KARYOTYPIC ANALYSIS OF THE *PODOCNEMIS* TURTLES

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KARYOTYPIC ANALYSIS OF THE *PODOCNEMIS* TURTLES.—The side-necked turtle genus *Podocnemis* (Pleurodira, Pelomedusidae) is usually thought to consist of eight living species, seven of them from northern South America and one from Madagascar (Williams, 1954a; Wermuth and Mertens, 1961; Pritchard, 1967; Mittermeier and Wilson, 1974). However, the status of two of these species has never been clear. Williams (1954b, c) and Smith and James (1958) placed the Madagascan species in its own genus, *Erymnochelys*, and Tronc and

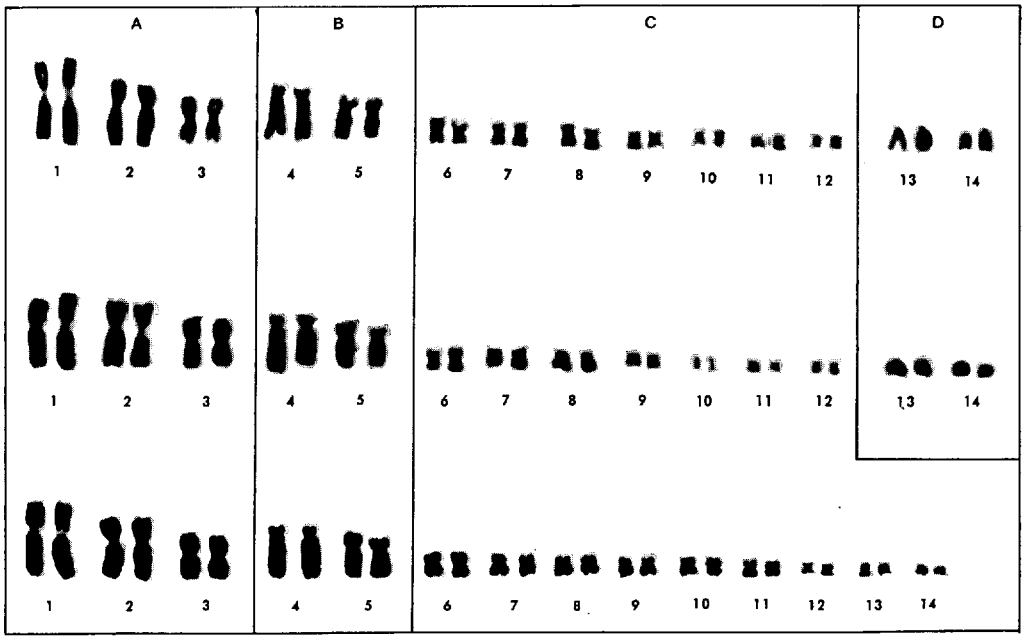

Fig. 1. Chromosomes of *Podocnemis lewyana* (top), *Podocnemis vogli* (middle), and *Podocnemis madagascariensis* (bottom). Chromosomal pairs are indicated by number and are divided into Groups A, B, C and D.

Vuillemin (1974) recently resurrected that name once again. In addition, the South American species usually referred to as *Podocnemis dumeriliana* (Siebenrock, 1902; Williams, 1954a) has sometimes been placed in the genus *Peltocephalus* (Williams, 1954b, c; Fretey, 1975, 1977).

Five *Podocnemis* species have already been karyotyped. Ayres et al. (1969) reported the karyotypes of *Podocnemis expansa, P. unifilis, P. sextuberculata, P. dumeriliana* and *P. erythrocephala* (for which they used the name *P. cayennensis*) and Huang and Clark (1969) described *P. expansa* and *P. unifilis* as well. In this paper, we present the karyotypes of *P. lewyana, P. vogli* and *P. madagascariensis,* the three species that have not yet been reported, and reassess the relationships of these eight turtles on the basis of our findings.

P. lewyana is restricted to the Magdalena and Sinú drainages in northern Colombia (Medem, 1964); *P. vogli* occurs in small rivers, streams and lagoons in the llanos (savannas) of Venezuela and eastern Colombia (Alarcon Pardo, 1969); and *P. madagascariensis* is restricted to western Madagascar (Tronc and Vuillemin, 1974).

Materials and methods.—Single specimens of *P. vogli, P. lewyana* and *P. madagascariensis* were studied. The *P. vogli* and *P. lewyana* specimens were both males and are now preserved in the collection of the Instituto Roberto Franco, Villavicencio, Meta, Colombia. The *P. vogli* (IRF 418) was from Pozo Llano Grande, Peralonzo, Meta, Colombia; the *P. lewyana* (IRF 528) from the Río Cocorna, Antioquia, Colombia. The *P. madagascariensis* was a female without locality data and is still alive in the collection of Rhodin (AGJR L305).

Subcutaneous connective tissue from the axillary region of the *P. madagascariensis* was cultured in McCoy's 5A medium with 20% fetal calf serum (Gibco) at 30 C for 10 days. Harvesting was preceded by 30 min of standard colcemid treatment. Cells were exposed to hypotonic (0.075 molar) KCl, fixed in 3:1 ethanol and glacial acetic acid, air dried, and stained with Giemsa. The c-metaphase chromosomes of *P. vogli* and *P. lewyana* were processed by the in vivo colchicine-hypotonic citrate technique described by Patton (1967), except for the following modifications: each turtle received a 1.5 cc intraperitoneal injection of .05% colchicine; in vivo incubation time was

TABLE 1. KARYOTYPIC CHARACTERISTICS OF THE *Podocnemis*. Diploid number ($2n$), fundamental number (FN), and number of pairs of chromosomes per group for all species of *Podocnemis*. Groups are defined in text. Numbers in parentheses refer to pairs as identified in Fig. 1.

Species	$2n$	FN	Chromosomal group			
			A	B	C	D
P. lewyana	28	52	3 (1–3)	2 (4, 5)	7 (6–12)	2 (13, 14)
P. vogli	28	52	3 (1–3)	2 (4, 5)	7 (6–12)	2 (13, 14)
P. unifilis	28	52	3 (1–3)[a]	2 (4, 5)[a]	7 (6–12)[a,c]	2 (13, 14)[a,c]
P. sextuberculata	28	52	3 (1–3)[a]	2 (4, 5)[a]	7 (6–12)[a]	2 (13, 14)[a]
P. expansa	28	52	3 (1–3)[a]	2 (4, 5)[a]	7 (6–12)[a,c]	2 (13, 14)[a,c]
P. erythrocephala[b]	28	54	3 (1–3)[a]	2 (4, 5)[a]	8 (6–13)[b]	1 (14)[b]
P. madagascariensis	28	56	3 (1–3)	2 (4, 5)	9 (6–14)	0
P. dumeriliana	26	52	4 (1–4)	0	9 (5–13)	0

[a] Ayres et al. (1969): Group A = pairs 1, 2, and 4; Group B = pairs 3 and 5; Group C = pairs 6–9 and 12–14; Group D = pairs 10 and 11.
[b] Ayres et al. (1969): reported as *P. cayennensis*; Group C = pairs 6–10 and 12–14; Group D = pair 11.
[c] Huang and Clark (1969): Group C = pairs 6–9 and 12–14; Group D = pairs 10 and 11.

two hours; and finely minced spleen and testes were exposed to 10 ml hypotonic (0.8%) sodium citrate in individual watch glasses for 20 min. Slides were flame dried and stained with Giemsa. Approximately 20 metaphase spreads were counted from each turtle and representatives were photographed.

In order to facilitate comparisons, chromosomes were segregated according to their morphology into four groups. These groups are defined as: A) large to medium-sized metacentrics and submetacentrics—length greater than 50% of the longest chromosome; B) large to medium-sized subtelocentrics; C) small to very small metacentrics and submetacentrics; D) small acrocentrics and subtelocentrics (subacrocentrics of Gorman, 1973).

Results.—The karyotypes of *P. lewyana* and *P. vogli* ($2n$ = 28, FN = 52, Fig. 1) are nearly identical to one another and to those reported for *P. unifilis, P. expansa* and *P. sextuberculata* (Ayres et al., 1969; Huang and Clark, 1969). The karyotypes of these five taxa (Tables 1 and 2) have 3 pairs of Group A, 2 pairs of Group B, 7 pairs of Group C and 2 pairs of Group D chromosomes. The chromosomal pair D13 of *P. lewyana* is noticeably larger than the corresponding pair in the other species. The karyotype of *P. erythrocephala,* reported by Ayres et al. (1969) as *P. cayennensis,* differs from these in having 8 pairs of Group C and 1 pair of Group D chromosomes. This difference is apparently derived from the morphology of chromosomal pair C9 (Table 2), which is comparable in relative size to the acrocentric pair D13 of *P. lewyana, P. vogli, P. unifilis, P. sextuberculata* and *P. expansa,* but possesses short second arms. Otherwise, with the exception of achromatic zones or secondary constrictions, the karyotypes of these six species are fundamentally alike (see Huang and Clark, 1969, for a discussion of secondary constrictions).

Podocnemis madagascariensis ($2n$ = 28, FN = 56; Fig. 1) differs from the six species described above in having a totally biarmed karyotype (no Group D chromosomes).

The karyotype reported by Ayres et al. (1969) for *P. dumeriliana* ($2n$ = 26, FN = 52) is unique among *Podocnemis* turtles in that it consists of 13 pairs of chromosomes instead of 14 and lacks Group B chromosomes. Like *P. madagascariensis, P. dumeriliana* also lacks Group D chromosomes.

Discussion.—Now that the karyotypes of all living *Podocnemis* turtles are known, we believe that certain tentative conclusions can be drawn concerning relationships within this group of animals.

One "typical" *Podocnemis* karyotype characterizes *P. expansa, P. lewyana, P. sextuberculata, P. unifilis* and *P. vogli.* The *P. erythrocephala* karyotype is very similar since its pair C9 differs relatively little from pair D13 of the other five species. The basic similarity of these six taxa indicates that they are probably more closely related to one another than to the other two species in the group.

Ayres et al. (1969) suggested that diagnostic specific differences existed in the D13 chromosomes of *P. expansa, P. sextuberculata* and *P. unifilis* and the C9 pair of *P. erythrocephala.* The large D13 pair of *P. lewyana* is also distinctive and indicates that this suggestion may

TABLE 2. COMPARISON OF ARM LENGTHS OF *Podocnemis* CHROMOSOMES. Arm and total lengths of chromosomes expressed as percentage of total length of largest chromosome. Measurements from photoidiograms (present study and Ayres et al., 1969).

	Chromosomal group																	
	A				B		C										D	
	1	2	3	4	4	5	5	6	7	8	9	10	11	12	13	14	13	14
	41	32	27		11	14		16	14	14	9	8	7	5			0	0
P. lewyana	59	49	27		54	38		16	14	14	9	8	7	5			30	22
	100	81	54		65	52		32	28	28	18	16	14	10			30	22
	38	38	31		14	16		19	13	11	6	8	6	6			0	0
P. vogli	62	56	41		69	47		19	16	14	11	8	6	6			22	20
	100	94	72		83	63		38	29	25	17	16	12	12			22	20
	39	35	26		13	17		13	9	9	9	6	4	3			0	0
P. unifilis	61	61	39		57	39		13	15	13	9	6	6	5			22	20
	100	96	65		70	56		26	24	22	18	12	10	8			22	20
	35	30	26		13	17		13	9	9	9	4	4	3			0	0
P. sextuberculata	65	52	35		57	35		13	13	11	9	4	4	5			17	15
	100	82	61		70	52		26	22	20	18	8	8	8			17	15
	38	33	25		13	17		13	8	8	8	4	6	3			0	0
P. expansa	62	50	33		54	38		13	15	15	8	8	6	6			17	14
	100	83	58		67	55		26	23	23	16	12	12	9			17	14
	31	27	23		12	15		12	12	10	6	8	6	6	3			0
P. erythrocephala	69	46	31		65	31		12	12	10	11	8	6	6	5			17
	100	73	54		77	46		24	24	20	17	16	12	12	8			17
	39	33	28		11	17		17	11	11	11	11	11	8	7	3		
P. madagascariensis	61	50	33		56	42		17	17	17	17	11	11	8	8	8		
	100	83	61		67	59		34	28	28	28	22	22	16	15	11		
	38	38	31	31			19	15	12	12	8	8	8	8	6			
P. dumeriliana	62	54	50	31			19	15	12	12	12	8	8	8	6			
	100	92	81	62			38	30	24	24	20	16	16	16	12			

have some validity. However, *P. vogli* and *P. sextuberculata* have D13 chromosomes that are similar in appearance, so use of banding techniques would be necessary to confirm the diagnostic value of pair D13 for all *Podocnemis* species.

The basic similarities of the Group A, B and C chromosomes of *P. madagascariensis*, the five species with the "typical" karyotype, and *P. erythrocephala* suggest fairly close phylogenetic affinity. However, *P. madagascariensis* differs from the other six species in lacking Group D (uniarmed) elements, a feature that may have arisen from pericentric inversions, unequal reciprocal translocations, or heterochromatin additions, deletions or combinations thereof. The differences that exist are difficult to interpret at this time and may be clarified by detailed analyses using C and G banding techniques. However, the fact that *P. madagascariensis* does differ from the other six species adds to the morphological and biochemical data that favor recognition of a distinct genus, *Erymnochelys* for this turtle (Mittermeier et al., in press; B. Van Valkenburgh and R. C. Wood, pers. comm.; Frair et al., 1978).

P. dumeriliana, although possessing the same fundamental number as five of the *Podocnemis*, is grossly different karyotypically. The karyotypic differences appear to have involved major

chromosomal repatterning (inversions and/or translocations) and not the presumably simpler additions or deletions of heterochromatin. Ayres et al. (1969) claimed that only two rearrangements were needed to convert a "typical" *Podocnemis* karyotype to that of *P. dumeriliana,* and did not attribute any special phylogenetic significance to the different karyotypes. However, we believe that more than two rearrangements must have been involved, unless both karyotypes evolved from a common primitive pattern.

In view of the stability and allegedly conservative nature of chelonian karyotypes, we believe that the differences displayed by *P. dumeriliana* have considerable phylogenetic significance. Stock (1972) was of the same opinion and recommended a review of the generic relationships among these turtles, but neither Ayres et al. (1969) nor Killebrew (1975) believed that the differences in *Podocnemis* karyotypes supported separation at the generic level. We feel that the distinct *P. dumeriliana* karyotype indicates a divergent evolutionary history for this turtle and strongly supports recognition of a distinct genus for which the name *Peltocephalus* Duméril and Bibron is available. As in the case of *P. madagascariensis,* morphological and biochemical data also support generic separation (Mittermeier et al., in press; Frair et al., 1978).

Although we feel that *Peltocephalus* and *Erymnochelys* are probably distinct genera, we believe that they share enough features in common to warrant their inclusion with *Podocnemis* in the same subfamily. One of the most striking features they share is the unusually low diploid number ($2n \leqslant 28$), which differs from other pelomedusids ($2n = 34$) and especially from the high diploid numbers of non-pelomedusid chelonian genera ($2n \geqslant 50$) (Killebrew, 1975).

Several earlier authors also recognized these animals as a distinct assemblage above the generic level. Gray (1873) and Baur (1888, 1890, 1893), for instance, placed them together in their own family, and Williams (1954b, c) recognized them as a group or subsection of the Pelomedusidae. The subfamilial name Podocneminae was first proposed by Smith and James (1958), but their definition included only *P. dumeriliana* and the six other South American species. *Erymnochelys* was placed in its own subfamily, Erymnochelyinae. The karyotypic data presented here, and the morphological and biochemical work already cited, definitely do not support recognition of a separate subfamily for *Erymnochelys,* and we therefore include it in the Podocneminae, as first suggested by Wood (1971) and later by Albrecht (1976).

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