A Karyological Study of Two Species of Tortoises from the Amazon Region of Brazil

M. M. Sampaio,[1] R. M. Barros,[2] M. Ayres[3] and O. R. Cunha[3,4]

Received September 23, 1969

According to Williams (1960) the two species of tortoises *G. carbonaria* and *G. denticulata* distinguished by Thomas Bell in 1825, are still customarily synonymized, though it has been suggested that they may be distinct. Williams (*loc. cit.*) puts the two above mentioned species into the subgenus *Chelonoidis* of the genus *Geochelone*.

In Brazil the two species *G. carbonaria* (Spix) and *G. denticulata* (Linnaeus) occur from the Amazon Region in the North to Rio de Janeiro in the South.

The present study describes the karyotypes of the species *G. carbonaria* and *G. denticulata*.

Materials and methods

Three males and one female of *G. carbonaria* and one male and one female of *G. denticulata* were studied.

Each animal received 0.015 ml of 1% colchicine solution subcutaneously for every 10 g of animal weight four hours before examination. The spleen and gonads were removed and put on separate Petri dishes containing 0.7% sodium citrate solution, where they were minced in order to obtain a fine cellular suspension. This was pipetted into a 15 ml tube, which remained for 30 minutes at 37°C. After this hypotonic treatment the material was centrifuged. The supernatant was then removed and the cells resuspended in the fixative (methanol and glacial acetic acid 3 : 1); this operation was repeated three times and the final preparation of the slides followed the usual routine. All preparations were stained with Giemsa.

Results

The results are presented in Tables 1 and 2 and Figs. 1 to 3. The two species have a diploid number of 52 chromosomes, which is confirmed by the

[1] Departamento de Histologia e Embriologia, Faculdade de Medicina, Universidade Federal do Pará, Belém-Pará-Brasil.

[2] Laboratório de Genética, Faculdade de Filosofia, Ciências e Letras, Universidade Federal do Pará, Belém-Pará-Brasil.

[3] Museu Paraense "Emílio Goeldi", Belém-Pará-Brasil.

[4] Fellow. Conselho Nacional de Pesquisas.

presence of 26 bivalents in diakinesis, and 26 half-bivalents in metaphase II. The first 15 larger pairs are arranged in decreasing order of size; the other smaller chromosomes were difficult to pair and could be considered micro-

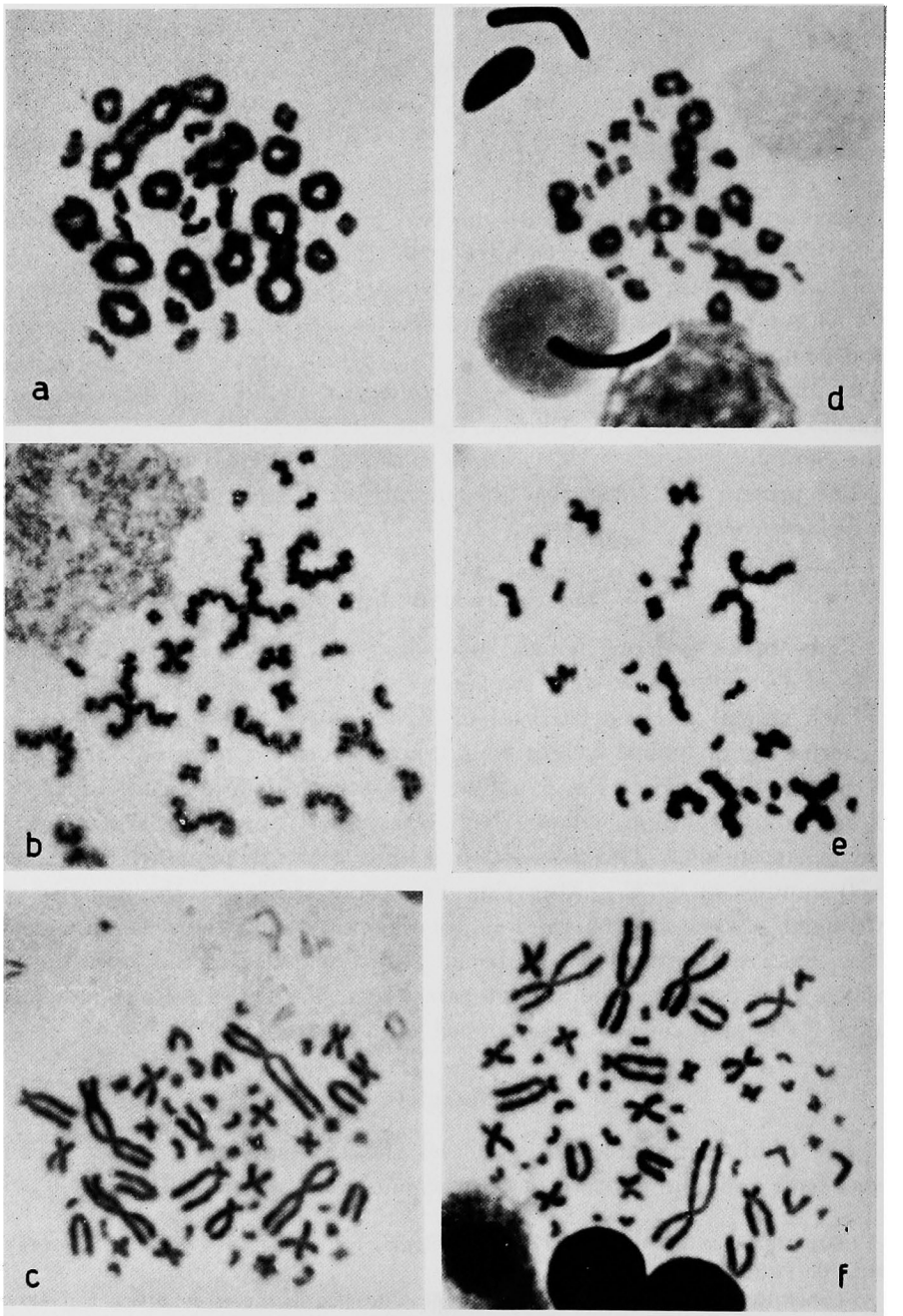

Fig. 1. Meiotic chromosomes of *G. carbonaria*. (a, diakinesis; b, metaphase II) and *G. denticulata*. (d, diakinesis; e. metaphase II). Mitotic chromosomes of *G. carbonaria* (c)

chromosomes.

The chromosome of the two species are of about the same size and have the same morphological aspect exception for the 12th pair, which is acrocentric in *G. denticulata* and metacentric in *G. carbonaria.*

The chromosomes of pairs one and two present the same morphology, but members of pair one are slightly larger. The 3rd and 4th pairs are

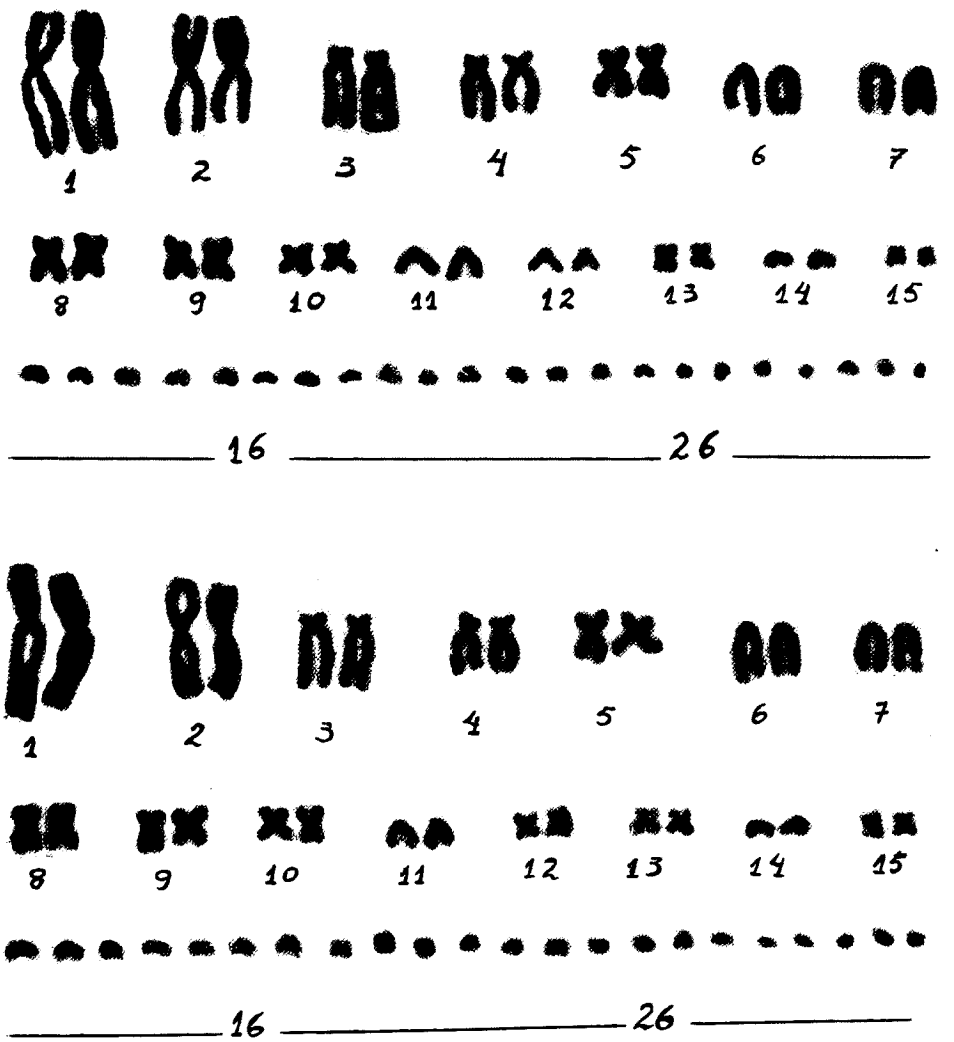

Fig. 2. Karyotypes of *G. denticulata* (♂) and *G. carbonaria* (♀), from spleen cells.

composed of large submetacentrics however, the 3rd pair is definitely larger than the 4th. The 6th and 7th pairs have medium acrocentrics. The 5th, 8th, 9th and 10th pairs show medium metacentrics. The 11th and 14th pairs present small acrocentrics and 13th and 15th pairs are small metacentrics.

Very slight secondary constrictions were observed in the first four pairs

Table 1. Number of mitotic and meiotic chromosomes of the animals examined

Species	Sex	Mitosis			Meiosis						Total
					Diakinesis (Bivalents)			Metaphase II (Half-bivalents)			
		≤51	52	≥53	≤25	26	≥27	≤25	26	≥27	
G. carbonaria	male	3	27	—	1	14	—	—	5	—	50
G. carbonaria	male	1	28	1	2	12	—	—	6	—	50
G. carbonaria	male	3	10	—	—	10	—	—	9	—	32
G. carbonaria	female	1	24	1	—	—	—	—	—	—	26
G. denticulata	male	1	27	1	—	11	—	—	7	—	47
G. denticulata	female	—	6	—	—	—	—	—	—	—	6

Table 2. Karyotypes of tortoises of the genus *Geochelone**

Species	Sex	Modal no. (2n)	Metacentrics no.	Sub-metacentrics no.	Acrocentrics no.
G. carbonaria	male	52	18	4	8
G. carbonaria	female	52	18	4	8
G. denticulata	male	52	16	4	10
G. denticulata	female	52	16	4	10

* The morphological identification includes the first 15 pairs only, since the others are too small and cannot be classified properly.

of chromosomes of both species.

The meiotic cells in diakinesis show 26 bivalents. The first four pairs can be easily identified: the 1st pair presented 5 chiasmata, the 2nd four, and the 3rd and 4th three in the 47 cells examined. The medium size bivalents showed a ring form with two terminal chiasmata.

Fig. 3. Pachytene of *G. denticulata* (♂). ×1,400.

The smaller bivalents form one cross with one chiasma (Fig. 1 a–d). In metaphase II (Fig. 1 b–e) there are 26 half-bivalents and 12 can be easily identified. Figure 3 shows the pachytene stage, with complete pairing of the homologues. No differential condensation, heteropycnosis, presence of unequal bivalents or early separation of any pair during the meiotic process were observed. No morphologically distinct sex chromosomes could be distinguished.

Discussion

The two species, *G. carbonaria* and *G. denticulata*, show 52 chromosomes. There is a striking similarity in the morphology of the first 15 pairs of the two species, with the only exception of the 12th pair which is a metacentric in *G. carbonaria* and acrocentric in *G. denticulata*. This difference, we believe, justifies the acceptance of *G. carbonaria* and *G. denticulata* as two different species, which is made otherwise on morphological grounds (Sampaio *et al.* 1969).

The other 22 chromosomes exhibit slight morphological differences in both species concerning the position of the centromeres, but the pairing of the homologues of the remaining chromosomes present great difficulties in the majority of the cells so far examined.

Secondary constrictions were observed in the chromosomes of the first four pairs of both species, but their number was not high in the cells studied and they are, no doubt, less frequent than the numerous and conspicuous secondary constrictions found in the mitotic chromosomes of species of the genus *Podocnemis* of the Brazilian Amazon Region (Ayres *et al.* 1969, Huang and Clark 1969).

A high number of chiasmata was observed in each bivalent of both species studied here. This fact was also verified by Ayres *et al.* (1969) in *Podocnemis unifilis*.

With reference to the 12th pair which is of the same size but has different morphology in two species studied here, the difference may have occurred through a pericentric inversion. It is interesting to recall that *Clemmys japonica* and *Geoclemys reevesii* (Sasaki and Itoh 1967) present an almost identical karyotype with the two species studied here and that the 12th pair of *G. carbonaria* has the same morphology as the one of equivalent size found in the species studied by Sasaki and Itoh (1967).

The absence of heteromorphic chromosomes seems to be the most frequent condition among the *Chelonia* (Mathey 1951, Sasaki and Itoh 1967, Ayres *et al.* 1969).

Summary

The two morphologically distinct species *G. carbonaria* and *G. denticulata* have 52 chromosomes. The same number found in *Clemmys japonica* and *Geoclemys reevesii*. This number is, however, about double that of the number described in species of genus *Podocnemis* of the Amazon Region of Brazil. The two species show marked morphological similarities in their chromosomes but the 12th pair is dissimilar, being metacentric in *G. carbonaria* and acrocentric in *G. denticulata*.

Acknowledgments

The authors wish to express their gratitude to Professor Francisco Mauro Salzano, Chefe da Seção de Genética e Diretor do Instituto de Ciências Naturais da Universidade Federal do Rio Grande do Sul, Brasil, for his many suggestions ; to Doctor J. J. Shaw from the Wellcome Parasitology Unit of Institute Evandro Chagas, for his help with the manuscript ; to Mr. Candido Plácido Sodré of the Museu Paraense E. Goeldi for the photomicrographies.

References

Ayres, M., Sampaio, M. M., Barros, R. M., Dias, L. B. and Cunha, O. R. 1968. Estudos preliminares sôbre o número cromosômico de quelônios do gênero *Podocnemis*. Ciência e Cultura 20: 174-175.

—, —, —, — and —. A karyological study of turtles from the Brazilian Amazon Region. Cytogenetics (in press).

Huang, C. C. and Ciark, N. P. 1969. Chromosome studies of the cultured cells of two species of side-necked turtles (*Podocnemis unifilis* and *Podocnemis expansa*). Chromosoma (Berl.) 26: 245-253.

Matthey, R. 1951. The chromosomes of the vertebrates, pp. 159-180; in Advances in Genetics, Vol. 4, Ed. M. Demerec, Academic Press. New York.

Sampaio, M. M., Barros, R. M., Ayres, M. and Cunha, C. R. 1969. Constituiçao cromossômica de quelônios da Regiao Amazônica Brasileira II. Estudos preliminares do gênero *Geochelone* Fitzinger, 1835. Ciência e Cultura 21: 276.

Sasaki, M. and Itoh, M. 1967. Preliminary notes on the karyotype of two species of turtles, *Clemmys japonica* and *Geoclemys reevesii*. Chromosome Information Service 8: .

Williams, E. E. 1960. Two species of tortoises in Northern South America. Breviora Museum of Comparative Zoology 120: 1-15.