



Short Communication

## The lowest diploid number in Testudines: Banding patterns, telomeric and 45S rDNA FISH in *Peltocephalus dumerilianus*, $2n = 26$ and $FN = 52$ (Pleurodira, Podocnemididae)

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### Abstract

The karyotype of the big-headed Amazon River turtle, *Peltocephalus dumerilianus*, is characterized based on a sample of seven juveniles from Reserva Biológica do Rio Trombetas, Pará State, Brazil (1°30' S, 56°34' W). Here we present the first results on GTG and CBG-banding patterns, Ag-NOR staining and FISH, with telomeric and 45S rDNA sequences as probes. A cytogenetic comparison with related Podocnemididae is also provided.

**Key words:** big-headed Amazon River turtle, molecular cytogenetics, Pelomedusoides.

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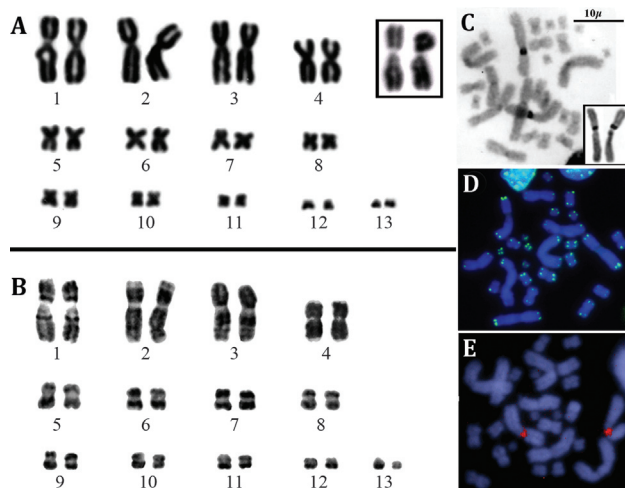
The big-headed side-neck river turtle, *Peltocephalus dumerilianus* (Schweigger, 1812), occurs in the Amazon region and belongs to the superfamily Pelomedusoides (approximately 24 living species), which comprises the families Pelomedusidae, with two living genera: *Pelomedusa* and *Pelusios* represented by one, and at least 15 species, respectively; and Podocnemididae, with three living genera: the monotypic *Erymnochelys* and *Peltocephalus*, and *Podocnemis* comprising six species (Ayres *et al.*, 1969; Vitt and Caldwell, 2009). In Podocnemididae cytogenetic data are scarce and based mostly on conventional staining. The *Podocnemis* and *Erymnochelys* species (*P. erythrocephala*, *P. expansa*, *P. lewyana*, *P. sextuberculata*, *P. unifilis*, *P. vogli* and *E. madagascariensis*) present a diploid number ( $2n$ ) of 28, with a karyotype composed of five macrochromosomes (M) and nine microchromosomes (m) (Ayres *et al.*, 1969; Huang and Fred Clark 1969; Rhodin *et al.*, 1978; Bull and Legler, 1980; Fantin and Monjeló, 2011; Gunski *et al.*, 2013). The exception is *Peltocephalus dumerilianus* that presents  $2n = 26$  ( $FN = 52$ ) with 4 M and 9 m, the lowest diploid number in Testudines (ranging from  $2n = 26$  to  $2n = 96$ ) (Ayres *et al.*, 1969; Bull and Legler, 1980). The available cytogenetic data for this species report a karyotype that is similar to those of other Podocnemididae, in which differentiated sex chromosomes are absent and a conspicuous secondary constriction is observed

on the proximal region of chromosome 1p (Ortiz *et al.*, 2005; Fantin and Monjeló, 2011).

Herein, based on a sample of seven juveniles from Reserva Biológica do Rio Trombetas (1°30' S, 56°34' W), Amazonian forest of Pará State, Brazil, the karyotype of *P. dumerilianus* was characterized for the first time using routine differential techniques, such as GTG, CBG-banding and Ag-NOR staining (Seabright, 1971; Sumner, 1972; Howell and Black, 1980, with modifications) and molecular tools employing FISH with telomeric and 45S rDNA sequences as probes in metaphases obtained from *in vivo* bone marrow preparations (Ford and Hamerton, 1956).

Briefly, FISH with telomeric probes was performed using a Telomere PNA FISH Kit/FITC (K 5325, Dako) following the manufacturer's protocol. The FISH procedures for the 45S rDNA were followed the method adapted by Cabral-de-Mello *et al.* (2010). The 45S rDNA probes were biotinylated by nick-translation (Invitrogen, San Diego, CA, USA) and detected with avidin-Cy3 (Life-Technologies). All slides were counterstained with DAPI diluted in Vectashield (Vector) and analyzed using a fluorescence microscope (Zeiss Axiophot) equipped with software for image capture Isis karyotyping system, MetaSystems).

For all individuals, at least 20 metaphases were analyzed for determining the  $2n = 26$  and  $FN = 52$  karyotype, as described by Ayres *et al.*, 1969, with a conspicuous secondary constriction on pair 1 (Figure 1A). GTG-banding patterns allowed the identification and the pairing of all chromosomes (Figure 1B). CBG-bands were tenuous at the pericentromeric region of most pairs, except for pair 1



**Figure 1** - Karyotype (A and B) and metaphases (C-E) of *Peltocephalus dumerilianus*,  $2n = 26$  and  $FN = 52$ . (A) Conventional staining. Inset, pair 1 from other metaphase showing the conspicuous secondary constriction. (B) GTG-banding pattern. (C) CBG-banding pattern. Note the conspicuous C-positive bands on pair 1. Inset, pair 1 bearing positive Ag-NORs. Scale bar = 10  $\mu$ m. (D) FISH with telomeric probes. Positive signals (green) are seen at the termini of all chromosomes. (E) Mapping of 45S rDNA. Positive FISH signals (red) are at the secondary constriction region of pair 1.

which exhibited a large block of constitutive heterochromatin at the secondary constriction region. Ag-NOR staining also showed positive NORs at the same region on chromosome 1p (Figure 1C). The FISH analysis showed telomeric signals restricted to the ends of the chromosomes (Figure 1D), and 45S rDNA sites were localized exclusively at the secondary constriction region of pair 1 (Figure 1E).

In Podocnemididae, the NOR-bearing pair was identified in three of the six living species of *Podocnemis* (*P. expansa*, *P. sextuberculata* and *P. vogli*) (Ortiz *et al.*, 2005; Fantin and Monjeló, 2011) and the staining region corresponds, as in *Peltocephalus*, to the secondary constriction of chromosome 1, characterizing until now a conserved condition for the family. On the other hand, CBG-banding patterns are quite specific when compared to the chromosomes of *Podocnemis vogli* which present larger amount of heterochromatin distributed at pericentromeric and interstitial regions of the chromosomes, as described by Ortiz *et al.* (2005). Both data suggest that despite the similar diploid number, chromosomal morphology and the NOR-bearing pair, the CBG-banding pattern allows to identify species-specific karyotypes, revealing chromosomal differences that accumulated during karyotype evolution. At present, a more adequate interspecific chromosomal comparison is not possible, as the differences in chromosomes condensation presented in the published data render unreliable the establishment of similarities in banding patterns. Future chromosomal comparisons using CBG and GTG-banding patterns, as well as mapping of the 45S rDNA and telomeric sites in other representatives, could be useful to identify the

rearrangements involved in karyotypic differentiation of Podocnemididae.

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