Comparative cytogenetics of eight species of Cycloramphus (Anura, Cycloramphidae)

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Abstract

Several aspects of the biology of Cycloramphus species of the Atlantic Forest are still poorly known, which makes it difficult to understand their historical relationships. Therefore, we were stimulated to promote a comparative cytogenetic analysis of several species of the genus Cycloramphus. The study of Cycloramphus acangatan, C. boraceiensis, C. brasiliensis, C. carvalhoi, C. eleutherodactylus, C. fuliginosus, C. lutzorum, and C. rhyakonastes, revealed that these eight species share a diploid number $2n = 26$. Cycloramphus fuliginosus presented the most distinct karyotype, due to the presence of subtelocentric chromosomes in pairs 1 and 4. The main diagnostic feature observed in the other species was the presence of one pair of telocentric chromosomes in C. boraceiensis, C. carvalhoi, and C. eleutherodactylus, while the remaining species presented karyotypes composed exclusively of biarmed chromosomes. Constitutive heterochromatin was predominantly located in pericentromeric regions in all species,
although additional C-bands detected on telomeric and/or interstitial regions were partially species-specific. Silver staining revealed Ag-NORs located on pair 6 in six species, whereas C. acangatan presented it on pair 1 and a multiple pattern was observed in C. fuliginosus with three Ag-NOR bearing chromosomes. Fluorescent in situ hybridization using rDNA probe was performed in specimens of C. eleutherodactylus from Paraná, C. lutzorum, and C. rhyakonastes, which did not reveal inactive NOR. Despite the apparent highly conserved diploid number, data on the karyotype microstructure characterize the cytogenetic profile of the genus and may contribute to clarify the phylogenetic relationships among Cycloramphus, the Cycloramphinae, or even the family Cycloramphidae.

**Keywords:** Amphibian cytogenetics; Cycloramphus; Karyotypes; Ag-NORs; C-banding; FISH

1. Introduction

The genus *Cycloramphus* (Tschudi, 1838) comprises 27 species of frogs, all restricted to the Atlantic forest domain (Ab’Sáber, 1970), occurring from the southern portion of the State of Bahia down to the State of Santa Catarina, reaching their highest diversity in the mountain chains in southeastern and southern Brazil (Heyer, 1983, 1988; Haddad and Sazima, 1989; Verdade and Rodrigues, 2003, 2008; Brasileiro et al., 2007).

Despite the lack of available data for the most Brazilian cycloramphids, natural history coupled with morphology can be used to classify *Cycloramphus* species into two ecomorphological groups, which so far have not been tested by an explicit phylogeny. The forest litter dwellers comprising *Cycloramphus acangatan* (Verdade and Rodrigues, 2003), *Cycloramphus bolitoglossus* (Werner, 1897), *Cycloramphus carvalhoi* (Heyer, 1983), *Cycloramphus diringshofeni* (Bokermann, 1957), *Cycloramphus eleutherodactylus* (Miranda-Ribeiro, 1920), *Cycloramphus faustoi* (Haddad Sawaya and Sazima, 2007), *Cycloramphus miguelii* (Heyer, 1988), and *Cycloramphus stejnegeri* (Noble, 1924), lay their eggs in the moist forest floor, and present terrestrial endotrophic tadpoles (Heyer and Crombie, 1979; Verdade, 2005; Brasileiro et al., 2007). The stream dweller group includes the remaining 18 species, which breed in streams in forested areas, lay their eggs on rocks in the splash zone, (except for *Cycloramphus bandeirensis* (Heyer, 1983) that live in high open areas and lay their eggs under rocks), and present semiterrestrial exotrophic tadpoles (Heyer, 1983; Haddad and Sazima, 1989; Giaretta and Cardoso, 1995; Giaretta and Facure, 2003; Lima et al., 2010; Verdade et al., unpublished data).

Their distribution, usually associated with areas of sharp relief, and the stealthy habits of both leaf litter and stream dwellers, make it difficult to find and collect them. This, in turn, might explain the scarce information on vocalization, tadpoles, and distribution (see Heyer and Maxon, 1983; Verdade, 2005; Lingnau et al., 2008), so most of these species are placed into the data deficient category of IUCN (IUCN, 2008). Such scarcity of data is also reflected in chromosomal information, since only four species of *Cycloramphus* had their karyotypes described and most of them were limited to the description of diploid number (Brum-Zorrilla and Saez, 1968; Bogart, 1970; Bečak et al., 1970; Silva et al., 2001; Lima et al., 2010). The data for most reveal karyotypes with $2n = 26$ chromosomes and a fundamental number (FN) equal to 50 due to the presence of one pair of telocentric chromosomes, or FN = 52, with all biarmed chromosomes. In order to contribute to the general knowledge on and evolution of the genus we present a cytogenetic analysis and comparison of eight species: *Cycloramphus acangatan*, *Cycloramphus boraceiensis* (Heyer, 1983), *Cycloramphus brasiliensis* (Steindachner, 1864), *Cycloramphus carvalhoi*, *Cycloramphus eleutherodactylus*, *Cycloramphus fuliginosus* (Tschudi, 1838), *Cycloramphus lutzorum* (Heyer, 1983), and *Cycloramphus rhyakonastes* (Heyer, 1983) based on conventional staining, Ag-NOR detection, C-banding, and fluorescent in situ hybridization using rDNA probes.

2. Materials and methods

2.1. Specimens

Cytogenetic analyses were carried out on 23 specimens of eight species of *Cycloramphus* collected in the states of Bahia, Paraná, Rio de Janeiro, and São Paulo (Table 1). Voucher specimens are deposited in the Célio F.B. Haddad Amphibian Collection, Universidade Estadual Paulista, in Rio Claro, São Paulo (CFBH); Capão da Imbuia National History Museum, in Curitiba, Paraná (MHNCI); and Museum of Zoology, Universidade São Paulo, São Paulo (MZUSP), all in Brazil.

2.2. Chromosome preparation and techniques

The procedures used in the current study were in accordance with the Animal Experimentation Ethics Committee recommendations (UFPR 01/03BL) and the current Brazilian legislation (CONCEA 1153/95). Chromosome preparations were made directly from bone marrow and liver according to Baldissera et al. (1993), or from intestinal epithelium as described by Schmid (1978). Chromosomes were classified by visual inspections according to the nomenclature proposed by Green and Sessions (1991).

Conventional staining was performed using 5.0% Giemsa diluted in sodium-phosphate buffer (pH 7.0, for 10 min).
The Ag-NOR and C-banding techniques were carried out according to Howell and Black (1980) and Sumner (1972), respectively. Fluorescent in situ hybridization (FISH) was performed using 18S rDNA probe from the fish Prochilodus argenteus (Spix and Agassiz, 1829) (Hatanaka and Galetti, 2004). The probes were labeled with biotin 14-dATP by nick translation, following the manufacturer’s instructions (BionickTM DNA Labeling System – Invitrogen). The detection and amplification of hybridization signals were carried out using conjugated streptavidin-FITC (Molecular Probes™ – Invitrogen). The metaphases were examined on a Zeiss Axioshot epifluorescence microscope and the chromosome images were captured using the software Case Data Manager Expo 4.0 (Applied Spectral Imaging), or viewed under an Olympus BX51 microscope with a digital camera Olympus D71, and the images captured using the software DPController.

3. Results

3.1. Karyotype

All species analyzed showed karyotypes composed of 26 chromosomes (Fig. 1), the first six pairs being medium and large-sized chromosome pairs, and the remaining seven pairs of small sizes. Cycloramphus acangatan, C. brasiliensis, C. fuliginosus, C. lutzorum, and C. rhyakonastes presented karyotypes that consist exclusively of biarmed chromosomes with FN = 52, while C. boraceiensis, C. carvalhoi, and C. eleutherodactylus have one pair of telocentric chromosomes showing FN = 50 (Fig. 1, Table 2).

The karyotype of Cycloramphus acangatan presented metacentric pairs 1, 5, 7 to 10, submetacentric pairs 2, 3, 4, 6 and 12 and subtelocentric pairs 11 and 13 (Fig. 1a). Cycloramphus boraceiensis has metacentric pairs 1, 5, 7 to 11 and 13, submetacentric pairs 2, 3, 4 and 12, and telocentric pair 6 (Fig. 1b). Cycloramphus brasiliensis and C. rhyakonastes showed similar karyotypes with metacentric pairs 1, 5 to 13, and submetacentric pairs 2, 3 and 4 (Fig. 1c and i, respectively). Cycloramphus carvalhoi and C. eleutherodactylus have a karyotype similar to those observed in C. brasiliensis and C. rhyakonastes, except for the submetacentric pair 5 in C. eleutherodactylus and the telocentric pair 13 in both species (Fig. 1d–f, respectively). The karyotype of C. lutzorum is also similar to C. brasiliensis and C. rhyakonastes, except the pair 11 which is submetacentric (Fig. 1b). C. fuliginosus presented the most distinctive karyotype within the genus, with subtelocentric pairs 1 and 4, submetacentric pairs 2, 3, 5, 8 and 12 and metacentric pairs 6, 7, 9 to 11 and 13 (Fig. 1g). Heteromorphic sex chromosomes were not identified in the present samples including males and females.

Secondary constrictions were observed at proximal regions of the long arm of telocentric pair 6 in C. boraceiensis, in an interstitial region in the long arm of the pair 6 in C. brasiliensis, and in the interstitial regions of short arms of pair 6 in C. eleutherodactylus and C. lutzorum (Fig. 1b, c, e, f, and h, respectively).

3.2. Nucleolar organizer region (NOR)

Except for C. fuliginosus, which showed Ag-NOR in the interstitial region of long arms of pairs 1 and 4, the other species presented only one Ag-NOR bearing chromosome pair (Fig. 2). Cycloramphus acangatan showed Ag-NOR in the interstitial region of the short arm of pair 1 and the remaining species presented Ag-NOR in pair 6 in the interstitial region of the short arm in C. eleutherodactylus, C. lutzorum, and C. rhyakonastes, and interstitial and proximal region of the long arm in C. boraceiensis, C. brasiliensis, and C. carvalhoi, respectively (Fig. 2 and Table 2).

One specimen of C. eleutherodactylus from Estação Biológica de Boracéia (Salesópolis, São Paulo) presented

Table 1. Cycloramphus species, number of individuals, sex, and sampled localities. F: female; M: male; SP: São Paulo state; RJ: Rio de Janeiro state; PR: Paraná state; BA: Bahia state.

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<th>Species</th>
<th>Sample</th>
<th>Locality</th>
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</tr>
<tr>
<td></td>
<td>1F</td>
<td>Paranaípaca, SP (23°46’S, 46°18’W)</td>
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<tr>
<td></td>
<td>1F</td>
<td>Ribeirão Grande, SP (24°05’S, 48°21’W)</td>
</tr>
<tr>
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<td>2M, 1F</td>
<td>São Sebastião, SP (23°45’5S, 45°24’W)</td>
</tr>
<tr>
<td></td>
<td>1F</td>
<td>Ubatuba, SP (23°22’S, 44°50’W)</td>
</tr>
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<td>2M, 1F</td>
<td>Guapimirim, RJ (22°42’S, 42°58’W)</td>
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<tr>
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<td>Iporanga, SP (24°35’S, 48°35’W)</td>
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<td>1M</td>
<td>São José da Vitória, BA (15°09’S, 39°18’W)</td>
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<td>Cycloramphus rhyakonastes</td>
<td>2M, 2F</td>
<td>Morretes, PR (25°23’S, 48°51’W)</td>
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</tbody>
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Fig. 1. Giemsa-stained karyotypes of (a) Cycloramphus acangatan, (b) C. boraceiensis, (c) C. brasiliensis, (d) C. carvalhoi, (e) C. eleutherodactylus from São Paulo, (f) C. eleutherodactylus from Paraná, (g) C. fuliginosus, (h) C. lutzorum, and (i) C. rhyakonastes. Bar = 10 μm.

Table 2. Karyotypic features in Cycloramphus species (m: metacentric; sm: submetacentric; st: subtelocentric; t: telocentric; p: short arm; q: long arm).

<table>
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<td>m</td>
<td>1p</td>
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<td>C. boraceiensis</td>
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<td>m</td>
<td>6q</td>
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<td>C. fuliginosus</td>
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<td>C. fuliginosus</td>
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<td>C. lutzorum</td>
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<td>C. rhyakonastes</td>
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<td>m</td>
<td>6p</td>
<td>52</td>
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*a* Beçak et al. (1970).
*b* Bogart (1970).

only one active Ag-NOR in one homologue of pair 6. Heteromorphism in size of Ag-NOR was frequently observed in C. boraceiensis, C. brasiliensis, C. eleutherodactylus, and C. lutzorum and the secondary constrictions detected in these species corresponded to the Ag-NOR sites.

FISH using 18S rDNA probes was carried out in C. eleutherodactylus from Morretes, Paraná, C. lutzorum and C. rhyakonastes, confirming the previous results detected by silver staining and not revealing the presence of inactive NOR (Fig. 3).

3.3. Constitutive heterochromatin

The C-banding in C. acangatan and C. lutzorum had an exclusively centromeric and pericentromeric heterochromatin pattern (Fig. 4a and g, respectively); in C. boraceiensis the constitutive heterochromatin is distributed in pericentromeric region on all chromosomes and additional C-bands were detected in the telomeric regions of pairs 3 and 4, in the short arms of pair 13 and in the same region of secondary constriction of the pair 6 (Fig. 4b). Besides the pericen-
Fig. 2. Ag-NOR bearing chromosomes of (a) Cycloramphus acangatan, (b) C. boraceiensis, (c) C. brasiliensis, (d) C. carvalhoi, (e) C. eleutherodactylus from Paraná, (f) C. lutzorum, (g) C. rhyakonastes, and (h) C. fuliginosus.

tromeric pattern, C. brasiliensis showed an additional C-band in the long arms in pair 3 (Fig. 4c). Cycloramphus carvalhoi presented a similar pattern of C. brasiliensis and interstitial C-bands in the long arm of pairs 4 and 6 (Fig. 4d). In C. rhyakonastes faint interstitial C-bands were also detected in the proximal region of long arm of pairs 1, 2 and 3, as well as telomeric faint bands on both arms of pair 1 (Fig. 4h).

In C. eleutherodactylus intraspecific variation of heterochromatin distribution was observed. Individuals from three locations in the state of São Paulo showed the pericentricromeric pattern and positive C-bands in the short arms of pair 6, that correspond to the secondary constriction (Fig. 4e), while a specimen from Sengés, in the state of Paraná, showed conspicuous telomeric bands in the short arm of pair 2 (Fig. 4f). The C-banded idiograms, including Ag-NOR bearing chromosomes, of all species are shown in Fig. 5.

4. Discussion

Considering the data available for Cycloramphus species, all presented 26 chromosomes with two distinct fundamental numbers. C. acangatan, Cycloramphus asper (Werner, 1899), C. brasiliensis, C. fuliginosus, C. lutzorum, and C. rhyakonastes have a FN = 52 composed exclusively of biarmed chromosomes, whereas a FN = 50 is characteristic of C. boraceiensis, C. carvalhoi, Cycloramphus dubius (Miranda-Ribeiro, 1920), and C. eleutherodactylus, due to

Fig. 3. Metaphase chromosome spreads of after FISH with an 18S probe: (a) Cycloramphus eleutherodactylus from Paraná, (b) C. lutzorum and (c) C. rhyakonastes. The arrows indicate the hybridization signals in the short arm of pair 6. Bar = 10 μm.

Fig. 4. C-banded karyotypes of: (a) Cycloramphus acangatan, (b) C. boraceiensis, (c) C. brasiliensis, (d) C. carvalhoi, (e) C. eleutherodactylus from São Paulo, (f) C. eleutherodactylus from Paraná, (g) C. lutzorum and (h) C. rhyakonastes. Bar = 10 μm.
Fig. 5. Idiograms of the karyotypes of: (a) *Cycloramphus acangatan*, (b) *C. boraceiensis*, (c) *C. brasiliensis*, (d) *C. carvalhoi*, (e) *C. eleutherodactylus* from PR, (f) *C. lutzorum* and (g) *C. rhyakonastes*. Solid blocks: dark C-bands; Gray blocks: faint C-bands; Circles: Ag-NORs.

the occurrence of one telocentric pair (Brum-Zorrilla and Saez, 1968; Bečak et al., 1970; Bogart, 1970; Silva et al., 2001; Lima et al., 2010; present study). Most karyological studies on the genus have described the chromosomal number and morphology based only on conventional staining. The only exception is the study carried out by Silva et al. (2001), where chromosomal banding patterns in *C. boraceiensis* were described.

The individuals of *C. boraceiensis* studied here showed a karyotype similar to those described by Silva et al. (2001). However, our specimen of *C. fuliginosus* was obtained at São José da Vitória, Bahia, in the furthest northern part of the species distribution area. It has a karyotype distinct from that described by Bogart (1970) for individuals from Tijuca forest, Rio de Janeiro. The main differences between these specimens are due to the presence of two pairs of subtelocentric chromosomes (pairs 1 and 4) in the individual from Bahia, while in all specimens from Rio de Janeiro these pairs are submetacentric. It is presently admitted that *C. fuliginosus*, a stream-adapted species has a disjunct distribution throughout its area of occurrence, confined to the states of Bahia, Espírito Santo, and Rio de Janeiro (Heyer and Maxon, 1983). Based on the above considerations, there are two possibilities to explain the karyological differences among these specimens. The first one is that the specimen karyotyped by Bogart (1970) may have been misidentified because the karyotype described by Bogart (1970) is very similar to the karyotype described for *C. brasiliensis* in the present study, and this latter species is morphologically similar to *C. fuliginosus* occurring also in Rio de Janeiro. Another hypothesis is that these differences might be indicative of two distinct closely related taxa presently identified under the name *C. fuliginosus*, or either that karyotypic differentiation occurs among different populations of this species. The external and internal morphology of specimens belonging to these populations were studied by Heyer (1983) and Verdade (2005), who did not find significant differences. Additional studies are needed to address these issues.

Bogart (1973) and Heyer and Diment (1974) suggested that a $2n = 26$ was the primitive chromosome number for the family “Leptodactylidae” (*sensu* Lynch, 1971), which included the current family Cycloramphidae. However, amphibian systematics has recently undergone major changes and the former Leptodactylidae are now spread into many different families (Frost et al., 2006; Grant et al., 2006; Heinicke et al., 2007; Hedges et al., 2008). The lack of a complete phylogeny for Cycloramphidae limits our interpretation on the origin of $2n = 26$. *Cycloramphus* is currently included in the subfamily Cycloramphinae, which also includes *Crossodactylodes* (Cochran, 1938) ($2n = 26$), *Rhinoderma* (Duméril and Bibron, 1841) ($2n = 26$), and *Zachaenus* (Cope, 1866) ($2n = 26$) (Bečak et al., 1970; Bogart, 1970, 1973; Formas, 1976; Campos, 2010). Cycloramphinae is the sister-group to subfamily Alsodinae, including *Alsodes* (Bell, 1843) ($2n = 22, 26, 30, 34$), *Eup-
sophus (Fitzinger, 1843) (2n = 28, 30), Hylorina (Bell, 1843) (2n = 26), Insectophrynus (Barrio, 1970) (2n = 26), Limnomedusa (Fitzinger, 1843) (2n = 26), Macrognathochlorus (Carvalho, 1946) (2n = 22), Odontophrynus (Reinhardt and Lütken, 1862) (2n = 22, 44), Proceratophrys (Miranda-Ribeiro, 1920) (2n = 22), and Thoropa (Cope, 1865) (2n = 26) (King, 1990; Formas, 1991; Formas et al., 2002; Cuevas and Formas, 1996, 2001, 2003; Silva et al., 2003; Cuevas, 2008; Campos, 2010). This intrafamily variation in diploid number precludes characterizing an ancestral karyotype hypothesis for this group.

Although the diploid number is conserved in the genus Cycloramphus, a difference in chromosome formulae is observed among the karyotypes. Cycloramphus acangatan, C. asper, C. brasiliensis, C. lutzorum, and C. ryakonastes (FN = 52) present karyotypes composed only of biarmed chromosomes. In contrast, karyotypes with one telocentric chromosome pair are found in C. boraceiensis, C. carvalhoi, C. dubius, and C. eleutherodactylus. One possible explanation for these differences would be the occurrence of gross chromosomal rearrangements mainly pericentric inversions, although other rearrangements, like deletions, transpositions, amplifications of satellite DNAs, among others, must also have occurred leading to differentiation of the chromosome morphology (Fig. 1, Table 2).

The karyological differences detected in Cycloramphus are difficult to interpret considering the phylogenetic information available (Heyer, 1983; Heyer and Maxon, 1983; Verdade, 2005). As for the current phylogenies, there is low taxon and character sampling (Heyer, 1983; Heyer and Maxon, 1983) and species groups are poorly supported, except for the relationships between the forest litter dwellers (Verdade, 2005). The species of the genus Cycloramphus are divided into five groups according to morphological similarities (sensu Heyer, 1983). We have studied the species from three of these groups: C. acangatan and C. carvalhoi which are allocated in the C. boliagoglossus group, C. eleutherodactylus allocated in the C. eleutherodactylus group, and the remaining species that are allocated in the C. fuliginosus group. Neither the species of the C. boliagoglossus group, nor those of the C. eleutherodactylus group, which are forest litter dwellers, or the species of the C. fuliginosus group that are stream dwellers have shown chromosomal differences correlated with these habits. The differences observed in chromosome morphology, in the pattern of localization of Ag-NORs, and distribution of constitutive heterochromatin are not consistent with any of these groups, morphologically or eco-morphologically determined. Thus, this framework does not allow us to draw definite conclusions.

Different staining techniques indicated the occurrence of species-specific markers in Cycloramphus (Figs. 2, 4 and 5). Except for C. acangatan and C. lutzorum, which showed only a pericentromeric pattern of distribution of constitutive heterochromatin, the other species exhibited interstitial and telomeric C-bands that characterize each species (Figs. 4 and 5). Cycloramphus boraceiensis presented differences in the pattern of distribution of constitutive heterochromatin. Besides pericentromeric C-bands, the assessed individuals had additional bands in the telomeric regions of pairs 3 and 4, in the short arm of pair 13 and in the region of secondary constriction of pair 6 (Figs. 4b and 5b). Specimens described by Silva et al. (2001) showed additional interstitial bands in pairs 2 and 5 and a much more conspicuous telomeric band in the short arm of pair 4. Interpopulation differences of distribution of constitutive heterochromatin have been described for some anurans, e.g. in Bufo japonicus (Temminck and Schlegel, 1838), Haddadus binotatus (Spix, 1824), Eupemphix nattereri (Steindachner, 1863), Leptodactylus latrans (Steffen, 1815) and Leptodactylus fuscus (Schneider, 1799) (Miura, 1995; Silva et al., 2000; Amaro-Ghilardi et al., 2004; Ananias et al., 2007; Campos et al., 2008) and were related to processes of population differentiation, or even an indicative of a species complex.

Interpopulation polymorphism of C-bands was also observed in C. eleutherodactylus (Fig. 4e and f): the individual from Sengs, Paraná exhibited a conspicuous C-band in the telomeric region of the short arms of pair 2, absent in populations of this species from São Paulo. Cycloramphus eleutherodactylus is the species with the widest distribution in the genus (Heyer, 1983). The differences observed in C-band pattern must be added to the morphological variation considered so far to be a cline (Heyer, 1983; Verdade, 2005; Matos, unpublished data).

The C-banding pattern of pair 3 also deserves to be highlighted. Cycloramphus brasiliensis and C. ryakonastes showed a proximal C-band in the long arm, while C. boraceiensis showed C-bands in the short arm of this pair, indicating the occurrence of pericentric inversion events. A similar pattern was observed in the genus Alsodes (Cuevas, 2008), reinforcing the importance of the role of pericentric inversions in the differentiation of karyotypes of cycloraphids, either followed or not by heterochromatin amplification.

According to the results using FISH and chromosome banding, the NORs were found to be adjacent or embedded in C-banded heterochromatin (Figs. 2–5). In six out of eight Cycloramphus species analyzed here, chromosome 6 was identified as the Ag-NOR bearing, but the position of these Ag-NORs varied: interstitial portion of the short arm of C. eleutherodactylus, C. lutzorum, and C. ryakonastes; interstitial region of the long arm of C. brasiliensis and C. carvalhoi and in the proximal region of the long arm of C. boraceiensis. These changes in the localization of Ag-NORs could be explained by intrachromosomal rearrangements, like pericentric and paracentric inversions.

A different situation was observed in C. acangatan, which showed Ag-NOR in the short arm of pair 1, while C. fuliginosus showed multiple Ag-NOR in pairs 1 and 4 (Fig. 2a and h, respectively). This condition could be due to interchromosomal rearrangements, e.g. transposition or
translocation, which may lead to the ribosomal gene loci to other chromosomes, mainly when there is constitutive hetereochromatin associated with NOR. This situation has already been reported for the majority of cases of NOR variability in the genome of several vertebrates groups (Rui
e et al., 1981; Reed and Phillips, 1995; Woznicki et al., 2000; Datson and Murray, 2006).

A single pair of Ag-NOR in the genome represents the primitive condition for most vertebrate species (Hsu et al., 1975; Schmid, 1978; Amemiya and Gold, 1988). Therefore, the occurrence of more than one Ag-NOR-bearing chromosome pair, as observed in C. fuliginosus can be considered an apomorphy (Hsu et al., 1975). Additional analysis by FISH will be useful to confirm the number and location of the true NOR in the species. Besides, size heteromorphism between homologous is frequent in some species. Rearrange-
ments, such as spontaneous deletions, duplications or unequal recombination are important evolutionary processes that act over repetitive sequences in tandem (Dover, 1986), which may originate heteromorphisms. Heteromorphic NORs could also be related to differences in genetic activity (Schmid, 1978; Amaro-Gihlardi et al., 2008).

The apparently highly conserved diploid number within Cycloramphus species would fit as an outcome of karyo-
typic orthoselection evolution, characterized by pericentric inversions, which has conserved the basic karyotype in the species. Our comparative study, including various Cycloram-
plus species, revealed species-specific karyotypes both in conventional and differential staining, which improves our understanding of the karyotype evolution within this genus and may contribute to future phylogenetic studies.

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