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A karyological study of turtles from the Brazilian Amazon Region

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Abstract. The karyotypes of five turtles of the genus *Podocnemis* from the Amazon Region of Brazil were determined. Four species, *P. cayennensis*, *P. expansa*, *P. sextuberculata* and *P. unifilis* have 28 (2n) chromosomes and one, *P. dumeriliana*, has 26 (2n). No morphologically distinguishable sex chromosomes could be observed. The difference in number in *P. dumeriliana* can be explained by the occurrence of a centric fusion. The chromosome numbers in this genus are extremely low when compared with the range previously found in Chelonia (2n = 50 to 64).

Introduction

This report is concerned with the five species of the turtles of the Amazon Region of Brazil belonging to the genus *Podocnemis* (Testudines, Pelomedusidae). Their systematic position based on morphological grounds is, at the present time, as follows (WILLIAMS, 1954):

P. cayennensis (SCHWEIGGER)
P. dumeriliana (SCHWEIGGER)
P. expansa (SCHWEIGGER)
P. unifilis (TROSCHEL)
P. sextuberculata (CORNALIS)

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These turtles, with the exception of *P. cayennensis*, are widely distributed and fairly abundant all over the Amazon Region. *P. cayennensis* is apparently restricted to the Negro River, tributary of the left margin of the Amazon. Since the Negro River communicates with the Orinoco of Venezuela through the Cassiquiari channel, this turtle is also found in that country.

To our knowledge there have not been made, up to now, any investigations concerning the karyotypes of these turtles. The chromosome numbers reported by MATTHEY (1951) and WHITE (1954) on Chelonia (2n = 50 to 64) are very different from the ones we found in *Podocnemis*. We were able to study representatives of all the species presently classified in this genus living in our country.

Materials and methods

Information about the animals studied is presented in Table I. They were bled from their hind legs through an incision made with a razor blade. No anesthesia was used. Blood was allowed to drain or extracted by means of a pipette directly from the cut vessels.

A microtechnique for culturing peripheral blood leukocytes, modified from the method of MOORHEAD *et al.* (1960) as described by GORMAN *et al.* (1967), was used to obtain metaphase plates. Leukocytes were cultured for four days at 37°C. Six hours prior to harvesting, Colcemid was added to the cultures to accumulate cells in metaphase.

A few animals received Colcemid after their blood was taken for culture. Four to six hours afterwards, direct preparations of the spleen and gonads were made for mitotic and meiotic studies according to the technique described by GORMAN *et al.* (1967).

Fifty metaphases of each animal were analysed to determine the modal number (Table I), and pairing of chromosomes was made according to their size and centromere position.

Results

The results are presented in Table II and Figures 1 and 2. All species studied have 28 chromosomes (2n) with the exception of *P. dumeriliana*, which has 26.

If the 10th pair of *P. cayennensis* is considered as a submetacentric chromosome, the fundamental number in this species will be equal to 54; however, if we consider this 10th pair as an acrocentric, with the short arm a little longer than that found in the homologous pair

TABLE I

Data related to the turtles examined

Species	No. of the animal studied	Sex	Weight (g)	Tissue	Total No. of cells examined	Modal No. (2n)
P. unifilis	1	undetermined	90	spleen	50	28
P. unifilis	2	male	450	leukocyte	50	28
P. unifilis	3	female	800	leukocyte	50	28
P. unifilis	5	male	120	spleen	50	28
P. unifilis[1]	14	male	1000	spleen	50	28
P. expansa	4	undetermined	30	leukocyte	50	28
P. expansa	10	undetermined	100	leukocyte	50	28
P. expansa	17	male	3000	leukocyte	50	28
P. expansa	20	female	2500	leukocyte	50	28
P. cayennensis	7	undetermined	16	leukocyte, spleen	50	28
P. cayennensis	9	undetermined	16	leukocyte	50	28
P. dumeriliana	8	female	4000	leukocyte	50	26
P. dumeriliana	21	female	1000	spleen	50	26
P. sextuberculata	11	male	130	leukocyte	50	28
P. sextuberculata	12	undetermined	40	leukocyte	50	28
P. sextuberculata	13	male	1000	leukocyte	50	28

[1] The gonads were examined for meiotic study.

TABLE II

Karyotypes of turtles of the genus Podocnemis

Species	Modal No. (2n)	No. of metacentrics	No. of sub-metacentrics	No. of acrocentrics	Fundamental number
P. cayennensis[1]	28	22	4	2	54
P. expansa	28	22	2	4	52
P. sextuberculata	28	22	2	4	52
P. unifilis	28	22	2	4	52
P. dumeriliana	26	26	–	–	52

[1] If we consider the 10th pair as having acrocentric chromosomes, the fundamental number will be equal to 52.

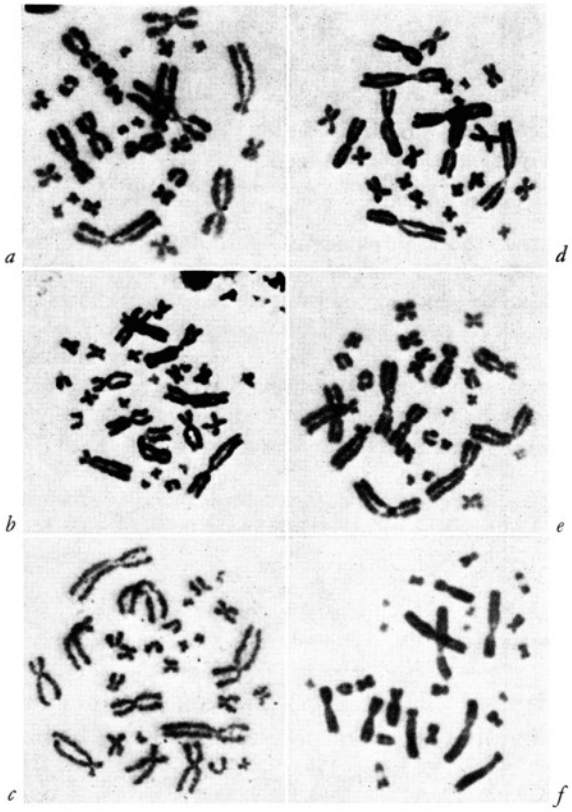

Fig. 1. Metaphase plates of the somatic-cell chromosomes of turtles from the Brazilian Amazon Region: *(a) P. cayennensis; (b) P. expansa; (c) P. sextuberculata; (d) P. dumeriliana; (e) P. unifilis*, and *(f) P. unifilis*. Giemsa stain; reproduced at 800 ×.

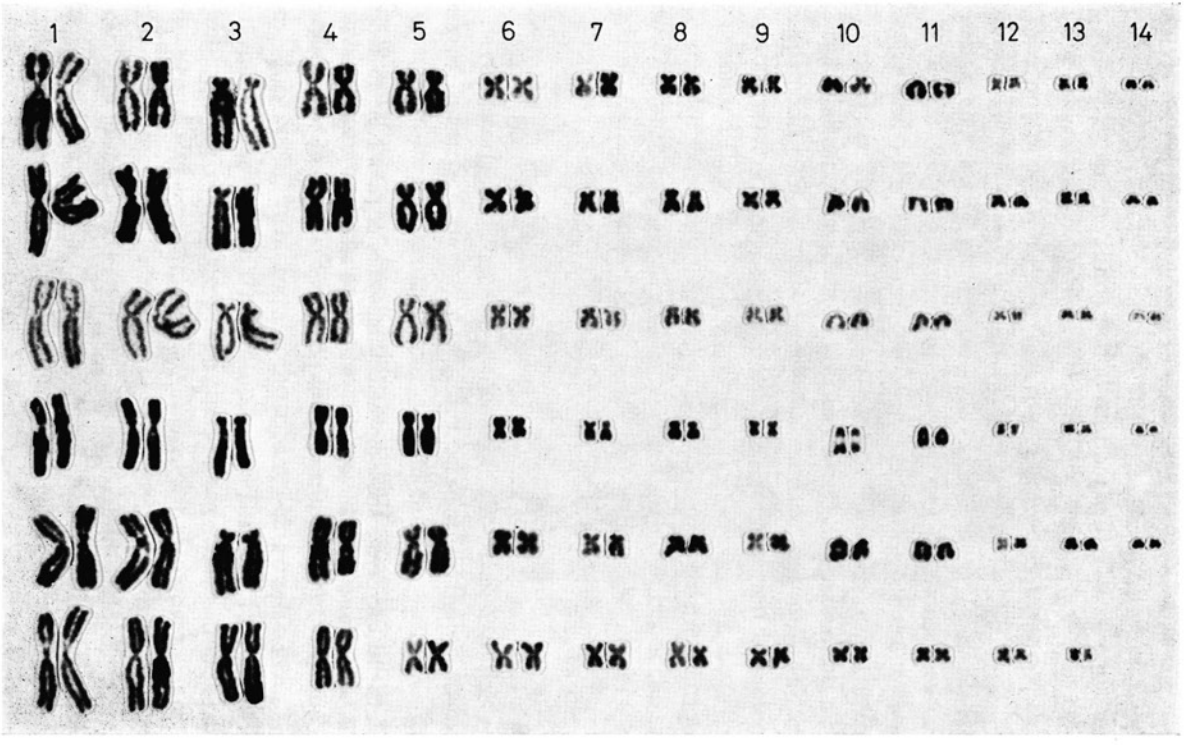

Fig. 2. Karyotypes of the metaphase cells shown in Figure 1.

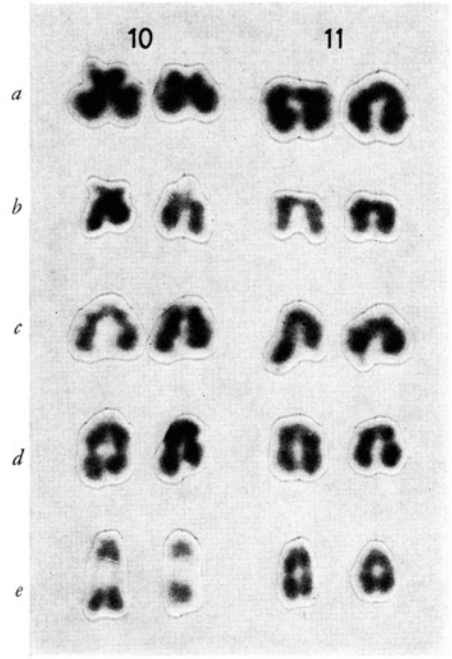

Fig. 3. Partial karyotypes showing the chromosomes of pairs Nos. 10 and 11 of *(a) P. cayennensis* (sex unknown), *(b) P. expansa* (female), *(c) P. sextuberculata* (male), *(d) P. unifilis* (female) and *(e) P. unifilis* (male). Giemsa stain.

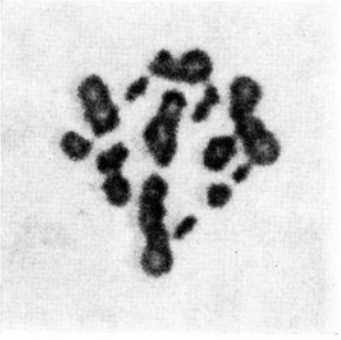

Fig. 4. Male meiotic cell from *P. unifilis* showing 14 bivalents. Giemsa stain.

of *P. expansa*, then the fundamental number will also be equal to 52, as in the other four species studied in this paper.

It was not possible to find heterochromosomes in any specimen examined.

Four species, including *P. cayennensis*, *P. expansa*, *P. sextuberculata* and *P. unifilis*, have presumably homologous chromosomes of the same size and identical form, with the exception of the 10th pair. In *P. cayennensis* this pair is submetacentric or acrocentric; in *P. expansa* it can also be classified as an acrocentric but presenting a short visible arm; in *P. sextuberculata* it is acrocentric, and in *P. unifilis* it is acrocentric with a well defined secondary constriction in almost all cells examined (Fig. 3).

We could obtain only one male cell in the meiotic preparations of *P. unifilis*. Figure 4 shows 14 bivalents with a high number of chiasmata in each bivalent.

Discussion

We have determined the karyotypes of all species of the genus *Podocnemis* occurring in the Amazon Region with the exception of *P. vogli* and *P. lewyana*, which occur outside Brazil.

These karyotypes are fairly uniform in chromosome number and size in all species with the exception of the 10th pair, as stated earlier. *P. dumeriliana*, however, has a diploid number of only 26 chromosomes, all of them, including the third pair, being metacentric.

It is interesting to note that DUMERIL and BIBRON (1835), based on morphological grounds, proposed that *P. dumeriliana* should belong to the genus *Peltocephalus* and not *Podocnemis*. However, since this form is not sufficiently different from the other species of the group, we can still classify it in the genus *Podocnemis*. Indeed, only two rearrangements are needed to explain the changes which occurred in the chromosomes of this species when compared to those with a 2n equal to 26, namely, a centric fusion of the two acrocentric pairs of the latter and a pericentric inversion changing the submetacentric pair into a metacentric chromosome.

In relation to the taxonomy of this group, *P. cayennensis* was first described by SCHWEIGGER (apud WILLIAMS, 1954) as *Emys cayennensis*. SPIX (1824) apparently described the same species as *Emys erithrocephala*. The species types of both authors were unfortunately lost. GOELDI (1886) described a young form of *P. cayennensis* as a new

species under the name of *P. coutinhii (P. coutinhoi)*. In this paper we refer to *P. cayennensis*, giving, obviously, priority to SCHWEIGGER.

It should be noted that the secondary constrictions are well marked in the acrocentric pairs, especially in *P. unifilis*, and a little less in the large metacentric pairs. The secondary constrictions seen in the species studied here may represent nucleolar organizer sites or be due to temperature shocks. In this event they would merely represent achromatic regions. Plans are being made to investigate this in more detail using different temperature and staining techniques. If more such regions are found, we could be able to reconstruct in a more precise way the past evolutionary events which occurred in the history of these forms.

At least five chiasmata were seen in some large pairs (Fig. 4). The metacentric of medium size has a chiasma in each arm. The high frequency of chiasmata observed may be related to the biology of these turtles.

We can only conjecture about why there was such a reduction in chromosome number in this genus. The most common diploid number in Chelonia is 52, the range previously found being 50 to 64. Since we have found in *Podocnemis* chromosome numbers equal to 26 and 28, we will have to postulate extensive rearrangements to explain this reduction by half of the chromosomes. An obvious alternative would be that the number found here would represent an ancestral condition which led by duplication to the present high numbers found in other genera. New studies in related forms, including determination of the DNA content per nucleus, are needed to settle this question.

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