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Karyotype of the Yellow-Bellied Sea Snake, *Pelamis platurus*

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ABSTRACT—In this paper we describe the karyotype of the yellow-bellied sea snake, *Pelamis platurus* from Costa Rica. The diploid number is 38 chromosomes, with 20 macrochromosomes and 18 microchromosomes. The pairs 1 and 2 are metacentrics, pair 3 is subtelocentric and pairs from 4 to 9 have the centromere in a terminal position. Females have a pair of slightly heteromorphic chromosomes identified as sex chromosomes Z and W; both are metacentrics but have different centromeric index and W is slightly smaller. The pattern of heteromorphism in sex chromosomes and the secondary constriction present in some sea snake karyotypes could be useful parameters in determining evolutionary relationships between the sea snakes.

* * *

INTRODUCTION

The sea snakes constitute a very specialized group of reptiles, with about twelve genera and forty-eight species (Hecht, et al., 1974) whose place of origin was probably the Indo-Australian area (Dunson, 1975). At this time their distribution ranges from South Africa and Japan in the Indian and West Pacific Oceans, respectively, eastward to the region of the East Pacific between Mexico and Ecuador (Dunson, 1975). They have been traditionally treated as Hydrophiidae (Barme, 1968; Smith, 1926) but McDowell (1969) believes that they belong within the Elapidae. He divides the sea snakes into two elapid subfamilies: Laticaudinae and Hydrophiinae.

Pelamis platurus, the yellow-bellied sea snake, is of particular interest for zoogeographic and evolutionary studies because it is the only species found in both the Indo-Malasian and Australian regions and the east Pacific, reaching American tropical waters, being the only one in this region (Kropach, 1975); it appears in great numbers during the dry season at the Pacific coast of Costa Rica (Bolaños, et al., 1974). The phylogenetic relationships of sea snakes are being assessed by various workers (e.g. Voris, 1977) and study of the karyotype of *Pelamis platurus* and assessment of chromosomal evolution may aid our understanding of the evolutionary history of this radiation.

MATERIALS AND METHODS

Two males and two females were collected from Playas del Coco and Matapalo, Guanacaste, Costa Rica. These specimens are in the Zoology Museum of the University of Costa Rica (UCR N°7933–7936). In order to obtain karyotypes the snakes were subcutaneously injected with raw phytohemagglutinin isolated from the seed of *Phaseolus lunatus* (Taylor and Bolaños, 1975). One ml of 5% phytohemagglutinin was administered for each 34 g body weight. The snakes were then immediately injected with colchicine by the same route (0.5% solution, one ml for each 100 g body weight). Blood samples were taken at 48 h from the tail with heparinized capillaries; a 1% sodium citrate was then used to cause a hypotonic shock. The cells were fixed with methanol: acetic acid (3:1) and then stained with Giemsa diluted 1:60 with a phosphate buffer at pH 6.8.

The chromosomes were classified, according to the centromere position, as metacentrics, submetacentrics, subtelocentrics and acrocentrics (Levan, et al., 1964). They were ordered on the basis of centromere position and then, within these groups, in decreasing length, according to

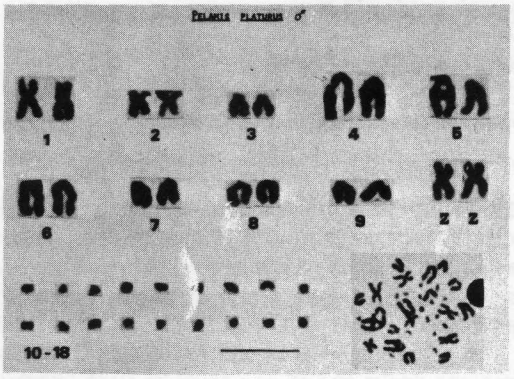

FIGURE 1. Karyotype of male *Pelamis platurus*. One chromosome of the fourth pair shows a small constriction. The bar corresponds to ten micrometers.

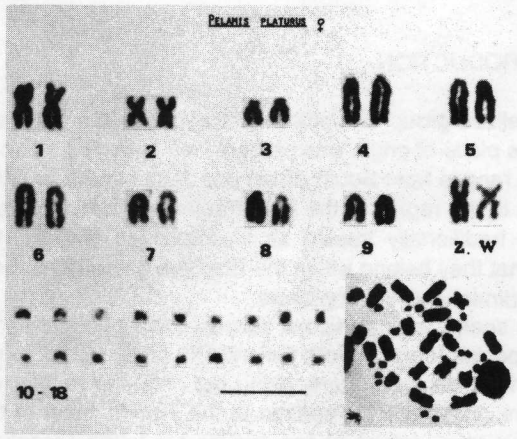

FIGURE 2. Karyotype of female *Pelamis platurus*. The bar corresponds to ten micrometers.

Singh (1972a, 1974). The morphometric analyses of karyotypes were done using the relative length and centromeric index as parameters. To calculate the relative length of chromosomes we included the microchromosomes in order to facilitate the comparison with other karyotypes described by Singh (1972a, 1974) in earlier papers.

RESULTS

Sixty-five metaphase plates were examined. The diploid number was 38 chromosomes; 20 macrochromosomes and 18 microchromosomes. The autosome pairs one and two are metacentrics, pair three has the centromere in a subterminal position and pairs from four to nine have a terminal centromere (Fig. 1 and 2). Three of the 4 specimens analyzed show a small constriction in one of the chromosomes of the fourth pair. The morphometric data of these and other sea snake karyotypes are listed in Table 1. The karyotypes of the females show a pair of slightly heteromorphic chromosomes. Both are metacentrics, but one of them has the centromere in a more submedian position and is slightly smaller than the other. These chromosomes were identified as Z and W sex chromosomes respectively; this identification is corroborated by similarity in relative length and centromeric position of Z chromosome with that of other species of this subfamily. Furthermore, it has a greater number of acrocentric chromosomes than those of other species of sea snakes whose karyotypes have been reported.

DISCUSSION

All of the sea snakes whose karyotypes have been studied belong to the subfamily Hydrophiinae, with the exception of *Laticauda semifasciata* (Table 2). The fact that all the species studied in this subfamily have 18 microchromosomes establishes that the chromosomal evolution has taken place within the macrochromosomes (Singh, 1972a). *Laticauda semifasciata,* Laticaudinae, has 24 microchromosomes according to Nakamura (1935); nevertheless, Gorman (personal communication) studied this karyotype with modern techniques and found a diploid number of 38, with 18 macro and 20 microchromosomes. According to Minton and da Costa (1975), *L. semifasciata* is clearly dissociated from other species of sea snakes as it is apparently a very primitive one.

Based on immunoelectrophoretic analysis of blood serum, Minton and da Costa (1975) reached the conclusion that *Lapemis, Astrotia, Hydrophis* and *Pelamis* make up a group with similar patterns. The biochemical and immunological analysis of venoms from several snakes shows a high similarity between them. The main toxin of *Lapemis hardwicki* and a toxin of

TABLE 1. Morphometric data of the chromosomes of the sea snakes karyotypes described. Relative length and Centromeric Index are given as fraction L^r/I^c*.

Species	1	2	3	4	5	6	7	8	9	Z	W_1	W_2	Microchromosomes	References
Pelamis platurus	12/48	8/48	5/22	13/6	10/6	9/4	7/5	6/8	5/7	10/48	9/42	—	16/0	Present report
Hydrophis ornatus	21/49	12/48	10/44	7/47	18/35	5/20	—	—	—	10/41	9/0	—	16/0	Singh (1974)
Hydrophis spiralis	20/49	11/44	9/45	6/45	18/35	4/22	—	—	—	8/39	8/21	—	14/0	Singh (1974)
Kerilia jerdoni	20/46	12/47	10/47	7/46	18/35	5/17	—	—	—	10/42	?	—	17/0	Singh (1974)
Hydrophis cyanocinctus	20/50	11/47	10/45	6/44	17/36	5/24	—	—	—	9/40	3/0	2/0	16/0	Singh (1972a)
Enhydrina schistosa	19/49	11/48	9/44	7/45	15/39	5/30	—	—	—	8/44	4/0	2/0	19/0	Singh (1972a)
Hydrophis fasciatus	19/48	14/48	6/43	17/34	4/25	5/0	4/0	—	—	10/47	4/46	3/34	14/0	Singh (1972a)
Microcephalophis gracilis	19/49	12/48	6/43	17/38	5/23	5/0	4/0	—	—	8/49	5/22	4/35	15/0	Singh (1972a)

$$*L^r = \frac{\text{Length of chromosome X 200}}{\text{Total length of karyotype (2n)}} \qquad I^c = \frac{\text{Length of short arm}}{\text{Length of chromosome}}$$

TABLE 2. Review of the karyotypes described from sea snakes.

Species	2n (macro. + microchromosomes)	Constriction	Sex heteromorphic patterns	Reference
Laticauda semifasciata	38 (18 + 20)	?	?*	Gorman (Pers. Comm.)
Pelamis platurus	38 (20 + 18)	yes	Z W	Present report
Hydrophis ornatus	32 (14 + 18)	yes	Z W	Singh (1972a, 1974)
Hydrophis spiralis	32 (14 + 18)	no	Z W	Singh (1972a)
Kerilia jerdoni	32 (14 + 18)	yes	?	Singh (1974)
Hydrophis cyanocinctus	males: 32 (14 + 18) females: 33 (15 + 18)	yes	Z W_1 W_2	Singh (1972a)
Enhydrina schistosa	males: 32 (14 + 18) females: 33 (14 + 19)	yes	Z W_1 W_2	Singh (1972b)
Hydrophis fasciatus	males: 34 (16 + 18) females: 35 (17 + 18)	no	Z W_1 W_2	Singh (1972a)
Microcephalophis gracilis	males: 34 (16 + 18) females: 35 (17 + 18)	no	Z W_1 W_2	Singh (1972a)

*According to Gorman, the probable pattern is Z W.

Enhydrina schistosa are identical (Fox, et al., 1977); another toxin of *E. schistosa* is identical to the pelamitoxin a of *Pelamis platurus* (Wong, et al., 1976). Also, an antivenom against *E. schistosa* cross-reacts with the venoms of *Hydrophis cyanocinctus, P. platurus* and *L. hardwicki* (Tu and Ganthavarn, 1969), when tested by immunodiffusion. Furthermore, the antivenin of *E. schistosa* neutralizes the venoms of *Praescutata viperina, P. platurus* and *L. laticauda* (Tu and Salafranca, 1974). These facts indicate a strong homology of these venoms. This situation makes the use of biochemical and immunological criteria difficult in the studies of the phylogenetic relationships within this group.

Singh (1972a, 1972b, 1974) reported the karyotypes of seven species of sea snakes and concluded that, at a chromosomal level, the evolution of this group has taken place on the fourth pair of autosomes and on the W-sex chromosome; centric fissions and inversions are the postulated mechanisms involved in these rearrangements.

There are two parameters that could be useful in order to establish evolutionary relationsh within the karyotypes of sea snakes: (a) The pattern of heteromorphism of sex chromosomes. T karyotypes of *Laticauda semifasciata, Hydrophis ornatus, Hydrophis spiralis* and *Pelamis platurus* have a two sex-chromosomes pattern (ZW) in females, with various degrees of variation. In the case of *Pelamis,* the variation between Z and W is smaller than in the others. On the other hand, the karyotypes of *Hydrophis cyanocinctus, Enhydrina schistosa* and *Microcephalophis gracilis* show a multiple sex chromosome system with ZW_1W_2 in females (Table 2). (b) The secondary constriction is present in one chromosome pair in several species of sea snakes and absent in others. This constriction is present in the first pair of *H. ornatus, H. cyanocinctus, E. schistosa* and *K. jerdoni* and in the fourth pair of *P. platurus* (Table 2).

At this time further research is needed on the karyotypes of additional sea snake species; application of modern techniques of chromosome banding could help to determine homologies. It is also important to study the karyotypes of specimens of *Pelamis platurus* from other latitudes to see if there are intraspecific variations. These studies, together with molecular and cytogenetic data will assist in the understanding of a topic so controversial as the evolution of sea snakes.

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