The first investigation record of threatened horseshoe crabs in the Banyuasin estuarine, South Sumatra, Indonesia

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Abstract
The first investigation of the Asian horseshoe crabs in the Banyuasin Estuarine (Indonesia) was carried out during July 16-31, 2019. Sampling was conducted together with local fishermen using a trammel net. Two species of the Asian horseshoe crabs have been identified from 52 obtained specimens, namely Carcinoscorpius rotundicauda (♂ = 18, ♀ = 27) and Tachypleus gigas (♂ = 5, ♀ = 6). The prosoma width for the two species was 100-150 mm and 115-210 mm respectively, with body weights of 72-285 grams and 99-918 grams, respectively. All specimens of C. rotundicauda were adults (their prosomal width ≥ 8 cm). Female was larger in size than male for both species. Its geographical distribution was from the waters near mangroves up to 4.2 km towards the sea. These results indicated that Banyuasin Waters was included in the geographical distribution coverage for both species. Further studies were needed to assess population structure, abundance, spawning ground, spawning activity, growth aspects as well as morphometric and DNA studies.

Key words: Banyasin estuarine, Carcinoscorpius rotundicauda, distribution, Indonesia, Tachypleus gigas.

Introduction
The estuarine ecosystem provides an important habitat for many economically and ecologically fish resources, including horseshoe crabs (Chen et al., 2015). Horseshoe crab juveniles usually forage on the estuarine waters close to mangrove area (Almendral and Schoppe, 2005; Chen et al., 2004; Sekiguchi et al., 1988). Four species of horseshoe crabs still exist in the world, namely, Carcinoscorpius rotundicauda, Tachypleus tridentatus, and Tachypleus gigas found in Asia (from India to Japan and south to Malaysia and Indonesia), whereas Limulus polyphemus found along the Atlantic coast of North America (Almendral and Schoppe, 2005; Cartwright-Taylor et al., 2011; Lee and Morton, 2005).
The horseshoe crabs habitats are generally found in coastal, intertidal (sand or mudflat) and deeper waters (Jawahir et al., 2017). Muddy and brackish areas are a common habitat for *C. rotundicauda* while *T. gigas* usually existed in the sand to muddy areas (Robert et al., 2014; Tan et al., 2012). In addition, *C. rotundicauda* commonly found in mangrove swamps or sometimes found in shallow waters (Cartwright-Taylor et al., 2012). The existence of the three Asian horseshoe crabs was declared near threatened by IUCN (2010), threatened (2014) and data deficient (2015). Whereas *T. tridentatus* was an endangered based on the red list category and criteria of IUCN (2019). Decree of the Indonesian Ministry of Forestry No. 12/KPTS.II/1987 and Government Regulations No. 7/1999 were to protect the three Asian horseshoe crabs. Furthermore, these crabs have been established as an endangered marine biota since 2013 by the Indonesian Ministry of Marine Affairs and Fisheries.

Banyuasin estuarine with highly potential mangroves can be possible as a habitat for the Asian horseshoe crabs. This hypothesis is in line with the previous studies results that these horseshoe crabs were a discarded catch by the trammel net fishing in Banyuasin Coastal Waters (Fauziyah et al., 2018). In Indonesia waters, *C. rotundicauda* was found in the coastal area of Bintan Bay, Riau Islands Province (Anggraini et al., 2017), *T. gigas* was found in coastal waters of Northern Java Island (Meilana et al., 2016), meanwhile the three Asian horseshoe crabs were found in Muara Badak Waters, Kutai Kartanegara Regency, East Kalimantan Province (Ahmad et al., 2017). In the eastern Indonesian Waters (Dolejš and Vaňousová, 2015), the horseshoe crabs were reported in Maluku waters in 1898. In addition, the geographic distribution information of horseshoe crabs in Indonesian waters was very limited (John et al., 2018).

Research on horseshoe crab in Indonesian waters is still rarely conducted and in fact, this research is the first investigation of the horseshoe crab in the Banyuasin Waters. The study's aim was to investigate the occurrence of the Asian horseshoe crabs, and confirmed the first investigation record in the Banyuasin estuarine, South Sumatra, Indonesia.

### Materials and Methods

The investigation survey was conducted in the Banyuasin estuarine of South Sumatra Province, Indonesia on July 19-31, 2019. Sampling sites were determined based on the fishermen experience capturing horseshoe crabs. Sampling was carried out using a trammel net together with the local fisherman.

The horseshoe crabs catch were identified according to morphological characteristics such as the form of telson, color, and opisthosoma spines. For *T. tridentatus* and *T. gigas* have triangle shape of telson, however, *T. tridentatus* has special characteristics namely three spines on the rear part of the opisthosoma (only one spine for *T. gigas*) and many tiny spines on opisthosoma, whereas *C. rotundicauda* has the smallest size among the other two Asian horseshoes crabs, the cross-section of the tail is rounded, and the anterior telson jagged (Cartwright-Taylor et al., 2009; Dolejš and Vaňousová, 2015; Tanacredi et al., 2009).

All species of the identified horseshoe crabs were recorded and photographed for the occurrence of evidence. Sampling locations were recorded as a basis for mapping the geographic distributions. The horseshoe crabs samples that have recorded then released to the waters.

### Results and Discussions

Table 1 showed 56 specimens were recorded during study period consisting of 45 *C. rotundicauda* ($\varphi = 18$, $\delta = 27$) and 11 *T. gigas* ($\varphi = 5$, $\delta = 6$). The photographic evidence presented in Figure 1 for *T. gigas* and Figure 2 for *C. rotundicauda*. Both species were also found around Singapore's main island (Cartwright-Taylor et al., 2011). The males *C. rotundicauda* had prosomal width between 10 and 13 cm while the female had prosomal width between 10 and 15 cm. These prosomal widths were larger in size than *C. rotundicauda* found in Bintan Bay of Riau Islands Province (Anggraini et al., 2017). The body weight for both males and females ranged 85 - 165 grams and 72 - 285 grams, respectively. The male *T. gigas* had the prosomal width between 11.5 and 17 cm whereas the female had the prosomal width between 15 and 21 cm. In the Balasore coast (the Bengal Bay, India), the prosomal width of *T. gigas* for both sexes ranged between 12.0 to 24.5 cm (Sahu and Dey, 2013). The specimen of *T. gigas* in this study smaller in size than the species found in Balok Beach of Pahang, Malaysia with the largest prosomal width for both sexes were 24 and 32 cm respectively (Tan et al., 2012). The body weight for both males and females ranged 99 - 359 grams and 196 - 918 grams,
respectively. The mean body weight of *C. rotundicauda* and *T. gigas* were 169 and 370 grams whereas its prosomal widths were 12 and 16 cm respectively. These results indicated that *T. gigas* larger in size than *C. rotundicauda* as well as females larger in size than males. The adult females were larger in size than males due to differences in molting activities for their growth and numerous egg in prosomal cavity for females (Graham et al., 2009; Tan et al., 2012).

Juvenile and adult differences can be determined based on the size of the prosomal width. For the *C. rotundicauda* and *T. gigas*, their juveniles had the prosomal width less than 8 cm whereas if their prosomal width size 8 cm and above were considered as adults (Cartwright-Taylor et al., 2009). Thus, all specimens that recorded during the study period were adults (their prosomal width ≥ 8cm). The sex ratio for both species was 0.67 and 0.83 respectively.

**Table 1.** Identification records two Asian horseshoe crabs from Banyuasin Estuarine Waters, South Sumatra, Indonesia.

| Parameters                | *C. rotundicauda* | *T. gigas* |
|---------------------------|-------------------|------------|
|                           | Male (♂)         | Female (♀) | Male (♂) | Female (♀) |
| Body Weight (grams)       |                   |            |          |            |
| min                       | 85                | 72         | 99       | 196        |
| max                       | 165               | 285        | 359      | 918        |
| mean                      | 120               | 202        | 223.4    | 493        |
| Prosoma Width (cm)        |                   |            |          |            |
| min                       | 10                | 10         | 11.5     | 13         |
| max                       | 13                | 15         | 17       | 21         |
| mean                      | 11                | 13         | 14.8     | 17.25      |
| Number specimen           | 18                | 27         | 5        | 6          |
| Sex ratio                 | 0.67              | 0.83       |

**Figure 1.** *Tachypleus gigas* from Banyuasin Estuarine Waters, South Sumatra, Indonesia. This species has a triangular telson shape and only one spine (yellow arrow) on the rear part of the opisthosoma. Female has a chelate clasper like scissors (red oval) while the male has a hemicelately clasper like hooks on the first and second walking legs (yellow oval).
Figure 2. *Carcinoscorpius rotundicauda* from Banyuasin Estuarine Waters, South Sumatra, Indonesia. This species is smaller than the others and the only species where the telson cross-section is rounded. Female has a chelate clasper like scissors (red oval) while the male has a hemichelate clasper like hooks on the first and second walking legs (yellow oval).

Figure 3. The geographic distribution map of horseshoe crabs in Banyuasin Estuarine, South Sumatra, Indonesia.
Geographic distribution for both Asian horseshoe crabs in Banyuasin Estuarine was presented in Figure 3. The specimens of *C. rotundicauda* were found at every sampling site (0.2 - 4.2 km from mangrove) whereas *T. gigas* were found close to the sea waters (station 1, 3, 12). The horseshoe crabs distribution was influenced by environmental parameters such as temperature, salinity, pH, DO, and substrate types (Jawahir et al., 2017). The horseshoe crabs prefer the waters with temperatures more than 20°C (Zaleha et al., 2012). High salinity habitat was preferred by *T. gigas* on the contrary, *C. rotundicauda* prefers low salinity habitat (Jawahir et al., 2017). The sediment with medium grain size (sandy substratum) was preferred by *T. gigas* (Tan et al., 2012) due to suitable for their larvae survival (Jackson et al., 2005) while *C. rotundicauda* prefers muddy sediment (Avissar, 2006).

The horseshoe crabs are a protected marine biota by Indonesia Government, thus prevention efforts are needed to reduce these species caught by gillnet, trammel net or other fishing gear. Some prevention efforts that probably can be carried out include: 1) avoiding the fishing gear operation around spawning and nursing ground; 2) Reducing the use of bottom gillnet or trammel net; 2) Experimental fishing by installing LED lights on gillnet to reduce these species as by-catch or discard; 3) release immediately after these species being caught by fishing gear. Further detail study is needed to investigate marine nursery area, spawning area, population structure, abundance, growth aspects as well as morphometric and DNA studies.

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