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First Record of the Ghost Shrimp *Karumballichirus karumba* (Poore and Griffin, 1979) (Decapoda, Callichiridae), Found in the Intertidal Zone of the Madura Strait, Indonesia

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ABSTRACT

The research objective is to describe the morphological characteristics of ghost shrimp found in the Madura Strait, Indonesia, and support the genetic profile through comparison with the NCBI database. Research on ghost shrimp from the waters of the Madura Strait shows similarity to the morphological description of *Karumballichirus karumba* (Poore and Griffin, 1997), which has been reported from Australia, Singapore, Taiwan, Papua New Guinea, and Bangka. Differences between males and females are indicated by the location of the gonopore: in males, it is located on the fifth pereiopod, while in females, it is located on the third pereiopod. The major cheliped of male ghost shrimp is larger than that of females. Another difference is found in the shape of the first pleopod: males have a curved tip, while females have a shoulder on the second segment. Ovigerous females have a total length (TL) of 78.8– 109.3mm, carapace length (CL) of 19– 24.5mm, carapace width (CW) of 9.9– 13.4mm, and body weight of 13.15– 26.7g. The embryo diameter ranges from 0.6– 2.2mm and is divided into two phases: with eyespots and without eyespots. The major cheliped in small individuals has a smoother surface, and there are no teeth on the dactylus. The results of genetic analysis and phylogenetic tree reconstruction show that the ghost shrimp specimen (BIOSUB249 001) has an identity value of 93.57% with *K. karumba* (Accession Number MN237832.1) and is closely related to specimens from Taiwan. *K. karumba* found in the Madura Strait inhabits muddy and rocky sand substrates, as indicated by the presence of fine sediment at the outlet.

INTRODUCTION

The general term “ghost shrimp” usually refers to taxa of the axiidean Callianassidae, Dana, 1852 (Callianideidae Kossmann, 1880 and Ctenochelidae Manning & Felder, 1991). Callianassidae and Ctenochelidae ghost shrimp (Gourrettiidae Sakai, 1999) have soft bodies, pleons longer than the carapace, and are usually found in shallow intertidal and subtidal marine environments, especially in tropical and subtropical waters (**Dworschak, 2005, 2008**). Recent research on ghost shrimp in Indonesia has focused on

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hepatopancreas damage due to heavy metal pollution in the Madura Strait (**Kristiani, 2016**).

In the Indo-Pacific, ghost shrimp are distributed in Australia (Norman River, Karumba, Queensland; type locality of *C. karumba* Poore & Griffin, 1979); Papua New Guinea (estuary near Lea); Taiwan (Changhua County, Shengang and Lugang); Singapore (Lim Chu Kang); Bangka, Indonesia (type locality of *N. kempi* Sakai, 1999); and Bangkok, Thailand (**Dworschak, 2008**). Their distribution on the west coast of North America extends from Mutiny Bay, Alaska, to the mouth of the Tijuana River, San Diego County, California (**MacGinitie, 1934**). **Ricketts and Calvin (1968)** also reported the discovery of specimens along the southern El Estuario de Punto Banda, Baja California Norte, Mexico. The structure of ghost shrimp burrows does not follow a specific pattern and is considered random (**MacGinitie, 1934**).

Morphological characters are traits based on simple Mendelian inheritance, including shape, color, length, and weight (**Suryo, 2008**). Meanwhile, genetic traits are segments of DNA that can show polymorphism among individuals (**Liu, 2007**). Among mitochondrial DNA (mtDNA) markers, 16S and COI have been widely used to determine phylogenetic relationships among shrimp species. Previous studies have concluded that the 16S region has a relatively low evolutionary rate (**Meyer, 1994**), making it particularly useful for species-level differentiation. The 16S gene can therefore also be used to establish evolutionary relationships among shrimp at different taxonomic levels (**Mata, 2009**).

Research on the genetic and molecular analysis of ghost shrimp discovered in the Madura Strait has not yet been conducted. Therefore, this study aims to examine and compare morphological characters and genetic profiles of ghost shrimp found in the Madura Strait, as well as their biological aspects.

MATERIALS AND METHODS

This research was conducted from July 2023 to June 2024. A total of 902 ghost shrimp samples were collected from the intertidal zone of the Madura Strait at latitude −7°09′34″S and longitude 112°47′32″E (Fig. 1). The sampling location was determined based on the first recorded discovery of ghost shrimp in the area. Sample collection was carried out during low tide, as shown in Fig. (2).

Representative samples were collected by examining all visible ghost shrimp burrows using the method described by **Hernaez (2018)**. The entrances to burrows constructed by ghost shrimp are easily identified by their distinctive volcano-shaped mounds on the sediment surface (Fig. 2a).

The collected ghost shrimp specimens were rinsed with seawater, placed in sample bottles, and preserved in 97% ethanol until the DNA analysis stage. The preserved samples were then transported to the Indonesian Biodiversity Laboratory (BIONESIA) for further analysis.

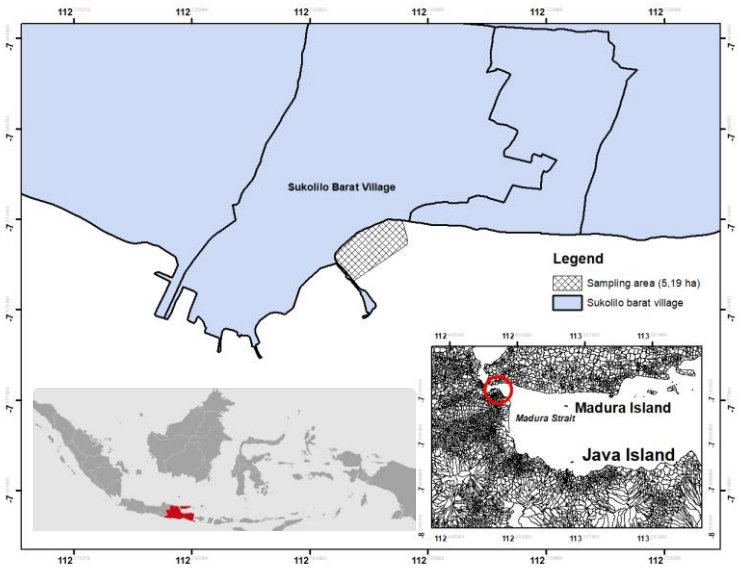

Fig. 1. The white dot is the sampling location for Ghost Shrimp (*Karumballichirus karumba*).

Fig. 2. Ghost shrimp (*Karumballichirus karumba*) sampling process. a) the main burrow of the ghost shrimp is indicated by the presence of light sediment at the mouth of the hole, b) digging 10-15cm (each burrow differs in depth between locations), c) after finding the main burrow and inserting a finger that serves as bait, the shrimp can be caught.

Morphological Identification

Measurement and identification of morphometric characters were conducted based on the references of **Pillai (1954)**, **Biffar (1971)**, **Forbes (1973)**, **Sakai (2005)**, and **Dworschak (2008)**. Species identification followed the descriptions of *Neocallichirus karumba* (Poore & Griffin, 1979) (Figs. 1–6); *Callianassa karumba* (Poore & Griffin, 1979) (Figs. 30 & 31); *Neocallichirus kempi* (**Sakai, 1999**) (Figs. 24a–e); *Karumballichirus karumba* (*Poore et al.*, 2019) (Figs. 13e, 13l, & 13s; Figs. 14b & 14l);

Neocallichirus karumba **(*Poore et al.*, 2019)** (Figs. 14c & 14p); and (**Dworschak & Anker, 2022**) (Fig. 1b).

Molecular Identification

Tissue samples (approximately 10g) were collected, and DNA extraction was performed following the Qiagen protocol. The extracted DNA was then amplified using the Polymerase Chain Reaction (PCR) method according to the BIONESIA laboratory protocol.

The primers used for amplification were 16Sar (5′-CGC CTG TTT ATC AAA AAC AT-3′) and 16Sbr (5′-CCG GTC TGA ACT CAG ATC ACG-3′) (**Palumbi *et al.*, 1991**). The total PCR reaction volume was 26µL, consisting of 2µL of extracted DNA template, 1.25µL of each primer (10 mM concentration), 9µL of ddH_2O, and 12.5µL of ready mix.

Amplification was performed using an Applied Biosystems™ 2720 Thermal Cycler. The PCR thermal profile consisted of an initial denaturation at 94°C for 3 minutes; followed by 38 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 60 seconds; with a final extension at 72°C for 2 minutes.

PCR products were visualized on 1% agarose gel stained with GelRed®. Samples showing positive DNA bands were subsequently sequenced using the Sanger dideoxy method at PT Genetika Science, Jakarta.

Data Analysis

Sequencing results in the form of AB1 files were analyzed using computer software. The obtained sequences were edited and aligned using the ClustalW method in MEGA XI software. Each nucleotide base was manually checked to ensure data quality. Samples with poor sequence quality were re-amplified and re-sequenced.

The sequences were compared with reference data in the GenBank database (NCBI) using the Basic Local Alignment Search Tool (BLAST). Similarity and identity values were recorded for each sample.

In addition to BLAST analysis, phylogenetic reconstruction was performed to determine the evolutionary relationships among samples and to confirm species-level identification. The phylogenetic tree was constructed using the Neighbor-Joining (NJ) method with 1000 bootstrap replications in MEGA XI software and further visualized using iTOL.

RESULTS AND DISCUSSION

Morphological Identification

Based on the results of morphological identification, the ghost shrimp found in the Madura Strait were identified as *Karumballichirus karumba* (Poore & Griffin, 1979), according to the World Register of Marine Species (WoRMS). The morphological characteristics of this species are presented in Figs. (3–6).

Order Decapoda Latreille, 1802
Suborder Pleocyemata Burkenroad, 1963
Family Callichiridae Manning & Felder, 1991
Genus *Karumballichirus Poore*, Dworschak, Robles, Mantelatto & Felder, 2019
Species *Karumballichirus karumba*, Poore & Griffin, 1979

Diagnostic features

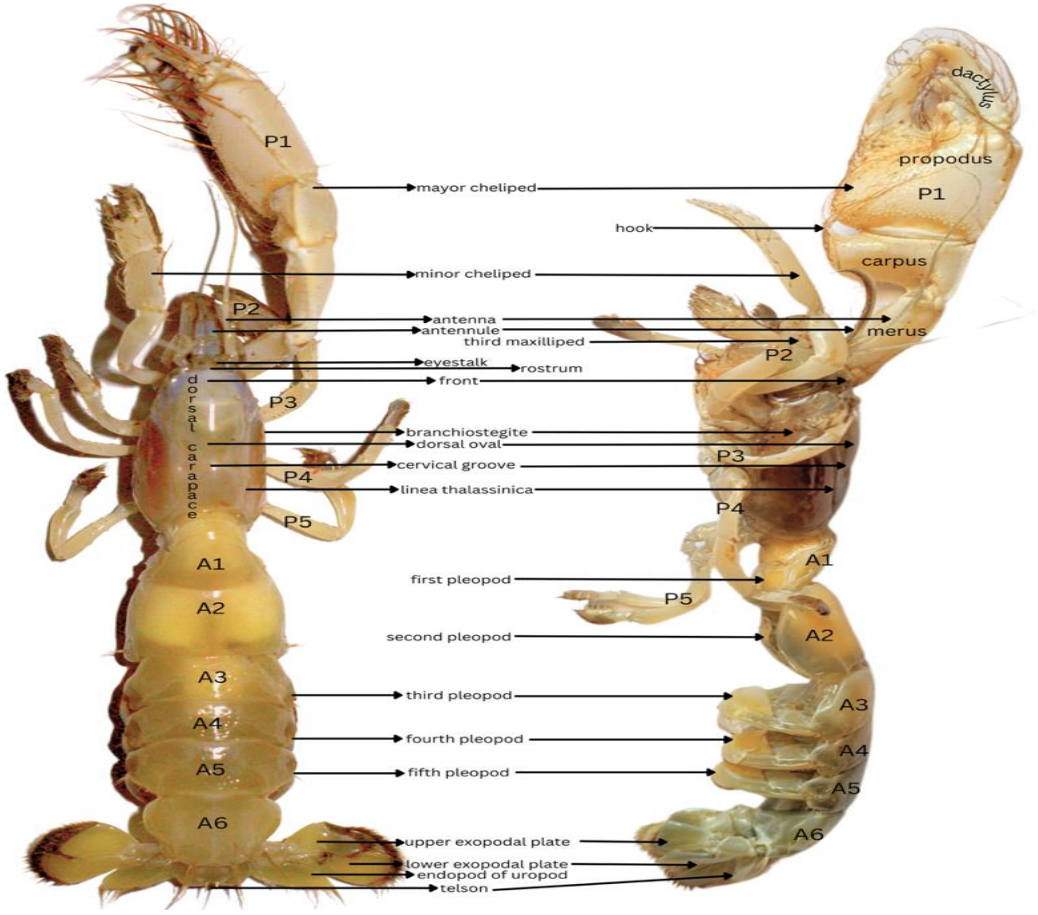

Fig. 3. General morphology of *Karumballichirus karumba* found in the intertidal zone of Madura Strait, dorsal and side views. Abdominal parts are indicated by A1, A2, and etc; walking legs/pereopods are indicated by P1, P2, etc.

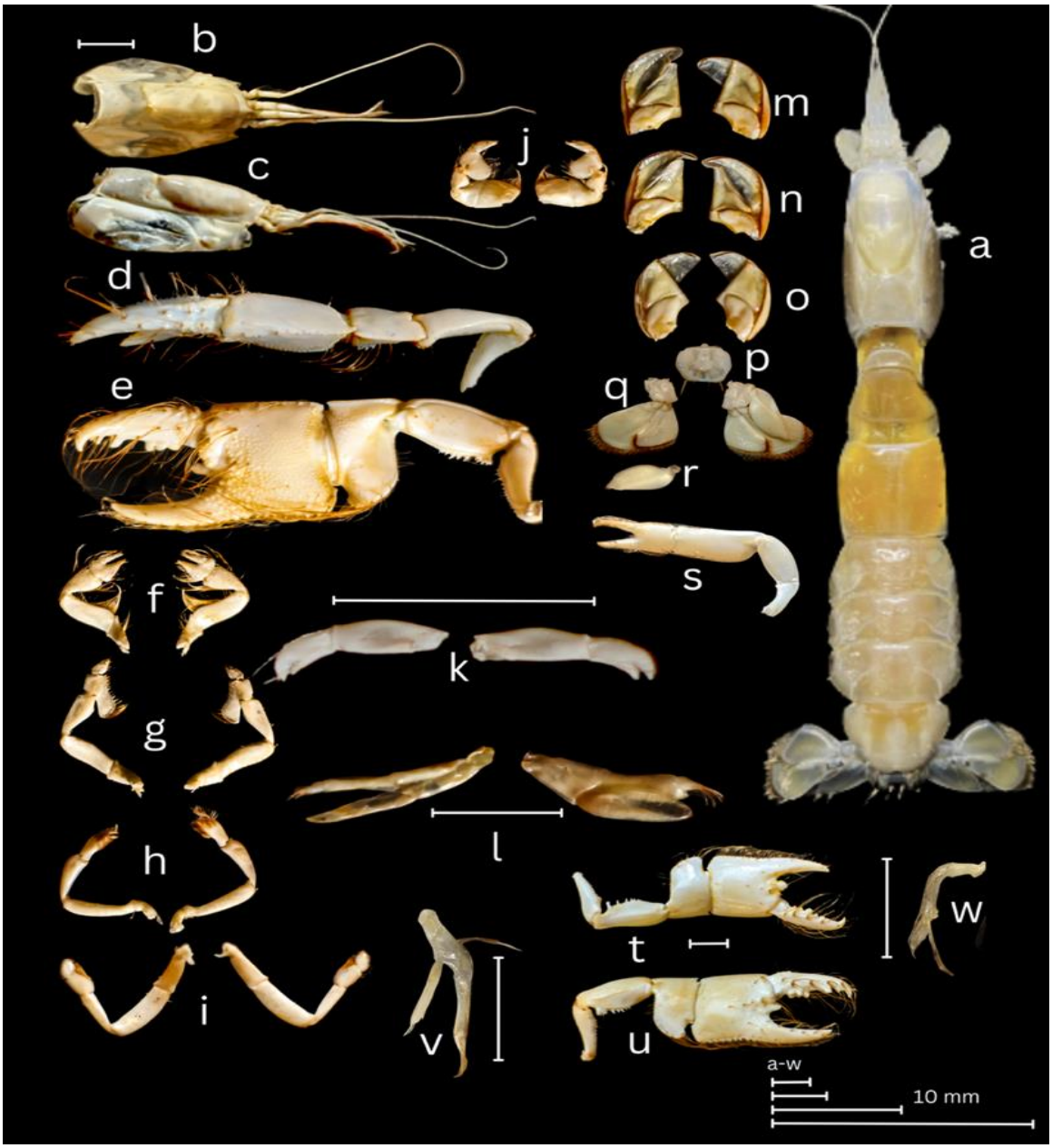

Fig. 4. *Karumballichirus karumba* Madura Strait. a. Body dorsal view; b. Carapace dorsal view; c. Carapace lateral view; d. Cheliped mayor dorsal view; e. Cheliped mayor lateral view; f. Second pereiopod; g. Third pereiopod; h. Fourth pereipod; i. Fifth pereiopod; j. Third maxilliped; k. First pleopod in male; l. Second biramous pleopod in male; m-o. Third to fifth pleopods; p. Telson; q. Lower & upper exopodal; r. Endopod of uropod; s. Cheliped minor; t. Left cheliped mayor; u. Right cheliped mayor; v. Second biramous pleopod in female; w. First pleopod in female.

Male-Female Difference

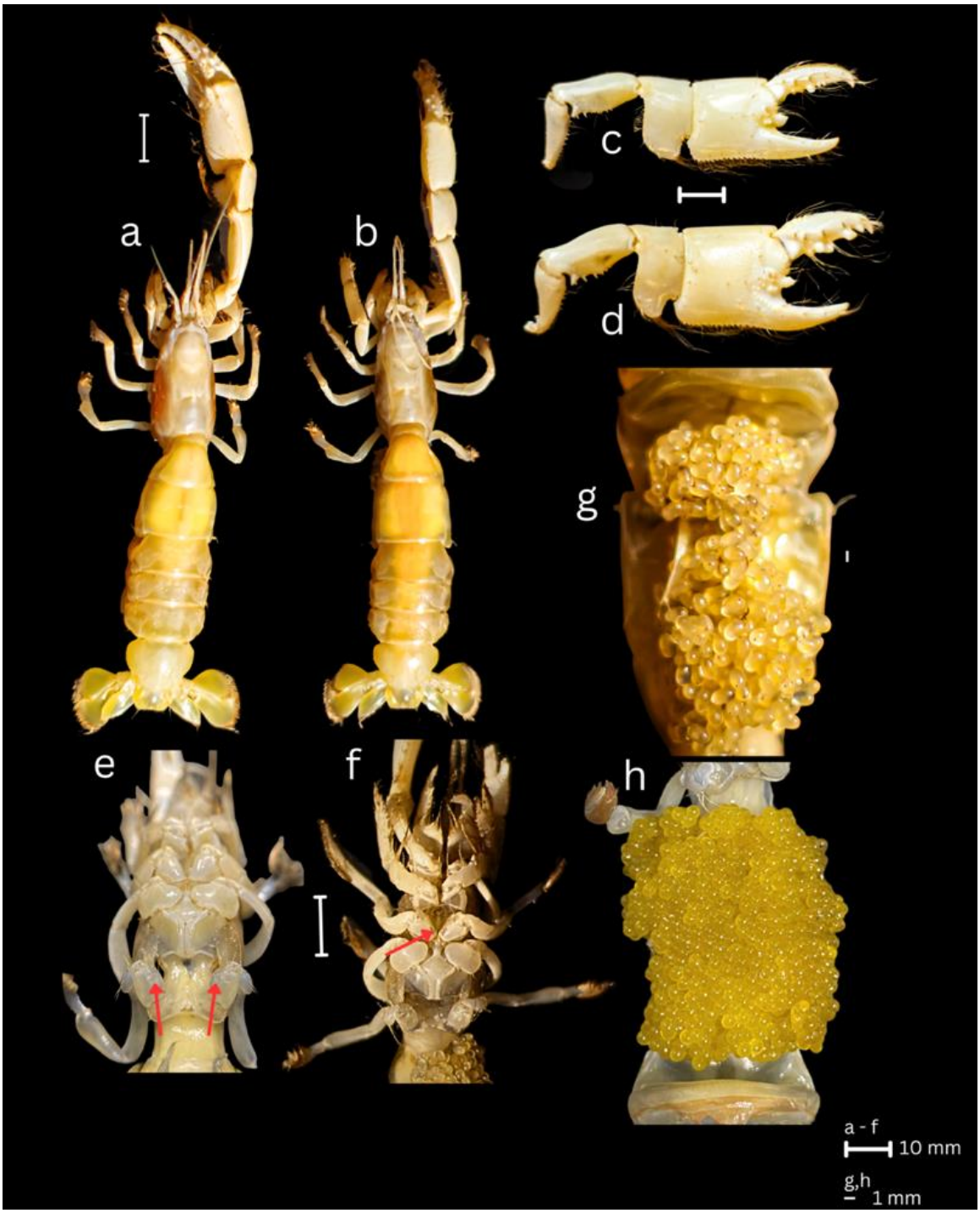

Fig. 5. Schematic representation of male-female differences in dimorphism and embryo diameter in the *Karumballichirus karumba.* Red arrows indicate gonopore location.

The difference between male and female *Karumballichirus karumba* ghost shrimp is indicated by differences in the size and shape of the major cheliped, in addition to the slender shape of the female propodus, as well as differences in the number and shape of

teeth on the propodus and dactylus (Fig. 5c, d). Another difference lies in the presence of the gonopore where the male is located in the fifth pereiopod (Fig. 5e) and the female is located in the third pereiopod (Fig. 5f).

Morphological Differences Based on Sample Size

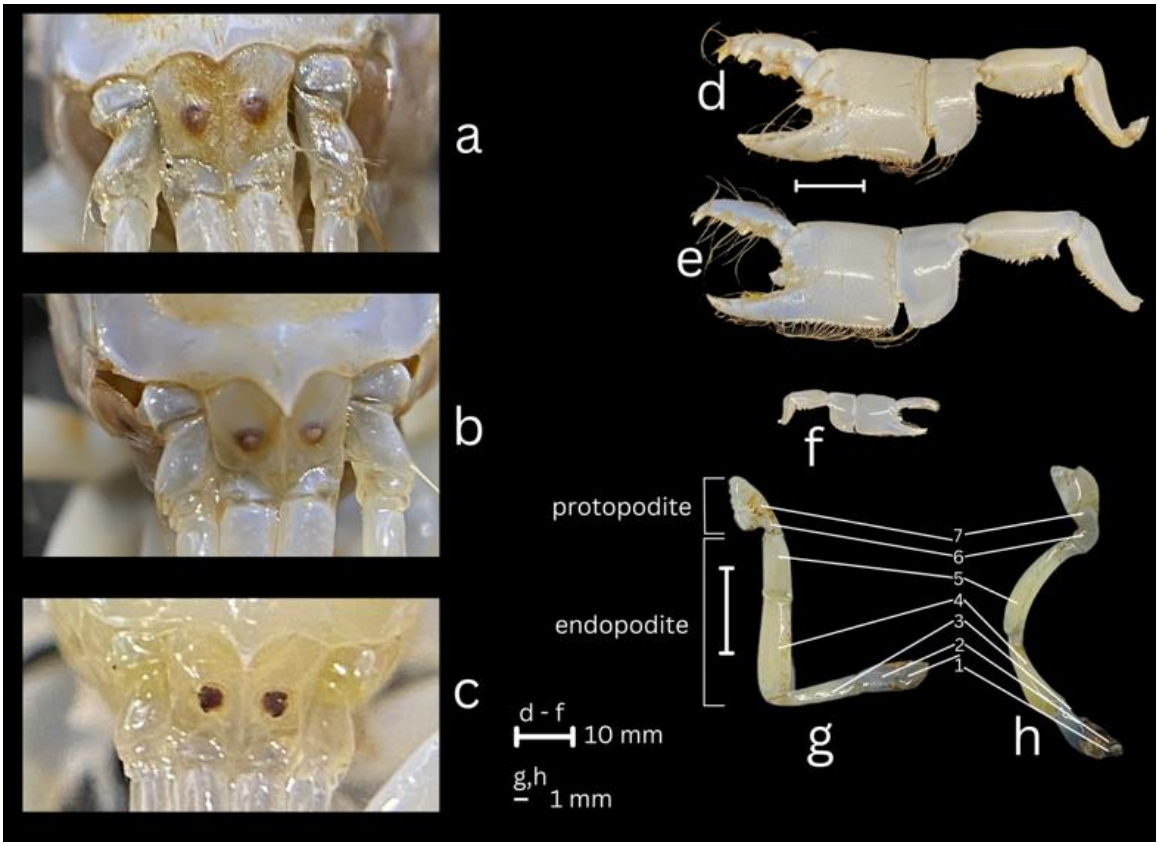

Fig. 6. Morphological differences of ghost shrimp *Karumballichirus karumba* based on sample size and parts of fourth periopod (h) and fifth periopod (g) 1. Dactylus, 2. Propodus, 3. Carpus, 4. Merus, 5. Ischium, 6. Basium, 7. Coxa.

Ghost shrimp (*Karumballichirus karumba*) samples examined included the following measurements:

- **Fig. 6a and d**: TL (total length) 100.2 mm; CL (carapace length) 22.4 mm; CW (carapace width) 15.1 mm.
- **Fig. 6b and e**: TL 76.1 mm; CL 17.7 mm; CW 11.1 mm.
- **Fig. 6c and f**: TL 44.1 mm; CL 11.9 mm; CW 6.1 mm.

Several morphological characters appear to be related to growth. In small specimens (Fig. 6c), the eyestalks have solid black corneas, likely due to the more transparent body of smaller individuals. In medium and large specimens (Fig. 6a, b), the cornea appears more faded (gray) and slightly bulging. In all specimens, regardless of size, the eyestalks extend beyond the length of the rostrum.

In small specimens, the major cheliped surface is generally smooth, although the propodus near the dactylus bears tubercles, and the dactylus lacks teeth (Fig. 6f). In medium-sized specimens (Fig. 6e), the surface shows more tubercles, and the dactylus has fewer teeth compared to large specimens. Large specimens (Figs. 6d, d, 4e, u, and 3) exhibit a rough surface with tubercles present on nearly all segments, including the dactylus, propodus, carpus, and merus.

Small specimens generally show no noticeable differences in size or shape between males and females. However, in larger specimens, sexual dimorphism is evident. Male individuals possess a longer and taller propodus on the major cheliped compared to females of similar size (Fig. 5c and d). The ischium and merus surfaces are tuberculated, with low tubercles near the inferior margin. The propodus bears numerous tubercles, and the dactylus has several proximal tubercles (Figs. 4d, e, t, u, 5c, d). Overall, the merus, carpus, and propodus are tuberculated, with variation in density and pattern among individuals.

Specimens size

The specimens observed in the study had a size range of TL; 43 - 117 mm, CL; 11.4 - 36 mm, CW; 5.5 - 25.5 mm. Reports from the literature range from tl 15 (10) to tl 131/cl 30 (22).

Fig. 7. Size variation of ghost shrimp Karumballichirus karumba caught in Madura Strait

Sample Measurement

The examined specimen of ghost shrimp (*Karumballichirus karumba*) had a total length (TL) of 109.3mm; carapace length (CL) of 24.5mm; carapace width (CW) of 13.4mm; body weight of 26.7g; telson length of 8.2mm; and telson width of 9.3mm. The uropod endopod measured 11.9mm in length and 5mm in width.

Measurements of the major cheliped were as follows: ischium length 18.2mm and width 7.2mm; merus length 20.1mm and width 10.1mm; carpus length 12.7mm and width 20.9mm; propodus length 20.1mm and width 22mm; and dactylus length 20.4mm.

Embryonic Development of *Karumballichirus karumba*

Ovigerous females had TL ranging from 78.8– 109.3mm, CL from 19– 24.5mm, CW from 9.9– 13.4mm, and body weight from 13.15– 26.7g.

The stages of embryonic development were observed visually based on the external appearance of the eggs. According to Hailstone and Stephenson, embryonic development is divided into five stages: white or transparent (stage 1), yellow (stage 2), yellow/orange (stage 3), orange (stage 4), and dark red (stage 5).

However, further research by Hailstone and Stephenson indicated that the yellow and orange stages already exhibit eyespots. Therefore, classification based on the presence or absence of eyespots (eyed and eyeless embryos) is considered more reliable than classification based solely on color changes.

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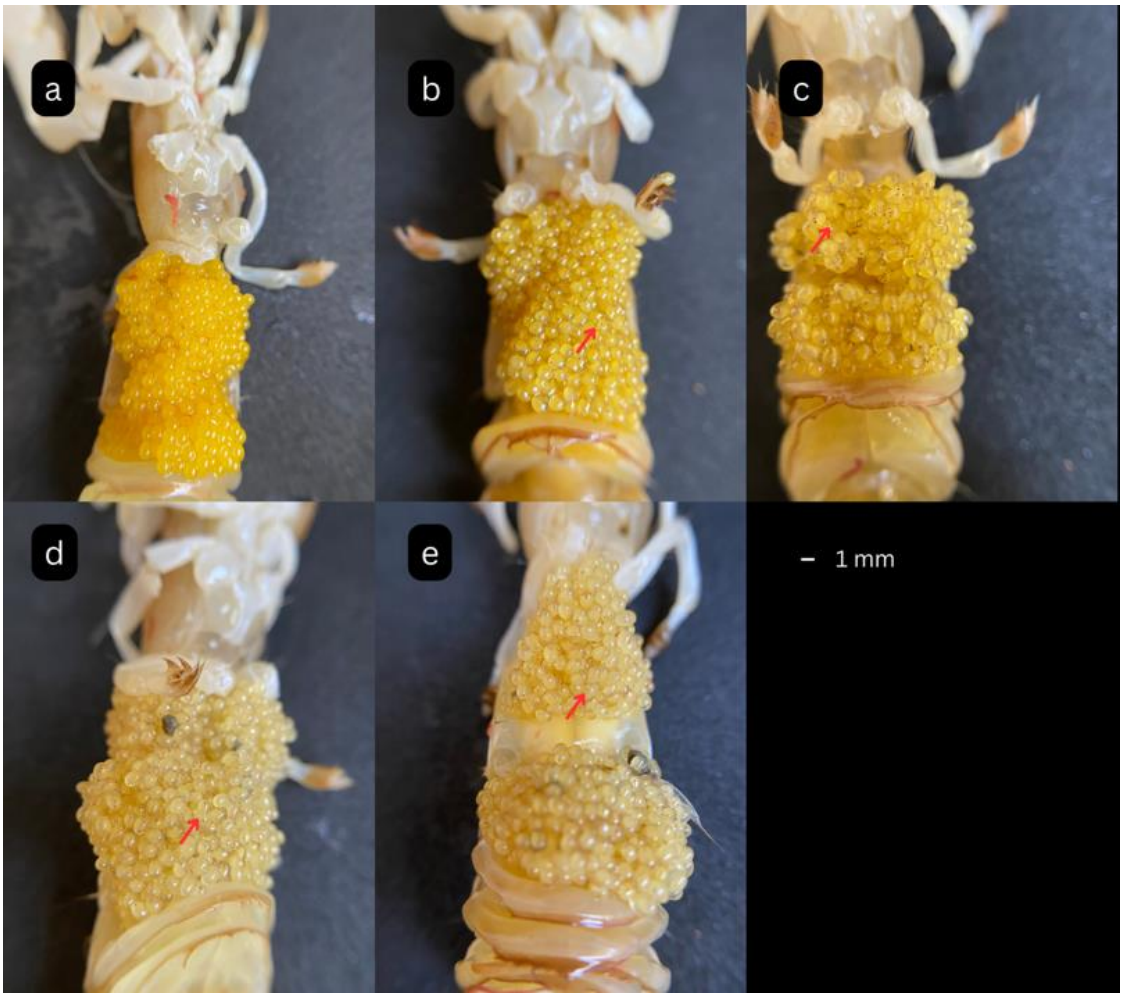

Fig. 8. Presence of eye spots on ghost shrimp *Karumballichirus karumba* samples found in the Madura Strait. Red arrows indicate eye spots on embryos.

Observations showed that eye spots appeared on yellow to cloudy white embryos (Fig. 8b, c, d, e) while bright yellow (Fig. 8a) and transparent yellow (Fig. 5h) embryos did not have eye spots. The results of the female ghost shrimp sample obtained embryo size (Fig. 5g and h) that ranged from 0,6-2,2mm, with the most diameter size on 1,2-1,3mm (Fig. 9).

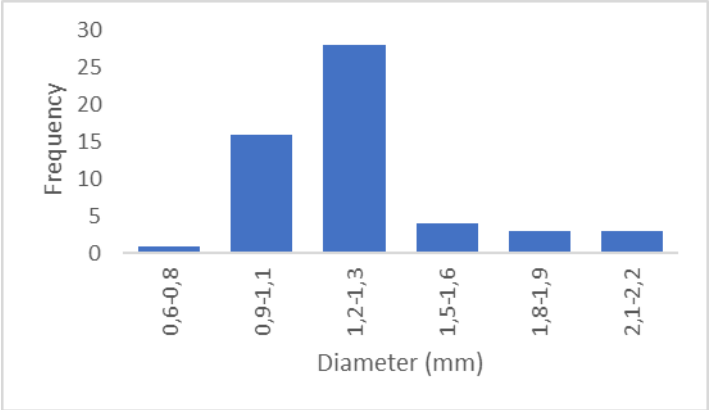

Fig. 9. Frequency distribution of egg diameters *K. karumba*.

Egg size is an important aspect of shrimp reproduction, as it is related to the duration of embryogenesis and the size of larvae at hatching. Several studies on ghost shrimp and mud lobster have demonstrated a clear relationship between egg size and the type of larval development (**Forbes, 1973; Steele, 1975; Felder, 1994**).

Habitat

Karumballichirus karumba is found on muddy and rocky sand substrates in the Madura Strait. The presence of ghost shrimp burrows is indicated by light sediment mounds on the surface. The burrow structure widens at a depth of approximately 10–15cm below the surface. The burrows are irregular in shape and have diameters ranging from approximately ±1 cm to ±3 cm.

Based on shrimp sampling results, burrow diameter appears to correspond to the size of the ghost shrimp inhabiting it; large-diameter burrows are unlikely to be occupied by small individuals.

Ghost shrimp are found in the intertidal zone at distances ranging from approximately 1– 75m from the shoreline of the Madura Strait. Beyond 75m, ghost shrimp are rarely found or may be absent. Juvenile to adult individuals (TL 70– 109mm) were primarily found at a distance of around 50m from the shoreline.

Fig. 10. Ghost shrimp *Karumballichirus karumba* sampling sites in the Madura Strait

In Singapore, specimens were collected from hard sand near the Lim Chu Kang mangrove system. Burrow openings are characterized by small mounds or simple pits, often surrounded by lighter sediments. In Taiwan, specimens were found in small numbers on muddy sandy tidal flats dominated by the upogebiid *Austinogebia edulis* (**Ngoc-Ho & Chan, 1992**). In India, this species is found in soft, silty sand with salinities between 15 and 36ppm (**Kemp, 1915**). **Daniel (1981)** reported this species as a pest in saline areas of India.

Remarks

The male dactylus of specimens from Papua New Guinea (*N. karumba*; **Dworschak, 2008**: 82, pl. 8, Fig. 6b–c) lacks interior teeth. In contrast, specimens from the Madura Strait show the presence of teeth on the male dactylus beginning at TL 50mm (Fig. 7).

The lower third pereiopod is not flattened in specimens from Singapore and Papua New Guinea (**Dworschak, 2008**: 78–79, pl. 4–5, Figs. 2r & 3n; no image available for Bangka specimens). In contrast, it tends to be flattened in specimens from Australia and Taiwan (**Dworschak, 2008**: 77–81, pl. 3, figs. 1; pl. 6–7, Figs. 5–6), as well as in Madura specimens (Fig. 4g). This variation may be influenced by the burrowing behavior of ghost shrimp, as adaptation to digging could reduce damage to the lower portion of the third pereiopod and potentially affect identification results.

The telson is pointed at the external margin in specimens from Singapore and Bangka (**Sakai, 1999**: 102, Fig. 24b; **Dworschak, 2008**: 79, pl. 5, Fig. 3a), whereas Madura specimens exhibit a more quadrate (box-shaped) telson (Figs. 3 and 4p). No telson images are available for the Taiwan and Papua New Guinea specimens.

Morphological comparisons between specimens from the Madura Strait and previously described material from Australia, Singapore, Taiwan, Papua New Guinea, and Bangka reveal only minor differences within *Karumballichirus karumba*. Madura specimens appear morphologically similar to Australian specimens in telson shape but differ from Singaporean and Bangka specimens.

Pillai (1954) reported adult-sized female specimens based on reproductive characteristics. Later, **Sakai (1999)** stated that female specimens from Bangka were adults, as evidenced by remnants of moulted eggs on pleopods 1–2; these females had a major chela reaching 33mm in length. The largest female specimen found in the Madura Strait had a chela length of 35mm (TL 114.6mm) and was ovigerous at TL 80.4mm. These similarities suggest that Bangka and Madura specimens may belong to the same species, particularly based on adult chela size and the size at which females become ovigerous. However, further studies integrating detailed morphological comparison and molecular data are required to confirm this assumption.

Dworschak (2008) considered specimens from Taiwan and Singapore to represent the same species and regarded *Neocallichirus kempi* **Sakai (1999)** as a junior synonym of *Neocallichirus karumba* (Poore & Griffin, 1979). However, ***Robles et al.***

(2020) suggested that *Karumballichirus karumba* specimens from Taiwan and Singapore may represent two distinct species.

Distribution

Early records from India include Nalbano Island and Barhampur Island, Lake Chilka, and areas near Madras (**Kemp, 1915**); Kayamkulam Lake, Central Travancore (**Pillai, 1954**); and Voyalur (Chingleput District, Tamil Nadu) and Manginapudi (Krishna District, Andhra Pradesh) (**Daniel, 1981**).

According to **Sakai (1999)**, *Neocallichirus kempi* was reported from N. Parur, Travancore, India, and Bangka, Indonesia. Subsequently, **Dworschak (2008)** documented the distribution of *Neocallichirus karumba* (under the name *C. karumba* [Poore & Griffin, 1979]) in Australia (Norman River, Karumba, Queensland), Papua New Guinea (estuary near Lea), Taiwan (Changhua, Shengang, and Lugang districts), Singapore (Lim Chu Kang), Bangkok (Thailand), and India.

In the Madura Strait, *Karumballichirus karumba* was found along approximately 2.6km of the intertidal zone (field survey data), between coordinates 7°09′58″S, 112°46′39″E and 7°09′32″S, 112°47′46″E.

Genetic Identification

Molecular data for *Karumballichirus* in the NCBI database remain limited. **Dworschak (2008)** recognized *Neocallichirus kempi* **Sakai (1999)** as a junior synonym of *Neocallichirus karumba* (Poore & Griffin, 1979), treating them as a single species. In contrast, **Robles *et al.* (2020)** suggested that specimens identified as *Karumballichirus karumba* from Singapore and Thailand (Thailandcallichirus) may represent two distinct species.

According to the World Register of Marine Species (WoRMS), the name *Neocallichirus karumba* is currently listed as "unaccepted > superseded combination" (AphiaID: 477736), with the accepted name being *Karumballichirus karumba*.

DNA analysis of the examined samples produced sequences with an average length of 530 base pairs (bp) (Table 1). Nucleotide similarity analysis and phylogenetic reconstruction against the GenBank database yielded the results presented in Table (1) and Fig. (11).

Table 1. BLAST barcoding results of samples using primers 16Sar/16Sbr

Sample Code	Species	Length (bp)	Marker Gene	Accession Number	Query Cover (%)	Identity (%)
BIOSUB249.001	*Karumballichirus karumba*	530	16S	MN237832.1	90	93.57

Table 2. Nucleotide sequence of each sample

Sample Code	Sequence DNA result
BIOSUB249.001	GCCGGTGTAAACTCAGATGACGTCGCCTGGTTTTAC AAAAACATGTCTGCTTGACAAATTAAAAGCAGTCTG GCCTGCCCACTGATTTTTTTTTAAAGGCCGCGGTATC CTGACCGTGCGAAGGTAGCATAATAATTAGTCTTTT AATTGAAGACTGGAATGAAGGGTCGGACAAAAAGC AAGCTGTCTCAGCTTCAACTATTGAAATTAACTTTT AAGTGAAAAGGCTTAAATAAACTTAAAGGACGATA AGACCCTATAAAACTTTATACTTAATGTTATAATCT CCTATTTATTTATATAATAGTTGCTCATAACTTTATG TATTACGCTGGGGCGGCGAAAGCAAAAAAATAACT GCTTTTTAATAAAAACAATTATATTTGAACATAATG ATCCTTTAACCTAGATTTCAAGAAAAAGTTACTTTA GGGATAACAGCGTTATTTTTCTTTAGAGTCCACATC GACAGAAAAGTTTGCGACCTCGATGTTGAATTAAAC TATCCTTTCAGTGCAGCAGCTGCAAAG

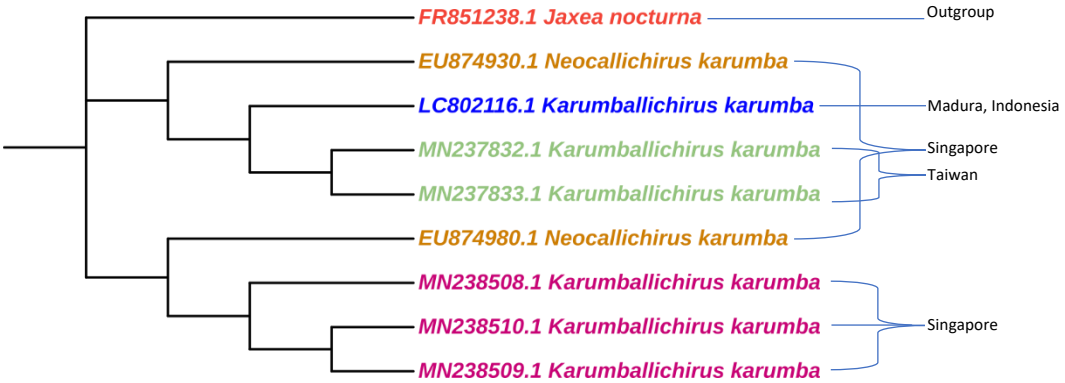

Fig. 11. Phylogenetic results of samples using 16Sar/16Sbr primers

The phylogenetic tree (Fig. 11) shows that the ghost shrimp specimen BIOSUB249 001 has an identity value of 93.57% (Table 1) with *Karumballichirus karumba* (GenBank Accession Number MN237832.1) and forms a monophyletic group with *Neocallichirus karumba*. This value is slightly below the commonly reported intraspecific identity threshold for the 16S gene in Decapoda (≥95%).

The observed genetic divergence may reflect intraspecific variation among local populations of *K. karumba*, potentially resulting from geographic isolation or the limited availability of comparative reference sequences in public databases. Alternatively, the relatively low identity value may indicate the presence of cryptic diversity or even the possibility of an undescribed species within the *Karumballichirus* complex.

Phylogenetic reconstruction based on 16S rRNA sequences revealed generally low to moderate bootstrap support at several internal nodes, indicating limited resolution of deeper branching relationships. Such low nodal support is common in 16S-based analyses

of callianassid taxa and likely reflects the limited phylogenetic signal of this mitochondrial marker for resolving older divergences.

Despite these limitations, BIOSUB249 (LC802116.1) consistently clustered within the *Karumballichirus karumba* clade, demonstrating close genetic affinity with previously published sequences. Comparative phylogenetic analysis including specimens from Singapore, Taiwan, and Madura indicates that the Madura specimen is more closely related to specimens from Taiwan than to those from Singapore.

CONCLUSION

The results showed that ghost shrimp found in Madura Strait has morphological similarities with *Karumballichirus karumba* Poore & Griffin, 1979. The results of genetic testing on the NCBI database showed a percent identity of 93.57%. The construction of a phylogenetic tree gave the result that *K. karumba* found in the Madura strait is closely related to samples from Taiwan. The DNA sequence was deposited into GenBank with the accession number LC802116.1.

Ethical Clearance

The authors state that sampling of the ghost shrimp species studied was done with consideration of ecological impacts, including the number of samples and the condition of the sampled species. Species that have been recorded under normal conditions are returned to the habitat.

Declaration of Competing Interest

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

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