# Cytogenetics of a new species of *Paratelmatobius cardosoi* group (Anura: Leptodactylidae), with the description of an apparent case of pericentric inversion

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**Abstract.** The karyotype of a new species of *Paratelmatobius* from the *P. cardosoi* group is described. As with other *Paratelmatobius* and *Scythrophrys* karyotypes, *Paratelmatobius* sp. (aff. *cardosoi*) shows a diploid number of 24 chromosomes, in addition to other similarities with the former karyotypes. The *Paratelmatobius* sp. (aff. *cardosoi*) karyotype differs from that of *P. cardosoi* in the morphology of pair 4, the NOR location and the C-bands in pairs 3 and 8 (exclusive to *Paratelmatobius* sp.) and those of pairs 7 and 9 (exclusive to *P. cardosoi*). Both karyotypes also differ in the amount of heterochromatin in pair 1. The presence of interstitial heterochromatin in the long arm of pair 1 and the interstitial C-bands in both arms of chromosome 5 are apparently synapomorphic characters of *P. cardosoi* and *Paratelmatobius* sp. (aff. *cardosoi*), since they are absent in the other *Paratelmatobius* and *Scythrophrys* karyotypes. In *Paratelmatobius* sp. (aff. *cardosoi*), the nucleolus organizer region is on the short arm of a small metacentric chromosome (pair 9), an arrangement similar to the NOR-bearing chromosome pair in the karyotype of *P. poecilogaster* and in karyotype II of *Scythrophrys*. A conspicuous heteromorphism unrelated to the sex determining mechanism was also observed and probably arose from a pericentric inversion.

## Introduction

The genus *Paratelmatobius* Lutz and Carvalho comprises five species placed in two groups (Pombal Jr. and Haddad, 1999). The *P. cardosoi* group includes *P. cardosoi* and *P. mantiqueira* while the *P. lutzii* group contains *P. lutzii*, *P. poecilogaster*, and probably *P. gaigeae*. All *Paratelmatobius* species are endemic to Brazil and *P. mantiqueira*, *P. lutzii* and *P. gaigeae* were considered as missing species by those authors.

Comparative cytogenetic analyses of living species of *Paratelmatobius* (Lourenço et al., 2000), *P. lutzii* (De Lucca et al., 1974) and genus *Scythrophrys* (Lourenço et al., in press) have shown several similarities between these genera and have allowed the evolutionary study of some chromosomal characters in this group.

In the present paper, we describe the karyotype of a new species of *Paratelmatobius* in the *P. cardosoi* group and reassert the evolutionary hypothesis proposed in our previous studies of *Paratelmatobius* and *Scythrophrys* (Lourenço et al., 2000, in press). An interesting case of an autossomal inversion is also described. This new species, here called *Paratelmatobius* sp. (aff. *cardosoi*), was found in the Serra do Mar in the State of Paraná, Brazil, and differs from *P. cardosoi* by its advertisement call.

## Materials and methods

Six males and two females of *Paratelmatobius* sp. (aff. *cardosoi*) collected from Piraquara, Paraná, Brazil, in October 1999 were studied cytogenetically. Chromosome preparations were obtained from intestinal and testes cell suspensions, as described by Schmid (1978) and Schmid et al. (1979). Conventional staining with a 10% Giemsa solution, the C-banding technique (King, 1980) and the Ag-NOR method (Howell and Black, 1980) were done. Chromosomes were classified according to Green and Sessions (1991). All the specimens were deposited in Célio F.B. Haddad collection (CFBH), Departamento de Zoologia, Universidade Estadual Paulista, Rio Claro, SP, Brasil.

## Results

The diploid complement of *Paratelmatobius* sp. (aff. *cardosoi*) consists of 24 chromosomes, with six pairs of metacentric chromosomes and six of submetacentric chromosomes (figs 1-3, 5; table 1). In all the specimens analyzed, a conspicuous secondary constriction was observed in the short arm of homologue 9 in Giemsa-stained metaphases (fig. 1) and was identified as an NOR site in silver-stained preparations (fig. 2). Additionally, a smaller secondary constriction was observed interstitially in the long arm of chromosome 5 in more decondensed metaphases (fig. 1a). All the centromeric regions of the *Paratelmatobius* sp. (aff. *cardosoi*) karyotype showed a small amount of constitutive heterochromatin (fig. 3). Non-centromeric heterochromatic bands were easily visualized in pairs 1, 3, 5, and adjacent to the NOR in pair 9 (fig. 3). In some metaphases, a small telomeric C-band was observed in the short arm of pair 8 (fig. 3). Considering the small size of this band, it was probably not detected in all the metaphases because of technical problems in performing C-banding.

An interesting dimorphism was noted for pair 1 in one of the females. In this specimen, pair 1 was deeply heteromorphic in all metaphases analyzed (figs 1-3). While one homologue (1a) was a submetacentric chromosome with an arm ratio of 1.72, the other homologue (1b) could almost be considered a subtelocentric chromosome since its arm ratio was 2.88 and, according to the classification system of Green and Sessions (1991), the

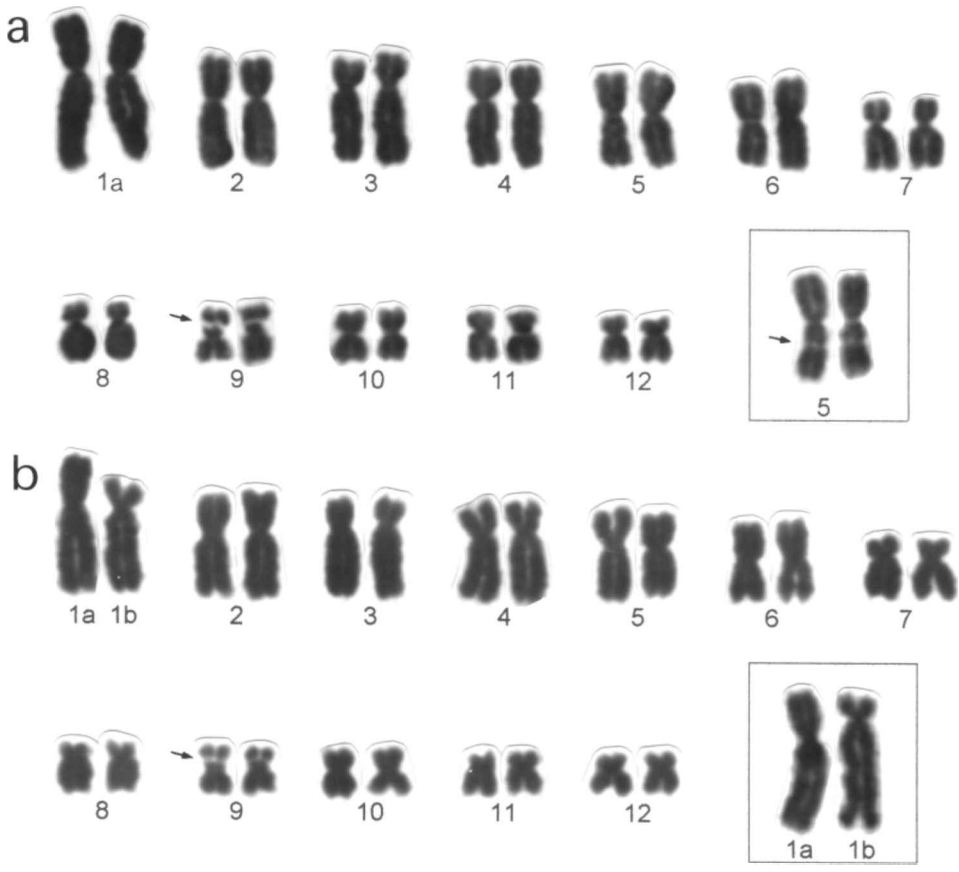


**Figure 1.** *Paratelmatobius* sp. (aff. *cardosoi*) karyotype. In **a**, a specimen with a homomorphic pair 1. The inset shows a secondary constriction in pair 5 (arrow). In **b**, a specimen with a heteromorphic pair 1. The inset shows another pair 1 of the same specimen. Note the variable size of morph 1b relative to 1a. The secondary constriction of the NOR in pair 9 is also shown in **a** and **b** (arrow).

arm ratio of a subtelocentric chromosome is 3.01-7.00. These morphs of pair 1 also differed in their C-banding pattern. Thus, while morph 1a showed interstitial heterochromatin in its long arm and nearly all its short arm was heterochromatic, in morph 1b, the long arm was heterochromatic and no heterochromatin was detected in the short arm (fig. 3). Intercellular variation was observed in the size of chromosome 1b relative to its homologous chromosome 1a. While in some metaphases morphs 1a and 1b showed almost the same size, in others, chromosome 1b was clearly smaller than 1a (fig. 1b).

The meiotic analysis of four males showed that, in 92% of 119 diakineses, the homomorphic pair 1 had a clear rod-like configuration, probably the result of a terminal association of the long arms (fig. 4). Such an association was particularly identifiable in

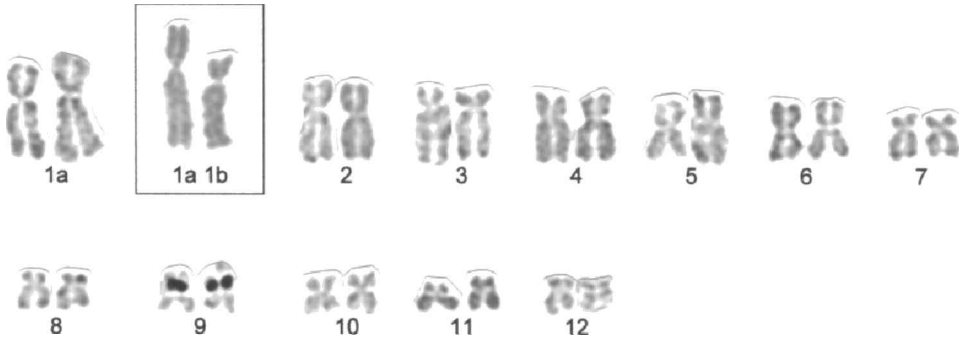


**Figure 2.** Silver-stained karyotype. Note the active NOR in pair 9. The inset shows the heteromorphic pair 1 in one female specimen.

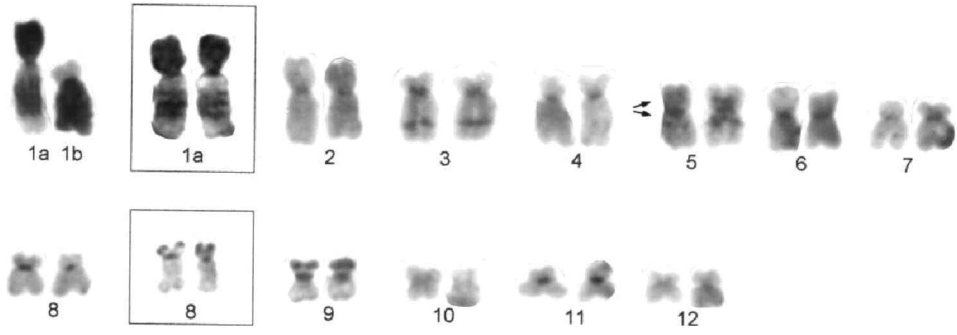


**Figure 3.** C-banded karyotype. The insets show a homomorphic pair 1, and a pair 8 with a clear telomeric C-band in the short arm. The small interstitial bands of pair 5 are indicated by arrows.

**Table 1.** Morphometric data based on measurements from 39 metaphases of *Paratelmatobius* sp. (aff. *cardosoi*).

| | Chromosomes | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 1b | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| r.l. | 14.44 | 11.96 | 11.47 | 10.66 | 10.42 | 9.40 | 8.69 | 6.98 | 5.72 | 5.54 | 5.32 | 4.94 | 4.77 |
| a.r. | 1.72 | 2.88 | 1.58 | 2.30 | 1.96 | 1.25 | 1.15 | 1.88 | 1.91 | 1.08 | 1.26 | 1.71 | 1.32 |
| c.c. | *sm* | *sm* | *m* | *sm* | *sm* | *m* | *m* | *sm* | *sm* | *m* | *m* | *sm* | *m* |

r.l.: relative length (%); a.r.: arm ratio; c.c.: centromeric classification; m: metacentric; sm: submetacentric; st: subtelocentric.

C-banded diakinesis in which the heterochromatic markers of chromosome 1 (almost the whole arm and an interstitial band in the long arm) could be seen (fig. 4b).

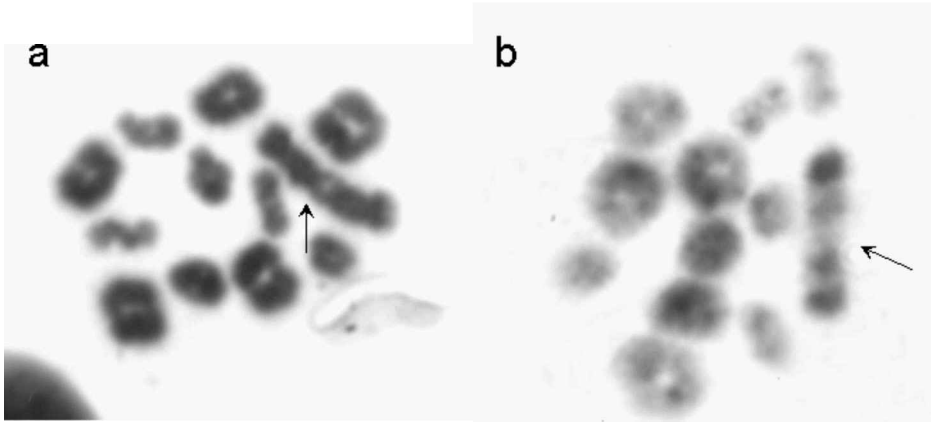


**Figure 4.** Giemsa-stained (**a**) and C-banded (**b**) diakineses from a specimen with homomorphic pair 1. Note the rod-like configuration of this pair and the position of the short arms. The terminal association of the long arms is indicated (arrow).

## Discussion

*Interspecific analysis*

The diploid number of *Paratelmatobius* sp. (aff. *cardosoi*) is the same as in *P. cardosoi*, *P. poecilogaster* (Lourenço et al., 2000), and *P. lutzii* (De Lucca et al., 1974). The morphology of several chromosome pairs is also very similar among these species (fig. 5). The *Paratelmatobius* sp. (aff. *cardosoi*) karyotype differs from those of *P. poecilogaster* and *P. lutzii* especially in pair 6, which is metacentric in *Paratelmatobius* sp. (aff. *cardosoi*) and subtelocentric in *P. poecilogaster* and *P. lutzii.* On the other hand, pair 4 of *Paratelmatobius* sp. (aff. *cardosoi*) differs morphologically from that pair 4 of *P. cardosoi.* The location of the NOR also distinguishes *Paratelmatobius* sp. (aff. *cardosoi*) from *P. cardosoi*, since in *P. cardosoi* pair 7 is the NOR-bearing chromosome pair. Apart from the heterochromatic bands of pair 4 of *P. cardosoi*, which are characteristic of this species, the non-centromeric bands of pairs 7 and 9 of *P. cardosoi* are also not found in the *Paratelmatobius* sp. (aff. *cardosoi*) karyotype. The interstitial band in pair 3 and the telomeric band in pair 8 that are present in *Paratelmatobius* sp. (aff. *cardosoi*) do not occur in *P. cardosoi.* Additionally, while in *P. cardosoi* the short arm of chromosome 1 has an interstitial C-band, nearly all the short arm of chromosome 1a in *Paratelmatobius* sp. (aff. *cardosoi*) is heterochromatic. All of these differences provide a clear karyological distinction between the specimens studied here and *P. cardosoi*, and corroborate that *Paratelmatobius* sp. (aff. *cardosoi*) is an undescribed species.

Both karyotypes have interstitial heterochromatin in the long arm of pair 1 (morph 1a in *Paratelmatobius* sp.), although the amount of this heterochromatin is greater in *Paratelmatobius* sp. (aff. *cardosoi*) than in *P. cardosoi.* In addition, pair 5 of *Paratelmatobius* sp. (aff. *cardosoi*) strongly resembles pair 5 (especially morph 5b) of *P. cardosoi.* The

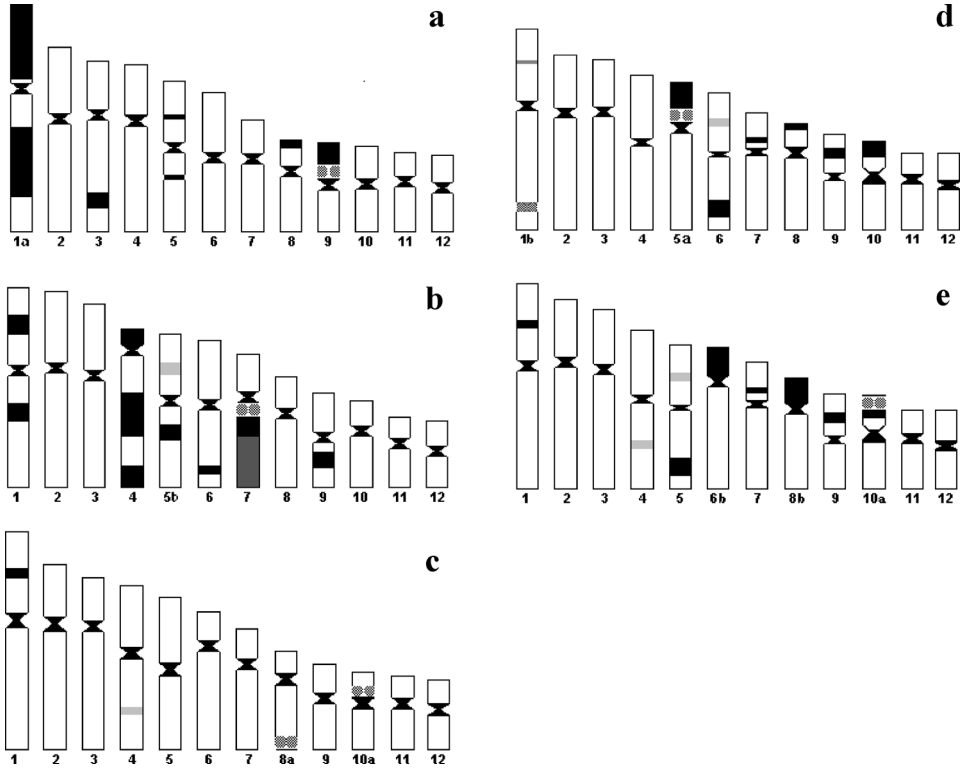


**Figure 5.** Idiograms of the karyotypes of *Paratelmatobius* sp. (aff. *cardosoi*) (**a**), *P. cardosoi* (**b**; based on Lourenço et al., 2000), *P. poecilogaster* (**c**; based on Lourenço et al., 2000), group I and II of *Scythrophrys* (**d** and **e**; based on Lourenço et al., 2001, and manuscript in preparation). Solid blocks: dark C-bands. Gray blocks: faint C-bands. Open regions: secondary constrictions. Checkered circles: NORs.

similar morphology and C-banding pattern in both arms of these chromosomes is a further evidence of their homeology. The long arm of both these chromosomes also has a secondary constriction. Interstitial heterochromatin in the long arm of chromosome 1 and the C-banding pattern of chromosome 5 mentioned above were not found in *Paratelmatobius poecilogaster* (Lourenço et al., 2000). Also, such characteristics were not seen in the genus *Scythrophrys* (Lourenço et al., in press; fig. 5), which is closely related to *Paratelmatobius* (Heyer, 1975; Garcia, 1996; Lourenço et al., in press). Although one specimen of *Scythrophrys* showed a faint C-band in the long arm of one homologue of pair 1, it differed from the interstitial heterochromatin in the long arm of pair 1 of *P. cardosoi* and *Paratelmatobius* sp. (aff. *cardosoi*) also because it was associated with a NOR in that *Scythrophrys*. So, we conclude that those characteristics of pairs 1 and 5 of *P. cardosoi* and *Paratelmatobius* sp. (aff. *cardosoi*) can be synapomorphic characters that assemble these species.

The NOR-bearing chromosome pair of *Paratelmatobius* sp. (aff. *cardosoi*) is probably homeologous to pair 10 of *P. poecilogaster* and to pair 10 of karyotype II of *Scythrophrys*, both of which carry a NOR (Lourenço et al., 2000, in press). The classification of this pair as 9 in the *Paratelmatobius* sp. (aff. *cardosoi*) karyotype and not as pair 10 (as in the other karyotypes) is probably because of a conspicuous C-band between the NOR and the telomere of the short arm of this chromosome, which is absent in pair 10 of *P. poecilogaster* and of karyotype II in *Scythrophrys*. This heterochromatic band is apparently responsible for the greater size of the short arm of that chromosome in *Paratelmatobius* sp. (aff. *cardosoi*).

The intrachromosomal location of the NOR in pair 10 of karyotype II of *Scythrophrys* differs slightly from that in pair 10 of *P. poecilogaster* and pair 9 of *Paratelmatobius* sp. (aff. *cardosoi*). In *Scythrophrys*, the NOR is more terminal, while in *Paratelmatobius* sp. (aff. *cardosoi*) and in *P. poecilogaster* it is proximal to the centromere. This variation may have been generated by a hypothetical paracentric inversion. Anyway, the location of a NOR in a small metacentric/submetacentric chromosome is an ancestral characteristic relative to the other NOR site present in *Paratelmatobius* (in pairs 7 and 8; Lourenço et al., 2000) and *Scythrophrys* (in pairs 1 and 5), as proposed elsewhere (Lourenço et al., in press). Thus, the location of the NOR in pair 10 of *P. poecilogaster* and in the homeologous pair 9 of *Paratelmatobius* sp. (aff. *cardosoi*) cannot be used to group these species separately from *P. cardosoi*, which does not have this NOR.

The comparison between the *Paratelmatobius* sp. (aff. *cardosoi*) and *Scythrophrys* karyotypes also showed that, as in *Paratelmatobius* sp. (aff. *cardosoi*), karyotype I of *Scythrophrys* has a telomeric C-band in the short arm of pair 8, whereas in karyotype II the entire arm of this chromosome is heterochromatic. If the presence of heterochromatin in this region is considered a homeologous characteristic, then the common ancestor of *Paratelmatobius* and *Scythrophrys* must have this heterochromatin before being lost in *P. poecilogaster* and *P. cardosoi*. An alternative hypothesis is that the telomeric C-band in pair 8 of *Paratelmatobius* sp. (aff. *cardosoi*) is not homeologous to the non-centromeric heterochromatin of the *Scythrophrys* karyotypes so that these heterochromatin arose independently in these genera.

Overall, these cytogenetic data agree with the two groups of *Paratelmatobius* proposed by Pombal Jr. and Haddad (1999), and allow the inclusion of *Paratelmatobius* sp. (aff. *cardosoi*) in the *P. cardosoi* group.

*Intraspecific variation in chromosome pair 1*

Of the eight specimens of *Paratelmatobius* sp. (aff. *cardosoi*) examined, one female showed heteromorphism in pair 1 that was easily detected in all preparations. While chromosome 1a of *Paratelmatobius* sp. (aff. *cardosoi*) was similar in morphology to chromosome 1 of the other *Paratelmatobius* and *Scythrophrys* species (see above), the unusual morph 1b of the karyotype described here was observed only in *Paratelmatobius*

sp. (aff. *cardosoi*). One possible mechanism to explain the appearance of morph 1b could be a pericentric inversion in morph 1a. This hypothesis is supported by the variation in the arm ratios of the morphs and by their distinct patterns of heterochromatin distribution. However, this mechanism cannot fully explain the decrease in the amount of euchromatin in morph 1b compared to 1a, particularly since this decrease may have arisen from a deletion or heterochromatinization.

The influence of this heteromorphism on the meiotic pairing/crossing of pair 1 was not evaluated because this phenomenon was seen in only one female. However, the meiotic analysis of homomorphic pairs of chromosome 1 suggested that the large amount of heterochromatin present impaired correct pairing/crossing and prevented the ring configuration. Such an influence of heterochromatin has been reported for several organisms (see review by John, 1988).

The heteromorphism in pair 1 of *Paratelmatobius* sp. (aff. *cardosoi*) was not related to the sex determining mechanism since in another female examined the morphs were homomorphic, as was the case for males.

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