 2 or in one pair of microchromosomes. The male specimen from São Luís (MA) presented a prominent secondary constriction at the distal end of the long arm of macrochromosome pair 2, which is the location of the Ag-NORs (Figure 2b, 2c). In the female from Jacobina (BA), the secondary constriction was absent in pair 2 and

Figure 1 - Karyotypes after Giemsa staining. (a) Female of *Tropidurus mucujensis* (2n=36); (b) Female of *Tropidurus montanus* with 2n=36. Inset: XY sex chromosomes of male. Bar = 10 µm.

Figure 2 - Karyotype of a male of *Tropidurus hispidus* (2n=36) with XY microchromosomes. (a) After Giemsa staining. Inset: sex chromosomes of female; (b) Pair 2 of the specimens from S. Luis (MA) with the distal secondary constriction (Karyotype A); (c) Ag-NORs in the secondary constriction of pair 2; (d) Pair 2 of the specimen from Jacobina (BA) without secondary constriction (Karyotype C). Bar = 10 µm.
the Ag-NORs were located in one pair of microchromosomes (Figures 2d, 5b). The C-band patterns were similar in Karyotypes A and C (Figures 6a, 6b). Pair 2 exhibited positive C-bands in both the centromeric and distal regions of the long arm while pair 5 presented only centromeric C-bands. In Karyotype A, pair 6 also showed centromeric constitutive heterochromatin, which was absent in the same pair of Karyotype C. Pair 2 of Karyotype C exhibited heteromorphism of centromeric C-bands. In both karyotypes, some larger microchromosomes showed light centromeric C-bands.

_**T. montanus**_ presented a small secondary constriction in pair 9 which was Ag-NOR positive (Figures 1b, 5a). In some metaphases, Ag-NORs of different sizes in the homologs were observed. Two different C-banding patterns were detected among specimens from Rio de Contas and Pico das Almas. In a specimen from Rio de Contas, we identified light C-bands corresponding to centromeric regions of the macrochromosomes and some microchromosomes. Two heterochromatic blocks were identified. One situated in the distal part of the long arm of pair 2 and the other in the secondary constriction of pair 9 (Figure 7a). In the specimen from Pico das Almas, the C-banding pattern revealed a small amount of constitutive heterochromatin in the centromeric regions of both macro and microchromosomes. The conspicuous block in the distal end of the pair 2 was absent (Figure 7b).

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Figure 3 - Karyotypes obtained by Giemsa staining. (a) Male of _Tropidurus spinulosus_ (2n=36); (b) Male of _Tropidurus semitaeniatus_ (2n=36). Bar = 10 μm.

Figure 4 - Karyotype after Giemsa staining of _Uranoscodon superciliosus_. (a) Female karyotype with XX microchromosomes; (b) Set of male microchromosomes showing the XY sex chromosomes. Observe the presence of many metacentric microchromosomes in this karyotype. Bar = 10 μm

Figure 5 - Ag-NORs in five species of Tropiduridae. (a) Metaphase of _Tropidurus montanus_ showing Ag-NORs in a pair of microchromosomes; partial metaphases of (b) _Tropidurus hispidus_; (c) _Uranoscodon superciliosus_ and (d) _Tropidurus semitaeniatus_ with Ag-NORs in microchromosomes pairs. In (e) Ag-NORs in pair 6 of macrochromosomes of _Tropidurus spinulosus_. The Ag-NORs are indicated by arrows. Bar = 10 μm.
The R-banding pattern allowed the identification of the macrochromosome pairs in the karyotypes of the specimens from Rio de Contas and Pico das Almas. Although the C-band patterns were different, the specimens from both localities shared a common R-banding pattern (Figure 10). Meiotic analysis of a male specimen from Pico das Almas, revealed 18 bivalents at diplotene and 18 chromosomes in metaphase II cells (Figure 11a, 11b). The presence of a small heteromorphic bivalent in diplotene was interpreted as the X and Y chromosomes. In a metaphase II there were six macrochromosomes and 12 microchromosomes, including the dot-like Y chromosome (Figure 11b).

Among the microchromosomes of *T. semitaeniatus*, there were three pairs of metacentrics (pairs 8, 13 and 15) and nine pairs of acrocentrics (pairs 7, 9, 10, 11, 12, 16, 17 and 18). The Ag-NORs were restricted to one pair of metacentric microchromosomes (Figures 3b, 5d). The light C-bands occurred in the centromeric regions of macrochromosomes and the majority of the microchromosomes. A proximal C-band was observed in one of the chromosome arms of pair 4 (Figure 8a). Some minor C-bands in the telomeric regions of microchromosomes were also observed.

In *T. spinulosus*, the Ag-NORs were observed at the telomeric region of the long arm of pair 6 (Figure 5e). C-banded metaphases exhibited a small amount of constitutive heterochromatin in the centromeric regions of macrochromosomes. On the other hand, the majority of the microchromosomes presented conspicuous centromeric blocks (Figure 8c).

All specimens of *U. superciliosus* presented a karyotype constituted of the metacentric pairs 1, 2, 3, 4, 5 and the submetacentric pair 6. Among the microchromosomes, eight pairs of metacentrics (7, 8, 9, 11, 12, 13, 14, 15) and three pairs of acrocentrics (10, 16, 17) were identified. Pair 12 showed a large secondary constriction which was Ag-NOR positive (Figure 4a, 5c). After C-banding procedures only slightly stained centromeric C-bands could be observed in some macro and microchromosomes (Figure 8b). Meiotic studies of a male specimen revealed 18 bivalents in diplotene cells (Figure 11c). One of the
Figure 8 - C-banded metaphases. (a) Male of *Tropidurus semitaeniatus*. The arrows indicate the proximal C-bands in pair 4; (b) Male of *Uranoscodon superciliosus*; (c) Male of *Tropidurus spinulosus*; (d) Female of *Tropidurus mucujensis*. Bar = 10 μm.

Figure 9 - R-banding pattern after BrdU incorporation in female of *Tropidurus mucujensis* (2n=36). Note the late-replicating region in the distal part of the long arm of pair 2. Bar = 10 μm.

Figure 10 - R-banding pattern after BrdU incorporation in the set of male macrochromosomes of *Tropidurus montanus* (2n=36) from Rio de Contas (BA). Pair 2 showed a late-replicating region in the distal part of the long arm. Inset: pair 2 of the specimen from Pico das Almas (BA). Bar = 10 μm.

Figure 11 - Meiotic cells in two tropidurid species. (a) and (c) Diplotenes with 18 bivalents including the XY sex heteromorphic bivalent (arrows); (b) and (d) Metaphases II with 6 macrochromosomes and 12 microchromosomes, including the dot-like Y (arrows) in *Tropidurus montanus* and *Uranoscodon superciliosus*, respectively. Bar = 10 μm.
small bivalents was constituted by two microchromosomes of different sizes which were interpreted as the X and Y chromosomes. Metaphase II showed six macrochromosomes and 12 microchromosomes, including the dot-like Y chromosome (Figure 11d).

**DISCUSSION**

Although the same basic karyotype of 2n=36 (12M+24m) was shared by the six species, the comparative morphological analyses of the macro and microchromosomes, location of Ag-NORs, amount and distribution of constitutive heterochromatin, led us to characterize most of them. The analyses of macrochromosomes stained by Giemsa showed that the metacentric or submetacentrics pairs 1, 3, 4 and 6 are conservative among the six species. On the other hand, pairs 2 and 5 were submetacentric in *T. mucujensis*, *T. hispidus*, *T. montanus*, *T. semitaeniatus* and *T. spinulosus* while in *U. superciliosus* these pairs were clearly metacentric.

In our sample of *T. hispidus*, we found Karyotype A in specimens from S. Luis (MA) and Karyotype C in specimen from Jacobina (BA). These kind of karyotypic variants were considered as intraspecific geographical chromosome variations (Kasahara et al., 1987a). Although no correlation had been established between the three karyotypes and the morphological traits of the animals, the authors suggested an association with altitude. Karyotype A in specimens from *S. strobilurus*, *T. hispidus*, *T. montanus*, *T. semitaeniatus* and *T. spinulosus* while in *U. superciliosus* these pairs were clearly metacentric.

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In the majority of the *Tropidurus* species, the Ag-NORs have been described in a pair of microchromosomes, but in some cases, these were found in the distal region of the long arm of pair 2 (Kasahara et al., 1983; 1987a,b, 1989; Yonenaga-Yassuda et al., 1988; Rodrigues et al., 1989). The presence of the Ag-NORs in the telocentric region of the long arm of pair 6 is now considered a synapomorphy of the species of *nanuquae* group: *Tropidurus maniizae*, *T. amathites* and *T. divaricatus*. The occurrence of a pericentric inversion which displaced the Ag-NORs to the short arm of pair 6 in *T. divaricatus* could have an important role in the differentiation of *T. amathites* and *T. divaricatus* (Kasahara et al., 1987b). More recently, in situ hybridization (FISH) has been employed to determine the localization of ribosomal DNA sequences in chromosomes of squamate reptiles (Porter et al., 1991; 1994). The position of rDNA in lizards provides systematic information and these data can help to establish the systematic relationships between different taxa.

The C-banding pattern of *T. spinulosus* with microchromosomes showing conspicuous centromeric blocks is also typical of this species. The constitutive heterochromatin distribution pattern, as well as the Ag-NORs location, led us to suggest a species specific karyotype for *T. spinulosus*.

The karyotype of *U. superciliosus* is described here for the first time and differs from all the karyotypes found in tropidurid group by the morphology of macro and microchromosomes and the C-banding pattern.

The R-banding pattern was only obtained in *T. mucujensis* and *T. montanus*. All pairs of macrochromosomes were precisely identified while microchromosome identification was difficult. The distal end of the long arm of submetacentric pair 2 was a late-replication DNA region in all specimens. This pattern has also been found in *T. hispidus* (Kasahara et al., 1987a), *T. montanus* and *T. torquatus* (Yonenaga-Yassuda et al., 1988), *Tropidurus strobilurus*, formally *Strobilurus torquatus* (Rodrigues et al., 1989), *T. erythrocephalus*, *T. etheridgei*, *T. hygomi*, *T. itambere* and *T. oreadicus* (Kasahara et al., in preparation).

A chromosomal mechanism of sex determination of the XX:XY type was found in *T. hispidus*, *T. montanus* and *U. superciliosus*. These species exhibited a dot-like microchromosome only in male cells, which was interpreted as a Y chromosome. The X was not morphologically recognizable. It probably was a middle size acrocentric microchromosome. These conclusions are supported by the fact that an asymmetric small bivalent in diplotene cells of male specimens was observed. This kind of sex chromosomal mechanism in which the Y is a dot-like microchromosome has already been described in *T. hispidus* (Kasahara et al., 1987a), *T. torquatus* (Yonenaga-Yassuda et al., 1988) and *S. torquatus* (Rodrigues et al., 1989).

**ACKNOWLEDGMENTS**

The authors are grateful to Dr. Tien Hsi Chu for the fibroblast cultures and to Miriam Romeo and Cristina Maria de Lourdes Barnabé for technical assistance. We are indebted to Ana Carolina C. Barbosa for her assistance.
for a critical reading of the manuscript. José Manuel Martins, Gabriel Skuk and Rosana Moraes gave unvalued aid in the field.

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). Publication supported by FAPESP.

RESUMO

Estudos citogenéticos empregando técnicas de coloração convencional e diferencial (bandas C-, R- e Ag-RONs) foram utilizadas na caracterização cariotípica de seis espécies de lagartos da família Tropiduridae: Tropidurus mucujensis, T. hispidus, T. montanus, T. semitaeniatus, T. spinulosus e Uranoscodon superciliosus.

Todas as espécies apresentaram 2n = 36 e um cariótipo básico constituído de 12 macrocromossomos metacentéricos ou submetacentéricos e 24 micromcromossomos (12M + 12m). Embora um cariótipo padrão esteja presente nessas espécies, é possível caracterizá-las com base em análises morfológicas comparativas de macro e microcromossomos, localização dos Ag-RONs, quantidade e distribuição da heterocromatina constitutiva. Um mecanismo cromossômico de determinação do sexo do tipo XX : XY foi encontrado em T. hispidus, T. montanus e U. superciliosus.

A presença de cariótipos do tipo espécie-específicos é sugerida para T. spinulosus e U. superciliosus.

REFERENCES


(Received June 27, 1994)