Amphibia-Reptilia 8 (1987): 171-178, E.J. Brill, Leiden

Karyotype of the sea-turtle *Dermochelys coriacea* (Vandelli, 1761)

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Abstract. Chromosomes of *Dermochelys coriacea* were prepared from kidney, spleen and lung cells of three neonates hatched from eggs incubated at 26.5 ± 0.5° C (a temperature yielding 100% phenotypic males). A study of the karyotype shows a diploid number of 56 chromosomes consisting of 14 meta- and submetacentrics, 10 telo- and subtelocentrics, and 32 microchromosomes. This karyotype, which is similar to those previously described for *Chelonia mydas, Caretta caretta*, and *Eretmochelys imbricata*, has been considered to be primitive for cryptodiran turtles. The implication of our results for the phylogenetic classification of *Dermochelys coriacea* is discussed.

Résumé. Des chromosomes de *Dermochelys coriacea* ont été préparés à partir de cellules rénales, spléniques et pulmonaires de trois nouveau-nés issus d'œufs incubés à 26,5 ± 0,5°C (température donnant 100% de mâles phénotypiques). L'étude caryologique montre que le nombre diploïde de chromosomes est de 56, se répartissant en 14 métacentriques et submétacentriques, 10 télocentriques et subtélocentriques et 32 microchromosomes. Ce caryotype qui est similaire à celui décrit chez d'autres tortues marines (*Chelonia mydas, Caretta caretta, Eretmochelys imbricata*), a été considéré comme primitif pour les tortues cryptodires. L'implication de nos résultats pour la classification phylogénétique de *Dermochelys coriacea* est discutée.

Introduction

Over the past decade, karyological studies have been carried out in numerous species of the two suborders of living turtles, Pleurodira and Cryprodira (Bickham, 1983). Karyotypes for 13 of 14 genera of pleurodiran, side-necked turtles, have recently been described (Bull and Legler, 1980). Among cryptodiran turtles, karyological data are available for 55% of the species including members of all but one family, the Dermochelyidae (Bickham, 1983; Bickham and Carr, 1983). This family is represented by only one extant marine species, *Dermochelys coriacea*, the largest of living turtles.

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Knowledge of the karyotype of this species is of special interest, since its phylogenetic relationship with the other sea-turtles is still questioned (Rhodin et al., 1981). We have examined the karyotype of *Dermochelys coriacea* and present here the C-, Q- and R-banding patterns of the chromosomes.

Materials and Methods

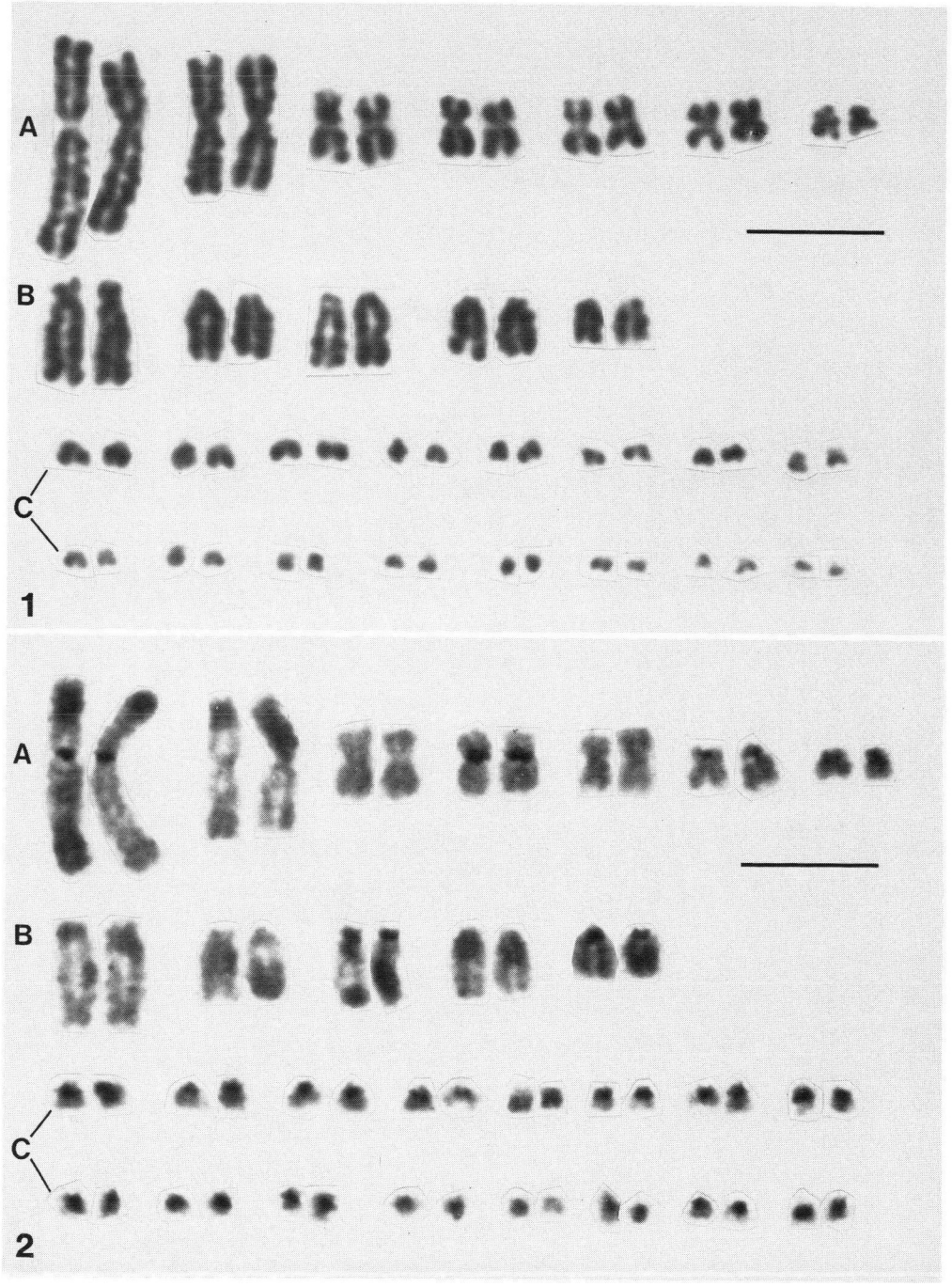
Eggs of *Dermochelys coriacea* were collected immediately after being laid in July 1984, on the beach of "les Hattes", in French Guiana. They were placed in styrofoam boxes with moist sand and transported to the Muséum National d'Histoire Naturelle in Paris, France. Incubations were performed in the laboratory at 26.5 ± 0.5° C. At this temperature all individuals acquire a male phenotype (Rimblot et al., 1985). The duration of embryonic development was 84 days.

Three animals from the same clutch were sacrificed by decapitation two days after hatching, and karyotypes were prepared for each individual. Kidney, spleen and lung cell suspensions were obtained by mechanical disruption of the tissues through metallic grids. Kidney cells were used directly for spreading to visualize metaphase plates. Spleen cells were cultured at 1 to 5 × 10^6 cells/ml in medium 199 (Gibco) supplemented with 20% fetal calf serum and 5% phytohemagglutinin P for three to five days at 30° C. Lung cells were used to establish fibroblast cultures which were incubated at 30° C in RPMI 1640 medium (Gibco) containing 10% fetal calf serum. These cells were subcultured every two weeks.

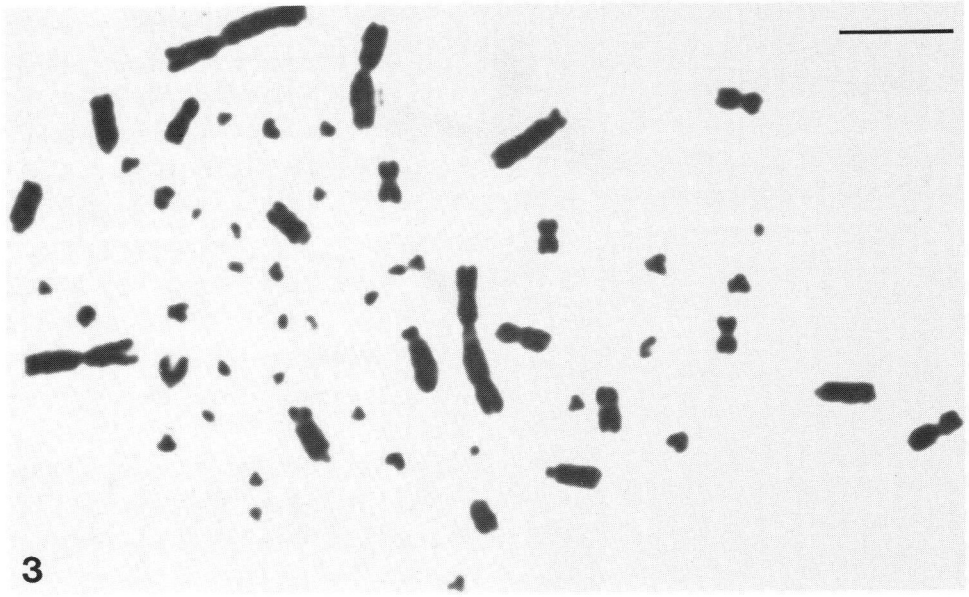
The cultured spleen and lung cells were arrested in metaphase by exposure for 90 minutes to colcemid at 0.1 μg/ml. They were treated with hyptonic solution (0.075 M KCl), fixed in Carnoy's solution (methanol/acetic acid, 3/1 vol/vol), and dropped onto clean slides and air dried.

C-bands (heterochromatin) were visualized on the chromosomes using a modification of the technique of Sumner (1972). Slides were successively treated with 0.2 M HCl for 20 minutes, rinsed in distilled water, immersed for 5-10 minutes in 5% BaOH, rinsed in distilled water, incubated for 30 minutes at 60° C in 2 × SSC, and stained with 2% giemsa in phosphate buffer.

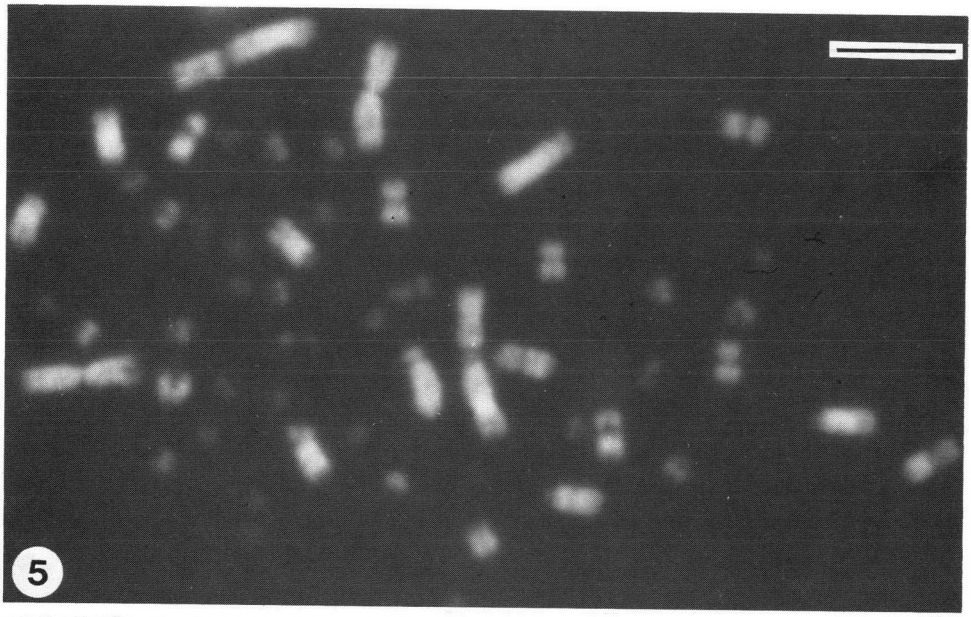
Conventional Giemsa staining, Q-banding (corresponding to A-T-rich regions), and R-banding (corresponding to G-C-rich regions) were performed on the same preparations so that individual chromosomes could be relocated. Slides were first stained with Giemsa and the metaphases photographed using Agfaortho 25 film. They were subsequently destained in 70%, then 50% ethanol, rinsed in distilled water, and immersed for 20 minutes in quinacrine mustard (Sigma) at 0.05 mg/ml. They were mounted in phosphate buffer, pH 6.8, and the metaphases photographed for quinacrine fluorescence. Chromosomes were then submitted to partial heat denaturation by immersing the slides in Earle's solution, pH 6.5, at 87° C, for one to ten minutes, until centromeric heterochromatin became apparent. They were stained with acridine orange and again photographed.

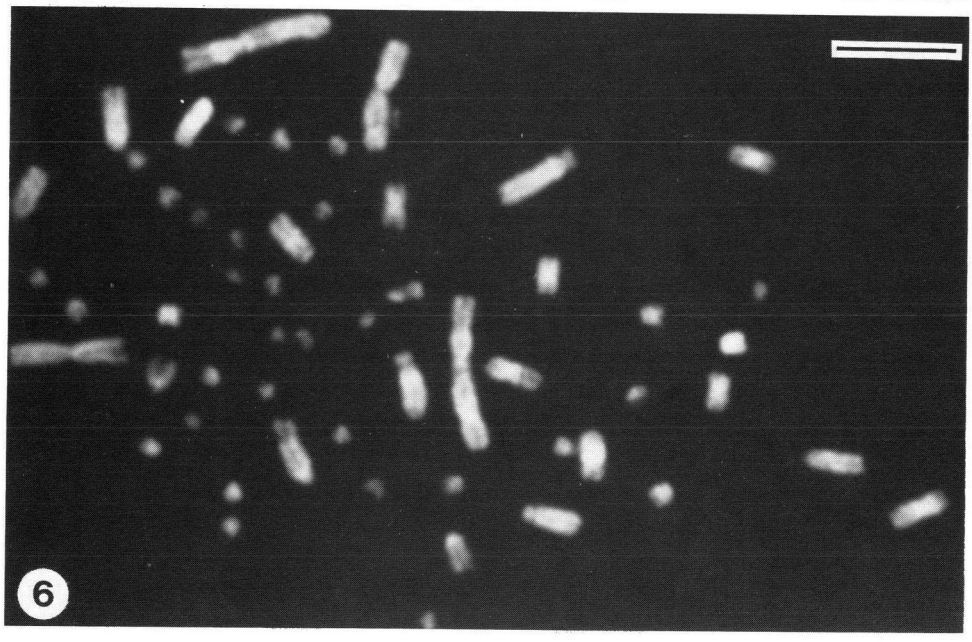


Figures, 1 and 2 - Karyotype of two phenotypic male neonates of *Dermochelys coriacea* with the chromosomes arranged in groups A, B and C. Fig. 1: Giemsa staining. Fig. 2: C-banding. Bars represent 10 micrometers.



Figures, 3 to 6 - Metaphasic chromosomes of *Dermochelys coriacea* stained successively with Giemsa (Fig. 3), quinacrine (Fig. 5), and acridine orange (Fig. 6). In figure 4, one chromosome of each pair stained using the three techniques is presented. Bars 10 represent micrometers.





To facilitate comparison of the karyotypes of *Dermochelys coriacea* with those of turtles previously studied, the chromosomes have been arranged according to Bickham (1975). Group A is composed of metacentric and submetacentric chromosomes, group B of telocentric and subtelocentric chromosomes, and group C of microchromosomes.

Results

Standard karyotype. Dermochelys coriacea has a diploid number of 56 chromosomes consisting of 7 pairs of group A macrochromosomes, 5 pairs of group B macrochromosomes, and 16 pairs of group C microchromosomes (Fig. 1). No heteromorphic sex chromosomes were distinguishable in any of the three animals examined.

C-band pattern. The C-band pattern of the chomosomes is shown in figure 2. The centromeric region is positive in macrochromosomes 1A and 4A. C-bands adjacent to the centromere are also shown in the macrochromosomes 6A, 7A, 3B (the two short arms are positive) and 5B (in some, but not all metaphases). Macrochromosomes 2A, 3A, 5A, 1B, 2B and 4B do not display conspicuous C-banded regions. Most of the microchromosomes are partially C-banded.

Q- and R-band patterns. Figures 3 to 6 show a single metaphase stained successively with Giemsa (fig. 3), quinacrine [Q-bands (Fig. 5)] and acridine orange [R-bands (Fig. 6)]. In figure 4, one chromosome of each pair stained by the three techniques is presented and the haploid set of chromosomes is arranged into groups A, B and C. Among the macrochromosomes, the centromeric and juxtacentromeric regions are generally dark after staining by quinacrine, wheras they are fluorescent after heat denaturation at 87° C and acridine orange staining. This is especially marked in chromosomes 1A, 3A, 4A, 5A, 6A and 7A and in chromosomes 2B, 3B and 5B. The arms of the macrochromosomes are fluorescent after each of the two stainings. The brightest regions after quinacrine treatment are usually less fluorescent after acridine orange, and vice-versa. This is not as clear however, as for centromeric regions. Most of the microchromosomes appear brightly fluorescent after acridine orange and weakly fluorescent after quinacrine staining.

Discussion

The karyotype of *Dermochelys coriacea* is composed of 56 chromosomes consisting of 14A and 10B macrochromosomes and 32C microchromosomes. C-heterochromatin is detected on most microchromosomes but only on half of the macrochromosomes. When it is visible, it is restricted to the centromeric region or to the region adjacent to the centromere, as is the case in many turtles (Bickham and Baker, 1976; Bull and Legler, 1980). A relatively good correlation exists between C- and R-banding patterns (Figs. 2 and 6) suggesting that the C-banded regions are rather G-C-rich. In particular, it appears that the centrometric region of most chromosomes is mainly composed of heterochromatin of the G-C-rich type.

Implications of this work for the phylogenetic classification of sea-turtles is also of interest. *Dermochelys coriacea* has the same number (2n = 56) of chromosomes as other sea-turtles (*Chelonia mydas, Caretta caretta, Eretmochelys imbricata*) previously studied (Bickham, 1979; Bickham et al., 1980; Bickham and Carr, 1983). Moreover, chromosomal morphology and number of chromosome pairs in groups A, B and C

are similar in *Dermochelys coriacea* and in *Chelonia mydas* (Bickham et al., 1980). In both species, the short arms of chromosome 3B are clearly C-banded.

A close relationship between *Dermochelys coriacea* and other living sea-turtles has been previously deduced from behavioral (Carr and Ogren, 1959), morphological (Nick, 1912; Zug, 1966) and biochemical (Frair, 1979, 1982; Chen et al., 1980; Chen and Mao, 1981) studies. Serological tests and results of serum electrophoresis led Frair (1982) to place all the living marine turtles in the family Cheloniidae and to distinguish *Dermochelys coriacea* from other species only at the subfamily level. However, this distinction does not appear to be based on the analysis of derived characters and does not take into account extinct families.

According to Bickham (1981, 1983), the 2n = 56 (7:5:16) karyotype can be considered to be primitive for the suborder Cryptodira. It has been conserved in all living marine turtles, including *Dermochelys coriacea*, and in the monotypic family Dermatemydidae (Carr et al., 1981). Consequently, this karyotype likely does not constitute a synapomorphy. Adult morphological characters of *Dermochelys coriacea*, such as shell constitution (Romer, 1956) and chondro-osseous morphology (Rhodin et al., 1981) which differ notably from those of other marine turtles, represent derived characters. For these reasons, we agree with the classifications of Gaffney (1975, 1984) and Bickham and Carr (1983) in which living marine turtles are placed in two distinct families, Dermochelyidae, including *Dermochelys coriacea* and Cheloniidae, including all other species. These two families are grouped in Chelonioidea which is considered as a superfamily (Gaffney, 1975; Bickham and Carr, 1983) or a microorder (Gaffney, 1984) of the cryptodiran turtles.

Acknowledgments. We thank J. Lescure and J. Fretey who collected turtle eggs in French Guiana and sent them to Paris, France. We are grateful to B. Dutrillaux for his expert advice for preparation and staining of the chromosomes. We also wish to thank F. De Broin, S. Elsevier and R. Bour for critical reading of the manuscript. Financial support was provided by A. T. P. Ifremer-Cnrs "Bases biologiques de l'aquaculture", n° 82/2780.

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Received: February 9, 1986