Comparative cytogenetics and supernumerary chromosomes in the Brazilian lizard genus Enyalius (Squamata, Polychrotidae)

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Cytogenetical analyses based on conventional and differential staining were performed for the first time on five species of the Brazilian lizard genus Enyalius: E. bibronii, E. bilineatus, E. iheringii, E. leechii, and E. perditus. The species share a similar 2n = 36 (12M + 24m) karyotype, comprising of 12 metacentric or submetacentric macrochromosomes, except for an acrocentric pair 6 that characterizes E. bibronii. The 24 microchromosomes were acrocentrics, but in E. perditus two meta/submetacentric microchromosome pairs were unambiguously identified. Karyotypes with 2n = 37 and 2n = 37/38 chromosomes were also observed in some specimens of E. bilineatus as a result of the presence of supernumerary chromosomes (Bs). Ag-NORs were always located at the distal region of the long arm of the submetacentric pair 2. The constitutive heterochromatin was mostly restricted to the pericentromeric regions of some microchromosomes and microchromosomes. A XX:XY mechanism of sex determination with a dot-like Y microchromosome occurs in E. bilineatus, E. leechii, and E. perditus.

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Lizards of the genus Enyalius are predominantly inhabitants of eastern and central forested habitats of Brazil. Jackson (1978) recognized a total of six species, two of them polytypic: E. bilineatus, E. brasiliensis (2 ssp.), E. catenatus (3 ssp.), E. iheringii, E. leechii, and E. perditus. A more recent inspection of additional specimens by one of us (MTR) suggests that a new revision for the genus is needed, and although we here adopt Jackson’s scheme, we ignore subspecies and consider tentatively E. bibronii (a subspecies of E. catenatus) as a good species. Except for E. leechii, which occurs in the Amazon basin, all the others species are distributed throughout a wide range of the Brazilian Atlantic Forest, extending from state of Rio Grande do Norte (northeast) to Rio Grande do Sul (southern) (Jackson 1978; Etheridge and De Queiroz 1988). Occasionally, some populations occur in gallery forests in the Cerrados of Central Brazil (E. bilineatus) or in isolated patches of forest in the Caatingas (E. bibronii).

Cytogenetic studies of pleurodont Iguania (former Iguanidae, Estes et al. 1988; Frost and Etheridge 1989) suggest that there are two distinct trends in chromosome evolution in these lizards. Considerable karyotype variability is found in species of the highly diverse genus Anolis (2n = 25 to 2n = 48), and among those of the genus Liolaemus (2n = 30 to 2n = 44). At the other extreme, there are species from several different families that share the same 2n = 36 (12M + 24m) karyotype with very similar macrochromosome complements, which has been considered as ancestral for pleurodont Iguania, which includes the family Polychrotidae (Olmo 1986). However, the use of differential staining reveal that these conventionally-stained conservative karyotypes are distinct with respect to C-banding patterns, Ag-NORs localization, morphology of microchromosomes, among other cytogenetic aspects (Bertolotto et al. 1996; Kasahara et al. 1996). Here, we describe the karyotypes of five species of the Brazilian lizard Enyalius based on banding patterns, and compare them with those reported for other pleurodont Iguania taxa.

MATERIAL AND METHODS

A total of 20 individuals from five species of Enyalius collected from different Brazilian localities were cytogenetically studied (Table 1), and deposited at the Museu de Zoologia of the Universidade de São Paulo (MZUSP), Brazil. Chromosome spreads were obtained from bone marrow, liver, spleen, testes according to routine techniques, or from fibroblast cultures (Yonenaga-Yassuda et al. 1988). Mitotic and meiotic chromosomes were studied after standard
Table 1. Species, specimen number, sex, locality, diploid number and number of metaphases analysed for five species of Enyalius in this study. The regions of Brazil are: BA = Bahia, SP = São Paulo, MG = Minas Gerais, DF = Distrito Federal; MT = Mato Grosso. M = male and F = female; 2n = diploid number

<table>
<thead>
<tr>
<th>Species</th>
<th>Specimen number</th>
<th>Sex</th>
<th>Locality</th>
<th>2n</th>
<th>Number of metaphases</th>
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<tr>
<td><em>E. bibronii</em></td>
<td>LG 1377</td>
<td>F</td>
<td>Serra da Jibóia (BA)</td>
<td>36</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>LG 427</td>
<td>F</td>
<td>São José do Rio Preto (SP)</td>
<td>36</td>
<td>21</td>
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<tr>
<td></td>
<td>LG 814</td>
<td>F</td>
<td></td>
<td>37</td>
<td>69</td>
</tr>
<tr>
<td></td>
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<td>M</td>
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<td></td>
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<td>52</td>
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<tr>
<td></td>
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<td>M</td>
<td></td>
<td>37/38</td>
<td>59/7</td>
</tr>
<tr>
<td></td>
<td>LG 1467</td>
<td>F</td>
<td>Brasilia (DF)</td>
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<td>26</td>
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<tr>
<td><em>E. iberingii</em></td>
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<td>Santana de Parnaiba (SP)</td>
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</tr>
<tr>
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<td>F</td>
<td>Picinguaba (SP)</td>
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<td>LG 929</td>
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<td></td>
<td>LG 1223</td>
<td>M</td>
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<td>14</td>
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<tr>
<td><em>E. leechii</em></td>
<td>LG 1249</td>
<td>F</td>
<td></td>
<td>36</td>
<td>33</td>
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<tr>
<td></td>
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<td>F</td>
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<tr>
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<td>LG 1505</td>
<td>M</td>
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<tr>
<td>Total</td>
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Giems and Ag-NOR staining, and C-banding. Replication R-banding (RBG), after in vitro treatment with 5-bromodeoxyuridine (BrDU) and 5-fluorodeoxyuridine (FudR) followed by FPG staining (Dutrillaux and Couturier 1981) was carried out on metaphases of *E. bilineatus*

RESULTS

Comparative analyses after conventional staining

A similar karyotype of 2n = 36 (12M + 24m) comprised of 12 macrochromosomes and 24 microchromosomes characterized the five species of *Enyalius* (Fig. 1a–e, Table 1). The macrochromosome pairs 1, 3, 4, and 5 are metacentrics, and the pairs 2 and 6 are submetacentrics in all species, except for *E. bibronii* which has an acrocentric pair 6 (Fig. 1a). An aberrant pair 6 formed by a submetacentric and an acrocentric was detected in one specimen (LG 1223) of *E. leechii* (data not shown). Pair 2 exhibits a typical secondary constriction at the distal end of the long arm. Some microchromosomes seem to be acrocentrics, and there are indubitably two distinct metacentric or submetacentric pairs in *E. perditus* (Fig. 1e).

Supernumerary chromosomes (Bs) were observed in two specimens of *E. bilineatus* (Fig. 2a). The specimen LG 814 had a karyotype of 2n = 37 chromosomes, while the specimen LG 919 was a mosaic composed of a major 2n = 37 (one B) and a minor 2n = 38 (two Bs) lineages, the latter representing about 10% of its cells (Table 1).

A mechanism of sex determination of XX:XY type was found in *E. bilineatus*, *E. leechii* and *E. perditus*. The Y is a dot-like microchromosome and the X is a medium-sized microchromosome which is indistinguishable from the other chromosomes of the same size (Fig. 1b, d, e and Fig. 2a). An evident heteromorphic microbivalent corresponding to the pairing of the X and Y was observed in some diakinesis cells from males of *E. bilineatus* and *E. perditus* (Fig. 2b). At metaphase II cells, 6 macrochromosomes and 12 microchromosomes were visualized. In the aberrant specimen of *E. leechii* (LG 1223), metaphase II cells contained either the submetacentric or the acrocentric macrochromosome 6. Analysis of seven diakinesis cells from the mosaic specimen LG 919 showed the presence of 6 macrobivalents, 12 microbivalents (one of them representing the pairing XY), and a probable univalent (Fig. 2b).

Analyses after differential staining

The Ag-NORs were detected at the distal end of the long arm of pair 2, corresponding to the conspicuous secondary constriction in all five species of *Enyalius* (Fig. 3b–e). Out of 45 Ag-stained metaphases of one specimen of *E. bilineatus* (LG 815), 37 were heteromorphic with respect to the size of the NOR (Fig. 3b).
Fig. 1a–e. Karyotypes of species of *Enyalius* after conventional staining (2n = 36, 12M + 24m). a *E. bibronii* female; b *E. bilineatus* male; c *E. iheringii* female; d *E. leechii* female; e *E. perditus* female. For the sex chromosomes of the opposite sex, see the inset in b, d and e. Bar = 10 μm.
Fig. 2a and b. Cells of the mosaic specimen of *Enyalius bilineatus*, male, after conventional staining. 

- **a** Mitotic metaphase with $2n = 37$ (12M + 24m + 1B). Note the supernumerary chromosome (small arrow) and the dot-like Y microchromosome (large arrow);
- **b** Diakinesis cell presenting 6 macrobivalents, 12 microbivalents (one of them heteromorphic: small arrow), and one univalent (B chromosome: large arrow).

Fig. 3a–e. Chromosomes of species of *Enyalius* after differential staining. 

- **a** C-banded metaphase of *E. bilineatus*. The labeled arrows indicate: (a) interstitial constitutive heterochromatin on pairs 1 and 2; (b) constitutive heterochromatin at the distal end of the long arm of pair 2. The supernumerary chromosome is indicated by a B;
- **b** Ag-NORs in *E. bilineatus*. Note the heteromorphism of size (arrows);
- **c**–**e** Pairs 2 of *E. perditus*, *E. iheringii* and *E. leechii* after Ag-staining, respectively.

All five species of *Enyalius* were characterized by a small amount of constitutive heterochromatin after C-banding, which was mostly restricted to the pericentromeric region of some chromosomes (Fig. 3a). The region of the secondary constriction of pair 2 was also positively stained in some metaphases of *E. bibronii*, *E. bilineatus*, and *E. perditus*. A proximal C-band equidistantly located on pair 1 and 2 was detected in the karyotypes of *E. bilineatus* and *E. bibronii* (Fig. 3a). The B chromosome detected in two specimens of *E. bilineatus* exhibits an intermediate staining between the darkest and the lightest C-positive blocks of the autosomes (Fig. 3a).

RBG pattern was obtained for *E. bilineatus*, and allowed all its macrochromosomes to be unambiguously paired (Fig. 4a). A late replicating region at the distal end of the long arm of pair 2 was also observed. The B chromosome is late-replicating (Fig. 4a, b), and, interestingly, in two metaphases of the mosaic specimen LG 919 ($2n = 37/38$; Table 1) bearing two Bs, only one of them was late replicating (Fig. 4c).

**DISCUSSION**

The five species of *Enyalius* share a common $2n = 36$ (12M + 24m) karyotype which has been described among several taxa within pleurodont Iguania (BICKHAM 1984; KASAHARA et al. 1996; PELLEGRINO et al. 1999). There is similarity among the macrochromo-
somomes of the *Enyalius* species, with the exception of *E. bibronii* that present an acrocentric pair 6, making it distinct from its congeners, all other Polychrotidae, and related families within pleuroid Iguaia that share the conservative karyotype usually with metacentric or submetacentric pair 6. We also detected the presence of a heteromorphic pair 6 in both somatic and meiotic cells of one specimen of *E. leechii*. This chromosome aberration could be explained by a deletion affecting the short arm of one homologue of this pair. It seems that the macrochromosome pair 6 might be a “hot spot” for karyotypic evolution in the genus *Enyalius*. A parallel example was reported in the genus *Tropidurus* (Tropiduridae) with three closely related species: *T. nanuzae* and *T. amathites* having a secondary constriction and NORs in the long arm of pair 6, while in *T. divaricatus* these regions are located in the short arm of the same pair due to the occurrence of a pericentric inversion (KASAHARA et al. 1987).

All five species of *Enyalius* were very similar with respect to location of the NORs and the amount and distribution of constitutive heterochromatin. The distal end of the long arm of pair 2 bearing the NORs and a positive C-band has been described across several species of pleuroid Iguania families: Tropiduridae (*Tropidurus*, KASAHARA et al. 1987, 1996; *Strobilurus*, RODRIGUES et al. 1989; *Liolaemus*, BERTOLLOTTO et al. 1996), Polychrotidae (*Pristidactylus*, PELLEGRINO 1993; *Urostrophus*, PELLEGRINO et al. 1999), and Phrynosomatidae (*Sceloporus*, PORTER et al. 1994), indicating that it is a conservative region shared by these related families.

Studies involving comparative analyses of microchromosomes are usually limited because their reduced size prevents precise definition of their morphology in most cases, with few exceptions (PINNA-SENN et al. 1987; PELLEGRINO et al. 1994; BERTOLLOTTO et al. 1996; KASAHARA et al. 1996). In all species of *Enyalius*, the 24 microchromosomes seem to be acrocentrics, but *E. perditus* has at least two biarmed pairs. Increase in quality of the chromosome preparations should allow these chromosomes to be used more often in comparative analyses, and indeed facilitate the detection of mechanisms of sex determination that involve these microchromosomes.

A mechanism of the XX:XY type was detected in *E. bilineatus*, *E. leechii*, and *E. perditus*. A heteromorphic microbivalent, representing the pairing of X and Y, was observed in meiotic cells of *E. bilineatus* and *E. perditus*, and allowed us to evaluate the size of the X chromosome. In pleuroid Iguania, both simple (XX:XY) and multiple (X,X,X,X,Y) mechanisms of sex determination involving microchromosomes occur, including the polychrotids *Pristidactylus* (PELLEGRINO 1993), *Anolis* (GORMAN and ATKINS 1968; GORMAN and STAMM 1975; our unpublished data), *Polychrus* (BERTOLLOTTO et al. 2001) and *Urostrophus* (PELLEGRINO et al. 1999).

Fig. 4a–c. RBG pattern from the mosaic specimen of *E. bilineatus*. a Partial karyotype showing the six pairs of macrochromosomes. A late-replicating B chromosome from a different metaphase is shown (inset); b Metaphase with one B chromosome (arrow); c Metaphase with two Bs (arrows), with only one of them late-replicating (large arrow).
Supernumerary chromosomes of intermediate size between the microchromosomes and macrochromosomes are present in two specimens of *E. bilineatus*. They do not exhibit a heterochromatic pattern, but they are almost entirely late-replicating after R-banding, which is typical for the heterochromatin of the Bs. Few reports of occurrence of supernumerary chromosomes have been made among lizards, compared to those from plants and other animals. Besides the present study, these chromosomes have been found in ten other genera, which represent eight different families including the polychrotid *Anolis* (Gorman et al. 1968).

The function, composition, and origin the supernumerary chromosomes are not completely known, with the most intriguing questions regarding their origin. Several molecular cytogenetic studies utilizing restriction endonucleases, fluorochromes, in situ hybridization, and chromosome microdissection techniques have been used to elucidate the composition, structure, origin and evolution of the supernumerary chromosomes (Lopez-Leon et al. 1994; Brinkman et al. 2000; Maistro et al. 2000).

The present study extends the knowledge on karyotypes of the yet poorly known lizard family Polychrotidae. Considering that the relationships within this family are still unresolved, studies employing banding techniques on the remained taxa should allow the identification of synapomorphies that can be useful in a phylogenetic context.

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