Arthropoda Selecta 34(1): 41–50

First record of *Clausidium sarii* (Copepoda: Clausidiidae) from the coastal waters of Pakistan, with the first description of the male and a new host

Первая находка *Clausidium sarii* (Copepoda: Clausidiidae) из прибрежных водах Пакистана, с первым описанием самца и нового хозяина

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KEY WORDS: diversity, association, parasitism, symbiosis, morpho-taxonomy, Indo-Pacific.

КЛЮЧЕВЫЕ СЛОВА: разнообразие, ассоциация, паразитизм, симбиоз, морфотаксономия, Индо-Тихоокеанский регион.

ABSTRACT. The article represents a new record and host association of the symbiotic copepod *Clausidium sarii* Sepahvand et Kihara, 2018 (Copepoda: Clausidiidae) with the burrowing axiid shrimp *Corallianassa martensi* (Miers, 1884) (Decapoda: Axiidea: Callichiridae), which were collected from the intertidal zone of the coast of Buleji, Karachi, Pakistan. Morphological description of a male of this species is also presented for the first time. This is the first discovery of this copepod species and host association in the coastal waters of Pakistan, and the second record of the species in the northern part of the Arabian Sea since the original description, which was based on a single holotype female. Our study also shows that the species of the genus *Clausidium* may not always be strictly specific to a single host, and can instead be found on several different species within the same taxonomic group (genus, family).

How to cite this paper: Ahmed Q., Mohammad Ali Q., Marin I.N., Mubarak S., Baloch A. 2025. First record of *Clausidium sarii* (Copepoda: Clausidiidae) from the coastal waters of Pakistan, with the first description of the male and a new host // Arthropoda Selecta. Vol.34. No.1. P.41–50. doi: 10.15298/arthsel.34.1.03

РЕЗЮМЕ. В статье представлена новая находка и симбиотическая ассоциация паразитической копеподы *Clausidium sarii* Sepahvand et Kihara, 2018 (Copepoda: Clausidiidae) с роющей креветкой-аксиидой *Corallianassa martensi* (Miers, 1884) (Decapoda: Axiidea: Callichiridae), собранной в приливной зоне побережья Буледжи, Карачи, Пакистан. Впервые представлено морфологическое описание самца этого вида. Находка представляет собой первое обнаружение этого вида копепод и ассоциации в прибрежных водах Пакистана, а также только вторую находку вида в северной части Аравийского моря с момента первоописания, основанного на единственной самке (голотипе). Наше исследование также показывает, что виды рода *Clausidium* не всегда строго специфичны для единственного хозяина, и могут обитать на нескольких хозяевах из одной таксономической группы (рода, семейства).

Introduction

There is scarce information available on the biology of marine parasitic copepods, their ecology and associations with the ghost shrimps (Decapoda: Axiidea). According to Huys & Boxshall [1991], almost half of all known species of copepods have lived in close associations with other phyla, at least since the lower Cretaceous period. The burrows as well as the body of ghost shrimps are usually used by symbiotic copepods for living [Kinoshita *et al.*, 2010; Kihara, Rocha, 2013; Itoh, Nishida, 2013; Marin, Antokhina, 2020; Sepahvand *et al.*, 2020]. However, only a few associations between copepods of ghost shrimps have been described and recorded worldwide [Pillai, 1959; Corset, Strasser, 2003; Kihara, Rocha, 2013; Sepahvand *et al*., 2017a, b, 2018, 2019]. Many researchers suggested these relationships as symbiotic commensalism [Corset, Strasser, 2003; Kihara, Rocha, 2013] or parasitism [Wilson, 1935, 1937; Pearse, 1947; Humes, 1949; Pillai, 1959; Conradi *et al.*, 2012].

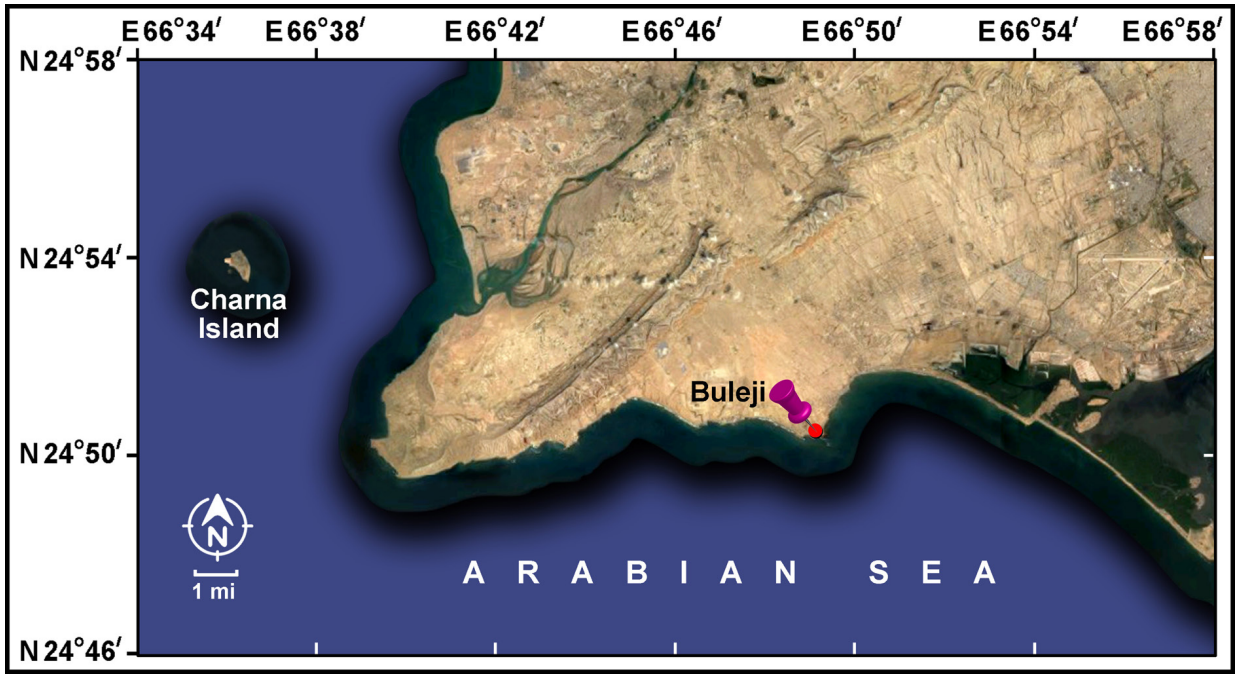

Fig. 1. The location of the sampling area at Buleji coast (Pakistan) of the Northern Arabian Sea.
Рис. 1. Местоположение района отбора проб на побережье Белиджи (Пакистан) в Северной части Аравийского моря.

The genus *Clausidium* Kossman, 1874 (Copepoda: Cyclopoida: Clausidiidae) includes the species usually associated with the mud burrowing shrimps of the families Callianassidae, Callichiridae and Upogebiidae (Crustacea: Decapoda) [Boxshall, Halsey, 2004; Kihara, Rocha, 2013]. Moreover, representatives of the genus *Clausidium* are recorded rarely because of the cryptic lifestyle of their burrowing hosts, as well as the hard sampling. Currently 15 species of *Clausidium* are presented in the WoRMS database [Walter, Boxshall, 2024]. It is hypothesized that each species shows a preference for a specific host [Sepahvand *et al*., 2019], and it can be assumed that the diversity of this genus is much higher.

The diversity of Axiidea in the coastal waters of the Indian Ocean comprises 19 species belonging to 16 genera and 5 families [Vadher *et al.,* 2021]. According to Sepahvand *et al*. [2013], 2 of 11 burrowing shrimp species, recorded from the littoral zone of Iran, are associated symbiotic or parasitic copepods. Among them, the ghost shrimp *Corallianassa martensi* (Miers, 1884) (Axiidea: Callichiridae) was previously also recorded by Tirmizi [1974] (as *Callianassa martensi*) from West Pakistan, northern part of the Arabian Sea, while detailed information related to a specific sampling area of this species is not available, and this species has not been found in coastal area of Pakistan since this discovery 50 years ago.

Corallianassa martensi (Miers, 1884) is a representative of the infraorder Axiidea (de Saint Laurent, 1979) (Crustacea: Decapoda), also known as ghost shrimps, mud shrimps or burrowing shrimps [Dworschak *et al*., 2012]. They are closely related to Anomura and Brachyura (crabs) than to Caridea (true shrimp), and currently comprising of 11 valid families (after WoRMS [2020a]), mostly living in shallow intertidal and subtidal waters, but reaching the abyssal depths [Marin, 2022]. These burrowing animals, usually build complex of deep burrows, are an important benthic component of sandy/muddy intertidal and shallow subtidal habitats (e.g., Golubinskay *et al.* [2016]). The sediment filtration and mixing the substratum as a result of their burrowing activity signify them as one of the ecologically important members of benthic intertidal and shallow subtidal infaunal communities. Their burrows are occupied by a variety of different smaller marine organisms, including copepods [Dworschak *et al*., 2012; Dworschak, 2015]. At the same time, these cryptic animals are still poorly studied, but are extremely interesting to scientists in terms of their behavior, ecology, and classification and morphologically adapted to a burrowing life style.

The present study provides information on the presence of symbiotic copepod *Clausidium sarii* Sepahvand et Kihara, 2018 found in association with the ghost shrimp *Corallianassa martensi* (Miers, 1884) along the coastal line of Pakistan, with the taxonomic description on both animals. The male of *C. sarii* is described for the first time during present study. Previously, species was recorded by a single holotype ♀ collected from the large chelipeds of the ghost shrimp *Neocallichirus natalensis* (Barnard, 1947) (Axiidea: Callichiridae) [Sepahvand *et al.*, 2018].

Materials and Methods

Sampling was carried out during the low tide on 23 January 2023 (Tide –0.20 m; 5:47 pm) at Buleji, 24°50′20.41″ N 66°49′24.15″ E, along the Karachi coast, Pakistan, Northern Arabian Sea (Fig. 1). A single specimen (♂) of the burrowing axiid shrimp *Corallianassa martensi* (Miers, 1884) (Decapoda: Axiidea: Callichiridae) was found hidden under a rock and was

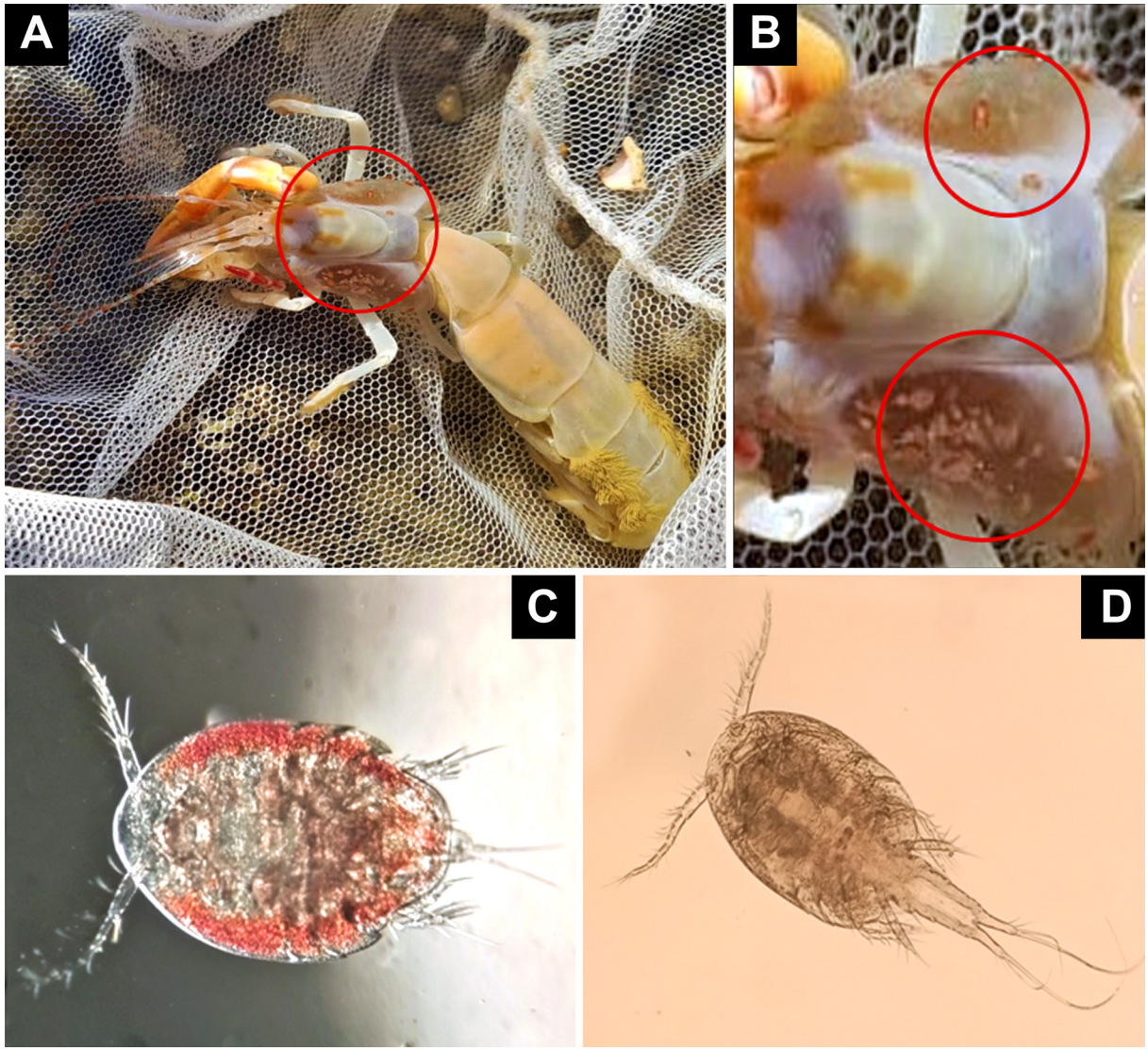

Fig. 2. A — photo of alive specimen of axiid shrimp *Corallianassa martensi* after sampling; B — parasitic copepods *Clausidium sarii* inside the gill chamber of axiid shrimp *Corallianassa martensi*; C — *Clausidium sarii*, ♀; D — *C. sarii*, ♂.

Рис. 2. A — фотография живого экземпляра креветки-аксииды *Corallianassa martensi* после отбора проб; B — паразитические копеподы *Clausidium sarii* внутри жаберной полости креветки-аксииды *Corallianassa martensi*; C — *Clausidium sarii*, ♀; D — *C. sarii*, ♂.

collected by using a hand net (Fig. 2A). Parasitic copepods were discovered in the brachial chamber of a collected specimen of a ghost shrimp (Fig. 2B). Ghost shrimp was relaxed with a 70% ethanol solution added to seawater, and thus symbiotic copepods were separated from their host. A total of 123 specimens of *Clausidium sarii* were found from the brachial chamber of ghost shrimp. Then, the host shrimp and symbiotic copepods were fixed in 90% ethanol for further study.

In the laboratory, the specimens were mounted on slides with glycerin and dissected under upright Nikon Labophot 2 microscope. Microphotography of the studied specimens were also performed with digital camera; morphological drawings were made with the help of drawing tube (Nikon drawing tube 1.25X). Specimens were identified to the lowest possible taxonomic level based on morphological features. Only primary synonyms are given.

All collected specimens were deposited in the repository of the Marine Reference Collection and Resource Centre, University of Karachi, Pakistan (MRCC).

ABBREVIATIONS: P — pereopod; Pp — pereopods.

Results

Class Malacostraca Latreille, 1802
Order Decapoda Latreille, 1802
Family Callichiridae Manning et Felder, 1991
Genus *Corallianassa* Manning, 1987
***Corallianassa martensi* Miers, 1884**
Figs 2A, B, 3.

MATERIAL EXAMINED. ♂, Cat no. MRCC-UOK-THAL-02, Northern Arabian Sea, Pakistan, along the Karachi coast, Buleji, 24°50′20.41″ N 66°49′24.15″ E, soft bottom habitats, collected with a hand net under rock boulder, 23 January 2023.

BRIEF DESCRIPTION. Body long with yellowish translucent. Carapace whitish with tinge of yellowish on rostrum to post-rostral area and transverse band of similar color medially on

Fig. 3. Axiid shrimp *Corallianassa martensi,* ♂: A — dorsal view; B — ventral view C — carapace; D — rostrum; E — antenna; F — larger cheliped II; G — pleomeres III–V; H — telson and uropods; I — telson; H — uropods.

Рис. 3. Креветка-аксиида *Corallianassa martensi*, ♂: A — вид сверху; B — вид снизу; C — панцирь; D — рострум; E — антенна; F — большая клешня (халипеда) II; G — плеомеры III–V; H — тельсон и уроподы; I — тельсон; H — уроподы.

dorsal oval; lateral spines are stout, well developed and articulate. Rostrum pointed at tip and extends beyond midlength of eyes. Eyes stout, not reaching distal margin of basal antennular segment; cornea dark, bulging and sub-terminal. Antennular peduncle extends only trifle beyond penultimate segment of antenna; terminal segment of antennule longer than second segment. Left cheliped I wanting; right cheliped I strong and unarmed. Chelae and carpi of chelipeds II mottled with white and yellow-brown; merus with tinge of yellow-brown distodorsally; fingers of cheliped II longer than palm. First abdominal segment bell-shaped; second segment large, being half as long as first one. Telson less than half length of last abdominal segment. Inner margin of exopod of uropod deeply concave; with well-developed truncate apically (not sharp spine) process near proximal end of posterior margin; endopod short and more or less triangular.

SIZE. The total body length of the collected ♂ is 12 cm.

HABITAT. The ghost shrimp was collected in a shallow intertidal area, hiding in the muddy sand under the small rock boulder.

DISTRIBUTION. Widely distributed Indo-Western Pacific species, presently known from Mauritius (type locality), Chabahar Iran, Gulf of Oman, Pakistan (Northern Arabian Sea), Sri Lanka, southern Japan, Cocos Islands, Indonesia (Ambon) and Australia (Queensland).

Class Copepoda Milne Edwards, 1840
Order Cyclopoida Burmeister, 1834
Family Clausidiidae Embleton, 1901
Genus *Clausidium* Kossmann, 1874
***Clausidium sarii* Sepahvand et Kihara, 2018**
Figs 4–7.

MATERIAL EXAMINED. 5♀♀ (dissected), 5♂♂ (dissected), Cat no. MRCC-UOK-COPE-05, Northern Arabian Sea, Pakistan, along the Karachi coast, Buleji, 24°50′20.41″ N 66°49′24.15″ E, extracted from brachial cavity of the axiid ghost shrimp *Corallianassa martensi*, 23 January 2023.

Additional material: 40♂♂ and 73♀♀, same as indicated above.

DESCRIPTION. ADULT FEMALE (Figs 4, 5). Body dorsoventrally compressed and oval in shape. Mean body length measured from frontal margin of cephalothorax to posterior length of urosomal segment about 2.25–2.7 mm (10 specimens measured). Thoracic somite I fused with cephalosome and formed cephalothorax. Cephalothorax is much wider than long. Rostrum fused into cephalothorax with broad postrolateral margin. Antenna I 7-segmented. Antenna II 4-segmented. Labrum wider than long and slightly form a hexagonal shape. Mandible well developed. Maxillula unsegmented with 7 setae. Maxilla 2-segmented. Maxilliped 4-segmented. Epimera of thoracic somite II–III expanded posteriorly. Thoracic somite IV anteriorly broader and posteriorly narrower, forming posterior rounded protuberance of somite. Urosome 3-segmented, comprised of with thoracic somite V, genital double somite and anal somite. Caudal ramus about 4 times longer than wide, and armed with 6 setae. PI biramous and well developed. Coxa and basis fused forming well-developed segment with 1 naked seta on outer corner near exopod insertion; large blade-like seta on inner corner, with lines and apex. Endopodal segment 3 elongated, irregular segment ending in a lobe armed with 1 seta and 2 sucking discs. PpII–IV biramous, with 3 segmented endopod and 3 segmented exopod.

Armature formula of PpII–IV as follows (Roman numerals representing spines, Arabic numerals representing setae):

	Coxa	Basis	Exopod	Endopod
PII	0–1	1–0	I–0; I-1; III, I, 4	0–1; 0–2; I, II, 3
PIII	0–1	1–0	I–0; I–1; III, I, 4	0–1; 0–2; I, II, 3
PIV	0–1	1–0	I–0; I–1; III, I, 5	0–1; 0–2; I, II, 2

PV 2-segmented and uniramous. Exopodal segment is elongated, with 2 pinnate setae along the outer margin, 1 small or vestigial naked subterminal seta and 1 long terminal pinnate apical seta. PVI with 2 setae.

ADULT MALE (Figs 6, 7). Body cyclopiform in shape. Mean body length measured from frontal margin of cephalothorax to posterior length of urosomal segment about 1.5–1.9 mm (10 specimens measured). Thoracic somite I fused with the cephalothorax. Thoracic somite VI broader than long, posteriorly formed bilobed and not completely covered thoracic somite V. Prosome longer than urosome. Labrum expanded and broader than wider. Antenna I, antenna II, mandible, maxilla I and maxilla II are similar as in ♀♀. Maxilliped is modified for prehension with a large basal segment; lobe formed near inner edge of distal border of segment 1; segment 2 armed with a moveable process/claw with 3 blunt teeth. Abdomen with 4 segments: 1 genital segment, 2 abdominal segments and 1 anal somite. Anal somite clearly distinct from caudal rami on ventral side. Caudal furca as similar as in ♀♀. PI biramous as in ♀♀; endopodal segment 2 have 1 large pectinate stout seta and 2 small setae in replacement of blade like process and blunt median process as observed in PI female. 2 sucking disks present, with smaller distal disc have a bifurcate element. Armature formula for exopod I–0; I–0; III–2–2. PpII–IV have only 1 inner seta on endopodal segment 2.

Armature formula of PpII–IV as follows:

	Coxa	Basis	Exopod	Endopod
PII	0–1	1–0	I–0; I–1; III, I, 4	0–1; 0–1; I, II, 3
PIII	0–1	1–0	I–0; I–1; III, I, 4	0–1; 0–1; I, II, 3
PIV	0–1	1–0	I–0; I–1; III, I, 5	0–1; 0–1; I, II, 2

PV is uniramous and shorter than PV in ♀♀. Exopodal segment with 4 well developed setae: 2 along the outer margin, 1 subterminal seta and 1 long terminal pinnate apical seta. PVI with 1 seta present on genital segment.

HABITAT. The species was found living in the brachial cavity of the ghost shrimp *Corallianassa martensi* Miers, 1884 (Axiidea: Callichiridae), which represents a new host recorded for the species. Totally, 84 specimens were extracted from left and 39 specimens from right brachial cavity of the collected ghost shrimp. Previously, the species was recorded from the large chelipeds of the ghost shrimp *Neocallichirus natalensis* (Barnard, 1947) (Axiidea: Callichiridae) [Sepahvand *et al.*, 2018].

DISTRIBUTION. Gulf of Oman, Chabahar, Iran (type locality) and Pakistan (present study).

REMARKS. The species has been previously known by a single holotype ♀ [Sepahvand *et al.*, 2018]. The species could be referred to *C. sarii* by the specific distinguishing morphological features of free exopodal segment of PV, which is elongated and about 5 times longer than wide; the presence of 3 well developed and 1 vestigial seta on exopodal segment of PV in ♀♀, similar as illustrated by Sepahvand *et al*. [2018].

Discussion

The host ghost shrimp *Corallianassa martensi* is a widely distributed Indo-Pacific species, which comprehensive morphological descriptions corresponding were provided by Miers [1884] from Mauritius, by Tirmizi [1974] from the Arabian Sea (Pakistan), by Sakai [1983] from Queensland (Australia), by Dworschak [1992, 2014] from Sri Lanka and Cocos Islands, by Komai *et al*. [2015] from Ryukyu Islands, Japan, and by Sepahvand & Tudge [2019] from the Iranian waters. The present specimen of *C. martensi* agrees well with the previous descriptions [Tirmizi, 1974; Dworschak, 2014; Komai *et al*., 2015; Sepahvand, Tudge, 2019]; the coloration of the specimen agrees with Komai *et al*. [2015] and Sepahvand & Tudge [2019], who described it as "*whitish or yellowish translucent body, carapace with tinge of yellowish brown on rostrum to postrostral area and transverse band of similar color medially on dorsal oval*". The second pleomere of the studied shrimp is characterized as "*large, being a little less than one and a half times as long as the first*" (after Tirmizi [1974]), which corresponds to previous published reports and the collected specimens. Our specimen fits well with the description, presented by Sepahvand & Tudge [2019], based on the morphology of cheliped II, namely propodus and dactylus of the minor cheliped, which is longer than the carpus, and the cutting edge of the fixed finger is unarmed, except of a single triangular tooth situated medially.

Copepods have the potential to inhabit ghost shrimp as hosts. The space between the ghost shrimp's carapace and

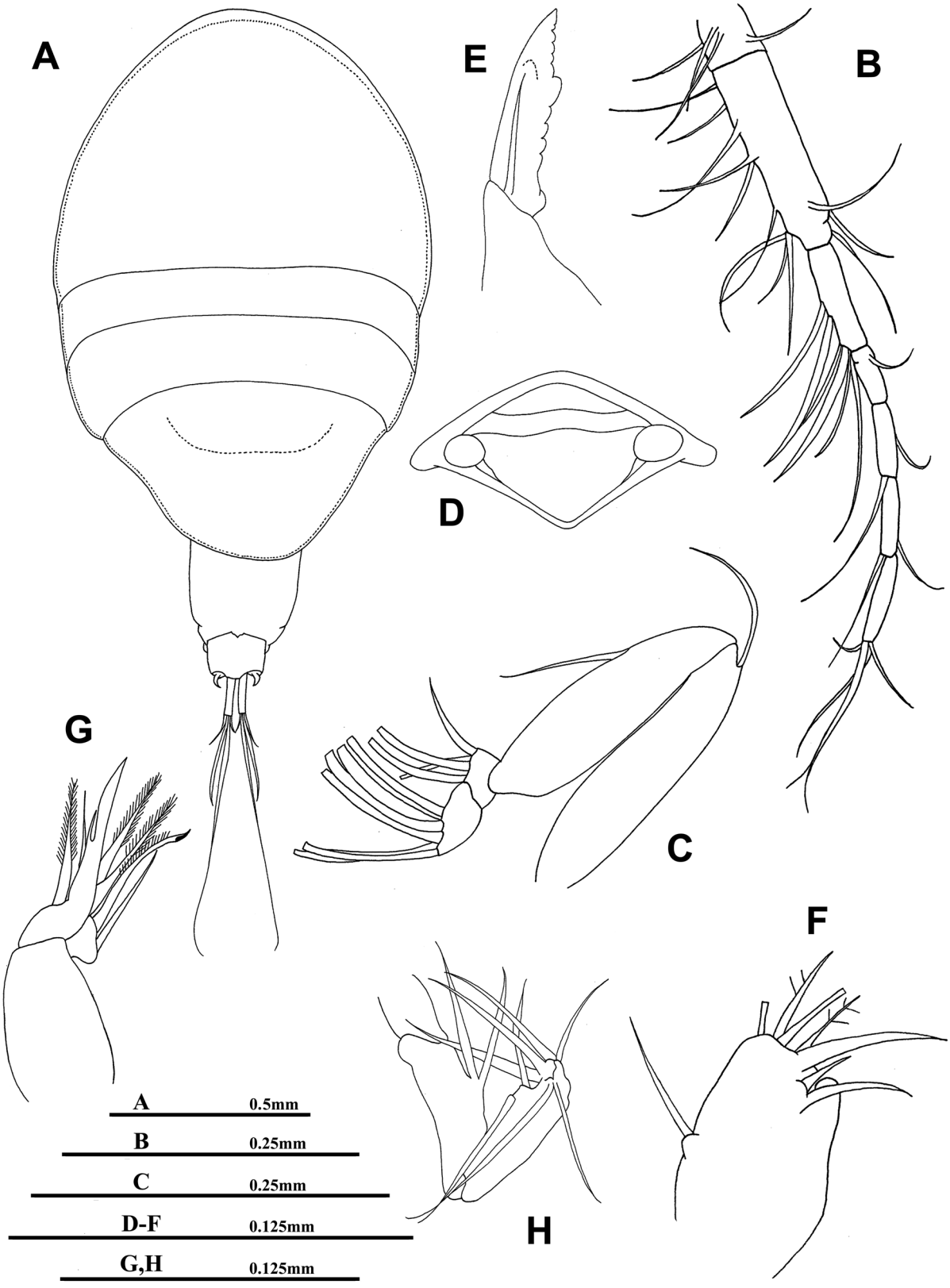

Fig. 4. Symbiotic copepod *Clausidium sarii*, ♀; A — habitus; B — antenna I; C — antenna II; D — labrum; E — mandible; F — maxillule; G — maxilla; H — maxilliped.

Рис. 4. Симбиотическая копепода *Clausidium sarii*, ♀; A — общий вид; B — антенна I; C — антенна II; D — нижняя губа (лабрум); E — мандибула; F — максиллула; G — максилла; Y — максиллипеда.

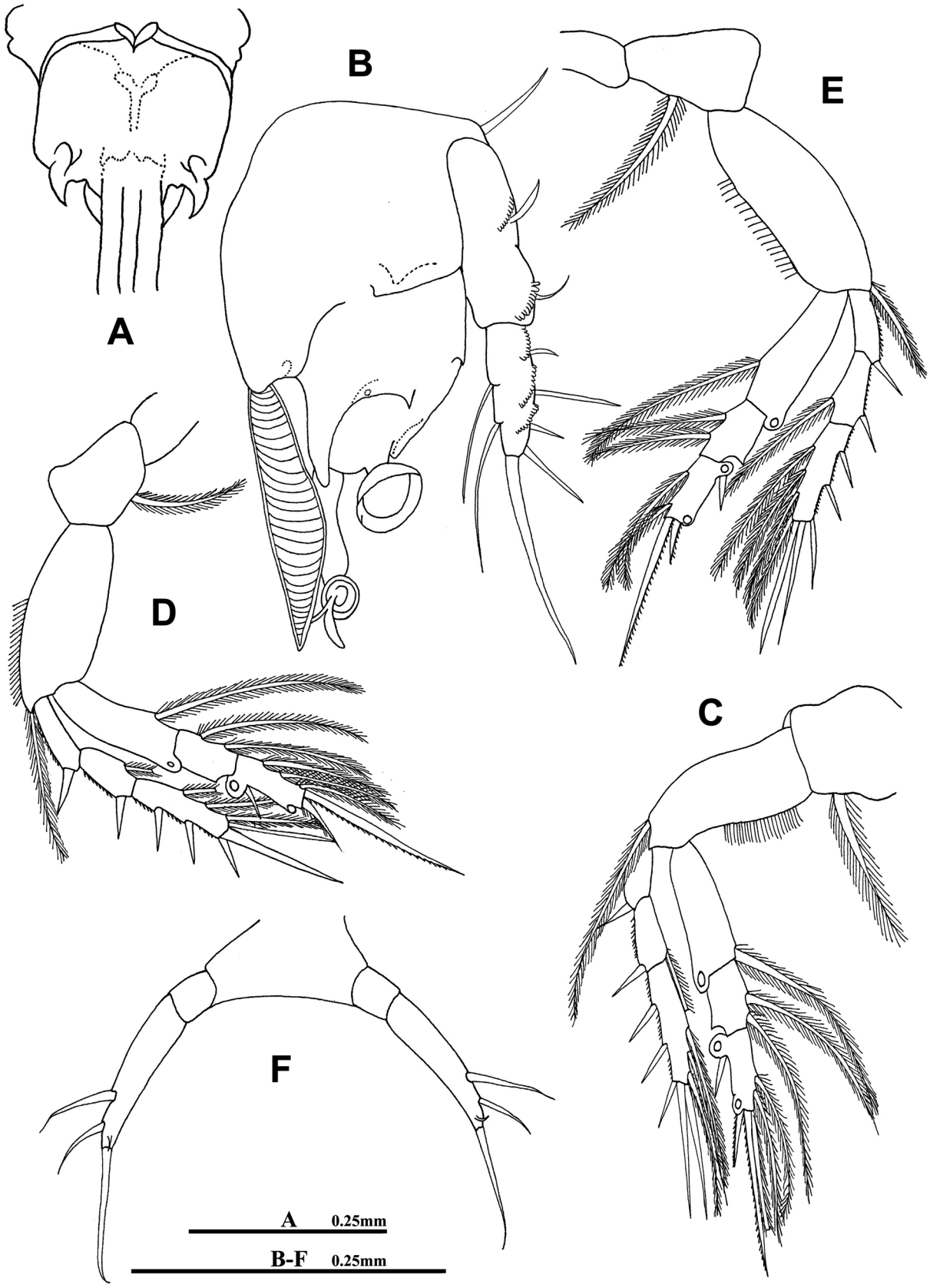

Fig. 5. Symbiotic copepod *Clausidium sarii*, ♀; A — anal somite (ventral); B — PI; C — PII; D — PIII; E — PIV; F — PV.
Рис. 5. Симбиотическая копепода *Clausidium sarii*, ♀; A — анальный сомит (вентральный); B — PI; C — PII; D — PIII; E — PIV; F — PV.

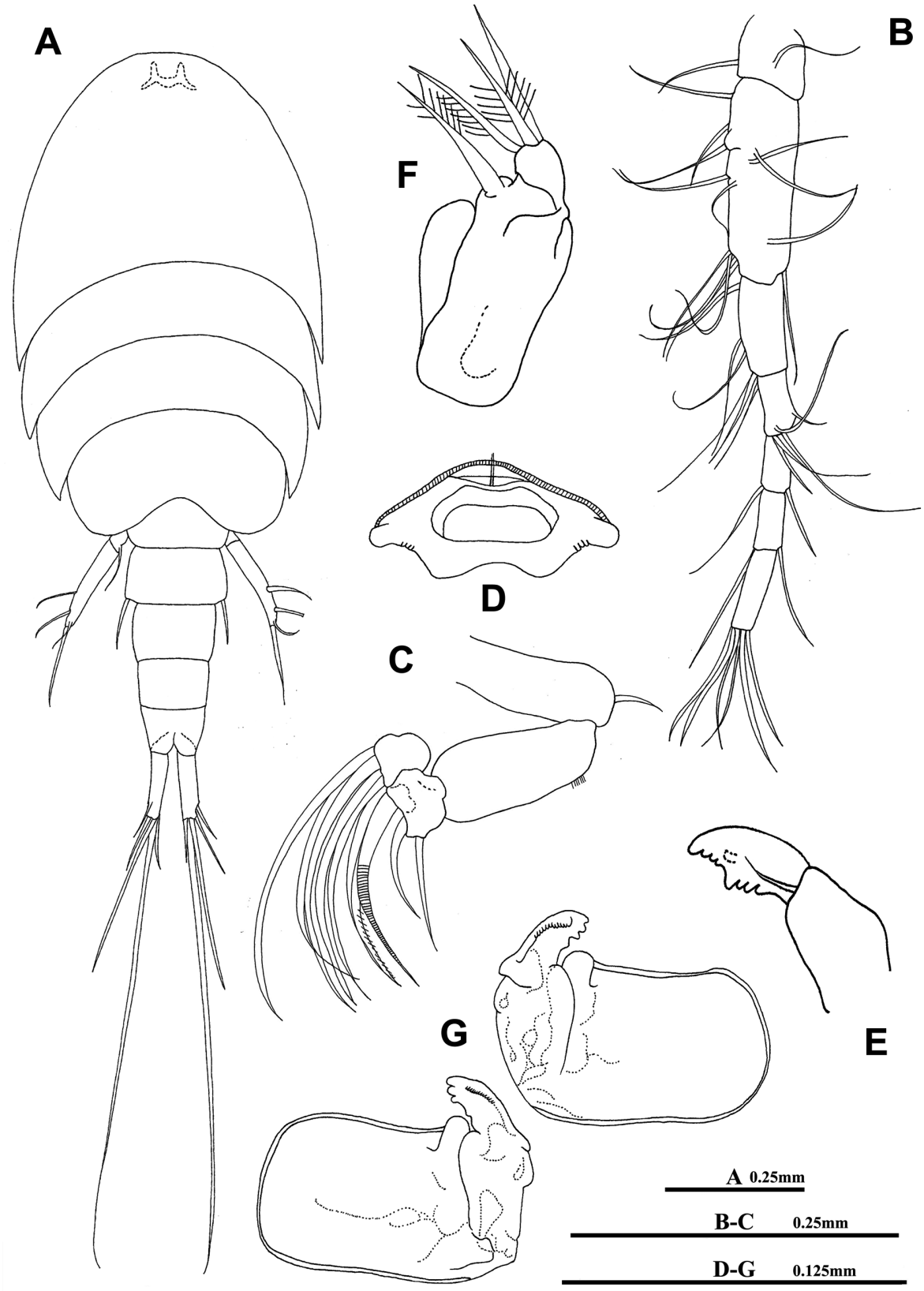

Fig. 6. Symbiotic copepod *Clausidium sarii*, ♂: A — habitus; B — antenna I; C — antenna II; D — labrum; E — mandible; F — maxilla; G — maxilliped.

Рис. 6. Симбиотическая копепода *Clausidium sarii*, ♂: A — общий вид; B — антенна I; C — антенна II; D — нижняя губа (лабрум); E — мандибула; F — максилла; G — максиллипеда.

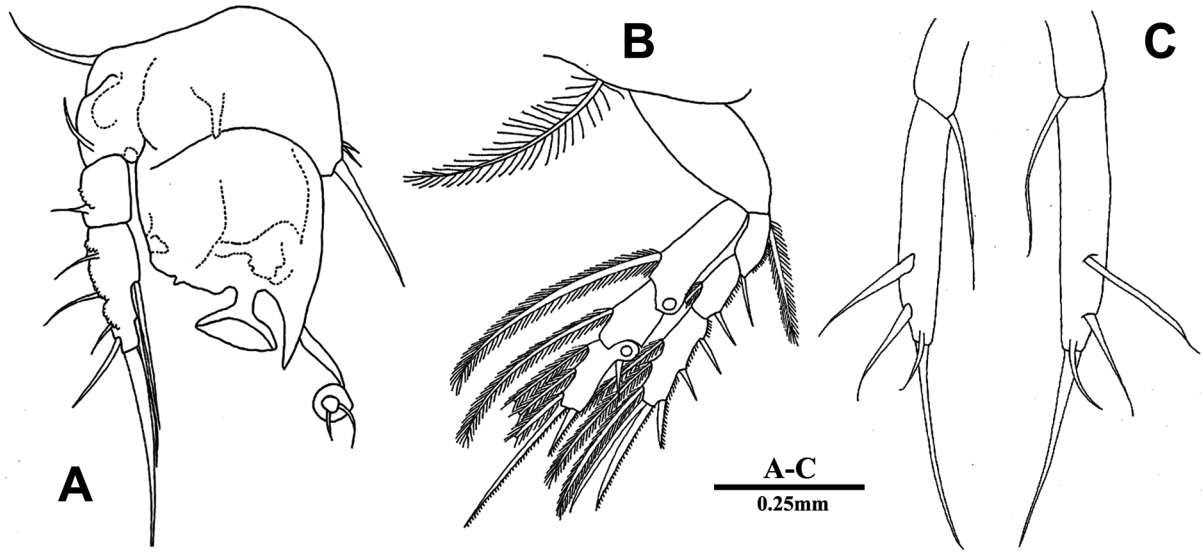

Fig. 7. Symbiotic copepod *Clausidium sarii*, ♂: A — PI; B — PII; C — PV.
Рис. 7. Симбиотическая копепода *Clausidium sarii*, ♂: A — PI; B — PII; C — PIII.

its body, actually the branchial chamber, offers a favorable location for these associated arthropods, while they can live of the surface of the body as well. The known hosts of the species of *Clausidium* have been described till the present time are: *Clausidium searsi* Wilson C.B., 1937 is known from *Callianassa* sp. (Axiidea: Callianaassidae) along the Peruvian coast [Wilson, 1937]; *Clausidium senegalense* Humes, 1957 on *Callianassa* sp. from the South Atlantic [Humes, 1957]; *Clausidium chelatum* Pillai 1959 on *Callianassa* sp. and *Clausidium travancorense* Pillai, 1959 on *Neocallichirus maxima* (A. Milne-Edwards, 1870) (Axiidea: Callichiridae) from India [Pillai, 1959]; *Clausidium saldanhae* Kensley, 1974 on *Pestarella rotundicaudata* (Stebbing, 1902) (Callianassidae) from South Africa [Kensley, 1975] and *Clausidium apodiformis* (Philippi, 1839) is recorded from the burrowing callianassid shrimp *Gilvossius candidus* (Olivi, 1792) (Callianassidae) in the northern Black Sea [Marin, Turbanov, 2016; Marin, 2021]. Kihara & Rocha [2013] established a new species *Clausidium rodriguesi* Kihara et C.E.F. Rocha, 2013 from Brazil, and *Clausidium maximus* Hwang, Lee et I.H. Kim, 2016 from *Callianassa* sp. was reported from Korea by Hwang *et al*. [2016].

Recent taxonomic studies revealed the presence of 4 species from the Iranian coastal water of the Persian Gulf and Gulf of Oman, namely *Clausidium makranensis* Sepahvand *et al*. 2018 from *Corallianassa martensi* and *Clausidium sarii* Sepahvand *et al*., 2018 from *Neocallichirus natalensis* (Barnard, 1947) [Sepahvand *et al*., 2017a], while *Clausidium persiaensis* Sepahvand et Kihara, 2017 from the gill chamber of *Callianidea typa* H. Milne Edwards, 1837 [Sepahvand *et al*., 2017b], and *Clausidium iranensis* Sephavand, Kihara et Boxshall, 2019 from *Neocallichirus jousseaumei* (Nobili, 1904) [Sepahvand *et al*., 2019]. Vadher *et al*. [2021] also discovered *Corallianassa coutierei* (Nobili, 1904) infested with copepods *Clausidium* sp. on the carapace.

The studied specimens fit well with the descriptions *Clausidium sarii* collected from the ghost shrimp *Neocallichirus natalensis* [Sepahvand *et al*. 2018]. The studied individuals of *C. sarii* remains attached to the gills of the ghost shrimp during the sampling and processing, clearly showing the symbiotic, probably parasitic, lifestyle. Usually, male specimens remain attached to the female's abdomen, maintaining its grip even when specimens are placed in the preserving solution. Similar observations were presented by Pillai [1959] and Marin & Turbanov [2016] for other species of the genus *Clausidium*.

Our study also shows that many species of the genus *Clausidium* are not always strictly specific to their host and can inhabit several species of the same taxonomic group (genus, family).

Compliance with ethical standards

Conflict of interests: The authors declare that they have no conflict of interest.

Ethical approval: No ethical issues were raised during our research.

Acknowledgements. Authors would like to acknowledge the Marine Reference Collection and Resource Centre, University of Karachi, for providing research facilities and field trips. We would like to acknowledge the field assistance by Mr. Kahsif and Mr. Umer for data collection. Special thanks are sending to Mr. Abrar Ali for the assistance and guidance in the field of illustrations and photography.

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Responsible editor K.G. Mikhailov