MANGROVE
Mangrove Watch
MANGROVE 2(2): 78–96
ISSN 3048-2000

Institute for Advanced Science, Social and Sustainable Future
MORALITY BEFORE KNOWLEDGE

Morphogenetic and molecular insights into coastal crab species: Implications for mangrove biodiversity and conservation

Putri Nurul Pratiwi Abas[1], Dewi Wahyuni K. Baderan[1,*], Regina Valentine Aydalina[1], Magfiratul Jannah[1], Marini Susanti Hamidun[2]

[1] *Biology Study Program, Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Gorontalo, Bone Bolango, Gorontalo, 96119, Indonesia;*

[2] *Population and Environmental Studies Program, Postgraduate Program, State University of Gorontalo, Gorontalo, 96128, Indonesia.*

*Correspondence: dewi.baderan@ung.ac.id2

Received Date: February 19, 2025 Revised Date: July 14, 2025 Accepted Date: August 27, 2025

ABSTRACT

Background: There are crab species that live in sandy, rocky and muddy coastal areas. One of the locations with these characteristics is Tabongo Village, Dulupi District, Boalemo Regency, this location has a lot of crab species diversity that has not been well confirmed. Identification of crab species was carried out by morphological and genetic characterization of crab species suspected to be *Baptozius vinosus* and *Ocypode ceratophthalmus*. This study aims to characterize the morphological and molecular characteristics of both species to understand their variations and phylogenetic relationships. **Methods:** Sampling used a handpicking method with purposive sampling to select individuals that have similar characters to the target species. Samples were then analyzed through DNA extraction, amplification, sequencing, and measurement of environmental data (temperature, salinity, pH). **Findings:** 16S rRNA gene sequence alignment results showed the nucleotide length of B. vinosus 568 bp and O. ceratophthalmus 567 bp. BLAST analysis showed 99.81% similarity between the O. ceratophthalmus specimen and the reference sequence (LC150355.1). Genetic distance analysis showed the closeness of the specimen to the population in Pakistan based on the Neighbor-Joining method (Kimura 2-parameter, bootstrap 1000 times). However, the phylogeny of B. vinosus could not be constructed due to limited sequence data. **Conclusion:** This study shows that morphogenetic characterization and molecular analysis can reveal the variation and phylogenetic relationships of coastal crab species**. Novelty/Originality of this Article:** This study provides insights into the morphogenetic characterization of coastal crabs and its implications for conservation and biodiversity of mangrove ecosystems.

KEYWORDS: *Baptozius vinosus; c*haracterization; mangrove*;* morphogenetics; *Ocypode ceratophthalmus;* 16S rRNA.

1. Introduction

Mangroves are a special type of ecosystem that grows along tropical and subtropical coasts that act as a barrier between land and sea. In contrast to terrestrial forests, mangrove forests have a more specific habitat due to the interaction between complex and complicated ecosystem components (Rosyid, 2020). Mangrove ecosystems have an important role for humans, including as an economic function, physical function and ecological function. As an economic function, mangrove ecosystems are used as firewood,

Cite This Article:
Abas, P. N. P., Baderan, D. W. K., Aydalina, R. V., Jannah, M., & Hamidun, M. S. (2025). Morphogenetic and molecular insights into coastal crab species: Implications for mangrove biodiversity and conservation. *Mangrove Watch, 2*(2), 77-96. https://doi.org/10.61511/mangrove.v2i2.2025.1723

building materials, batik dyes, and medicines. As a physical function, mangrove ecosystems are used for sediment traps and wave barriers (Rahman et al., 2020). And the ecological function of this ecosystem is useful as a nursery ground, spawning ground, and feeding ground, as well as supporting the lives of various biota such as fish, shrimp, and shellfish, And other biota (Rahman et al., 2020). Mangroves have extensive benefits from ecological, biological and economic aspects. Mangroves have an important role for ecology, including maintaining coastal stability and as a bird habitat. As a biological function of mangroves as a natural hatchery for fish, shrimp and plankton-eating marine biota and artificial fish hatcheries, while the economic function is as a place of recreation and a source of wood (Katili et al., 2017).

One of the mangrove areas in Gorontalo Province is located on the coast of Tabongo Village, Dulupi District, Boalemo Regency. Based on the National Mangrove Map by BPDAS Bone Bolango (2023), it is known that the total mangrove area in Tabongo Village is 116.963 Ha. Djamadi et al. (2024) revealed that there are six mangrove species found on the coast of Tabongo Village, Dulupi District, Boalemo Regency, namely *Sonneratia alba, Rhizophora apiculata, Rhizophora mucronata, Ceriops tagal, Bruguiera gymnorrhiza,* and *Avicennia alba.* These mangrove species are said to grow on different substrate conditions; some are muddy, sandy, silt, silty clay, and some are mixed with mud and sand. Katili et al. (2017) also revealed that in Tabongo Village there are mangrove stands of other species, namely *Rhizophora stylosa* stands.

Baderan et al. (2023) stated that the mangrove ecosystem of Tabongo Village is a dense mangrove with muddy and sandy soil substrate. In line with that, Saragi & Desrita (2018) revealed that the denser the mangrove, the more biomass of fallen leaves will also increase. The availability of mangrove leaf litter, which is the food of crabs, will also increase, which affects the abundance of crabs in an area. Research related to crab species was conducted by Katili et al. (2017), who found that in Tabongo Village there are crab species *Uca annulipes, Uca dussumieri, Uca triangularis,* and *Uca vocans.* In addition, research conducted by Faqih & Juramang (2023) also found other species, namely the crab species *Uca forcipata, Exanthus dentatus, Parathelphusa convexa,* and *Metacarcinus magister.*

Based on observations made by researchers on July 15, 2024, crabs with characters similar to those of *Baptozius vinosus* and *Ocypode ceratophthalmus* were found. However, until now there have been no reports related to the presence of these two species in Tabongo Village or in other areas in Gorontalo Province. Literature studies show that both species are found in Indonesia but in different areas, namely *Ocypode ceratophthalmus* species found in the waters of Pondang Beach and Lopana, North Minahasa, North Sulawesi Province (Lepa et al., 2022), in the mangrove area of Gili Sulat Lombok (Putri et al., 2022), and in the Opak River, Yogyakarta Special Region (Eprilurahman et al., 2015). Meanwhile, the crab species *B. vinosus* is found in the Natuna Islands (Pratiwi & Elfidasari, 2020).

The discovery of certain species in an area, especially those that have never been reported before, requires an identification process. Identification is an activity to recognize and classify based on general characteristics of body shape, color, and other morphological characters, which is important to understand the diversity and distribution of crab species. Identification can be done based on several aspects, including morphological and molecular approaches. Morphological identification is done by observing the body characteristics of species similar to *B. vinosus* and *O. ceratophthalmus,* while molecular identification is done by analyzing the DNA of crabs suspected to be *B. vinosus* and *O. ceratophthalmus.* Therefore, to ensure the presence and confirmation of *B. vinosus* and *O. ceratophthalmus* species, it is necessary to conduct research related to the morphological and genetic characterization of the two species in the mangrove area of Tabongo Village, Dulupi District, Boalemo Regency.

2. Methods

The research was conducted from October to December 2024. Identification of morphological characters as well as isolation and preservation of DNA samples were carried out at the Agrotechnology Laboratory, Faculty of Agriculture, Gorontalo State University. Furthermore, DNA isolation and amplification were performed at PT Griya Sains, East Java, while DNA sequencing was completed at 1st BASE Laboratories SDN Bhd.

The research method used is descriptive method with the aim describe the morphological and genetic characters of crabs suspected as species *of Baptozius vinosus and Ocypode ceratophthalmus* species found in the mangrove area of Tabongo Village, Dulupi District, Boalemo Regency. With primary data consisting of morphological data, genetic data and secondary data including morphological descriptions of both species. The object of this research is crabs found in the mangrove area of Tabongo Village.

2.1 Data collection technique

Observation activities were carried out directly by observing the condition of the research location and the object to be studied. This activity aimed to gain a comprehensive understanding of the physical conditions and environmental characteristics that would be the focus of the research. In addition, environmental parameters such as soil pH, ambient temperature, and water salinity were measured carefully to support the description of the research site. These initial steps were essential to ensure that the sampling process was conducted in representative and controlled environmental conditions.

Sampling was carried out using the hand-picking method by catching the crabs directly. *Baptozius vinosus* crab samples were collected using a claw, while *Ocypode ceratophthalmus* crabs were collected using a net because their mobilization is quite high. The hand-picking method was used to identify the presence of both species directly in their natural habitat. A total of 30 individuals were collected through purposive sampling, consisting of 15 males and 15 females, and morphological identification was carried out on all crabs, while molecular identification was performed on one individual from each species.

The samples obtained included both specimens for morphological characterization and for molecular characterization. Morphological samples were preserved in 70% alcohol, while molecular samples were kept alive during transfer to the Agronomy Laboratory, Faculty of Agriculture, Gorontalo State University. All samples were initially kept in containers and maintained alive to preserve their condition before being processed. The laboratory handling ensured that the crabs remained suitable for both morphological and genetic analysis.

Morphological identification was conducted based on several references, including Eprilurahman & Tejo Baskoro (2015), Yang et al. (2022), Putri et al. (2022), Pratiwi & Elfidasari (2020), Milla et al. (2022), Kurnia et al. (2023), Lepa et al. (2022), Pratiwi & Susilohadi (2019), and Komarpant et al. (2018). Meanwhile, molecular identification referred to Barua et al. (2021), Suherman & Arsad (2020), Abbas et al. (2016), Triandiza & Madduppa (2018), and Siahaan et al. (2023), in addition to references from books and existing species literature. The primers used to amplify the sequences were 16S rRNA, which served as the main genetic marker for molecular analysis. Molecular identification was then carried out as the final stage to confirm the species identity of *Baptozius vinosus* and *Ocypode ceratophthalmus.*

2.1.1 Sample DNA preparation, extraction, and amplification procedures

DNA samples used for molecular analysis were taken from the muscle tissue of the first and fifth street legs of the crab. The tissue samples were collected specifically from the right front and back legs using a sterile scalpel and adjusted to the size of the crab. After collection, the tissue was placed into a cryo tube with the addition of absolute ethanol solution and stored in the freezer to prevent damage during transportation. These samples were then sent to PT G.S, East Java, for further DNA extraction and amplification required in the molecular analysis process.

DNA extraction was performed using the NexPrep Kit isolation procedure. Crab tissue samples weighing approximately 20 mg were crushed until smooth and placed into a 1.5 ml tube, after which 200 µl of GT1 buffer was pipetted into the tube and mixed using a vortex. The next step involved adding 200 µl GT2 buffer and 20 µl Proteinase K, which were again mixed thoroughly with a vortex. The mixture was then incubated at 56 °C for 10 minutes, with the tube inverted every 5 minutes, and subsequently 200 µl of absolute ethanol was added and vortexed briefly before transferring the mixture onto a Spin Column for centrifugation at 13,000 rpm for 1 minute.

After centrifugation, the flow-through was discarded, and 500 µl of buffer W1 was added to the Spin Column, followed by centrifugation for 1 minute at 13,000 rpm. The flow-through was removed, and 700 µl of buffer W2 (with ethanol added) was introduced and centrifuged again for 1 minute at the same speed. The flow-through was discarded, and an additional centrifugation step was carried out for 2 minutes at 13,000 rpm to ensure complete removal of liquid. The Spin Column containing DNA was then placed into a new 1.5 ml tube, followed by the addition of 50–100 µl Elution Buffer, incubation at room temperature for 1 minute, and centrifugation for 1 minute at 13,000 rpm. The Spin Column was discarded, and purified DNA was obtained, which was stored at –20 °C for short-term preservation or –70 °C for long-term storage.

The amplification process was carried out using a Biorad666t7 PCR machine in a 30 µl reaction mixture consisting of 15 µl PCR Master Mix Nexpro, 3 µl template DNA (100 ng/µl), 6 µl nuclease-free water, and 3 µl primers (5 pmol each for forward and reverse primers). The primers used to amplify the sequences were 16S rRNA (forward: 5'CGC CTG TTT ATC AAA AAC AT 3'; reverse: 5'CCG GTC TGA ACT CAG ATC ATGT 3') (Barua et al., 2021). The thermal cycling conditions for PCR were set as follows: pre-denaturation at 95 °C for 5 minutes, followed by 41 cycles of denaturation at 95 °C for 30 seconds, annealing at 54 °C for 30 seconds, and extension at 72 °C for 1 minute. The final post-extension step was performed at 72 °C for 5 minutes to complete the amplification. PCR products were analyzed by electrophoresis on 2% agarose gel to verify successful amplification, and the confirmed PCR products were then sequenced using the sequencing services of 1st BASE Laboratories Sdn Bhd.

2.2 Data analysis

Morphological characteristics are described quantitatively based on morphological and meristic characters. The measurements were carried out using the ImageJ application to ensure accuracy and consistency in data collection. The results of these measurements are presented systematically and illustrated in the following figure to provide a clearer description of the morphological traits observed.

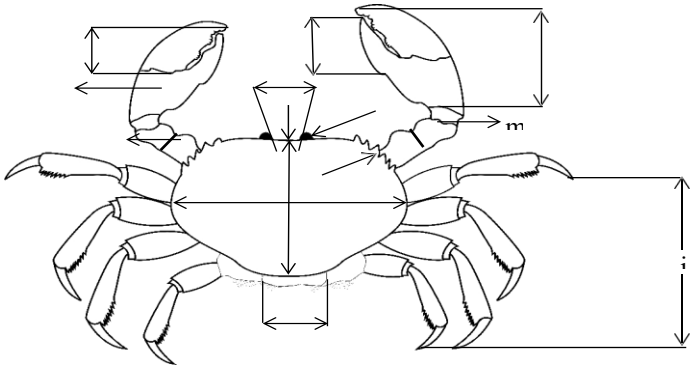

Fig. 1. Morphology of Baptozius vinosus crab

Figure 1 shows the morphological measurement of *Baptozius vinosus* crab, which includes several main characters of the body. The measurements consist of carapace width, carapace length, claws, dactyl, pollex, eye gap, abdomen, eye, suborbital border, walking

foot, orbit, proplodus, carpus, and merus. These morphological characters are important for quantitative description and serve as diagnostic features to differentiate crab species accurately.

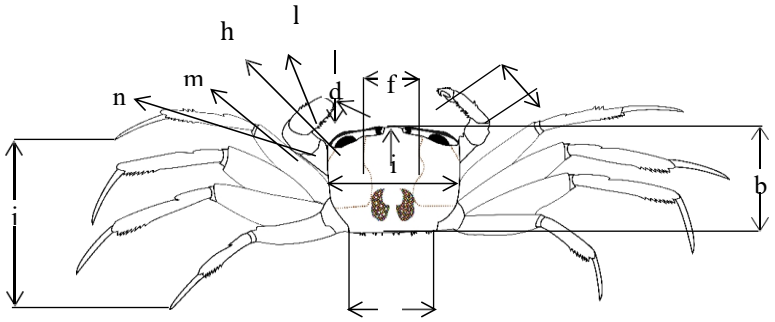

Fig. 2. Morphology of *Ocypode ceratophthalmus* crab.

Environmental data collected in this study included water temperature, salinity, and pH, which were measured to describe the abiotic conditions of the habitat. Sequence data analysis was conducted using BioEdit software to process and align the obtained sequences. The similarity analysis of sequence data was carried out by comparing the sequences obtained with the existing sequence data available in NCBI for *Baptozius vinosus* and *Ocypode ceratophthalmus* species. Furthermore, genetic distance analysis was performed using the Kimura 2-parameter model in MEGA 12, followed by phylogenetic relationship analysis through the Neighbor-Joining method, also using MEGA 12, to determine the evolutionary relationships between the studied species.

3. Results and Discussion

3.1 Results

Based on morphological identification, a total of 41 crab individuals were found at the research site. These findings indicate the presence of more than one species in the area, reflecting the diversity of crab populations within the habitat. The data obtained serve as a foundation for further morphological characterization and ecological analysis.

Table 1. Number of Crabs Found in the Research Site

No.	Species name	Number of Individuals		Total individuals
		Male	Female	
1.	*Baptozius vinosus*	5	1	6
2.	*Ocypode ceratopthalmus*	20	15	35
Total		25	16	41

3.1.1 Classification of Baptozius vinosus

The following are the results of morphological characterization based on observations of several main body characteristics, including the carapace, eyes, claws, abdomen, and legs. The classification of *Baptozius vinosus* is as follows: Kingdom Animalia, Division Arthropoda, Class Malacostraca, Order Decapoda, Family Oziidae, Genus *Baptozius*, and Species *Baptozius vinosus*. The individuals of *Baptozius vinosus* found in the research site exhibited distinct morphological characteristics, which are described in detail and illustrated in the following figure.

Fig. 3. Eye of *Baptozius vinosus*

The eyes of *Baptozius vinosus* are round and convex, protruding slightly with short stalks extending from the lateral part of the carapace. They exhibit suborbital maturation, and the eye color is distinctly dark red with a compound structure. In addition, small spots are visible along the margins, with 25 spots on the upper edge and 27 spots on the lower edge.

Fig. 4. *Carapace* of *Baptozius vinosus*

The carapace of *Baptozius vinosus* is oval in shape with a rough surface texture. It is equipped with four sharp spines along the antero-lateral edges, where the first, second, and third spines are angular and broad, while the fourth spine appears more curved. The carapace color generally tends to be dark, with slight variations of lighter shades on the pollex and abdomen, and it does not display any striking color or pattern variations.

Fig. 5. Abdomen of *Baptozius vinosus*

The abdomen of *Baptozius vinosus* is flattened in shape and composed of several distinct segments with clear boundaries adjacent to the legs. Its coloration contrasts with the darker tone of the carapace, displaying a generally lighter shade and a smooth texture. A distinctive feature is the presence of a purplish line beneath the eyes, which emphasizes the ventral part of the abdomen, as illustrated in Figure 5.

Fig. 6. Capitulum of *Baptozius vinosus*

The claws of *Baptozius vinosus* are strong and large, showing clear asymmetry with one claw larger than the other. Each claw consists of two main parts, namely the dactylus and propodus, both of which display dense fine grains similar to those on the carapace. The claws are generally brown to tawny in color, with spines present on both the dactylus and pollex; the spines are brownish black, and the tip of the claw is shaped like pliers, as illustrated in Figure 6.

Fig. 7. Whole morphology of *Ocypode ceratophthalmus*

The feet of *Baptozius vinosus* consist of four walking legs that are symmetrically arranged on both sides of the body. Each leg has a reddish-brown tip and is covered with fine hairs along its segments, giving it a slightly textured appearance. The legs are relatively long and slender with tapered tips, which support efficient locomotion, as illustrated in Figure 7.

3.1.2 Classification of Ocypode ceratopthalmus

The species *Ocypode ceratophthalmus* belongs to the Kingdom Animalia and is classified under the Phylum Arthropoda, Class Malacostraca, and Order Decapoda. It is further categorized within the Family Ocypodidae and the Genus *Ocypode*. The identified species, *Ocypode ceratophthalmus*, is one of the commonly recognized ghost crabs that exhibit distinctive morphological features described in the following section.

Fig. 8. Eye of *Ocypode ceratophthalmus*

The eyes of *Ocypode ceratophthalmus* are positioned upward with long stalks that give them a prominent appearance. The cornea is oval in shape and brown in color, supported

by an orbital anchor and sometimes accompanied by a shorter eye horn. These are compound eyes, showing distinct patterns with 36 spots on the upper eye and 34 spots on the lower eye, as illustrated in Figure 8.

Fig. 9. Carapace of *Ocypode ceratophthalmus*

The carapace of *Ocypode ceratophthalmus* is square-shaped and convex with a rough surface texture. Its frontal region appears narrow and displays a distinctive butterfly-like pattern, accompanied by irregular spots across the surface. The overall coloration is pale yellowish-brown, and the antero-lateral edges are sharply structured, as illustrated in Figure 9.

Fig. 10. Abdomen of *Ocypode ceratophthalmus*

The abdomen of *Ocypode ceratophthalmus* is brownish in color and divided into several distinct segments. Its dominant coloration is yellowish-white, with some parts appearing more transparent, and it displays clear bilateral symmetry on both sides of the segments. The abdomen is separated by a triangular structure known as the telson, and fine hairs are present on the lower segment located between the third and fourth walking legs, as illustrated in Figure 10.

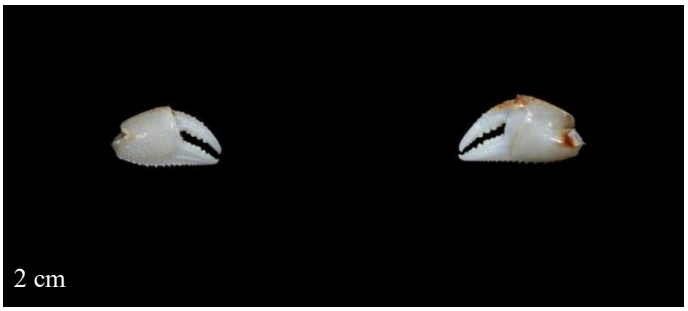

Fig. 11. Claw of *Ocypode ceratophthalmus*

The claws of *Ocypode ceratophthalmus* are asymmetrical, with one claw larger than the other, and are predominantly white in color. Spines are present on the tips of the claws, and the larger claw has additional spines along the merus. The claw tips are hook-shaped, while the pollex contains a distinctive triangular structure, as shown in Figure 11.

Fig. 12. Whole morphology of *Ocypode ceratophthalmus*

The feet of *Ocypode ceratophthalmus* consist of four pairs of walking legs that are equal in length and arranged symmetrically on both sides of the body. Each leg is segmented, covered with fine hairs, and ends with a pointed tip that supports efficient movement. The legs exhibit a grayish-white coloration with an irregular sand-like pattern, as illustrated in Figure 12.

Table 2. Morphological identification results of *Baptozius vinosus* and *Ocypode ceratopthalmus* crabs.

No.	Character		*Baptozius vinosus*	*Ocypode ceratopthalmus*
1.	Karapas	Carapace length	2.974	2.628
		Carapace width	4.632	3.356
2.	Anterior	Eye gap	1.595	1.377
		Suborbital boundary	1.023	2.072
3.	Eye	Eye	0.500	0.860
4.	Claw	Claw	3.085	1.789
		Daktilus	1.658	0.810
		Pollex	1.566	0.758
5.	Abdomen	Abdomen	1.825	2.032

Furthermore, molecular characterization, gel electrophoresis results that have been analyzed and visualized using UV-Transluminator are shown in Figure 9. Meanwhile, the 16S rRNA electrophoresis results used in this study are shown in Figure 10. Comparison of sequences using BLAST-NCBI is shown in Table 3, then the genetic distance analysis of Ocypode ceratophthalmus crabs was carried out using the Kimura 2 paramteter method with the results shown in Table 4, then the kinship relationship between species was visualized through the phylogeny tree.

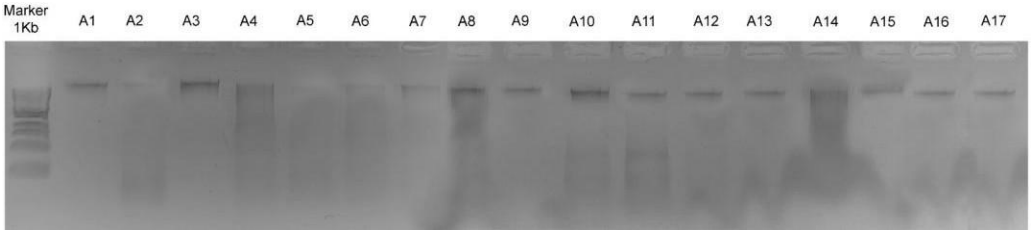

Fig. 13. Electrophoresis of isolated DNA

Figure 13 presents the electrophoresis profile of isolated DNA obtained from the samples. The gel visualization demonstrates clear DNA bands in multiple lanes, confirming that the extraction process was successfully carried out and yielded intact genomic material. Variations in band thickness and intensity are observable, which may indicate differences in DNA concentration or purity among the samples, yet overall the results verify that the isolated DNA is of sufficient quality for subsequent molecular analyses.

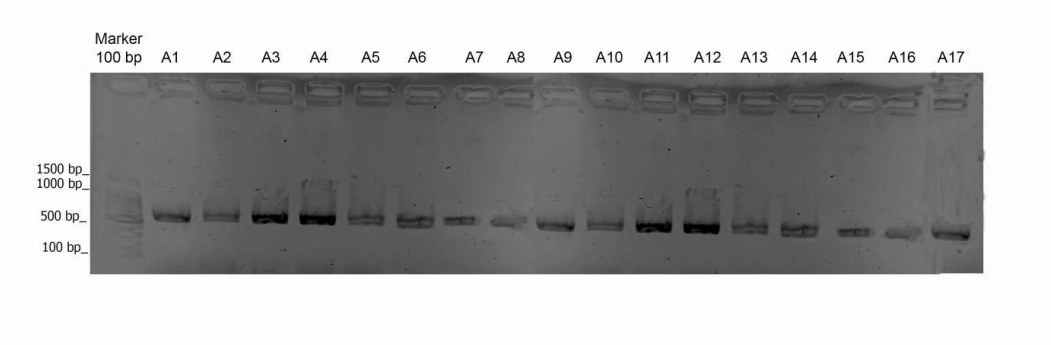

Fig. 14. Electrophoresis of 16S rRNA results

Figure 14 illustrates the phylogenetic tree constructed from the 16S rRNA gene sequences of *Ocypode ceratophthalmus* using the Kimura 2 parameter model with the Neighbor-Joining method. The branching patterns clearly demonstrate the genetic relationships among the analyzed specimens, where closely clustered branches indicate a high degree of similarity and genetic proximity. This phylogenetic reconstruction not only confirms the taxonomic placement of the species but also provides insight into its evolutionary relationship with other decapod crabs included in the analysis.

Table 3. Sequence comparison using NCBI blast

No.	Sample	Identification	% Identity	Seq ID
A15	Tabongo SP.L	*Baptozius vinosus*	99,06%	HM637963.1
			99,81%	LC150355.1
			97.79%	LC150357.1
		Ocypode	98.46%	LC150356.1
A16	Tabongo SP.P	*ceratophthalmus*	97.56%	MF509787.1
			97.56%	MF509786.1
			97.37%	MF495679.1
			97.37%	MF509785.1
Outgroup		*Ocypode mortoni*	92.04%	ON379461.1

Table 3 presents the results of sequence comparison using BLAST-NCBI for the 16S rRNA gene. Sample A15 from Tabongo SPL was identified as *Baptozius vinosus* with a similarity value of 99.06% against sequence HM637963.1 and above 98% with other related sequences, confirming the accuracy of morphological identification. Meanwhile, Sample A16 from Tabongo SPP was identified as *Ocypode ceratophthalmus*, showing similarity values ranging from 97.37% to 97.79% with multiple reference sequences, which supports the molecular confirmation of species identity. The use of *Ocypode mortoni* as an outgroup, with 92.04% similarity, further strengthens the comparative framework for phylogenetic analysis.

Table 4. Genetic distances of 16S rRNA in Ocypode ceratophthalmus species using the 2- parameter kimura method

	TA05001	MF509785	MF509786	MF509787	MF495679	LC150355	LC150356	LC150357	ON379461
TA05001									
MF509785	0.00								
MF509786	0.00	0.00							
MF509787	0.00	0.00	0.00						
MF495679	0.00	0.00	0.00	0.00					
LC150355	0.00	0.00	0.00	0.00	0.00				
LC150356	0.01	0.01	0.01	0.01	0.01	0.01			
LC150357	0.01	0.01	0.01	0.01	0.01	0.01	0.02		
ON379461	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	

Table 4 displays the genetic distance matrix of *Ocypode ceratophthalmus* species calculated using the Kimura 2 parameter method. The results show very low genetic distance values, ranging from 0.001 to 0.008, indicating a close genetic relationship among the analyzed sequences. These small divergence values confirm that the examined specimens of *Ocypode ceratophthalmus* share a high level of genetic similarity, which is consistent with the BLAST results and reinforces their placement within the same species group.

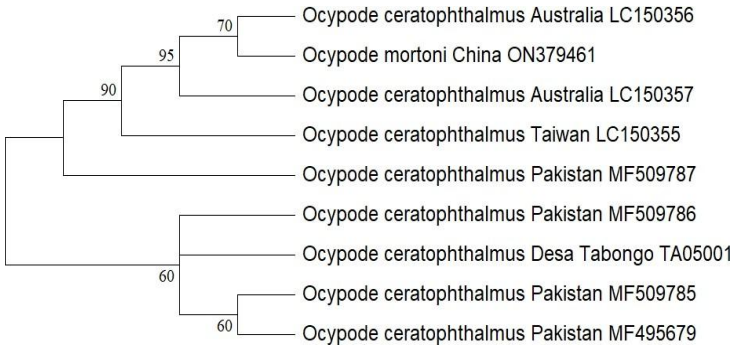

Fig. 15. Phylogeny tree of the crab species *Ocypode ceratophthalmus*

Figure 15 illustrates the phylogenetic tree of *Ocypode ceratophthalmus* constructed from 16S rRNA sequences using the Kimura 2 parameter and Neighbor-Joining method. The tree demonstrates clustering of *O. ceratophthalmus* specimens from different geographic locations, including Australia, Taiwan, Pakistan, and Indonesia, which are grouped closely with high bootstrap values, indicating strong genetic relatedness. The outgroup *Ocypode mortoni* from China is positioned separately, confirming its evolutionary distance and validating the phylogenetic structure obtained.

Table 5. Environmental parameter measurement results

No.	Environmental parameters waters	Average
1	Degree of acidity (pH)	6.75
2	Salinity (ppt)	9.76
3	Temperature (^{0}C)	32.7
4	Humidity (%)	0.54
5	Light Intensity (cd)	2976
6	Wind Speed (m/s)	1.4

Table 5 summarizes the measurement results of environmental parameters at the research site. The waters exhibited a slightly acidic condition with a pH of 6.75 and a salinity level of 9.76 ppt, reflecting a brackish habitat. The recorded temperature was relatively high at 32.7°C, with low humidity (0.54%), moderate wind speed (1.4 m/s), and light intensity reaching 2976 cd, all of which describe the ecological conditions influencing the distribution and survival of *O. ceratophthalmus* in the studied area.

3.2 Discussion

Tabongo Village in Dulupi Sub-district, Boalemo Regency, is one of the locations with mangrove ecosystems in Gorontalo Province. This mangrove ecosystem has a very important role in maintaining environmental balance, protecting the coastline, and providing habitat for various types of flora and fauna species. The total area of mangroves in Tabongo Village is 116,963 Ha (BPDAS, 2023). The mangrove area of Tabongo Village shows a significant diversity of substrate types, consisting of muddy, mixed mud and sand and sandy substrates. This substrate diversity affects the type of vegetation that can grow in the area. Muddy substrates usually have a higher nutrient content, thus supporting the growth of more lush mangrove vegetation.

According to Masruroh & Insafitri (2020), mangroves can grow well on substrates of sand, mud or coral. Most types of mangroves grow well on muddy substrates, but some grow well on sandy substrates, even broken coral substrates. According to Kordi (2012), the type of substrate on a beach plays an important role in mangrove growth. Soil types of silt (dust) and clay (clay) are the main factors that support the regeneration process, because clay particles in the mud are able to accommodate mangrove fruits that fall when they are ripe. This regeneration process directly affects the density of mangroves in an area. Mangrove vegetation found in the study site includes several genera, namely Rhizopora, Sonneratia, Avicennia and Bruguiera. Furthermore, species characterization is the process of identifying and classifying the traits or characteristics of an object based on certain characters. Characterization involves observation and analysis of various aspects to understand the object as a whole. Species characterization that is often used is morphological characterization, because it only involves analyzing the physical form and structure of organisms such as shape, size, color, texture and body structure (Putri et al., 2022).

The most commonly used species is morphological characterization, because it only involves the analysis of the physical form and structure of organisms such as shape, size, color, texture and body structure (Putri et al., 2022). Morphological characterization of crabs has limitations, which are easily affected by conditions in the field (Subositi et al., 2013). Species identification often experiences errors caused by *cryptic species*, so identification techniques are needed with a molecular approach, namely *DNA barcoding* (Zamroni et al., 2024).

Several studies related to DNA barcoding with genes encoding the *Cythrocrome c Oxidase* subunit I (COI) gene have been conducted by Siahaan et al. (2023), Triandiza & Madduppa (2018), Abbas et al. (2016). In addition to studies that focus on the use of COI encoding genes, there are other approaches that combine two encoding genes to get more comprehensive results. Barua et al. (2021) used two DNA barcodes, namely the COI and 16S rRNA genes, which revealed that COI is superior in distinguishing closely related crab species, while 16S rRNA is more appropriate for phylogenetic tree construction.

3.2.1 Morphological characteristics

Based on the data in Table 4, the total number of crabs found in Tabongo Village, Dulupi Subdistrict, Boalemo Regency is 41 individuals. The identification results show that the crabs found have characteristics that match the crab species *Baptozius vinosus* and *Ocypode ceratophthalmus*. The dominant species found in the study site was *Ocypode cerathopthalmus*, with 35 individuals and a male to female ratio of 4:3. Meanwhile, the *Baptozius vinosus* species found was dominated by males with a total of 5 individuals. To understand more about the characteristics of the two crab species found, morphometric measurements taken to analyze the morphological differences between the crab species *Baptozius vinosus* and *Ocypode cerathopthalmus* presented in Table 2.

Although the crabs *Baptozius vinosus* and *Ocypode cerathopthalmus* are from different families, they both show special adaptations to the coastal environment. The adaptation shown by the crab species *Baptozius vinosus* is with a transverse oval- shaped carapace, the front of the carapace has a rough double edge with the front side of the carapace having four spines with sharp edges in Figure 6. Based on their habitat, these crabs are generally found in marine habitats, especially areas with hard substrates such as coral reefs or rocks. Hard structures such as coral or rocks allow the crab species *Baptozius vinosus* to hide in crevices and cavities, away from the reach of larger predators (Putri et al., 2024). It has claws with a rough texture with a reddish brown color at the end of the dactylus and white in the *pollex* and has walking legs whose outer surface has a little fine hair. , the species *Ocypode ceratphthalmus* is a semi- terrestrial crab that lives in the tidal zone with a pair of upturned eyes, a red-brown carapace and a dimorphic claw size (the left claw is larger than the right claw) with white claw tips. This crab has four pairs of equally long walking legs shown in Figure 9. This species has muscular legs that can run very fast to avoid predators. In addition, this crab performs camouflage and makes burrows as hiding places, which makes no

difference between the color of the crab's carapace and the sand where it lives (Pratiwi & Susilohadi, 2019). This is in line with Eprilurahman & Baskoro (2015), who stated that the genus Ocypode has a habitat in tropical coastal areas. They also added that this crab can only be found in the estuary because the substrate at that location is dominated by sand, which is a natural habitat for this crab.

Baptozius vinosus crabs have a number of morphological features that distinguish them from other crab species. The most striking difference lies in the unique pattern under the eyes of this crab that resembles Kumadori makeup in Kabuki, a character in traditional Japanese theater with makeup that follows the muscles of the performer. The texture of the carapace is rough and pitted, much different from the smoother or regularly patterned surface of many other crab species (Figure 5). In Vermeij (1977), this crab has an average width for male individuals of 5.3 mm and female individuals of 7.5 mm. The shape of the carapace itself tends to be oval and wider than it is long, with the antero-lateral edges dotted with several sharp spines, especially laterally, and the number and size of these spines can vary between individuals.

The chelipeds or claws are large and strong, showing asymmetry with one claw being dominant or larger. The surface of these claws is also rough and pitted, matching the texture of the carapace. Other differences can be found in the proportions and shape of the abdomen, which in females is wider and rounded to accommodate the eggs, in contrast to the abdomen of males which is narrower and folded under the carapace. In line with Huxley (1924), this wide abdominal shape allows for effective attachment and incubation of eggs, protecting and ensuring better development before release. Overall, the combination of the rough texture of the carapace, the shape and size of the claws, and the variety of colors that match the environment make *Baptozius vinosus* easily distinguishable from other crab species that inhabit the same habitat. The crab species *Baptozius vinosus* is found to be widespread in the Indo-West Pacific region, namely found scattered in the Natuna Islands by Pratiwi & Elfidasari (2020) and more in the Philippines, namely in Sta. Maria, Davao Occidental, Philippines by Milla et al. (2022), in Pagbilao and Catanauan of Quezon Province, Philippines (Bandibas & Hilomen, 2016).

Furthermore, the *Ocypode ceratophthalmus* crab is a beach dweller with a striking morphology that reflects its adaptation to the coastal environment. This crab is known for its habit of making burrows that function as a place to live, shelter, and as a location to find food (Kurnia et al., 2023). In line with Pratiwi & Elfidasari (2020), it is known that the crab species *Ocypode ceratophthalmus* can adapt to various habitat types including mangroves with mud substrates, seagrasses with sand and sand-mud substrates, and in sandy beach areas. The carapace is almost square in shape, the carapace surface is rough, and the color varies from grayish white to pale yellowish, providing effective camouflage among the sand on the coastal plain (Figure 11).

The carapace of *Ocypode ceratophthalmus* has a distinctive shape and adaptations that favor its life in sandy beach environments. The carapace is almost square in shape with a slightly convex and rough surface. The carapace varies in size, with the width reaching about 6 cm in adult individuals. This color can vary depending on the local environment and the age of the individual, with some crabs showing darker shades or patches and having a unique H like pattern on the posterior. This is in line with what Odhano et al. (2023) stated that the carapace of *Ocypode ceratophthalmus* is grayish yellow with maroon spots in the posterior region.

This crab has a long horn-shaped eye stalk, in line with Odhano et al. (2023) who stated that the cornea of this crab's eye is located at the base of the eye and extends to form a horn. The five pairs of legs that it has, namely the first pair of legs are modified into chelipeds or claws, with one claw larger or asymmetrical. The other four pairs of legs are long and slender, used for speed and efficiency in running on sand.

In Odhano et al. (2023), the crab *Ocypode ceratophthalmus* is described as having five pairs of legs that are pale yellow on the lateral side while the ventral side is darker in color. This crab is known for its speed of movement, which allows it to quickly traverse sandy habitats with minimal resistance from body weight (Weinstein, 1994). The abdomen, which

is relatively narrow and folded under the carapace, functions to protect vital organs. Sexual dimorphism is evident in the abdomen, where males possess a narrower and tapered structure while females have a wider and rounded form. This difference is directly related to reproduction, since the female abdomen is adapted to carry eggs attached to the pleopods of the swimming legs.

In addition to protecting internal organs, the carapace of *Ocypode ceratophthalmus* has a lightweight yet strong structure, enabling it to support and hold eggs during development. This crab's ability to move rapidly across sand represents a key morphological adaptation that makes it an agile predator and highly effective at surviving in dynamic coastal environments. Although the abdomen is not the primary organ of locomotion, it contributes to body balance and plays an important role in digging burrows. Such adaptation allows the crab to protect itself from predators, maintain body moisture, and survive in hot and dry coastal habitats.

The distribution of *Ocypode ceratophthalmus* is wide, with records in the mangrove area of Tanjung Panjang Nature Reserve, Pohuwato District, Gorontalo (Lapolo et al., 2018), in Lukupang and Tanawangko (Wada, 2019), across regions extending from East Africa to Australia (Kurnia et al., 2023), and in the waters of Pondang and Lopana Beach, South Minahasa (Lepa et al., 2022). To further confirm the species that have been identified morphologically, molecular identification is carried out to obtain more accurate results. This is in accordance with Butet et al. (2019), who performed both morphological and genetic identification using 16S rRNA primers to study marine snail species. The 16S rRNA subunit, with a length of 1500–2000 bp, provides sufficient information on evolutionary relationships between species and is widely used for species identification (Suherman & Arsad, 2020).

3.2.2 Molecular characteristics

Molecular characterization, or better known as DNA barcoding, is the analysis of specific gene sequences to identify species that are difficult to distinguish morphologically (Antil et al., 2023). Based on genetic analysis using 16S rRNA molecular markers (Figure 15), it was confirmed that the specimens found belong to the species *Baptozius vinosus* and *Ocypode ceratophthalmus*. The presence of DNA bands indicates that DNA amplification was successful, visualizing the sample band with code A15 for *Baptozius vinosus* and A16 for *Ocypode ceratophthalmus* (Figure 12). Novitasari et al. (2014) revealed that intact DNA can be observed from thick and clear bands with no smear, and according to Yılmaz et al. (2012), differences in band thickness are strongly influenced by the DNA condition. Furthermore, DNA concentration, purity, and contamination may affect the band intensity, while Yang et al. (2022) emphasized that taxonomy-related studies can utilize multi-source data such as morphology, DNA barcoding, genetics, and ecological niches. In line with Triandiza & Madduppa (2018), cryptic species phenomena in marine biota were studied using a combined morphological and genetic approach, where crabs can be identified using markers such as COI, 16S rRNA, 12S rRNA, and 18S rRNA.

Tissue isolation was successful with sizes matching the target for *Baptozius vinosus* coded A15 and *Ocypode ceratophthalmus* coded A16, while PCR results on the 16S rRNA gene produced fragments ranging from 500–600 bp as shown in Figure 13. The results of sequence alignment showed that *Baptozius vinosus* had a nucleotide length of 568 bp and *Ocypode ceratophthalmus* 567 bp. The obtained sequences were validated using BLAST, where validation was based on percentage similarity (%Identity) compared to database sequences. Kumar et al. (2018) stated that similarity between sequences can be measured by sequence identity, where a higher number of identical nucleotides in alignment positions indicates a higher level of similarity. The BLAST results showed that *Ocypode ceratophthalmus* from Tabongo Village had a similarity level of 99.81% with *Ocypode ceratophthalmus* (LC150355.1). Other sequences also showed similarity, though slightly lower, including LC150357.1 (97.79%), LC150356.1 (98.46%), MF509787.1 and MF509786.1 (97.56%), and MF495679.1 and MF509785.1 (97.37%).

For *Baptozius vinosus*, BLAST was not performed due to only one comparator sequence being available in the GenBank database. This limitation prevented comprehensive sequence matching, as BLAST requires multiple references for accurate analysis (Madden, 2013). The results of genetic distance analysis showed that *Ocypode ceratophthalmus* had the farthest distance with *Ocypode mortoni* at 0.08 and the closest distance with *Ocypode ceratophthalmus* sequences with accession codes MF509787.1, MF509786.1, MF495679.1, MF509785.1, and LC150355.1. According to Hebert et al. (2003), species are considered different if their genetic distance exceeds 3%. This analysis was conducted using the Neighbor-Joining method with the Kimura 2-parameter model, and the phylogenetic tree constructed using MEGA 12 with 1000 bootstrap replicates showed that *Ocypode ceratophthalmus* from Tabongo is closely related to populations from Pakistan with the same accession codes (Figure 14).

For *Baptozius vinosus*, phylogenetic analysis could not be performed because only one 16S rRNA sequence was available in the database, which prevented reconstruction of the phylogenetic tree and calculation of genetic distance. Butet et al. (2019) explained that phylogenetic trees are built from genetic distance values to describe kinship between species and subspecies, while Pertiwi (2022) stated that the branching pattern of a phylogenetic tree reflects the closeness of samples based on their genetic distance. This finding highlights both the success and limitations of molecular identification, where *Ocypode ceratophthalmus* could be clearly resolved in phylogenetic context, while *Baptozius vinosus* was constrained by limited reference sequences.

4. Conclusions

Based on the research conducted, it can be concluded that the morphological characteristics of *Baptozius vinosus* and *Ocypode ceratophthalmus* samples found in Tabongo Village are consistent with theoretical descriptions of these species. Molecular analysis using 16S rRNA markers further confirmed that the two specimens collected from Tabongo Village indeed belong to *Baptozius vinosus* and *Ocypode ceratophthalmus*. These findings strengthen the validity of combining morphological and molecular approaches to accurately identify crab species in coastal ecosystems.

Acknowledgement

The authors very grateful to the Tabongo Village government for granting permission to conduct this research. Their support and cooperation have played an important role in completing my studies in Biology.

Author Contribution

P.N.P.A, contributed to field sampling, data collection, and preparation of the initial manuscript draft. D.W.K.B, supervised the overall research design, provided guidance on methodology, and contributed to data interpretation and critical revision of the manuscript. R.V.A, was responsible for molecular analysis, including DNA extraction, amplification, sequencing, and contributed to drafting the results. M.J, assisted in morphological measurements, image documentation, and supported statistical analysis. M.S.H, contributed to environmental data analysis, literature review, and manuscript editing. All authors have read and approved the final version of the manuscript, and agree to be accountable for all aspects of the work.

Funding

This research received no external funding.

Ethical Review Board Statement

Not available.

Informed Consent Statement
Not available.

Data Availability Statement
Not available.

Conflicts of Interest
The authors declare no conflict of interest.

Open Access

References
Abbas, E. M., Abdelsalam, K. M., Mohammed-Geba, K., Ahmed, H. O., & Kato, M. (2016). Genetic and morphological identification of some crabs from the Gulf of Suez, Northern Red Sea, Egypt. *Egyptian Journal of Aquatic Research, 42*(3), 319–329. https://doi.org/10.1016/j.ejar.2016.08.003

Antil, S. A. (2023). DNA barcoding, an effective tool for species identification: A review. *Molecular Biology Reports, 50*, 761–775. https://doi.org/10.1007/s11033-022-08015-7

Baderan, D. W. K., Aydalina, R. V., & Hamidun, M. S. (2023). Morphological characteristics and biodiversity of mudskipper fish (*Periophthalmus*: Gobiidae) in mangrove ecosystem of coastal Bay of Tomini, Boalemo, Gorontalo Province, Indonesia. *Biodiversitas, 24*(1), 498–507. https://doi.org/10.13057/biodiv/d240158

Bandibas, M. B., & Hilomen, V. V. (2016). Crab biodiversity under different management schemes of mangrove ecosystems. *Global Journal of Environmental Science and Management, 2*(1), 19–30. https://doi.org/10.7508/gjesm.2016.01.003

Barua, A., Afrin, T., Akhand, A. A., & Ahmed, M. S. (2021). Molecular characterization and phylogenetic analysis of crabs (Crustacea: Decapoda: Brachyura) based on mitochondrial COI and 16S rRNA genes. *Conservation Genetics Resources, 13*(3), 291–301. https://doi.org/10.1007/s12686-021-01212-9

Butet, N. A., Dewi, I., & Zairion, H. A. A. (2019). Species Validation of Mole Crabs Based on Molecular Marker of 16s rRNA from Bantul and Purworejo waters. *Journal of Tropical Fisheries Management, 3*(2), 28–35. https://doi.org/10.29244/jppt.v3i2.30434

BPDAS Bone Bolango. (2023). *National mangrove map*. Balai Pengelolaan Daerah Aliran Sungai Bone Bolango.

Milla, M. R. C., Fernandez, E. B., & Elemino, M. M. (2022). Distribution of decapods in the selected mangrove sites in Sta. Maria, Davao Occidental, Philippines. *Asian Journal of Fisheries and Aquatic Research, 19*(1), 27–36. https://doi.org/10.9734/ajfar/2022/v19i130466

Djamadi, D. A., Faqih, A., SM, F., Safitri, I., & Baderan, D. W. K. (2024). Analysis of mangrove forest vegetation structure in Tabongo coastal district, Dulupi, Boalemo Regency. *Journal of Marine Research, 13*(2), 319–327. https://doi.org/10.14710/jmr.v13i2.42138

Eprilurahman, R., & Tejo Baskoro, W. (2015). Diversity of crab species (Decapoda: Brachyura) in Opak River, Yogyakarta Special Region. *Biogenesis, 3*(2), 100–108. https://doi.org/10.24252/bio.v3i2.934

Faqih, A., & Juramang, R. R. (2023). Diversity and abundance of crustacean species in the coastal mangrove forest area of Langala, Dulupi District, Boalemo Regency. *Jambura Edu Biosphere Journal, 5*(2), 65–71. https://doi.org/10.34312/jebj.v5i2.22077

Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 270*(1512), 313–321. https://doi.org/10.1098/rspb.2002.2218

Katili, A. S., Ibrahim, M., & Zakaria, Z. (2017). Degradation level of mangrove forest and its reduction strategy in Tabongo Village, Boalemo District, Gorontalo Province, Indonesia. *Asian Journal of Forestry, 1*(1), 18–22. https://doi.org/10.13057/asianjfor/r010102

Katili, A. S., Utina, R., & Mopangga, N. L. (2017). Short communication: Crab species distribution under mangrove stands in Tabongo, Gorontalo Province, Indonesia. *Biodiversitas, 18*(2), 520–524. https://doi.org/10.13057/biodiv/d180211

Komarpant, S., Padate, V. P., & Rivonker, C. U. (2018). Morphology, growth and behavior of *Ocypode ceratophthalma* (Pallas, 1772) (Decapoda: Brachyura: Ocypodidae) from Goa, west coast of India. *Journal of the Marine Biological Association of India, 60*(1), 71–76. https://doi.org/10.6024/jmbai.2018.60.1.1927-11

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution, 35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Kurnia, K. A., Satria, R., Arya, F., Nugraha, D., & Kardiman, R. (2023). Review of the diversity of crab (Ocypodidae) in Indonesia which can be used as a bioindicator of environmental pollution. *Serambi Biologi, 8*(3), 321–328. https://serambibiologi.ppj.unp.ac.id/index.php/srmb/article/view/217/140

Lapolo, N., Utina, R., & Baderan, D. W. K. (2018). Diversity and density of crabs in degraded mangrove area at Tanjung Panjang Nature Reserve in Gorontalo, Indonesia. *Biodiversitas, 19*(3), 1154–1159. https://doi.org/10.13057/biodiv/d190351

Lepa, B. G., Paransa, D. S. J., Mantiri, D. M. H., Boneka, F. B., Lumoindong, F., & Tilaar, F. F. (2022). Identification and diversity of crabs in Pondang and Lopana coastal waters of southern Minahasa. *Platax Scientific Journal, 10*(1), 85–91. https://doi.org/10.35800/jip.v10i1.38004

Madden, T. (2013). The BLAST sequence analysis tool. In *The NCBI handbook* (2nd ed., pp. 425–436). National Center for Biotechnology Information.

Masruroh, L., & Insafitri, I. (2020). The effect of substrate type on density *Avicennia marina* vegetation in Gresik District. *Juvenil: Marine Scientific Journal and Fisheries, 1*(2), 151–159. https://doi.org/10.21107/juvenil.v1i2.7569

Novitasari, D. A., Elvyra, R., & Roslim, D. I. (2014). Total DNA isolation and electrophoresis technique in *Kryptopterus apogon* (Bleeker 1851) from Kampar Kiri and Tapung Hilir Rivers, Kampar Regency, Riau Province. *Jurnal Online Mahasiswa FMIPA Universitas Riau, 1*(2), 258–261. https://www.neliti.com/publications/185210/teknik-isolasi-dan-elektroforesis-dna-total-pada-kryptopterus-apogon-bleeker-185

Odhano, S., Rosenberg, M. S., Saher, N. U., Zhang, G. Y., & Kamal, M. (2023). Comparative biochemical and genetic analysis of two species ghost crabs, *Ocypode rotundata* and *Ocypode ceratophthalmus* from the coast of Pakistan. *Pakistan Journal of Zoology, 57*(1), 117–131. https://doi.org/10.17582/journal.pjz/20220721190738

Pertiwi, N. P. D. (2022). Identification of pelagic fish species sold in Denpasar City Market using mitochondrial control region (mtDNA) markers. *Undiksha Journal of Biology Education, 9*(1), 95–102. https://ejournal.undiksha.ac.id/index.php/JJPB/article/view/45584

Pratiwi, E., & Susilohadi. (2019). Camouflage and antipredation strategies in *Ocypode* ghost crabs. *Biospecies, 12*(1), 1–10. https://doi.org/10.22437/biospecies.v12i1.6598

Pratiwi, R., & Elfidasari, D. (2020). Short communication: The crustaceans fauna from Natuna Islands (Indonesia) using three different sampling methods. *Biodiversitas, 21*(3), 1215–1226. https://doi.org/10.13057/biodiv/d210349

Putri, H., Mahatma, R., & Muhammad, A. (2022). Inventory and characterization of Biola crab (Ocypodidae) in the intertidal environment of Bengkalis Regency, Riau. *Sriwijaya Bioscientia, 3*(3), 1–8. https://doi.org/10.24233/sribios.3.3.2022.333

Rahman, Wardiatno, Y., Yulianda, F., & Rusmana, I. (2020). Distribution of species and density status of mangrove ecosystems on the coast of West Muna Regency, Southeast Sulawesi. *Journal of Natural Resources and Environmental Management, 10*(3), 461–478. https://doi.org/10.29244/jpsl.10.3.461-478

Rosyid, N. U. (2020). *Mangrove ecoliteration*. SPASI Media.

Saragi, S. M., & Desrita, D. (2018). Mangrove ecosystem as a habitat for mangrove crabs (*Scylla serrata*) in Kampung Nipah, Sei Nagalawan Village, Perbaungan Sub-district, Serdang Bedagai, North Sumatra Province. *Depik, 7*(1), 84–90. https://doi.org/10.13170/depik.7.1.8742

Siahaan, T. A. G., Paransa, D. S. J., Paulus, J. J. H., Wullur, S., Mangindaan, R. E. P., & Pangemanan, P. (2023). Morphological and biomolecular of the *Atergatis floridus* crab in the intertidal zone of the Minanga coast, Manado City. *Scientific Journal of Science, 22*(2), 135–143. https://doi.org/10.35799/jis.v22i2.46363

Huxley, J. S. (1924). The variation in the width of the abdomen in immature fiddler crabs considered in relation to its relative growth-rate. *The American Naturalist*, *58*(658), 468-475. https://doi.org/10.1086/279998

Subositi, D., & Mujahid, R. (2013). Genetic characterization of tempuyung (*Sonchus arvensis* L.) based on sequence-related amplified polymorphism molecular marker. *Indonesian Journal of Biology, 9*(2), 167–174. https://jurnalbiologi.perbiol.or.id/home/article/0aafcece-913c-44c9-97fe-77d4074f488b

Suherman, S. P., & Arsad, S. (2020). Phylogenetic and DNA barcode analysis of *Octolasmis cor* ectoparasites infested with mangrove crabs *Scylla* spp. *Jambura Fish Processing Journal, 2*(2), 94–100. https://doi.org/10.37905/jfpj.v2i2.6969

Triandiza, T., & Madduppa, H. (2018). Application of morphological analysis and DNA barcoding to determine the type of porcelain crab (*Pisidia* sp.) originating from Tunda Island, Banten. *Jurnal Perikanan dan Kelautan, 2*(2), 50–57. http://www.ejournalfpikunipa.ac.id

Vermeij, G. J. (1977). Patterns in crab claw size: The geography of crushing. *Systematic Biology, 26*(2), 138–151. https://doi.org/10.1093/sysbio/26.2.138

Yang, B., Zhang, Z., Yang, C. Q., Wang, Y., Orr, M. C., Wang, H., & Zhang, A. B. (2022). Identification of species by combining molecular and morphological data using convolutional neural networks. *Systematic Biology, 71*(3), 690–705. https://doi.org/10.1093/sysbio/syab076

Yılmaz, M., Ozic, C., & Gok, İ. (2012). Principles of nucleic acid separation by agarose gel electrophoresis. In *Gel electrophoresis – Principles and basics* (pp. 33–52). InTechOpen.

Zamroni, A., Boer, M., Butet, N. A., & Zairion, Z. (2024). Dominant species of squid in the waters of Western Kalimantan and Northern Java identified by DNA barcoding method. *Egyptian Journal of Aquatic Research, 50*(1), 121–126. https://doi.org/10.1016/j.ejar.2023.12.007

Biographies of Authors

Putri Nurul Pratiwi Abas, Biology Study Program, Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Gorontalo, Bone Bolango, Gorontalo, 96119 , Indonesia.
- Email: putrinurulabas@gmail.com
- ORCID: N/A
- Web of Science ResearcherID: N/A
- Scopus Author ID: N/A
- Homepage: N/A

Dewi Wahyuni K. Baderan, Biology Study Program, Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Gorontalo, Bone Bolango, Gorontalo, 96119 , Indonesia.
- Email: dewi.baderan@ung.ac.id
- ORCID: 0000-0003-3014-0832
- Web of Science ResearcherID: N/A
- Scopus Author ID: 57202264799
- Homepage: https://fmipa.ung.ac.id/formasi/people/197909142003122003

Regina Valentine Aydalina, Biology Study Program, Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Gorontalo, Bone Bolango, Gorontalo, 96119 , Indonesia.
- Email: aydalinaregina@ung.ac.id
- ORCID: 0000-0002-7256-5591
- Web of Science ResearcherID: N/A
- Scopus Author ID: 58093433200
- Homepage: https://ung.ac.id/formasi/people/199002032019032013

Magfiratul Jannah, Biology Study Program, Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Gorontalo, Bone Bolango, Gorontalo, 96119, Indonesia.
- Email: magfirahtuljannah@ung.ac.id
- ORCID: N/A
- Web of Science ResearcherID: N/A
- Scopus Author ID: N/A
- Homepage: https://ung.ac.id/formasi/people/198906152022032003

Marini Susanti Hamidun, Population and Environmental Studies Program, Postgraduate Program, State University of Gorontalo, Gorontalo, 96128, Indonesia.
- Email: marinish70@ung.ac.id
- ORCID: 0000-0003-3282-4496
- Web of Science ResearcherID: N/A
- Scopus Author ID: 57208315828
- Homepage: https://ung.ac.id/formasi/people/197005042001122001