

LIFE CYCLE OF THE PEA CRAB *PINNOTHERES HALINGI* SP. NOV., AN OBLIGATE SYMBIONT OF THE SEA CUCUMBER *HOLOTHURIA SCABRA* JAEGER

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ABSTRACT

A new species of pea crab, *Pinnotheres halingi* sp. nov. (Pinnotheridae), found encysted in the right respiratory tree of the sea cucumber, *Holothuria scabra* (Holothuridae), from Solomon Islands, is described. The reproduction, infestation and pairing behaviour of the crabs were investigated through field observations and experiments. Infestation frequency in 8 monthly samplings of 25-30 holothurians was  $98.6 \pm 2.6\%$  in Kogu Halingi bay and 0% in two nearby sites. Of 403 pea crabs, 91.4% were found in pairs of opposite sex, 7.9% were single females and <1% were single males. The embryos developed on the female pleopods over ca. 30 days from fertilisation to the release of first zoeae and subsequently went through five pelagic zoeal stages. Infestation occurred at the megalops stage after about 59 days of development. A single pea crab (male or female) per host was found three months after larval infestation. Young males appeared to be strongly attracted to hosts that sheltered a single female, suggesting that pairing occurred as a male <6 mm carapace width joined a female. Larger crabs could not enter the host. Copulation was observed within the female cyst, preceding or overlapping oviposition. The male then progressed away from the female and from the anus, forming its own cyst along the way. Both larvae and small sub-adults invaded *H. scabra* with a minimum length of 80 mm, exclusively. *P. halingi* induced atrophy of the right respiratory tree of its host.

INTRODUCTION

Symbiotic crabs belonging to the family Pinnotheridae, commonly called pea crabs, are frequently found in the gut, the cloaca and the respiratory tree of sea cucumbers. Besides holothurians, they also regularly associate with asteroids and echinoids (Schmitt et al. 1973; Jangoux 1987) as well as with bivalves and gastropods (Pregenzer 1978, 1983; Campos 1990; Fernando Felix Pico 1992; Stevens 1992; Palmer 1995).

Twelve species of pinnotherid crabs are known to be symbiotic with holothurians in the Indo-Pacific (Schmitt et al. 1973). With two possible exceptions, all of them are obligate symbionts and are not known from other hosts. The ten known Indo-West Pacific species of *Pinnotheres* associated with holothurians are all characterised by dilated and spatuliform dactylus and propodus of the third maxilliped palp. Three species of holothurian-dwelling *Pinnotheres* are known only from the Indian Ocean, i.e. *P. deccanensis* (southern India), *P. pilumnoides* (Red Sea, Gulf of Aden), and *P. setnai* (Andaman Islands). Six species are known only from Southeast Asia and the Indo-West Pacific, i.e. *P. flavus* (Philippines), *P. holothuriae* (Philippines), *P. holothuriensis* (South Australia), *P. ortmanni* (Philippines, Peninsular Malaysia, Singapore), *P. semperi* (Java, Sumatra, Sulawesi, Peninsular Malaysia, Singapore) and *P. tenuipes* (Philippines) (Schmitt et al. 1973). Only one species, *P. villosissimus*, occurs in both the Indian and Pacific oceans (Sumatra, Andaman Islands, New Guinea) (Chopra 1931; VandenSpiegel & Jangoux 1989). Manning (1993) recently recognised the genus *Holothuriophilus* (which had long been synonymised under *Pinnotheres*) for two species of east Pacific pinnotherids which are obligate symbionts, *H. trapeziformis*, and *H. pacificus*.

Pinnotherid crabs associated with holothurians have been classified as commensal, inquilistic, parasitic or undetermined symbionts (Jones & Mahadevan 1965; VandenSpiegel & Jangoux 1989; VandenSpiegel et al. 1992). They are reported to have no effect or only a slight effect on their host. For example, Tao (1930), Jones & Mahadevan (1965), Jangoux (1987), VandenSpiegel & Jangoux (1989) and VandenSpiegel et al. (1992) mentioned that these crabs did not feed on host tissues, nor cause any other detrimental effects, except to slightly wound the wall of the respiratory tree or of the cloaca, forming a membranaceous cyst.

Data on the biology and life cycle of pinnotherid crabs remain scarce, focusing largely on bivalve-dwelling species. Post-embryonic development of mussel pea crabs *Pinnotheres pisum* and *P. pinnotheres*, was described by Atkins (1926, 1955), while the oyster pea crab, *P. ostreum*, was studied by Stauber (1945), Sandoz & Hopkins (1947) and Christensen & McDermott (1958). Pearce (1964) and Costlow & Bookhout (1966) reported on *P. maculatus* from bivalves, while Pearce (1966) investigated the life history of *Fabia subquadrata* from echinoids. More recently, Otani et al. (1996) described the development and breeding of the swimming pea crab, *Tritodynamia horvathi*, whereas Takeda et al. (1997) studied the ecology of *Pinnixa tumida*, a crab that lives in the alimentary canal of the sea cucumber *Paracaudina chilensis*.

A number of symbiotic crabs, including pinnotherids, have previously been found in the tropical sea cucumber *Holothuria scabra*. Pea crabs *Pinnotheres deccanensis* were observed in the respiratory tree of specimens collected in Indian coastal waters (Chopra 1931; Jones & Mahadevan 1965), whereas *P. semperi* was described as a parasite of animals collected around Singapore by Lanchester

(1900) and Chuang (1961). VandenSpiegel & Ovaere (1992) and VandenSpiegel et al. (1992) reported the eumedonid crab, *Hapalonotus reticulatus*, from the respiratory tree of *H. scabra* from Papua New Guinea. The portunid crab *Lissocarcinus orbicularis* has also been reported (Crosnier 1962) in the same species. Other symbionts of *H. scabra* have also been observed: Humes (1980) reported associations with copepods, whereas Jangoux (1987), in his review, mentioned that carrapid fish (i.e. *Encheliophis gracilis*) were observed in the coelomic cavity of this holothurian.

*Holothuria scabra* is a deposit-feeding holothurian that can be found throughout the Indo-Pacific. During an extensive study on the reproductive cycle of the species conducted by the International Centre for Living Aquatic Resources Management (ICLARM) in the Solomon Islands, we discovered that almost all animals collected at a specific site harboured an undescribed species of pea crab. The present study serves to describe this new *Pinnotheres* species, as well as to investigate its reproduction, embryonic development and infestation behaviour. The benefits of the association to the crabs, and the possible deleterious effects on the host are also investigated. To our knowledge, this is one of the very few detailed behavioural portraits of a pea crab in association with a holothurian host. This study is also the first to present evidence of larval infestation and pairing formation in symbiotic pea crabs.

We wish to thank Dr. Ilan Paperna of the University of Jerusalem in Israel for conducting the histological investigation of the respiratory tree tissues. The assistance of Dr. Stephen C. Battaglione, Rayner Pitt, Christain Ramofafia, Maxwell Saurungo, Evizel Seymour, Thomas Tabounaba and Tan Swee Hee was also greatly appreciated. Finally, we are thankful to Drs Peter Gardiner and Johann Bell for their comments on the early draft of the manuscript. This research was supported by a grant from the Canadian International Development Agency (CIDA) under CGIAR-Canada Linkage Fund Programme. This is ICLARM contribution # 1447.

## MATERIALS AND METHODS

### *Study sites and sampling procedures*

The first batch of pea crabs *Pinnotheres halingi* sp. nov. was found in the respiratory tree of sea cucumbers *Holothuria scabra* collected in the bay of Kogu Halingi between 1-3 m depth in August 1997 (Fig. 1, site 1). The bay is located in the Vonavona lagoon, along Kohinggo Island, Western Province of Solomon Islands (8°10'S 157°11'E). Monthly samplings of *H. scabra* (N = 20-30) were subsequently conducted at the same site between August 1997 and March 1998 to study the crab's reproductive biology. Pea crabs obtained were kept alive for subsequent experiments. Additional sea cucumbers were collected from two other nearby sites: Kogu Veke (site 2) and Malmalagiri Inlet (site 3) (Fig. 1) in August and September 1997 for the study.

To assess the habitat which characterises the hosts of the pea crabs, each of the three sampling sites was described with respect to tides (Australian Nation-

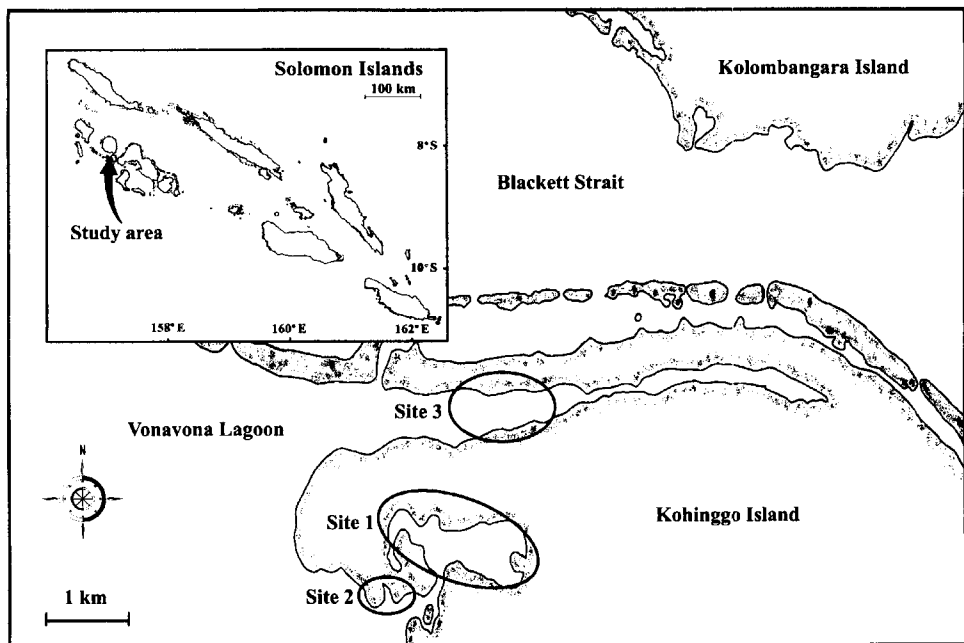


Fig. 1. Map of Kohinggo Island, Western Province of Solomon Islands, illustrating sampling site 1 (Kogu Halingi), site 2 (Kogu Veke) and site 3 (Malmaragiri Inlet).

al Tide Tables 1997-1998), currents, temperature, dissolved oxygen, organic matter (OM hereafter) and grain size of the sediment. Temperature, dissolved oxygen and salinity were monitored at 1 m depth using a U-10 Horiba Water Checker. Water movements and current direction were measured using stains. The proportion of OM in the sediment was determined by comparing the weight of a given sample after the following treatments: drying to constant weight in an oven at 55°C and burning in a muffle furnace at 500°C for 24 h. OM contents were expressed as ash-free weight/dry weight  $\times 100$ . The grain size of the sediment was established using successive sieving and measurement under a light microscope. The infestation status of other species of sea cucumbers collected was assessed.

#### *General observations and reproductive cycle of pea crabs*

The respiratory tree was removed from all sea cucumbers collected at site 1 (Fig. 1) between August 1997 and March 1998. The position of each pea crab within the respiratory tree was measured with respect to all other pea crabs and to the anus. The respiratory tree was then opened and the crabs were extracted. The proportion of different embryonic stages was noted in every ovigerous female. Each pea crab was then weighed to the nearest 0.0001 g. Measures of car-

apace width and length to the nearest mm were also taken, using callipers. Most of the crabs were kept alive for experimentation, although some were fixed in 10% neutral buffered formalin for identification purposes.

Pea crabs collected in August and September 1997 were also used to determine the relationships between crab and host sizes. The number of eggs or embryos present on each ovigerous female was ascertained under a light microscope at 40x. Embryos were measured when fully extended with a micrometer ocular (100x). The surrounding membrane was thus removed when needed. The pooled data were expressed as Mean $\pm$ SD.

#### *Macroscopic and microscopic effects of crabs on their host*

Morphometric measures of several organs removed from infested and non-infested sea cucumbers *Holothuria scabra* collected in August and September 1997 were compared to assess if the presence of crabs had a significant impact. The sex, weights of the body walls, gonads and respiratory trees of each sea cucumber were recorded. The gonad and respiratory tree indices were determined using the ratio of the wet weight of the gonad and dry weight of the respiratory tree to the wet weight of the body wall, respectively. The volume of each cyst was estimated by displacement in a graduated cylinder and the thickness of the respiratory tree epithelium was measured in different areas with a micrometer ocular at 40x. The pooled data were again expressed as Mean $\pm$ SD.

To establish the more subtle impact of the crab on the respiratory tree of the sea cucumbers, 25 mm<sup>2</sup> tissue samples were collected from 10 male and 10 female infested hosts. Similar samples were collected from the respiratory tree of non-infested sea cucumbers, in approximately the same geographical area. The samples were kept in 10% neutral buffered formalin for 4 weeks. They were subsequently embedded in a glycol-methacrylate resin (GMA of Agar Scientific Ltd. UK). Series of transversal and longitudinal sections of 3-4  $\mu$ m were made on each block using a JB4 Sorvall glass-knife microtome. The sections were stained with Meyer's haemalum, using eosin as counterstain.

#### *Embryonic development of pea crabs*

Thirty-six live ovigerous female crabs presenting a similar level of maturity were extracted from different sea cucumbers *Holothuria scabra* in August 1997. They were maintained separately, with their respective male, in 1.5-l bowls supplied with running seawater (ca. 3 l h<sup>-1</sup>) and natural photoperiod (12 h light: 12 h dark) with a light intensity of 25  $\mu$ mol photons m<sup>-2</sup> s<sup>-1</sup>. The salinity fluctuated between 33 and 34‰ and the temperature between 26 and 29°C. Each bowl was filled with a sandy substrate and contained a few rock shelters. At the beginning of the experiment, the broods of the selected females ranged from undeveloped eggs to 4-cell stage embryos. Embryos were sampled from all females every 6 h for the first 5 days, and then daily until the end of the experiment.

The embryos were gently collected from the females' abdomens with a pipette. To reduce the shock of this daily operation, the females were kept underwater at all times and manipulated with dissecting pincers for less than 10-15 seconds. A new stage of development was considered attained when more than 50% of the embryos had progressed from one stage to the next. Controls for this experiment consisted of ovigerous females of comparable maturity in which the embryos were sampled only at the beginning and at the end of the experiment. The development of embryos from regularly sampled and undisturbed females were subsequently compared.

Three female crabs with late gastrulae to developing prezoëa were individually placed in 1.5-l culture bowls provided with running seawater as described above. These females released their embryos within 48 h. The subsequent pelagic stages of development were examined and they were used in the infestation experiments described below. Larvae were reared in seawater filtered with ultraviolet light and 20 µm mesh at a density of about 50 larvae l<sup>-1</sup>. They were fed throughout the experiments, three times a day, with 25 ml of a mix of two phytoplanktonic diatoms, *Chaetoceros simplex* and *Chaetoceros muelleri* at a concentration of ca. 80 000 cells ml<sup>-1</sup>. A few specimens of each larval stage were periodically placed in presence of adult *Holothuria scabra* to determine at which stage they would be ready to infest their host.

#### *Choice of host for larval infestation*

Experiments were conducted with megalopal larvae described above to verify whether the larval crabs could select a host based on the species and size of sea cucumbers. The trials were conducted in a 500-l tank supplied with running seawater (ca. 150 l h<sup>-1</sup>) under the above mentioned regimes of photoperiod, salinity and temperature. Approximately 300 megalopae were simultaneously exposed to seven species of crab-free adult sea cucumbers: two *Actynopiga mauritiana*, two *Holothuria scabra*, two *Holothuria atra*, two *Holothuria leucospilota*, two *Holothuria fuscopunctata*, two *Bohadschia marmorata*, and two *Stichopus variegatus*. The sea cucumbers were maintained in the experimental tank until no more larvae were found in the water. They were then transferred to a larger tank submitted to identical flow-through conditions for the next three months before they were anaesthetised in MgCl<sub>2</sub> and dissected. The cloaca, respiratory tree and last third of the intestine of each sea cucumber were examined for juvenile crabs using a dissecting microscope. All crabs found were sexed and measured.

Another experiment was conducted concurrently to ascertain whether the larvae would show a size preference for the host. About 300 megalopal larvae were used under the same conditions as described above. This time, they were simultaneously exposed to various sizes of *Holothuria scabra*: eight specimens <10 mm, six between 10-40 mm, six between 40-80 mm, four between 80-120 mm, four between 120-150 mm, and three >200 mm. Three *H. scabra* >200 mm

from site 1 were also used. These were presumably infested since the proportion of infested *H. scabra* in site 1 had been determined to be close to 100% over 8 months of sampling. The prospective hosts were examined three months later, as described above. To complete the investigation and determine the reaction and delay of settlement of the larvae in the absence of a host, ca. 150 larvae were distributed in three 50-l tanks covered with sand and left to develop concurrently with the above experiments.

Complementary observations were conducted in order to understand how the propagation of the crabs could occur. During the experiments described above, the behaviour of the larvae observed in the water column, and in contact with the sea cucumbers, was noted until the time when all larvae had disappeared from the water column.

### *Behaviour*

These experiments were designed to: 1) determine if adult pea crabs were attracted to and could re-infest a host after they had been removed from the respiratory tree; 2) ascertain the sensitivity of adult and sub-adults with regard to the status of their host; i.e. infested or non-infested and 3) examine the sequence of male-female pair formation.

The first experiment was conducted with adult pea crabs, i.e. ovigerous females and their male companions, which respectively averaged 15 and 7 mm in carapace width. Twelve males and twelve females were placed in a 500-l tank supplied with flow-through seawater together with two sea cucumbers of the species described above in the first larval infestation experiment. The movements of the crabs in the presence of the different sea cucumbers were noted. In a second experiment, the response of adult crabs toward infested or non-infested *Holothuria scabra* was examined. Sea cucumbers collected from crab-free areas were identified by a small scratch on the dorsal part of their body wall, whereas specimens from site 1 were left untouched. The behaviour of the crabs, including any attempts to re-enter a host, was recorded.

For the third experiment, sea cucumber hosts harbouring pea crabs of known sex and size were needed. This was achieved by placing sub-adult pea crabs, either male or female, close to the anus of non-infested *Holothuria scabra*. These crabs usually entered the host within an hour. The following interactions between non-mature sub-adult pea crabs <6 mm carapace width and infested *H. scabra* were observed: 1) a female crab exposed to a sea cucumber harbouring a single male crab; 2) a female crab exposed to a sea cucumber harbouring a single female crab; 3) a male crab exposed to a sea cucumber harbouring a single female crab; 4) a male crab exposed to a sea cucumber harbouring a single male crab. Four other interactions were also tested: a female or male pea crab exposed to a sea cucumber harbouring an adult, or sub-adult male-female pair. Each type of interaction was repeated a minimum of 6 times.

Another experiment was designed to verify if crabs were able to move from

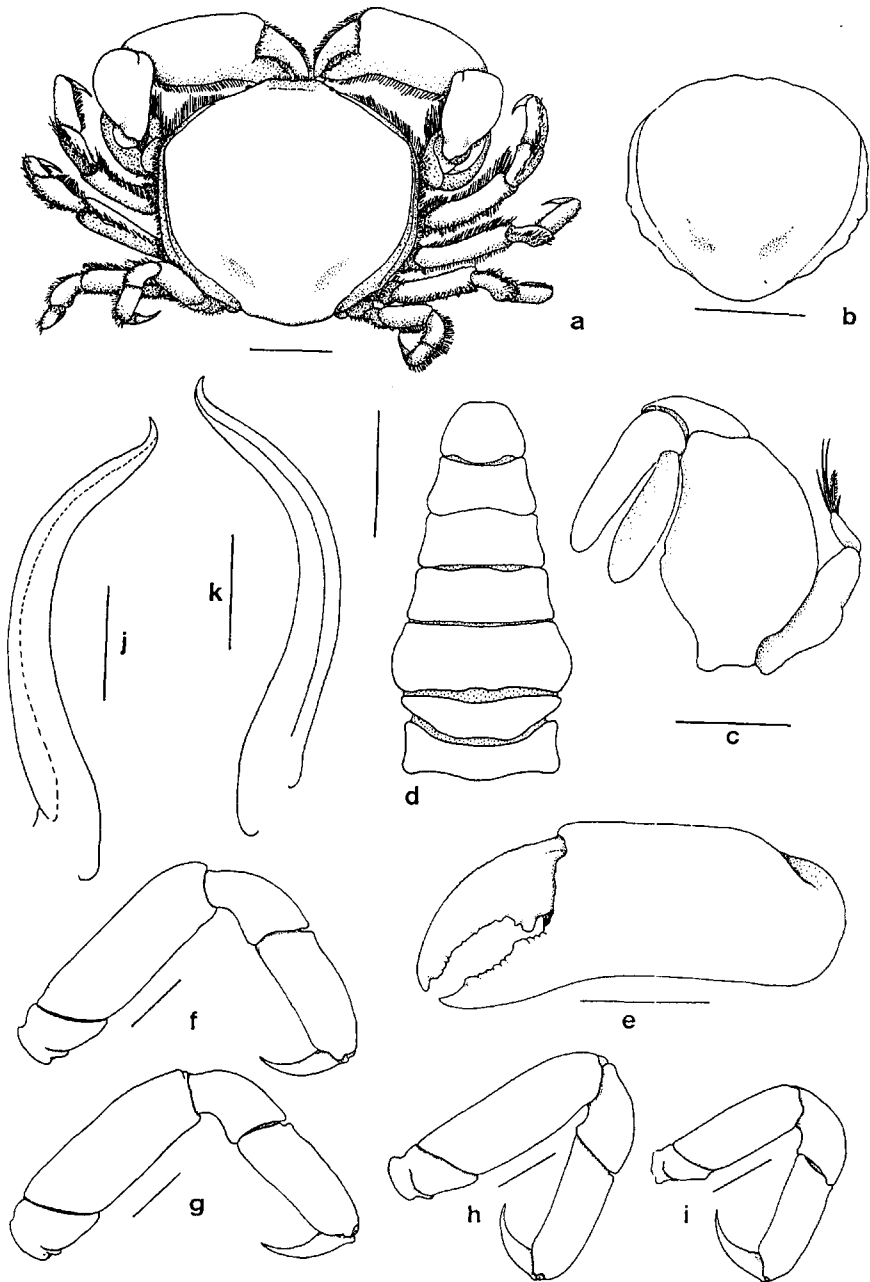


Fig. 2. *Pinnotheres halangi* sp. nov.-a, overall dorsal view of holotype male (8.0 by 8.0 mm) (ZRC), bar = 2.5 mm; b, carapace of allotype female (11.1 by 11.1 mm) (ZRC), bar = 5.0 mm; c-k, paratype male (7.9 by 7.8 mm) (ZRC), bars = 1.0 mm; c, left third maxilliped; d, male abdomen, bar = 2.0 mm; e, left male chela, bar = 2.0 mm; f-i, P2-P5 respectively; j & k, left male first pleopod. All structures (except a) denuded of setae.



one host to another. In this experiment, six sea cucumbers harbouring a single female, and six harbouring a single male, were used. Each host was identified by a specific mark on its body wall. The sea cucumbers were placed in a 4000-l tank for a week. All sea cucumbers were then anaesthetised in  $MgCl_2$  and dissected; the presence or absence of pea crabs, their sexes and locations in the respiratory tree of the host were noted.

### DESCRIPTION

In the following description, the abbreviations P1-5 refer to the respective pereopods (P1 = cheliped and P2-5 = first to fourth ambulatory legs) and G1 to the male first pleopod. Measurements provided are of the carapace width and length, respectively. Specimens are deposited in the Zoological Reference Collection (ZRC), School of Biological Sciences, National University of Singapore; and National Museum of Natural History (USNM), Smithsonian Institution, Washington D.C.

#### *Pinnotheres halingi* sp. nov. (Fig. 2)

*Material examined.* – Holotype, male (8.0 by 8.0 mm) (ZRC), Kogu Halingi bay, Kohinggo Island, Western Province, Solomon Islands, ca. 2 m depth, coll. September 1997.

Allotype, female (11.1 by 11.1 mm) (ZRC), same data as holotype.

Paratypes, 3 males (7.9 by 7.8 mm, 6.9 by 6.8 mm, 7.7 by 7.8 mm), 3 females (10.4 by 10.5 mm, both ovigerous, 10.2 by 10.3 mm, spent, 9.7 by 9.8 mm, ovigerous) (ZRC), 1 male (7.9 by 7.8 mm), 1 female (10.3 by 10.2 mm, ovigerous) (USNM), same data as holotype.

*Description.* – Carapace width equal to subequal to length, male subsquarish in shape, female longitudinally ovate; dorsal surface smooth, inflated; posterior branchial regions gently depressed. Lateral margins almost straight, subparallel; posterior carapace margin angular. Third maxilliped with ischium and merus completely fused, without trace of suture, subovate, with median notch on outer margin; exopod very stout, outer margin with submedian cleft, with well developed flagellar setae; palp 3-segmented, propodus slightly longer than dactylus, dactylus spatuliform, attached to base of propodus. Chelipeds subequal; fingers distinctly shorter than palm; base of cutting edge of dactylus with large submolariform tooth, rest of cutting edge and that of propodus with blunt teeth and denticles; distal part of fingers excavated on inner surface. Ambulatory legs relatively stout,  $P2 > P3 > P4 > P5$  in length (basis-ischia to tip of dactylus); dactyli of ambulatory legs with relatively long, sharply tapering dactylus, all dactyli similar in size and shape. Male abdomen with all 6 segments distinct, freely articulating, telson broadly triangular, lateral margins gently convex, tip subtruncate. G1 relatively slender, sinuous, tip distinctly curving upwards.

*Etymology.* – The name is derived from the type locality, Kogu Halingi bay. The name is used as a noun in apposition.

*Remarks.* – Of the Indo-Pacific species of *Pinnotheres* which are symbiotic in holothurians, *P. halingi* sp. nov. is perhaps most similar to *P. semperi*, *P. setnai*, *P. holothuriae* and *P. ortmanni* with regards to the general carapace shape and more projecting front. *P. holothuriae* and *P. ortmanni*, however, are easily distinguished from *P. halingi*. Compared to *P. halingi*, *P. ortmanni* has a more rounded carapace with the lateral margins distinctly convex (vs. straight and subparallel), generally more slender and longer ambulatory legs (vs. stout and short), the dactylus of P5 is long, slender and much longer than the other ambulatory dactyli (vs. proportionately shorter, with the dactyli of all the legs similar in size), and the dactylus of the third maxilliped palp is proportionately much shorter. *P. holothuriae* can be separated from *P. halingi* by the dactylus of P5 being long, slender and distinctly longer than the other ambulatory dactyli (vs. proportionately shorter, with the dactyli of all the legs similar in size), the dactylus and propodus of the third maxilliped palp is proportionately much shorter and the dactylus is distinctly shorter than the propodus (vs. slightly shorter) (cf. Bürger 1895).

*Pinnotheres halingi* is perhaps closest to *P. semperi* and *P. setnai*. The identity of *P. semperi*, however, poses some problems. In the original description, Bürger (1895: 382, pl. 9 Fig. 28, pl. 10 Fig. 27) described and illustrated (rather schematically) a female specimen in which the frontal margin is distinctly produced and triangular in shape from dorsal view. Schmitt et al. (1973) stated that the male and female syntypes of *P. semperi* (supposedly in the Berlin Museum) are no longer existent. A check with the Berlin Museum confirmed that the types of *P. semperi* (as well as those of *P. flavus*) are not there (O. Coleman, pers. comm.). In Singapore, however, *P. semperi* has been reported twice before (Lanchester 1900; Chuang 1960) and the ZRC has a good series of specimens.

The specimens of *Pinnotheres semperi* we have examined do not have the long rostrum figured by Bürger (1895). The females have a very narrow front with the margin distinctly sinuous, and the eyes are not visible from the dorsal view. The males have a more produced front which is subtruncate from dorsal view but even when they are positioned with the anterior part of the carapace arched upwards, they are still quite unlike that figured by Bürger (1895: pl. 9 Fig. 28a). In uncleaned male specimens in which the row of setae lining the edge of the frontal margin are still intact and covered with dirt, the front appears more produced anteriorly and more triangular from the dorsal view. After brushing, however, their fronts appear subtruncate. Female specimens never look like the female figured by Bürger (1895). It is thus quite possible that Bürger had figured a male (and not female) specimen which had not been properly cleaned. The third maxilliped figured by Bürger (1895: pl. 10 Fig. 27) is similar to those of the present specimens except that the outer margin is rounded (without any

distinct angle, Fig. 2C). Because Bürger's figure of this structure is so schematic, not much can be said. The male abdomen figured by Bürger (1895: pl. 9 Fig. 28b) resembles those of the present specimens, but it is too schematic to make detailed comparisons. When the taxonomy of the various holothurian-dwelling species is revised, a neotype for *P. semperi* should probably be designated (possibly from the Singapore series).

Direct comparisons of specimens of *Pinnotheres halingi* with *P. semperi* (as defined here) show that they differ in several distinct features. Compared to *P. semperi*, the carapace of *P. halingi* is slightly broader than long or the length and width are subequal (vs. male and female width to length ratio 0.99-1.01) and the male lateral margins are subparallel (vs. male and female carapace distinctly broader than long, width to length ratio 1.05-1.10, with male lateral margins slightly diverging); the posterior part of the female carapace projects strongly posteriorly (vs. normal); the surfaces of the appendages are covered with setae (but never dense enough to obscure the outline), while those on the carapace are relatively sparse (carapace and appendages densely covered with setae *P. semperi*, obscuring the outlines); the chelae are relatively longer and more slender (vs. relatively shorter and stouter); the male telson is semi-circular in shape (vs. telson subtriangular with the tip rounded); and the GI is more or less recurved distally. These differences are valid for specimens of equivalent sizes.

Compared with *Pinnotheres setnai* (fide Chopra 1931), *P. halingi* has a more glabrous cheliped and legs (vs. densely setose), the front of the female is proportionately broader and appears strongly sinuous from the dorsal view; the posterior part of the female carapace projects strongly posteriorly (vs. normal); the fused merus-ischium of the third maxilliped is proportionately broader; the dactylus of the palp of the third maxilliped is subequal in length to the propodus (vs. dactylus distinctly longer than propodus); and the male telson is semi-circular in shape (vs. telson subtriangular with the tip rounded).

Chopra (1931) commented that for the series of specimens he had of *Pinnotheres setnai*, the carapace width of smaller ovigerous females tends to be slightly greater than the length, but larger ones have proportionately greater carapace lengths, resulting in specimens at 10 mm carapace length having their carapace widths and lengths equal. Males on the other hand, always have carapace widths that are greater or equal to the lengths. This variation, however, was not observed for the present series of specimens of *P. halingi* (nor for *P. semperi*). Chopra (1931) also noted that smaller females tend to have proportionately stouter chelae. The differences observed here between *P. halingi*, and *P. semperi* and *P. setnai*, however, are for specimens of comparable sizes, and are reliable for the series examined.

## RESULTS

### *Habitat of infested sea cucumbers*

Sea cucumbers *Holothuria scabra* harbouring pea crabs *Pinnotheres halingi* were found only at site 1 and never at the two other locations (Fig. 1) or elsewhere in Solomon Islands.

Kogu Halingi (site 1, Fig. 1), is a bay less than 1 km<sup>2</sup>, which receives substantial but sporadic inputs of freshwater run-off during the rainy season. Salinity fluctuated between 15 and 36‰, remaining low for up to a week during the rainy season. The bay is encircled by an extensive mangrove swamp and connected to the sea by a single narrow channel ca. 30 m wide. The tidal current recorded in the channel fluctuated around 0.1 m sec<sup>-1</sup>. However, because the channel is very shallow compared to the bay, exchanges with the open sea seem to be minimal. The shallowest areas of the bay are covered by extensive seagrass beds in which many sea cucumbers were collected during the study. Elsewhere, the bottom consists of alternate zones of sand (0.42±0.05 mm) and coral fragments (9.73±2.45 mm) in the shallows and muddy sediment (0.06±0.01 mm) in deeper locations. The largest sea cucumbers were found on the muddy substrate. The OM content of sediments ranged from 12 to 55%. Visibility within the bay was usually <1 m during the 8 months of observation. Water temperature at the site varied between 30 and 36°C. None of the other holothurians, *Bohadschia marmorata*, *Stichopus variegatus*, *Opheodesoma* sp., *Holothuria leucospilota*, *H. fuscopunctata*, and *H. atra* were infested with *Pinnotheres halingi*.

Sites 2 and 3 (Fig. 1) were quite different, being much more exposed to currents (between 0.3 and 3.0 m s<sup>-1</sup>) and waves and consequently characterised by a higher level of energy. The OM content in the sediment (5-15%) was lower than at site 1, the temperature fluctuated between 26 and 30°C and the visibility varied between 1 and 12 m. Numerous patch reefs and other habitats surrounded both sites.

### *General observations*

Apart from experimental manipulations, pea crabs were never observed outside their host in the field or in the holding tanks. All pea crabs were found in the right respiratory tree of the sea cucumbers, inside a cyst that surrounded their body very tightly. The female cyst was generally located 21.0±6.0 mm from the anus, whereas the position of the male varied, as described below. The cysts of the largest females stretched the respiratory tree branch three to five times its original diameter. Males were always much smaller than females. The position of the legs, and the presence of a terminal hook, seemed to favour lateral contact more than bottom contact. In fact, when they were released on a sandy or rocky substrate, pea crabs were almost incapable of forward movement: their abdomen touched the substrate and their legs scarcely reached the bottom.

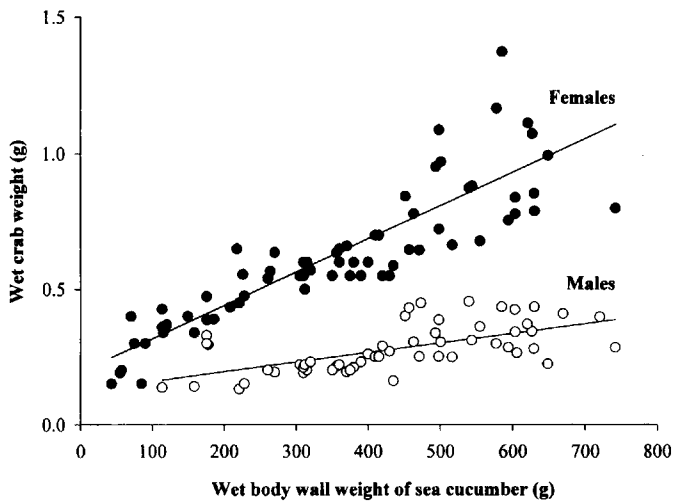


Fig. 3. Relationships between the wet weight of male and female pea crabs *Pinnotheres halingi* and the wet body wall weight of their sea cucumber host, *Holothuria scabra* (N = 75).

Large females were unable to progress in such circumstances. The survival of pea crabs outside their host never exceeded three months.

The infestation frequency in the eight monthly samplings of *Holothuria scabra* collected at site 1 (Fig. 1) was  $98.6 \pm 2.6\%$  (Mean  $\pm$  SD); only three out of 222 holothurians did not harbour any pea crab. Of the 403 pea crabs observed, 91.4% were found within a male-female pair, either in the same or in separate cysts, while 7.9% were single non-ovigerous females and 0.7% were single adult males. Pairs were thus found in 184 hosts or 82.9% of the samplings.

The wet weight of adult male pea crabs was relatively constant (0.13 to 0.46 g), whereas the wet weight of females (FWW) ranged from 0.15 to 1.65 g, increasing with the wet body wall weight of their host (WBW) according to the following equation (Fig. 3):

$$FWW = 0.19 + 1.23 \times 10^{-3} (WBW), r^2 = 0.66$$

The wet weight of ovigerous females increased with their volume as well as with the number of eggs they carried (Fig. 4).

Measurements of ovigerous females gave an average carapace width of  $13.7 \pm 2.1$  mm with the smallest ones measuring 9.0 mm (N = 178). The broods contained  $605 \pm 204$  embryos (N = 16). Single non-ovigerous females were always smaller ( $7.2 \pm 1.6$  mm, N = 32), while adult paired males had a carapace width of  $7.3 \pm 1.1$  mm (N = 177).

#### *Reproductive cycle of pea crabs*

A male and a female *Pinnotheres halingi* were found together in the same cyst on 32 occasions: their ventral surfaces were touching and the space available for

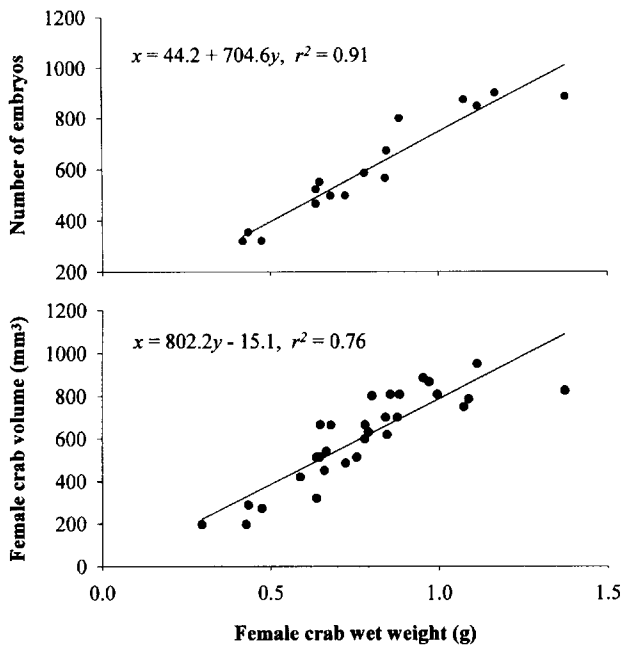


Fig. 4. Relationships between the wet weight of ovigerous female pea crabs *Pinnotheres halingi* and the number of embryos carried (N=16) or the female volume (N=30).

both crabs was quite reduced. Association of males and females was related to copulation, as the first pair of the male's pleopods, which conduct the sperm, were inserted into the openings of the female spermatheca. The duration of the copulation process could not be established. However, male and female re-

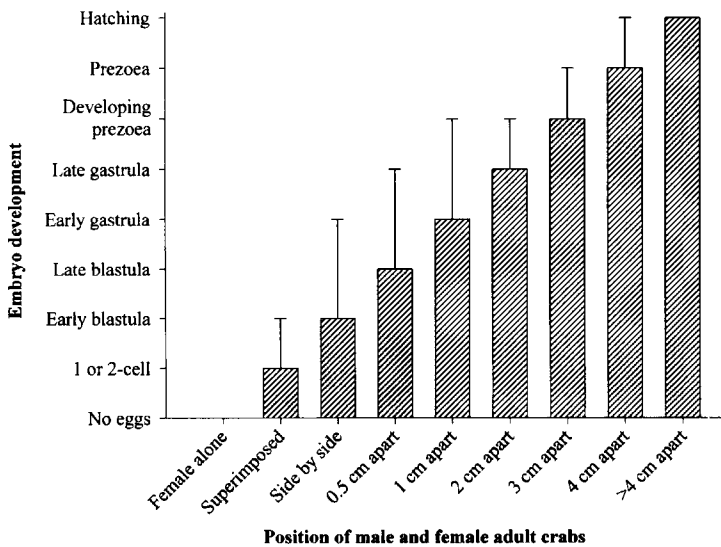


Fig. 5. Embryonic development of the female brood versus the position of male and female pea crabs *Pinnotheres halingi* with respect to each other inside the respiratory tree of their host. A total of 184 pairs were examined and the vertical lines represent the complete range of stages observed.

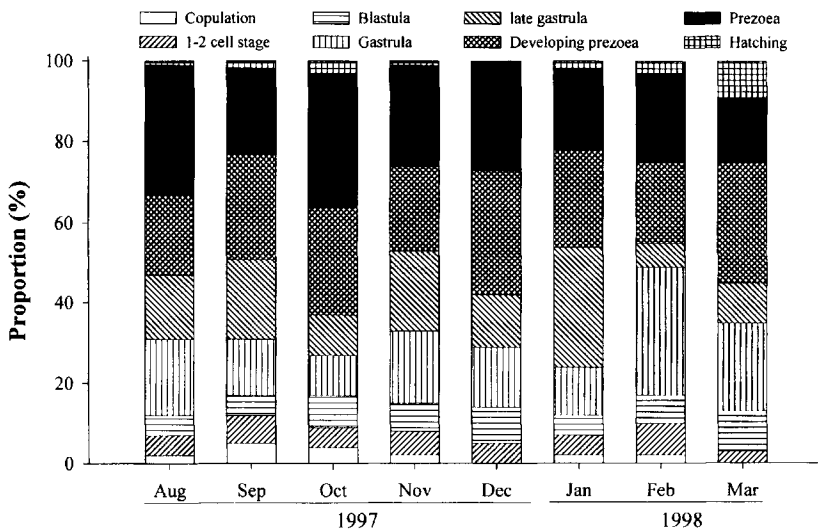


Fig. 6. Proportion of copulating crabs and different embryonic stages observed in the broods of female *Pinnothores hatingi* collected from August 1997 to March 1998.

mained associated long enough for some embryos to reach the blastula stage, which corresponds to a delay of about 5 days. In nine cases, the paired female harboured no egg or embryo.

When male and female were in the same host but in separate cysts, the female was always found closer to the anus than the male, except on three occasions when males measuring  $5.4 \pm 0.3$  mm were found at the very entrance of the cloaca with non-ovigerous females.

The sequence of events that precedes and follows copulation was determined by correlating the maturation of the female's embryos with the dispositions of male and female crabs in the respiratory tree (Fig. 5). While single females possessed either no or unfertilised oocytes, the occurrence of a couple was always associated with the presence of fertilised eggs or developing embryos, except in the nine cases stated earlier, where male and female were in the same cyst. The greater the distance between the male and the female, the more developed the embryos on the female's pleopods (Fig. 5).

There was no distinct pattern of maturation in the brood of ovigerous females over the monthly samplings (Fig. 6). While the development of embryos of a given female was homogeneous, the development of embryos was quite heterogeneous between females collected at the same date: some held unfertilised or recently fertilised eggs, while others had embryos in the early stages of development or were nearing the release period. There was always a small proportion of spent females, with residual cement on the pleopods. Late gastrula,

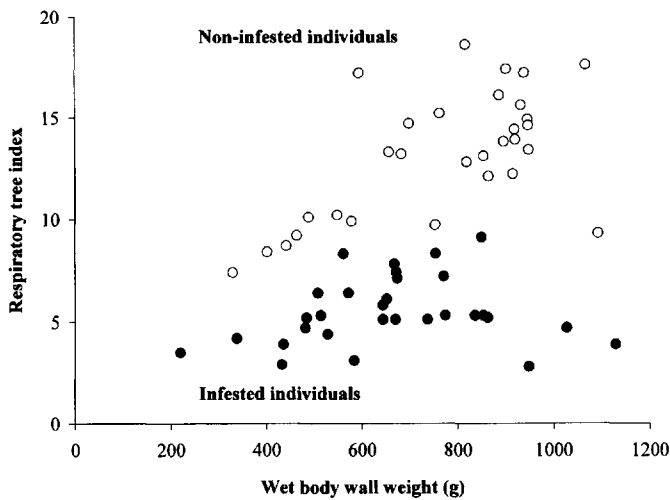


Fig. 7. Respiratory tree indices of infested and non-infested sea cucumber *Holothuria scabra* (N = 30).

developing prezoa and prezoa were the most abundant stages of development. (Fig. 6).

#### *Macroscopic and microscopic effects of pea crabs on their host*

**Macroscopic effects.** – Atrophy of the right respiratory tree was visible in all infested sea cucumbers. The thickness of the epithelium around the cyst of male and female crabs was  $1.7 \pm 0.3$  mm and  $3.3 \pm 0.6$  mm, respectively, whereas the thickness of non-infested tissue was  $0.8 \pm 0.4$  mm. Male crab cysts had a volume of  $809 \pm 204$  mm<sup>3</sup> and female cysts had a volume of  $1437 \pm 350$  mm<sup>3</sup>. The right respiratory tree was generally thickened between the male and female cysts.

No difference was noted between the gonad index of infested versus non-infested sea cucumbers (Kolmogorov-Smirnov test,  $P > 0.05$ ). However, the respiratory tree indices of infested animals were significantly lower (Kolmogorov-Smirnov test,  $P < 0.001$ ) (Fig. 7). Considering male and female sea cucumbers together, the respiratory tree index was almost three times lower in infested (N = 30) compared to non-infested individuals (N = 30) collected in August and September 1997 (Fig. 7).

**Microscopic effects.** – The membranaceous cyst that formed around the male and female pea crabs looked like massive cellular proliferations of fibrous cells (parenchymatic cells) in the lining of the inner epithelium and the connective tissue layer of the respiratory tree. The thickness of the respiratory tree wall was consequently increased by up to 550% for female cysts and 275% for male cysts. The glandular epithelium area was affected by anarchic cellular proliferation around a zone that appeared to be altered by successive lesions inflicted by the



Table 1. Development of the pea crab *Pinnotheres halingi* collected from the respiratory tree of sea cucumbers *Holothuria scabra* in Kogu Halingi, Solomon Islands. Development was deemed to have progressed to another stage once >50% of individuals were involved. The time taken to reach a given stage is the average for 28 broods. The size of eggs and embryos is given as Mean  $\pm$  SD (N = 15-58).

Stage of development	Time	Size ( $\mu$ m)
Unfertilized oocytes	-	435 $\pm$ 15
1-cell	0	482 $\pm$ 21
2-cell	6 h	488 $\pm$ 19
4-cell	14 h	487 $\pm$ 22
8-cell	1 d	475 $\pm$ 12
16-cell	2 d	472 $\pm$ 15
64-cell	3 d	482 $\pm$ 11
128-cell	3.6 d	479 $\pm$ 9
256-cell	4.4 d	480 $\pm$ 12
Late blatula	8 d	522 $\pm$ 19
Early gastrula (blastopore)	10 d	543 $\pm$ 23
Late gastrula (slight axial elongation)	11 d	526 $\pm$ 14
Developing prezoaea	14 d	545 $\pm$ 21
	28 d	667 $\pm$ 32
Hatching of prezoaea	30 d	701 $\pm$ 22
First zoeal stage	31 d	701 $\pm$ 15
Second zoeal stage	35 d	775 $\pm$ 23
Third zoeal stage	40 d	907 $\pm$ 35
Fourth zoeal stage	42 d	1112 $\pm$ 34
Fifth zoeal stage	45 d	1215 $\pm$ 41
Megalopal stage	50 d	1175 $\pm$ 35
Host infestation	59 d	1275 $\pm$ 25

pea crab legs. The longitudinal and circular muscle bands and outer peritoneal epithelium remained unaffected.

#### *Embryonic development of pea crabs*

Because the pea crabs cannot survive long outside their host, eight of the 36 females that were maintained for the purpose of this study died before the end of the experiment. Nonetheless, the development of ca. 600-700 embryos was successfully monitored until the time they were released by the females (Table 1). Time 0 presented in the development does not correspond to fertilisation, but rather to the stage 1-cell, as we could not assess the exact time of fertilisation.

The development of the embryos from the 1-cell stage to late gastrula took ca. 11 days (Table 1). The egg mass developed an orange tinge over this period. In the following days, the first differentiation of appendices appeared during the development of the prezoaea. Hatching occurred ca. 30 days after the beginning of brooding when the first zoeae were fully developed. Five successive zoeal stages of increasing size developed over the next 19 days. The subsequent

Table 2. Choice of host by megalopal larvae of *Pinnotheres halingi* exposed to different species of adult sea cucumbers. The results obtained with different sizes of *Holothuria scabra* are also illustrated. Width values as Mean  $\pm$  SD.

Sea cucumber species	Infestation frequency (%)	Location of crab	Crab width (mm)	Crab sex
<i>Actinopyga mauritiana</i>	0	-	-	
<i>Stichopus variegatus</i>	0	-	-	
<i>Bohadschia marmorata</i>	0	-	-	
<i>Holothuria leucospilota</i>	0	-	-	
<i>Holothuria fuscopunctata</i>	0	-	-	
<i>Holothuria atra</i>	0	-	-	
<i>Holothuria scabra</i> (<10 mm)	0	-	-	
<i>Holothuria scabra</i> (10-40 mm)	0	-	-	
<i>Holothuria scabra</i> (40-80 mm)	0	-	-	
<i>Holothuria scabra</i> (80-120 mm)	100	Right respiratory tree	2.4 $\pm$ 0.3	3 ♀ and 1 ♂
<i>Holothuria scabra</i> (120-150 mm)	100	Right respiratory tree	3.0 $\pm$ 0.4	2 ♀ and 2 ♂
<i>Holothuria scabra</i> (>200 mm)	100	Right respiratory tree	3.6 $\pm$ 0.4	1 ♀ and 2 ♂
<i>Holothuria scabra</i> (>200mm, already infested)	0	-	-	

megalopal stage lasted ca. 9 days (Table 1). Megalops were ready to infest a host ca. 59 days after the beginning of development. In the absence of a host, the megalops died after 10 more days without showing any settling behaviour. No difference in development of embryos was observed between those collected daily and those collected after 30 days in undisturbed females.

Subsequent development of young crabs occurred inside the hosts and were observed only randomly. While we are unable to present the precise development, we observed that specimens collected after three months of growth in the infestation experiment described below showed that males ca. 2.4 mm wide and females ca. 3.0-3.6 mm wide were at the pre-hard stage.

#### *Larval dispersion and infestation*

On one occasion (19 August 1997) pelagic first zoeae of *Pinnotheres halingi* were released during exhalation via the anus of a host sea cucumber *Holothuria scabra*.

During the course of the infestation experiments, the megalops repetitively touched the host and found their way to the anus within 3-4 h. They entered during an inhalation of the holothurian. The megalops were never observed to settle on the surrounding substrate.

The pelagic larvae of *Pinnotheres halingi* were highly selective (Table 2). When given a choice between different holothurian species, they only infested *Holothuria scabra*. This observation corresponds to the field results presented earlier. More specifically, the larvae only selected *Holothuria scabra* hosts measuring >80 mm (Table 2). In the infestation experiment, a total of five male and six female

Table 3. Infestation frequency of host (N = 5-22) by male and female sub-adult pea crabs *Pinnotheres halingi* exposed to sea cucumbers *Holothuria scabra* with different levels of infestation.

Level of infestation	Pea crab sex	Infestation frequency (%)	Location of pea crab in host
Non-infested	Female	32	Respiratory tree
Non-infested	Male	14	Cloaca
Infested with female	Female	0	-
Infested with male	Female	0	-
Infested with sub-adult couple	Female	0	-
Infested with adult couple	Female	0	-
Infested with male	Male	0	-
Infested with female	Male	94	Respiratory tree
Infested with sub-adult couple	Male	0	-
Infested with adult couple	Male	0	-

sub-adult pea crabs <4 mm were found, all located in the right respiratory tree. Their shape and colour after three months of growth were very similar to those of the adults and there was always only one crab per host. Larval infestation did not occur in hosts already infested with larger crabs (Table 2).

#### Behaviour

*Adults.* – Adult pea crabs demonstrated no ability to re-infest a host after being removed from their previous one. When exposed to a choice of different species of sea cucumbers, the adult crabs wandered among all species, unable to enter any, including crab-free and pre-infested *Holothuria scabra*.

*Sub-adults.* – Sub-adult pea crabs exposed to non-infested *Holothuria scabra* were not always able to colonise them (Table 3). Overall, 32% of the females tested were able to enter the respiratory tree of a non-infested host, whereas only 14% of the males had the same ability. Females >7 mm and males >6 mm wide were never observed to re-enter a host.

Sub-adult female pea crabs exposed to sea cucumbers already infested by a sub-adult female, male, or by a pair (either sub-adult or adult), did not enter the host (Table 3). Sub-adult males responded in the same way, except in the presence of a sea cucumber harbouring a single sub-adult female. In 94% of those instances, the male rapidly found its way through the anus of the sea cucumber to join the female in the right respiratory tree.

The final infestation frequency of sea cucumbers *Holothuria scabra* pre-infested with either a male or a female pea crab and maintained together for a week in the same tank was as follows: 100% of the sea cucumbers infested by a single sub-adult male were empty, whereas 65% of the hosts that initially harboured a single female contained a male-female pair. It appeared that two of the males died during the course of the experiment.

No aggressiveness was ever observed when more than one male crab was placed in the presence of a host infested by a single female. The first male to reach the anus entered the host and the others remained outside for the rest of the observation period.

#### Other associations observed in *Holothuria scabra*

Other symbiotic associations were observed in a few sea cucumbers *Holothuria scabra* collected in the bay of Kogu Halingi (site 1, Fig. 1). The carrapid fish *Encheliophis (Jordanica) gracilis* was found in the coelomic cavity of 2.5% of the sea cucumbers. On one occasion, two specimens were observed in the same host. The crabs *Hapalonotus reticulatus* and *Lissocarcinus orbicularis* were observed in the cloaca of five specimens. Finally, an undetermined species of bivalve was growing on the carapace of two pea crabs *Pinnotheres halingi* from two different hosts.

## DISCUSSION

### *General observations*

Pea crabs *Pinnotheres halingi* were mostly found in pairs of opposite sex in *Holothuria scabra* hosts, whereas previously described holothurian-dwelling crabs are reported to occur predominantly or exclusively alone, with a greater proportion of females (Chopra 1931; Wells & Wells 1961; Jones & Mahadevan 1965; VandenSpiegel & Jangoux 1989; VandenSpiegel et al. 1992; Takeda et al. 1997). However, Wells & Wells (1961) recorded five pairs out of hundreds of *Pinnaxodes floridensis* in *Theelothuria princeps*, Chopra (1931) observed that pairing occurred in *Pinnotheres setnai* from an unknown host and Chuang (1961) mentioned that pairs of *P. semperi* may occur in *H. scabra*.

Within a pair of pea crabs *Pinnotheres halingi*, males were always roughly half the carapace width of females. A similar disproportion has been observed in other crabs associated with holothurians (Chopra 1931; Wells & Wells 1961; VandenSpiegel & Jangoux 1989; VandenSpiegel et al. 1992). This is almost certainly associated with the egg-production role of the female.

The location of *Pinnotheres halingi* inside the respiratory tree is also similar to that observed in other pea crab-holothurian symbioses (Chopra 1931). In fact, all known crab symbionts of *Holothuria scabra* occur in this area: *P. semperi* (Lanchester 1900; Chuang 1961), *P. deccanensis* (Jones & Mahadevan 1965) and *Hapalonotus reticulatus* (VandenSpiegel et al. 1992). Both *P. semperi* and *H. reticulatus* are also said to form a membranaceous cyst (Lanchester 1900; VandenSpiegel et al. 1992) like the one observed during our study.

### *Reproduction and life cycle of pea crabs*

VandenSpiegel & Jangoux (1989) suggested that the proximity of hosts *Actinopyga mauritiana* would allow pea crabs *Pinnotheres villosissimus* to find a mate.

The reproduction of *P. halingi* and that of their hosts might be closely related since adult *Holothuria scabra* appear to exhibit an aggregative behaviour that could favour their own reproductive synchrony (Mercier & Hamel, unpub. data) as well as the pair formation of the symbiotic pea crabs they harbour. Couples seem to form early in the life history of *P. halingi* and soon after the infestation of a new host, as inferred by the fact that male-female pairs were observed in nearly 99% of the hosts collected. Furthermore, the crabs lose the ability to select and enter a host upon reaching a certain size.

Adult male crabs were usually found deeper in the respiratory tree than females. At first, we suspected that this was due to males entering first. However, the available evidence is against this hypothesis. First, single females were far more abundant than single males. Second, all the single females were non-ovigerous and smaller than the females found in a pair, suggesting they were immature, whereas all single males were mature adults. This observation suggests that single males remained in the host after the departure of their female companion. Third, females were always found roughly at the same distance from the anus, inferring that they are fixed in the host. Finally, small males <6 mm were found to be attracted to hosts that already harboured a female crab, whereas the inverse was not observed, confirming that the male was the motile partner. The thickness of female cysts also suggested that they remain immobile in the host for a longer period than males. Thus, we concluded that the female enters the host first, either at the megalop stage or as an immature crab. The male, which remains motile for a longer period seems to enter subsequently. The progression of the male from the anus to meet the female over an average distance of 21 mm must be rather rapid since only three males, all <6 mm, were found between a non-ovigerous female and the anus.

From the development of the embryos and the associated position of the male crabs inside the respiratory tree, we can estimate that, once it has found a female, the male can travel approximately 40 mm in a month. This coincides with the delay between copulation and the time the embryos attain the first zoeal stage. It is likely that the movements of males are sporadic rather than continuous because independent male and female cysts are formed as soon as the two sexes are 5 mm apart. This indicates that the cyst is synthesised quite rapidly. Its resorption also seems rapid, as only minimum traces of successive cyst formations were left by the progression of males from the anus to the end of the right branch of the respiratory tree.

The fact that females were occasionally found to be non-ovigerous during copulation suggests that mating may precede oviposition and that fertilisation may be delayed while the female stores sperm in a spermatheca for future use. However, we also observed male and female in the same cyst while the female was sheltering a developing mass of eggs. This indicates that oviposition must usually take place shortly after copulation. The fact that males apparently do not travel backwards after copulation, but continue toward the end of the res-

piratory tree is quite unexpected. No ovigerous or spent female was ever found alone. It would thus seem that males never leave the host after copulation. Furthermore, since small males were never seen to enter a host already occupied by a male-female pair, it would seem that pairing is permanent. This may be due to the fact that entrance into a host is possible only during larval and juvenile stages, an infestation behaviour that may have evolved because of the relative scarcity of crab-free *Holothuria scabra* in the bay. Previous reports usually stated that adult pea crabs freely moved from one host to another but also estimated much lower infestation frequencies (Wells & Wells 1961; Takeda et al. 1997).

Whether *Pinnotheres halingi* reproduces repeatedly remains unknown, and the evidence we have at present does not support the possibility. It would require that the male went back to the female after the release of the zoeae, however males were never found less than 40 mm away from a spent female. Nonetheless, considering the rapid development of the crabs, it would be possible for this species to reproduce more than once during its life cycle. Furthermore, female crabs seem to become ovigerous at a small size (ca. 9 mm wide) and remain able to reproduce when reaching 17 mm wide. Finally, the heterogeneous level of development of the broods of females collected at the same date suggests that copulation, embryonic development and release of zoeae occur all year round and do not follow a precise cycle. In contrast, pea crabs *Pinnixa tumida*, which live singly inside the alimentary canal of the holothurian *Paracaudina chilensis*, appear to reproduce synchronously on a yearly period (Takeda et al. 1997).

Owing mainly to the fact that specimens were usually scarce or immediately preserved, the reproductive cycle of crabs associated with holothurians have rarely been presented in such detail as has been done here for *Pinnotheres halingi*. The development of holothurian-dwelling *Pinnotheres* has apparently not been reported before. To our knowledge, development and rearing of pinnotherids have only been described for species associated with mussels and oysters (Atkins 1926, 1955; Stauber 1945; Sandoz & Hopkins 1947; Christensen & McDermott 1958; Pearce 1964, 1966; Soong 1997) and for *Tritodynamia horvathi* (Otani et al. 1996). Even though *T. horvathi* is a swimming species, it exhibits a kinetic of zoeal and megalopal stages development that is similar to the one we observed in *P. halingi*. Our observations are also comparable to those of Atkins (1955) for mussel crabs *P. pisum* and *P. pinnotheres*, although the latter often developed more slowly, presumably because they were reared at lower temperatures associated with the habitat of their host, *Mytilus edulis*. One factor that needs to be considered in our description of development is that although we left the embryos to develop on the female pleopods, removal of the female from the host may have influenced embryonic development.

In our study, most larvae settled after nine days in the megalopal stage when they found a suitable host. They were, however, able to survive for another 10 days in the absence of a host. The fact that no larvae settled elsewhere than in a

host indicates that infestation occurs early in the life cycle of *Pinnotheres halingi*. In contrast, bivalve-dwelling species were observed to invade the host as true crabs (Sandoz & Hopkins 1947; Christensen & McDermott 1958; Pearce 1966). Stauber (1945) proposed that invasion of the bivalve host in the crab stage would be facilitated by the carapace, as it offered protection against the crushing valves. However, it is not clear whether or not previous studies tried placing megalopal stages in contact with the hosts. Wells & Wells (1961), who studied *Pinnaxodes floridensis* in the holothurian *Theelothuria princeps*, found juvenile crabs in the anterior digestive system of some hosts, while adult symbionts occupied the cloaca and respiratory tree. They could not assess whether this was really a path of invasion, but believed that infestation could occur at the first crab stage. Takeda et al. (1997) found that only mature pea crabs *Pinnixa tumida* were able to enter their holothurian hosts, *Paracaudina chilensis*. We conclude that *P. halingi* either invades its holothurian host at an earlier stage of development than previously described species of pinnotherids, or that we have observed megalopal invasion for the first time. Nonetheless, small true crab stages of *P. halingi* were also observed to retain an invasive capacity until they reached ca. 6 mm in carapace width. Contrary to *P. chilensis* (Takeda et al. 1997), *Holothuria scabra* hosts were never observed to resist the entrance of the crabs.

The rarity of juvenile crabs in hosts collected from the wild does not necessarily mean that development to crab stage occurs outside the host as proposed by VandenSpiegel & Jangoux (1989). Even though juveniles were scarce in our samples, infestation experiments clearly demonstrated that *Pinnotheres halingi* could enter its host at the megalopal stage. The potentially rapid growth and maturation of newly settled crabs, or a climax with nearly 100% infestation frequency as observed in Kogu Halingi, could account for the scarcity of juvenile individuals in the wild.

Prior to this study, descriptions of pairing and copulation of pea crabs associated with holothurians were scarce and largely speculative. Based on the observation of two male and four female *Hapalonotus reticulatus* found individually in the respiratory trees of *Holothuria scabra* from Papua New Guinea, VandenSpiegel et al. (1992) proposed that copulation occurred in the cloaca or outside the hosts due to the crampedness of the cyst. Because the density of holothurian hosts was low, they also suggested that the crabs would have to move long distances to mate. However, as mentioned above, *H. scabra* aggregates periodically (Mercier & Hamel, unpub. data) and copulation of pea crabs inside a tight cyst was observed in this study. Therefore, these principles may also be applicable to *H. reticulatus*. For their part, Wells & Wells (1961) collected 174 *Pinnaxodes floridensis* from *Theelothuria princeps* in the Gulf of Mexico. Based on male:female ratio and characteristics of the samples, the authors tentatively concluded that males had to search for females, that copulation occurred in the host before the female reached maturity and that oviposition followed moulting. Al-

though the proposed sequence is similar to the one we observed, no evidence of moulting was found during our study and copulation occurred between well-developed mature adults.

#### *Host-symbiont specificity and geographical distribution*

Host recognition was precise in megalops of *Pinnotheres halingi*: they invaded hosts of a single species and a certain size and only those that did not already shelter a pea crab. In turn, sub-adult male *P. halingi* were only slightly attracted to non-infested *H. scabra*, but strongly attracted to those that harboured a single female. It is possible that water-borne chemicals play an important role in the life cycle of *P. halingi*: first during the infestation period and later during the pair formation. The role of pheromones in crustaceans has previously been described (Atema & Engstrom 1971; Atema et al. 1979; Gleeson 1980; Atema & Cowan 1986) while chemotaxis was reported between the pinnotherid *Dissodactylus mellitae* and its echinoid host (Johnson 1952), as well as between crinoid hosts and their symbionts, the crab *Harrovia longipes* (Eeckhaut et al. 1998) and the shrimp *Synalpheus stimpsoni* (VandenSpiegel et al. 1998). Conspecific recognition and host choice by pea crabs *Pinnixa chaetoptera* living in the tubes of polychaete *Chaetopterus variopedatus* have recently been tested by Grove & Woodin (1996). They showed that adult crabs were not attracted by cues from their host but rather by cues from conspecifics, although sex-specific recognition such as the one observed in this study was not detected.

A striking result of this study is that only populations of *Holothuria scabra* in Kogu Halingi were infested with pea crabs. Because the pelagic stages of pea crab larvae extend for nearly two months, allowing sufficient time for propagation, the physical characteristics of the bay, i.e. topography and currents, are apparently the main factors limiting larval distribution and infestation. It is thus likely that this new species of pea crab, *Pinnotheres halingi*, occurs only in sea cucumbers *H. scabra* from Kogu Halingi in the Solomon Islands. While host-specificity is a common feature of pea crabs associated with holothurians, their distribution, whenever more than one sample of hosts is examined, is always wider than so far observed for *P. halingi* (Chopra 1931; Wells & Wells 1961; Jones & Mahadevan 1965; Schmitt et al. 1973).

#### *Relationship between Pinnotheres halingi and Holothuria scabra*

There is no evidence that the relationship between *Pinnotheres halingi* and *Holothuria scabra* is useful to both parties. The benefits to the crab and its adaptation to the symbiotic way of life are the most obvious. No advantages to the host could be determined. Rather, a severe respiratory tree atrophy seems to be associated with the presence of pea crabs, probably because they partly interrupt water flow in the right portion of the organ.

Based on its behaviour and degenerate features, *Pinnotheres halingi* is appar-



ently totally dependent on its host. The fact that neither male nor female crabs >6 mm are able to re-infest a host, and that neither can subsist long outside their host, further points to a permanent symbiosis, which somewhat differs from previous observations of *Holothuria scabra* symbionts. VandenSpiegel et al. (1992) observed that even though *Hapalonotus reticulatus* induced the formation of a cyst, their ability to move out of the host in an aquarium, and their contrasted colours and well-developed eyes suggested that they became symbiotic only late in their lives. Jones & Mahadevan (1965) found female *P. decanensis* in the respiratory tree of *H. scabra*: again, the ability to re-enter the host, and the absence of juveniles led them to assume that the crabs were not born commensals. The authors further stated that they could not assess whether or not the relationship was obligatory. As for *P. semperi*, their presence in *H. scabra* was reported (Lanchester 1900; Chuang 1961) but their relationship with the host was never carefully studied.

All *Pinnotheres halingi*, juveniles and adults, were found exclusively in the right respiratory tree of *Holothuria scabra*. This part of the respiratory tree is the only internal organ that is never eviscerated when this species is disturbed. The adaptive significance of this for an obligate symbiont is obvious. Nevertheless, VandenSpiegel et al. (1992) found that the eumedonid crab *Hapalonotus reticulatus* also inhabited the right respiratory tree of *H. scabra* even though the crab could move in and out of its host. Thus, the choice of the right respiratory tree seems advantageous whether or not the crabs can survive outside the host. Other types of holothurian parasites do, however, occupy organs that can be eviscerated. For instance, Byrne (1985) showed that the gastropods *Thyonicola americana* were eviscerated seasonally with the intestine of *Eupentacta quinquesemita* and that the parasites were not able to survive outside the host. Stability is obviously not the only criterion in the choice of a location; food availability is likely to be equally important. *H. scabra* generally live in nutrient-rich littoral areas. As residents of the respiratory tree, pea crabs are thus provided with an abundance of particulate matter with each inhalation of their host.

#### Summary

Pea crabs *Pinnotheres halingi* sp. nov. possess distinct features that set them apart from all previously described Indo-Pacific species of *Pinnotheres*. After ca. 59 days of development through brooded and pelagic stages, larval *P. halingi* then infest sea cucumbers *Holothuria scabra* >80 mm in length, and only one crab survives per host. Male crabs may later move out of their initial host to find a female. This transfer could occur during the monthly aggregations of the sea cucumbers. During this period the male pea crab is able to detect the presence of a female located in a nearby host. The crabs copulate within the female cyst. The male remains with the female, but progressively moves to the end of the right respiratory tree as the embryos develop on the female pleopods. The in-

festation frequency observed in Kogu Halingi bay (ca. 99%) is, to our knowledge, the highest ever reported for a holothurian-dwelling crab. It suggests that the population of pea crabs has reached its climax in Kogu Halingi, that the mortality rate of *P. halingi* may be less than their reproductive output and that *H. scabra* recruits are infested as soon as they reach the proper size. This species of pea crab appears to be restricted to this one site. Either the hydrodynamics of the bay and its isolation from the open ocean restrict propagation of the crab larvae, or the particular physico-chemical properties of the bay favour the proliferation of *P. halingi* in Kogu Halingi.

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