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# Differential staining and microchromosomal variation in karyotypes of four Brazilian species of Tupinambinae lizards (Squamata: Teiidae)

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**Abstract** Kayotypes of four neotropical teiid lizard species (Tupinambinae) were herein studied after conventional as well as silver staining and CBG-banding: *Crocodilurus amazonicus* ($2n = 34$), *Tupinambis teguixin* ($2n = 36$), *Tupinambis merianae* and *Tupinambis quadrilineatus* ($2n = 38$). The karyological data for *T. quadrilineatus* as well as those obtained using differential staining for all species were unknown until now. The karyotypes of all species presented 12 macrochromosomes identical in morphology, but differed in the number of microchromosomes: 22 in *C. amazonicus*, 24 in *T. teguixin* and 26 in *T. quadrilineatus* and *T. merianae*. The Ag-NOR located at the secondary constriction at the distal end of pair 2 is shared by all species, contrasting with the variability observed for this character in species of the related Teiinae. CBG-banding revealed a species-specific pattern in *T. quadrilineatus* with conspicuous interstitial C-blocks at the proximal region of the long arm of pair 4 and the whole heterochromatic short arm of pair 6. The karyological data reported here corroborates the relationship hypothesis obtained for *Tupinambis* based on molecular characters. *T. teguixin* presents the putative ancestral karyotype for the genus with $2n = 36$ whereas *T. merianae* and *T. quadrilineatus* exhibit $2n = 38$, due to an additional pair of microchromosomes.

**Keywords** Teiidae · Tupinambis · Karyotypes · Differential staining · Lizards

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## Introduction

The Teiidae is a characteristic New World family of ten genera of extant lizards, assembling around 120 species occurring predominantly in South and Central America, with a single genus (*Aspidoscelis*) occurring in North America (Reeder et al. 2002). Two distint radiations with subfamilial rank are presently admitted. The Tupinambinae comprises *Callopistes*, *Crocodilurus*, *Dracaena*, and *Tupinambis* whereas the Teiinae comprises *Ameiva*, *Aspidoscelis* (the only extending to North America and recently separated from *Cnemidophorus*), *Cnemidophorus*, *Dicrodon*, *Kentropyx*, and *Teius* (Vanzolini and Valencia 1965; Gorman 1970; Presch 1974; Estes et al. 1988; Rieppel 1980; Denton and O'Neil 1995). Known informally as macroteiids, teiids are the sister group of Gymnophthalmidae (microteiids) and along with related fossil forms are included in Teiioidea. It has been widely accepted to assemble the Teiioidea (New World runners) and their sister group the Lacertidae (Old World runners) in a monophyletic Lacertiformes in the Infra-Order Scincomorpha. This view has been recently challenged after a comprehensive molecular analysis of squamates suggesting that the teiioids (Teiformata) are a basal lineage sister to amphisbaenians plus lacertids (Lacertibaenia) (Vidal et al. 2005; Fry et al. 2006; Townsend et al. 2004).

In spite of the unambiguous monophyly of Teiinae and Tupinambinae, the phylogenetic relationships of genera within them are still disputed. For example, even including only four genera four hypotheses were proposed for the Tupinambinae, and all are controversial (Gorman 1970; Presch 1974; Rieppel 1980; Denton and O'Neil 1995).

The diploid number in the Tupinambinae ranges from 34 to 38 chromosomes, with a clear distinction between 10–12 macrochromosomes and 22–26 "dot like" michrochromosomes (Gorman 1973).

The chromosomal formula of $2n = 36$ with 12 macrochromosomes and 24 microchromosomes (12M + 24m), pairs 1, 3, and 4 metacentrics and 2, 5, and 6 submetacentrics and the presence of a conspicuous secondary constriction at the distal region of the long arm of pair 2, is found in three out four traditionally admitted lizard Infra-Orders (Anguimorpha, Iguania and Scincomorpha) as well as in snakes, amphisbaenians and dibamids. This wide distribution of the $2n = 36$ (12M + 24m) karyotype led many authors to consider it as homologous and so ancestral within Squamata (Olmo 1986). In spite of its wide occurrence, very few studies using chromosomal banding techniques in species with the putative saurian ancestral karyotype have been performed so far (Pellegrino et al. 1994; Kasahara et al. 1987, 1996; Bertolotto et al. 2002). This approach would ideally allow the establishment of accurate homologies among karyotypes of close related species.

The genus *Tupinambis*, currently comprises six species according to the recent review by Péres and Colli (2004): *T. duseni* (Lönnberg 1910), *T. longilineus* (Ávila-pires 1995), *T. quadrilineatus* (Manzani and Abe 1997), *T. rufescens* (Günther 1871) and *T. teguixin* (Linnaeus 1758). Much confusion has plagued the taxonomy of *Tupinambis* especially attribution of names involving *T. teguixin* and *T. rufescens* (Ávila-pires 1995; Péres and Colli 2004) and *T. teguixin* and *T. merianae* (Ávila-pires 1995), leading to misapplications of names and erroneous conclusions following comparative studies. As an example, De Smet (1981) describes *T. teguixin* with a $2n = 36$ (12M + 24m) karyotype, whereas Beçak (1972) refers $2n = 38$ (12M + 26m). Similarly, *T. nigropunctatus*, presently a synonymous of *T. teguixin*, according to Ávila-pires (1995), was described as having a $2n = 36$ and $2n = 38$ karyotype (Gorman 1970; De Smet 1981). Veronese et al. (2003) found $2n = 38$ chromosomes in *T. merianae*. As there are no vouchers available, these identifications can only eventually be checked with further karyotypic analysis.

In this study, *Crocodilurus amazonicus, Tupinambis merianae*, *T. quadrilineatus* and *T. teguixin*, were analysed after conventional and silver staining plus CBG-banding. Considering that cytogenetic studies of these and other teiids have revealed conflicting results or are still scant in literature our study brings light to both taxonomy and chromosomal diversity within this group of lizards.

## Materials and methods

Cytogenetic analyses were carried out on a total of 20 specimens of *C. amazonicus*, *T. merianae*, *T. quadrilineatus*, and *T. teguixin*. Collecting localities are in Table 1. Voucher specimens were deposited in the herpetological collection of the Museu de Zoologia da Universidade de São Paulo (MZUSP).

**Table 1** Species, specimens ID and sex and locality of Tupinambinae lizards sampled for this study

| Species | Specimen number (LG)/sex | Locality |
|---|---|---|
| *Crocodilurus amazonicus* | 1411 (F), 1412 (F) | Reserva biológica do rio trombetas, PA (01°30′ S, 55°50′ W) |
| *Tupinambis merianae* | 1,964 (F), 1,983 (M), 2,038 (M), 2,039 (F) | Lajeado, TO (09°45′ S, 48°21′ W) |
| | L 85 (F) | Rio claro, SP (22°24′ S, 47°33′ W) |
| | 1,469 (F) | Rosal, ES (19°38′ S, 39°49′ W) |
| | 2,304 (F) | Reserva vale do rio doce, ES (19°23′ S, 40°04′ W) |
| *Tupinambis quadrilineatus* | 1,132 (M), 1,133 (F) | Niquelândia, GO (14°28′ S, 48°27′ W) |
| | 2,289 (F), 2,290 (F) | Peixe, TO (12°30′ S, 48°21′ W) |
| | 2,027 (F) | Lajeado, TO (09°45′ S, 48°21′ W) |
| *Tupinambis teguixin* | 1,548 (F), 1,549 (F) | Manso, MT (15°09′ S, 55°04′ W) |
| | 1,483 (M), 1,484 (F) | Parque Nacional do Araguaia, GO (10°81′ S, 50°19′ W) |
| | 2,284 (F), 2,285 (F), 2,286 (F) | Peixe, TO (12°30′ S, 48°21′ W) |
| | 1,984 (M), 2,029 (F), 2,030 (F), 2,031 (F) | Lajeado, TO (09°45′ S, 48°21′ W) |

ID numbers from the Laboratório de Citogenética de Vertebrados (IBUSP, São Paulo, Brazil)

M = male; F = female; ES = state of Espírito Santo; GO = state of Goiás; PA = state of Pará, PI = state of Piauí; SP = state of São Paulo; TO = state of Tocantins

Chromosomal spreads were obtained from bone marrow, intestine, liver and spleen according to Kasahara et al. (1987), or from fibroblast culture of caudal muscle following Yonenaga-Yassuda et al. (1988). Meiotic analyses were performed on testis preparations. The diploid number and chromosome morphology were established after conventional staining. Mitotic chromosomes were analysed after CBG and silver staining according to routine techniques.

## Results

Three different karyotypes with diploid numbers ranging from $2n = 34$ to 38 were found after conventional staining.

### $2n = 34$ (12M + 22m)

This karyotype is exclusive of *C. amazonicus* which has 12 macrochromosomes, with pairs 1, 3–5 metacentrics and pairs 2 and 6 submetacentrics, plus 22 microchromosomes (pairs 7–17), most of them biarmed (Fig. 1a). In some metaphases, a secondary constriction at de distal end of the long arm of pair 2 was observed.

### $2n = 36$ (12M + 24m)

The karyotype of *Tupinambis teguixin* is formed by 12 macrochromosomes, with pairs 1, 3–5 metacentrics, pairs 2 and 6 submetacentrics and 24 microchromosomes (pairs 7–18), some of them biarmed (Fig. 1b). A secondary constriction at the distal end of the long arm of pair 2 was observed in some metaphases.

### $2n = 38$ (12M + 26m)

*Tupinambis merianae* and *T. quadrilineatus* present a karyotype composed of 12 macrochromosomes, with pairs 1, 3–5 metacentrics, pairs 2 and 6 submetacentrics and 26 microchromosomes (pairs 7–19), most of them biarmed. There is a conspicuous secondary constriction at the distal end of the long arm of pair 2 (Fig. 1c, d).

### Silver staining and CBG-banding

All species presented Ag-NORs located at the secondary constriction at the distal end of long arm of pair 2 (Fig. 2).

In *C. amazonicus* the CBG-banding revealed the presence of dark stained blocks of constitutive heterochromatin at pericentromeric regions of all macrochromosomes. Some macrochromosomes exhibited faint positive C-bands at their pericentromeric regions (Fig. 3a).

The CBG pattern found in *T. merianae* is characterized by conspicuous blocks at the pericentromeric regions of all macrochromosomes and of some microchromosomes, as well as at the region of the secondary constriction of pair 2. A single microchromosome appeared completely heterochromatic (Fig. 3b).

The karyotype of *T. quadrilineatus* showed small positive C-bands at pericentromeric regions of all macrochromosomes, except for pair 6 that has the whole short arm heterochromatic. Conspicuous interstitial heterochromatic blocks also occur at the proximal region of the long arm of pair 4. The region of the secondary constriction at the distal end of pair 2 is also C-band positively stained. All microchromosomes present constitutive heterochromatin on the telomeric or centromeric regions (Fig. 3c).

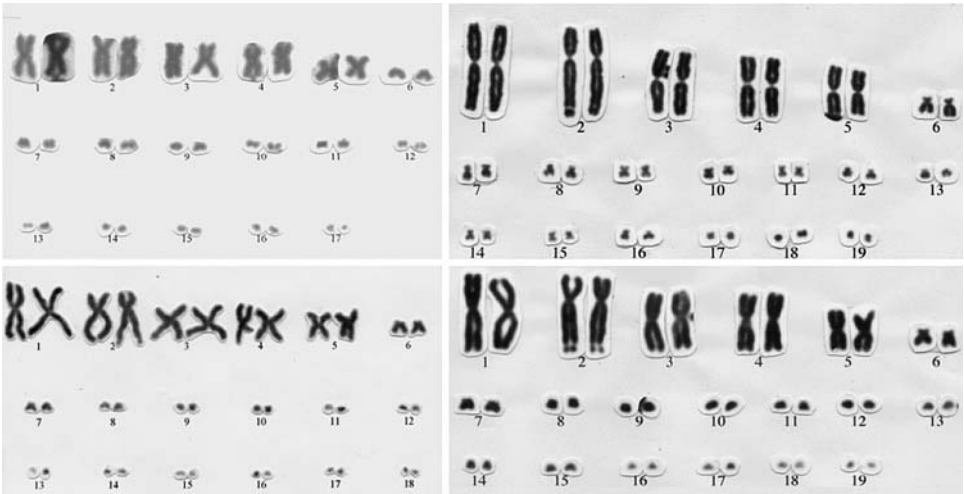

**Fig. 1** Karyotypes of Tupinambinae species, after conventional staining. (**a**) *Crocodilurus amazonicus* $2n = 34$ (12M + 22m), male; (**b**) Female of *Tupinambis merianae* ($2n = 36$ 12M + 24m); (**c**) *Tupinambis teguixini* ($2n = 38$, 12M + 26m), male; (**d**) Female of *Tupinambis quadrilineatus* ($2n = 38$, 12M + 26m). Observe the presence of the secondary constriction at de distal end of the long arm of the macrochromosome pair 2 in **a** and **b**

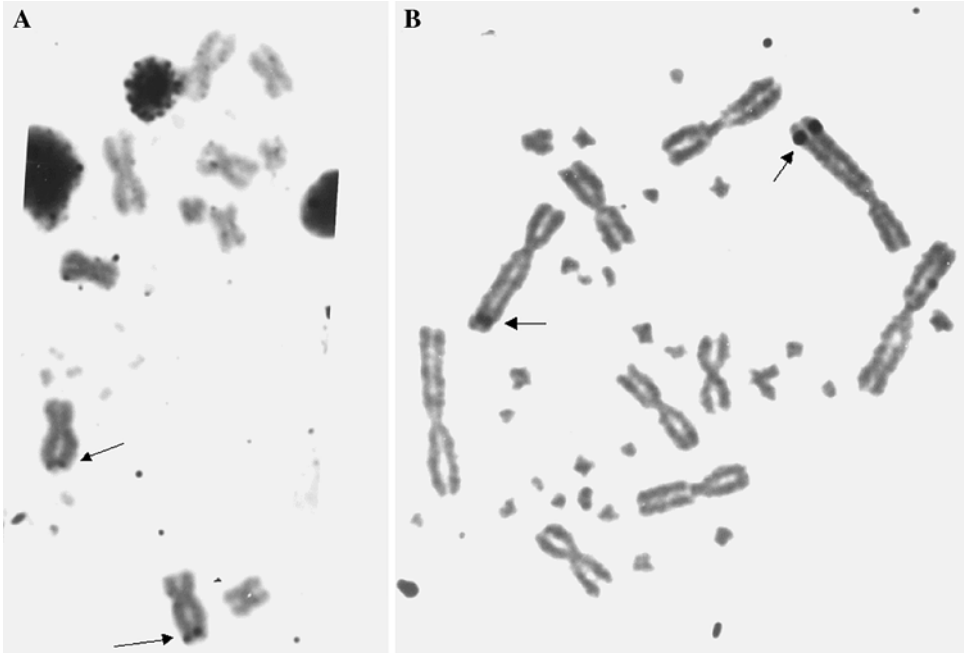


**Fig. 2** Ag-NORs of the Tupinambinae species located at secondary constriction at the distal end of the long arm of pair 2 (arrows)

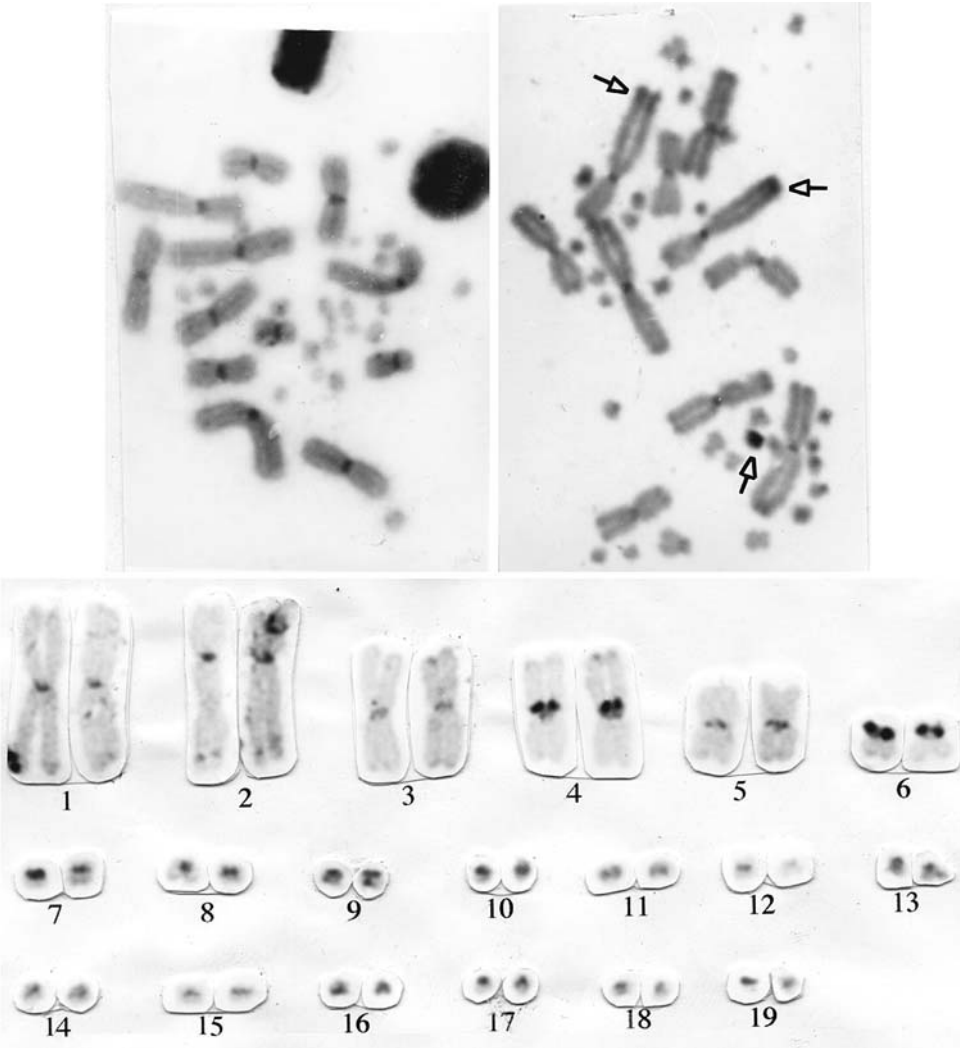


**Fig. 3** C-banding patterns of Tupinambinae species. (**a**) Metaphase of *Crocodilurus amazonicus* $2n = 34$ (12M + 22m); (**b**) metaphase of *Tupinambis merianae* ($2n = 36$ 12M + 24m) with positive C-bands at the secondary constriction of the long arm of pair 2 and a heterochromatic microchromosome (arrows); (**c**) karyotype of *Tupinambis quadrilineatus*. See conspicuous interstitial C-blocks at pair 4 and the heterochromatic short arm of pair 6

## Discussion

Karyotypes with $2n = 34$, 36, and 38 chromosomes were found among *C. amazonicus*, *T. merianae*, *T. quadrilineatus*, and *T. teguixin* studied herein. All karyotypes present 12 macrochromosomes metacentrics or submetacentrics, but differ in the number of microchromosomes, which varies from 22 to 26. The karyotype of *T. quadrilineatus* in conventional staining and the chromosomal data obtained after differential staining had been unknown for these four Tupinambinae species until now.

The karyotype of *C. amazonicus* ($2n = 34$, 12M + 22m) is identical to that described by Gorman (1970) for a Brazilian specimen (locality not mentioned) who suggested that the $2n = 34$ karyotype derived from the ancestral karyotype of $2n = 36$ (12M + 24m) through the loss a microchromosome pair. Also, he argued that the conserved morphology of macrochromosomes and the presence of the secondary constriction at the distal end of pair 2, which bears the Ag-NORs, were shared by several teiids of his *Dracaena* group (present Tupinambinae), which indicates their conservative status. This conservative pattern of Ag-NORs distribution in Tupinambinae contrast with that described for Teiinae, that exhibit considerable intergeneric variation in the character (Santos et al. 2007).

Two different karyotypes with $2n = 36$ and 38 were described for *Tupinambis*, after conventional staining. The occurrence of these two karyotypes has been already reported in the genus (Beçak 1972; Gorman 1970; De Smet 1981). Nevertheless their association with the correct species are doubtful considering the confused taxonomy and the chaotic attribution of names which characterized the systematics of *Tupinambis* until recently. Considering this and in absence of precise localities and of voucher specimens we rely on karyotypic analysis to confirm such identifications. Our data clearly demonstrate that *T. teguixin* presents $2n = 36$, like described by De Smet (1981) and Gorman (1970), and *T. merianae* and *T. quadrilineatus* share a $2n = 38$ karyotype. A karyotype with $2n = 38$ was also found in *T. merianae* by Veronese et al. (2003). This is to say that the $2n = 38$ karyotype reported by Beçak et al. (1972) for *T. teguixin*, should be attributed to *T. merianae*, and not to the Amazonian species presently recognized as *T. teguixin* (Peres), which present a $2n = 36$. Similarly, we believe that the $2n = 38$ karyotype reported by De Smet (1981) for *Tupinambis nigropunctatus* (presently a junior synonym of *T. teguixin*) should be attributed to *T. merianae* or to *T. quadrilineatus*, both with a $2n = 38$ karyotype. Our data also suggests that Veronese et al. (2003) identification was correct.

Fitzgerald et al. (1999) studied the phylogenetic relationships of the genus *Tupinambis* using mitochondrial DNA sequences and found two distinct clades: one formed by *T. longilineus* and *T. teguixin*, the other one including *T. merianae*, *T. rufescens*, and *T. duseni*. Contrarily to Peres et al. conclusions he did not found molecular differences supporting the distinctiveness between *rufescens* and *duseni*, a result that can again be due to misidentification.

The two groups of *Tupinambis* recognized in the above analysis are also supported by karyotypic data: by one side *T. teguixin* with $2n = 36$, by the other *T. merianae*, *T. quadrilineatus,* and *T. rufescens* (data for the last species according to Hernando, http://www1.unne.edu.ar/cyt/biologia/b-019.pdf), all with an additional pair of microchromosome leading to a $2n = 38$ karyotype.

Besides the differences related to the diploid number in *Tupinambis*, there are two diferent C-banding patterns in $2n = 38$ karyotypes. Specimens of *T. quadrilineatus* present a clear interstitial C-block at the proximal region of the long arm of pair 4 and the whole short arm of pair 6 is heterochromatic. By contrast, *T. merianae* exhibit C-bands predominantly at pericentromeric regions of most chromosomes, similar to the pattern found in *C. amazonicus*. We suggest that the C-banding pattern observed in *T. quadrilineatus* is species-specific.

In the Gymnophthalmidae and Lacertidae, C-banding patterns have been considered phylogenetically informative. The gymnophthalmids *Procellosaurinus erythrocercus*, *P. tetradactylus* and *Vanzosaura rubricauda* (Yonenaga-Yassuda et al. 1996), present very similar karyotypes in both conventional and R-banding, but distinct patterns of constitutive heterochromatin. This was a useful character that corroborated the split into two different genera, proposed on the basis of morphology.

After a wide revision of the C-banding and Ag-NORs patterns in lacertid species, Olmo et al. (1990) described many species-specific patterns published by other authors (Capula et al. 1989; Cardone et al. 1990) and along with data on restriction enzymes, aimed to achieve a better comprehension about the phylogenetic relationships of this lizards.

The variability detected at the C-banding patterns within Lacertidae and Teiidae families indicates that this character may be useful for citotaxonomy and chromosomal evolution of groups of lizards.

The present work is the second to report differential stained karyotypes in Teiidae (Santos et al. 2007), and it also contributes to clarify some conflicting reports of karyotypes for Tupinambinae in the literature.

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