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ORIGINAL ARTICLE

Species diversity and molecular taxonomy of symbiotic crustaceans on *Portunus pelagicus* (Linnaeus, 1758) in Vietnam, with remarks on host records and morphological variation

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ABSTRACT

The blue swimming crab (*Portunus pelagicus* (Linnaeus, 1758)) is an economically and ecologically important species in Vietnam, and a potential subject for aquaculture as well. More than 400 individual crabs were collected along the Vietnamese coastline and examined for ectosymbiotic crustaceans. Two molecular markers (28S rRNA and COI mtDNA) were applied for species delineation. Seven species were reported and described, of which six are cirripede barnacles (Thecostraca, Thoracia); and one parasitic copepod *Choniosphaera indica* Gnanamuthu, 1954 (Copepoda, Podoplea). Four species (*Chelonibia testudinaria* (Linnaeus, 1758), *Semibalanus* sp., *Octolasmis neptuni* (MacDonald, 1869), and *Dianajonesia tridens* (Aurivillius, 1894)) were the first records for both host and location. The symbiotic crustaceans occupy specific niches on the crab body, and vary in their infestation status. Molecular taxonomy of symbiotic crustaceans was classified and confirmed based on sequence similarity and phylogenetic analyses.

KEYWORDS

Crustaceans, infestation, phylogeny, Portunidae, symbionts

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Introduction

The swimming crab, *Portunus pelagicus* (Linnaeus, 1758) is a major economic species throughout the Indo-Pacific and to the coast of Africa (Galil and Innocenti, 1999). In Vietnam, it is widely distributed throughout the coastal waters and nearby islands (Vu *et al.*, 2014). Throughout its native range, it is a valued market organism with numerous reports of its commercial value as a multi-million dollar export commodity between Vietnam and Japan and the United States of America (VASEP, 2021). Crabs play ecological important roles, through complex food webs, in coastal and marine ecosystems, especially in mangrove forests, seagrass beds and coral reefs (Kunsook *et al.*, 2014a; 2014b). Additionally, their carbonate carapaces are widely known as a living substrate for many epibiont/symbiont organisms (Abelló and Corbera, 1996; Gaddes and Sumpton, 2004; Dvoretsky, 2012; Machado *et al.*, 2013).

Reciprocal selection pressure between the host and its symbiotic species can potentially alter species diversity, ecological function, and community dynamics (Galil and Innocenti, 1999). The diversity of the symbiotic community on *P. pelagicus* is known to depend on its geographic distribution (Shields, 1992; Shields and Wood, 1993). Among the symbiotic crustacean species, the barnacles frequently found attached to decapod crustaceans have received the most attention. Their symbiotic association depends on the host's biological characteristics, such as distribution, sex, maturity stage, molt cycle, and size (Weng, 1987; Gaddes and Sumpton, 2004; Klinbunga *et al.*, 2007; Babu *et al.*, 2012; Machado *et al.*, 2013). Growing numbers of symbiotic species have been reported on portunid decapod species (Hudson and Lester, 1994; Isaeva *et al.*, 2005; Babu *et al.*, 2012). In Vietnam, several symbiotic species have so far been detected on *P. pelagicus* and *Portunus trituberculatus* (Miers, 1876). Among these, two species (*Carcinonemertes mitsukurii* Takakura, 1910 and *Choniosphaera indica* Gnanamuthu, 1954), recognized egg eating parasites, are known to have a negative impact on host populations (Vo *et al.*, 2013; Le *et al.*, 2018a; 2018b).

In the past decade, molecular markers have been increasingly applied to investigate species diversity and phylogenetic relationships, including research examining the phylogenetic position and evolution of Cirripedia (Mizrahi *et al.*, 1998; Wu, 2011; Kwiatkowski *et al.*, 2012; Yusa *et al.*, 2012; Filipiak *et al.*, 2016). So far, no research has focused on elucidating the phylogenetic relationships of the symbiotic crustacean community on the blue swimming crab.

The current research conducts the first comprehensive study of species diversity, infestation status, and molecular taxonomy of symbiotic crustacean associations occurring on *P. pelagicus* distributed along the Vietnamese coastline.

Material and Methods

Symbiotic crustacean sampling, identification, and infestation status

Blue swimming crabs (*P. pelagicus*) were collected from the Vietnamese coastline from Cat Ba Island - Hai Phong, Ha Long Bay - Quang Ninh in the North; Nha Trang and Van Phong Bay - Khanh Hoa, Song Cau and Tuy Hoa - Phu Yen in the Center, and Phu Quoc Island and Rach Gia - Kien Giang in the South. The crabs were transported alive in aerated sea water to the laboratory where they were kept in aquaria until dissected. Sampling information of crab individuals collected is presented in Tab. 1.

Crabs were examined externally for symbiotic crustaceans. Each crab was divided into 6 separate parts: (1) Carapace; (2) Limbs (maxillipeds, chelipeds, and swimming legs); (3) Abdomen, including egg clutches (if any); (4) Mouth parts; (5) Gills; and (6) Sternum (see Appendix, Fig. A1). Each part was placed separately into petri dishes containing clean seawater for inspection by the naked eye and a stereoscope (Olympus SZX9).

Freshly collected symbiotic crustacean species were used for the descriptions and identification. The individuals intended as whole-mounts were transferred to a vial of 70 % alcohol, and those for DNA analysis were preserved in 95 % EtOH, and stored at -20 °C. Symbiotic crustaceans were identified using various keys and species descriptions follow Jeffries and Voris (1996); Jeffries *et al.* (2005); Cheang *et al.* (2013). Infestation status of symbiotic species were examined by prevalence and mean intensity, as defined in Margolis *et al.* (1982) and Rózsa *et al.* (2000).

Table 1. Sampling site information and number of individuals of *Portunus pelagicus* collected along the Vietnam coastline.

Ecoregion	Sampling location	Longitude	Latitude	No. of Individuals
North	Cat Ba Island - Hai Phong	107°00'57.25"E	20°45'01.745"N	91
	Ha Long Bay - Quang Ninh	107°04'59.42"E	20°56'55.43"N	58
Central	Nha Trang and Van Phong Bay - Khanh Hoa	109°13'02.39"E 109°21'31.60"E	12°12'46.85"N 12°29'40.12"N	100
	Song Cau and Tuy Hoa - Phu Yen	109°13'46.54"E 109°18'48.98"E	13°27'36.21"N 13°05'02.596"N	100
South	Phu Quoc Island – Kien Giang	103°57'11.07"E	10°13'23.06"N	41
	Rach Gia - Kien Giang	105° 04'28.85"E	90°59'23.39"N	89

Molecular taxonomy

DNA was extracted from individuals of each collected symbiont species using DNeasy Tissue Extraction Kit (Qiagen) in accordance with the manufacturer's instructions. 28S rRNA and COI (Cytochrome c oxidase subunit I) mitochondrial DNA were amplified using primer LSU 5 and 1500R (Olson *et al.*, 2003), and LCO1490 and HCO2198 (Folmer *et al.*, 1994), respectively.

PCR reactions were performed using a total volume of 50 µl with components at the following concentrations: 10 µL of DreamTaq buffer 10X (Thermo Fisher Scientific), 2 µL dNTP (10 mM), 2 µL each primer (10 mM), 1.25 unit of DreamTaq polymerase (5U/µl), 5 µl DNA template and distilled water to the final volume. Amplification was implemented using the following PCR profile: a preliminary denaturation at 94 °C for 3 minutes (min), followed by 35 cycles of 94 °C for 45 seconds (s), annealing for 45 s (for 28S rRNA, COI mtDNA genes at 56 °C, 42 °C, respectively), and then 72 °C for 45 s. This was followed by a final extension period at 72 °C for 7 min before the samples were cooled to 4 °C. PCR products were run on a 1.5 % agarose gel for confirmation of equal length against an appropriate size marker. The PCR products were purified using DNA purification kits (Promega) and pre-sequenced using dye– labels dideoxy terminator (Big Dye Terminator 3.1, Applied Biosystems) with the same primer as the PCR reactions at the following temperatures: 96 °C for 30 s, 50 °C for 30 s and 60 °C for 4 min. Sequences of both strands were generated on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) using the amplification primers.

Sequence contigs were assembled using the Geneious Pro 5.5.7 (Kearse *et al.*, 2012). The resulting sequences were confirmed by the Basic Local Alignment Search Tool (BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences were aligned, analyzed using BioEdit 7.0.5.3 (Hall, 1999), and submitted to GenBank. Sequence identity matrix was used to investigate similarity/identity values. Information on gene application, and Genbank accession numbers are presented in Tab. 2.

To confirm the molecular taxonomy, phylogenetic analyses were conducted using obtained 28S rRNA and COI mtDNA of collected symbiotic crustaceans (7 sequences of two genes and 17 available Genbank sequences (Tab. 2). Phylogenetic trees were constructed using Bayesian inference (BI) approaches. Prior to BI analysis, best-fit models of nucleotide substitution were selected by the Akaike Information Criterion as implemented by MrModeltest 2.2 (Nylander, 2004). The best selected models were GTR+G for 28S rRNA and GTR+I+G for COI data sets, respectively. BI analyses were conducted in MrBayes 3.2.6 (Huelsenbeck and Ronquist, 2001) under the selected best-fit models and parameters. Four chains were used, and the analysis was run for 1 million generations, with the sampling frequency of 100. Each analysis was repeated twice to check for similarity of the likelihood plateau. Additionally, parameter values were evaluated for convergence throughout the run by using the "sump" command in MrBayes and by examining results in Tracer 1.3 (Drummond *et al.*, 2005). Plots from Tracer were used to determine the appropriate number of trees to be discarded in the "burn in" and a final 50 % majority-rule consensus tree was constructed from the remaining trees.

Table 2. Species list, infestation status (*Portunus pelagicus*, n = 479), and Genbank accession numbers for all sequences of symbiotic crustaceans used in the phylogenetic analysis. (1) Carapace, (2) Limbs (maxilliped, cheliped, and swimming legs), (3) Abdomen, including egg clutches (if any), (4) Mouth parts, (5) Gills, and (6) Sternum. * - sequences from Genbank; x - sequences not available; NI: no information

Species	Prevalence Mean Intensity	Infection sites	COI mtDNA	28S rRNA	Host/substrata
I. Class: Thecostraca, Subclass: Cirripedia, Infraclass: Thoracica					
I.1. Order: Lepadiformes					
Octolasmis angulata	75.57 57.26±93.06	(2), (3), (5)	MH753551	MH727736	Swimming crab (*Portunus pelagicus*)
Octolasmis neptuni	11.48 21.55±30.26	(2), (3), (5)	MK541906	MH727737	Swimming crab (*Portunus pelagicus*)
Octolasmis warwicki	15.45 23±31.12	(1), (2), (3), (4)	MH753552	MH727739	Swimming crab (*Portunus pelagicus*)
*Octolasmis cor**			KC138499	EU082326	NI
Octolasmis sp.*			x	EU082327	NI
*Octolasmis unguisiformis**			LC467960	LC467957	Sea urchin (*Echinothrix diadema*)
Dianajonesia tridens	19.00 7.95±8.49	(2), (3), (4), (5)	MH753553	MH727738	Swimming crab (*Portunus pelagicus*)
I.2. Order: Sessilia					
Chelonibia testudinaria	14.82 9.32±9.96	(1), (2), (6)	MH753554	MK087648	Swimming crab (*Portunus pelagicus*)
*Chelonibia testudinaria**			KF042514	AB723914	Green sea turtle (*Chelonia mydas*)
*Chelonibia testudinaria**			KF042515	KM217527	Hawksbill sea turtle (*Eretmochelys imbricate*)
*Chelonibia patula**			JF823674	EU082295	Mud crab (*Scylla serrata*)
*Chelonibia manati** (= *C. testudinaria*)			JN589813	AB723917	West Indian manatee(*Trichechus manatus*)
*Chelonibia caretta**			KF042512	AB723915	Hawksbill sea turtle (*Eretmochelys imbricate*)
*Chelonibia caretta**			KF042513	KM217526	Loggerhead Sea turtle (*Caretta caretta*)
*Semibalanus cariosus**			KM611728	AY520593	NI
*S. balanoides**			MF748337	EU370440	NI
Semibalanus sp.	0.21/6	(1)	MH753555	MH727740	Swimming crab (*Portunus pelagicus*)
*Sacculina carcini**			KT209175	AY520622	NI
II. Class: Copepoda, Superorder: Podoplea					
II.1. Order: Siphonostomatoida					
Choniosphaera indica	2.09 20.90±4.97	(3)	MK541907	MK087649	Swimming crab (*Portunus pelagicus*)
*Asterocheres lilljeborgi**			KR049050	KR048868	NI
*Parabrachiella hugu**			KT030285	KR048861	NI
II.2. Order: Harpacticoida					
*Canthocamptus staphylinus**			MF077881	MF077853	NI
*C. coreensis**			KT030277	KR048886	NI

Results

Crustacean symbiont diversity and infestation status

In total, seven symbiotic crustaceans were found, with the most common symbiont being the pedunculate barnacle *Octolasmis angulata* Aurivillius, 1894 (Prevalence 75.57 %, Mean intensity 57.26 ± 93.06). Its two congeners (*O. neptuni* and *Octolasmis warwicki* Gray, 1825), and the closely related species *Dianajonesia tridens* Aurivillius, 1894 (WoRMS, 2020; Young, 2001) were found to have moderate abundance (> 10 %). The external morphology of these four species (Fig. 1) was consistent with previously published descriptions (Jeffries *et al.*, 2005).

Octolasmis spp. showed the greatest variety of preferred infection sites. *Octolasmis warwicki* infected most of the body parts of the crabs, except for the gills, while its two congeners (*O. angulata* and *O. neptuni*) do not appear on the carapace. *Dianajonesia tridens* was found on most body parts, but absent from the carapace (Tab. 2, Fig. 1).

Taxonomic records

***Chelonibia testudinaria* (Linnaeus, 1758)**

Host and location: *Portunus pelagicus*, Ha Long and Kien Giang, new record for Vietnam.

Material examined and measured. Ten live specimens, including 4 specimens from a female crab (87 mm CW, 25 August 2016, locality Ha Long, collector T. Q. Sang) and 6 from a male crab (100 mm CW, 14 May 2016, locality Kien Giang, same collector).

Morphological description (Fig. 2A–D). Shell conical or evenly rounded, heavy, flat and smooth with a diameter of 7.09 ± 4.75 (1 – 15) mm (n = 10), with 6 calcium plates (1 carina, 1 rostrum, and 4 lateral), solidly joined with each other forming a hard shell surrounding the body. The orifice opening 3.33 ± 1.98 (0.5 – 6) mm long (n = 10), partially covered by 2 tergum and 2 triangular patella (Fig. 2A). Dwarf males found settled on the plates and are distributed randomly (Fig. 2B).

Infestation status. *Chelonibia testudinaria* is recorded as a moderately abundant species (Prevalence 14.82 %, mean intensity 9.32 ± 9.96 (Tab. 1)) and it occupied the outer surface (carapace and limbs), and inside of the sternum (Fig. 2C, D).

Remarks: *Chelonibia testudinaria* is well-known as a successful generalist epibiotic barnacle. It is found on a wide range of marine hosts. Three species of *Chelonibia* Leach, 1817 have been described: *C. testudinaria* on sea turtles (Rawson *et al.*, 2003; Zardus and Hadfield, 2004; Cheang *et al.*, 2013), *Chelonibia manati* Gruvel, 1903 on Sirenians, and *Chelonibia patula* (Ranzani, 1818) on crustaceans, e.g. blue crab (Frazier and Margaritoulis, 1990; Bakır *et al.*, 2010; Udoh and Otoh, 2017). Based on genetic characteristics, these three species were later identified as morphotypes of the same species and synonymized under *C. testudinaria* (see Cheang *et al.*, 2013; Zardus *et al.*, 2014). Geographically, specific clades were also detected for *C. testudinaria* throughout its distribution range such as in the Atlantic, Indian - West Pacific, and Eastern Pacific Oceans (Rawson *et al.*, 2003; Zardus *et al.*, 2014). *Chelonibia testudinaria* was previously recorded on *Portunus pelagicus* (see Pasternak *et al.*, 2002; Bakır *et al.*, 2010; Babu *et al.*, 2012; Sami, 2018); this, however, is the first record in Vietnam.

***Semibalanus* sp.**

Host and Location. *Portunus pelagicus,* at Khanh Hoa, Vietnam, new record for the genus to both host and location.

Material examined and measured. All specimens (n = 4) from a female crab (141 mm CW, 17 Mar 2016, locality Nha Trang Bay, collector L. T. K. Oanh).

Morphological description (Fig. 3A, B). The shell is ovoid, diameter of 3.35 ± 1.04 (2 – 4.5) mm (n = 4), with 6 plates (1 carina, 1 rostrum, 2 carinolateral and 2 lateral) truncated pyramidal, grey-white, thin, fragile, and rather smooth surface. Parietes tubiferous with a single row of major tubes. Carina trapezoidal-shaped; lateral borders overlap and do not close up

with the carinolateral plates. Rostrum wide, curved trapezoidal-shaped, borders overlap the border of the laterals. Carinolateral narrow with border overlapped by carina and laterals. Orifice wide, diamond-shaped; operculum cover, diamond-shape; scutum wide, triangular with horizontal striations parallel with the basal edge and inconspicuous adductor ridge; tergum narrow and beaked with a narrow and long spur (Fig. 3A). The basis is membranous and very closely cemented to the crab carapace. The tissue inside is white (Fig. 3B).

Infestation status. *Semibalanus* sp. occurred with low prevalence (0.21 %), and was only found on the carapace of one individual host crab.

Remarks. *Semibalanus* Pilsbry, 1916 is the genus of acorn barnacle most abundant on tropical intertidal zones. Four species are currently recorded for this genus. The two common ones (*Semibalanus balanoides* (Linnaeus, 1767), and *Semibalanus cariosus* (Pallas, 1788)) are found mostly attached to rock (Takeda *et al.*, 1998; Brousseau and Goldberg, 2007; Gyory, 2011), and rarely on crabs (McDermott, 2007); *Semibalanus madrasensis* (Daniel, 1958) was found on a fishing craft (Daniel, 1958), and *Semibalanus sinnurensis* (Daniel, 1962) on a mollusc shell (Daniel, 1962). This *Semibalanus* specimen shared some similar characters with *S. balanoides* (parallel grooves on the opening surface), and *S. sinnurensis* (parietes provided with minute calcareous projections), however, the observed external morphological characters (of only two individuals), and the DNA sequences are not enough for species identification. Moreover, the occurrence of *Semibalanus* sp. on *Portunus pelagicus* is assumed to be incidental.

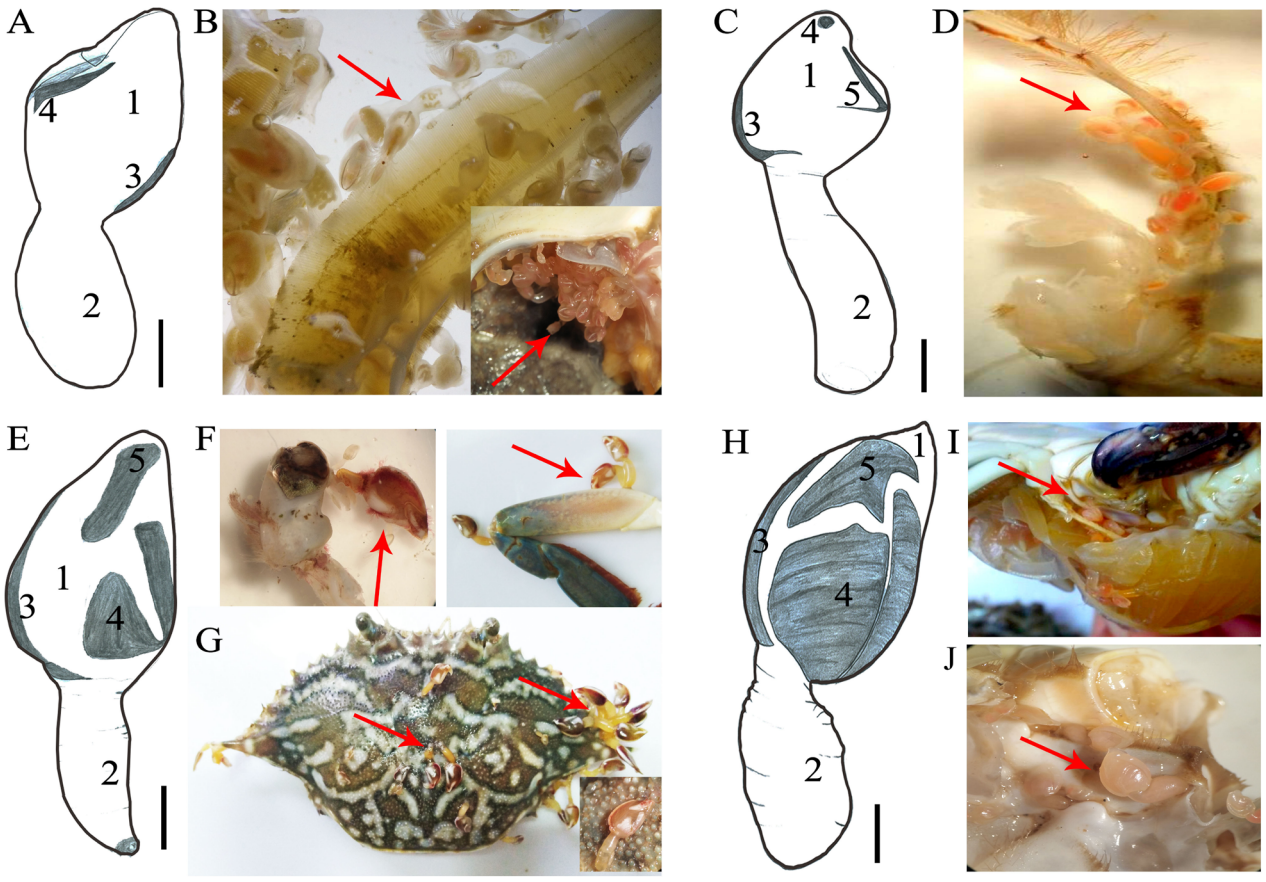

Figure 1. Line drawings and photo images for description and distribution of infected sites of symbiotic crustaceans on *P. pelagicus*. *Octolasmis angulata* (**A**) and infected sites (gill lamella (above) and gill chamber (inset) (**B**); *Octolasmis neptuni* (**C**), on the maxilliped (**D**); *Octolasmis warwicki* (**E**), and on the eye (**F**, left), walking leg (**F**, right), carapace (**G**); *Dianajonesia tridens* (**H**), and on the coxa of cheliped (**J**), gill (**I**). Arrows indicate the symbionts. 1: Capitulum, 2: Peduncle, 3: Carina; 4: Scutum; 5: Turgum. Scale bar = 1mm.

***Choniosphaera indica* Gnanamuthu, 1954**

Host and Location. *Portunus pelagicus* in Khanh Hoa, Vietnam.

Material examined and measured. All specimens (n = 5) from an ovigerous female crab (125 mm CW, 17 Mar 2016, locality Nha Trang Bay, collector L. T. K. Oanh).

Morphological description (Fig. 4A–C). The adult female is seed-like and ellipsoid in outline, size 800 – 1200 µm (1014 ± 67.04) × 400 – 700 µm (552 ± 119.57) (n = 5), and the posterior part projects with the caudal styles. The abdomen is fused with the cephalothorax, and the esophagus occupies most of the abdominal cavity. *Choniosphaera indica* body is yellow or light pink with two black eyes visible on the head. The cup-like mouth tube is surrounded by the maxillae and the maxillipeds. The mouth is at the bottom of the cup and has a membranous lip (Fig. 4A1). The maxilliped is a long appendage of four unequal segments with the second one longer than the others; it has two short recurved spines and a long, comb-shaped spine on its fourth segment. Two antennas; the first antennae has five articles. The first article bears a spine, the fourth has three spines, and the terminal article carries three spines (Fig. 4A2). The second antennae are five-segmented. The third article has a spine, and the terminal article carries two long slender spines (Fig. 4A3). The tail forms two branches, assisting attachment to the crab egg (Fig. 4A, B).

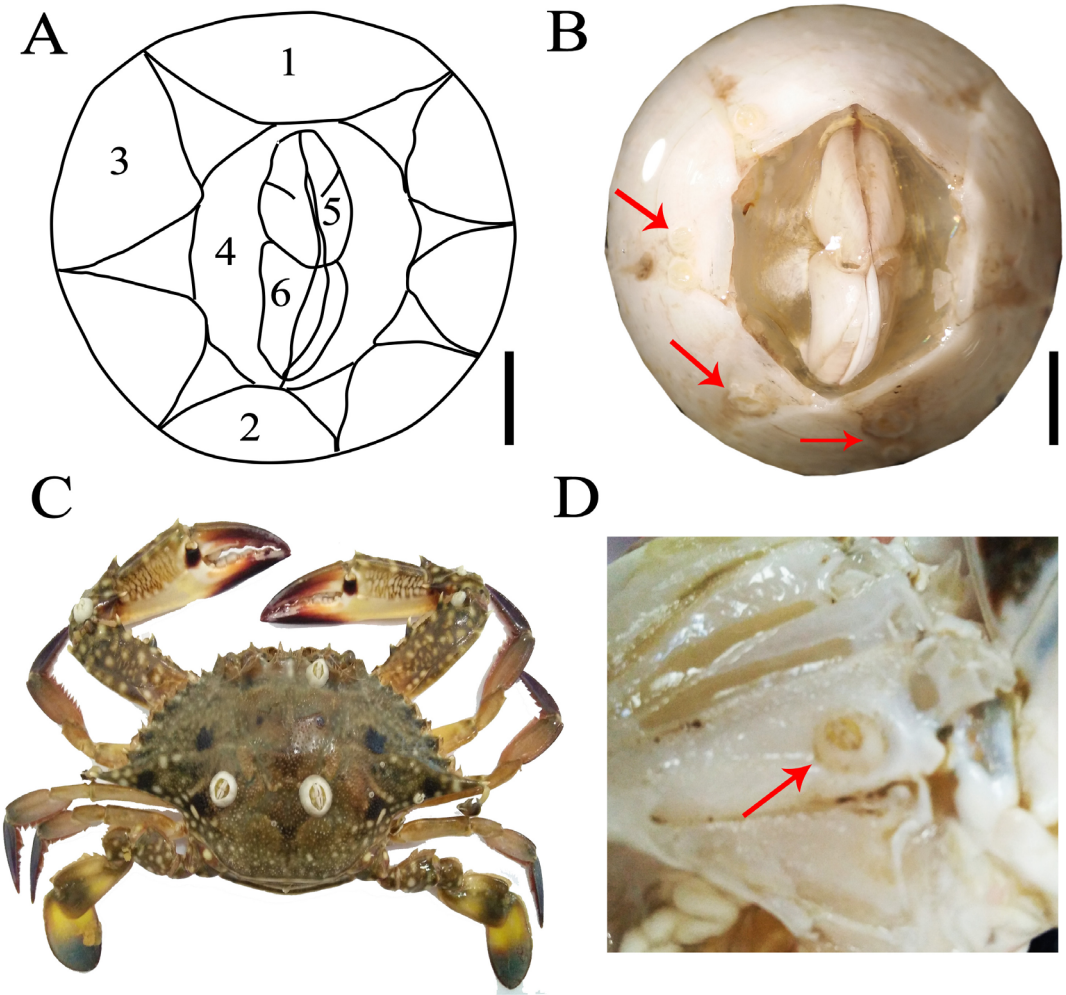

Figure 2. Line drawing (**A**) and photo image (**B**) of a large individual of *Chelonibia testudinaria* on *Portunus pelagicus*. Note: Arrows indicate dwarf male distributed to the radii. Infestation sites on the host: carapace and limbs (**C**), and inside of the sternum (arrow) (**D**). 1: Carina; 2: Rostrum; 3: Lateral; 4: Orifice Opening; 5: Tergum; 6 Scutum; Scale bar = 2 mm (for **A** and **B** only).

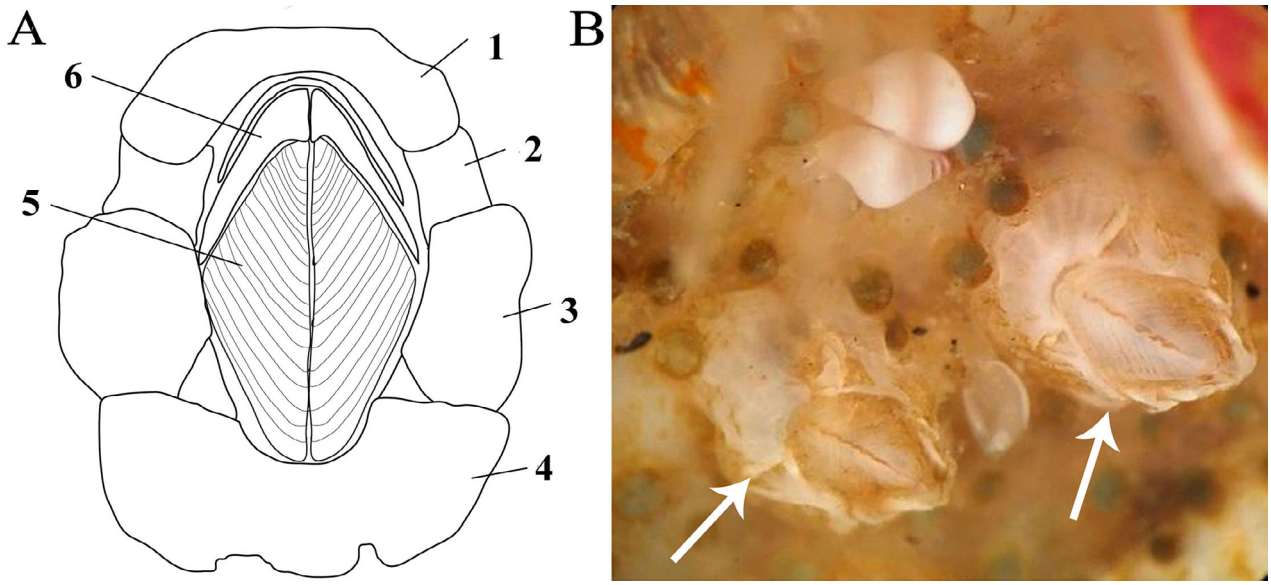

Figure 3. Line drawing (**A**) and photo image (**B**) of *Semibalanus* sp. individuals (arrows) attached to the carapace of *Portunus pelagicus*. Note: two unidentified gastropod snails in image. 1: Carina; 2: Carinolateral; 3: Lateral; 4: Rostrum, 5: Scutum;6: Tergum. Scale bar = 1mm for **A**, and 0.5 mm for **B**.

Infestation status: *Choniosphaera indica* occurred with low prevalence (0.29 %), and moderate intensity (20.90 ± 4.97), and was found strictly on the egg mass of the female crab host (Tab. 2, Fig. 4C), no larvae of this species were found.

Remarks. *Choniosphaera* spp. are specific egg eating parasites on decapod crabs. Three species have so far been recorded (Costello *et al*., 2001; Connolly, 2010), and among them *C. indica* is the most common. *Choniosphaera indica* was previously reported on *Portunus pelagicus* (Shields, 1992; Vo *et al*., 2013), and considered as a significant contributor to mortality of the crab host, and affecting the host population (Shields and Wood, 1993). Despite being one of the parasites capable of affecting the portunid host species population, no gene sequence of this species is available on GenBank for reference. The present study provides descriptions and images of this species, and also registers two gene sequences (28S rRNA and COI mtDNA) into GenBank.

Molecular taxonomy

In total, seven sequences were generated from each gene region of seven species of symbiotic crustacean on *P. pelagicus* distributing along the Vietnamese coastline. The aligned data contained unambiguous 598 bp and 921 bp of COI mtDNA and 28S rRNA genes, respectively. Compared to GenBank sequences (28S rRNA and COI mtDNA), two species (*O. warwicki* and *C. testudinaria)* showed a 100 % (EU082328 and KC138501) and 99.7 – 99.8 % (AB723914 and AY174338) identity, respectively. As for *O. angulata*, there is no 28S rRNA reference sequence on Genbank, however, the current COI mtDNA sequence shows 100 % matching. The remaining four species have no comparable sequences available in GenBank, therefore, comparisons were conducted with species of the same genus, and/or different genera with high proportion of similarity in a blast search (as is the case with *C. indica*) (see Appendix, Tab. A1). Conflicting results were obtained for the two genes for *Semibalanus* sp., with the COI sequence showing a 100 % identity match to *S. cariosus* (MH753555 and KM611728), but the 28S rRNA gene showing a match of only 93.4 % between the two species (MH727740 and AY520593).

Phylograms from obtained sequence data sets (28S rRNA and COI mtDNA) provided broader view of molecular taxonomy of studied symbiotic crustaceans. The BI approach applied to both data sets produced almost similar tree topologies, except the unidentified position of *C. indica* (Copepoda, Siphonostomatoida) from the COI tree, while it is clustered with Siphonostomatoid species (*Asterocheres* Boeck, 1859 and *Parabrachiella* C.B. Wilson, 1915) on the 28S rRNA phylogram (Fig. 5).

In the 28S rRNA topology, three *Octolasmis* Gray, 1825 species (*O. angulata, O. warwicki,* and *Octolasmis cor* (Aurivillius, 1892)) were clustered together, as a sister clade to *O. neptuni* + *Octolasmis* sp., and *Octolasmis unguisiformis* Kobayashi and Kato, 2003 + *D. tridens* (Fig. 5A). Minor differences were observed in the COI tree, such as *O. angulata, O. warwicki*, and *O. neptuni* grouped as sister species, and *O. cor* and *O. unguisiformis* forming a clade (Fig. 5B). In both cases, *D. tridens* was grouped within the *Octolasmis* species, either as a sister clade to *O. unguisiformis* (28S rRNA) or maintained as separate clade (COI mtDNA).

The sequence differences between *D. tridens* and *Octolasmis* species ranged from 6.9 % (*O. neptuni)* to 9.7 % (*O. unguisiformis)* for 28S rRNA, and 15.7 % (*O. warwicki*) to 21.3 % (*O. unguisiformis)* for COI mtDNA. The differences between the *Octolasmis* species ranged from 0.7 % (*O. neptuni* and *Octolasmis* sp.) to 12.2 % (O. *unguisiformis* and *O. warwicki*), and from 14.5 % *(O. angulata* and *O. warwicki)* to 20.6 % *(O. neptuni* and *O. unguisiformis)* for 28S rRNA and COI, respectively (Appendix, Tab. A2). *Dianajonesia tridens* was previously placed in the genus *Octolamis* and later moved to *Dianajonesia,* represented by nine species (Koçak and Kemal, 2008).

Moderate differences were also seen between the COI and 28S rRNA trees in the relative position of *Semibalanus* sp., which was either identical to *S. cariosus* (COI phylogram) or sister clade to other *Semibalanus* species (28S rRNA phylogram). The sequence differences were 7.6 % and 7.9 % to *S. cariosus,* and *S. balanoides,* respectively (Appendix, Tab. A2). The current *C. testudinaria* specimen clustered in the same clade with previous *C. testudinaria* and conspecifics *C. patula,* and *C. manati*. They all formed a clade with *Chelonibia caretta* (Spengler, 1790).

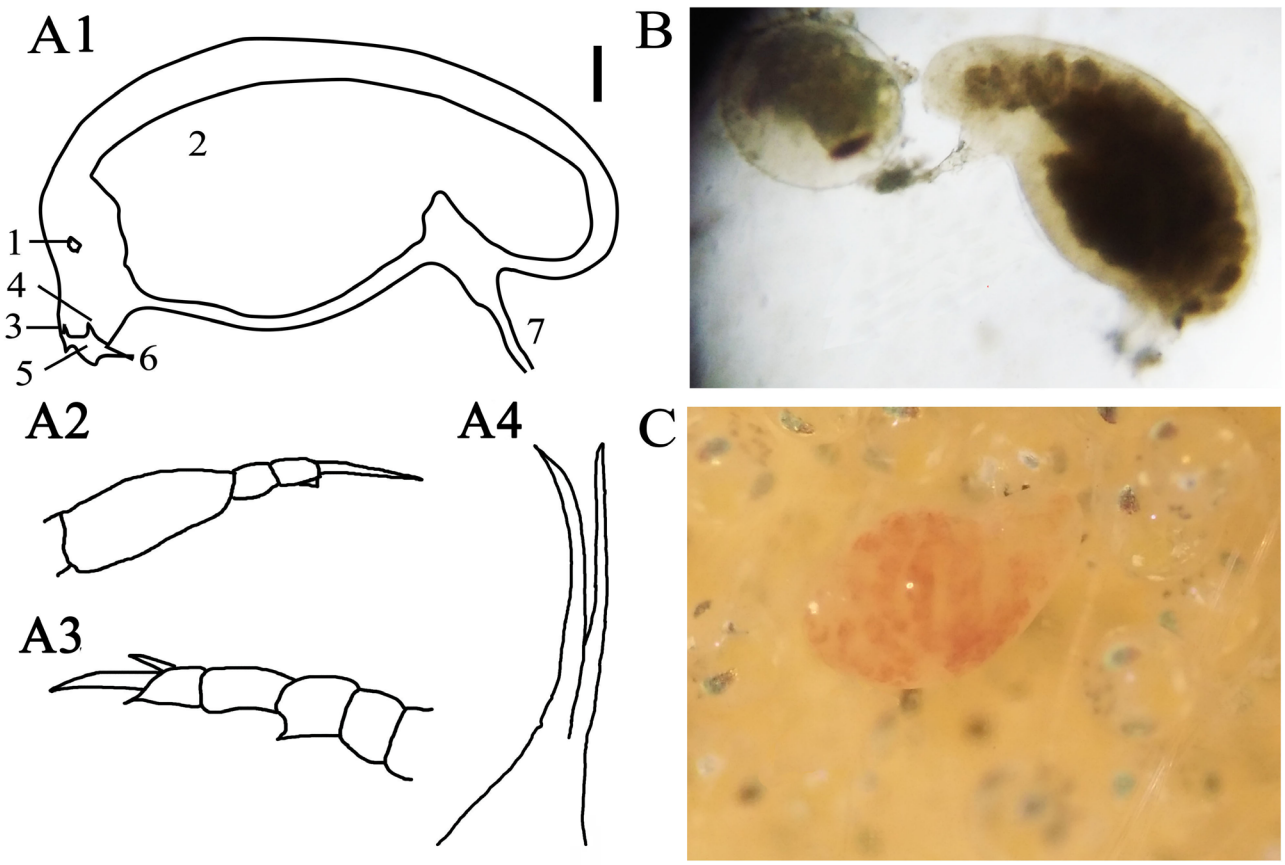

Figure 4. Line drawings (**A**1–4) and photo images (**B**, **C**) of *Choniosphaera indica* on *Portunus pelagicus.* External morphology (**A**1), first antenna (**A**2), second antenna (A3), and the tail (**A**4). *C. indica* eating the egg (**B**) and inside the egg mass (**C**). 1: Eyes; 2: Esophagus; 3: First antennae; 4: Second antennae; 5: Mouth tube; 6: Maxilliped; 7: Caudal portion. Scale bar = 100 mm.

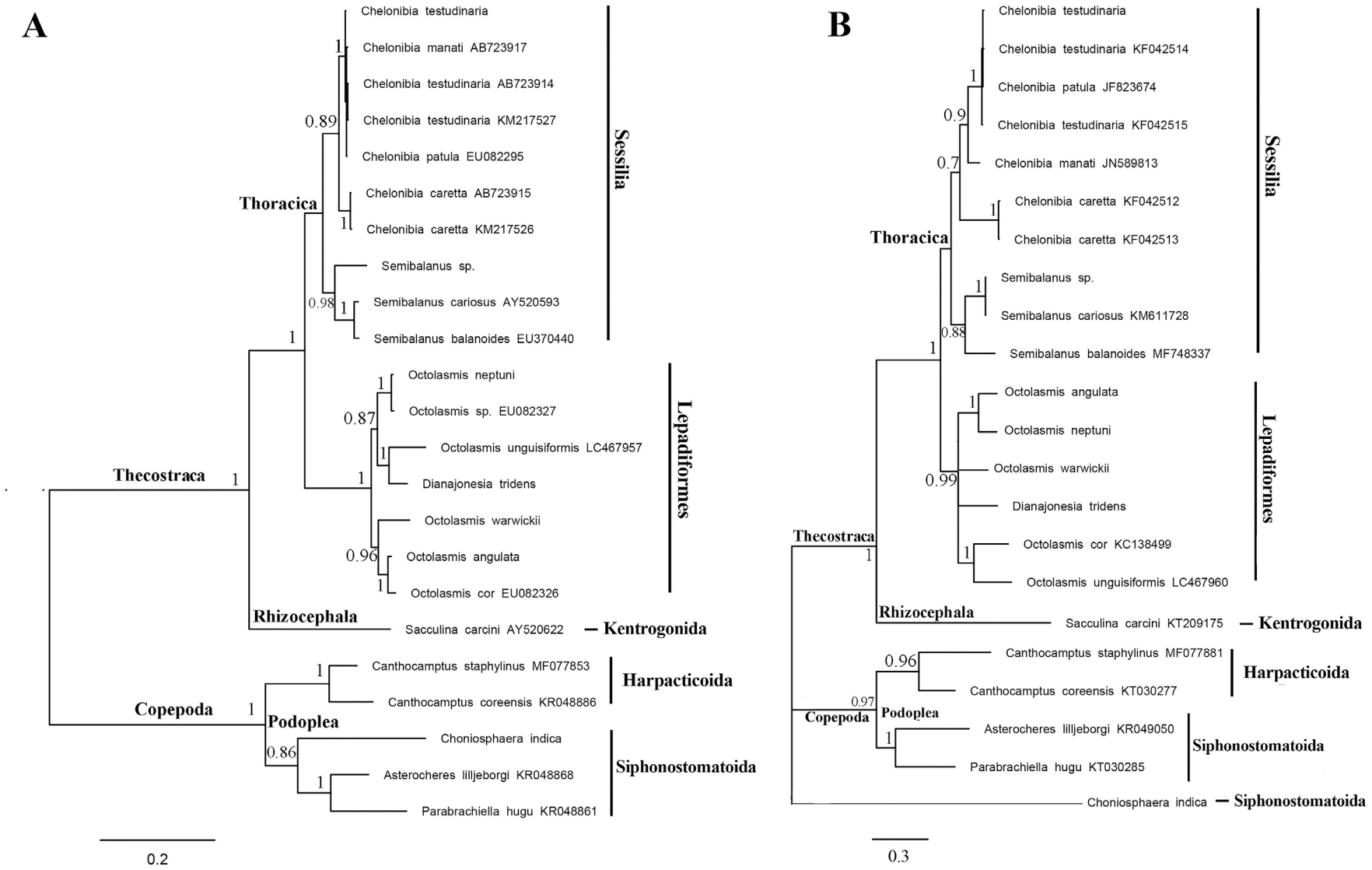

Figure 5. Phylograms of Bayesian Interference trees of the symbiotic crustacean species using 28S rRNA (**A**) and COI mtDNA (**B**) data sets. The values at the tree nodes indicate Bayesian posterior probabilities. Scale bars show number of substitutions per site in the alignment.

Discussion

Seven symbiotic crustaceans (belonging to 2 suborders, 3 families, and 5 genera) were detected on *P. pelagicus* in Vietnam. Of these, four species (*Semibalanus* sp., *C. testudinaria, D. tridens,* and *O. neptuni*) are recorded in Vietnam for the first time on *P. pelagicus*. Despite the intensive sampling (over 400 individual crabs), the rhizocephalan, *Sacculina* Thompson, 1836, was not found. This is similar to the previous reports by Vo *et al.* (2013) on the same crab species, and Le *et al.* (2018b) on *Portunus sanguinolentus* (Herbst, 1783).

Octolasmis spp. and the genus *Dianajonesia* have been documented in previous studies on various host crustaceans (Shields and Overstreet, 2003; Dvoetsky and Dvoretsky, 2010; Dvoretsky, 2012; Ekanem *et al.*, 2013) including swimmer crabs (Weng, 1987; Shield, 1992; Jeffries *et al.*, 2005). These species were found to mainly colonize the outer surface of the host, such as the carapace, gills and gill chamber. The high density of symbionts is thought to affect the respiration and movement of hosts, exposing them to predators (Gaddes and Sumpton, 2004; Yusa *et al.*, 2012). Although in the current study, *Octolasmis* species were found on almost every part of *P. pelagicus* body surface (Tab. 2), definite niche(s) were observed among the different species. The specific distribution may be related to the external body structure of each species. For example, *O. angulata* and *O. neptuni* have capitular plate coverage of 10 % and 16.4 %, with 3 and 5 reduced capitular plates, respectively, are found primarily on the gills (Fig. 1A, C), while *O. warwicki* and *D. tridens,* which are covered by 5 robust capitular plates (coverage 43 % and 71 %, respectively) are mainly distributed on the carapace. These findings are concordant with previous studies in terms of the number of *Octolasmis* species detected on swimming crabs (4 species), in the specific infestation sites, and in the structure and percent coverage of the capitular plates/capitulum (Jeffries *et al.*, 2005).

The *Semibalanus* species was morphologically similar to *S. balanoides*; however, the molecular analysis shows it as a different taxon. We could not identify it, or describe it as a new species, due to the conflicting results from 28S rRNA and COI mtDNA sequences and limited taxonomic characters. We also found *C. testudinaria,* which has a well-known global distribution, and a wide variety of substrate-hosts species. Intraspecific morphological variation has previously been considered to be due to host substrate habitat differences (Rawson *et al.*, 2003; Torres-Pratts *et al.*, 2009; Cheang *et al.*, 2013), and in fact, the three species of this genus reported here (*C. testudinaria*, *C. patula*, and *C. manati*), usually from different host taxa (marine turtles, decapod crustaceans, snakes, and Sirenia), have been combined by some (Zardus *et al.*, 2014).

The current study provides new insights on the host and symbiont associations of swimming crabs in Vietnam and further studies could focus on the ecology of these ectosymbiont species.

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Appendix

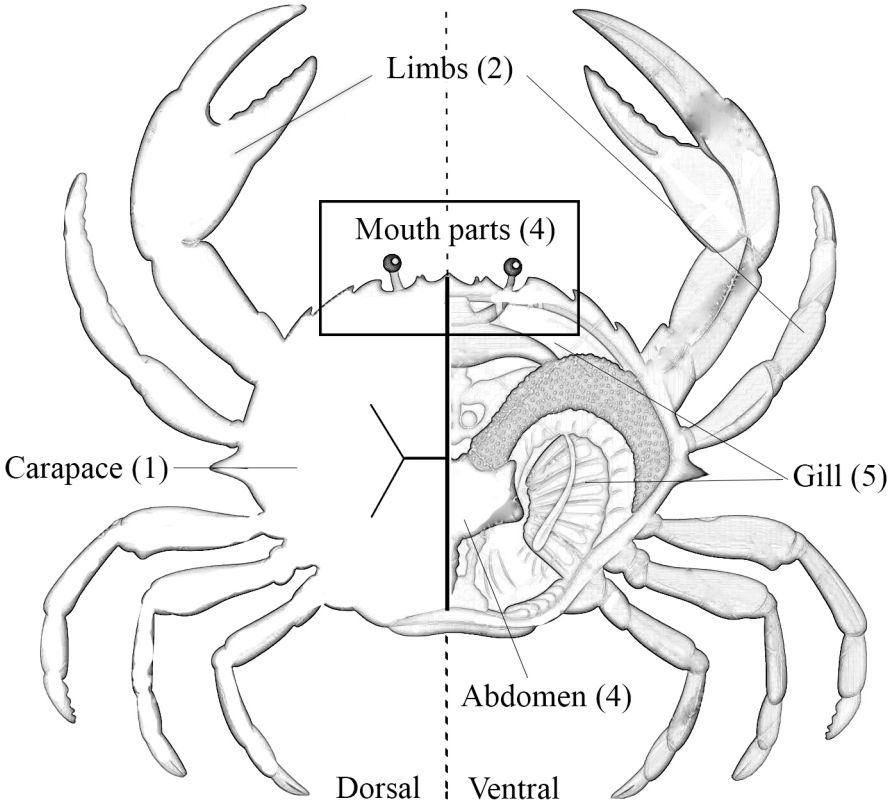

Figure A1. Line drawing showing the external morphology of the blue swimming crab (half dorsal and cut-away views) with dissected body parts (numbered from 1–5) used for recording the symbiotic organisms.

Appendix

Table A1. Results of Blast Nucleotide search for 28S rRNA and COI mtDNA gene sequences of studied species with the sequences retrieved from Genbank. GB – GenBank; X - sequences not available

No.	Studied species	28S rRNA				COI mtDNA		
		Reference species	Query cover (%)	% Identity	GB Accession no.	Query cover (%)	% Identity	GB Accession no.
1	*Octolasmis angulata*	*Octolasmis cor*	100	98.1	EU082326	X	X	
		Octolasmis unguisiformis	99	91.5	LC467957	X	X	
		Octolasmis angulata	**X**	**X**		**100**	**100**	**KC138498**
2	*Octolasmis neptuni*	*Octolasmis* sp.	100	99.2	EU082327	X	X	
		Octolasmis unguisiformis	99	91	LC467957	X	X	
		Octolasmis cor	X	X		100	98.9	KC138500
3	*Octolasmis warwicki*	***Octolasmis warwicki***	**100**	**100**	**EU082328**	**100**	**100**	**KC138501**
		Octolasmis cor	100	93	EU082326	X	X	
4	*Dianajonesia tridens*	*Octolasmis cor*	100	93	EU082326	X	X	
		Octolasmis sp.	100	93	EU082327	X	X	
		Dichelaspis hawaiense	X	X		100	84.5	KF484230
5	*Chelonibia testudinaria*	*Chelonibia patula*	100	99.9	EU082295	100	99.8	JF823668
		Chelonibia manati	100	99.7	AB723917	X	X	
		Chelonibia testudinaria	100	99.7	AB723914	100	99.8	AY174338
6	*Semibalanus* sp.	*Semibalanus cariosus*	100	93.4	AY520593	100	100	KM611728
		Semibalanus balanoides	100	93.4	EU370440	X	X	
7	*Choniosphaera indica*	*Asterocheres aesthetes*	69	90.3	KR048857	X	X	
		Asterocheres lilljeborgi	66	91	KR048868	X	X	

Appendix

Table A2. Percentage (%) of sequence differences (above diagonal) and identities (below diagonal) of 28S rRNA (A) and COI mtDNA (B) genes following the taxonomic families of study species. The largest different/identity values are bolded and highlighted in red.

A. 28S rRNA

I. Subclass: Thecostraca

I.I. Superorder: Thoracica, Order: Lepadiformes

Family: Poecilasmatidae

	Seq→	1	2	3	4	5	6	7
1	*Octolasmis angulata*	ID	6,1	7,1	6,5	1,9	10,1	7,3
2	*Octolasmis neptuni*	93,9	ID	8,3	**0,7**	6,3	10,4	6,9
3	*Octolasmis warwicki*	92,9	91,7	ID	8,4	7,4	**12,2**	9,2
4	*Octolasmis* sp. EU082327	93,5	**99,3**	91,6	ID	6,6	10,7	7,3
5	*Octolasmis cor* EU082326	98,1	93,7	92,6	93,4	ID	11	7,3
6	*Octolasmis unguisiformis* LC467957	89,9	89,6	**87,8**	89,3	89	ID	9,7
7	*Dianajonesia tridens*	92,7	93,1	90,8	92,7	92,7	90,3	ID

I.2. Superorder: Thoracica, Order: Sessilia

Family: Balanidae

	Seq→	1	2	3
1	*Semibalanus* sp.	ID	7,6	**7,9**
2	*Semibalanus cariosus* AY520593	92,4	ID	**1,3**
3	*Semibalanus balanoides* EU370440	**92,1**	**98,7**	ID

Family: Chelonibiidae

	Seq->	1	2	3	4	5	6	7
1	*Chelonibia testudinaria*	ID	0,3	0,4	2,7	**0,2**	4,4	6,1
2	*Chelonibia manati* AB723917	99,7	ID	0,4	2,7	**0,2**	4,7	**6,4**
3	*Chelonibia testudinaria* AB723914	99,6	99,6	ID	2,3	0,3	4,4	6,1
4	*Chelonibia testudinaria* KM217527	97,3	97,3	97,7	ID	2,5	5,7	4
5	*Chelonibia patula* EU082295	**99,8**	**99,8**	99,7	97,5	ID	4,6	6,3
6	*Chelonibia caretta* AB723915	95,6	95,3	95,6	94,3	95,4	ID	2,4
7	*Chelonibia caretta* KM217526	93,9	**93,6**	93,9	96	93,7	97,6	ID

II. Subclass: Copepoda

Superorder: Podoplea

Order: Siphonostomatoida

	Seq→	1	2	3	4	5
1	*Choniosphaera indica*	ID	24,9	27,3	29,7	**30,3**
2	*Asterocheres lilljeborgi* KR048868	75,1	ID	18,6	20,1	21,8
3	*Parabrachiella hugu* KR048861	72,7	81,4	ID	24,6	25,8

Order: Harpacticoida

4	*Canthocamptus staphylinus* MF077853	70,3	79,9	75,4	ID	**10,2**
5	*Canthocamptus coreensis* KR048886	**69,7**	78,2	74,2	**89,8**	ID

B. COI mtDNA

I. Subclass: Thecostraca

I.I. Superorder: Thoracica, Order: Lepadiformes

Family: Poecilasmatidae

	Seq→	1	2	3	4	5	6
1	*Octolasmis angulata*	ID	16	**14,5**	17,6	18,7	17,8
2	*Octolasmis neptuni*	84	ID	14,7	19,7	20,6	18,1
3	*Octolasmis warwicki*	**85,5**	85,3	ID	16,9	17,8	15,7
4	*Octolasmis cor* KC138499	82,4	80,3	83,1	ID	19,4	18,8

Appendix

Table A2. Cont.

B. COI mtDNA								
I. Subclass: Thecostraca								
I.I. Superorder: Thoracica, Order: Lepadiformes								
Family: Poecilasmatidae								
5	*Octolasmis unguisiformis* LC467960	81,3	79,4	82,2	80,6	ID	**21,3**	
6	*Dianajonesia tridens*	82,2	81,9	84,3	81,2	**78,7**	ID	
I.2. Superorder: Thoracica, Order: Sessilia								
Family: Balanidae								
	Seq→	1	2	3				
1	*Semibalanus* sp.	ID	**0**	**14,7**				
2	*Semibalanus cariosus* KM611728	**100**	ID	**14,7**				
Family: Chelonibiidae								
	Seq→	1	2	3	4	5	6	7
1	*Chelonibia testudinaria*	ID	10,3	1,1	1,3	0,7	**17,6**	17,4
2	*Chelonibia manati* JN589813	89,7	ID	10	9,6	9,8	16,7	16,6
3	*Chelonibia testudinaria* KF042514	98,9	90	ID	0,9	**0,4**	17,1	16,9
4	*Chelonibia testudinaria* KF042515	98,7	90,4	99,1	ID	0,6	17,3	17,1
5	*Chelonibia patula* JF823674	99,3	90,2	**99,6**	99,4	ID	17,3	17,1
6	*Chelonibia caretta* KF042512	**82,4**	83,3	82,9	82,7	82,7	ID	0,7
7	*Chelonibia caretta* KF042513	82,6	83,4	83,1	82,9	82,9	99,3	ID
II. Subclass: Copepoda								
Superorder: Podoplea								
Order: Siphonostomatoida								
	Seq→	1	2	3	4	5		
1	*Choniosphaera indica*	ID	42,2	**44,5**	43,3	43,3		
2	*Asterocheres lilljeborgi* KR049050	57,8	ID	26,6	29,5	28,3		
3	*Parabrachiella hugu* KT030285	**55,5**	73,4	ID	30,2	**21,4**		
Order: Harpacticoida								
4	*Canthocamptus staphylinus* MF077881	56,7	70,5	69,8	ID	22,6		
5	*Canthocamptus coreensis* KT030277	56,7	71,7	**78,6**	77,4	ID		