Prevalence, reproduction and morphology of the parasitic isopod Athelges takanoshimensis Ishii, 1914 (Isopoda: Bopyridae) from Hong Kong hermit crabs

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ORIGINAL ARTICLE

Prevalence, reproduction and morphology of the parasitic isopod *Athelges takanoshimensis* Ishii, 1914 (Isopoda: Bopyridae) from Hong Kong hermit crabs

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Abstract
The bopyrid isopod *Athelges takanoshimensis* is a relatively common abdominal parasite of hermit crabs from Asia. This study investigated the prevalence, reproduction, and morphology of *A. takanoshimensis* from over 1560 hermit crab specimens collected in Hong Kong between 2000 and 2004. Among these collections, *A. takanoshimensis* was on ∼7% of the hermit crab *Pagurus angustus* and was also recorded from 5% of *Pagurus hedleyi*; no hermit crabs from the genus *Clibanarius* were infested. The male–female ratio of parasitized *P. angustus* was approximately 3:7, in contrast to a 3:2 ratio for uninfested hosts, suggesting that parasitism has an influence on host sex ratio. *Athelges takanoshimensis* produced up to 5031 embryos (average brood size = 2852). Estimates of body size (head length, pereon length, and total length) were analysed as predictors of fecundity but a significant correlation was only found between brood size and pereon length. The morphology of the life history stages of *A. takanoshimensis* is described using scanning electron microscopy (SEM), including the first investigation of epicaridium larvae for this species. Notes on the behaviour of ovigerous females and the release of their larvae are provided, thus providing a better understanding of the natural history and morphology of *A. takanoshimensis*.

Key words: *Bopyrid, epicaridium, fecundity, isopod,* Pagurus, *parasite*

Introduction

Isopods are a diverse group of crustaceans, with over 10,300 described species (Wilson 2008). Most isopods are free-living, but many (∼20%) are exclusively parasitic on a range of hosts, including fish and crustaceans (Williams & Boyko 2012). Those that exclusively parasitize crustacean hosts are in the Epicaridea and belong to two superfamilies: Bopyroidea Rafinesque, 1815 and Cryptoniscoidea Kossman, 1880 (Williams & Boyko 2012; Boyko et al. 2013; WoRMs 2013). Within Bopyroidea, members of the family Bopyridae Rafinesque, 1815 are all ectoparasites, parasitizing crustacean hosts by invading their branchial chamber or attaching to their abdominal region. There are five subfamilies within the Bopyridae that parasitize the branchial chambers of their hosts (Argeiinae, Bopyrinae, Keponinae, Orbioniinae, Pseudioninae) and three that are abdominal parasites (Hemiarthinae, Phyllodurinae, Athelginae;

Markham 1986; Williams & Boyko 2012; Boyko et al. 2013). The evolutionary relationships of the subfamilies have been investigated and it is hypothesized that the abdominal parasites do not form a monophyletic group, but instead are convergent in their abdominal parasitism (Boyko et al. 2013).

Bopyrids exhibit sexual dimorphism where the dwarf male is of typical isopod form while the female is much larger with more modified morphology (Anderson 1990; Williams & Boyko 2012). Whereas the male's body is symmetrical, the female's is typically dextrally or sinistrally distorted to varying degrees. Bopyrids affect host reproduction such that it may be reduced or they may cause complete castration by feeding on the hosts' blood (Tucker 1930; Hiraiwa & Sato 1939; Dall et al. 1991; McDermott 1991, 2002; Jordá & Roccatagliata 2002; González & Acuña 2004; Calado et al. 2005; Romero-Rodríguez & Román-Conteras 2008, 2013; McDermott et al. 2010; Dumbauld et al. 2011). Calado et al. (2005) indicated

that host reproduction is inhibited in both sexes, with parasitized females not being able to develop mature gonads and parasitized males being feminized. Similarly, Romero-Rodríguez & Román-Contreras (2011) found feminization of the shrimp *Thor floridanus* Kingsley, 1878 by the branchial parasite *Bopyrinella thorii* (Richardson, 1904). Other effects may include inhibition of host metabolism (Anderson 1975a, 1975b, 1977), physiology (Bursey 1978; Schuldt & Rodrigues-Capítulo 1987), and changes in behaviour (Bass & Weis 1999). Although the impacts of bopyrids on hosts have been studied in some detail, the ecology and reproduction of bopyrids are in need of investigation.

Bopyrid life cycles involve two crustacean hosts: a decapod definitive host and a copepod intermediate host (Figure 1; see also McDermott et al. 2010; Williams & Boyko 2012). After mating and brooding eggs on a definitive host, a sexually mature female releases epicaridium larvae. The larvae first parasitize an intermediate copepod host in the water column, typically a calanoid copepod; see Owens & Rothlisberg (1995). While attached, the larvae metamorphose into microniscus larvae, and later cryptoniscus larvae. After becoming cryptoniscus larvae, they detach from the intermediate hosts and infest a definitive decapod host. The first juvenile bopyridium to attach to the definitive host develops into a female, while the subsequent juveniles will be male and attach to the female on the ventral surface of the pleon and will fertilize the eggs (Reinhard 1949; but see Hiraiwa 1936 for an example of a bopyrid in which sex appears to be determined early in development). The female houses developing embryos within a brood chamber covered (at least partially) by oostegites, while residing in the branchial chamber of the host or on the host's abdomen (Beck 1980a). The cycle repeats itself when larvae are
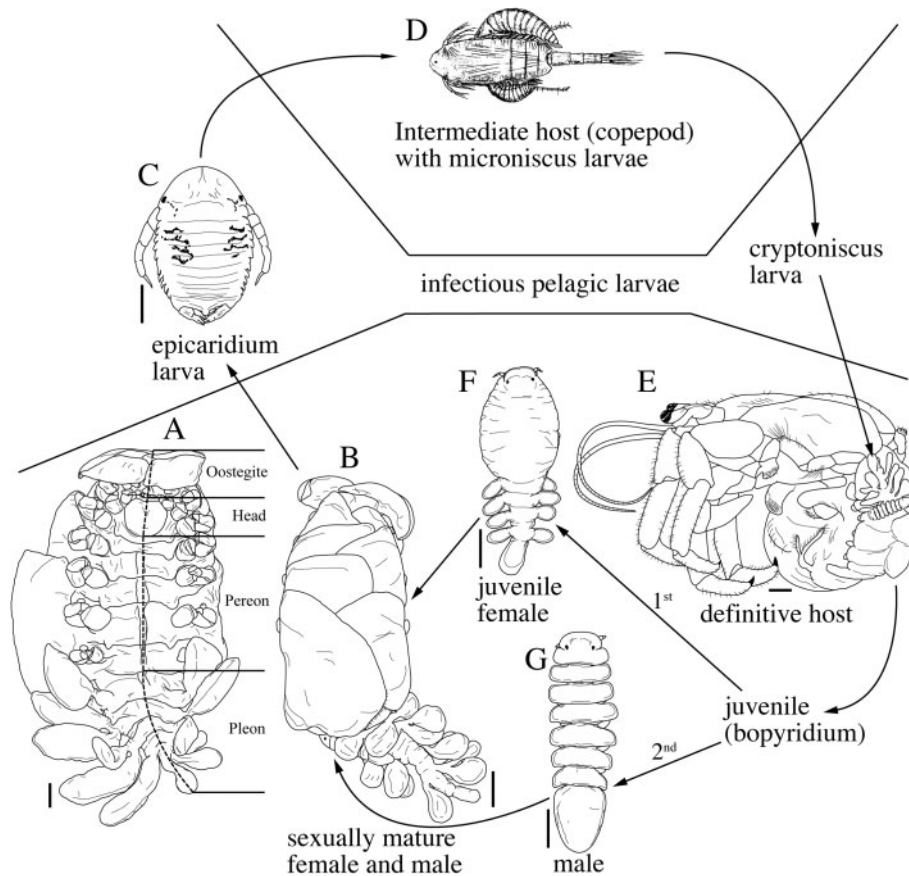
Figure 1. Life cycle stages and morphology of *Athelges takanoshimensis*. (a) Dorsal view of mature female parasites showing body measurements (oostegite = extension of first oostegites beyond anterior head margin, head length, pereon length, pleon length; total body length was calculated from the sum of all four measures); (b) Ventral view of ovigerous female. Female releases epicaridium larvae (c) from oostegites, which move through the water column and parasitize calanoid copepod intermediate hosts (d), subsequently metamorphosing into microniscus larvae. Cryptoniscus larvae detach and parasitize a definitive host hermit crab (e). At this point, the larvae have developed into juveniles; the first juvenile to parasitize the host becomes a female (f), and any subsequent juvenile that settles on parasitized hosts becomes a dwarf male (g), which lives and grows on the female while fertilizing her eggs. The cycle repeats itself when the ovigerous female (a,b,e) releases her larvae. Scale bars = 1 mm (a,b,e), 0.5 mm (f,g), 0.05 mm (c). Intermediate host (d) is not drawn to scale. Modified from McDermott et al. (2010).

released from the chamber (Figure 1; McDermott et al. 2010; Williams & Boyko 2012). Although some bopyrids are known to have the ability to change sex (i.e. male to female if the primary female dies), this has not been shown definitely in most species (Reinhard 1949).

Many studies have shown that parasite size correlates positively with host size (Wenner & Windsor 1979; Jay 1989; Cash & Bauer 1993; Poulin 1995; Jordá & Roccatagliata 2002; Miranda & Mantelatto 2010). Wenner & Windsor (1979) found a positive linear relationship between the size of the squat lobster hosts *Munida iris* A. Milne Edwards, 1880 and the bopyrid parasite *Anuropodione carolinensis* Markham, 1974. This relationship appears to be the result of isopods parasitizing the squat lobsters when they are both juveniles, with both growing towards sexual maturity together. Similar relationships have been shown in other hosts (Owens & Glazebrook 1985), including hermit crabs (Reinhard 1949; Pike 1961; see figure 12 in McDermott et al. 2010). The life span of most hermit crabs is estimated at approximately 2–5 years (Pike 1961; Mantelatto et al. 2005), but the longevity of bopyrids is poorly known. For some bopyrids that have been investigated, the life span appears to match that of the host (Pike 1961; Beck 1980b), whereas in others they may be shorter-lived (Cañete et al. 2008). Infested hosts may not live as long as uninfested conspecifics due to the expense of energy required to host a parasite (Pike 1961; Cañete et al. 2008).

Less is known about the relationship between size and fecundity of ovigerous female bopyrid parasites. Romero-Rodríguez & Román-Contreras (2008) investigated reproductive attributes of *B. thorii* parasitizing *T. floridanus*. The authors found that brood size correlated positively with total body length under natural conditions and discussed the literature on reproduction in bopyrids. However, data on the reproduction of bopyrids remain limited (Table I) and analysing trends between parasite size and eggs per brood is difficult because of the asymmetrical structure of females and potential distortion of the body during fixation. Head and pereon lengths of females are easier to measure and may serve as better proxies than total body length for examining these relationships, expecially in abdominal bopyrids which can exhibit extensive distortion or twisting of the pleon. Review of the literature on the fecundity of bopyrids shows that several studies have investigated the brood sizes of branchial species, but far fewer exist for abdominal species (Table I). Although there are over 40 species of athelgine bopyrids that parasitize hermit crabs (McDermott et al. 2010), only two studies to date have reported on the reproduction of abdominal bopyrids associated with hermit crab

hosts (Table I; McDermott 1998, 2002). McDermott (1998) showed that the abdominal bopyrid *Anathelges hyptius* (Thompson, 1902), parasitizing the hermit crab *Pagurus longicarpus* Say, 1817, had a maximum fecundity of 3437 embryos. The abdominal bopyrid *Stegias clibanarii* Richardson, 1904, parasitizing *Clibanarius tricolor* (Gibbes, 1850), showed a positive correlation between the numbers of embryos and the length of ovigerous females with a maximum of 667 embryos (McDermott 2002). Reproduction of branchial bopyrids has been studied in more detail because they parasitize hosts of commercial importance (e.g. shrimp) and have been shown to negatively impact host reproduction.

Determining the effects of abdominal bopyrids on hermit crab hosts is also needed because hermit crabs are ecologically important members of marine systems. Most hermit crabs are omnivorous scavengers (Schembri 1982), often attaining large population sizes and representing key components of food webs, including serving as prey for commercially important fish (Yang 2004). In addition, hermit crabs are ecologically important because they act as ecosystem engineers, providing a substrate for whole communities of species (Williams & McDermott 2004). In fact, hermit crabs are hosts for over 700 symbionts including commensal, mutualistic, and parasitic species such as bopyrids (Williams & McDermott 2004; McDermott et al. 2010).

Most hermit crabs live in gastropod shells and are prone to predation, temperature stress, and brood mortality when not in their shells (Lancaster 1988). Shells are thus very important in the life cycle of hermit crabs and shell supply may limit the population sizes of some hermit crab species (e.g. Scully 1979; but see Peura et al. 2013). The factors that influence shell choice by hermit crabs have been extensively studied (see Arce & Alcaraz 2012; Peura et al. 2013; Wooi & Ching 2013) and it is has been documented that they will utilize suboptimal shells (e.g. too heavy, not large enough, or easily cracked) if others not are available. The impacts of shell attributes on hermit crab fecundity have been investigated (Wait & Schoeman 2012), including studies on the impacts of symbionts on host reproduction (see review in Williams & McDermott 2004). However, the impacts of parasites such as athelgine bopyrids on the biology of hermit crabs are in need of investigation. In addition, the shell choice by hermit crab hosts may impact the reproduction of the parasitic isopods because they attach to the abdomens of the hermit crabs and could be limited by the available space in shells occupied by hosts.

Recently, the abdominal species *Athelges takanoshimensis* Ishii, 1914 was discovered on a new species of hermit crab host from Hong Kong (An et al. 2011),

Table I. Review of studies on the fecundity and prevalence of bopyrid isopods and their host associations★.

Host species and taxonomic authority	Higher taxon of host	Parasite species and taxonomic authority	Abdominal or branchial (A or B)	Sexually mature female size (mm)	Prevalence of parasitism	Fecundity range (#eggs/ embryos)	Location	Reference
Pagurus longicarpus Say, 1817	Anomura	*Anathelges hyptius* (Thompson, 1902)	A	–	0.15%	1149–3437	Cape May, NJ	McDermott (1998)
Pagurus angustus (Stimpson, 1858)	Anomura	*Athelges takanoshimensis* Ishii, 1914	A	6.0–14.0	7.20%	1078–5031	Hong Kong, China	Current study
Clibanarius erythropus (Latreille, 1818)	Anomura	*Parathelges cardonae* R. & M. Codreanu in Codreanu, 1968	A	Up to 7.0	0.30%	–	Southeast Spain	Williams et al. (2011)
Clibanarius tricolor (Gibbes, 1850)	Anomura	*Stegias clibanarii* Richardson, 1904	A	2.0–4.8	3.71%	191–667	Whalebone Bay, Bermuda	McDermott (2002)
Munida iris A. Milne Edwards, 1880	Anomura	*Anuropodione carolinensis* Markham, 1974	B	5.0–16.0	4.70%	9500–28,000	Norfolk, VA	Wenner & Windsor (1979)
Crangon franciscorum Stimpson, 1856	Caridea	*Argeia pugettensis* Dana, 1853	B	2.7–9.3	2.92%	1600–38,300	Humboldt Bay, CA	Jay (1989)
Hippolyte zostericola (Smith, 1873)	Caridea	*Bopyrina abbreviata* Richardson, 1904	B	1.2–2.4	0.37%	131–1548	Laguna de Términos, Mexico	Romero-Rodríguez & Román-Contreras (2013)
Hippolyte obliquimanus Dana, 1852	Caridea	*Bopyrina ocellata* (Czerniavsky, 1868)	B	–	–	61–596	Brazil	Tsukamoto (1981)
Thor floridanus Kingsley, 1878	Caridea	*Bopyrinella thorii* (Richardson, 1904)	B	1.9–3.5	–	83–1036	Bahía de la Ascención, Mexico	Romero-Rodríguez & Román-Contreras (2008)
Clibanarius tricolor (Gibbes, 1850)	Anomura	*Bopyrissa wolffi* Markham, 1978	B	Up to 2.2	0.16%	236–388	Whalebone Bay, Bermuda	McDermott (2002)
Penaeus semisulcatus De Haan, 1844	Decapoda	*Epipenaeon ingens* Nobili, 1906	B	–	2.90%	–	Queensland, Australia	Owens & Glazebrook (1985)
Neotrypaea uncinata (H. Milne Edwards, 1837)	Decapoda	*Ionella agassizi* Bonnier, 1900	B	Up to 12.0	26.77%	Up to 1500	Lenga, Chile	Muñoz & George-Nascimento (1999)
Pachygrapsus transversus (Gibbes, 1850)	Decapoda	*Leidya bimini* Pearse, 1951	B	4.4–7.1	8.98% WB, 29.45% FR	4,479–21,171	Whalebone Bay (WB) and Ferry Reach (FB), Bermuda	McDermott (1991)
Latreutes fucorum (Fabricius, 1798)	Decapoda	*Probopyrinella latreuticola* (Gissler, 1882)	B	–	–	60–90,000	Gulf of Mexico	Trilles (1999)
Macrobrachium ohione (Smith, 1874)	Caridea	*Probopyrus pandalicola* (Packard, 1879)	B	5.5–13.5	3.00% (on average, estimated)	Up to 5,241	Atchafalaya River Basin, LA	Truesdale & Mermilliod (1977)
Palaemonetes paludosus (Gibbes, 1850)	Caridea	*Probopyrus pandalicola* (Packard, 1879)	B	At least 3.0	–	350–11,850	Wakulla County, FL	Beck (1980b)
Palaemon concinnus Dana, 1852	Caridea	*Pseudione elongata africana* Kensley, 1968	B	4.2–4.6	10–30%	89–357	Mozambique	Penha-Lopes et al. (2013)

★Taxon names and authorities based on WoRMS (2013).

although known from a wide range of hosts in the Indo West Pacific (Markham 2009; McDermott et al. 2010; An et al. 2011). Little is known about the natural history of the species within *Athelges*, including the reproduction of *A. takanoshimensis*. Thus, the purpose of this study was to investigate aspects of the natural history of *A. takanoshimensis*, including: (1) host range and prevalence on hermit crabs from Hong Kong; (2) reproduction and behaviour; and (3) morphology of life stages (females, males and epicaridium larvae).

Materials and methods

Hermit crab examination

Host hermit crab specimens were haphazardly collected by hand from rocky intertidal habitats during the spring and summer months of 2000–2004 at four sites in Hong Kong (Figure 2): Discovery Bay, Lantau Island (22°18′0.74″N, 114°01′0.84″E), Nim Shue Wan, Lantau Island (22°17′29.49″N, 114°01′11.76″E), Siu Kau Yi Chai (island near Peng Chau; 22°17′16.70″N, 114°03′28.65″E), and Silver Mine Bay, Lantau Island (22°16′20.44″N, 114° 0′11.78″E). Some hermit crabs were examined while alive and maintained in aerated natural seawater for behavioural observations; all other hermit crabs were fixed in 70% ethyl alcohol for later study. To determine the prevalence of *Athelges takanoshimensis*, shells were gently cracked and hermit crabs were extracted. Abdomens and branchial chambers were then examined for parasites (bopyrid isopods and rhizocephalan barnacles). Hermit crab carapace length was determined using an ocular micrometer or microruler tool (Electron Microscopy Sciences Co.) and sex was determined based on observing the presence or absence of gonopores.

Host species were identified based on McLaughlin et al. (2007). Prior reports on subsamples of the current specimens identified the predominate host of *A. takanoshimensis* as *Pagurus minutus* Hess, 1865 (An et al. 2011; Cericola 2013). We have identified the host here as *Pagurus angustus* (Stimpson, 1858); both species have a prominent tubercle on the meri of the chelipeds, but in *P. angustus* the dactyls of the ambulatory legs are shorter than the propodi and have < 9 spines (compared to *P. minutus* with longer dactyls and > 9 spines). However, the row of tubercles on the propodus of the left third pereopod, which are characteristic for *P. angustus*, are obscure or lacking in our specimens and thus our host identification remains tentative until molecular data can be used to confirm this. The only other host of *A. takanoshimensis* in our samples was *Pagurus hedleyi* (Grant & McCulloch, 1906), which is similar to *Pagurus kulkarnii* Sankolli, 1962. Apart from coloration patterns, *P. hedleyi* can be distinguished from *P. kulkarnii* based on the presence of spines on the dactyl and carpus on the right cheliped (Haig & Ball 1988; McLaughlin 2002).

Parasite fecundity

Parasitic isopods were measured by taking digital images using an Olympus DP11 microscope camera on an Olympus SZX12 stereo microscope. Digital and SEM images, as well as drawing tube attachment outlines, were analysed using Image J software to measure the lengths of the female after calibration with scale micrometers. For females, five measurements of length were made: oostegite 1 extension beyond the head; head; pereon; pleon; and total body length (Figure 1a). For males, only total length was measured. Damaged isopods were removed from length measurements. Embryo volumes were determined by measuring their diameters with a Olympus CX31 compound microscope and using the formula $V = \frac{4}{3}\pi r^3$; larval volumes were determined using the formula:

$$V = \frac{\pi(d_1^2)(d_2)}{6}$$

where d_1 and d_2 are the width and length of the larvae, respectively (Romero-Rodríguez & Román-Contreras 2013).

Brood sizes were quantified in females containing embryos through stage 2 of development (see Beck 1980b for developmental stages). Embryos were removed from the brood chamber (using pipettes) and counted individually through the use of Image J software and/or by hand. To prepare embryos for counting with Image J, embryos were placed in a 2.3 cm^2 circular cavity slide and were digitally photographed with an Olympus DP11 microscope camera. Digital images of the broods were then uploaded to Image J, counted using the software's automated cell counter, and counts verified manually with the multipoint tool. The cell counter detected embryos by the contrast of their colour against the image background. Contrast was optimal when embryo images were converted to 8 bit grayscale so that the cell counter could most easily recognize embryos against the background of the image. The cell counter was unable to distinguish embryos from lipids or tissue fragments that had similar relative areas to the embryos. In addition, embryos that were bunched together could have been miscounted by Image J. Thus, fragments and aggregates of embryos were corrected and removed from counts during manual inspection with the multipoint tool.
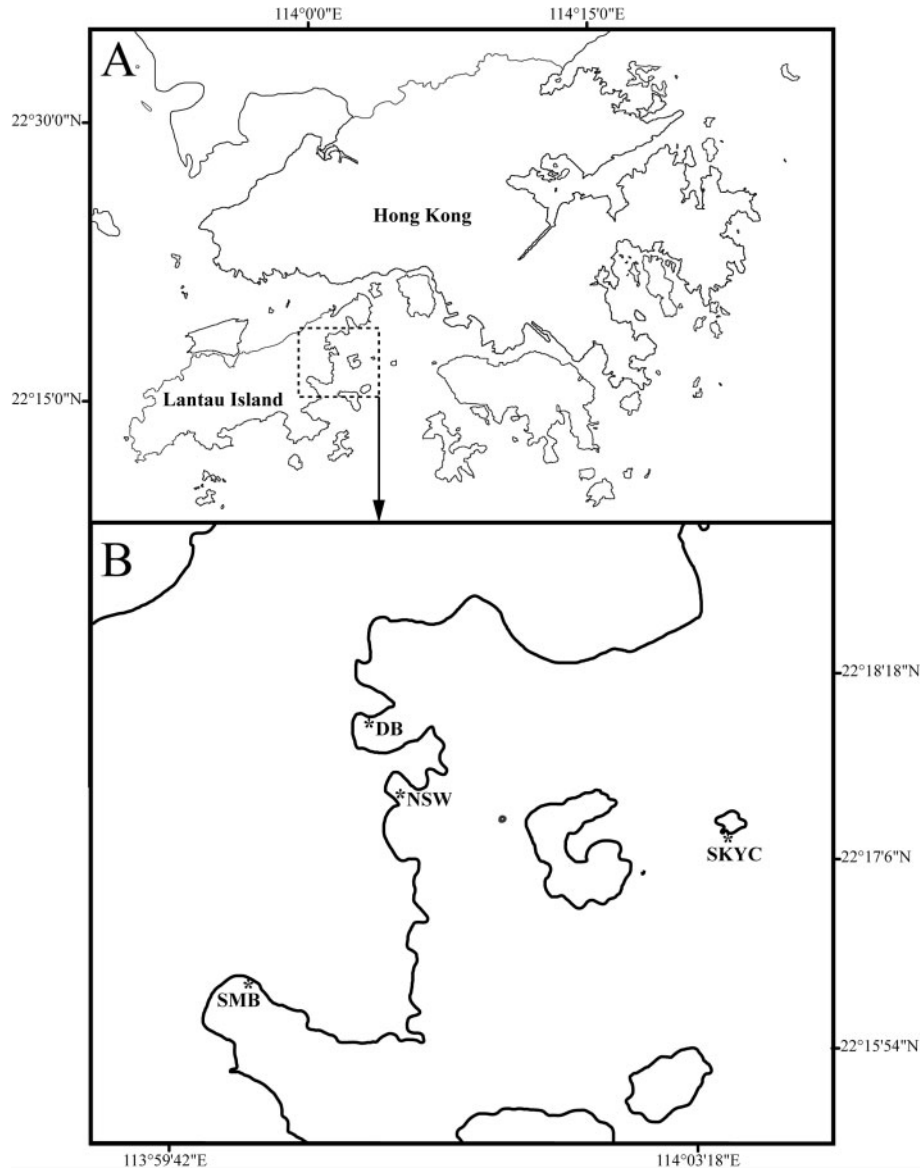
Figure 2. Map of Hong Kong (Special Administrative Region of the People's Republic of China) showing sampling sites. (a) Overview of Hong Kong; (b) area around Lantau Island showing the four collecting sites: DB, Discovery Bay; NSW, Nim Shue Wan; SKYC, Siu Kau Yi Chai; SMB, Silver Mine Bay.

SEM

Larval and adult specimens were examined by scanning electron microscopy (SEM). Preparation of specimens followed protocols of Lee (1993) and Bozzola & Russell (1999), as modified by Williams & Madad (2010). Preparation began by placing specimens in perforated capsules (BEEM capsules for adults pierced with hot needles or microporous capsules) and dehydrating them for 10 min each in 75, 80, 85, 90, and 95% ethanol, followed by three sets of 100% for 15 min each. Specimens were then immediately placed in a Samdri−795 critical point dryer. Following placement in a desiccator overnight, dried specimens were fixed to aluminium SEM stubs with double-sided tape and coated with gold in an EMS-550 sputter coater. Viewing was done using an Hitachi 2460N SEM, images were captured with Quartz PCI v. 5.5 software, and Adobe Photoshop CS5 was used to construct the figures.

Data analysis

Descriptive statistics were reported as mean ± standard deviation and an Independent Samples t-test was used to compare means. Chi-square tests of independence were used to determine whether the distribution of parasites was different from expected values of equal distribution among male and female hosts. Measures of parasite size and brood size were compared using linear regression analysis.

Results

Prevalence

In total, 1563 hermit crabs were examined, including: *Pagurus angustus* ($n = 1404$); *Pagurus hedleyi* ($n = 79$); *Clibanarius virescens* (Krauss, 1843) ($n = 40$); *Clibanarius infraspinatus* (Hilgendorf, 1869) ($n = 38$); and unidentified species ($n = 2$). Of the *P. angustus* collected, 92 were parasitized by *Athelges takanoshimensis* (7.2%; Table II). Of the *P. hedleyi* samples collected, four were parasitized by *A. takanoshimensis* (5.1%). None of the other hermit crabs hosted *A. takanoshimensis*. Of all infested *P. angustus* ($n = 92$), the male to female ratio was approximately 3:7, with 28 males and 64 females infested. In contrast, the uninfested specimens of *P. angustus* had an opposing trend with a male to female ratio of 3:2, with 766 males and 546 females found (Table II). Female hosts were found more often to be parasitized than predicted (based on expected equal distribution of parasites among female and male hosts: $\chi^2 = 27.4$, $n = 1404$, $P < 0.001$). The average percentage of infested *P. angustus* across collections was 5.3% ± 7.0 ($n = 17$), 10.3% ± 12.6 ($n = 17$), and 7.1% ± 5.8 ($n = 17$) for males, females and overall, respectively (Table II). No females of *P. angustus* were found to be ovigerous; four uninfected females of *P. hedleyi* were ovigerous (8.5%; 4/47).

Eighteen *P. angustus* were found with rhizocephalans (1.47%; 18 of 1404 hermit crabs). Each hermit crab had one externa, similar in morphology to *Peltogaster* sp., on the abdomen (10 of the hosts were female, 8 were male).

Reproduction and notes on behaviour

Approximately 66.0% of *Athelges takanoshimensis* females were mature with embryos, 19.3% were mature without embryos, and 14.7% were immature juveniles. Minimum and maximum mature female body lengths were 3.99 and 13.95 mm; the average total body length was 9.78 ± 2.15 mm ($n = 72$; Table III). Average head length was 1.07 ± 0.21 mm ($n = 69$) and was positively correlated with total body length for females ($R^2 = 0.50$, d.f. = 66, $P < 0.01$; Figure 3a). Minimum and maximum body lengths for males were 0.60 and 3.44 mm; average length was 2.17 ± 0.61 mm ($n = 67$; Table III) and was positively correlated with total length of females ($R^2 = 0.35$, d.f. = 65, $P < 0.01$; Figure 3b). The total body length of females was positively correlated with host size ($R^2 = 0.12$, d.f. = 75, $P < 0.01$; Figure 3c). Juvenile females of *A. takanoshimensis* were significantly smaller than sexually mature females (3.38 ± 1.230 mm ($n = 11$) and 9.78 ± 2.15 mm ($n = 72$), respectively; $t_{81} = −9.6$, $P <$

0.001). Males of *A. takanoshimensis* associated with juvenile females were significantly smaller (1.37 ± 0.33 mm ($n = 8$)) than males associated with mature females (2.28 ± 1.06 mm ($n = 59$)) ($t_{65} = −4.5$, $P < 0.001$).

Of the 96 infested hermit crabs, 91.7% had a mature female and male bopyrid pair, 6.3% had a mature female only, and 2.0% only had an immature female attached to the abdomen. In one instance, the host *Pagurus angustus* had two pairs of parasitic isopods (Figure 4a): one pair with a mature female (10.7 mm) and a juvenile male (0.6 mm), and another pair with an immature female (3.2 mm) and a mature male (1.9 mm); both pairs were attached to the abdomen. One *P. angustus* had an immature female in the gill chamber (as opposed to the abdomen in all other cases).

Athelges takanoshimensis was found to have minimum and maximum fecundities of 1078 and 5031 embryos, respectively; the mean number of embryos per brood was 2851.55 ± 1141.04 ($n = 29$). Brood size was positively correlated with pereon length ($R^2 = 0.26$, d.f. = 27, $P = 0.01$; Figure 3d); average pereon length of ovigerous females was 3.6 ± 1.0 mm ($n = 55$). No other measurements of body size were significantly correlated with brood size. The smallest ovigerous female specimen was 5.75 mm in total length.

Average diameter of embryos was 144.7 ± 17.4 μm ($n = 141$); average volume of embryos was approximately 1.7 ± 0.6 μm^3 ($n = 141$). Average dimensions of epicaridium larvae were: length = 262.1 ± 12.7 μm ($n = 20$); width = 169.1 ± 5.0 μm ($n = 20$); and volume = 3.9 ± 0.3 μm^3 ($n = 20$).

In life, female *A. takanoshimensis* were positioned dorsolaterally on the abdomen of the hermit crab hosts between the second and fourth pleopods (the dactyli and propodi of the female pereopods were used to attach to the cuticle of the host abdomen or pleopods), with the anterior end facing toward the telson of the host. The pleon of the isopod would sometimes extend anteriorly on the host so that it reached beyond the posterior margins of the gill chamber (Figure 4a). The head of the female was closely applied to the abdomen of the host but the mouthparts of the females were not found to be penetrating the cuticle of the host. In life, the body of the females was an opaque white to orange colour (orange representing developing eggs within the body). Male *A. takanoshimensis* were positioned immediately posterior to the fifth oostegites or among the pleopods of the females (Figure 4a,b). Males were mobile on the surface of the females, and in one case a female was found off the host after being maintained in the lab overnight (presumably the female was damaged during the shell removal

Table II. Prevalence of *Athelges takanoshimensis* on *Pagurus angustus* collected in Hong Kong between 2000 and 2004.[*] Date format: mm/dd/yy.

Collection sites (see Figure 1)	Collection date	Total *P. angustus* collected	# Uninfested males	# Uninfested females	# *P. angustus* infested	% *P. angustus* infested	# Infested males	% Infested males	# Infested females	% Infested females
Discovery Bay, Lantau Island	6/9/01	166	107	37	22	13.3	3	2.7	19	33.9
Discovery Bay, Lantau Island	7/24/02	15	5	8	2	13.3	2	28.6	0	0.0
Discovery Bay, Lantau Island	7/25/02	19	14	5	0	0.0	0	0.0	0	0.0
Discovery Bay, Lantau Island	7/29/02	122	57	58	7	5.7	2	3.4	5	7.9
Discovery Bay, Lantau Island	7/31/02	93	58	35	0	0.0	0	0.0	0	0.0
Discovery Bay, Lantau Island	8/2/02	100	44	56	0	0.0	0	0.0	0	0.0
Discovery Bay, Lantau Island	8/3/02	63	38	24	1	1.6	1	2.6	0	0.0
Discovery Bay, Lantau Island	8/4/02	183	88	89	6	3.3	1	1.1	5	5.3
Discovery Bay, Lantau Island	5/25/04	130	66	61	3	2.3	2	2.9	1	1.6
Silver Mine Bay, Lantau Island	5/29/04	8	6	1	1	12.5	1	14.3	0	0.0
Discovery Bay, Lantau Island	6/1/04	59	27	22	10	16.9	1	3.6	9	29.0
Discovery Bay, Lantau Island	6/2/04	106	40	52	14	13.2	3	7.0	11	17.5
Siu Kau Yi Chai (Island near Peng Chau)	6/3/04	79	53	19	7	8.9	2	3.6	5	20.8
Nim Shue Wan, Lantau Island	6/5/04	53	30	21	2	3.8	2	6.3	0	0.0
Nim Shue Wan, Lantau Island	6/6/04	21	16	4	1	4.8	0	0.0	1	20.0
Discovery Bay, Lantau Island	6/8/04	132	80	44	8	6.1	5	5.9	3	6.4
Discovery Bay, Lantau Island	6/14/04	55	37	10	8	14.5	3	7.5	5	33.3
Totals		1404	766	546	92		28		64	
Means						7.1 ± 5.8		5.3 ± 7.0		10.3 ± 12.6

[*]Additional 159 hermits surveyed: 75 uninfested *Pagurus hedleyi* (Grant & McCulloch, 1906) (30 males/45 females), four infested *P. hedleyi* (one male/three females); plus 80 other hermit crabs, none infested (including: 40 *Clibanarius virescens* (Krauss, 1843) (three with *Pseudostegias setoensis* Shiino, 1933; see An et al. 2011), 38 *Clibanarius infraspinatus* (Hilgendorf, 1869) and two unidentified hermit crabs).

Table III. Measurements of body length and fecundity of *Athelges takanoshimensis* (see Figure 1a for schematic diagram of how body measurements were made).

Measurements	Minimum	Maximum	Mean ± SD	n
Mature ♀ first oostegites length (above head) (mm)	0.62	1.83	1.14 ± 0.25	69
Mature ♀ head length (mm)	0.49	1.63	1.07 ± 0.21	69
Mature ♀ pereon length (mm)	1.91	5.98	4.21 ± 0.97	70
Mature ♀ pleon length (mm)	1.29	5.85	3.54 ± 1.00	71
Mature ♀ total body length (mm)	3.99	13.95	9.78 ± 2.15	72
Brood size (number of eggs or embryos)	1078	5031	2851.55 ± 1141.04	29
Immature ♀ total body length (mm)	1.74	6.04	3.38 ± 1.23	11
♂ total body length (mm)	0.6	3.44	2.17 ± 0.61	67

process) and the male had repositioned to the abdomen of the host. In life, the body of the males was an opaque white colour.

Females were observed in life during brooding of eggs and release of epicaridium larvae. During this time, females pumped water into the brood chamber through the use of the maxillipeds. The muscular attachment of the maxillipeds was contracted approximately two times/s, causing water to be drawn into the brood chamber through the opening at the posterior end of the fifth oostegites, up through the brood chamber and out through the first oostegites (Figure 4b,c). The pumping of the maxillipeds caused a slight flapping motion of the anterior lobe of the first oostegites; additionally, the pumping caused movement of hemolymph in the body of the female, most clearly observed in the dark red central region at the base of the pleon. Less frequently (~once every 10–20 s), females would sometimes appear to contract the entire body (contraction of body muscles such that brood chamber volume was reduced and then expanded upon muscle
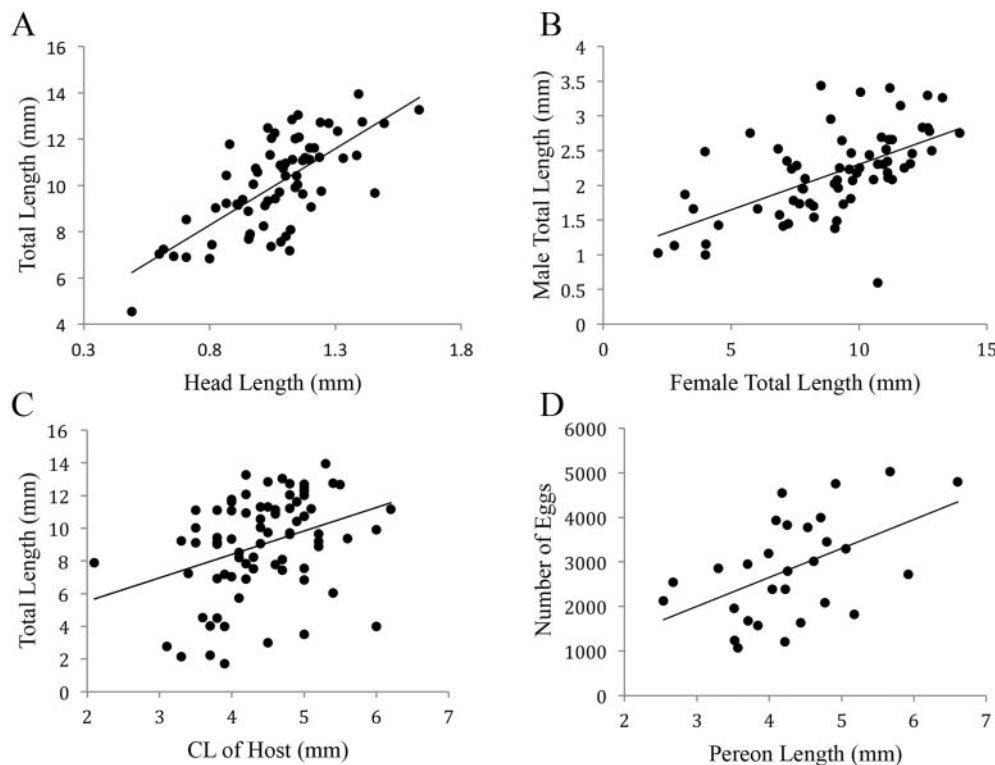

Figure 3. (a) Relationship between female total body length and female head length of *Athelges takanoshimensis* ($y = 6.61x + 2.99$; $R^2 = 0.50$; $n = 68$; $P < 0.01$); (b) relationship between male total body length and female total body length of *Athelges takanoshimensis* ($y = 0.13x + 0.99$; $R^2 = 0.35$; $n = 67$; $P < 0.01$); (c) relationship between female total body length of *Athelges takanoshimensis* and length of its host hermit crab *Pagurus angustus* (as measured by carapace length = CL; $y = 1.43x + 2.66$; $R^2 = 0.12$; $n = 77$; $P < 0.01$); (d) relationship between female brood size (number of embryos or larvae) and female pereon length of *Athelges takanoshimensis* (regression equation for embryos and larvae: $y = 650.42x + 50.83$; $n = 29$; $R^2 = 0.26$; $P < 0.01$).
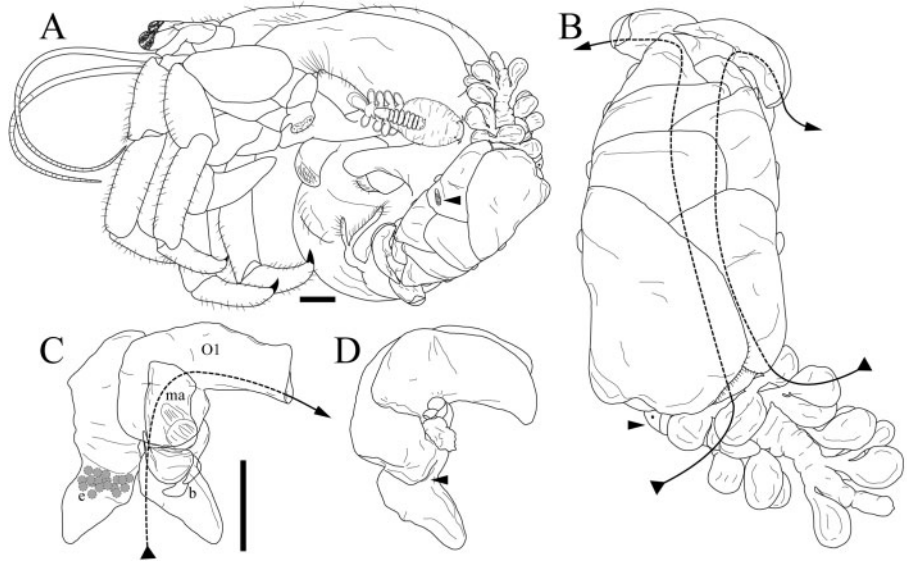
Figure 4. Morphology and behaviour of *Athelges takanoshimensis*. (a) Lateral view of host *Pagurus angustus* with two pairs of *A. takanoshimensis*, one mature female with a juvenile male (shown by arrowhead) and one immature female with a mature male. (b) Ventral view of mature female with male attached at posterior end (shown by arrowhead); movement of water through brood chamber shown by dashed lines. (c) External view of first oostegites (O1); movement of water over oostegites shown by dashed line (right side only); position of maxilliped (ma) and digitate extensions of barbula (b) shown on right side; position of eggs (e) held back by internal ridge of oostegites shown on left side. (d) Internal view of left first oostegite, with internal ridge shown by arrowhead. Scale bars = 1 mm.

relaxation). The long setae on the posterior margin of the fifth oostegites (Figure 4b) presumably function in preventing debris from entering the brood chamber during the pumping process, as evidenced by the accumulation of materials on them in some instances. The water washed over the eggs (orange in colour) or epicaridium larvae (black pigmented) contained in the brood chamber and flowed out of the sides of the first oostegites (Figure 4b,c). Eggs and larvae were blocked from being swept out of the brood chamber by the internal ridge of the first oostegites (Figure 4c,d) and perhaps by the extensions of the barbula (Figure 4c). Eggs and larvae accumulated at the ridge formed by the oostegites (Figure 4c), but larvae were released when the oostegites were raised enough so that the larvae could pass under the ridge and over the top of the maxillipeds, ultimately exiting through the sides of the first oostegites (Figure 4b,c). Epicaridium larvae were released over a 12–24 h period. After release, the larvae were noted to be positively phototactic.

Morphology of parasite life stages

Reference female (Figure 5). Sexually mature female *Athelges takanoshimensis* with male attached. Total body length 9.0 mm and maximal width 2.7 mm across pereomere 3. Head pentagonal in shape, 0.8 mm long by 0.7 mm wide, anterior edge (frontal lamina) straight, slightly expanded and curled, posterior edge extending to rounded point, eyes absent.

Head surrounded by pereomeres 1 and 2. First oostegites extending well beyond head (Figure 5a). Mouthparts consisting of two blade-like structures surrounding mandibles (Figure 5a,b). Antennule and antennae with three and five articles, respectively; antennae with scales on all articles and setae at the terminus of each article (Figure 5a,b). Maxilliped with extended spur medially on rounded posterior lobe, anterior lobe approximately twice as long as posterior; palp absent (Figure 4c). Barbula composed of two curved, digitiform extensions (Figure 4c).

Pereon with seven pereomeres, broadest across third pereomere, tapering anteriorly and posteriorly. Medial portion of pereomere 1 separated by posterior end of head, posterior margin of pereomeres 2–7 with approximately straight middle portion; sides of all pereomeres with round lobes (Figure 5a). Seven pereopods of nearly same size (Figure 5a,e). Dactylus short, curved and deeply set into propodus, setae present on propodus around insertion point; carpus with scales and row of approximately five short setae (Figure 5e). Oostegites completely enclosing highly vaulted brood pouch. First two oostegites extending beyond head, making up approximately one-tenth of total body length. Oostegite 1 more than twice as long as wide, proximal lobe subtriangular, distal lobe ovate (Figure 4c,d).

Pleon with six pleomeres. Pleomeres 1–4 with biramous pleopods, lacking lateral plates. Endopodites and exopodites of pleomeres similar in size and shape, oval with expanded centers (Figure 5c). Male
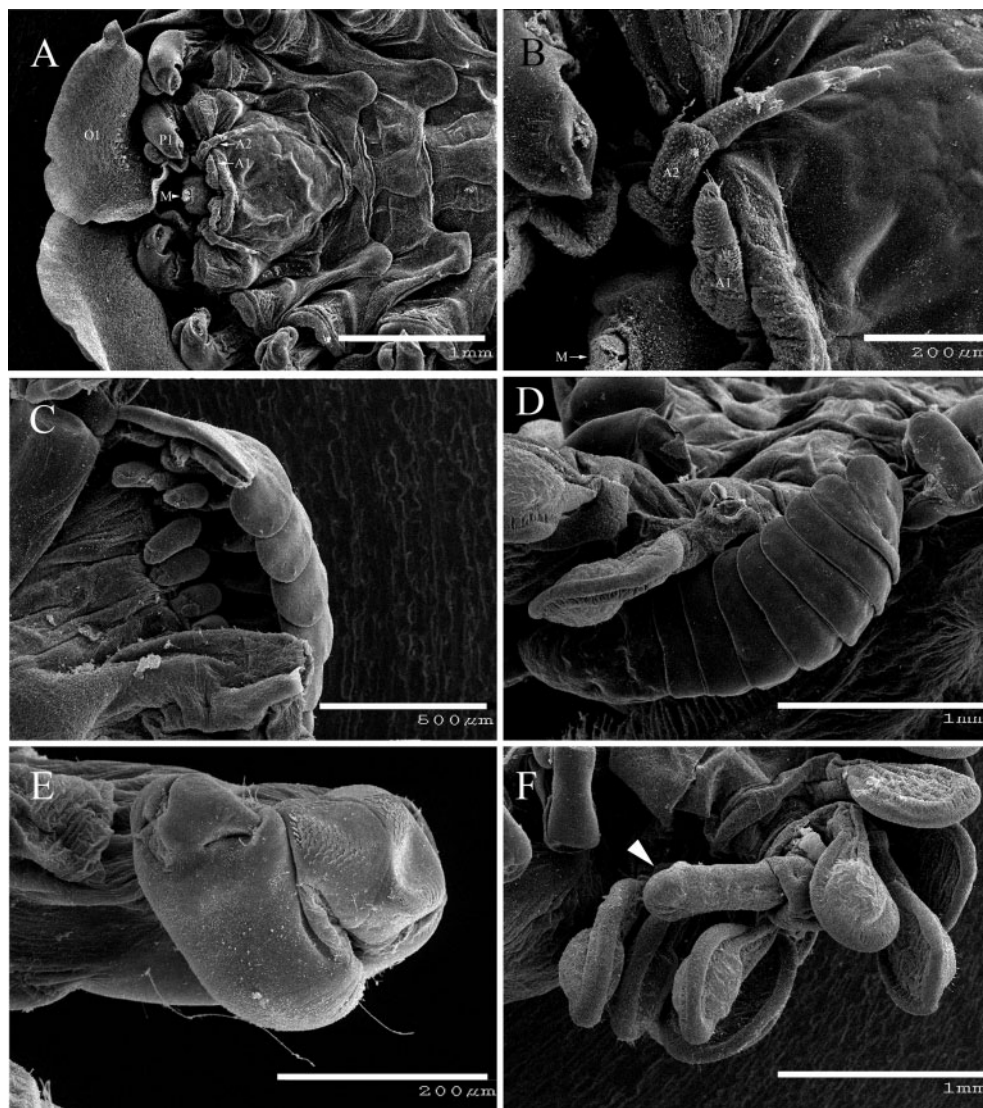
Figure 5. *Athelges takanoshimensis* Ishii, 1914, scanning electron micrographs of female. (a) Anterior dorsal view; (b) right antennae; (c) male attachment site, oblique view; (d) male attached among pleopods of female, oblique dorsal view; (e) 3rd left pereopod; (f) telson (indicated by arrowhead) and pleopods. A1, antenna 1; A2, antenna 2; M, mouth; P1, pereopod 1; O1, anterior portion of oostegite 1. Scale bars = 1 mm (a,d,f), 500 μm (c), 200 μm (b,e).

attached to right side of female, below seventh pereopod of female, near pleopods (Figure 5c,d). Terminal segment of the pleon forms a digitiform, club-like pleotelson (Figure 5f).

Reference male (Figures 5c,d, 6). Sexually mature male *Athelges takanoshimensis* with total body length of 1.8 mm, maximum width of 0.7 mm across pereomeres 3–4. Body elongate, tapering posteriorly to pleon (Figure 6a,b). Head elliptical, wider than long, separated from first pereomere. Small dark eyes near posterolateral margins. Cephalic slits (presumably sensory structures, see Bourdon et al. 1981), located laterally on the dorsal surface of head (Figure 6c). Antennule with three articles, with tuft of setae on terminal article; antennae with five articles, with

tuft of setae on distal end of last two articles. All pereopods of similar structure and proportion, pereopod 3 largest, sizes of pereopods slightly diminishing anteriorly and posteriorly. Pereopods with curved dactylus, insertion point of dactylus on surface of propodus surrounded by U-shaped ring of stout scales; carpus with scales and irregular row of setae (Figure 6b). Pleon fused into single tapering piece extending to a rounded posterior margin (Figure 6a), uropods absent. Filamentous structures on pleon are presumably thalli of an unidentified mesomycetozoan (see McDermott et al. 2010 for review of their associations with hermit crabs).

Epicaridium larvae (Figure 7). Epicaridium larvae approximately 250 μm in length. Body ovate in
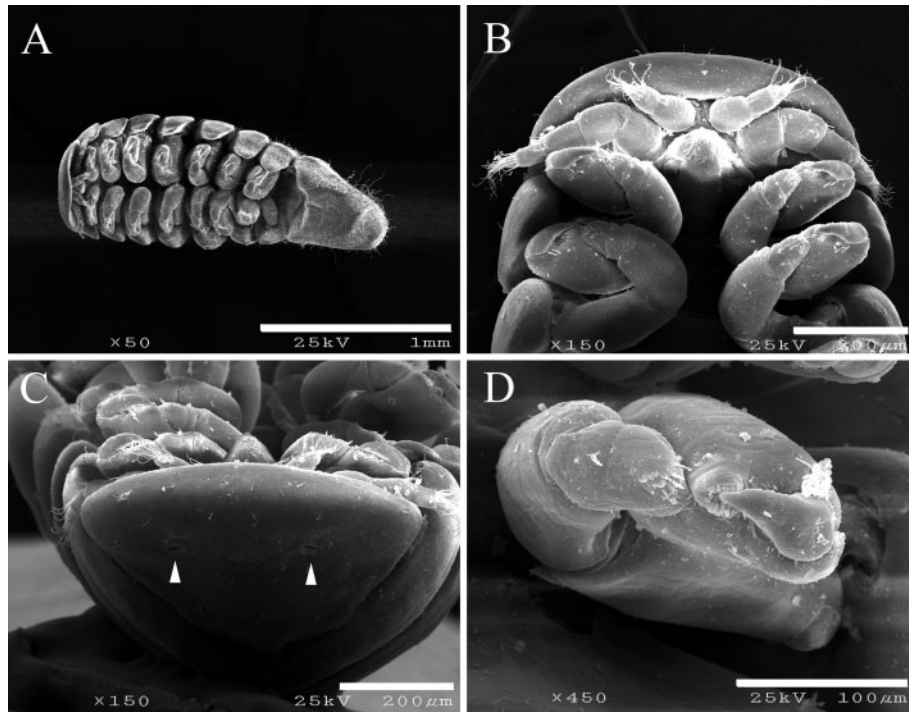
Figure 6. *Athelges takanoshimensis* Ishii, 1914, scanning electron micrographs of male. (a) Ventral view; (b) anterior end, ventral view; (c) anterior end, *en face* view, showing cephalic slits (indicated by arrowheads); (d) right pereopod 7. Scale bars = 1 mm (a), 200 μm (b,c), 100 μm (d).

shape (Figure 7a), with black-pigmented eyespots, irregular in shape, and pigment bands on dorsal surface of pereon. Anterior margin of head rounded (Figure 7a–c). Antenna 1 appears to be composed of two articles, first article broad, with three large acutely tapering projections, second article digitiform, tapering to point (Figure 7b,d,e). Antennae 2 elongate, nearly three-quarters the length of body; composed of five articles, with broad, long first article, ending with stout terminal setae next to insertion point of article 2, articles 2–5 short, subequal in size, terminal article with two stout setae, inner seta approximately twice as long as outer seta (Figure 7a,b). Mouthparts consist of small mandibles surrounded by three pairs of digitiform extensions, interpreted to be maxilla 1 (short, with rounded tips to sides of mandibles), maxilla 2 and maxilliped (more elongate, with tapered distal ends, below mandibles) (Figure 7d; note: mouthparts and homology of these structures are poorly known in bopyrid isopods (see Calman 1898; Dale & Anderson 1982; Boyko & Wolff 2014)).

Six gnathopodal pereopods, subequal in size, first pair oriented perpendicular to axis of body, rest oriented posteriorly until sixth pair, which is oriented parallel with axis of body. Pereopods with stout, slightly curved dactylus extending beyond margin of propodus, short triangular extension on propodus at base of dactylus (Figure 7e,f).

Pleon with five biramous pleopods (Figure 7f); exopods with three stout, tapering setae, endopods with one stout, tapering seta. Uropods biramous, similar in shape to pleopods but stouter and slightly longer; endopods and exopods of uropods ending in two short, broad setae (Figure 7f). Short anal tube between uropods (Figure 7a,b).

Discussion

The prevalence of *Athelges takanoshimensis* in *Pagurus* spp. from Hong Kong is generally less than 10% of host specimens collected. Whereas the prevalence of branchial bopyrids is better known (0.16–29.45%), the prevalence of abdominally parasitizing isopods (0.15–7.20%) remains understudied (Table I). Although *A. takanoshimensis* was not found on specimens of *Clibanarius* spp. in the present study, the parasite has a broad host range and has been documented from hermit crabs in four genera (*Clibanarius*, *Diogenes*, *Pagurodofleinia*, *Pagurus*; An et al. 2011). In addition to the parasitic isopod, a rhizocephalan barnacle was found on ~1.5% of the *Pagurus angustus* collected; further morphological and molecular work will have to be completed to identify this parasite to species (see Yoshida et al. 2011, 2012 for recent work on the taxonomy of rhizocephalans from hermit crabs in Asia).
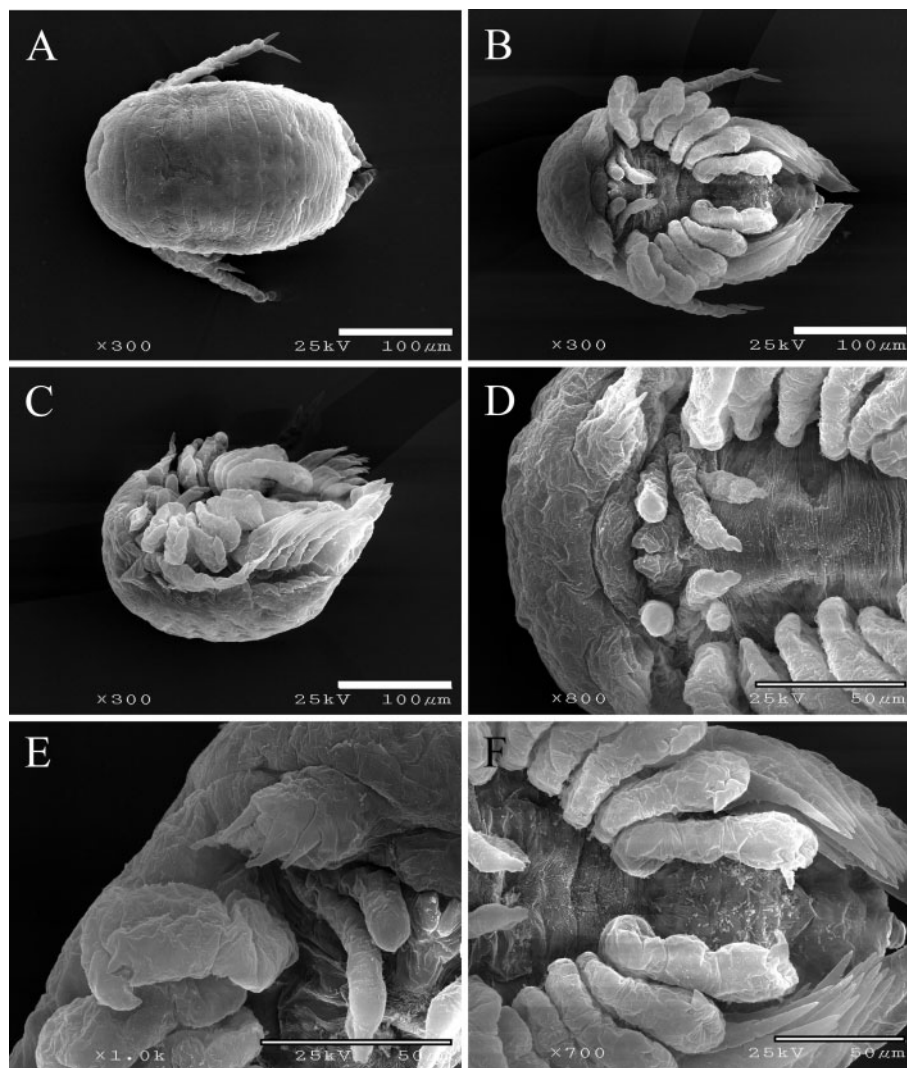
Figure 7. *Athelges takanoshimensis* Ishii, 1914, scanning electron micrographs of epicaridium larva. (a) Dorsal view; (b) ventral view; (c) oblique lateral view; (d) ventral view of anterior end showing mouthparts; (e) right antenna 1 and pereopod 1; (f) ventral view of posterior pereopods and pleopods. Scale bars = 100 μm (a–c), 50 μm (d–f).

Live observations on the behaviour of bopyrid isopods have rarely been reported, particularly in reference to reproduction (Tucker 1930; Reinhard 1949; Beck 1980b; Cash & Bauer 1993); however, Cash & Bauer (1993) documented aspects of the reproduction and behaviour of a branchial bopyrid in relation to host ecdysis. They noted ventilatory movements of the anterior oostegites during host intermoult periods; it this likely that the movement of the oostegites they observed were caused by the maxillipeds underlying the oostegites, as noted in the present work. The role of maxillipeds in ventilation of the brood chamber has been documented in other isopod groups (see Table 1 in Johnson et al. 2001). The finding that one hermit crab had an immature female in the gill chamber supports Pike's (1961) observation that abdominal bopyrids may initially settle on the gills of hosts and eventually move onto the abdomen. Whether this is the typical pattern for athelgines remains to be investigated.

Previous studies have documented negative impacts of bopyrids on the reproduction of hosts (Tucker 1930; Hiraiwa & Sato 1939; Lancaster 1988; Jordá & Roccatagliata 2002; McDermott 1991, 2002; González & Acuña 2004; Calado et al. 2005; Romero-Rodríguez & Román-Conteras 2008, 2013; McDermott et al. 2010; Dumbauld et al. 2011). Impacts of *A. takanoshimensis* on the reproduction of *Pagurus* spp. from Hong Kong cannot be assessed because samples were collected during spring and summer months when females were generally not ovigerous. Although the reproduction of *Pagurus* spp. from Hong Kong has not been studied, *Pagurus minutus* in Japan are known to breed mainly from October through April (Wada et al. 2007). Future studies should be conducted

during the breeding season of hosts to examine the impacts of *A. takanoshimensis* on the reproduction of hermit crabs. Other bopyrids such as *Pseudostegias setoensis* Shiino, 1933 have been found to leave a hole in the host cuticle from the mouthparts (An et al. 2011); *A. takanoshimensis* was not observed to penetrate the cuticle in the present study. Additional research should examine feeding in this and other bopyrid species to determine patterns of hemolymph uptake (i.e. do they feed only during development or intermittently after reaching sexual maturity?). Whether males feed at all remains a mystery in bopyrids (Tucker 1929; Reinhard 1949). Another intriguing finding is that eggs of *A. takanoshimensis* are distinctly orange in colour, whereas those of *P. setoensis* collected from *Clibanarius virescens* from Hong Kong (An et al. 2011) are distinctly pink. Research could address whether these colour differences in eggs result from differences in host physiology or diet through uptake of hemolymph (containing carotenoproteins) by the parasitic isopod and transfer to the eggs (as lipovitellins), or if this variation in colour reflects developmental changes in lipovitellins or otherwise (Goodwin 1951; Cheesman et al. 1967; Ghidalia 1985; Mantiri et al. 2004).

There was extensive variation in brood size. Such variation has been reported in other studies on bopyrid reproduction and in other isopods (Table I; Klapow 1970; Jay 1989; McDermott 1998, 2002). Unlike in some previous studies (Truesdale & Mermilliod 1977; Wenner & Windsor 1979; Beck 1980b; Jay 1989; McDermott 1991, 1998, 2002; Muñoz & George-Nascimento 1999; Romero-Rodríguez & Román-Contreras 2008, 2013), brood size did not significantly correlate with female total body length. However, pereon length was significantly correlated with brood size (Figure 3d). The bodies of female isopods can become distorted during fixation (Truesdale & Mermilliod 1977; Beck 1980b), especially the pleon and oostegite extensions beyond the head. Thus, total body length might be a poor proxy for size to correlate with fecundity, especially for abdominal bopyrids. In contrast, pereon length is a good substitute because (1) it is less prone to contraction; and (2) is a closer estimate of brood chamber volume.

The fecundity of *A. takanoshimensis* appears to be limited by female size and the capacity to harbour developing embryos, consistent with other studies correlating reproduction with size (Wenner & Windsor 1979; Beck 1980b; Jay 1989; McDermott 1991, 2002; Muñoz & George-Nascimento 1999; Romero-Rodríguez & Román-Contreras 2008, 2013). Extensive variation in brood sizes of branchial bopyrids has been found and several researchers have suggested that it may be due to the variation in outpocketing size

of the host's branchiostegite (Beck 1980b; Jay 1989; Romero-Rodríguez & Román-Contreras 2008). Whereas these studies focused on branchial hosts, brood size variation of abdominally parasitizing isopods of hermit crabs may be limited by the size of the shells occupied by the host. Impacts of a host's shell size or morphology on brood size of athelgine bopyrids could be studied in future experiments where parasitized hosts are reared in shells of varying internal volumes and parasite broods are analysed accordingly. Shell use and choice by hermit crabs have been extensively studied (Reese 1962, 1963; Lancaster 1988; Bulinski 2007), but no one has investigated potential impacts on the reproduction of their parasites. Researchers have utilized 3D printers to produce artificial shells that allow for studies on the factors that influence shell choice (Gravel et al. 2004), and such shells could be used to investigate potential impacts on bopyrid reproduction. In addition, clear artificial shells could be used to examine movement of isopod larvae upon release from the brood chamber and whether they are transported out of the shell by the respiratory currents of hosts in an analogous manner to how the larvae of branchial bopyrids are released from the gill chamber of hosts (Cash & Bauer 1993).

Variation in brood size could also be due to natural loss of embryos during incubation, as found in other crustaceans including free-living, commensal, and parasitic isopods (Kuris 1991). Copepods of the genus *Paranicothoe* Carton, 1970 are known predators of bopyrid embryos (Carton 1970; Kuris 1991), but have not been found with *Athelges* spp. Nevertheless, the presence of copepod predators and other symbionts in the shells of hermit crabs are worth investigation as possible causes for brood loss. In addition, loss of embryos could be an artefact resulting from handling during collection, extraction, or counting. Loss during collection and extraction of hermit crabs from shells is unlikely since no brood counts were made on bopyrids that exhibited damage. However, embryo counts were difficult and it is possible that recorded brood sizes are slightly different from actual counts due to human error. It is likely that miscounting produced a lower number than the actual total because instances of undercounting resulting from accidental loss (e.g. embryos stuck in syringe, failing to extract all embryos from the brood chamber, or embryos drifting away) were difficult to avoid, whereas overcounting (e.g. due to presence of lipids/tissue fragments in images) was relatively easy to correct during manual check. Thus, brood size estimates are likely to be conservative.

Although *A. takanoshimensis* was originally well-described by Ishii (1914) and has subsequently been reported from multiple localities and hosts (see

review in An et al. 2011), this is the first report to provide a detailed redescription of the male and female of the species and to utilize SEM for description of its epicaridium larval stage. Few descriptions of epicaridium larvae of bopyrids exist (see Anderson & Dale 1981; Dale & Anderson 1982; Williams & An 2009) and larval stages may provide important data for future taxonomic and systematic studies on bopyrids. The cryptoniscus larval stage remains unknown in *A. takanoshimensis* and studies on their use of intermediate copepod hosts are needed to fully understand the life history of this bopyrid. In addition, research should be conducted to test the hypothesis that the function of the internal ridge of the oostegites and extensions of the barbula is to block exiting of developing embryos during brooding and to test whether setae of the posterior oostegites function to prevent debris from entering the brood chamber and/or loss of embryos.

Acknowledgements

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