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CHROMOSOMAL ANALYSIS OF *CHELONOIDIS CARBONARIA* AND *CHELONOIDIS DENTICULATA* KEPT IN CAPTIVITY

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Introduction: Chelonians, an order of the class *Reptilia* are mostly long lived animals with relatively small capacity for rapid population growth (POUGH; HEISER; MCFARLAND, 1999). The most widely distributed genus is the *Chelonoidis*, which comprises 22 species found in South America, Africa, Asia and the Oceanic Islands. In Brazil, two species of turtles come from humid forests, the *Chelonoidis carbonaria* and *Chelonoidis denticulata*. The devastation and deforestation of these forests are pointed out as one of the reasons for species of this genus to spread and even mate with each other. Hybrids may compete for resources with parental species, favored by "hybrid vigor" or, if they are fertile, have an impact on the genetic integrity of wild populations due to the potential risk of backcrossing with consequent introgression. Hybridization can pose a threat to small populations, even when the gene sets do not mix. There are records of hybrids involving species of the same suborder, with the genus *Trachemys* (JACKSON, 2010). In order to provide subsidies for the improvement of sustainable biological management and conservation programs as well as for future projects to be developed with chelonians, this work aimed the characterization of the karyotypes by means of conventional staining, chromosome banding and mapping of some repetitive DNAs in two *Chelonoidis* kept in captivity. **Methods and Materials:**This work analyzed 28 specimens of the genus *Chelonoidis* kept in captivity at the Wildlife Medicine and Research Center from Botucatu/SP and at the Municipal Ecological Park from Americana/SP, Brazil. The morphological characterization between the two species was carried out according to Siqueira, Silva and Moral (2004). The material collection was performed in order to provide the lowest possible risk to the physical integrity of the animal. For the cytogenetic analyzes, samples of 3 mL of peripheral blood were obtained by puncturing the caudal vein. To obtain metaphase chromosomes, it was applied the technique described by Moorhead *et al.* (1960). The banding techniques employed included C-banding by Sumner (1972), the sequencial staining CMA_3/DA/DAPI by Schweizer *et al.* (1983) and Ag-NOR-banding by Rufas *et al.* (1987). In addition, slides were submitted to the fluorescence *in situ* hybridization (FISH) procedure employing the 18S rDNA and telomeric motif (TTAGGG)n as probes. The 18S rDNA probe was obtained from the genomic DNA of *C. carbonaria* using the protocol by Sambrook and Russel (2001). The Polimerase Chain Reaction (PCR) was performed by using the primers Sca18SF and Sca18SR (CABRAL-DE-MELLO; MOURA; MARTINS, 2010). Telomeric probe was obtained by PCR using the complementary primers (TTAGGG)5 and (CCCTAA)5 according to (IJDO *et al.*, 1991). The procedures and animal handling were authorized by the Ethical Committee for Animal Research of the São Paulo State University (Unesp), Brazil (protocol 217/10-CEEA). **Results:** By means of morphological analyzes, 20 specimens were defined as *C. denticulata* and eight as *C. carbonaria*.

Five metaphases from each animal were analysed with standard Giemsa staining. The karyotype of *C. denticulata* presented eight pairs of macrochromosomes (MACs) of submetacentric and metacentric morphology (A); six pairs of MACs of acrocentris and telocentric morphology (B); 12 pairs of microchromosomes (MICs) (C) (Figure 1). For the *C. carbonaria*, the karyotype presented nine pairs of MACs of submetacentric and metacentric morphology (A); five pairs of MACs of acrocentric and telocentric morphology (B); 12 pairs of MICs (C) (Figure 1). The presence of MICS as well as the absence of heteromorphic sex chromosomes is quite common in this group of vertebrates.

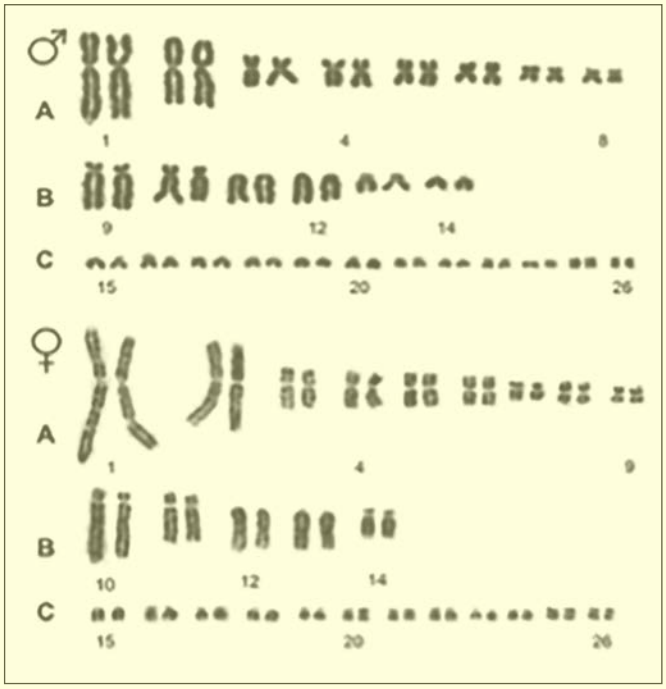

Figure 1 - Karyotype of *Chelonoidis denticulata* (male) 2n=52, 8:6:12 and karyotype of *Chelonoidis carbonaria* (female) 2n=52, 9:5:12.
Source: Personal file.

Differential chromosomal analysis and mapping of repetitive DNA sequences were performed on *C. carbonaria* specimens. The C-banding technique allowed the identification of small constitutive heterochromatin blocks located in the centromeric regions of almost all chromosomes. Moreover, an additional heterochromatic block was observed in one of the arms of a pair of MICs that probably corresponds to the rDNA sites (Figure 2a). CMA3 positive blocks were observed, besides in the centromeric heterochromatin, in the terminal region of some chromosomes, including a large block in a MIC pair, which probably correspond to the rDNA site (Figure 2b). DAPI staining did not revealed signals (Figure 2c). The 18S rDNA was located in a MIC pair, like observed under silver sitrate analysis, that located the NORs (Figure 2d, 2e). Telomeric probe revealed terminal sites corresponding to telomeres. Additionally, in a pair from Group B, centromeric labeling corresponding to ITS (Internal Telomeric Sequence) regions was observed, suggesting occurrence of chromosomal rearrangement or occurrence of heterochromatin rich in TTAGGG sequence (Figure 2f).

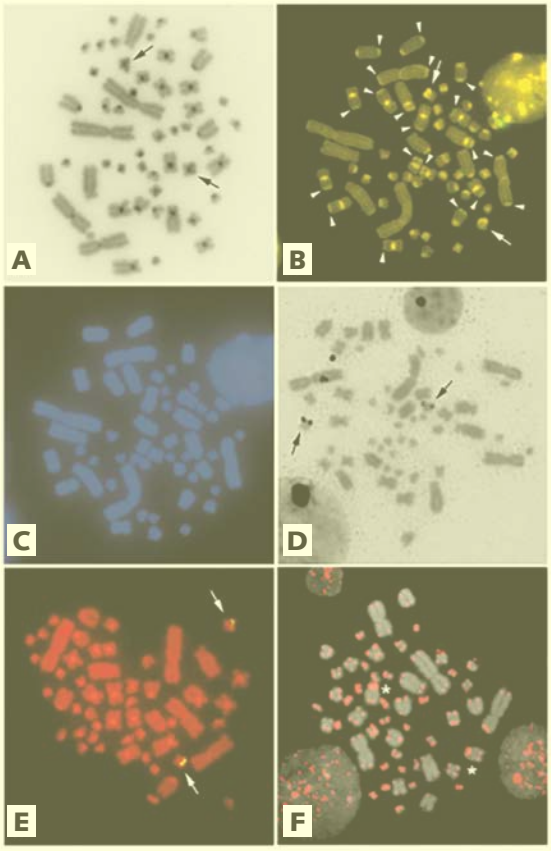

Figure 2 - Metaphase of *Chelonoidis carbonaria* differentially stained (A-D) and hybridized with repetitive DNA probes (E, F). (A) C-banding, (B) CMA_3, (C) DAPI, (D) Ag-NOR, (E) 18S rDNA, (f) Telomeric sequence. The arrows in (A, B, D, E) indicate the pair harboring NORs and the arrowheads in (B) indicate some terminal CMA_3^+ blocks.
Source: Personal file.

Discussion and conclusion: The diploid number observed for the two species were also observed in some species from the same genus. On the other hand, the quantity of MACs and MICs and chromosomal morphologies differs between species, suggesting chromosomal reorganization in the genus. It is evident in the two species studied here, in which the number of chromosomes of group A and B are different. For wild animals, the cytogenetics analysis reflect a more descriptive aspect describing chromosomal number and morphology, identification of possible chromosomal markers and applications of staining techniques. The present study revealed that it is possible to study the karyotype of chelonians using economical and viable protocols without harming the animal and preserving it in its own habitat. However, in programs aimed at the reproduction of these animals, it is necessary to identify the species correctly, in such a way that their genetic patrimonies coul be preserved and not decharacterized by hybridization.

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CRIMINALISTICS IN VETERINARY MEDICINE. CLASS WITH GRADUATE STUDENTS

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Introduction: The Chair of Legislation of the Catholic University of Cordoba, Argentina, in the unit: Veterinary Legal, implemented as an organizational teaching modality: practical classes carried out in the field, on the theme scene of crime scene. The purpose of these classes is to contribute and to obtain competences in academic knowledge related to the forensic sciences and their ethical application in legal causes; instruct the veterinarian to act as an expert in the face of judicial or extrajudicial requirements, incorporate