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**RESEARCH ARTICLE**

# Identification and Morphology of the Two Horseshoe Crab Species, *Tachypleus tridentatus* (Leah, 1819) and *Carcinoscorpius rotundicauda* (Latreille, 1802) (Merostomata: Limulidae) from Honda Bay, Palawan, Philippines

**Juliana C. Baylon[1] and Lota B. Alcantara-Creencia[2]**

[1]*Division of Biological Sciences, College of Arts and Sciences, University of the Philippines Visayas, 5023 Miagao, Iloilo, Philippines*
[2]*College of Fisheries and Aquatic Sciences, Western Philippines University, 5300 Puerto Princesa City, Philippines*

ABSTRACT

There were reports of the existence of horseshoe crabs in Palawan, Philippines, but there is a need to identify and confirm these species. A total of 128 adult horseshoe crabs were collected from Honda Bay, Palawan, Philippines. These were identified as *Tachypleus tridentatus* (68 males, 40 females) and *Carcinoscorpius rotundicauda* (2 males, 18 females). Morphology, morphometric measurements, and ratios of male and female of each species were described. Morphological characteristics in differentiating a male from a female crab involve examination of the appearance of the genitalia. Morphological characteristics in identifying the species include the presence or absence of notches in the frontal margin, arching in the frontal view, appearance of the chelae of the 2nd and 3rd prosomal appendages, number of lateral spines and the immovable spines in the opisthosoma, the presence of spinnerets on the surface of the telson, and shape of the telson and its cavity in cross-section. This study confirmed the identification of the two species found in Palawan, Philippines, and the morphological characteristics of the male and female of each species. In addition, a comparison was made of the size of the horseshoe crab species in Palawan with those found in other locations in the Asia-Pacific region.

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## 1. INTRODUCTION

Horseshoe crabs belong to an ancient group of aquatic merostome arthropods that have maintained their morphology almost unchanged for the past 200 million years (Xia 2000; Shuster and Anderson 2003; Akbar John et al. 2018). These ancient organisms first appeared in the Ordovician seas during the Paleozoic era about 450 million years ago (Rudkin et al. 2008; Van Roy et al. 2010). Their static morphology and adaptable physiology enabled the horseshoe crabs to survive in various environmental conditions over long periods (Sekiguchi and Shuster 2009).

Horseshoe crabs are important economically. In the USA, these crabs are primarily harvested as bait for fishing industries. Also, their blood contains a chemical called *Limulus* amoebocyte lysate (LAL) that can be used to detect pathogens and their endotoxins. The growing interest in their use for research, education, biomedical applications, and habitat loss has threatened the wild horseshoe crab populations (Carmichael and Brush 2012). The horseshoe crabs are now included in the International Union for the Conservation of Nature's (IUCN) red list of threatened species (Laurie et al. 2019).

At present, there are four recognized extant species of horseshoe crabs: the American horseshoe crab, *Limulus polyphemus* (Linnaeus 1758) and the three Asian species consisting of the tri-spine horseshoe crab, *Tachypleus tridentatus* (Leach 1819), the coastal horseshoe crab, *Tachypleus gigas* (Müller 1785), and the mangrove horseshoe crab, *Carcinoscorpius rotundicauda* (Latreille 1802).

The ancestors of the extant species were believed to have originated from the Tethys Sea, an ancient body of water that existed during the Mesozoic era (250 - 65 million years ago). One ancestral type moved westward, giving rise to *Limulus*, while the other antecedent group went eastward, giving rise to the three Asian species (Shuster and Anderson 2003; Obst et al. 2012). The three Asian species were believed to originate from a marine stem group that inhabited the shallow coastal waters between the Andaman Sea, Vietnam, and Borneo. From this region, *C. rotundicauda* may have separated from the *Tachypleus* stem group by invading estuarine habitats, while *T. tridentatus* and *T. gigas* migrated northeast along the southern coast of China and towards Japan (Obst et al. 2012).

The American horseshoe crab, *L. polyphemus* occurs mainly on the eastern coast of North America, while the three Asian species are found generally in the coastal waters of the Indo-Pacific. *L. polyphemus* has been reported to be present in the North American Atlantic coast from Maine to Florida and along the Gulf States, USA with a genetically distinct population on the northern east coast of the Yucatan Peninsula in Mexico (Anderson and Shuster 2003; Sekiguchi and Shuster 2009). For the Asian species, the *T. tridentatus* is distributed along the coasts of Borneo, Malaysia, in the islands of Java, Sulawesi, and Sumatra in Indonesia, Vietnam, Philippines, Hongkong, Taiwan, Southern China, Korean Peninsula, and Japan (Sekiguchi and Shuster 2009; Akbar John et al. 2018; Vestbo et al. 2018). *T. gigas* occurs in the waters of Southeast Asia along the coasts of Indonesia and the Bay of Bengal (Sekiguchi and Shuster 2009; Noor Jawahir et al. 2017; Vestbo et al. 2018). *C. rotundicauda* occurs along the coasts of Indonesia, the Thai-Malay Peninsula, Singapore, Brunei, Cambodia, Vietnam, Philippines, Hongkong, Southwestern China, Eastern part of India, and Bangladesh (Chiu and Morton 2003; Sekiguchi and Shuster 2009; Cartwright-Taylor 2015; Chen et al. 2015; Vestbo et al. 2018). *T. tridentatus and T. gigas* inhabit open sandy beaches, while *C. rotundicauda* prefers estuaries and mangrove mudflats (Cartwright-Taylor et al. 2009; Manca et al. 2017; Vestbo et al. 2018).

In identifying the three Asian species, the sex of the individual needs to be determined first before the species can be identified. A male could be differentiated from a female through the appearance of the genitalia. The morphological traits used in the identification of the species include the presence or absence of notches in the frontal margin; the presence or absence of arching in the frontal view; presence or absence of modification in the chelae of the second and third prosomal appendages; the number of movable lateral spines in the opisthosoma; the number of small immovable spines in the posterior end of the opisthosoma; the apex of posterior outer lobes of genital operculum; the presence or absence of tiny spines on the telson surface; the appearance of the cross-section of the telson; and the shape of the cavity of the telson (Sekiguchi and Nakamura 1979; Yamasaki et al. 1988; Chiu and Morton 2003).

In the *T. tridentatus* species, the male exhibits morphological characteristics that differ from the female. In the male *T. tridentatus,* there are two indentations in the frontal margin of the prosoma, while the female has none. The frontal view of the prosoma is arched in males but flattened in females. The chelae of the second and third appendages of the prosoma are swollen, looking like boxing gloves with a hemichelate clasper, but these appendages are unaltered in the female. There are six marginal spines in the opisthosoma of a male but only three in a female. There are also similarities between the sexes. In both male and female crabs, there are three immovable small spines on the posterior end of the opisthosoma. The apex of the outer lobes of their genital operculum extends beyond the inner lobes. On the surface, the telson has a row of small spinnerets on the dorsal and lateral ridges. In cross-section, the telson is triangular and has a cavity that likewise exhibits the same shape (Sekiguchi and Nakamura 1979; Yamasaki et al. 1988; Chiu and Morton 2003).

In *C. rotundicauda,* both the male and female share similar morphological characteristics, such as the absence of indentations in the frontal margin and absence of arching in the frontal view of the prosoma, the presence of six marginal spines in the opisthosoma and three small, immovable spines on the posterior end of the opisthosoma. In addition, the apex of the outer lobes of the genital operculum extends beyond the inner lobes, and there are soft white hairs fringing the ventral ridge of the telson, which is subtriangular in cross-section and with a triangular cavity. The only difference in morphology between a male and a female *C. rotundicauda* aside from the appearance of the genitalia was the slight swelling in the chelae of the second and third prosomal appendages bearing claspers in the male, which is absent in the female (Sekiguchi and Nakamura 1979; Yamasaki et al. 1988; Chiu and Morton 2003).

Waterman (1958) first reported the presence of *T. tridentatus* and *C. rotundicauda* on Palawan Island in the Philippines, but his descriptions were just repetitions of previous reports. This was followed thirty years later by Sekiguchi (1988), whose identification of *T. tridentatus* and *C. rotundicauda* species was only based on one picture of a Japanese who visited the Philippines and was holding two horseshoe crabs. Fourteen years later, Schoppe (2002) interviewed fishers along the coast of Puerto Princesa, Palawan, about their sightings of horseshoe crabs and examined a few exoskeletons of dead adult specimens of crabs and concluded that all of the exoskeletons belonged to *T. tridentatus* species. She did not mention the presence of the species *C. rotundicauda*. Since the crabs were already dead, the genitalia was not examined, which could have enabled her to identify the sex. In identifying the *C. rotundicauda*, the sex has to be determined first before the species can be identified.

Since no actual collection and examination of live specimens of adult horseshoe crabs had been previously done, there is a need to confirm the species found in Palawan, Philippines. Therefore, this study aimed to identify the species of horseshoe crab samples collected from Honda Bay, Palawan, Philippines; compare the morphological characteristics of males versus females of each species; and compare the sizes of the species found in Palawan, Philippines with those in other locations in Asia-Pacific region.

## 2. MATERIALS AND METHODS

### 2.1 Collection of samples

Adult crabs were collected for one year, from September 2015 to August 2016. Every first week of the month, approximately ten crab samples were obtained from local fishers in Honda Bay which is located on the eastern shore of the island of Palawan in southwestern Philippines. Honda Bay occupies 9°50'21 "N latitude and 118°46'12 "E longitude and is approximately 12 km from the city proper of Puerto Princesa (Figure 1). This bay is used for subsistence fishing, commercial fishing, and recreation. The collectors of horseshoe crabs were fishers of the blue swimming crab, *Portunus pelagicus*, their target fishing species. However, the horseshoe crabs were caught up in their nets, either thrown away by the fishers back to the sea or brought home and allowed to die by drying under the sun. The horseshoe crabs are considered pests because they destroy the nets of the *Portunus'* crab fishers. After the fishers collected the horseshoe crabs, they were brought to the biology laboratory at the Western Philippines University campus in Puerto Princesa for measurements.

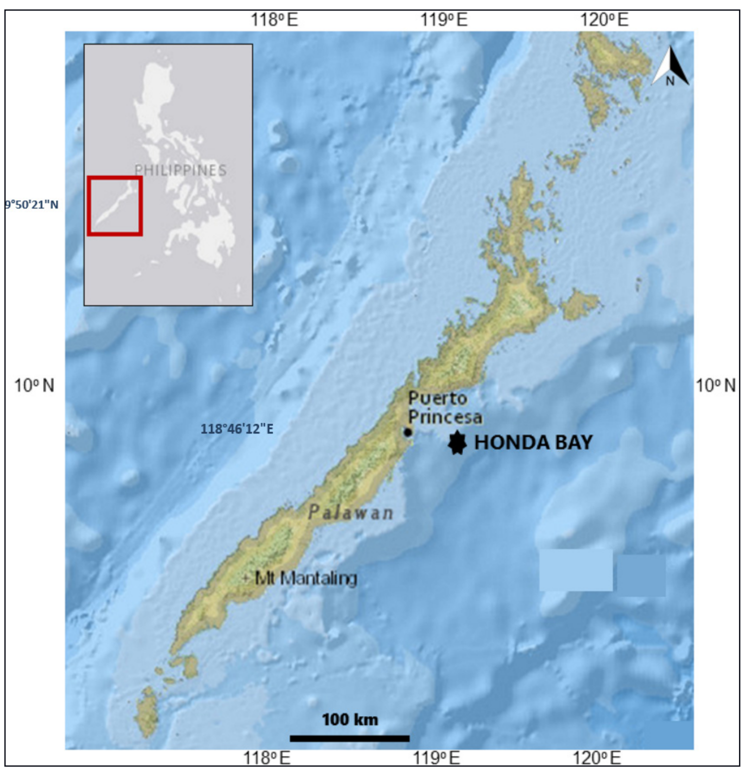


Figure 1. Map that shows the location of Honda Bay, Palawan, Philippines, where the two species of horseshoe crabs (*Tachypleus tridentatus, Carcinoscorpius rotundicauda*) were collected from September 2015 to August 2016. This is a coastal area with sandy beaches used for subsistence fishing, commercial fishing, and recreation.

### 2.2 Morphometric measurements

Each crab was measured for body weight (BW) and recorded to the nearest 0.01 g using a digital electronic balance. Prosoma length (PL) was measured as the distance from the anterior tip to the posterior end of the prosoma; opisthosoma length (OL) was from the anterior tip to the posterior end of the opisthosoma; carapace length (CL) was from the anterior tip of prosoma to posterior end of opisthosoma; carapace width (CW) was the widest part of the prosoma; and telson length (TL) was from anterior tip to the posterior end of the telson. These are standard measurements reported by Yamasaki et al. (1988) and Chiu and Morton (2003). (Figure 2). Measurements were done using a modified caliper up to the nearest 0.1 cm.

### 2.3 Sex and species identification

To be able to identify the species of the horseshoe crab, the sex of the crab sample needs to be determined first. Therefore, the male crab was differentiated from the female upon examination of the appearance of the genitalia. The appearance of the chelae on the prosoma's second and third appendages was another criterion used to distinguish a male horseshoe crab from a female. After the sex had been determined, the species of each crab sample were then identified using the characteristics reported by Sekiguchi and Nakamura (1979), Yamasaki et al. (1988), and Chiu and Morton (2003).

Morphometric ratios such as PL/OL, PW/PL, CL/TL, and CL/PW were computed using the obtained data. Comparisons were then made between male and female *Tachypleus tridentatus* and between male and female *Carcinoscorpius rotundicauda*.

### 2.4 Statistical analyses

Statistical data analysis was done using SPSS software version 20 (2011). Levene's test was used to determine the equality of variances. Data with unequal variances were square root transformed to attain homogeneity of variance. A comparison of means was made using the t-test in comparing the male and female *T. tridentatus.* However, no statistical test was done comparing the male and female *C. rotundicauda* due to insufficient male samples. All results were considered statistically significant at a 0.05 level of significance.

## 3. RESULTS

### 3.1 Number of crab samples

A total of 128 adult horseshoe crabs were collected and examined. These were identified as *Tachypleus tridentatus* (68 males, 40 females) and *Carcinoscorpius rotundicauda* (2 males, 18 females).

### 3.2 General morphology of horseshoe crab

The general morphology of each horseshoe crab was examined. The body is divided into three sections: the prosoma, the opisthosoma, and the telson. The prosoma is the largest section shaped like a horse's shoe. The middle division with movable lateral spines is called the opisthosoma. It is attached to the prosoma with a hinge. The third body part is the telson, also known as the "tail," attached to the opisthosoma.

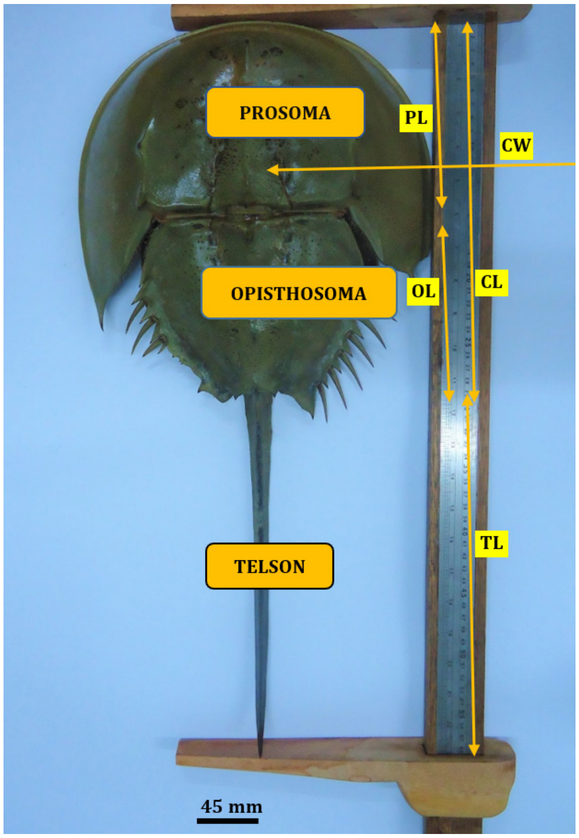


Figure 2. The horseshoe crab's body is divided into three sections: the prosoma, the opisthosoma, and the telson. Prosoma length (PL) was measured as the distance from the anterior tip to the posterior end of prosoma; opisthosoma length (OL) was from the anterior tip to the posterior end of opisthosoma; carapace length (CL) was from the anterior tip of the prosoma to posterior end of opisthosoma; carapace width (CW) was the widest part of the prosoma; and telson length (TL) was from anterior tip to the posterior end of the telson.

The underside of the prosoma of the horseshoe crab has six pairs of appendages or pedipalps (Figure 3). On the ventral side of the opisthosoma are six pairs of book gills which are respiratory organs. The first page of the six book gills is called the genital operculum because it covers the genital openings (Figure 4).

### 3.3 Morphological characteristics of male versus female

On the underside of the genital operculum, a male horseshoe crab exhibited a pair of genital papillae of hard raised bumps with the genital pore at the top of each papilla. On the other hand, a female crab had a pair of softer bumps with transverse slits in them (Figure 5). The chelae of the prosoma's second and third appendages in a male crab were either with modified clasper or unaltered, depending on the species (Figure 6).

### 3.4 Morphological characteristics of male and female *Tachypleus tridentatus*

The male *T. tridentatus* had two indentations in the frontal margin of the prosoma, while the female had none (Tables 1 and 2) (Figure 7). The male had an arched frontal view of the prosoma but flattened in the female (Figure 8). The chelae of the prosoma's second and third appendages in the male were swollen like a boxing glove with a hemichelate hook, but these appendages were unaltered in the female. There were six marginal spines in the opisthosoma of a male but only three in the female, with the other three either degenerate or missing. In both male and female crabs, the number of immovable small spines on the opisthosoma's posterior end ranged between 0-3 but mostly 3. The apex of the outer lobes of the genital operculum extended beyond the inner lobes. The telson, which was triangular in cross-section, has a row of small spinnerets found on the anterior part of the dorsal and lateral ridges only because most of the spinnerets have fallen off.

### 3.5 Morphological characteristics of male and female *Carcinoscorpius rotundicauda*

Except for the differences in the appearance of their genitalia, both the male and female *C. rotundicauda* had similar morphological characteristics (Tables 3 and 4). There were no indentations in the frontal margin and no arching in the frontal view of the prosoma. There was also no differentiation in the chelae of the prosoma's second and third appendages, and there were six marginal spines in the opisthosoma. In both male and female crabs, the number of immovable small spines on the opisthosoma's posterior end ranged between 0-3 but mostly 3. The apex of the outer lobes of the genital operculum extends beyond the inner lobes. The telson, which was triangular in cross-section, has a row of small spinnerets found on the anterior part of the dorsal and lateral ridges only because most of the spinnerets have fallen off.

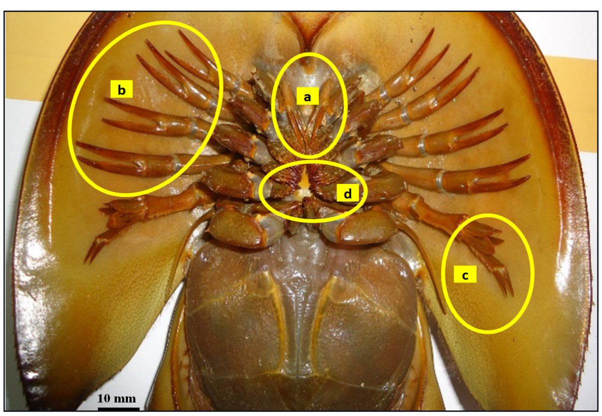


Figure 3. The underside of the prosoma of the horseshoe crab contains six pairs of appendages. The 1st pair called the 'chelicera' is used for placing food in its mouth (a). The 2nd to 5th pairs called the 'pedipalps' are used for walking (b). The 6th pair called the 'pushers legs' are used for clearing away sediments as the burrow into marine bottom and for pushing when walking on the floor of the ocean (c). The base of each of all the legs called 'coxa' is recovered with inward pointing spines called 'gnathobases' that move food towards the mouth located between the legs (d). As the legs are moving. food is crushed and macerated (Sekiguchi and Nakamura 1979; Yamasaki et al. 1988)

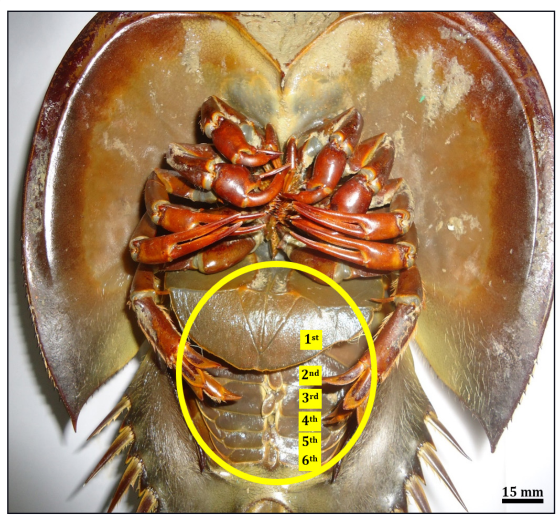


Figure 4. Ventral to the opisthosoma are six pairs of book gills that resemble the pages of a book. The crab uses them for propulsion when swimming and exchanging gases. The first pair is called the genital operculum because it covers the genital openings, while the second to sixth pairs are respiratory organs (Sekiguchi and Nakamura 1979; Yamasaki et al. 1988).

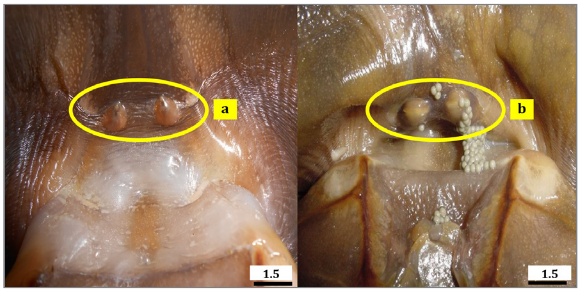


Figure 5. The sex of the horseshoe crab can be determined by observing the underside of the genital operculum, where the genital pores are located. The male has a pair of genital papillae or hard raised bumps (a), while the female has a pair of soft bumps with transverse slits in them (b). Sperms and eggs come out of the genital pores (Sekiguchi and Nakamura 1979; Yamasaki et al. 1988).

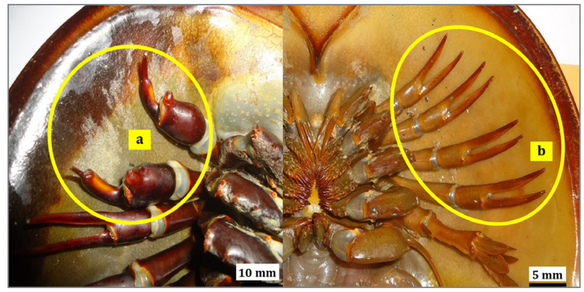


Figure 6. Comparison of chelae of the appendages of male and female *Tachypleus tridentatus*. In males, the chelae of the second and third appendages are swollen that look like "boxing gloves" with one hook-like clasper (hemichelate) (a). In females, the chelae of the second and third appendages are unaltered (chelate) (b) (Sekiguchi and Nakamura 1979; Yamasaki et al. 1988).

Table 1. Morphological characteristics used in the identification of male *Tachypleus tridentatus* from Honda Bay, Puerto Princesa, Palawan based on the description by Sekiguchi and Nakamura (1979), Yamasaki et al (1988), and Chiu and Morton (2003)

| **Morphological characteristic** | **Sekiguchi and Nakamura (1979)** | **Yamasaki et al (1988)** | **Choi and Morton (2003)** | **Present study** |
|---|---|---|---|---|
| Appearance of genitalia | A pair of hard raised bumps | A pair of hard raised bumps | A pair of hard raised bumps | A pair of hard raised bumps |
| Frontal margin | Presence of 2 indentations | Presence of 2 indentations | Presence of 2 indentations | Presence of 2 indentations |
| Frontal view | Arched | Arched | Arched | Arched |
| Chelae of 2nd and 3rd prosomal appendages | Swollen with hemichelate clasper | Swollen with hemichelate clasper | Swollen with hemichelate clasper | Swollen with hemichelate clasper |
| Apex of posterior outer lobes of genital operculum | Extends beyond the inner lobes | Extends beyond the inner lobes | Not reported | Extends beyond the inner lobes |
| Number of movable marginal spines in opisthosoma | 6 | 6 | 6 | 6 |
| Number of small immovable spines on posterior end of opisthosoma | Not reported | 3 | 3 | 0-3, but mostly 3 |
| Surface of telson | Not reported | Row of spinnerets on dorsal and lateral ridges | Row of spinnerets on dorsal and lateral ridges | Row of spinnerets on dorsal and lateral ridges |
| Cross section of telson | Triangular with a cavity of the same shape | Triangular with a cavity of the same shape | Triangular with a cavity of the same shape | Triangular with a cavity of the same shape |

Table 2. Morphological characteristics used in the identification of female *Tachypleus tridentatus* from Honda Bay, Puerto Princesa, Palawan, based on the description by Sekiguchi and Nakamura (1979), Yamasaki et al. (1988), and Chiu and Morton (2003)

| **Morphological characteristic** | **Sekiguchi and Nakamura (1979)** | **Yamasaki et al (1988)** | **Choi and Morton (2003)** | **Present study** |
|---|---|---|---|---|
| Appearance of genitalia | A pair of soft bumps with transverse slits | A pair of soft bumps with transverse slits | A pair of soft bumps with transverse slits | A pair of soft bumps with transverse slits |
| Frontal margin | Absence of indentation | Absence of indentation | Absence of indentation | Absence of indentation |
| Frontal view | Flattened | Flattened | Flattened | Flattened |

Continuation of Table 2

| Morphological characteristic | Sekiguchi and Nakamura (1979) | Yamasaki et al (1988) | Choi and Morton (2003) | Present study |
|---|---|---|---|---|
| Chelae of 2nd and 3rd prosomal appendages | Unaltered | Unaltered | Unaltered | Unaltered |
| Apex of posterior outer lobes of genital operculum | Extends beyond the inner lobes | Extends beyond the inner lobes | Not reported | Extends beyond the inner lobes |
| Number of movable marginal spines in opisthosoma | 3 | 3 | 3 | 3 |
| Number of small immovable spines on posterior end of opisthosoma | Not mentioned | 3 | 3 | 0-3, but mostly 3 |
| Surface of telson | Not mentioned | Row of spinnerets on dorsal and lateral ridges | Row of spinnerets on dorsal and lateral ridges | Row of spinnerets on dorsal and lateral ridges |
| Cross section of telson | Triangular with a cavity of the same shape | Triangular with a cavity of the same shape | Triangular with a cavity of the same shape | Triangular with a cavity of the same shape |

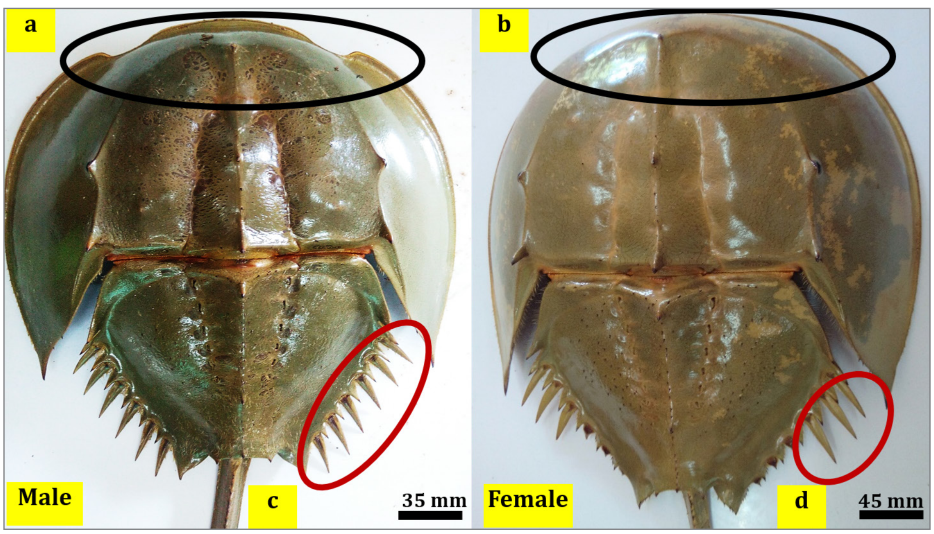


Figure 7. Morphological differences between male and female *Tachypleus tridentatus*. There are two indentations in the frontal margin of the prosoma of male *T. tridentatus* (a) while the female has none (b). The frontal view of the prosoma is arched in males but flat in females (not shown). There are six lateral spines in the opisthosoma of a male (c) but only three lateral spines in the opisthosoma of a female where the other three are either degenerate or missing (d) (Sekiguchi and Nakamura 1979; Yamasaki et al. 1988).

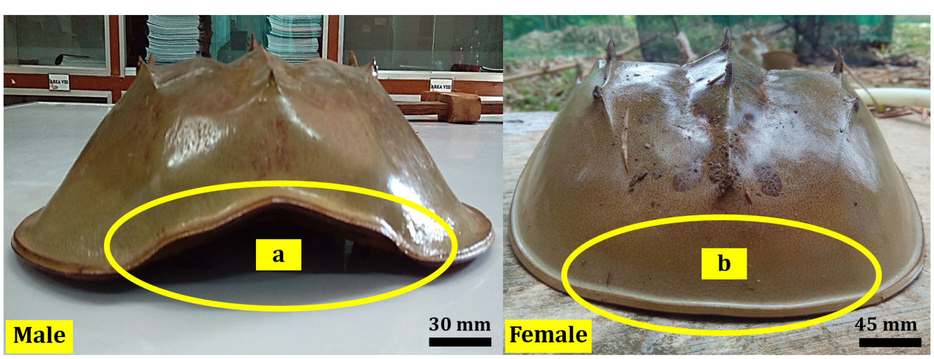


Figure 8. Frontal view of the prosoma between male and female *Tachypleus tridentatus*. It is arched in males (a) but flattened in females (b) (Sekiguchi and Nakamura 1979; Yamasaki et al. 1988).

Table 3. Morphological characteristics used in the identification of male *Carcinoscorpius rotundicauda* from Honda Bay, Puerto Princesa, Palawan, based on the description by Sekiguchi and Nakamura (1979), Yamasaki et al. (1988), and Chiu and Morton (2003)

| **Morphological characteristic** | **Sekiguchi and Nakamura (1979)** | **Yamasaki et al (1988)** | **Choi and Morton (2003)** | **Present study** |
|---|---|---|---|---|
| Appearance of genitalia | A pair of hard raised bumps | A pair of hard raised bumps | A pair of hard raised bumps | A pair of hard raised bumps |
| Frontal margin | Absence of indentation | Absence of indentation | Absence of indentation | Absence of indentation |
| Frontal view | Flattened | Flattened | Flattened | Flattened |
| Chelae of 2nd and 3rd prosomal appendages | Slightly swollen with chelate claspers | Slightly swollen with chelate claspers | Swollen into chelate claspers | Unaltered |
| Apex of posterior outer lobes of genital operculum | Extends beyond the inner lobes | Extends beyond the inner lobes | Not reported | Extends beyond the inner lobes |
| Number of movable marginal spines on opisthosoma | 6 | 6 | 6 | 6 |
| Number of small immovable spines on posterior end of opisthosoma | Not reported | 3 | 0-1 | 0-3, but mostly 3 |
| Surface of telson | Not reported | With spinnerets on dorsal and lateral ridges | Smooth | With spinnerets on dorsal and lateral ridges |
| Cross section of telson | Subtriangular with round edges and a triangular cavity | Subtriangular with round edges and a triangular cavity | Subtriangular with round edges and a triangular cavity | Subtriangular with round edges and a triangular cavity |

Table 4. Morphological characteristics used in the identification of female *Carcinoscorpius rotundicauda* from Honda Bay, Puerto Princesa, Palawan, based on the description by Sekiguchi and Nakamura (1979), Yamasaki et al. (1988), and Chiu and Morton (2003)

| **Morphological characteristic** | **Sekiguchi and Nakamura (1979)** | **Yamasaki et al (1988)** | **Choi and Morton (2003)** | **Present study** |
|---|---|---|---|---|
| Appearance of genitalia | A pair of soft bumps with transverse slits | A pair of soft bumps with transverse slits | A pair of soft bumps with transverse slits | A pair of soft bumps with transverse slits |
| Frontal margin | Absence of indentation | Absence of indentation | Absence of indentation | Absence of indentation |
| Frontal view | Flattened | Flattened | Flattened | Flattened |
| Chelae of 2nd and 3rd prosomal appendages | Unaltered | Unaltered | Unaltered | Unaltered |
| Apex of posterior outer lobes of genital operculum | Extends beyond the inner lobes | Extends beyond the inner lobes | Not reported | Extends beyond the inner lobes |
| Number of movable marginal spines in opisthosoma | 6 | 6 | 6 | 6 |
| Number of small immovable spines on posterior end of opisthosoma | Not reported | 3 spines | 0-1 spine | 0-3 but mostly 3 |
| Surface of telson | Not reported | With spinnerets on dorsal and lateral ridges | Smooth | With spinnerets on dorsal and lateral ridges |
| Cross section of telson | Subtriangular with round edges and a triangular cavity | Subtriangular with round edges and a triangular cavity | Subtriangular with round edges and a triangular cavity | Subtriangular with round edges and a triangular cavity |

### 3.6 Comparison of morphometrics between male and female *T. tridentatus*

There was sexual dimorphism between the male and female *T. tridentatus*. The female was larger than its male counterpart (Table 5). The female *T. tridentatus* had significantly higher values ($P < 0.05$) in all morphometric parameters, such as BW, PL, OL, TL, PW, and CL, compared to the male *T. tridentatus*. In terms of morphometric ratios, there was no significant difference ($P > 0.05$) in the PL/OL and PW/PL, and CL/PW but a significant difference in the CL/TL and CL/PW ($P < 0.05$) between female and male *T. tridentatus*.

### 3.7 Comparison of morphometrics between male and female *C. rotundicauda*

There was sexual dimorphism between the male and female *C. rotundicauda*. The female *C. rotundicauda* was larger than its male counterpart in all morphometric parameters such as BW, PL, OL, TL, PW, and CL, compared to the male *C. rotundicauda* (Table 6). In terms of morphometric ratios, no trend was observed between male and female *C. rotundicauda*.

Table 5. Morphometric parameters and ratios of male and female *Tachypleus tridentatus* from Honda Bay, Palawan, Philippines. Values in brackest represent the ranges.

| Morphometric parameter | Male (n=68) | Female (n=40) |
|---|---|---|
| Body weight, BW (kg) | $1.13 \pm 0.03^{a}$ (0.46-1.65) | $2.40 \pm 0.11^{b}$ (1.02-4.33) |
| Prosoma length, PL (mm) | $139.7 \pm 2.8^{a}$ (120-240) | $177.7 \pm 3.4^{b}$ (129-250) |
| Opisthosoma length, OL (mm) | $112.5 \pm 2.3^{a}$ (72-203) | $136.9 \pm 3.7^{b}$ (80-210) |
| Telson length, TL (mm) | $263.3 \pm 4.7^{a}$ (141-371) | $305.9 \pm 8.2^{b}$ (122-400) |
| Prosoma width, PW (mm) | $252.1 \pm 2.8^{a}$ (181-297) | $302.9 \pm 5.2^{b}$ (208-360) |
| Carapace length, CL (mm) | $247.0 \pm 2.7^{a}$ (172-285) | $311.5 \pm 5.6^{b}$ (187-367) |
| Morphometric ratio | | |
| PL/OL | $1.27 \pm 0.03^{a}$ | $1.32 \pm 0.03^{a}$ |
| PW/PL | $2.06 \pm 0.26^{a}$ | $1.72 \pm 0.02^{a}$ |
| CL/TL | $0.96 \pm 0.02^{a}$ | $1.05 \pm 0.04^{b}$ |
| CL/PW | $0.98 \pm 0.01^{a}$ | $1.03 \pm 0.01^{b}$ |

Means (± Standard Error of the Mean) with different superscript letters within the same row are significantly different ($P < 0.05$).

Table 6. Morphometric parameters and ratios of male and female *Carcinoscorpius rotundicauda* from Honda Bay, Palawan, Philippines. Values in brackets represent the ranges.

| Morphometric parameter | Male (n=2) | Female (n=18) |
|---|---|---|
| Body weight, BW (kg) | 0.74 ± 0.12 (0.62-0.85) | 1.14 ± 0.07 (0.70-1.76) |
| Prosoma length, PL (mm) | 116.5 ± 3.5 (113-120) | 135.2 ± 3.2 (114-160) |
| Opisthosoma length, OL (mm) | 90.0 ± 24.0 (66-114) | 103.6 ± 2.8 (85-123) |
| Telson length, TL (mm) | 260.0 ± 30.0 (230-290) | 244.7 ± 13.2 (55-310) |

Continuation of Table 6

| Morphometric parameter | Male (n=2) | Female (n=18) |
|---|---|---|
| Prosoma width, PW (mm) | 201.5 ± 4.5<br>(197-206) | 240.5 ± 4.3<br>(207-277) |
| Carapace length, CL (mm) | 202.0 ± 13.0<br>(189-215) | 244.3 ± 5.2<br>(211-282) |
| Morphometric ratio | | |
| PL/OL | 1.38 ± 0.33 | 1.31 ± 0.03 |
| PW/PL | 1.73 ± 0.01 | 1.78 ± 0.02 |
| CL/TL | 0.78 ± 0.04 | 1.15 ± 0.19 |
| CL/PW | 1.00 ± 0.04 | 1.01 ± 0.01 |

## 4. DISCUSSION

### 4.1 Occurrence of two species in Honda Bay, Palawan

A higher number of *T. tridentatus* individuals were collected from Honda Bay compared with *C. rotundicauda*. This difference in the number of crab species collected could be attributed to the characteristic of the collection site in Honda Bay, Palawan, which is an open coastal area with sandy shores and far from mangrove areas. It has been reported that *T. tridentatus* lay their eggs on sandy beaches while *C. rotundicauda* prefer mangroves and mudflats (Cartwright-Taylor et al. 2011; Manca et al. 2017; Vestbo et al. 2018).

### 4.2 Morphology and function

The horseshoe crab has a hard carapace to protect the vulnerable ventral parts of the body and to deter predators (Mikkelsen 1988). The prosoma contains the digestive, nervous, and circulatory systems. The underside of the prosoma has six pairs of appendages for various functions, such as placing food in its mouth, walking on the ocean floor, and pushing to clear away sediments as the crab walks or burrows into the ocean floor. The opisthosoma contains the muscles for the operation of the book gills. On the ventral side of the opisthosoma is the genital operculum, which covers the genital pores where the sperms and eggs are released. The crab uses the telson to turn itself right when overturned (Sekiguchi et al. 1988; Yamasaki et al. 1988).

### 4.3 Characteristics used in the identification of *T. tridentatus* species

The presence of indentations and arching in the anterior margin of the prosoma were features used in the identification of the male *T. tridentatus* species. These modifications promote effective clasping of the male *T. tridentatus* with the dorsal opisthosoma of the female *T. tridentatus* during the mating process or amplexus (Yamasaki et al. 1988). The indentations and arching in the anterior margin of the prosoma were absent in the female *T. tridentatus*.

The swelling of the chelae and its modification to form claspers in the second and third prosomal appendages was another morphological feature used in the identification of the male *T. tridentatus*. This modification is used to grasp the margins of the female opisthosoma during pairing firmly. Yamasaki et al. (1988) described the clasper of male *T. tridentatus* with a movable and immovable hook. The immovable hook, however, breaks easily and falls off during its first pairing, leaving the movable clasper with just one hook (hemichelate). The was no modification in the appendages of the female *T. tridentatus* where the second and third appendages remain unaltered. The last feature used in identifying *T. tridentatus* species was the presence of six lateral spines in the opisthosoma of the males but only three lateral spines in the females.

The presence of three immovable spines on the posterior edge of the opisthosoma, as reported by Yamasaki et al. (1988), was not consistently seen in the present samples of *T. tridentatus* and could not be used as a basis for species identification. In this study, the number of spines varies between 0-3 but mostly 3. According to Yamamoto et al. 1988, as the horseshoe crab molt into adulthood, these spines become smaller and generally worn down in the process of aging, hence, the variation in the number of spines.

Yamasaki et al. (1988) reported that the telson surface of *T. tridentatus* bears a row of small spinnerets on both the dorsal and lateral ridges. In this study, the hairs were mostly present only on the telson's most anterior because most of the hairs had already fallen off.

### 4.4 Characteristics used in the identification of *C. rotundicauda* species

There were no indentations and arching in the anterior margin of the *C. rotundicauda* male and female prosoma. The slight swelling in the second and third appendages of male *C. rotundicauda* as reported by Sekiguchi and Nakamura (1979), Yamasaki et al. (1988) and used by Chiu and Morton (2003) in the identification of male *C. rotundicauda* was not observed in all the three samples of *C. rotundicauda* that were collected in this present study. For the *C. rotundicauda*, both the males and females have six lateral spines in the opisthosoma. The telson surface could not be used as a reliable indicator in species identification. Choi and Morton (2003) reported the telson surface of *C. rotundicauda* to be smooth on the dorsal ridge with soft white hair fringes on the entire ventral edge. In this present study, the telson in *C. rotundicauda* bears very small spinnerets on the anterior part of the dorsal and lateral ridges, most of which have already fallen off, making the surface of the rest of the telson smooth.

### 4.5 Sexual dimorphism in horseshoe crabs

In both species, the female *T. tridentatus* and female *C. rotundicauda* collected from Honda Bay, Palawan, Philippines, were larger than their male counterparts, similar to what has been reported for all four extant species of horseshoe crabs (Yamasaki et al. 1988; Chiu and Morton 2003; Brockman and Smith 2009; Cartwright-Taylor et al. 2009; Manca et al. 2017). The sexual dimorphism in horseshoe crabs, where females are larger than males, has been directly attributed to females that need one more molt and one additional year of growth to reach sexual maturity compared to males. Sekiguchi et al. (1988) reported that to reach sexual maturity, *L. polyphemus* females require 17 molts (10 years), while males require only 16 molts (9 years). *T. tridentatus*, on the other hand, takes longer to reach sexual maturity, where females require 16 molts (14 years) while males require 15 molts (13 years). The larger body size of female horseshoe crabs may have evolved in response to the necessity to tow around the smaller-sized male that clings to the opisthosoma of the female (amplexus) during the spawning season (Botton and Loveland 1992). This sexual dimorphism in horseshoe crabs resulted in a higher degree of success in reproduction. Smith and Brockman (2014) associated larger females with higher egg production and predicted that for every centimeter increase in size, the female would lay 1,600 more eggs per spawning. Early maturation at a smaller size for males, on the other hand, would mean increased lifetime reproductive success (from mating an additional year). This will also provide a demographic advantage of their offspring hatched earlier and reproducing earlier too (Stearns 1992).

### 4.6 Comparison of morphometric ratios between male and female *T. tridentatus*

For *T. tridentatus* from Palawan, there was no significant difference in PL/OL and PW/PL between males and females, but a significant difference was found in CL/TL and CL/PW. No significant difference was found for *T. tridentatus* collected from Hongkong when the four morphometric ratios (PL/OL; PW/PL; CL/TL; and CL/PW) between males and females were compared. This suggests that *T. tridentatus* from Palawan and Hongkong belong to different populations of horseshoe crabs (Shuster 1979; Riska 1981; Yamasaki et al. 1988; Sekiguchi and Shuster 2009).

### 4.7 Comparison in size of horseshoe crabs from different locations in the Asia-Pacific region

Geographic variations in morphological characteristics of the four extant horseshoe crabs have been described in numerous studies (Sekiguchi et al. 1978; Riska 1981; Yamasaki et al. 1988; Chiu and Morton 2003). This variation was also observed when horseshoe crabs from Palawan were compared with those reported from other locations in the Asia-Pacific region (Figure 9). Based on the mean prosomal width, males of *T. tridentatus* collected from Palawan (252 mm) were comparable in size to those reported from Imari, Japan (244 mm), Tawau, Sabah, Malaysia (257 mm), Xiamen, China (260 mm), Hongkong (261 mm), larger than male *T. tridentatus* from Semporna, Sabah, Malaysia (217 mm), but much smaller than the male *T. tridentatus* recorded from Kudat, Sabah, Malaysia (277 mm), Kota Kinabalu, Sabah, Malaysia (285 mm) and Kunak, Sabah, Malaysia (289 mm) (Table 7).


Figure 9. Locations in the Asia-Pacific region where the presence of *Tachypleus tridentatus* (•) and *Carcinoscorpius rotundicauda* (◆) was reported.

Table 7. Prosomal width (mm) of male and female *Tachypleus tridentatus* from different Asia-Pacific regions. Values in brackets represent the ranges.

| MALE | FEMALE | LOCATION | REFERENCE |
|---|---|---|---|
| 252.1 ± 2.8*<br>(181-297) | 302.9 ± 5.2*<br>(208-360) | Palawan, Philippines | Present study |
| 260.9<br>(237-308) | 328.6<br>(265-367) | Hongkong | Chiu and Morton 2003 |
| 277.5 ± 26.8**<br>(230-297) | 332 ± 46.8**<br>(288-417) | Kudat,Sabah,Malaysia | Mohamed et al 2021 |
| 289.1 ± 14.8**<br>(189-320) | 294.4 ± 54.7**<br>(189-388) | Kunak, Sabah,Malaysia | Mohamed et al 2021 |
| 217.3 ± 13.4**<br>(158-250) | 244.2 ± 13.6**<br>(228-278) | Semporna, Sabah,Malaysia | Mohamed et al 2021 |
| 257 ± 16**<br>(200-298) | 312 ± 23**<br>(242-362) | Tawau,Sabah, Malaysia | Manca et al 2017 |
| 285<br>(252-318) | 376<br>(350-390) | Kota Kinabalu,Sabah, Malaysa | Yamasaki et al 1988 |
| 260<br>(230-294) | 320<br>(292-348) | Xiamen,China | Yamasaki et al 1988 |
| 244<br>(214-288) | 280<br>(254-298) | Imari, Japan | Yamasaki et al 1988 |

* Means (± Standard Error of the Mean)
** Means (± Standard Deviation)

On the other hand, the female *T. tridentatus* from Palawan (306 mm) were larger than those *T. tridentatus* reported from Semporna, Sabah, Malaysia (244 mm), Imari, Japan (280 mm), comparable in size to those collected from Kunak, Sabah, Malaysia (294 mm), Tawau, Sabah, Malaysia (312 mm), Xiamen, China (320 mm), but smaller than those females from Hongkong (329 mm), Kudat, Sabah, Malaysia (332), and Kota Kinabalu, Sabah, Malaysia (376 mm) (Table 7).

This present study also revealed that the male and female *C. rotundicauda* collected in Palawan were larger than their counterparts reported in different locations of Asia, such as Indonesia, Thailand, Hongkong, India, and Malaysia (Table 8). The male *C. rotundicauda* from Palawan, with mean prosomal width of 201 mm, was 36% larger than males from Johor, Malaysia (148 mm) and 79% larger compared to their counterparts from Indonesia (112 mm), while the female *C. rotundicauda* from Palawan (241 mm) was 52% larger than the female *C. rotundicauda* from Johor, Malaysia (159 mm) and 88% larger compared to those reported from Indonesia (128 mm). Morphological variations were similarly observed for the American horseshoe crab, *L. polyphemus,* where the prosomal width of males from Delaware Bay, New Jersey (220 mm) were 31% larger from *L. polyphemus* males reported from Pleasant Bay, Cape Cod, Massachusetts, and 86% larger compared to males from Plum Island Sound, Massachusetts. For female *L. polyphemus*, those from Delaware Bay, New Jersey (293 mm) were 32% larger than female *L. polyphemus* (222 mm) recorded from Pleasant Bay, Cape Cod, Massachusetts, and 89% larger than their counterparts found in Plum Island Sound, Massachusetts.

Studies on morphometric variations have concluded that there are distinct, discrete populations of the four extant species of horseshoe crabs (Shuster 1979; Riska 1981; Yamasaki et al. 1988; Sekiguchi and Shuster 2009). The morphological differences between nearby locations and widely separated populations could be attributed to environmental and genetic factors. Faurby et al. (2011), using discriminant analysis, have shown the existence of geographically-based intraspecific variation for *L. polyphemus*, *T. tridentatus,* and *C. rotundicauda.* The morphological variation was attributed to local adaptation to the different environmental conditions along the distribution of the three species. They also suggested that the studied horseshoe crab species were highly philopatric (tendency to stay in place) and had little interaction between the different populations, even without a barrier. Botton and Loveland (1992) found that adult *L. polyphemus* from Great Bay, New Hampshire were significantly smaller than *L. polyphemus* from Sandy Hook Bay and Delaware Bay, New Jersey, and Chincoteague Bay, Virginia, and suggested that Great Bay horseshoe crabs were restricted to colder waters with shorter warm season resulting to slower growth rate and selection for maturity at a smaller size. Srijaya et al. (2010) reported significant morphometric variation of two populations of *C. rotundicauda* from Peninsular Malaysia and attributed the smaller-sized horseshoe crabs to the unsuitable environmental conditions on the west coast of Malaysia, resulting to slow growth compared to the larger *C. rotundicauda* in the eastern coast which had more favorable environmental conditions.

Table 8. Prosomal width (mm) of male and female *Carcinoscorpius rotundicauda* from different Asia-Pacific regions. Values in brackets represent the ranges.

| MALE | FEMALE | LOCATION | REFERENCE |
|---|---|---|---|
| 201.5 ± 4.5*<br>(197-206) | 240.5 ± 4.3*<br>(207-277) | Palawan, Philippines | Present study |
| 138.6<br>(128-153) | 152.9<br>(142-167) | Hongkong | Chiu and Morton 2003 |
| 150.0 ± 8.1 ** | 172.7 ± 6.7** | Setiu, Malaysia | Srijaya et al 2010 |
| 148.1 ± 6.6 ** | 159.1 ± 7.0** | Johor, Malaysia | Srijaya et al 2010 |
| 142<br>(134-155) | 160<br>(142-174) | West Bengal, India | Yamasaki et al 1988 |
| 128<br>(100-133) | 133<br>(120-156) | Bangsaen, Thailand | Yamasaki et al 1988 |
| 112<br>(100-128) | 128<br>(117-135) | Belawan, Sumatra, Indonesia | Yamasaki et al 1988 |

* Means (± Standard Error of the Means)
** Means (± Standard Deviation)

Several genetic studies have also substantiated the presence of discrete populations of horseshoe crabs. DNA analysis of several populations of *Limulus* from New England to the Gulf of Mexico revealed genetic differentiation and distinct populations of the American horseshoe crabs (Saunders et al. 1986; King et al. 2003). Pierce et al. (2000) showed that the smaller-sized *L. polyphemus* from upper Chesapeake Bay, Maryland genetically differed from larger horseshoe crabs from Delaware Bay, New Jersey. With regard to the Asian horseshoe crabs, molecular analysis of the mtDNA gene (COI) showed genetic variation and discrete populations of *T. gigas* collected in India, South China, and Malaysia (Tudu et al. 2021). Similarly, mitochondrial marker sequences of COI confirmed a clear separation of *C. rotundicauda* population on the east coast from those of the west coast of Peninsular Malaysia (Fairuz-Fosi et al. 2021).

The mangrove horseshoe crab, *C. rotundicauda,* in this present study from Palawan, Philippines, was larger than those reported in locations such as India, Hongkong, Indonesia, Malaysia, and Thailand. Aside from differences in environmental conditions, one explanation is the production of horseshoe crab hybrids due to the possible interbreeding of *T. tridentatus* and *C. rotundicauda,* which co-exist in the same area. These hybrids resulting from the combination of the qualities of two organisms of different varieties, species, or genera could produce larger or better offspring than either parent (Bar-Zvi et al. 2017; Miyaji and Fujimoto 2018; Getahun et al. 2019). Sekiguchi and Sugita (1980) conducted an experimental hybridization of the three Asian horseshoe crabs and produced viable larvae or hybrids from crosses of *T. tridentatus* X *C. rotundicauda*, *T. tridentatus* X *T. gigas*, *C. rotundicauda* X *T. gigas*. However, no data on the resulting hybrid larvae size were reported. The presence of hybrids of *C. rotundicauda* in Palawan, therefore, needs further investigation through genetic studies.

## 5. CONCLUSION

This present study confirmed the presence of two species of horseshoe crabs *T. tridentatus* and *C. rotundicauda*, in Honda Bay, Palawan, Philippines. The females of both species were significantly larger than their male counterparts. The sex of the crab sample must first be determined to identify the species. The morphological characteristics used to identify the species include the appearance of the frontal margin and frontal view of the prosoma, modification of the second and third prosomal appendages, and the number of lateral spines in the opisthosoma. Other morphological characteristics, such as the number of small immovable spines on the posterior surface of the opisthosoma and the spines on the surface of the telson, are not reliable in identifying the species. The information obtained from this present study is a valuable contribution to the identification of the *T. tridentatus* and *C. rotundicauda* and the conservation of horseshoe crabs in Palawan, Philippines.

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## AUTHOR CONTRIBUTIONS

**Baylon JC**: Conceptualization, Methodology, Statistical analyses, Data interpretation, Writing of Final draft. **Alcantara-Creencia LB**: Collection of horseshoe crabs, Data collection, Data interpretation.

## CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## ETHICS STATEMENT

The researchers followed all institutional and national guidelines for the care and use of laboratory animals. The horseshoe crabs were not transported outside of Puerto Princesa. After measurements were done in the laboratory, they were immediately released into the sea.

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