

## Horseshoe crabs (King crabs) in the Bay of Bengal, South Asia

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### Abstract

We report all the species of horseshoe crabs in the Bay of Bengal, that is, South Asia. We found unique horseshoe crabs in mangroves of Sundarbans (the river-mouth of the Ganges) in Bangladesh and India. Also relationships of those horseshoe crabs and other horseshoe crabs in the world were examined. It was concluded that the Sundarbans Horseshoe crab is a sub-species of *Carinoscorpius rotundicauda*. However, we propose to call them the Sundarbans Horseshoe crabs from now on due to their uniqueness. Results suggested that there are two species namely *Carcinoscorpius rotundicauda* and *Tachypleus gigas*, found in the Bay of Bengal. Although they have no uniformity even in one species. In addition, we discuss the evolution, distribution and ecology of the Sundarbans Horseshoe crab and other ones in the Bay of Bengal.

key words: Horseshoe crab; South Asia; Bengal Bay; kinds; distribution; evolution

### Introduction

The horseshoe crabs, also known as king crabs are considered as the living fossils. They evolved from trilobites about 550 million years ago, and keep similar shapes for more than 200 million years. In the present days, they live only in east coast of North America and in Asia, although they lived all over the world long long ago (Itow et al., 2003). In Asia, they are distributed from the Bay of Bengal to South-West Japan. The horseshoe crabs in the Bay of Bengal are the ones at the most west end of distribution in Asia. Their species, distribution, shapes and behaviors are very interesting.

However, there are scanty informations about the horseshoe crabs in the Bay of

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Bengal, that is, in South Asia, especially in Bangladesh (Chowdhury and Hafizuddin, 1980). The report is limited to the East Bangladesh. Sekiguchi et al. (1976, 1978) have reported the horseshoe crabs in West Bengal of India, but they did not examine the horseshoe crabs of Bangladesh. In the present studies, we have investigated all regions of the Bay of Bengal including most seashores of Bangladesh, West Bengal and Orissa of India (Fig. 1). There are also several reports about the horseshoe crabs in Orissa and West Bengal in India (Roonwal, 1944; Chatterji et al., 1988, 1992; Debnath, 1991; Chatterji and Parulekar, 1992)

We found unique horseshoe crabs in the mangroves of the Sundarbans in Bangladesh, that is, in the river-mouth of the Ganges, which we compared with all the other horseshoe crabs from all over the world. This paper deals with the types of horseshoe crabs occurring in the Bay of Bengal with their characteristic features, distribution, ecology and evolution.

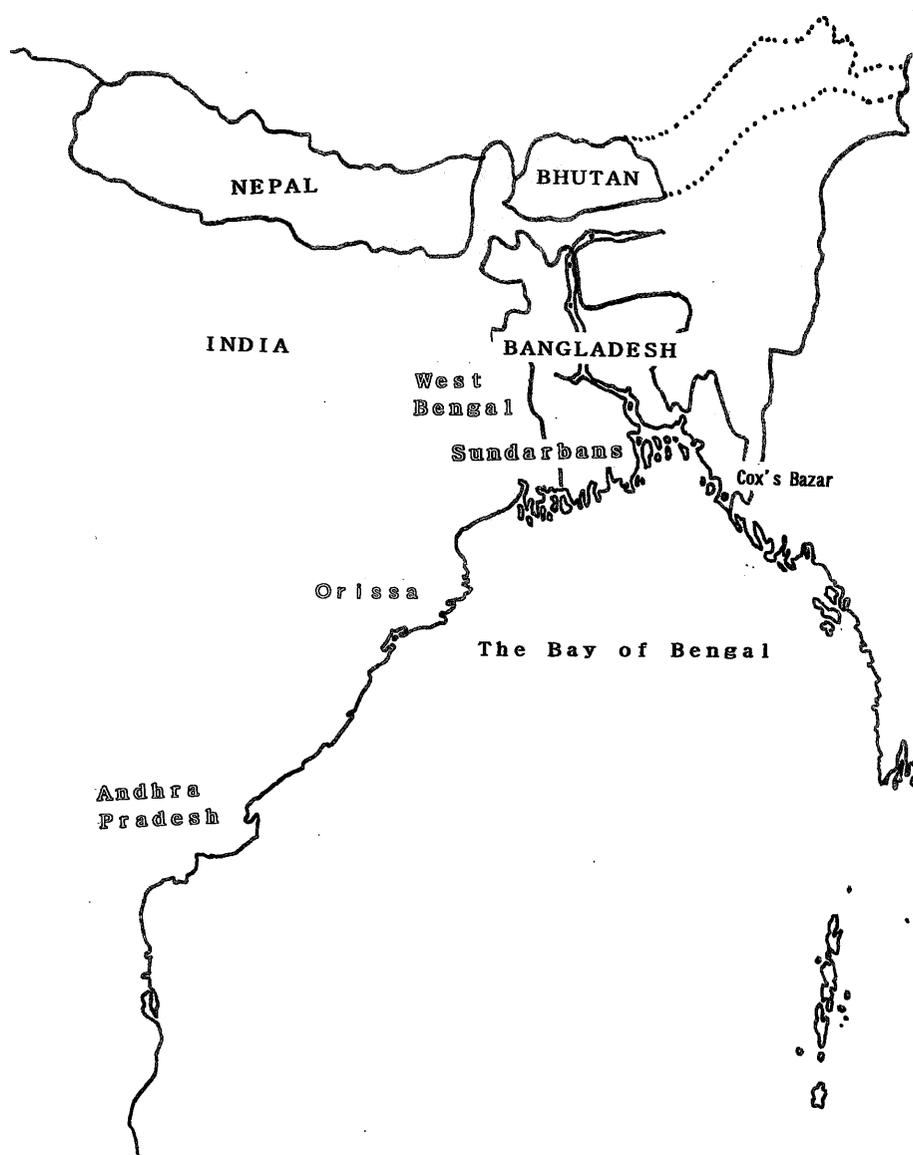


Fig. 1. The map of the Bay of Bengal

## Materials and Methods

### I. Materials

Horseshoe crabs at the Bay of Bengal region (Sundarbans, Cox's Bazar, St. Martin's Island, Orissa and West Bengal etc.), that is, South Asia mainly from April to June, 2002 were making examined. Most of the collected horseshoe crabs were examined in the field, and some were carried to Shizuoka University of Japan for further investigation.

Horseshoe crabs, *Tachypleus gigas* and *Carcinoscorpius rotundicauda* of South-East Asia from Bangsean in the Gulf of Siam (Gulf of Thailand) of Thailand were obtained from Prof. Suman of Srinakarin Virote Univesity, who kindly sent them to Shizuoka University, Japan.

American horseshoe crabs, *Limulus polyphemus* at the beach of Cape-May, New Jersey, USA were collected. Besides, we also got American horseshoe crabs from the Marine Laboratory of Woods Hole, Massachusetts, USA and the Gulf Specimen company in Florida, USA.

Japanese horseshoe crabs, *Tachypleus tridentatus* were collected from the beaches of South-West Japan.

Fossils of *Mesolimulus walchi* were found in Europe. We mainly examined them with the photographs which were taken at Solnhofen Museum in Bayern, Germany. Besides, we also examined some real fossils which were gotten from Germany.

### II. Methods

Different body parts of a number of horseshoe crabs were measured. Also the shapes, colors and body patterns were examined.

At Shizuoka University, some of the horseshoe crabs from the Bay of Bengal were artificially inseminated, and the eggs, embryos and larvae were obtained. Attempts were made to hybridize between all kinds of horseshoe crabs. Those eggs, embryos and larvae were observed in comparision with those of the horseshoe crabs of other places by using light microscopes.

The proteins of eggs, embryos and adults of the horseshoe crabs from the Bay of Bengal and other places were analyzed by the acrylamide electrophoresis in order to identify the species. The blood and whole unferilized eggs were examined by electrophoresis.

For the same purpose, the chromosome number of different kinds of horseshoe crabs were also examined. For this, embryos and the 1st and 2nd instar larvae were treated with 0.005% colchicine-2.9% NaCl<sub>2</sub> and the chromosomes of cephalothoracic epithelia were stained with Giemsa solution after fixation in 3:1 methanol-acetic acid mixture (Sekiguchi et al., 1988).

## Results

## I. The Sundarbans Horseshoe crabs

We found unique horseshoe crabs in the mangroves of the Sundarbans in Bangladesh, that is, at the river-mouth of the Ganges. There are wide forests of unique mangrove. Only one species of the animal was observed there, which we refer as Sundarbans Horseshoe crabs hereafter (Fig. 2). The shapes of their tails and their male claws are similar to those of the typical *Carcinoscorpius rotundicauda* of South-East Asia, such as in Thailand, Vietnam and south China. The sections of tails are round. The shapes of the 2nd and 3rd cephalothoracic appendages of males are a little modified, but claw-shapes. Whereas, the shapes of claws at same positions in case of *Tachypleus gigas* and *T. tridentatus* are very transformed, and hook-shaped. But there are also significant differences between the Sundarbans Horseshoe crab and the typical *Carcinoscorpius rotundicauda* of South-East Asia, which are as follows: ①The body size of the Sundarbans Horseshoe crab is larger than the typical *Carcinoscorpius rotundicauda* (Figs. 2 and 3, Table I). *Carcinoscorpius rotundicauda* is usually smaller than *Tachypleus gigas* in many places, whereas, Sundarbans horseshoe crabs are similar in sizes to *Tachypleus gigas* in the Bay of Bengal. Especially, the males are larger than their counter parts of *Tachypleus gigas* found in Western Bay of Bengal (TableII). ②The marginal (lateral) spines of abdomen of the Sundarbans Horseshoe crabs are relatively shorter than those



Fig. 2. The Sundarbans Horseshoe crab (center). left: typical *Carcinoscorpius rotundicauda* of South-East Asia (Thailand), right: typical *Tachypleus gigas* of South-East Asia (Thailand). The scale indicates 10 cm.

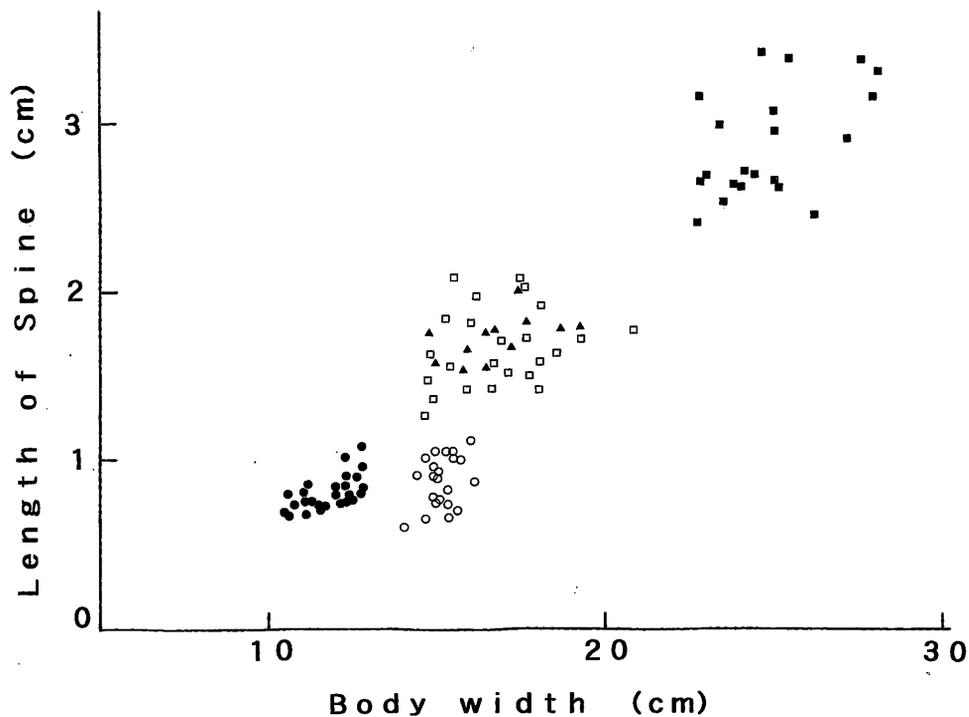


Fig. 3. The length of the 1st marginal spine (the ordinate) and the body width (the abscissa) of male horseshoe crabs in the world. Black circle: *Carcinoscorpius rotundicauda* of South-East Asia, Open circle: the Sundarbans Horseshoe crab, Black triangle: *Tachypleus gigas* of South-East Asia, Open square: *Limulus polyphemus*, Black square: *Tachypleus tridentatus* of Japan.

Table I

Measurement of the horseshoe crabs of the Bay of Bengal. The body width and length of the 1st marginal spine of males are shown in this table. Larvae of more than 6.5 cm of body width, or 0.3 cm of the 1st spine were used for the measurement, because the length of spines of smaller ones was not exactly measured.

cm (mean  $\pm$  standard deviation)

Place	Body width (A)	Length of spine (B)	B/A $\times$ 10
<i>Carcinoscorpius rotundicauda</i> and related horseshoe crabs			
Siam Gulf, Thailand	11.9 $\pm$ 0.7	0.81 $\pm$ 0.09	0.68 $\pm$ 0.06
East Bengal Bay	13.3 $\pm$ 0.8	0.78 $\pm$ 0.14	0.59 $\pm$ 0.08
Sundarbans	15.0 $\pm$ 0.7	0.88 $\pm$ 0.15	0.59 $\pm$ 0.09
West Bengal Bay	10.1 $\pm$ 2.0	0.69 $\pm$ 0.20	0.67 $\pm$ 0.10
<i>Tachypleus gigas</i>			
Siam Gulf, Thailand	17.3 $\pm$ 0.9	1.76 $\pm$ 0.06	1.01 $\pm$ 0.06
East Bengal Bay	14.9 $\pm$ 0.6	1.58 $\pm$ 0.06	1.07 $\pm$ 0.17
West Bengal Bay	13.9 $\pm$ 0.8	1.59 $\pm$ 0.06	1.15 $\pm$ 0.17

East Bengal Bay: = East Bangladesh including Cox's Bazar, St Martin's Island and neighbouring places in Bangladesh. Sundarbans: both Sundarbans of Bangladesh and India. West Bengal Bay: Orissa in India and neighbouring places.

Table II

Comparison of body width of *Carcinoscorpius rotundicauda* including related horseshoe crabs and *Tachypleus gigas* in each place of South-East Asia and South Asia, that is, the Bay of Bengal.

cm (mean  $\pm$  standard deviation)

Place		<i>Tachypleus gigas</i> (T.g.)	<i>Carcinoscorpius rotundicauda</i> and related horseshoe crabs (C. r)	T.g./C.r.
Siam Gulf, Thailand	♀	20.9 $\pm$ 1.3	13.2 $\pm$ 0.9	1.58
	♂	17.3 $\pm$ 0.9	11.9 $\pm$ 0.7	1.45
East Bengal Bay	♀	18.8 $\pm$ 0.7	14.7 $\pm$ 0.8	1.28
	♂	14.9 $\pm$ 0.6	13.3 $\pm$ 0.8	1.12
West Bengal Bay	♀	17.5 $\pm$ 0.6	15.9 $\pm$ 1.0	1.10
	♂	13.9 $\pm$ 0.8	15.0 $\pm$ 0.7	0.93

East Bengal Bay = East Bangladesh: Cox's Bazar in Bangladesh and the neighbouring places including the St. Martin's Island West Bengal Bay: Sundarbans both in Bangladesh and India, and Orissa of India. In Sundarbans, almost horseshoe crabs are Sundarbans Horseshoe crab which are related to *Carcinoscorpius rotundicauda*, and most horseshoe crabs in Orissa are *Tachypleus gigas*.

Table III

Measurement of the horseshoe crabs of the world. The body width and the length of the 1st marginal spine of males are shown in this table. The fossil *Mesolimulus walchi* were mainly measured with the photographs, therefore real lengths can not be shown. They are thought to be larvae.

cm (mean  $\pm$  standard deviation)

Species (Place)	Body width(A)	Length of spine(B)	B/A $\times$ 10
Female			
<i>Tachypleus tridentatus</i> (Japan)	28.2 $\pm$ 1.5	3.25 $\pm$ 0.33	1.15 $\pm$ 0.10
<i>Limulus polyphemus</i> (USA)	23.3 $\pm$ 1.8	2.24 $\pm$ 0.24	0.96 $\pm$ 0.10
<i>Tachypleus gigas</i> (Thailand)	20.9 $\pm$ 1.3	2.10 $\pm$ 0.19	1.00 $\pm$ 0.07
<i>Carcinoscorpius rotundicauda</i> (Thailand)	13.2 $\pm$ 0.9	0.94 $\pm$ 0.12	0.72 $\pm$ 0.07
Male			
<i>Tachypleus tridentatus</i> (Japan)	28.2 $\pm$ 1.5	3.25 $\pm$ 0.33	1.15 $\pm$ 0.10
<i>Limulus polyphemus</i> (USA)	23.3 $\pm$ 1.8	2.24 $\pm$ 0.24	0.96 $\pm$ 0.10
<i>Tachypleus gigas</i> (Thailand)	20.9 $\pm$ 1.3	2.10 $\pm$ 0.19	1.00 $\pm$ 0.07
<i>Carcinoscorpius rotundicauda</i> (Thailand)	13.2 $\pm$ 0.9	0.94 $\pm$ 0.12	0.72 $\pm$ 0.07
<i>Mesolimulus walchi</i> (Europe)			1.15 $\pm$ 0.20

of the typical *Carcinoscorpius rotundicauda* (Fig. 3, Table III). ③The color of the Sundarbans Horseshoe crab is dark green or greenish black. On the other hand, the color of typical *Carcinoscorpius rotundicauda* is brown or dark brown (Fig. 2). ④The carapace of the Sundarbans Horseshoe crab is thicker and swollen than that of the typical *Carcinoscorpius rotundicauda*, especially in abdomen region. ⑤The entire body shape except the tail of the Sundarbans Horseshoe crab is circular, the typical *Carcinoscorpius rotundicauda* is not perfectly circular (Fig. 2).

The horseshoe crabs in the Sundarbans of West Bengal in India, except *Tachypleus gigas*, have similar shapes to those of the horseshoe crabs in the Sundarbans of Bangladesh. There are also no remarkable differences between the size of both the groups. The Sundarbans of India and Bangladesh are not separated from each other, and belong to the river-mouth of the Ganges with same kind of mangrove forests. Thus we conclude that the Sundarbans Horseshoe crabs are distributed in the seas of Sundarbans of both Bangladesh and India. Most horseshoe crabs found in West Bengal of India were the Sundarbans Horseshoe crabs and we rarely found *Tachypleus gigas* there. The neighbouring place Orissa of India have wide sandy beaches, which are suitable for the living of *Tachypleus gigas*.

The Sundarbans Horseshoe crab is obviously different from *Tachypleus gigas*. The differences are observed in the shape of tail, the length of spine and the shape of male claws. The sections of tails of *Tachypleus gigas* are triangular and the spines are very long (Fig. 2, Tables I, II and III).

## II. The horseshoe crabs related to *Carcinoscorpius rotundicauda* in the East of the Bay of Bengal, that is, East Bangladesh

In the eastern Bangladesh (East Bangladesh), that is, East Bengal Bay including Cox's Bazar and Saint Martin's Island, there are two species of horseshoe crabs. One is *Carcinoscorpius rotundicauda*-like horseshoe crab and *Tachypleus gigas*, which are a little different from typical ones of South-East Asia. We shall describe the *Tachypleus gigas* later. At first, we would describe *Carcinoscorpius rotundicauda*-like horseshoe crab. These horseshoe crabs of East Bangladesh have the similar characteristics to Sundarbans Horseshoe crab. However, some characteristics are also similar to the typical *Carcinoscorpius rotundicauda* of South-East Asia. The shapes and colors are mainly similar to ones of Sundarbans Horseshoe crabs. But, the swelling of carapaces are a little weaker than ones of Sundarbans Horseshoe crabs, the color of some *Carcinoscorpius rotundicauda*-like horseshoe crabs of East Bangladesh included brown, and the spines of this species sometimes relatively longer than ones of Sundarbans Horseshoe crabs. The body sizes are smaller than the Sundarbans Horseshoe crab and larger than the typical *Carcinoscorpius rotundicauda* of South-East Asia (Table I, Figs. 2, 4 and 5).

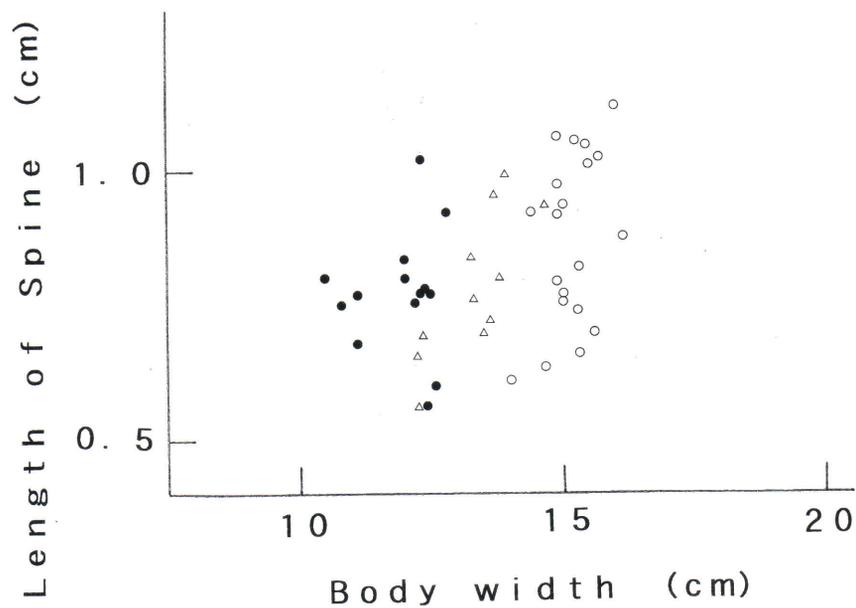


Fig. 4. The horseshoe crabs related to *Carcinoscorpius rotundicauda*. The length of the 1st marginal spine and the body width of males are shown in this figure. Black circle: typical *Carcinoscorpius rotundicauda* of Siam Bay in Thailand, Triangle: *Carcinoscorpius rotundicauda*-like horseshoe crab of East Bangladesh, Open circle: the Sundarbans Horseshoe crab.



Fig. 5. *Carcinoscorpius rotundicauda* and the related horseshoe crabs (female). From left; typical *Carcinoscorpius rotundicauda* of South-East Asia (Thailand), *Carcinoscorpius rotundicauda*-like horseshoe crab in East Bangladesh, and the Sundarbans Horseshoe crab. The scale indicates 10 cm.

In West Bengal of India, there are Sundarbans Horseshoe crabs in the mangrove forests as mentioned above. In the west of West Bengal and in many region of Orissa of India, there are wide sandy beaches. Mainly *Tachypleus gigas* lives there. In the north of Andhra Pradesh of India, we observed a few horseshoe crab also. However, in the south of Andhra Pradesh and other south and west regions of India, we could never find any horseshoe crabs. In the mangroves of south Orissa and north Andhra Pradesh, there are a few small *Carcinoscorpius rotundicauda*-like horseshoe crabs. Their characteristics are very similar to those of East Bangladesh.

### III. *Tachypleus gigas* in the Bay of Bengal

Concerning with *Tachypleus gigas*, they live in East Bangladesh and West Bengal Bay, that is, Orissa of India and neighbouring places. The *Tachypleus gigas* of East Bangladesh, that is, East Bengal Bay has no different characteristics from the *Tachypleus gigas* of West Bengal Bay. Although the body sizes of *Tachypleus gigas* of West Bengal Bay are a little smaller than the East Bengal Bay, and the marginal spines of *Tachypleus gigas* of West Bengal Bay are relatively longer than the those of the East Bengal Bay (Tables I and II).

Overall, the *Tachypleus gigas* in the Bay of Bengal is smaller than the typical *Tachypleus gigas* of South-East Asia (Tables I and II). However, the entire forms and patterns of *Tachypleus gigas* in the Bay of Bengal are very similar to the typical one in South-East Asia.

### IV. The eggs, embryos and larvae

We examined eggs, embryos and larvae after artificial inseminations (Fig. 6). The egg diameters of *Tachypleus gigas* ranges from 3.5 mm to 3.6 mm, while ones of the Sundarbans Horseshoe crab are about 2.5 mm. The diameter of the typical *Carcinoscorpius rotundicauda* eggs of Thailand are about 2.4 mm, whereas, the egg diameter of *Carcinoscorpius rotundicauda*-like horseshoe crabs in East Bangladesh are about 2.3 mm. In summary, the egg sizes of Sundarbans Horseshoe crab and *Carcinoscorpius rotundicauda*-like horseshoe crabs in East Bangladesh are similar to those of the typical *Carcinoscorpius rotundicauda* of South-East Asia (Table IV). The examination of shapes and sizes of the 1st instar larvae shows same results. The 1st instar larvae of the Sundarbans Horseshoe crab have same shapes and sizes with those of the typical *Carcinoscorpius rotundicauda* in Thailand and the *Carcinoscorpius rotundicauda*-like horseshoe crabs in East Bangladesh (Fig. 7, Tables II and V).

The marginal spines of larvae of Sundarbans Horseshoe crabs are relatively longer than those of the adults (Table I). It indicates that at the time of the last moulting, when sub-adults become adults, the marginal spines do not lengthen (Fig. 8).

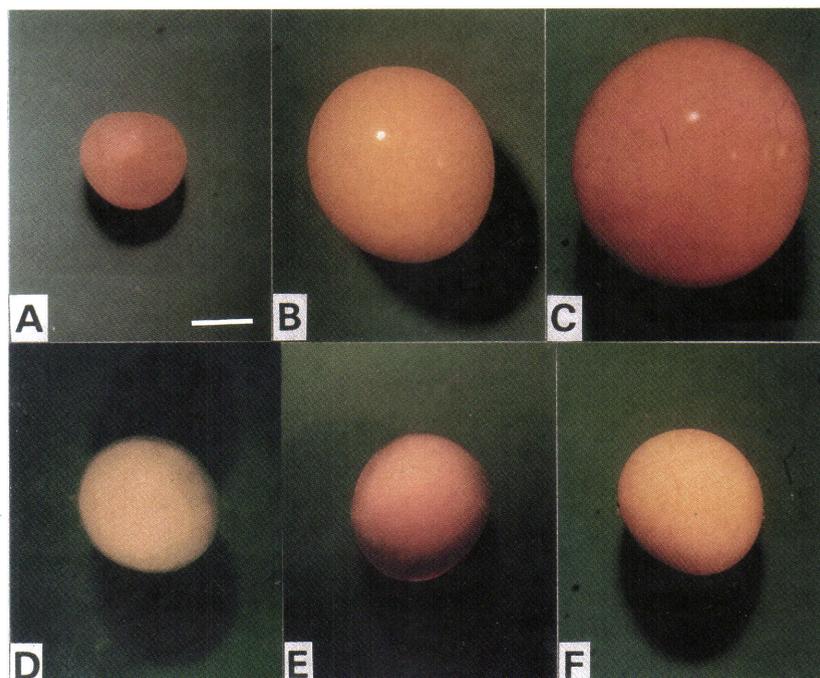


Fig. 6. The eggs of different kinds of horseshoe crabs. Same magnification. A: *Limulus polyphemus*, B: *Tachypleus tridentatus* of Japan, C: typical *Tachypleus gigas* of South-East Asia (Thailand), D: typical *Carcinoscorpius rotundicauda* of South-East Asia (Thailand), E: *Carcinoscorpius rotundicauda*-like horseshoe crab of East Bangladesh, F: the Sundarbans Horseshoe crab. The scale indicates 10 mm.

Table IV

Measurement of the egg diameter of horseshoe crabs in the world.

Species (Place)	Egg Diameter (mm) (mean $\pm$ standard deviation)
<i>Tachypleus tridentatus</i> (Japan)	2.86 $\pm$ 0.09
<i>Limulus polyphemus</i> (USA)	1.62 $\pm$ 0.07
<i>Tachypleus gigas</i> (Thailand)	3.61 $\pm$ 0.09
<i>Tachypleus giga</i> (East Bengal Bay)	3.51 $\pm$ 0.10
<i>Tachypleus gigas</i> (West Bengal Bay)	3.50 $\pm$ 0.06
<i>Carcinoscorpius rotundicauda</i> (Thailand)	2.36 $\pm$ 0.04
<i>Carcinoscorpius rotundicauda</i> -like (East Bengal Bay)	2.26 $\pm$ 0.05
Sundarbans Horseshoe crab	2.48 $\pm$ 0.02

East Bengal Bay = East Bangladesh: Cox's Bazar in Bangladesh and the neighbouring places including the St. Martin's Island. West Bengal Bay: Orissa in India and neighbouring places.

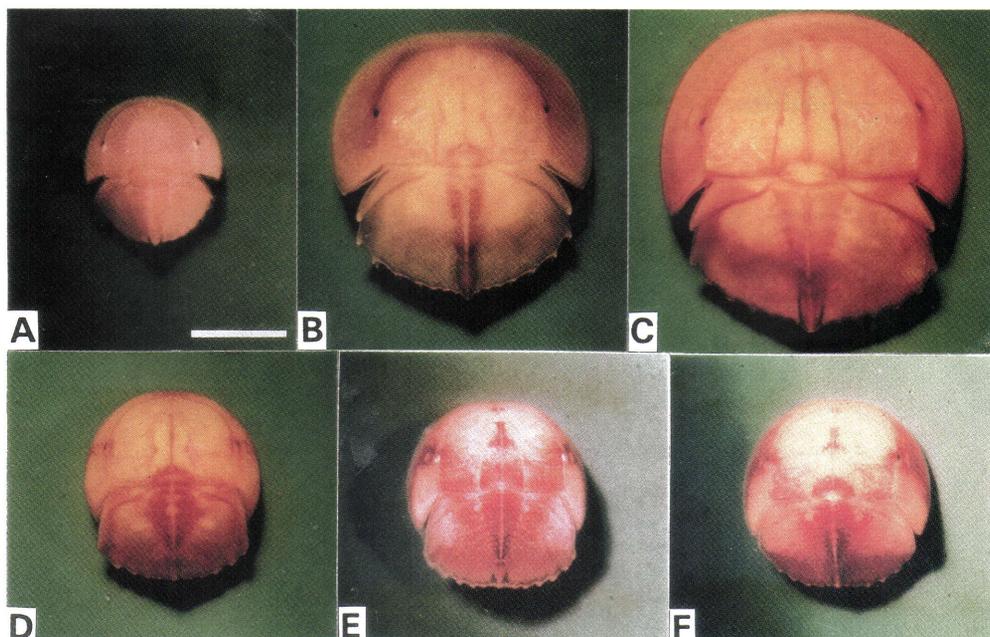


Fig. 7. The 1st instar larvae of different kinds of horseshoe crabs. Same magnification. A: *Limulus polyphemus*, B: *Tachypleus tridentatus* of Japan, C: typical *Tachypleus gigas* of South-East Asia (Thailand), D: typical *Carcinoscorpius rotundicauda* of South-East Asia (Thailand), E: *Carcinoscorpius rotundicauda*-like horseshoe crab of East Bangladesh, F: the Sundarbans Horseshoe crab. The scale indicates 20 mm.

Table V

Measurement of the body width of the 1st instar larvae of horseshoe crabs in the world.

Species (Place)	Egg Diameter (mm) (mean $\pm$ standard deviation)
<i>Tachypleus tridentatus</i> (Japan)	5.31 $\pm$ 0.06
<i>Limulus polyphemus</i> (USA)	3.00 $\pm$ 0.06
<i>Tachypleus gigas</i> (Thailand)	7.03 $\pm$ 0.26
<i>Tachypleus gigas</i> (East Bengal Bay)	6.93 $\pm$ 0.31
<i>Tachypleus gigas</i> (West Bengal Bay)	6.91 $\pm$ 0.30
<i>Carcinoscorpius rotundicauda</i> (Thailand)	3.96 $\pm$ 0.05
<i>Carcinoscorpius rotundicauda</i> -like (East Bengal Bay)	3.96 $\pm$ 0.24
Sundarbans Horseshoe crab	4.10 $\pm$ 0.08

East Bengal Bay = East Bangladesh: Cox's Bazar in Bangladesh and the neighbouring places including the St. Martin's Island. West Bengal Bay: Orissa in India and neighbouring places.

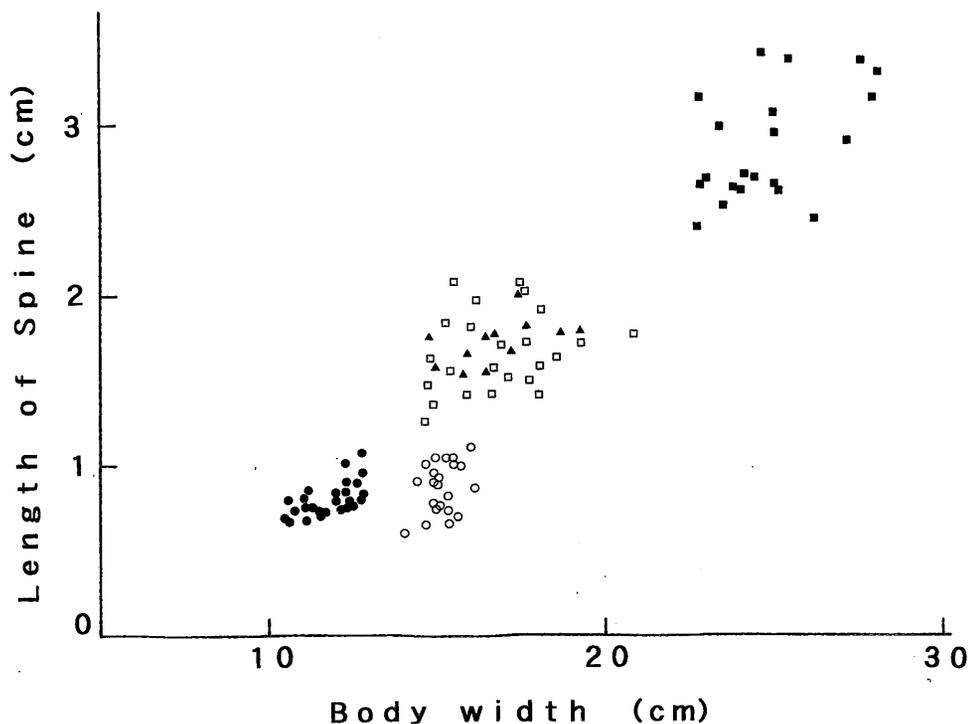


Fig. 8. The growth of larvae of the Sundarbans Horseshoe crab. The length of the 1st marginal spine and the body width are shown in this figure. Black circle: larvae, Open circle: adults (males). Larvae of more than 6.5 cm of body width, or 0.3 cm of the 1st marginal spine were recorded in this figure, because the length of spines of smaller ones was not exactly measured.

Concerning with the length of adult marginal spines of different horseshoe crabs, those of *Mesolimulus walchi* are relatively longest. In the present species, *Tachypleus tridentatus*, that is, the Japanese horseshoe crabs have relatively longest marginal spines (Table III).

#### V. Hybridization, electrophoresis and chromosome numbers

The results of hybridization are as follows: In all cases of hybridizations carried out between Asian species, the hybridized eggs developed normally, although the frequencies of embryonic developments became decreased. For example, the Sundarbans Horseshoe crab  $\times$  *Tachypleus gigas*, Sundarbans Horseshoe crab  $\times$  *T. tridentatus*, *T. gigas*  $\times$  *T. tridentatus*, *Carcinoscorpius rotundicauda* from Thailand  $\times$  *T. tridentatus*, and so on.

On other hand, the hybridized eggs between *Limulus polyphemus* and Asian horseshoe crabs never developed.

The results of electrophoresis showed that the molecular patterns of Sundarbans Horseshoe crab, *Carcinoscorpius rotundicauda*-like horseshoe crabs from East Bangladesh, and the typical *Carcinoscorpius rotundicauda* in South-East Asia were same. The pattern of *Carcinoscorpius rotundicauda* was remarkably different from that of *Tachypleus gigas*. While, the electrophoresis pattern of *Tachypleus gigas* from West

Bengal Bay, *Tachypleus gigas* from East Bangladesh, and the typical *Tachypleus gigas* from South-East Asia were same.

The chromosome numbers of Sundarbans Horseshoe crabs are 32. The number of *Carcinoscorpius rotundicauda*-like horseshoe crabs from East Bangladesh are also 32. These numbers are same with that of the typical *Carcinoscorpius rotundicauda* from South-East Asia. The chromosome numbers in *Tachypleus gigas* are 28, and the number of *Tachypleus tridentatus* was 26, and *Limulus polyphemus* 52.

## VI. Habitats and behaviors of horseshoe crabs of the Bay of Bengal

We could find many facts about the habitat and behavior of horseshoe crabs in the Bay of Bengal.

In the East Bangladesh (= East Bengal Bay), *Carcinoscorpius rotundicauda*-like horseshoe crabs and *Tachypleus gigas* lay eggs at the same beaches. The soil of the beaches is a mixture of silt (mud) and sand. The points of egg laying by *Carcinoscorpius rotundicauda*-like horseshoe crabs are in the vicinity of mangrove forests. On the other hand, the points of egg laying by *Tachypleus gigas* are bit away from the mangrove forests. The larvae of *Carcinoscorpius rotundicauda*-like horseshoe crabs live in the mud flat adjacent to the beach. Whereas, the larvae of *Tachypleus gigas* live a little offshore. The adults of *Carcinoscorpius rotundicauda*-like horseshoe crabs live in the silt (mud) of mangrove forests or near seashores. On the other hand, the adults of *Tachypleus gigas* live the offshore.

In the Sundarbans, there are unique and wide mangrove forests. Mangrove trees of other places of world are low and mainly have stilt roots, which are like the foot of octopus. But, the high trees of mangroves in the Bay of Bengal have respiratory roots (pneumatophores) which stand upright (Fig. 9). This type of mangrove is primitive. It was found that the Sundarbans Horseshoe crabs lay eggs on the beaches which constitutes a mixture of silt (mud) and sand. But the larvae after hatching live in the silty mudflat of the mangroves.

In the sandy beaches of Orissa in India, we found the egg laying of *Tachypleus gigas*, where many *Tachypleus gigas* come to the beaches in pairs to lay eggs at the time of highest high tide of full moon and new moon days. The larvae after hatching migrate a little offshore and live there.

In all the places through the Bay of Bengal region, that is, South Asia, horseshoe crabs were found to live near river-mouths. But the horseshoe crabs related to *Carcinoscorpius rotundicauda* live in mangrove forests. On the other hand, *Tachypleus gigas* live in relatively sandy seacoasts. There is the habitat segregation of those horseshoe crabs. There are a few reports on the ecology of horseshoe crabs in South-East Asia (Sekiguchi et al., 1976, 1978). It suggested that the ecology of horseshoe crabs of South-East Asia is not different from one found in the Bay of Bengal, that is,



Fig. 9. The respiratory roots of the mangrove forests in Sundarbans.

South Asia. The main difference of South-East Asia and South Asia is the type of mangroves.

VII. Geographical distribution of *Carcinoscorpius rotundicauda* and the related horseshoe crabs in all over the world

*Carcinoscorpius rotundicauda* live in South China, which is the region west of Macao, and do not live in the regions east and north of Hong Kong. *Carcinoscorpius rotundicauda* also found to live in the islands of the Orient Zone. Besides, they also live in the continental region of Vietnam, Cambodia (Kampuchea), Thailand and Malaysia. Those *Carcinoscorpius rotundicauda* of South-East Asia are similar to each other. In South Asia, that is, the Bay of Bengal, there are *Carcinoscorpius rotundicauda*-like horseshoe crabs and Sundarbans Horseshoe crabs as mentioned earlier.

VIII. Geographical distribution of *Tachypleus gigas* in all over the world

In the Tonkin Bay of South China and North Vietnam, there are many *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda*, but there were very rarely any *Tachypleus gigas* found. *Tachypleus gigas* live in the continental region west of South Vietnam.

Besides, *Tachypleus gigas* live in islands of the Oriental Zone. They are also found in the Bay of Bengal is mentioned above.

In summary, *Carcinoscorpius rotundicauda* and *Tachypleus gigas* live in the same regions except, the Tonkin Bay and the Sundarbans.

## Discussion

### I. Is Sundarbans Horseshoe crab a new species or sub-species of *Carcinoscorpius rotundicauda* ?

Judging from those characteristics mentioned earlier, we can say that the Sundarbans Horseshoe crab is either a new species or a sub-species of *Carcinoscorpius rotundicauda*. On the basis of the morphology studies, it can be said that Sundarbans Horseshoe crab is a new species. If they were fossils, we could have reached at the same conclusion.

However, we now conclude that it is a sub-species of *Carcinoscorpius rotundicauda* due to the reasons such as follows: (1) The Sundarbans Horseshoe crabs look like *Carcinoscorpius rotundicauda*-like horseshoe crabs in eastern Bangladesh (East Bangladesh), and some characteristics of *Carcinoscorpius rotundicauda*-like horseshoe crabs in East Bangladesh are similar to those of *Carcinoscorpius rotundicauda* in South-East Asia, especially those of Phuket Island where is at the west of Malay Peninsula. The *Carcinoscorpius rotundicauda* which live in Phuket Island and neighbouring places very resemble the *Carcinoscorpius rotundicauda* in the east of Malay Peninsula, which is, the typical *Carcinoscorpius rotundicauda*. But the horseshoe crabs of the west are a little larger than ones of the east (Sekiguchi et al., 1978). From these results, we presume the continuance of *Carcinoscorpius rotundicauda* and related horseshoe crabs. (2) The eggs, embryos and larvae of the Sundarbans Horseshoe crabs are very similar those of the typical *Carcinoscorpius rotundicauda* in South-East Asia (Figs. 6 and 7). (3) The molecular patterns of the Sundarbans Horseshoe crabs are same with those of the typical *Carcinoscorpius rotundicauda* of South-East Asia. Also the chromosome number of the Sundarbans Horseshoe crab is same with the typical *Carcinoscorpius rotundicauda* in South-East Asia, which is, 32 in numbers. However, we propose to call the horseshoe crabs of Sundarbans as the Sundarbans Horseshoe crab from now on, because they are very unique in their characteristics.

We also concluded *Carcinoscorpius rotundicauda*-like horseshoe crabs in East Bangladesh are the intermediate type from which the typical *Carcinoscorpius rotundicauda* in South-East Asia evolves to the Sundarbans Horseshoe crab. Both the Sundarbans Horseshoe crabs and *Carcinoscorpius rotundicauda*-like horseshoe crabs in East Bangladesh can become very important experimental model for analysing the origin and process of evolution of species.

## II. Cause of evolution of the Sundarbans Horseshoe crab

All the present horseshoe crabs in the world have a common ancestor, *Mesolimulus walchi*, which had long marginal spines and lived 200 million years ago in Europe (Table III). According to the separation of the continents, some of them moved to North America and others to Asia.

Judging from the length and chromosome numbers, *Tachypleus tridentatus* is most similar to *Mesolimulus walchi*. In Asia, *Mesolimulus walchi* may have become *Tachypleus tridentatus* whose chromosome number is 26. Then, *Tachypleus gigas* (chromosome number = 28) and *Carcinoscorpius rotundicauda* (chromosome number=32) appeared thereafter. *Tachypleus tridentatus* and *T. gigas* live in similar environment and the struggle of existence occurred between both species. In the tropical zone, *T. gigas* is stronger than *T. tridentatus*. Therefore, *T. tridentatus* now live in the periphery of South-East Asia and exceptionally in the Arctic zone, Japan. We suggest that the number of chromosome in *Mesolimulus walchi* was 26. It may have become the double, that is, 52 in the continent of North America, which became American horseshoe crabs, *Limulus polyphemus*. The possibility of hybridization between Asian species may show that they branched off from each other in the recent geologic time.

The origin of *Tachypleus gigas* and *Carcinoscorpius rotundicauda* is thought to be South-East Asia judging from the distribution pattern. If the Bay of Bengal were the place of origin, we could not explain the fact that they are never distributed in south and west India. *Tachypleus gigas* and *Carcinoscorpius rotundicauda* segregated the habitat. *Tachypleus gigas* lives in the original environment, that is, silt (mud) and sandy seacoast. On the other hand, *Carcinoscorpius rotundicauda* lives in the mangroves.

*Carcinoscorpius rotundicauda* evolved to match the environment of the mangroves of South-East Asia, which have stilt roots. The body size became smaller and the marginal spines became shorter.

Once upon a time, some of the *Carcinoscorpius rotundicauda* may have come to the Bay of Bengal. The mangroves of the Bay of Bengal are very different from those of South-East Asia, they are mainly tall trees having respiratory roots which stand upright (Fig.9). The horseshoe crabs began to evolve to match these unique mangrove environment. They became larger in body size, and some of them became larger than *Tachypleus gigas*. Especially in Sundarbans, where there are big mangrove forest with unique environment, they evolved into the Sundarbans Horseshoe crab. Their marginal spines became relatively shorter, although their larvae have relatively long spines (Fig. 8). The long spines of larvae show that their ancestor had long spines.

On the other hand, *Tachypleus gigas* may run away from Sundarbans forests after the defeat of the struggle for existence against Sundarbans Horseshoe crabs. Besides, *Tachypleus gigas* became smaller in all the Bay of Bengal regions.

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