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**THE INFESTATION STATUS OF SYMBIOTIC CRUSTACEANS  
ON THE SWIMMING CRAB *CHARYBDIS TRUNCATA* (FABRICIUS, 1798)  
FROM NHA TRANG BAY, VIETNAM (THE EAST SEA)**

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Symbioses are common relationships between organisms in marine ecosystems. Out of crabs belonging to the family Portunidae, an economically important one, *Charybdis truncata* (Fabricius, 1798) is a widely distributed species. However, the studies on its symbiotic assemblages are still limited. A total of 408 *C. truncata* were sampled in Nha Trang Bay (Khanh Hoa province, Vietnam, the East Sea) in January–August 2022. Symbionts were classified based on morphological characters. Statistical analysis was applied to compare the infestation status of symbionts. Six symbiotic species were detected, including four epibiotic barnacles [*Octolasmis angulata* (Aurivillius, 1894), *Octolasmis alata* (Aurivillius, 1894), *Octolasmis warwicki* Gray, 1825, and *Dianajonesia tridens* (Aurivillius, 1894)] and two obligate unidentified parasites [*Cancrion* sp. and *Sacculina* sp.]. Out of them, *O. alata* and *Cancrion* sp. were recorded on *C. truncata* for the first time. The overall prevalence of symbionts on the swimming crab hosts was 13%, and the number of infecting symbiont species on hosts ranged within 1–4. *O. angulata* was the dominant species, with the prevalence of 6.9%. The prevalence of this symbiotic species was significantly higher in ovigerous female crabs compared to non-ovigerous ones. Moreover, there was a noticeable rise in *O. angulata* prevalence rates as crab size increased. No significant differences were revealed in the mean intensity of symbionts by sex, reproductive status of females, and size of the crab hosts. The initial morphological modifications caused by infecting parasites, *Cancrion* sp. and *Sacculina* sp., were recorded and described. These findings provide data on the status of natural infection of symbiotic species on *C. truncata* as a basis for the management of commercial species and aquaculture development.

**Keywords:** *Charybdis truncata*, symbionts, epibionts, obligate parasites, Nha Trang Bay, Vietnam

Symbioses are common relationships between organisms in ecosystems which represent different associations, including mutualism, commensalism, and parasitism [Leung, Poulin, 2008]. Association between symbionts and their hosts is considered as one of the driving forces of evolution. In several cases, the finding of parasitic fauna on free-living organisms can provide new information for better understanding of host–symbiont interactions and clarify biodiversity data in the study region [Martinson, 2020].

In marine environments, crustaceans are the most diverse invertebrate organisms [Brusca, Brusca, 2003]. They are well-known to have symbiotic relationships [Ross, 1983]. Out of marine crustaceans, swimming crabs (Decapoda: Portunidae, an economically important family) are common hosts of symbiotic crustaceans. These symbionts of portunids include pedunculate barnacles (*Octolasmis* and *Dianajonesia* in the family Poecilasmatidae), acorn barnacles (*Chelonibia* in Chelonibiidae and *Semibalanus* in Balanidae), rhizocephalan barnacles (Sacculinidae and Thompsoniidae), isopods (Entoniscidae and Bopyridae), and copepods (Nicothoidae) [Jeffries et al., 1982; McDermott et al., 2019; Shields, 1992; Shields et al., 2015; Waiho et al., 2021].

Pedunculate and acorn barnacles are filter-feeding organisms attaching to the outer integument of crabs [Campos et al., 2022; Hosseini et al., 2023; Jeffries et al., 1982]. The host's respiratory currents supply them with food and oxygen [Jeffries et al., 1982]. In most cases, they are harmless to the host. However, when occurring at high intensity, they hamper the energetic condition, movement, and respiration of crabs. More seriously, the heavy infestation with epibiotic barnacles can lead to cumulative infection with rhizocephalan parasites causing serious effects on growth and development, even the death of their hosts [Campos et al., 2022; Gannon, Wheatly, 1992; Hudson, Lester, 1994].

Rhizocephalan barnacles are specialized parasites. Their body has two parts: a root-like system (interna) developing around the host's visceral organs for nutrient absorption and a female reproductive organ (externa) arising outside the host [Glenner, Hebsgaard, 2006; Walker, 2001]. The common resulting effects of rhizocephalan infection are alterations in morphological characters, physiology, and behavior of the crabs [Waiho et al., 2021]. Infected male crabs are usually found feminized – with an enlarged and more segmented abdomen, like in females. Meanwhile, the effects observed in female crabs are often unclear [Kristensen et al., 2012].

Isopods (Entoniscidae and Bopyridae) are obligate parasites within visceral cavities of crabs. Their life cycle goes through three larval stages. The eggs develop into epicardium larvae which then metamorphose into microniscus and finally into cryptoniscus larvae before becoming juveniles [Williams, Boyko, 2012]. These adult parasites are characterized by high sexual dimorphism: males are many times smaller in size than females [McDermott et al., 2019; Shiino, 1942]. Certain species could cause the castration of the host [Shiino, 1942].

Some egg-feeding copepod parasites of the family Nicothoidae were found on egg clutch and in branchial chambers of swimming crabs from India [Gnanamuthu, 1954], Australia [Shields, 1992], and Vietnam [Dang et al., 2022; Oanh et al., 2018]. Mostly, due to very low prevalence, the harmful effects of these parasites did not arouse much interest [Shields et al., 2015].

So far, eight pedunculate barnacles, two acorn barnacles, five rhizocephalans, three isopods, and one copepod species have been detected on ten species of swimming crabs on the coast of Vietnam. Out of them, both species composition and infection status of symbiotic crustaceans were studied in four commercial crabs: *Portunus pelagicus* (Linnaeus, 1758), *Portunus sanguinolentus* (Herbst, 1783), *Monomia haanii* (Stimpson, 1858), and *Charybdis feriata* (Linnaeus, 1758) [Dang et al., 2022; Oanh et al., 2018, 2022a; Vo et al., 2013]. In some cases, investigations were focused only on parasitic species. H. Boschma [1954] discovered four rhizocephalan species on six swimming crabs and described a new species, *Sacculina serenei* Boschma, 1954, on *C. feriata*. As for parasitic isopods, two new species, *Cancrion khanhensis* Oanh & Boyko, 2020 and *Stellatoniscus tentaculus* Oanh & Boyko, 2020, infesting *M. haanii* were recently described [Oanh, Boyko, 2020].

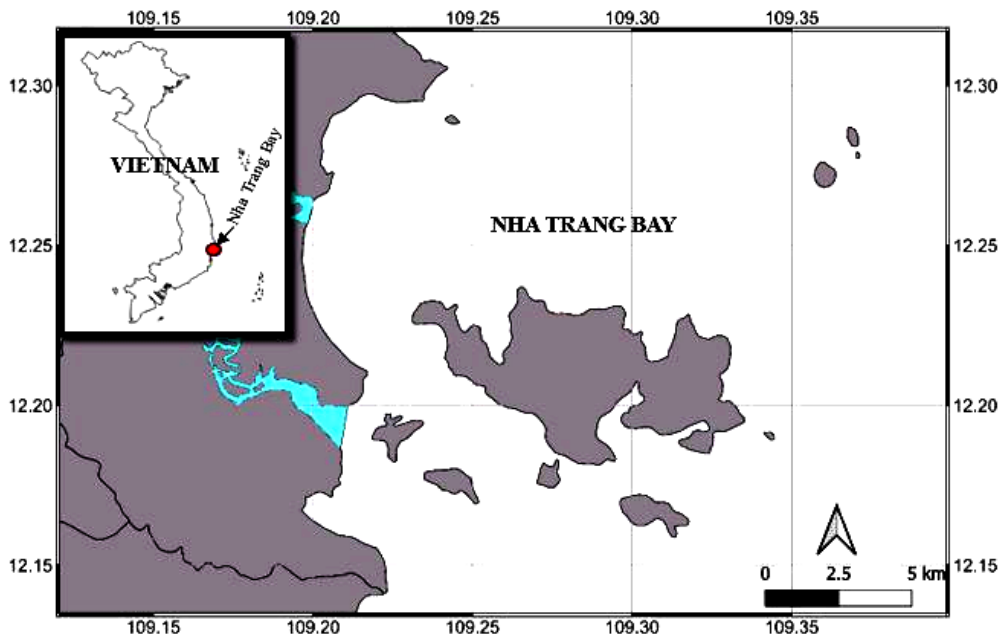
A portunid crab *Charybdis truncata* (Fabricius, 1798) is a widely distributed and commonly exploited species in the Indian Ocean (India) and the West Pacific region (Japan, China, Indonesia, Philippines, Malaysia, Singapore, and Australia) [Ng, 1998]. In Vietnam, it occurs in the Gulf of Tonkin, on central and southern coasts, in muddy or muddy-sandy seabed, at a depth of 5–60 m [Nguyen, 2002]. Several species of symbiotic crustaceans were revealed on *C. truncata*. Three epibionts,

*Octolasmis angulata* (Aurivillius, 1894), *Octolasmis warwicki* Gray, 1825, and *Dianajonesia tridens* (Aurivillius, 1894), were found on crabs from Hong Kong [Leung, Jones, 2000]. A parasitic rhizocephalan *Loxothylacus nierstraszi* Boschma, 1938 was recorded in Japan [Boschma, 1938], while *Sacculina scabra* Boschma, 1931 was registered in Indonesia [Chan, 2004]. Up to now, no known investigations have been carried out on symbiotic crustaceans on *C. truncata* in Vietnam.

This study aims at determining the species composition and infestation status of symbiotic crustaceans on *C. truncata* in Nha Trang Bay, Vietnam, and carrying out initial observations on the effect of obligate parasites on the crab hosts. This preliminary research contributes to filling knowledge gaps for this crab species in Vietnam and provides information for fisheries resource management programs.

## MATERIAL AND METHODS

**Crab sampling and symbiont examination.** Crabs were sampled in Nha Trang Bay (Khanh Hoa province, Vietnam, the East Sea) in January–August 2022 (Fig. 1). *C. truncata* specimens ( $n = 408$ ) were randomly sampled from fishing boats, transported alive to the laboratory, and temporarily kept in a fridge at the temperature of  $+4\text{ }^{\circ}\text{C}$ . The fresh crabs were examined externally; size (carapace width, hereinafter CW), sex, and reproductive status of females (ovigerous or non-ovigerous) were recorded. Following Li and co-authors [2014], crabs were divided into 3 size groups: small ( $< 20\text{ mm}$ ), medium ( $20\text{--}35\text{ mm}$ ), and large ( $> 35\text{ mm}$ ). Also, the host morphological modifications (if any) were carefully investigated and noted.



**Fig. 1.** Map of Nha Trang Bay (the East Sea), operation region of fishing boats from which crabs were sampled

**Рис. 1.** Карта залива Нячанг (Восточное море) — района эксплуатации рыболовных судов, с которых были собраны крабы

Symbionts were examined by the naked eye, under a stereo microscope Olympus SZ61 (Japan, magnification  $\times 10$  to  $\times 40$ ), and under a biological microscope Olympus CX41 (Japan, magnification  $\times 40$  to  $\times 100$ ). The entire integument of a crab (carapace, legs, ventral surface, and abdomen) was observed. Then, the carapace was detached to inspect gill chambers (ceiling, gills, and maxillipeds) and body cavity. The found symbiotic crustaceans were removed from the host by tweezers and placed in dishes

with seawater (for external symbionts) or saline solution of 9‰ (for endosymbionts). The abundance of individuals and infesting positions of symbionts were recorded. Fresh symbionts, after being examined and described, were stored in 70% alcohol.

**Symbiont identification and state of infestation.** Symbiotic crustaceans were identified following the diagnostic characteristics for each sampled group. Pedunculate barnacles were determined based on the body size and characters of calcareous plates on capitular [Aurivillius, 1894; Jeffries et al., 2005; Zevina, 1982] (see Fig. 2A). Size of mantle opening, as well as shape and position of male receptacles in the externa, are taxonomic features used to identify Rhizocephala [Boschma, 1938; Chan, 2004]. Both adults and larvae are often found for entoniscid species. Therefore, for species identification, we used the following characters: oostegite structure, number, and position of ovarian processes for a female; number of pereopod segments for a male; and structure of the 6<sup>th</sup> pereopods for epicardium larvae [Shields, Earley, 1993; Shiino, 1942].

The state of infestation by symbionts was characterized *via* prevalence and mean intensity. The prevalence of infestation of a particular symbiotic species is the number of hosts infested with that symbiont divided by the number of examined crabs. Intensity represents the number of individuals of a symbiotic species in a single infested crab. The mean intensity is the total number of symbionts found in examined hosts divided by the number of crabs infested with that symbiont [Bush et al., 1997]. The state of infestation by parasitic isopod was determined based on the occurrence of female individuals [Shields, Earley, 1993]. The chi-square test was applied to analyze the prevalence differences between the host groups (sex, reproductive status of females, and size group). The independent sample *t*-test was used to reveal the differences in mean intensity between the host sex and reproductive status of a female crab. To compare mean intensities between three host size groups, the one-way analysis of variance (ANOVA) was applied. All the analysis was carried out using Statistical Product and Service Solutions (SPSS) 24.0 (<https://www.ibm.com/support/pages/spss-statistics-240-fix-pack-1>) [Li et al., 2014]. Crabs were sampled not in all months of the year, and the number of samples in each month was uneven; accordingly, infestation status by symbionts was not assessed.

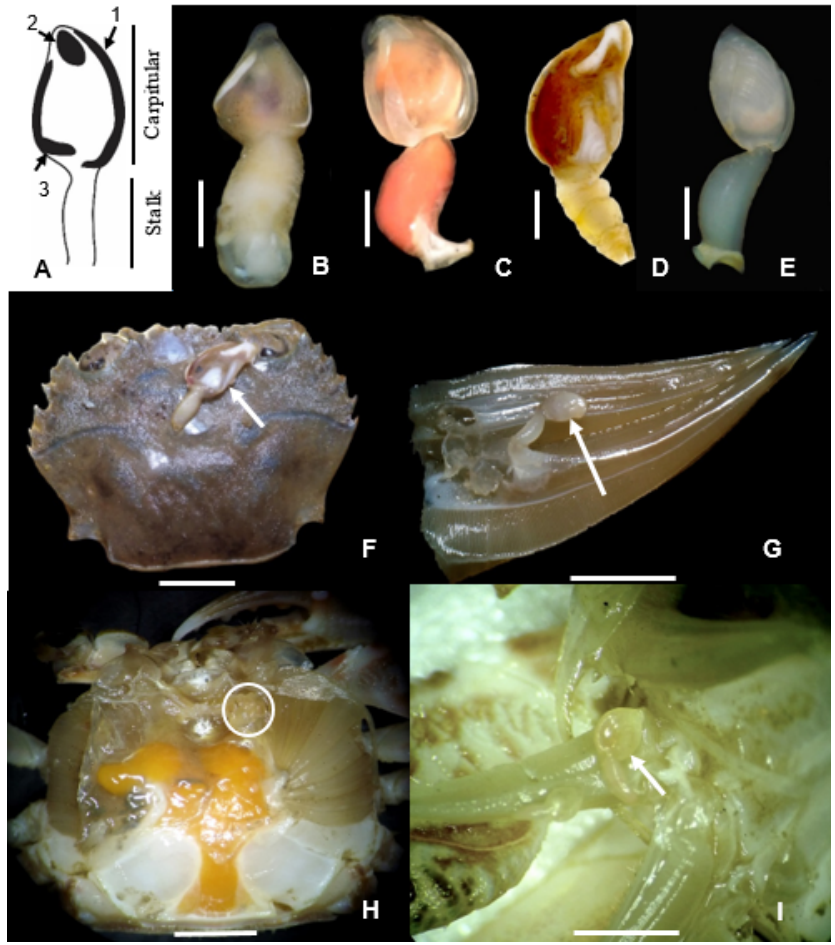
## RESULTS

**Species composition and infesting position.** In total, six symbiotic crustacean species were reported from 408 *C. truncata* crabs examined. Four epibiotic barnacles (Scalpellomorpha: Poecilasmidae) were found: *Octolasmis alata* (Aurivillius, 1894), *O. angulata*, *O. warwicki*, and *D. tridens* (Fig. 2). Two obligate endoparasites, *Sacculina* sp. (Rhizocephala: Sacculinidae) (Fig. 3) and *Cancrion* sp. (Isopoda: Entoniscidae) (Fig. 4), were revealed. *O. alata* and *Cancrion* sp. were the new records both on *C. truncata* and in Vietnam.

**Epibiotic barnacles.** Epibiotic pedunculate barnacles are common species found on swimming crabs. Sampled *Octolasmis* and *Dianajonesia* specimens were distinguished based on number, size, and shape of calcareous plates (Fig. 2B–E). Details of taxonomic characters of epibiotic species are provided in Table 1.

In terms of infesting positions, symbiotic species occurred on their hosts at different sites. Specifically, *O. warwicki* attaches to the carapace surface, while *O. angulata*, to the ceiling of gill chambers. *O. alata* was found on the proximal surface of gills, and *D. tridens*, on gills and maxillipeds (Fig. 2F–I).

**Taxonomy records.** *O. angulata* was distinguished from other epibionts by the lack of two terga calcareous plates on the capitular. All three remaining species had five calcareous plates (one carina, two terga, and two scuta). Out of them, *O. warwicki* possessed a fissure in the base of the carina, and its terga were ax-shaped; *O. alata* terga were maple leaf-shaped; and *D. tridens* terga were trapezoidal (Table 1).



**Fig. 2.** Epibiotic crustacean species and their infesting positions on the swimming crab *Charybdis truncata* in Nha Trang Bay (Vietnam, the East Sea): A, illustration of calcareous plate position of pedunculate barnacles of the family Poecilasmataidae (1, carina; 2, tergum; 3, scutum); B, *Octolasmis angulata*; C, *Octolasmis alata*; D, *Octolasmis warwicki*; E, *Dianajonesia tridens*; F, *O. warwicki* (an arrow) on the host’s carapace; G, *D. tridens* (an arrow) on gills; H, *O. angulata* (a circle) on the ceiling of the gill chamber; I, *O. alata* (an arrow) on gills. Scale bar: B–E, 1 mm; F–I, 5 mm

**Рис. 2.** Виды ракообразных-эпibiонтов и их локализация на крабе-плавунце *Charybdis truncata* из залива Нячанг (Вьетнам, Восточное море): А — иллюстрация положения известковых пластинок имеющих стемелёк морских уточек семейства Poecilasmataidae (1 — карина; 2 — тергум; 3 — скutum); В — *Octolasmis angulata*; С — *Octolasmis alata*; D — *Octolasmis warwicki*; E — *Dianajonesia tridens*; F — *O. warwicki* (стрелка) на панцире хозяина; G — *D. tridens* (стрелка) на жабрах; H — *O. angulata* (круг) на верхней части жаберной камеры; I — *O. alata* (стрелка) на жабрах. Масштабная линейка: В–Е — 1 мм; F–I — 5 мм

**Table 1.** Taxonomic characteristics of epibiotic species on *Charybdis truncata*

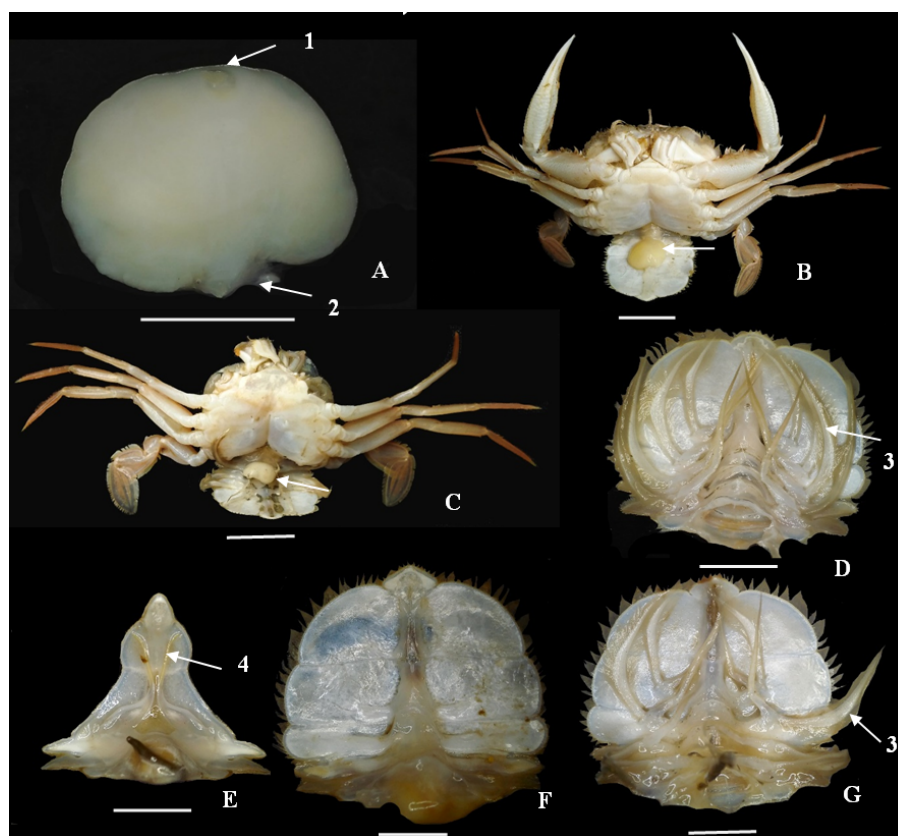
**Таблица 1.** Таксономическая характеристика видов-эпibiонтов на *Charybdis truncata*

Epibiotic species	Number of analyzed individuals	Body size, mm	Number of calcareous plates	Number and shape of carina	Number and shape of scutum	Number and shape of tergum
<i>Octolasmis angulata</i>	10	3.3 ± 0.57	3	1, thin, bar-shaped	2, bar-shaped	absent
<i>Octolasmis alata</i>	3	3.3 ± 0.82	5	1, robust, crescent-shaped	2, robust, two-part, with the first part bar-shaped and the second part L-shaped	2, maple leaf-shaped

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Epibiotic species	Number of analyzed individuals	Body size, mm	Number of calcareous plates	Number and shape of carina	Number and shape of scutum	Number and shape of tergum
<i>Octolasmis warwicki</i>	5	4.6 ± 1.48	5	1, robust, crescent-shaped with a fissure in the base	2, robust, two-part, with the first part bar-shaped and the second part fan-shaped	2, ax-shaped
<i>Dianajonesia tridens</i>	10	3.9 ± 0.90	5	1, robust, crescent-shaped	2, robust, two-part, with the first part bar-shaped and the second part trapezoid	2, wrench-shaped

***Sacculina* sp. Main morphological characters.** The externa (reproductive organ) ellipse-shaped, (5 ± 0.82) mm in length ( $n = 2$ ), with short stalk (see 2 in Fig. 3A); milk-white in color. Mantle opening wide, (0.7 ± 0.08) mm, located on the top of the externa (see 1 in Fig. 3A). The externa of *Sacculina* sp. was observed arising from the gut on the host abdomen (Fig. 3B, C).



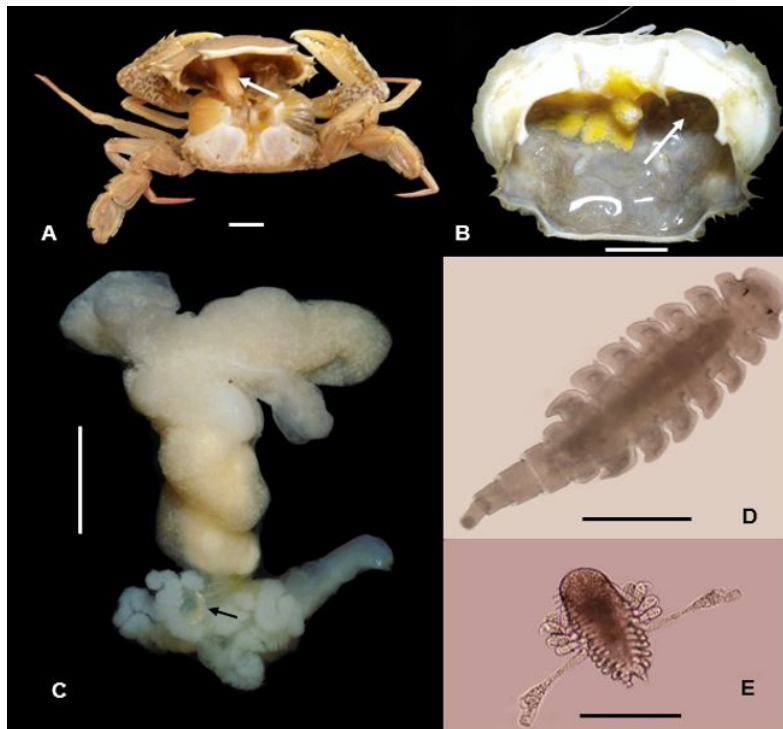
**Fig. 3.** Parasitic rhizocephalan *Sacculina* sp. infesting *Charybdis truncata* in Khanh Hoa, Vietnam: A, *Sacculina* sp. external view (1, mantle opening; 2, stalk); B, the externa of *Sacculina* sp. (an arrow) on a male crab's abdomen; C, the externa of *Sacculina* sp. (an arrow) on a female crab's abdomen; D, normal female abdomen (3, female pleopod); E, normal male abdomen (4, male pleopod); F, infested male abdomen without pleopods; G, infested female abdomen. Scale bar: A, 3 mm; B, C, 10 mm; D–G, 5 mm

**Рис. 3.** Паразитическая корнеголовая *Sacculina* sp., заражающая *Charybdis truncata* из провинции Кханьхоа, Вьетнам: А — внешний вид *Sacculina* sp. (1 — отверстие в мантии; 2 — ножка); В — экстерна *Sacculina* sp. (стрелка) на брюшке самца краба; С — экстерна *Sacculina* sp. (стрелка) на брюшке самки краба; D — нормальное брюшко самки (3 — плеопод самки); E — нормальное брюшко самца (4 — плеопод самца); F — брюшко заражённого самца без плеоподов; G — брюшко заражённой самки. Масштабная линейка: А — 3 мм; В, С — 10 мм; D–G — 5 мм

**Remark.** Parasitic Rhizocephala were found on two individuals only, a 26-mm male and 22.5-mm female. The above characteristics allow determining that this parasite belongs to *Sacculina*. Due to highly reduced morphological characters, microanatomical examination and molecular analysis need to be conducted for precise species identification.

***Cancrion* sp. Main morphological characters.** Total length of a female (cephalon, thorax, and abdomen) was  $(18.5 \pm 1.34)$  mm ( $n = 4$ ). Body recurved dorsally; first oostegite consists of three parts: ascendant, transverse, and recurrent; the lamellae of pleural segments well developed; the 1<sup>st</sup> and 2<sup>nd</sup> pleopods folded, while all the remaining simple; ovaries cream to orange; dorsal and dorsolateral ovarian processes present, while ventral processes absent (Fig. 4C). A male was found on pleopod of a female with five-segmented pereopod (Fig. 4C, D). The epicaridium larvae fully filled in the marsupium of a female (Fig. 4C); the 4<sup>th</sup> pereopod without spine on merus segment; the 6<sup>th</sup> pereopod elongated with highly modified dactyl process, and no spine on merus segment (Fig. 4E). *Cancrion* sp. was found to occupy the host hemocoel (Fig. 4A).

**Remark.** Entoniscidae is a family of parasitic crustaceans with high sexual dimorphism. There are combination characters of females, males, and epicaridium larvae representative for the genus *Cancrion*. To date, eight *Cancrion* species have been described. *Cancrion* sp. shared almost the same taxonomic characters with *Cancrion australiensis* [Shields, Earley, 1993], except for the shape of the first oostegite of a female (complex vs. simple), number of segments of male pereopods (5 vs. 3), and spine on merus segment of the 4<sup>th</sup> pereopod of epicaridium larvae (absent vs. occurring). These recorded differences suggest that *Cancrion* sp. discovered on the coast of Vietnam may be a new species for science and needs to be described.



**Fig. 4.** Parasitic isopod *Cancrion* sp. infesting *Charybdis truncata* in Vietnam (the East Sea): A, *Cancrion* sp. on the host hemocoel (an arrow); B, a gap (an arrow) in the body cavity after *Cancrion* sp. removal; C, female *Cancrion* sp. with a male (an arrow) and a lot of epicaridium larvae in female's marsupium; D, male *Cancrion* sp.; E, epicaridium larva of *Cancrion* sp. Scale bar: A–C, 5 mm; D, 1 mm; E, 50  $\mu$ m

**Рис. 4.** Паразитическая изопода *Cancrion* sp., заражающая *Charybdis truncata* во Вьетнаме (Восточное море). А — *Cancrion* sp. на гемоцели хозяина (стрелка); В — щель (стрелка) в полости тела после удаления *Cancrion* sp.; С — самка *Cancrion* sp. и самец (стрелка) и многочисленные личинки эпикаридий в сумке самки; D — самец *Cancrion* sp.; E — личинка *Cancrion* sp. из эпикарда. Масштабная линейка: А–С — 5 мм; D — 1 мм; E — 50 мкм

**Infestation status.** The overall prevalence of symbionts on the crab hosts was 13% (54 / 408 ind.). The number of infecting symbiont species on hosts ranged one to four. The percentage of hosts infected with one and two species was 10.5% (43 / 408) and 2.2% (5 / 408), respectively, while infection with three and four species occurred in one individual only accounting for 0.2%. At the species level, *O. angulata* and *D. tridens* were found to be infected with the prevalence of 6.9 and 6.6%, respectively; the values were higher than those for other symbionts, 0.5–1.2% (Table 2).

**Table 2.** Prevalence and mean intensity ( $\pm$  standard deviation) of symbionts found on *Charybdis truncata* in Nha Trang Bay, Vietnam. Values marked with different letters represent significant differences in prevalence between ovigerous and non-ovigerous crabs (a and b) and crabs of various size groups (x, y, and z)

**Таблица 2.** Встречаемость и средняя интенсивность ( $\pm$  стандартное отклонение) симбионтов, обнаруженных на *Charybdis truncata* в заливе Нячанг, Вьетнам. Значения, отмеченные разными буквами, показывают существенные различия во встречаемости между самками с кладкой икры и без неё (a и b) и крабами разных размерных групп (x, y и z)

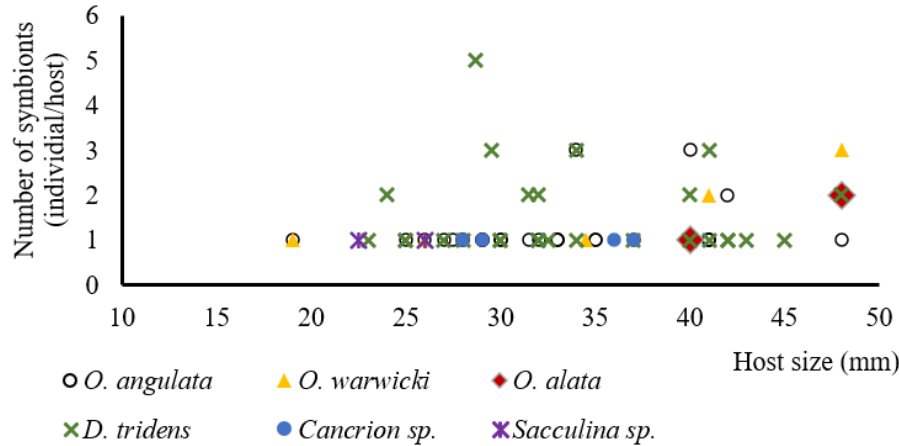
Symbiotic species	Crabs in total (n = 408)	Sex of crabs		Reproductive status of female crabs		Size of crabs		
		Male (n = 205)	Female (n = 203)	Ovigerous (n = 92)	Non-ovigerous (n = 111)	< 20 mm (n = 54)	20–35 mm (n = 302)	> 35mm (n = 52)
	Prevalence (%) Intensity (ind. per host)							
<i>Octolasmis angulata</i>	<u>6.9</u> 1.2 $\pm$ 0.54	<u>6.8</u> 1.4 $\pm$ 0.72	<u>6.9</u> 1	<u>12<sup>b</sup></u> 1	<u>2.7<sup>a</sup></u> 1	<u>1.9<sup>x</sup></u> 1	<u>5.6<sup>y</sup></u> 1.1 $\pm$ 0.47	<u>19.2<sup>z</sup></u> 1.3 $\pm$ 0.64
<i>Octolasmis alata</i>	<u>0.5</u> 1.5 $\pm$ 0.50	<u>1.0</u> 1.5 $\pm$ 0.5	–	–	–	–	–	<u>3.8</u> 1.5 $\pm$ 0.50
<i>Octolasmis warwicki</i>	<u>1.2</u> 1.6 $\pm$ 0.80	<u>2.0</u> 1.8 $\pm$ 0.83	<u>0.5</u> 1	–	<u>0.9</u> 1	<u>1.9</u> 1	<u>0.7</u> 1	<u>3.8</u> 2.5 $\pm$ 0.50
<i>Dianajonesia tridens</i>	<u>6.6</u> 1.6 $\pm$ 0.96	<u>5.9</u> 1.4 $\pm$ 0.64	<u>7.4</u> 1.7 $\pm$ 1.10	<u>10.9</u> 1.6 $\pm$ 1.2	<u>4.5</u> 1.8 $\pm$ 0.98	–	<u>5.6<sup>x</sup></u> 1.6 $\pm$ 1.08	<u>19.2<sup>y</sup></u> 1.3 $\pm$ 0.66
<i>Sacculina</i> sp.	<u>0.5</u> 1	<u>0.5</u> 1	<u>0.5</u> 1	–	<u>0.9</u> 1	–	<u>0.7</u> 1	–
<i>Cancrion</i> sp.	<u>1.1</u> 1	<u>1.5</u> 1	<u>0.5</u> 1	<u>1.1</u> 1	–	–	<u>0.7</u> 1	<u>3.8</u> 1

In terms of the host sex, no significant difference was observed in symbiont prevalence (chi-square test,  $P > 0.05$ ). *O. alata* was found to occur only on a male crab, with low prevalence (1%). As for sampled female crabs (92 ovigerous and 111 non-ovigerous individuals), *O. warwicki* and *Sacculina* sp. were not recorded on ovigerous crabs, while *Cancrion* sp. was not registered on non-ovigerous ones. The significant difference in the prevalence of infestation was noted for *O. angulata* alone (chi-square test, 6.71,  $df = 1$ ,  $P = 0.01$ ) (Table 2).

Data on the state of infestation by symbiotic crustaceans in terms of crab size are provided in Table 2 and Fig. 5. The smallest *C. truncata* bearing symbiotic crustacean was a 19-mm male, and the largest was a 48-mm male. Small crabs (CW < 20 mm) were infested by two epibiotic species, *O. angulata* and *O. warwicki*. *O. alata* was recorded on large crabs (> 35 mm), while *Sacculina* sp. was noted on medium-sized specimens (20–35 mm). Significant differences in the prevalence were revealed for *O. angulata* (chi-square test, 15.28,  $df = 1$ ,  $P < 0.01$ ) and *D. tridens* (chi-square test, 11.60,  $df = 1$ ,  $P < 0.01$ ).



The maximum intensity of infestation by a symbiont species was 5 ind. *per* host (Fig. 5). The mean intensity of epibiotic species was low: the value varied within 1.2–1.6 ind. *per* host, with the highest for *D. tridens* and the lowest for *O. angulata*. Both parasitic species, *Sacculina* sp. and *Cancrion* sp., infected the swimming crab with the intensity of 1 ind. *per* host (Table 2). No significant differences were recorded between all compared host groups (sex, reproductive status of females, and size group).



**Fig. 5.** Intensity of invasion by symbiotic crustaceans in the swimming crab *Charybdis truncata* of different size groups

**Рис. 5.** Интенсивность инвазии симбиотическими ракообразными крабов *Charybdis truncata* различных размерных групп

**Morphological modification of infested crabs.** In the present study, epibiotic species occurred at low prevalence and intensity. No changes in the host morphology were observed.

As noticed above, *Sacculina* sp. was found on two crab individuals (a male with CW of 26 mm and a female with CW of 22.5 mm). Due to appearance of the externa on a male crab, the abdomen broadened resembling that of an adult female. The abdominal segments the 3<sup>rd</sup> to 5<sup>th</sup> distinguished, and the pleopods completely disappeared (Fig. 3F). Compared to those of a normal crab, the abdominal segments the 3<sup>rd</sup> to 5<sup>th</sup> fused, and two pleopods were present (Fig. 3E). Unlike the male host, the female one infected with *Sacculina* sp. had no obvious morphological changes (Fig. 3D, G).

*Cancrion* sp. infected three males of sexually mature size (CW of 28, 36, and 37 mm) and one ovigerous female (CW of 29 mm). All infected specimens performed the swelling on the dorsal surface carapace corresponding to the position of which *Cancrion* sp. occupied the body cavity (Fig. 4B).

## DISCUSSION

The swimming crab *C. truncata* is a commonly exploited species in Khanh Hoa province, the south center of Vietnam. However, this species has not attracted much research interest. The present study is the initial survey on the fauna of symbiotic crustaceans of this swimming crab.

**Species composition.** On 408 *C. truncata* individuals randomly sampled from fishing boats operating in Nha Trang Bay, six symbiotic species were found, *inter alia* four epibiotic and two obligate parasite species. Three out of four registered pedunculate barnacles, *O. angulata*, *O. warwicki*, and *D. tridens*, are common epibionts recorded on *C. truncata* in Hong Kong [Leung, Jones, 2000] and several other crab species in Singapore, Thailand, and Pakistan [Jeffries et al., 1982, 2005; Kumaravel et al., 2009]. In Vietnam, these common barnacles were reported from four different swimming crab species: *P. pelagicus*, *P. sanguinolentus*, *M. haanii*, and *C. feriata* [Dang et al., 2022; Oanh et al., 2018, 2022a, b].

On the contrary, *O. alata* was only noted from gills of an ashore lobster *Palinurus* sp. in the Java Sea, Indonesia [Aurivillius, 1894; Zevina, 1982]. The present study recorded a new host and new geographical area of this rarely occurring barnacle. Moreover, acorn barnacles, *Chelonibia testudinaria* (Linnaeus, 1758) and *Semibalanus* sp., are epibionts occupying swimming crabs [Dang et al., 2022; Hosseini et al., 2023]. These species infestation on *C. truncata* have not recently been noted.

Rhizocephalan barnacles are specialized parasites infecting portunid crabs. Hitherto, there have been more than 50 swimming crab species infected by Rhizocephala, including six species recorded in Vietnam: *P. sanguinolentus*, *C. feriata*, *Charybdis anisodon* (De Haan, 1850), *Thalamita crenata* Ruppell, 1830, *Thalamita prymna* (Herbst, 1803), and *Podophthalmus vigil* (Fabricius, 1798) [Boschma, 1954; Lützen, ThiDu, 1999; Oanh et al., 2018, 2022a]. As already mentioned, on *C. truncata*, two parasitic barnacle species were reported, *L. nierstraszi* in Japan [Boschma, 1938] and *S. scabra* in Hong Kong [Chan, 2004]. In the present study, Rhizocephala representatives were noted from one male and one female *C. truncata*. The initial morphological analysis suggested that those belong to the genus *Sacculina*. For proper species identification, complex analysis is required.

Isopods of the family Entoniscidae, like Rhizocephala ones, are obligate parasites on anomurans, brachyuran crabs, and caridean shrimps [McDermott et al., 2019; Shiino, 1942]. Forty-one species of this family have been described. Five species, namely *Portunion moniezii* (Giard, 1878), *Priapion fraisei* (Giard & Bonnier, 1888), *Cancrion australiensis* (Shields & Earley, 1993), *Micippion asymmetricus* (Shiino, 1942), and *C. kxanhensis*, were reported on swimming crabs *Portunus puber* (Linnaeus, 1767), *Portunus holsatus* (Fabricius, 1798), *Thalamita sima* (H. Milne Edwards, 1834), *C. feriata*, and *M. haanii*, respectively [Giard, 1878; Giard, Bonnier, 1888; Mushtaq et al., 2016; Oanh, Boyko, 2020; Shields, Earley, 1993]. *Cancrion* sp. was possibly a new species to science. It may be the sixth entoniscid species that infected swimming crabs globally and the second one found in Vietnam.

**Infestation and modification.** Previous studies showed as follows: the number of epibiotic crustacean species *per* host ranges within 1–5; the prevalence and mean intensity by each pedunculate barnacle species varied within 22–100% and 2.5–75.6 ind. *per* host, respectively [Dang et al., 2022; Jeffries et al., 1982, 2005; Kumaravel et al., 2009; Oanh et al., 2018, 2022a, b]. Four epibiotic species infested *C. truncata* with low prevalence, 0.5–6.9%, and mean intensity of 1.2–1.6 ind. *per* host. The host's integument provides protective shelter, and respiratory currents supply epibionts with oxygen and nutrition [Jeffries et al., 1982]. *C. truncata* was smaller than other swimming crab hosts when comparing the body size; it means the space of gill chambers might be smaller, and respiratory currents might be weaker [Arudpragasam, Naylor, 1964]. These could limit the infestation by epibionts on this crab species.

*O. angulata* was the dominant symbiont on *C. truncata*, with the highest infestation percentage (6.9%). This value is consistent with data of studies on other swimming crab hosts [Oanh et al., 2018, 2022a, b]. The statistical analysis showed that *O. angulata* prevalence on ovigerous crabs (12%) was higher than on non-ovigerous ones (2.7%) and positively correlated with size of hosts. Epibiotic barnacle larvae reach inter-molt crabs, attach to them, and develop until being removed in the next molt of the host [Jeffries et al., 2005]. Generally, the bigger the crab, the longer the period between molts [Josileen, Menon, 2005]. Therefore, the prevalence of infestation by epibiont was usually higher in larger crabs.

The prevalence of infestation by *Sacculina* sp. (0.5%) was remarkably lower than by *Diplothylacus sinensis* (Keppen, 1877) on *P. sanguinolentus* (15%) [Lützen, ThiDu, 1999] and *S. serenei* on *C. feriata* (9.7%) [Oanh et al., 2022a] in the same region. Infection with rhizocephalan species often causes morphological changes in the crab host. Its organs may be affected by developing Rhizocephala: degradation of pleopods and appendages may occur. On the contrary, abdomen and marginal setae which help to protect parasites from external effect can grow more than usual [Waiho et al., 2021]. Morphological modifications were clearly observed in both male and female hosts, as in the case of *Sacculina beauforti* Boschma, 1949 infection on the mud crab *Scylla olivacea* (Herbst, 1796) [Waiho et al., 2017].

On the other hand, *Carcinus maenas* (Linnaeus, 1758) infected by *Sacculina carcini* Thompson, 1836 showed the changes in male crabs, while minor differences occurred in female ones [Kristensen et al., 2012]. In the present study, one infected male crab possessed a broadened abdomen, and pleopods degraded; this could lead to its castration. Despite the low infected rate (2 infected crabs out of > 400 randomly sampled ones), morphological alteration was also observed. Further studies are urgently required to determine the natural prevalence of infection, as well as possible effects on the host populations.

Parasitic isopods of the family Entoniscidae were reported to cause the vertical swelling of the crab carapace. The more profound effect may be the castration of a male and/or female in some cases [Shiino, 1942]. *Cancrion* sp. prevalence (1.1%) on Vietnamese *C. truncata* was low, compared to *C. australiensis* infection on *T. sima* (13.9%) [Shields, Earley, 1993] and *C. kanhensis* infection on *M. haanii* (10.1%) [Oanh, Boyko, 2020]. The morphological modifications which may cause the castration of infected *C. truncata* were not recorded herein. With only four infected crabs examined, it is impossible to fully assess the effects of parasitic isopods on the crab hosts. Further sampling is essential for species identification and examination of morphological and physiological changes/alterations.

### Conclusion:

1. Six symbiotic crustacean species, including four epibiotic and two parasitic ones, were detected on the swimming crab *Charybdis truncata* sampled in Nha Trang Bay, Khanh Hoa province, Vietnam, the East Sea.
2. *C. truncata* is a new host record for the pedunculate barnacle *Octolasmis alata* and parasitic isopod *Cancrion* sp.
3. The intensity of symbionts did not depend on the host sex, size, and reproductive status of female crabs.
4. The infection with a rhizocephalan *Sacculina* sp. caused morphological modifications in male swimming crabs that could result in a loss of reproduction capacity of male hosts.

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## СТАТУС ЗАРАЖЁННОСТИ СИМБИОТИЧЕСКИМИ РАКООБРАЗНЫМИ КРАБА-ПЛАВУНЦА *CHARYBDIS TRUNCATA* (FABRICIUS, 1798) ИЗ ЗАЛИВА НЯЧАНГ, ВЬЕТНАМ (ВОСТОЧНОЕ МОРЕ)

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Симбиоз — это довольно часто встречающиеся отношения между организмами в морских экосистемах. Среди крабов важного с экономической точки зрения семейства Portunidae широко распространён *Charybdis truncata* (Fabricius, 1798), однако его симбиотические комплексы всё ещё исследованы недостаточно. В январе — августе 2022 г. в заливе Нячанг (провинция Кхань-хоа, Вьетнам, Восточное море) собрано 408 особей *C. truncata*. Симбионты классифицированы на основе морфологических характеристик. Для сравнения статуса заражённости симбионтами применён статистический анализ. Обнаружено шесть симбиотических видов, в том числе четыре вида ракообразных-эпобионтов [*Octolasmis angulata* (Aurivillius, 1894), *Octolasmis alata* (Aurivillius, 1894), *Octolasmis warwicki* Gray, 1825 и *DianaJonesia tridens* (Aurivillius, 1894)] и два облигатных паразита, не идентифицированных до вида [*Cancrion* sp. и *Sacculina* sp.]. Из них *O. alata* и *Cancrion* sp. отмечены на *C. truncata* впервые. Общая встречаемость симбионтов на крабах-хозяевах составила 13 %, а количество видов симбионтов, заселяющих хозяев, варьировало от одного до четырёх. *O. angulata* был доминантом, его встречаемость составила 6,9 %. Встречаемость *O. angulata* была значительно выше у самок крабов с кладкой икры, чем у самок без неё. Кроме того, зарегистрирован существенный рост встречаемости *O. angulata* по мере увеличения размеров крабов. Достоверных различий в средней интенсивности симбионтов по полу, репродуктивному статусу самок и размерам крабов-хозяев не выявлено. Зафиксированы и описаны первоначальные морфологические изменения, вызванные заражением краба паразитами *Cancrion* sp. и *Sacculina* sp. Эти результаты предоставляют информацию о состоянии естественного заражения *C. truncata* симбиотическими видами, важную для менеджмента рыболовства и для развития аквакультуры.

**Ключевые слова:** *Charybdis truncata*, симбионты, эпобионты, облигатные паразиты, залив Нячанг, Вьетнам