Morphometry of Limulidae (Oscar E. Brown) in Leidong, Sei Berombang, and Tanjung Tiram, North Sumatera

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Abstract

Limulidae (Horseshoe crab), or Mimi in the local name, are known; there are four species, namely Limulus polyphemus in North America and Asia, namely Tachypleus tridentatus, T. gigas, and Carcinoscorpius rotundicauda. In Indonesia, these species are protected by the Decree of the Minister of Forestry Number 12/KPTS-II/1987; meanwhile, illegal fishing still occurs. This study aims to identify Limulidae species and analyze Mimi's morphometric characteristics and distribution in North Sumatra to conserve the species. This research was conducted using a quantitative descriptive method in Leidong, Sei Berombang, Tanjung Tiram - North Sumatera, from September to December 2021. Biometric measurements were performed on 98 Mimi, and the data were statistically analyzed using the Kruskal Wallis test, Mann-Whitney test, and Multinomial logistics. The results showed that *Mimi* in the study area was identified as *Tachypleus tridentatus, T. gigas,* and *Carcinoscorpius rotundicauda. The most commonly caught was T. tridentatus at the Tanjung Tiram. Based on the maximum width of the prosoma and body length, the largest to most petite sizes are T. tridentatus* at Leidong (10.31 cm)*, T. gigas* at Leidong, and Sei Berombang (10 cm) *and C. rotundicauda* at Sei Berombang (7.56 cm*) respectively. The cluster analysis based on 27 morphometric characters showed that T. tridentatus had almost the same characteristics as T. gigas, while C. rotundicauda had its features. It can be concluded that the largest to most petite sizes of Mimi in the study area were T. tridentatus*, *T. gigas, and C. rotundicauda,* respectively.

Keywords: *Carcinoscorpius rotundicauda, Horseshoe crab, Tachypleus gigas, Tachypleus tridentatus.*

Introduction

Limulidae, often called Horseshoe crabs, are living fossils and phylogenetic relics (Selander *et al*., 1970). Although the Horseshoe crab is a living fossil, many freshwater Limulidae species exist, such as in Australian waterways, even though Limulidae typically inhabit brackish or salt water (Kwan *et al*., 2017). Limulidae is a direct descendant of Trilobita (Subphylum Trilobitomorpha), which evolved into Subphylum Chelicherata within the Arthropoda phylum. Trilobites and marine scorpions simultaneously developed until their demise during the Paleozoic period (Bowden *et al*., 2020). During the Paleozoic period, trilobites and aquatic scorpions were extinct, leaving only the Limulidae alive today (Purba *et al*., 2017).

The Horseshoe crab plays a perfect role in the economic, ecological, and biomedical fields. In addition to carrying chitosan, Horseshoe crab shells can be processed into numerous goods such as contact lenses, skin treatments, and patching for head wounds. (Zhu *et al*., 2020). Biomedically, *Limulus polyphemus* blood cells are used to sterilize medical and pharmaceutical products because their blood contains Limulus Amebocyte Lysate (LAL), which may identify endotoxin in human blood. In addition, the Tachyleus genus also produces Tachyplesin Amoebocyt Lysate (TAL), which can locate endotoxins of Gram-negative bacteria, detect human blood endotoxins, and test drugs that are free from pathogenic bacteria before being consumed by humans (Aryawati *et al*., 2018).

Horseshoe Crab is one of the protected animals according to the Decree of the Minister of Forestry Number 12/KPTS-II/1987. The extinction of the Horseshoe crab can change the ecosystem's balance, which may have repercussions for humans in the future. Therefore, the species must be protected. *Tachypleus gigas, T. tridentatus,* and *Carcinoscorpius rotundicauda* can be found in Leidong, Sei Berombang, and Tanjung Tiram. People in Leidong, Sei Berombang, and Tanjung Tiram do not comprehend the Horseshoe crab's benefits, so the exploitation of the Horseshoe crabs is still uncontrolled and unrestricted. In the worst conditions

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it will lead to the extinction of these animals (Ambariyanto, 2017).

Horseshoe crab morphological research (Ismane, 2018) is insufficient to monitor the conservation status of these species, track emerging threats to the surviving Horseshoe crab populations in Indonesia, and justify the implementation of steps to preserve the species. Understanding their morphological traits, including the morphometric, is one method for identifying aquatic animals, such as Horseshoe crabs (Jawahir *et al*., 2017). Morphometric characteristics are an essential tool for studying changes and variations in the body form of organisms, particularly their size (Agustina *et al*., 2016). Changes and comparisons of quantitative data, primarily from body parts of organisms, are explained by morphometric research (Zheng *et al*., 2019). Identifying the population or genus of an organism can be aided by distinguishing morphological differences between species. Some previous study on horseshoe crab has been conducted, for example, on morphological characteristics, diversity of morphometric qualities, and characteristics of the blood (Meilana & Fang, 2020). Nevertheless, it is necessary to conduct research to complete the biological information for the conservation, in this case, research on horseshoe crab morphometry in Leidong, Sei Berombang, and Tanjung Tiram waters.

Materials and Methods

In this study, the material used was Horseshoe crab taken using *sero* net and *dupi dupi* in three locations: Leidong, Sei Berombang, and Tanjung Tiram waters in North Sumatera. This study used a quantitative descriptive method. The research location was determined using the purposive method, which selected the research area by considering the information and facts about the *Mimi* population known from the research area. The research was performed from September to December 2021. The research site map is in Figure 1.

Horseshoe crab sampling at the Leidong and Tanjung Tiram locations was conducted at night. The Horseshoe crab is nocturnal (active at night) (Yue *et al*., 2020). Meanwhile, Horseshoe crab sampling at the Sei Berombang location was performed in the morning following the schedule of local fishermen's habits. The selection was made using the census method; the only Horseshoe crab selected were those with intact limbs. If defects were, they would be returned to the waters because if the Horseshoe crab's condition were defective, the morphometric measurement would be constrained (Anggraini *et al*., 2017). *Mimi*'s morphometric measurements were carried out on the land with a modified technique from the measurement Technique by Sekiguchi *et al.* (1976). A practical method is used to study changes and variations in the body shape of an organism, namely through morphometric studies. The different colors of three species of *Mimi* are presented in Figure 2.

The species of *T. gigas* and *T. tridentatus* generally seemed the same, but the color and spines of the ophistoma could distinguish them. Additionally, *T. gigas* has a blackish-brown color, while *T. tridentatus* has a greenish-brown color. Meanwhile, the *C. rotundicauda* species has a smaller body size than the other two species (Bakker *et al*., 2016).

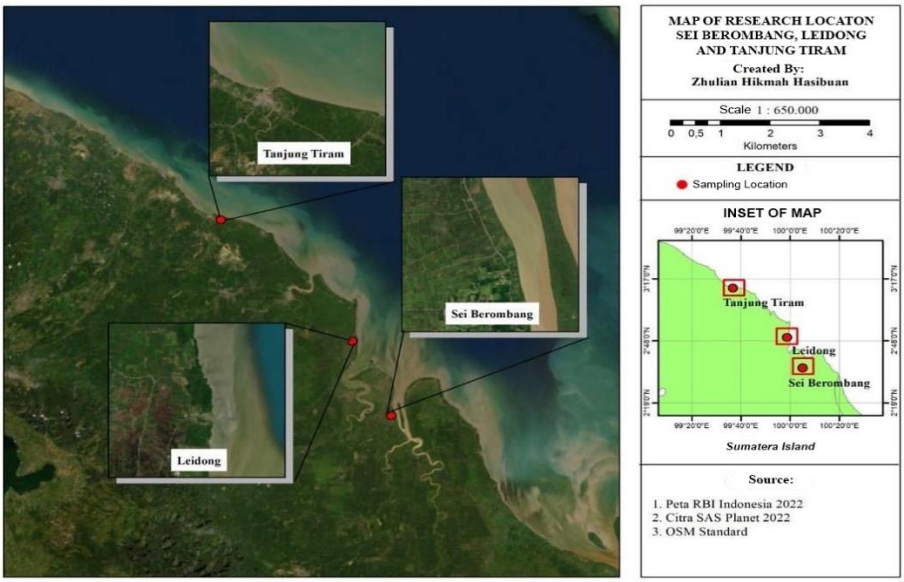

Figure 1. Research Sites Map of Leidong, Sei Berombang, and Tanjung Tiram, North Sumatera.

Kruskal Wallis test was used in this study with the application of Statistical Program for Social Science (SPSS) version 17. In addition, the Mann-Whitney test was done to determine the characteristics of Horseshoe crabs between populations. Meanwhile, multinomial logistic analysis was to test the relationship among the locations, which was done using SPSS 16. In addition, cluster analysis was done to determine the groups formed based on morphometric characters as measured by SPSS 16.

Result and Discussion

A total of 98 Horseshoe crabs were analyzed, and three species were found, namely *T. tridentatus, T. gigas, and Carcinoscorpius rotundicauda*, with details of males and females (Table 1.). Based on the maximum width of the prosoma and body length of the three species, *T. tridentatus, T. gigas, and Carcinoscorpius rotundicauda* were the largest, second most significant, and third largest species, respectively (Figures 3 and 4.). *Tachypleus tridentatus* from Leidong has the maximum prosoma width and body length, approximately same with *Tachypleus gigas* from Tanjung Tiram.

At the 5% significance level, three characters (X5, X10, and X12) of *T. tridentatus* (Sei Berombang, Leidong, and Tanjung Tiram) were not substantially different. In contrast, the remaining 25 characters (Sei Berombang, Leidong, and Tanjung Tiram) differed significantly. In this test, however, it is impossible to determine which characters have significant changes among the locations. H_0 was accepted at a significance level of 5% for all characteristics morphometric (X1-X28) of *Tachypleus gigas*. All characteristics of *Tachypleus gigas* did not differ significantly among the locations of Sei Berombang, Leidong, and Tanjung Tiram. At the 5% significance level, 27 characters of *C. rotundicauda* were not statistically different at the sites of Sei Berombang, Leidong, and Tanjung Tiram. In contrast, the characters of X21 were significantly different in all three areas. Nevertheless, it is impossible to determine precisely which characters have significant changes among the locations (Table 2.).

Figure 2. The color difference of *Tachypleus gigas* (left), *T. tridentatus* (middle), and *Carcinoscorpius rotundicauda* (right).

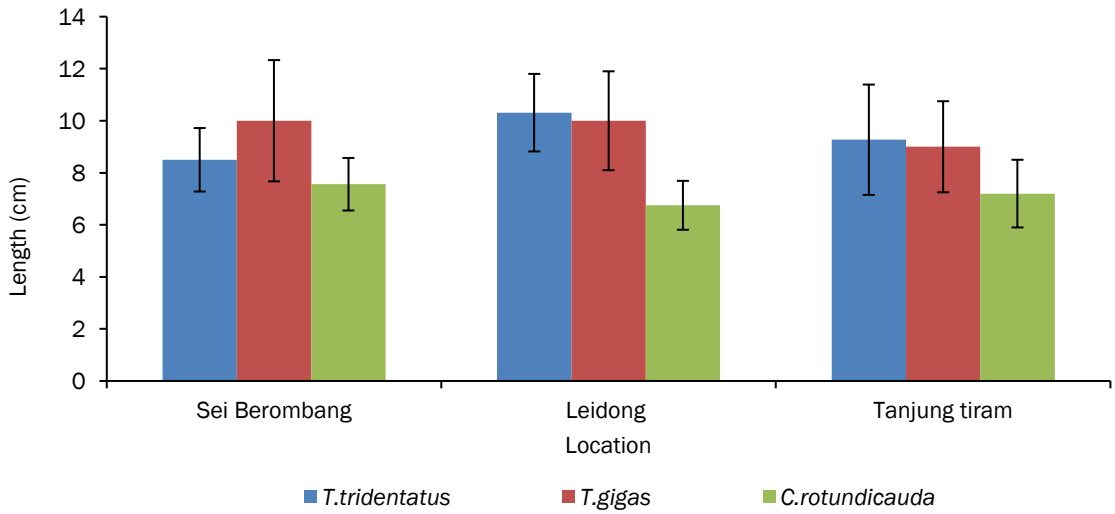

Figure 3. Horseshoe Crab's geographic variation in body size, measured in body length among the three species.

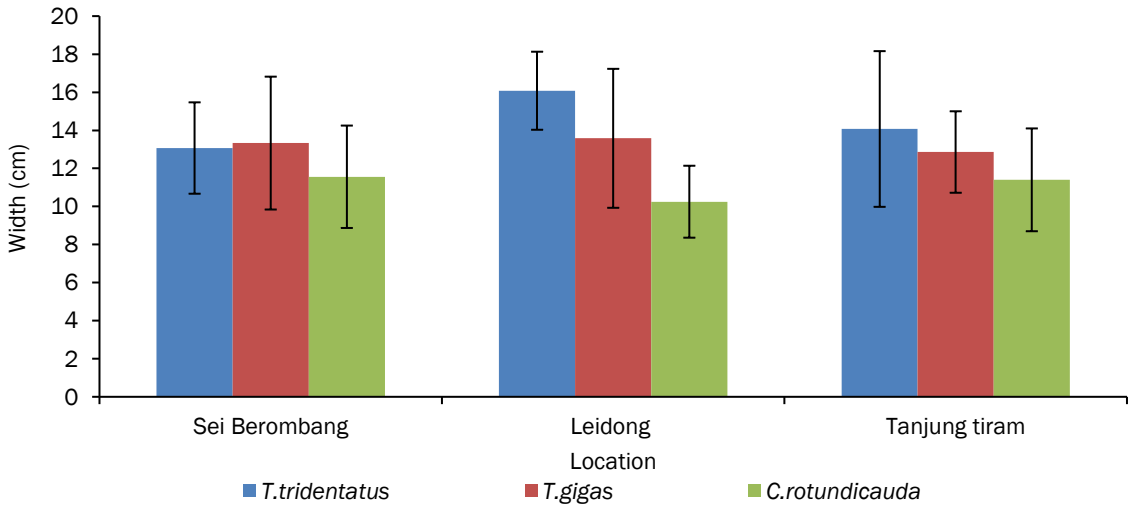

Figure 4. Geographical variation of Horseshoe Crab's body size, measured in the Prosoma Width of the three species.

Table 1. The number of Horseshoe Crab caught during the research

Sampling Location	*T. tridentatus*		*T. gigas*		*C. rotundicauda*		Total
	Male	Female	Male	Female	Male	Female	
Sei Berombang	7	7	7	5	4	5	35
Leidong	3	10	5	7	2	2	29
Tanjung Tiram	8	7	7	7	2	3	34

Table 2. Kruskal Wallis test of *T. tridentatus.*

Species	Unreal Different Characters	Real Different Characters
Tachypleus tridentatus	X5, X10, X12	X1-X4, X6-X9, X11, X13-X28
Tachypleus gigas	X1-X28	-
Carcinoscorpius rotundicauda	X1-X20, X22-X28	X21

The *T. tridentatus* species in the Sei Berombang location formed a separate group based on morphometric characters because it had the smallest body size and limbs compared to other sites. Meanwhile, the Leidong area has relatively large body and limb sizes compared to the Tanjung Tiram location (Figure 5). Figure 6 provides a dendrogram formed from cluster analysis based on 27 morphometric characters of *T. gigas,* which have been ratified at a similarity level of 41.33% in Sei Berombang and Leidong. At the same time, Tanjung Tiram obtained a similarity of 63.34% (Figure 7). Cluster analysis based on 27 morphometric characters of *C. rotundicauda* that have been compared at a level of similarity of 46.27% showed that Sei Berombang and Leidong form the same group. In contrast, Tanjung Tiram has a similarity level of 70.27%, which includes a separate group (Figure 7). Based on the correlation analysis between *Tachypleus tridentatus*, *T. gigas,* and *C. rotundicauda*, there is no relationship exists between species (<0.005).

This study was conducted in three locations, and the number of males and females of each species varied during data collection. First and foremost, *T. gigas and C. rotundicauda* species have the same number of males and females. In addition, *C. rotundicauda* females were more abundant than males, particularly in the water of Sei Berombang. Previous research revealed that the male Horseshoe Crabs without spouses assembled on the beach and acted as satellites while awaiting their turn to spawn. This accounted for the disparity between male and female ratios (Aini *et al*., 2020).

Meanwhile, *T. tridentatus* was the most dominant species in the Leidong location. In general, Leidong has a variety of food sources, such as plankton, allowing for more *T. tridentatus* species than in other areas. In the water of Leidong, the substratum is sandy and around 10 meters deep. Mangrove conditions in this location are likewise rated as good, making it easy for *T. tridentatus* to get food (Hanafi *et al*., 2020).

The result of Horseshoe crab catches in the Sei Berombang location is higher than in other sites. The variety of *T. tridentatus, T. gigas, and C. rotundicauda* is nearly identical. The substrate in this location is sandy, making it an ideal environment for Horseshoe crabs. Horseshoe crab generally builds a dune (*Boting*) as a dwelling in this location. According to local fishermen, numerous little organisms are trapped in the boat, so Horseshoe crab eats them. According to the study by Jawahir *et al*. (2017), Horseshoe crab is commonly detected in waterways with low salinity. The salinity in this location is 24 ppt, which is considerably lower than in other areas. The randomly distributed pattern performed by *T. tridentatus* existence shows an individual's ability to withstand natural resource availability. In Tanjung Tiram, three species were found, with the lack of *C. rotundicauda* likely due to adverse environmental circumstances. As Tanjung Tiram is a tourist destination, human activities can damage the water quality in this location. Tanjung Tiram's mangrove conditions are less extensive than those of Sei Berombang. Research by Manca *et al*. (2017) indicated that *C. rotundicauda* species preferred mangrove areas with muddy substrates, as it was a habitat rich with food supplies.

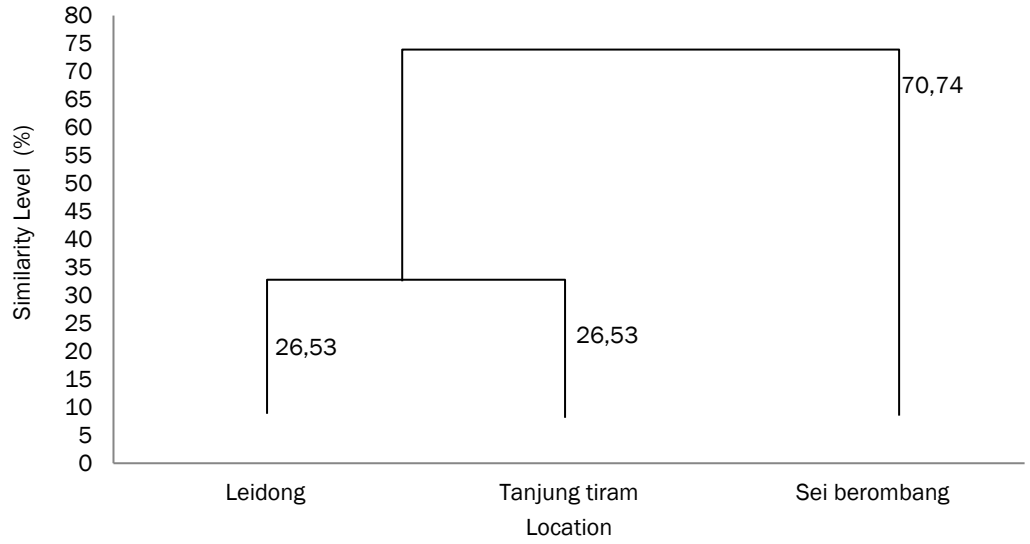

Figure 5. *Tachypleus tridentatus* Morphometric Character Dendrogram.

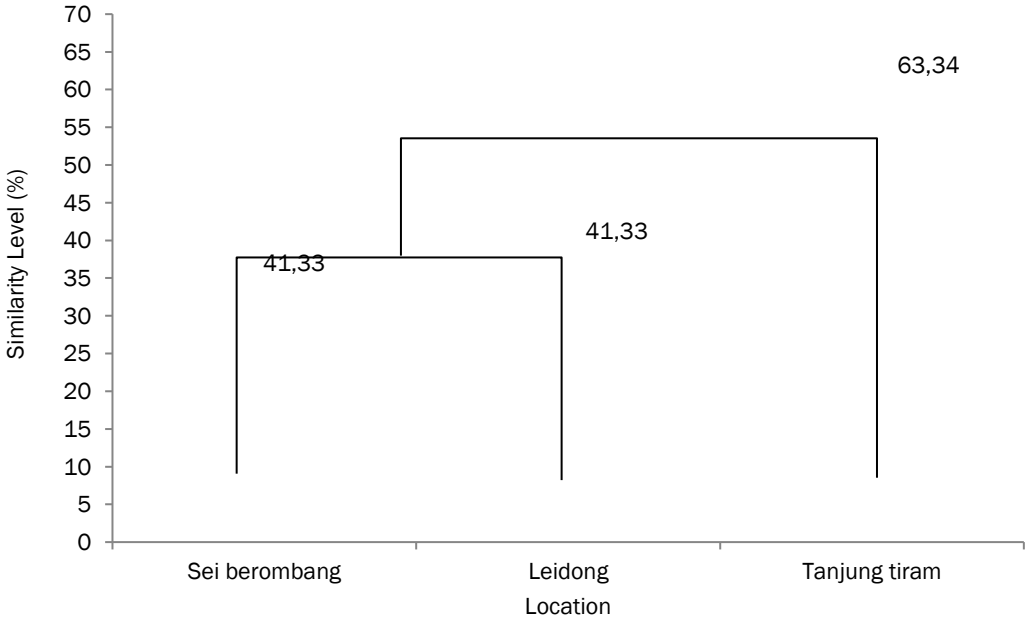

Figure 6. *Tachypleus gigas* Morphometric Character Dendrogram.

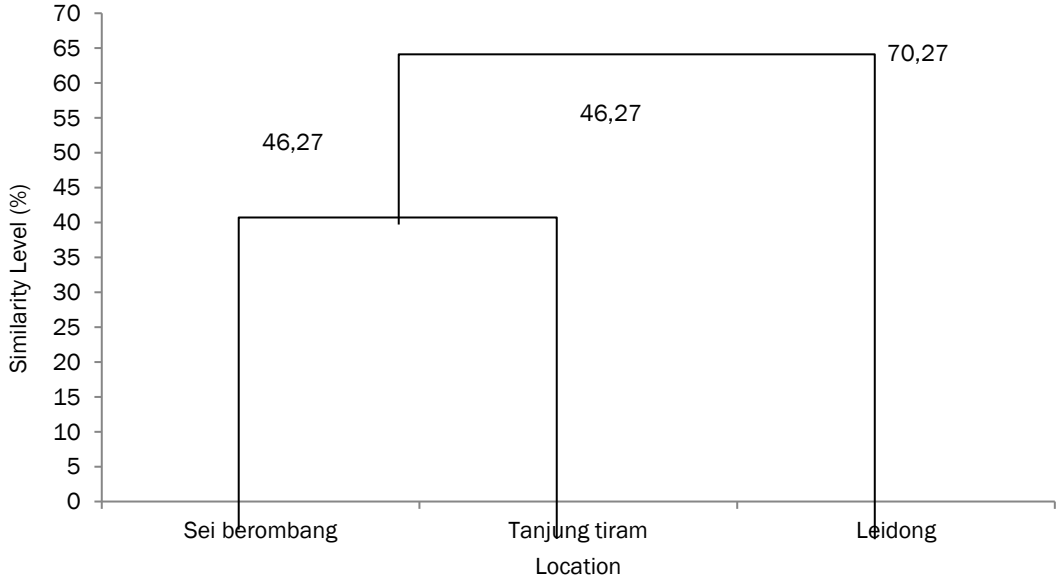

Figure 7. Dendrogram of Morphometric Character Dendogram of *C. rotundicauda*

Since the female Horseshoe crab had a different maximum displacement distance than the male, more of the female Horseshoe crab's composition was collected. Female Horseshoe crabs can travel up to 493.74 km, while males can only travel up to 363.7 km. It is inadequate to serve as a barrier between populations. Other factors, such as natural barriers, have a significant effect (Rehman *et al*., 2020).

Furthermore, based on the previously mentioned characteristics, the genotyping data suggest that male Horseshoe crabs have low heterozygosity values. The lower the heterozygosity value, the greater the ability to eliminate possible alleles, such as those connected to disease resistance, the formation of abnormalities, and growth issues (Monica *et al*., 2016). The value of heterozygosity in genetic diversity indicated the potential for environmental adaptability (Zhu *et al*., 2020). The lower the heterozygosity score, the fewer genes contribute to the fitness level of a population. Hence male Horseshoe crab has poor adaptation. In general, poor intrapopulation genetic diversity affects the low adaptation of a population's species to ensure its long-term survival (Nadiroh and Nurul, 2021).

Conclusion

Tachypleus gigas, T. tridentatus, and *Carcinoscorpius rotundicauda* are Horseshoe crab species found in Leidong, Sei Berombang, and Tanjung Tiram waters in North Sumatera. The largest to most petite sizes are *T. tridentatus, T. gigas,* and *C. rotundicauda,* respectively.

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