



The Crustacean Society

Journal of  
Crustacean Biology*Journal of Crustacean Biology* 40(1), 97–114, 2020. doi:10.1093/jcobiol/ruz088

Version of Record, first published online December 16, 2019, with fixed content and layout in compliance with Art. 8.1.3.2 ICZN.

# A new genus and species of parasitic isopod (Bopyroidea: Entoniscidae) infesting pinnotherid crabs (Brachyura: Pinnotheridae) on the Atlantic coast of the USA, with notes on the life cycle of entoniscids

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(Received 30 July 2019; accepted 1 November 2019)

## ABSTRACT

*Pinnixion sexdecennia* n. gen., n. sp. is described from three pinnotherid hosts: *Austinixa gorei* (Manning & Felder, 1989), *Pinnixa chaetopterana* Stimpson, 1860, and *Zaops ostreus* (Say, 1817). Females of the new species are distinguished from all other entoniscids by possessing two transverse lobes on oostegite 1 and fused oostegites 2–5; males have a pair of lateral expansions on each of the first two pleomeres. The epicaridium larvae of the new species also have a unique elongated, cylindrical terminal process on the pereopod 6 dactylus. *Pinnixion sexdecennia* n. gen., n. sp. exhibited highest prevalence (> 40%) in North Carolina and Florida. Examination of live specimens of *P. chaetopterana* infested with mature female isopods showed that larvae are liberated via a pore produced in the wall of the host's branchial chamber. Mature females of *P. sexdecennia* n. gen., n. sp. occupied nearly all available space within the host crab's hemocoel. Female *P. chaetopterana* had higher prevalence than males, and intensities of infestations were also greater in females. Evidence from all locations showed that mature parasites do not inhibit breeding or molting in the host. Our report is one of the few studies on the ecology and biology of entoniscids, a cryptic but ecologically important group of parasitic isopods. We also resolve long-standing nomenclatural issues involving *Entione* Kossmann, 1881 and *Entioninae* Codreanu, Codreanu & Pike, 1960, the subfamily to which the new genus and species belongs, by synonymizing *Grapsion* Giard & Bonnier, 1886 with *Entione*.

**Keywords:** *Austinixa*, *Entione*, *Entioninae*, *Grapsion*, hypersymbioses, parasitism, *Pinnixa*, symbiosis, *Zaops*

## INTRODUCTION

Most pinnotherid crabs (Brachyura, Pinnotheridae) have been recorded as obligate symbionts of a wide variety of marine invertebrate hosts as well as some species of urochordates (Schmitt *et al.*, 1973). Their host relationships appear to be primarily commensal but some may be functionally parasitic (Castro, 2015). The majority of species within the subfamily Pinnotherinae (e.g., *Zaops ostreus* (Say, 1817)) inhabit the mantle cavity of bivalve molluscs, but others are associated with gastropods and echinoderms. Individuals belonging to molluscan-dwelling species usually have poorly calcified and rounded carapaces, thus, their common

appellation of “pea crabs.” In contrast, members of the subfamily Pinnothereliinae (e.g., *Austinixa gorei* (Manning & Felder, 1989) and *Pinnixa chaetopterana* Stimpson, 1860) have more calcified and laterally elongated carapaces, a shape conducive to movement within tubes and burrows formed by their associates (McDermott, 2009). Species within Pinnothereliinae are morphologically adapted for a more free-living existence, and associate with hosts that occupy benthic tubes or burrows of polychaetes, echinoderms, and thalassinidean crustaceans.

There are more than 300 species of pinnotherid crabs (Schmitt *et al.*, 1973; Ng *et al.*, 2008; McDermott, 2009; WoRMS, 2019). Palacios Theil *et al.* (2009, 2016) provided molecular evidence

supporting the need for revisions of pinnotherid classification, especially with regard to the composition of subfamilies. Biological and life history information is better understood and more detailed for members of Pinnotherinae (e.g., Atkins, 1926, 1960; Stauber, 1945; Christensen & McDermott, 1958; Pearce, 1966; Beach, 1969; Kruczynski, 1973, 1975; Bierbaum & Shumway, 1988; Soong, 1997; Hamel et al., 1999; Hsueh, 2001a, b, 2003; Narvarte & Saiz, 2004; Becker & Türkay, 2010; Becker et al., 2011, 2012, 2013; Hernández et al., 2012), whereas such information is relatively scant for members of Pinnothereliinae (Gray, 1961; McDermott, 1962, 1981, 2005, 2006; Alves & Pezzuto, 1998; Grove & Woodin, 1996; Baeza, 1999; Sanford, 2006; Peiró & Mantelatto, 2011; Peiró et al., 2011, 2013).

Symbiotic pinnotherid crabs are themselves hosts for symbiotic organisms (i.e., hypersymbioses) (McDermott, 2009; Longshaw et al., 2012) but such relationships are poorly known. Among these, parasitic isopods (Isopoda: Epicaridea) infest pinnotherids as ectoparasites (Bopyridae) and endoparasites (Entoniscidae), but little is known of their natural history or host relationships except that some species may interfere with gonad development in host females and cause feminization of secondary sexual characteristics in male hosts (McDermott, 2009; Williams & Boyko, 2012; Shields et al., 2015).

The typical epicaridean isopod has three different larval stages and utilizes two hosts in its life cycle (see Williams & Boyko, 2012). Epicaridium larvae hatch from eggs that are brooded in the female's marsupium and then seek out appropriate intermediate hosts, which are, so far as is known, always calanoid copepods. The epicaridium larva then metamorphoses into a microniscus larva and feeds on the hemolymph of the copepod. After feeding, the microniscus larva leaves the copepod and transforms into a free-swimming cryptoniscus larva that seeks out an appropriate definitive host. The cryptoniscus then transforms into a juvenile that will develop into either a female (for most species, the fate of the first larva to encounter the host) or male (most, if not all, subsequent larvae that encounter a host already bearing a female parasitic isopod) (Boyko & Williams, 2009).

Entoniscid isopods (Entoniscidae) are endoparasitic in a variety of caridean, anomuran, and brachyuran decapods (Lester, 2005; Baeza & Piatoni, 2010). Presently 37 species of entoniscids have been described with the majority (28) known from brachyuran hosts (Williams & Boyko, 2012; Shields et al., 2015; Boyko et al., 2008 onwards). Their life cycles are similar to the general epicaridean species described above (Fig. 1; see reviews in Caullery, 1952; Wägele, 1989); however, entoniscid cryptoniscus larvae penetrate into the hemocoel of hosts and metamorphose into sexually undifferentiated juveniles inside the host. One of these parasites usually grows into an indistinctly segmented, asymmetrical, mature (ovigerous) female, although supernumerary females can also occur. One or more other cryptoniscus larvae develop into segmented males that attach to the female. The females possess large marsupia that may fill most of the host's hemocoel. Entoniscid epicaridium larvae follow the typical developmental pathway of epicarideans, although one species of entoniscid has been reported with abbreviated development in which the cryptoniscus larvae develop directly from the egg (Miyashita, 1940); this is the only documented case within Epicaridea that does not involve copepods as an intermediate host. Entoniscid infestations may cause castration of crab hosts and modify secondary sexual characteristics (Giard & Bonnier, 1889; Atkins, 1933; Reinhard & Buckeridge, 1950; Kuris et al., 1980; Brockerhoff, 2004) but, as we demonstrate here, this does not always occur.

Although entoniscids are endoparasitic, they are enveloped by a sheath formed by the host blood cells (Kuris et al., 1980) and possess a connection to the exterior of the host via the exit pore formed by the pleon. Additionally, although they reproduce inside their hosts, the next generation does not burden the same host as the parental one (i.e., unlike in bacteria, protozoans, and fungi). We therefore follow the terminology of others (e.g., Adkison,

1990; Shields et al., 2015) who use the term “infest” (rather than “infect”) for the entoniscid mode of parasitism.

The entoniscid isopod *Pinnotherion vermiforme* Giard & Bonnier, 1889 is the only described entoniscid previously known to infest pinnotherid crabs (Fig. 1); its host is the European pea crab *Pinnotheres pisum* (Linnaeus, 1767) (Pinnotherinae), which is primarily a symbiont of the blue mussel *Mytilus edulis* Linnaeus, 1758. Atkins (1933) showed that the mature female parasite caused complete gonadal atrophy of the female host. The only other known pinnotherid hosts for entoniscids are *Austinia gorei*, *P. chaetoptera*, and *Z. ostreus* (McDermott, 2005, 2006, 2009), but the parasite of these hosts was previously undescribed.

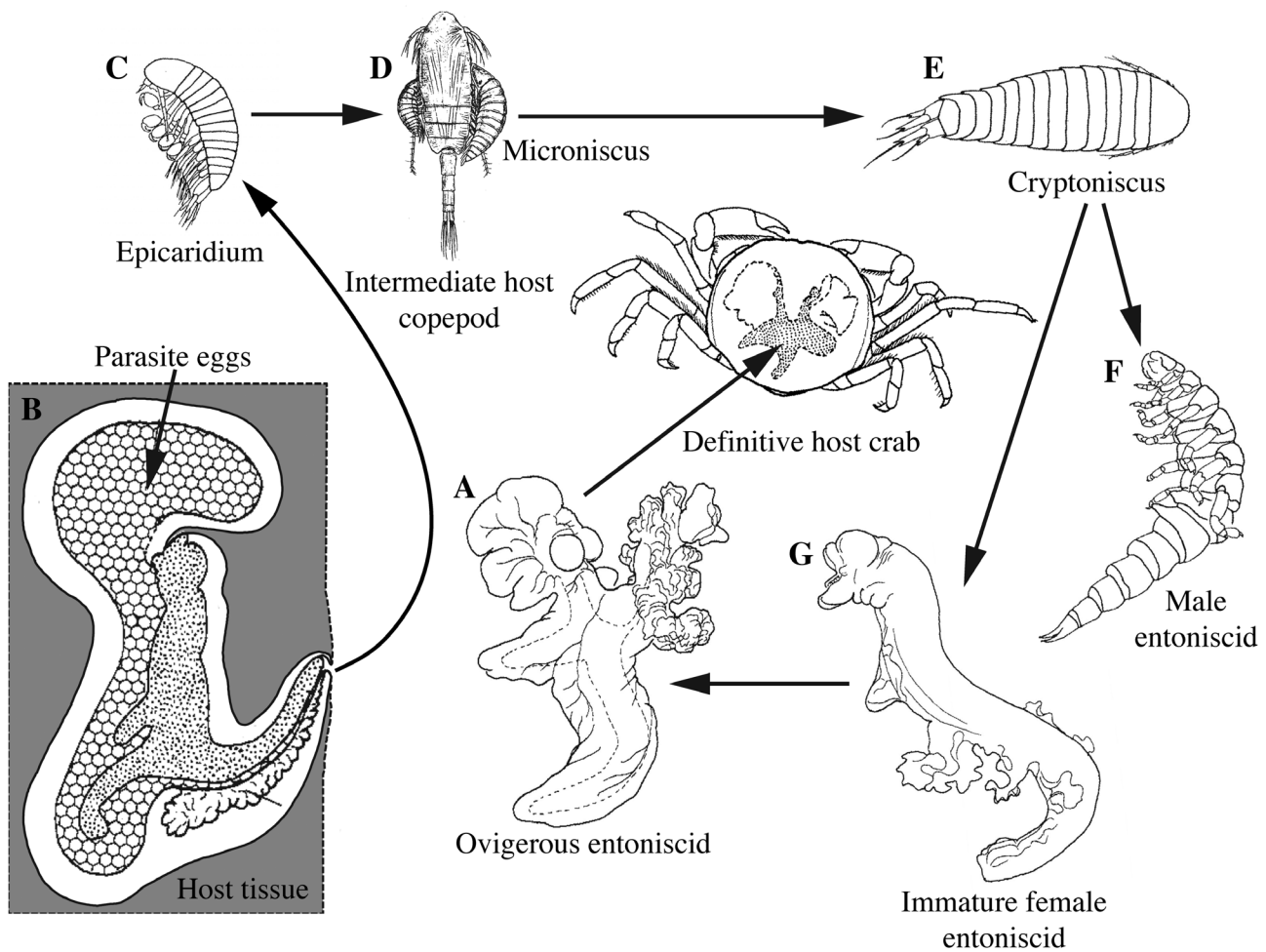
The objectives of our research were to: 1) describe a new genus and species of entoniscid isopod parasitizing intertidal populations of three species of pinnotherid hosts living along the Atlantic coast from New Jersey to Florida, USA (see McDermott, 2005); 2) provide data on the prevalence and intensity of the new species of entoniscid parasitizing these hosts, as well as information on the natural history and biology of the host-parasite relationships; and 3) resolve long-standing nomenclatural issues involving the validity of the subfamily Entoniscinae Codreanu, Codreanu & Pike, 1960, to which the new genus and species belongs, by synonymizing *Grapsion* Giard & Bonnier, 1886 (Giard & Bonnier, 1886a) with *Entione* Kossmann, 1881.

## MATERIALS AND METHODS

Specimens of *Pinnixa chaetoptera* were collected at three locations along the coast of New Jersey (NJ), North Carolina (NC), and Florida (FL), USA from 1963 to 1967. NJ crabs were obtained from the U-shaped tubes of *Amphitrite ornata* (Leidy, 1855) (Polychaeta) located in muddy sand on intertidal flats in Hereford Inlet (Cape May County; 39°02'N, 74°48'W); NC crabs were collected from the U-shaped, parchment-like tubes of *Chaetopterus variopedatus* (Renier, 1804) (Polychaeta) in muddy sand at intertidal locations in the vicinity of Beaufort (34°43'N, 76°40'W); FL crabs came from the tubes of *C. variopedatus* and other burrows of polychaetes and molluscan bivalves in sand flats on Virginia Key, Miami (25°40'N, 80°10'W) (see McDermott & Gibson, 1993 and McDermott, 2005 for further details concerning these locations). Crabs were isolated in the field or laboratory, sexed, and the carapace width (CW) was measured in mm with Vernier calipers; smaller juvenile hosts and parasitic isopods were measured using a microscope fitted with an ocular micrometer. Food (*Ulva* sp. fragments, *Artemia* nauplii, flakes of Pabulum™) was provided for those crabs maintained for weeks or months in the laboratory (McDermott, 2005).

Infestations of mature entoniscids in crabs from NJ were identified mainly by the emergence of epicaridium larvae in laboratory-maintained hosts. Crabs were seldom preserved and dissected because they were maintained for months in the laboratory for studies of reproduction, molting, and behavior. Comprehensive data on the prevalence of entoniscid infestations were therefore not recorded. Crabs from NC and FL, however, were dissected and examined shortly after collection to determine the prevalence and intensity of parasites. Data from the large collections of *P. chaetoptera* in NC (see McDermott, 2005: table 4) are emphasized herein. Dissected crabs were considered infested if any of the entoniscid life-history stages (sexually undifferentiated, immature males, immature females, or mature females) were found. The number of crabs infested with entoniscids is referred to as “prevalence” and is expressed as a percentage; “intensity” refers to the number of entoniscid life-history stages in the infested host and is expressed as the mean or average intensity of the parasite in crab populations (Bush et al., 1997).

Collections of *Zoops ostreus* were made by examining *Crassostrea virginica* (Gmelin, 1791) from the Beaufort NC, region (34°43'N, 76°40'W) in the summers of 1965, 1966, and 1967. Crabs were removed from infested oysters, their carapace widths (CW) measured



**Figure 1.** Life cycle of *Pinnotherion vermiforme*, a parasite of the pea crab *Pinnotheres pisum* that is a common symbiont of the blue mussel *Mytilus edulis*. Host crab shown in center with position of mature female indicated in shaded region. Ovigerous female removed from host (A); schematic cross-section of ovigerous female, showing eggs within brood and exit through which epicaridium larvae will be released (B); lateral view of epicaridium larva released into water column (C); copepod intermediate host with early microniscus larva (left side) and late microniscus larva (right side) (D); cryptoniscus larva that detaches from intermediate host and settle on a definitive host crab (center) (E); male entoniscid (F); immature female entoniscid (G). Modified from Sars (1899), Atkins (1933), and Wägele (1989).

with Vernier calipers to the nearest 0.1 mm, and examined internally for the presence of entoniscid parasites. The percent of parasite live weight *versus* crab weight was determined by subtracting dissected parasite weight from the combined weight of host and parasite. Excess fluid was removed prior to weighing. The majority of crabs were mature fifth-stage females, some were immature females, and one was a mature male hard stage (Christensen & McDermott, 1958; McDermott, 1998a). Smaller collections of *Austinia gorei*, a symbiont of the ghost shrimp *Gilvossius setimanus* (De Kay, 1844), were made from the northwest point of Bear Cut on Key Biscayne, FL (25°43'41"N, 80°09'27"W) in the summer of 1967 (McDermott, 2006).

Figures were produced using Adobe Illustrator to trace original drawing tube sketches or drawings made with a dissecting microscope and calibrated ocular grid. For SEM preparation, male entoniscids were dehydrated in an ascending ethanol (EtOH) series (70% to 100% EtOH; 10 min per dilution through 95% and 15 min  $\times$  3 for 100% EtOH). Drying was accomplished with a Samdri 795 critical point dryer (Tousimis, Rockville, MD, USA). Once dried, the entoniscids were mounted on aluminum stubs, coated with gold using an EMS-550 sputter coater (Electron Microscopy Sciences, Hatfield, PA, USA), and viewed with a FEI Quanta 250 SEM (Thermo Fisher Scientific, Waltham, MA, USA).

Although some authors (e.g., Shiino, 1942; Trilles, 1999) use the term “pygidium” to refer to the terminal pleomere of the

male, this term is otherwise used in crustaceans only for ostracods (McLaughlin, 1980), and we do not use it here. All host and parasite specimens are deposited in the United States National Museum of Natural History, Smithsonian Institution (USNM). References are provided for authors and dates of all parasitic taxa but not for those of hosts or other symbionts (see Schmitt *et al.*, 1973 for references of pinnotherid hosts; for current classification of pinnotheroids see WoRMS, 2019). We use the term “type host” to refer to the host species from which the holotype parasite was described (see Frey *et al.*, 1992).

## SYSTEMATICS

### Order Isopoda Latreille, 1817

### Superfamily Bopyroidea Rafinesque, 1815

### Family Entoniscidae Kossman, 1881

### Subfamily Entioninae Codreanu, Codreanu & Pike, 1960

*Status of Entione with designation of a type species* As discussed by Shiino (1942) and Adkison (1990), there are serious nomenclatural problems involving the present use of the subfamily name Entioninae, which was erected by Codreanu *et al.* (1960) for *Entione*

Kossmann, 1881 (not Kossmann, 1882 as cited by Adkison, 1990) as well as all the genera with species that “*infestent presque exclusivement les Brachyours*” (Codreanu et al., 1960: 441). The major issue is that, as currently used, *Entione* is technically unavailable because it does not contain any of its originally included species. Kossmann (1881) placed *Entoniscus cancrorum* Müller, 1864, *E. cavolinii* Giard, 1878, and *E. moniezii* Giard, 1878 in *Entione* but did not designate a type species. Giard & Bonnier (1886a) subsequently subdivided *Entione* into three subgenera. The first was *Grapsion*, consisting of only the type species *E. cavolinii*. The second was *Portunion*, with the type species *Entoniscus maenadis* Giard, 1886. *Portunion* also included: 1) *Portunion fraissii* [sic] Giard & Bonnier, 1886 (Giard & Bonnier, 1886a) (a *nomen nudum* from Giard & Bonnier, 1886a; later described in Giard & Bonnier, 1888 as *Priapion fraissii*), 2) *Entoniscus kossmanni* Giard & Bonnier, 1886 (Giard & Bonnier, 1886b) (a *nomen nudum* from Giard & Bonnier, 1886a), 3) *E. moniezii*, and 4) *Entione salvatoris* Kossmann, 1881 (a *nomen nudum* from Kossmann, 1881). *Entione salvatoris* is a *nomen nudum* despite the assertion of Giard & Bonnier, 1887 that Plate 8, Figure 1 of Kossmann (1881) represented this species; in fact, Kossmann (1881: 158) only proposed the name tentatively and even stated that it would probably not be sustainable (“*vermutlich wird diese aber doch nicht zu halten sein*”). Kossmann (1881) also labeled Plate 8, Figure 1 as *E. moniezii* without any mention of a host that could link the figure to his tentative name. *Entione salvatoris* is available, however, from Giard & Bonnier, 1887 as *Portunion salvatoris*, although Giard & Bonnier (1887) gave only a short description of the species and provided only a figure of an egg. The third subgenus was *Cancrion* into which Giard & Bonnier (1886a) placed: 1) *E. cancrorum* Müller, 1864 (“*probablement*”) (Giard & Bonnier, 1886a: 646), 2) *Cancrion floridus* Giard & Bonnier, 1886 (Giard & Bonnier, 1886a) (a *nomen nudum*, later described in Giard & Bonnier, 1887), and 3) *C. miser* Giard & Bonnier, 1886 (Giard & Bonnier, 1886a) (also a *nomen nudum*, later described in Giard & Bonnier, 1887). Giard & Bonnier (1886a) therefore did not leave any species in the nominotypical subgenus *Entione* and compounded the problem by later raising all three of their subgenera to genera (Giard & Bonnier, 1887) while also poorly describing a new species, *Entione achaii* Giard & Bonnier, 1887, which has subsequently remained the only species in this genus.

Another related problem that needs to be addressed is that whereas *Grapsion* and *Portunion* are available names from Giard & Bonnier (1886a), *Cancrion* is not. This is because two of the species names included in *Cancrion* Giard & Bonnier, 1886 (Giard & Bonnier, 1886a) are *nomina nuda* and as such unavailable, whereas the third, *E. cancrorum*, was only conditionally included and therefore cannot be considered an originally included species in the genus (fide International Commission on Zoological Nomenclature (ICZN) Article 67.2.5). *Cancrion* Giard & Bonnier, 1886 (Giard & Bonnier, 1886a) is therefore a *nomen nudum*; however, Giard & Bonnier (1887) used *Cancrion* as an available genus name that included *E. cancrorum*, *C. floridus*, and *C. miser* with the first two names being made available from 1887 and the third being unconditionally included in the genus.

Despite *Cancrion* being available from Giard & Bonnier (1887), no species was designated as the type of *Cancrion* at the time of its description or by any subsequent author(s) and we therefore select herein *Cancrion miser* Giard & Bonnier, 1886 (Giard & Bonnier, 1886a) as the type species of *Cancrion* because it is by far the best described and illustrated of the three originally included species. Adkison (1990) came to a different conclusion and stated that *E. cancrorum* should be the type species of *Cancrion*; his work, however, remains an unpublished dissertation with no bearing on type species selection.

There are three possible solutions for resolving the absence of any originally included species in *Entione*: 1) select *E. cavolinii* as the type species of *Entione*, thereby making *Grapsion* a junior objective synonym of *Entione*, 2) select *E. moniezii* as the type species, thereby making *Portunion* a junior subjective synonym of *Entione*, or

3) select *E. cancrorum* as the type species, thereby making *Cancrion* a junior subjective synonym of *Entione*. Adkison (1990) essentially advocated for the third option, although he stated that *E. cancrorum* should be the type species of both *Entione* and *Cancrion*. We disagree with this because of the aforementioned poor description and knowledge of *E. cancrorum*, as well as the fact that the seven species currently in *Cancrion* would now be combined with a different generic name. Likewise, selecting *E. moniezii* would be destabilizing because *Portunion* contains species that are the best studied entoniscids (e.g., Veillet, 1943a, 1943b, 1956; Kuris et al., 1980; Shields & Kuris, 1985; Brockerhoff, 2004) and the seven species currently in *Portunion* would also be combined with a different generic name. We therefore select *E. cavolinii* as the type species of *Entione*, as this species is well defined and described but not well studied beyond its taxonomy. *Grapsion* therefore becomes a junior objective synonym of *Entione* and only a single species will be affected by this synonymy. The only other species in *Entione*, aside from the type species now referred to as *Entione cavolinii* n. comb., is *E. achaii*, which is so poorly described (and never figured) that, although it is a *taxon inquirendum*, we prefer to leave in *Entione* until such time as new material is collected from the type locality (Desterro = Florianópolis, Brazil) and majoid host (*Achaeus* sp.). A summary of the changes to names of species originally or subsequently placed in *Entione* is given in Supplementary material Table S1.

### *Pinnixion* n. gen.

*Type species: Pinnixion sexdecennia* n. sp., by present designation. Monotypic.

*Etymology:* The generic name refers to the genus of the most common host crab (*Pinnixa* White, 1846); the gender is neuter.

*Diagnosis:* Female with sheath secreted by host surrounding entire parasite except for perforation at posterior end. Cephalon rounded, dorsally distinctly divided into 2 bulbous lobes, bearing 2 pairs of antennae, single pair of maxillipeds. Maxilliped with subquadrate coxopodite curved; lamellar exopodite. Pereon without ovarian processes, pereopods apparently absent. Five pairs of oostegites forming brood pouch, margins entire. Oostegite 1 with single ascendant lobe, 2 transverse lobes, single recurrent lobe; oostegites 2–5 fused in mature females, covering oostegite 1. Pleomeres 1–3 each with distinct uniramous flattened pair of pleopods that overlay one another; pleomeres 4, 5 with reduced pleopods. Two pairs of complex pleural lamellae positioned medially on pleon. Oval, bulbous heart in pleomere 3. Terminal pleomere with pair of anteroventral lobes, 2 long, tapered lateral projections, 4 distal finger-like projections.

Male with pereomere 1 ventrally fused with trapezoidal cephalon. Cephalon with pair of mediolateral cephalic slits, 2 minute eyes. Antennules as large ovate lobes fused medially; antennae absent. Pair of mitten-shaped maxillipeds at base of oral cone. Pereomere 7 with small, setose medioventral spine. Six pairs of pereopods with dactylus; propodus, carpus fused into single cylindrical segment. Pleomeres 1 and 2 with large hemispherical lobes laterally, lobes on pleomere 1 more than twice as large as those on pleomere 2. Pleotelson with 2 elongated tapered posterolateral lobes.

Epicaridium larva with antennule of 2 rounded articles, antenna elongated, approximately half length of body, composed of 6 articles. Sixth pair of pereopods greatly elongated with long, cylindrical process, articulated with propodus/carpus dorsal to dactylus.

*Remarks:* The new genus is included in Entoniscinae (now consisting of 12 genera) due to having the female marsupium formed in part by the oostegites, oostegite 1 divided into three parts, and well-developed pleural lamellae; males have segmented pereopods. In contrast, the females of Entoniscinae (five genera) have the marsupium formed entirely by host tissue, oostegite 1 not divided into parts, pleural lamellae absent, and male pereopods unsegmented.

Within Entioninae, females belonging to the new genus are most similar to those in *Entionella* Miyashita, 1941 in lacking any processes on the pereon. Females belonging to the new genus are distinct from those in *Entionella* by possessing two transverse lobes on oostegite 1 and fused oostegites 2–5 (one transverse lobe on oostegite 1 and separate oostegites 2–5 in *Entionella* spp.). Males belonging to the new genus have a pair of lateral expansions on each of the first two pleomeres, the only other genus containing a species with any pleomeres bearing lateral margin expansions is *Entionella* Miyashita, 1941, with *E. okayamaensis* Shiino, 1954, but this species has a pair of expansions only on pleomere 1. The epicaridium larvae belonging to the new genus have a long, cylindrical process, articulated with propodus/carpus on each of the sixth pereopods; no process with this form is known from epicaridium larvae of any other entoniscids (somewhat similar but distinctly different spoon-like processes are found in species of *Cancrion*).

Because the new genus and species parasitizes pinnotheroid crabs, we also compared it with *Pinnotherion* Giard & Bonnier, 1889, whose species are associated with pinnotheroid and majoid crabs. Females belonging to the new genus have two transverse lobes on oostegite 1 (absent in species of *Pinnotherion*) and two pairs of pleural lamellae on the pleon (four pairs in species of *Pinnotherion*). Males belonging to the new genus differ from those of *Pinnotherion* spp. by having two large lateral expansions on pleomeres 1 and 2 (no expansions in *Pinnotherion* species). The species of the new genus also has a medioventral spine on pereomere 7, whereas *Pinnotherion vermiforme* Giard & Bonnier, 1889 has a medioventral spine on both pereomere 7 and pleomere 1, and *P. setoensis* Shiino, 1942 has a medioventral spine only on pleomere 1. The epicaridium larvae belonging to the new genus differ from those of *Pinnotherion* spp. in having greatly elongated sixth pereopods each bearing a long, cylindrical process on the propodus/carpus (sixth pereopods subequal in size to other pereopods and without processes in *Pinnotherion* spp.).

We note that the coxopodite of the maxilliped in entoniscids is likely homologous with the anterior lobe of the maxilliped in bopyrids; both possess a muscular attachment that drives the beating of the maxilliped and allows for oxygenation of the brood (see Cericola & Williams, 2015).

**Nomenclatural statement:** A life science identifier (LSID) number was obtained for the new genus: urn:lsid:zoobank.org:pub:13A66D63-63CB-4F96-A9D8-195DEDD25282.

### *Pinnixion sexdecennia* n. sp.

Figs. 2–3

undescribed parasitic isopod – McDermott, 2005: 754.  
 unidentified entoniscid isopod – McDermott, 2005: 755, 761.  
 unidentified parasitic isopods (Entoniscidae) – McDermott, 2006: 345.  
 isopod parasites (Crustacea, Isopoda, Epicaridea, Entoniscidae) – McDermott, 2006: 355.  
 entoniscids recovered from *A. gorei* – McDermott, 2006: 359.  
 undescribed endoparasitic isopod (Entoniscidae) – McDermott, 2009: 787.  
 undescribed entoniscids – McDermott, 2009: 793, 800, table 2.  
 entoniscid parasite of pinnotherid crabs – Williams, 2018: 2.

**Type material:** mature female holotype (4.13 mm), infesting female *P. chaetoptera* (4.3 mm CL × 9.0 mm CW), Virginia Key, Miami, FL (25°40'N, 80°10'W), coll. J. J. McDermott, 11 or 21 April 1967 (specimen preserved on 11 May 1967) (USNM 1594834). 1 mature female paratype (3.98 mm), 1 immature female paratype (3.73 mm), 1 mature male allotype (2.12 mm), all infesting female *P. chaetoptera* (CL not measured [host lost] × 13.6 mm CW),

Beaufort, NC (34°43'N, 76°40'W), coll. J. J. McDermott, 23–24 Aug 1965 (specimen preserved on 4 Dec 1965) (USNM 1594841). Plus 90 paratypes (including females, males and larvae; USNM 1594835–1594886) from NJ, NC, and FL (see [Supplementary material S2 paratype list](#)).

**Type locality:** Virginia Key, Miami, Florida, USA (25°40'N, 80°10'W).

**Type host:** *Pinnixa chaetoptera* Stimpson, 1860 (Brachyura, Pinnotheridae).

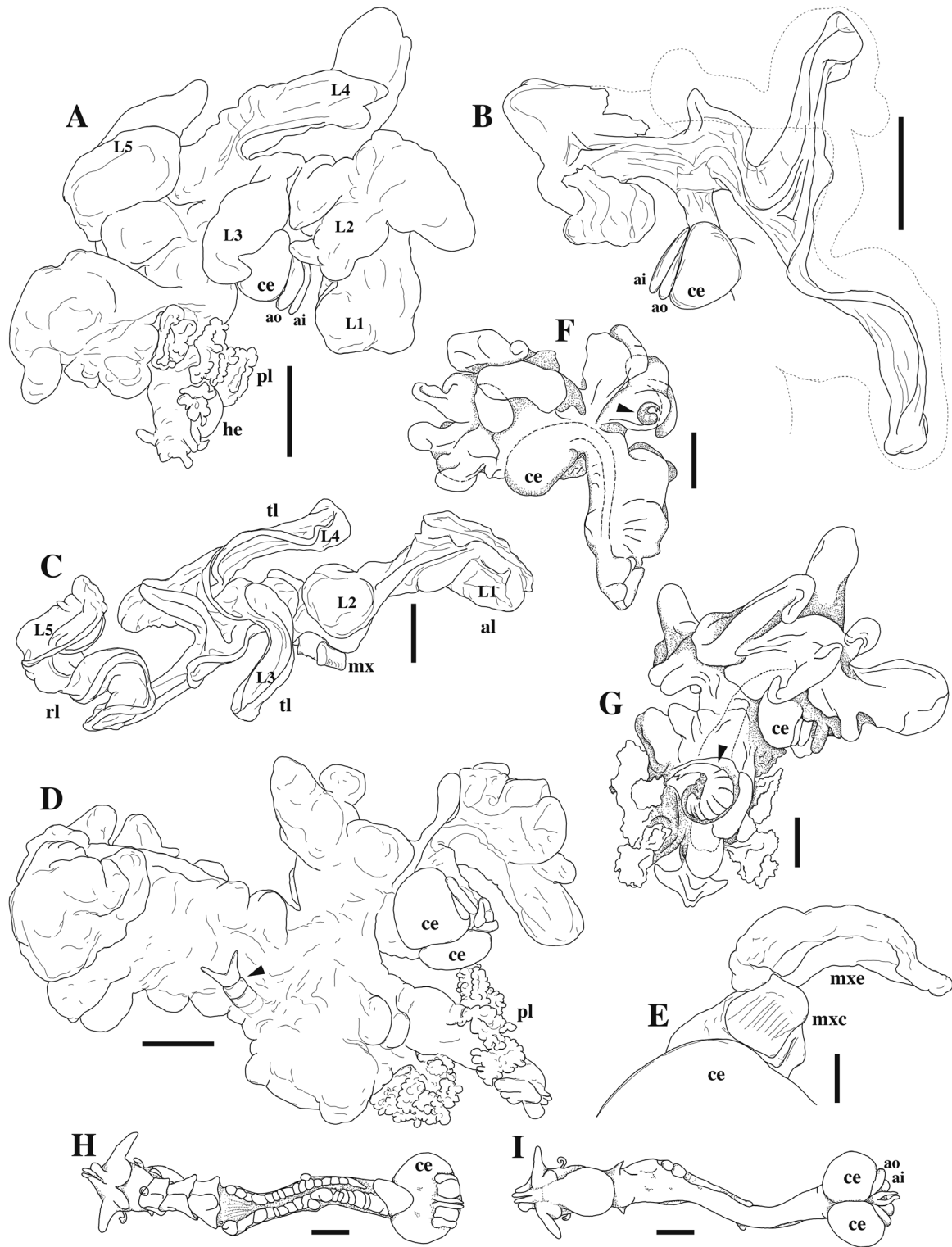
**Etymology:** The specific name refers to the six decades over which this study was accomplished. JJM collected the first specimens of the new genus and species in 1964 (see McDermott, 2005, 2006, 2009) and worked on the manuscript on and off until his death in 2017 (see Williams, 2018).

**Description of female** (Figs. 2, 3A–C, 4) (based primarily on holotype, 4.13 mm, USNM 1594834, unless examination of additional specimens or immature stages noted): Mature specimen occupying most of host hemocoel, brood chambers often extending into spaces within pleon, bases of walking legs. Sheath formed by host surrounds entire parasite except for perforation at posterior end (Figs. 2D, 3C), attached to oval opening into host's branchial chamber; opening surrounded by brownish ring (greater diameter  $341 \pm 80 \mu\text{m}$ ,  $N = 4$ ). One paratype specimen with ring extended into short tube protruding into branchial chamber.

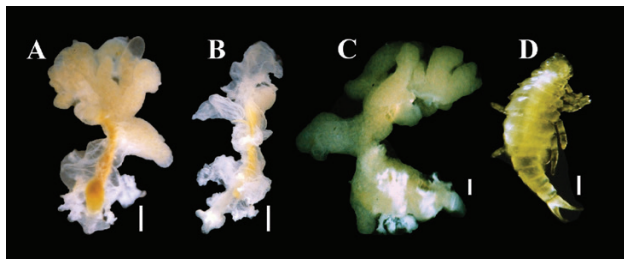
Body V-shaped from cephalon to posterior end of pleon, concave part (dorsal) facing anterior end of host (i.e., when posterior end attaches to right branchial wall of host, cephalon is on left side of hemocoel facing left branchial wall and parasite lying on right side; Fig. 2A, D). Juvenile females vermiform, S-shaped, without appendages (Fig. 4B, H–J). Cephalon rounded, dorsally distinctly divided into 2 bulbous lobes (Fig. 2A, B, D, I), ventrally undivided (Fig. 2H), bearing 2 pairs of antennae, pair of maxillipeds. Both pairs of antennae slender lobes, subequal in length (Fig. 2A, B), inner pair slightly broader (Fig. 4C). Maxilliped with subquadrate coxopodite (see Remarks above), curved, lamellar exopodite (Fig. 2E); endopodite absent. Pereon compact, ovarian processes (e.g., dorsal, ventral) absent, pereopods apparently absent except possibly for reduced pereopod 2 (Fig. 4D, E). Five pairs of oostegites forming brood pouch, margins entire. Oostegite 1 with ascendant lobe, 2 transverse lobes, one recurrent lobe (Fig. 2A–C). Oostegites 2–5 fused in mature females, covering oostegite 1 (Fig. 2B).

Pleon short, composed of 5 segments delineated ventrally, fused dorsally (Fig. 2H, I). Pleomeres 1–3 each with distinct uniramous flattened pair of pleopods that overlay one another, pleomeres 4 and 5 with reduced pleopods (Fig. 2H); in juveniles all pleopods distinct with pleopods 3–5 subequal (Fig. 4A, B). Two pairs of complex pleural lamellae positioned medially on pleon (Fig. 2A, D). Oval, bulbous heart in pleomere 3 (Figs. 2A, 4E). Terminal pleomere with pair of anteroventral lobes (possibly representing fused pleopod 5), two long tapered lateral projections (Figs. 2H, 4E), 4 distal finger-like projections (possibly uropods), tips with small scales, spines (Fig. 4F, G).

**Description of male** (Figs. 3D, 5, 6) (based primarily on allotype, 2.12 mm, USNM 1594841, unless examination of additional specimens noted): Body elongated, all pereomeres clearly separated dorsally, pereomere 1 ventrally fused with trapezoidal cephalon (Fig. 5A, 6A, E). Cephalon with pair of mediolateral cephalic slits (Fig. 6B); 2 minute eyes ( $\sim 25 \mu\text{m}$  in diameter) present dorsolaterally (Fig. 5A). Antennules as large ovate lobes fused medially, with stellate grouping of  $\sim 16$  short setae anteroventrally (Figs. 5B, 6B, D); antennae absent. Pair of mitten-shaped maxillipeds at base of oral cone (Fig. 6C). Maximal width at pereomeres 5 and



**Figure 2.** *Pinnixia sexdecennia* n. gen., n. sp. Left lateral view of mature female (USNM 1594843), internal branches of oostegite 1 shown with numbers 1–5 that correspond to 2C (A); left lateral, inner view of oostegite 1 (USNM 1594841), outer membrane that covers internal branches and fuses with posterior oostegites shown partially in dashed line (B); left lateral, outer view of oostegite 1 (USNM 1594843), showing internal branches with numbers 1–5 as in 2A (C); left lateral view of mature female (USNM 1594870), with host sheath surrounding body, position of one male shown by arrowhead (D); anterior portion of cephalon and maxilliped (E); living, ovigerous female removed intact from the hemocoel of a male *Pinnixia chaetoptera* (CW 12.2 mm), collected in North Carolina 21 June 1966; surrounded by host membrane. Dashed lines show the V-shape of the main body stem, male shown by arrowhead (F); mature female from ovigerous female *P. chaetoptera* (CW 15.5 mm) collected in North Carolina 25 June 1966; much of the host membrane is removed, dashed lines indicate the main body axis of the parasite, male shown by arrowhead (G); two views of dissected main body stems (oostegites and other pleural lamellae removed), of a mature ovigerous parasite from a female *P. chaetoptera* (CW 9.3 mm) collected in New Jersey 12 June 1963 (H, I); ventral view of cephalon, main body trunk, overlapping pairs of pleopods (1–4) and terminal pleomere with pair of laterally elongated lobes (possibly representing fused fifth pleopods) and



**Figure 3.** *Pinnixia sexdecennia* n. gen., n. sp. Living mature female removed from ovigerous female *Pinnixia chaetoptera* (CW 11.2 mm) collected in New Jersey 23 June 1964. Most of host encapsulating membranes removed. Parasite (6.8 mm total length) attached to crab's left branchial wall. Brood pouches full of mature epicaridium larvae (A); living mature female removed from same host as in A, viewed from their right side as appeared in host's hemocoel. Most of host's encapsulating membranes removed (B); living ovigerous female from an ovigerous *P. chaetoptera* (CW 9.3 mm) collected in New Jersey 12 June 1963. Pygidium was attached to pore in wall of right branchial chamber. Parasite partially covered by host sheath; extensive brood pouches filled with mature epicaridium larvae. The crab produced six broods of zoeae and molted three times during its year-long period in the laboratory (C); oblique dorsal view of living mature male from ovigerous female *P. chaetoptera* collected in North Carolina 6 August 1965, no female present (D). Scale bars: A–C = 500  $\mu$ m; D = 250  $\mu$ m.

6, gradually tapering anteriorly and posteriorly; pereomere 7 with small (length  $58 \pm 3 \mu$ m,  $54$  to  $60 \mu$ m,  $N = 3$ ), setose medioventral spine (Figs. 5B, 6E, J), pair of lateral gonopores (Fig. 6F, I). Six pairs of pereopods (no pereopods on pereomere 7) (Figs. 5A, B, 6A, E, F), pereopods increasing in size posteriorly, each with minute dactylus; propodus, carpus fused into single cylindrical segment, merus small, indistinctly separated from propodus/carpus; ischium, basis distinct (Fig. 6G, H). Pleon with 5 segments plus pleotelson. Pleomeres slightly narrower posteriorly, distinctly separated, pleomeres 1 and 2 with large hemispherical lobes laterally, lobes on pleomere 1 more than twice as large as those on pleomere 2 (Figs. 5A, B, 6F); pleomeres 3–5 cylindrical, pleopods absent. Heart mid-dorsal in pleomere 1; beating in living animal. Pleotelson with 2 elongated tapered posterolateral lobes (Figs. 3D, 5A, B, 6A); anal cone, uropods absent.

**Description of epicaridium larva** (Fig. 7) (USNM 1594839): Approximately 300  $\mu$ m in length (anterior margin of cephalon to end of telson). Body tear-drop shaped, with pigment bands on cephalon, lateral margins of some pereomeres, pleomeres, uropods (Fig. 7A). Anterior margin of head rounded, width approximately  $1.6 \times$  length; black-pigmented posterolateral eyespots, irregular in shape ( $13 \pm 0.4 \mu$ m diameter,  $N = 6$ ) (Fig. 7A). Antennule of 2 rounded articles, article 1 longer but scarcely broader than terminal article, 1 or 2 small setae; terminal article with multiple longer setae (Fig. 7B, C). Antenna elongated, approximately half length of body (Fig. 7B); composed of 6 articles, articles 1 and 2 subequal in size; articles 3 and 4 progressively longer, flagellar article 1 approximately  $1.5 \times$  as long as terminal article bearing few short setae, long medial seta nearly half as long as entire antenna (Fig. 7C); terminal seta appears divided (see Remarks).

Five anterior pairs of gnathopodal pereopods, subequal in size, each with slender, slightly curved dactylus extending approximately half length of propodus, tip of dactylus hooked; propodus, carpus fused, slightly protruding end of carpus with few short

setae; merus small, subtriangular, basis ovate, ischium elongated (Fig. 7B, D, E). Pereopod 6 greatly elongated, dactylus reduced with ventral seta; propodus, carpus fused, spherical, slightly smaller than other pereopods, with several long, filamentous setae dorsally and ventrally; long, cylindrical process articulated with propodus/carpus dorsal to dactylus, approximately twice as long as propodus/carpus, several long, filamentous setae extending from medial region, tip; merus, ischium, basis subequal in size and shape, merus approximately  $3 \times$  longer than on other pereopods; ischium, basis approximately  $2 \times$  longer than on other pereopods (Fig. 7B, F).

Pleon with 5 pairs of uniramous pleopods (Fig. 7B); triangular coxopodite bearing 2 long setae at inner distal point, ovate exopodite articulated with outer distal point of coxopodite bearing 3 long setae. Uropods biramous, stout cylindrical peduncle with one stout and one thin seta on outer angle, distally with 2 slender rami, endopod slightly longer than exopod, both ending in 2 short, robust setae (Fig. 7G).

**Remarks:** Because the new species is the sole taxon in its genus, a comparison of most of the important characters of the new species with other entoniscid species is given above under the description of the new genus.

In addition to parasitizing *P. chaetoptera*, the new entoniscid was found in *A. gorei* and *Z. ostreus*. The morphology of the parasites in these hosts matched those from *P. chaetoptera* (compare juvenile females in Figures 4 and 8). The epicaridium larvae from *P. sexdecennia* n. gen., n. sp. infesting *Z. ostreus* also appear identical to those from *P. chaetoptera*.

#### Observations on life-history stages

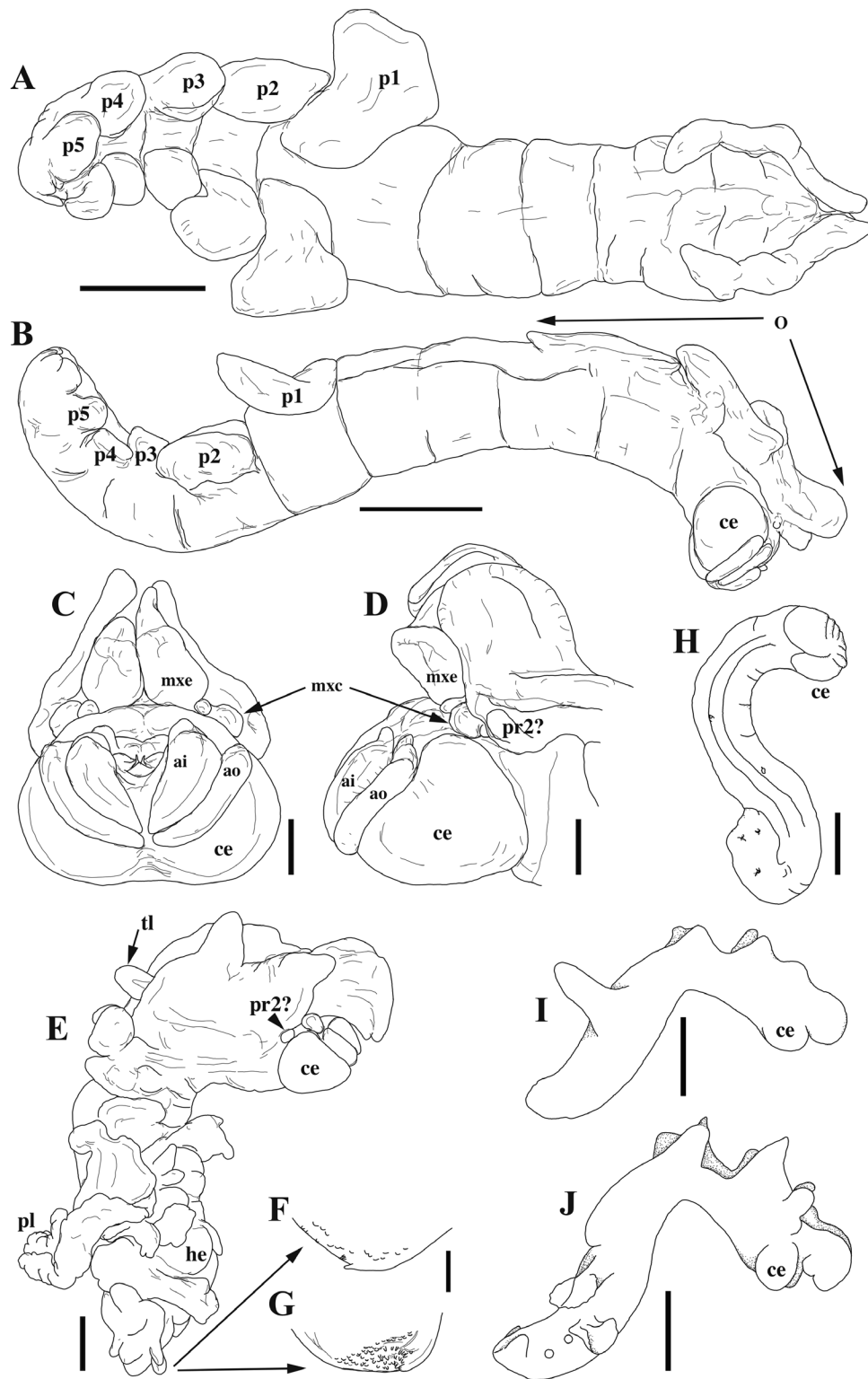
Embryos are sub-oval and become elongated as they develop within the marsupium of the female. Length and width of early stage embryos are  $112 \pm 5 \mu$ m  $\times$   $99 \pm 4 \mu$ m ( $N = 14$ ); intermediate stage (with eye development)  $176 \pm 9 \mu$ m  $\times$   $79 \pm 5 \mu$ m ( $N = 5$ ); near maturity  $287 \pm 15 \mu$ m  $\times$   $116 \pm 5 \mu$ m ( $N = 6$ ).

Epicaridium larvae freshly released from an infested female crab collected in FL had a length to end of telson of  $295 \pm 8 \mu$ m ( $287$  to  $301 \mu$ m,  $N = 8$ ); length including uropods was  $387 \pm 5 \mu$ m ( $381$  to  $397 \mu$ m,  $N = 7$ ); width at pereomeres 4 and 5 was  $141 \pm 2 \mu$ m ( $138$  to  $144 \mu$ m,  $N = 8$ ); cephalon width was  $122 \pm 3 \mu$ m ( $119$  to  $124 \mu$ m,  $N = 6$ ). Shiino (1942) stated that the epicaridium larvae of entoniscids have antennae with four basal articles and two terminal articles with one long seta. This appears to be the case in our specimens; however, the long seta appears divided approximately  $1/4$  along its length (Fig. 7C) and this could be interpreted as three flagellar articles followed by a somewhat shorter distal seta. Future SEM work is required to address this question in this species as well as in other entoniscids.

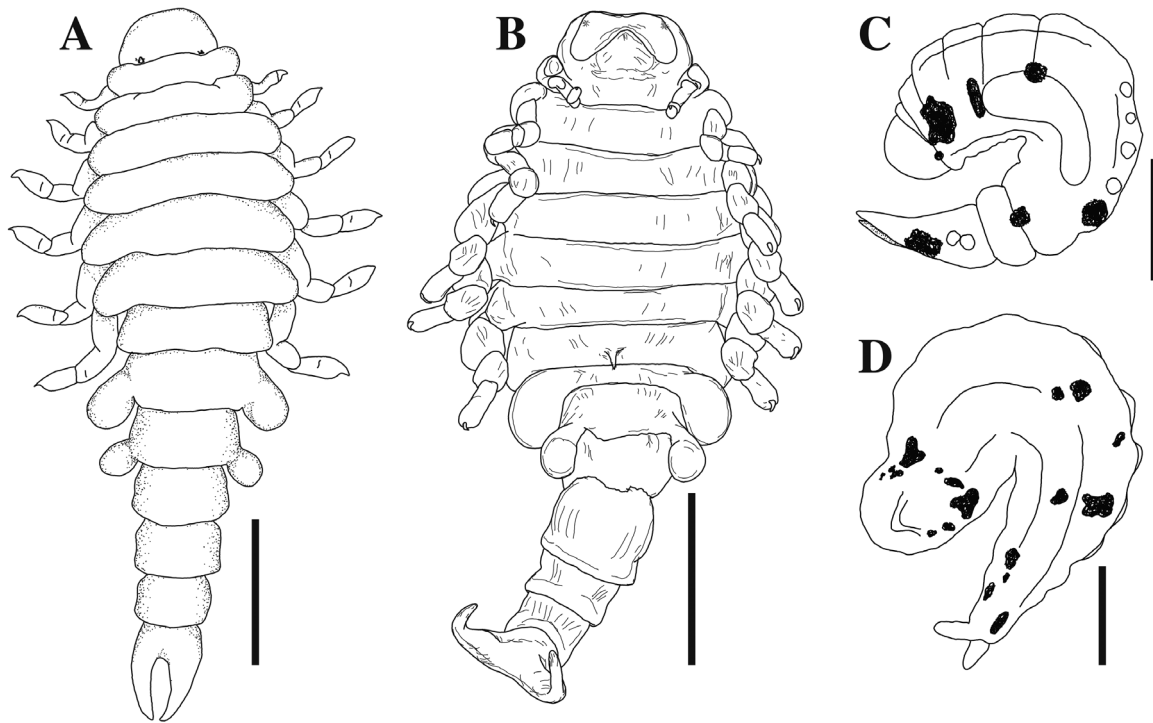
Cryptoniscus larvae were found externally attached in a few samples, either alive or as molts, to their *P. chaetoptera* hosts on the gills and oral appendages, as well as in the hemocoel on the crab's organs or attached to the external sheath of mature female parasites; the maximum number of cryptoniscus larvae per crab was four. Mean total length of cryptoniscus larvae (anterior end to most distal spine of uropods) was  $499 \pm 18 \mu$ m ( $475$ – $528 \mu$ m,  $N = 6$ ); width approximately  $1/3$  length.

Males were found *in situ* inside of the posterior region of the oostegites (Fig. 2D, F, G). Immature males were highly recurved

four distal finger-like projections. Minute attachments of some of the removed pleural lamellae appear laterally (H); dorsal view showing bilobed cephalon terminated by two pairs of antennae, main body trunk, posterior end with bulbous heart preceding terminal pleomere with pair of laterally elongated lobes and four distal finger-like projections. Minute attachments of some of the removed pleural lamellae appear laterally (I). Abbreviations: al, anterior lamella; ai, inner antenna; ao, outer antenna; ce, cephalon; he, heart; L1–L5, lobes of oostegite 1 so that their position (C) can be matched with that shown in (A); mx, exopodite of maxilliped; mxc, coxopodite of maxilliped; pl, pleural lamellae; rl, recurrent lamella; tl, transverse lamella. Scale bars: A, B, F, G = 1 mm; C, D, H, I = 500  $\mu$ m; E = 250  $\mu$ m.



**Figure 4.** *Pinnixion sexdecennia* n. gen., n. sp. (A–D: USNM 1594864; E–G: USNM 1594861). Immature female, ventral view (A); immature female, left lateral view (B); *en face* view of cephalon (C); cephalon, right lateral view (D); immature female, left lateral view (E); terminal portion of uropod, ventral view (F); terminal portion of uropod, dorsal view (G); dorsal view of living, non-flattened immature female from North Carolina in male *Pinnixa chaetoptera* (CW 13.3 mm) (H); lateral view of a living immature, non-flattened living female from *P. chaetoptera* collected in Florida in 1967 (I); lateral view of an immature living female from female *P. chaetoptera* (CW 2.7 mm) collected in Florida in 1967 (J). Abbreviations: ai, inner antenna; ao, outer antenna; ce, cephalon; he, heart; mx, exopodite of maxilliped; mxc, coxopodite of maxilliped; o, oostegites; p1–p5, pleopods; pl, pleural lamellae; pr2?, presumptive pereopod 2; tl, transverse lamella. Scale bars: A, B, I, J = 500 µm; C, D = 100 µm; E = 1.0 mm; F, G = 25 µm; H = 200 µm.



**Figure 5.** *Pinnixion sexdecennia* n. gen., n. sp. Dorsal view of mature male from female *Pinnixa chaetoptera* (CW 10.2 mm), collected in North Carolina 15 June 1966 (A); ventral view of male (USNM 1594841) (B); immature living segmented male entoniscids from hemocoel of *P. chaetoptera* specimens collected in North Carolina 26 July 1965 (C, D); lateral view in flexed condition from surface of crab's ovary (C); flattened, flexed specimen (D). Scale bars: A, B = 500  $\mu$ m; C, D = 100  $\mu$ m.

and with scattered pigmentation (Fig. 5C, D); adult males were similarly recurved. Mean total body length of males from NC was  $2.18 \pm 0.38$  mm (1.02 to 2.70 mm,  $N = 20$ ); mean cephalon width was  $0.44 \pm 0.08$  mm (0.26 to 0.58 mm,  $N = 14$ ). Mean total body length of preserved males from FL was  $1.78 \pm 0.02$  mm (1.44 to 2.21 mm,  $N = 11$ ); body width at pereomere 1 was  $0.34 \pm 0.06$  mm (0.25 to 0.42 mm,  $N = 12$ ), pereomeres 4 and 5 were  $0.55 \pm 0.11$  mm (0.38 to 0.66 mm,  $N = 8$ ), and  $0.59 \pm 0.10$  mm (0.40 to 0.70 mm,  $N = 7$ ), respectively.

#### Prevalence and intensity of parasites

**New Jersey.** Males and females of *Pinnixa chaetoptera* ( $N = 150$  and 182, respectively; females significantly larger) were obtained monthly from tubes of *Amphitrite ornata* during 1963 (May to November) and 1964 (January and April to October; McDermott, 2005: table 5, fig. 1). Overall prevalence of *P. sexdecennia* in 332 individuals of *P. chaetoptera* from NJ was 4.8%; 3 of 147 (2.0%) male hosts and 13 of 169 (7.1%) female hosts were infested. Female hosts were found to be parasitized more often than predicted based on expected equal distribution of parasites among female and male hosts ( $\chi^2 = 4.7$ ,  $N = 332$ ,  $P = 0.029$ ).

Two males and six females were infested with mature female *P. sexdecennia* n. gen., n. sp. detected via the emergence of epicaridium larvae and verified by subsequent dissection. Infestations in seven other females and one male were detected in individuals that died or were preserved in the laboratory before dissection. These crabs were infested only with males or immature female parasites. All 16 crabs were mature (CW  $11.1 \pm 0.9$  mm, 9.3–12.2 mm; males,  $10.3 \pm 0.8$ , 9.6–11.1 mm,  $N = 3$ ; females:  $11.1 \pm 0.8$  mm, 9.3–12.2 mm,  $N = 13$ ). Male and female crabs in NJ mature at  $\sim 7.0$  and 8.0 mm, respectively (McDermott, 2005).

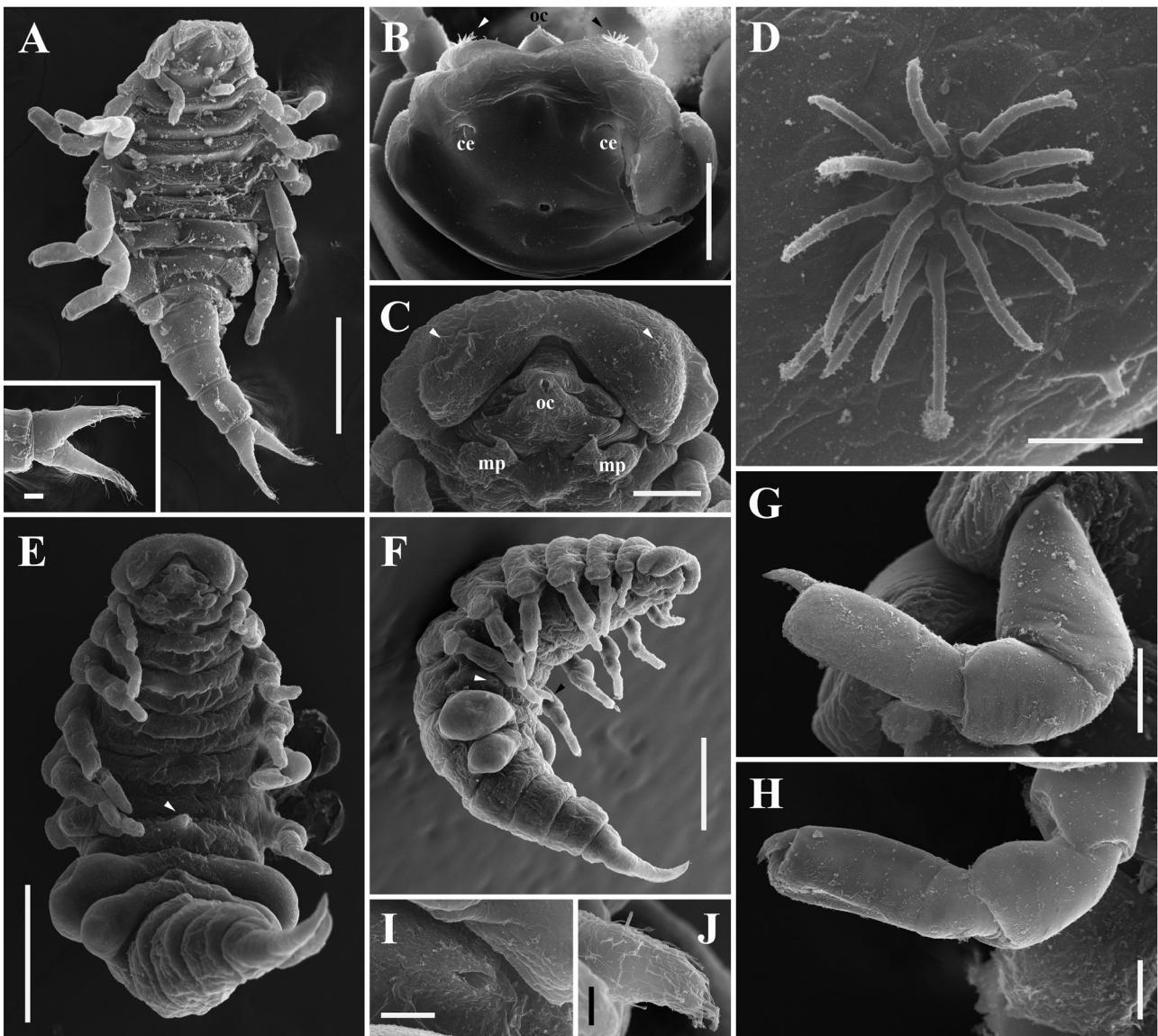
Nine of the 13 female crabs were ovigerous when collected, and five of these harbored mature female entoniscids, all of which

liberated epicaridium larvae. All of these ovigerous crabs produced one to two more viable broods of zoeae in the laboratory, and molting was not inhibited by infestations with these mature parasites. One of the 13 infested female crabs, non-ovigerous when collected and maintained in the laboratory for  $\sim 12$  months, produced two broods of zoeae and molted three times prior to the liberation of epicaridium larvae. One of the infested male crabs, maintained for nine months, molted three times and its parasite produced three broods of epicaridium larvae. Single male isopods were attached to the posterior half of four mature female entoniscids.

Emerging epicaridium larvae were not observed in some of the infested, originally ovigerous, crabs until they were in the laboratory in isolation for several months. It is thus possible that the female parasites were immature at the time of collection and had no effect on the functioning of the crabs' gonads. In one of the five ovigerous crabs, however, both crab zoeae and epicaridium larvae were emerging four days after the crab was collected. This crab was not dissected at that time, so the condition of its ovaries was unknown.

One of the crabs with a mature female parasite, detected by the liberation of epicaridium larvae, had, in addition to the mature female with a flaccid marsupium, another mature female filled with epicaridium larvae (Fig. 3). Both parasites had external pores in the left branchial wall of the host. Mature females of *P. sexdecennia* n. gen., n. sp. were all oriented inside of their host so that their posterior ends made contact with either the left or right branchial chambers via pores in the branchial walls.

**North Carolina.** Crabs were collected from the tubes of *Chaetopterus variopedatus* in the vicinity of Beaufort during June to August in 1965–1967, with in an additional collection in October 1966. Nearly equal numbers of males and females ( $N = 402$  and 407, respectively) were measured, dissected, and examined for entoniscid infestations. Females of *P. chaetoptera* were significantly larger



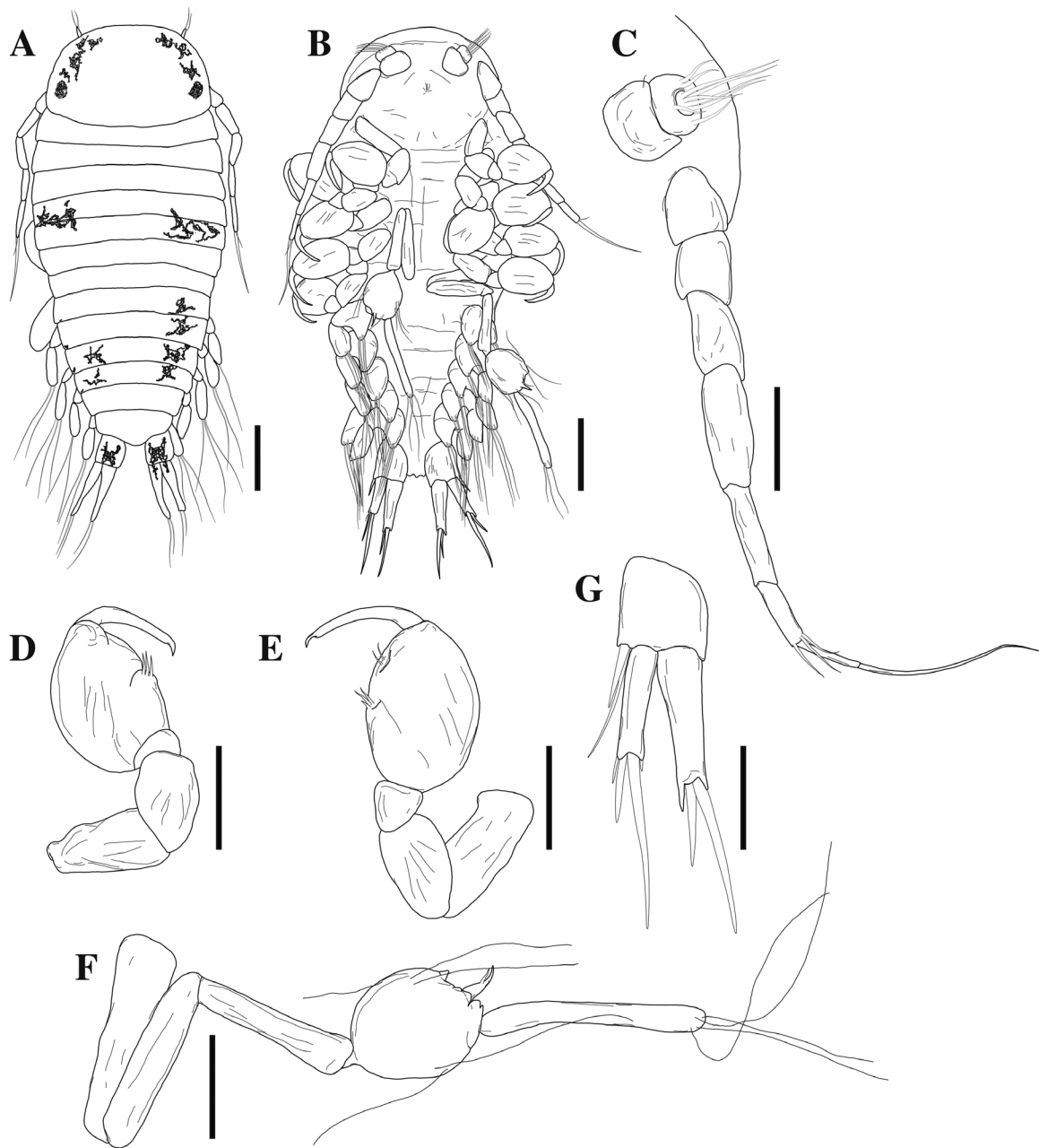
**Figure 6.** *Pinnixion sexdecennia* n. gen., n. sp. Ventral view of mature male (USNM 1594851), inset shows close-up view of telson (A); dorsal view of head showing cephalic slits (ce), oral cone (oc), and antennules (arrowheads) (USNM 1594880) (B); ventral view of head showing oral cone (oc), antennules (arrowheads), and maxillipeds (mp) (USNM 1594873) (C); right antennule (USNM 1594880) (D); ventral view of male showing midventral protuberance on pereopod 7 (white arrowhead) (USNM 1594873) (E); lateral view male showing position of gonopore on pereopod 7 (white arrowhead) and midventral protuberance on pereopod 7 (black arrowhead) (USNM 1594873) (F); right pereopod 2 (USNM 1594851) (G); right pereopod 6 (USNM 1594851) (H); right gonopore (USNM 1594873) (I); lateral view of midventral protuberance on pereopod 7 (USNM 1594873) (J). Scale bars: A, B = 200  $\mu$ m; B, C = 100  $\mu$ m; D = 10  $\mu$ m; E, F = 500  $\mu$ m; G, H, J = 25  $\mu$ m; I = 50  $\mu$ m; A inset = 25  $\mu$ m.

than males (see McDermott, 2005) and the mean carapace width for each sex each year was practically the same (Supplementary material Table S3). Larger, mature crabs of each sex were more highly infested, and mature female parasites were more prevalent in larger crabs (Fig. 9A, B). Some crabs collected from the tubes of *A. ornata* in NC were also infested with *P. sexdecennia* n. gen., n. sp.

Annual prevalence of *P. sexdecennia* n. gen., n. sp. in male and female *P. chaetoptera* from NC in 1965, 1966, and 1967 were 21.7%, 41.7%, and 36.3%, respectively (Supplementary material Table S4). Overall prevalence of *P. sexdecennia* n. gen., n. sp. in 809 individuals of *P. chaetoptera* from NC was 31.9%; 91 of 402 (22.6%) male hosts and 167 of 407 (41.0%) female hosts were infested (Fig. 9A, B). Female hosts were found more often to be parasitized than predicted based on expected equal distribution of

parasites among female and male hosts ( $\chi^2 = 31.5$ ,  $N = 809$ ,  $P < 0.0001$ ). Monthly and annual prevalences were greater in female crabs (Supplementary material Table S4) as were the intensities of entoniscid infestations (Supplementary material Table S5). Undifferentiated life-history stages of the parasite, adult males, and immature females were responsible for most of the recorded infestations, whereas reproductively mature females were relatively scarce (Fig. 9A, B; Supplementary material Table S6).

Mature male parasites were particularly numerous in the crab population, with up to eight individuals per crab. Usually one male was associated with the body of the mature female while the other males were found throughout the hemocoel of the same host. Males were also found with well-developed immature females. Some of these supernumerary males were dead and had become encapsulated or mummified with melanized host tissue



**Figure 7.** *Pinnixia sexdecennia* n. gen., n. sp. Epicaridium (A–C: USNM 1594839). Dorsal view of larva from a female infesting a male *Pinnixa chaetoptera* (CW 14.1 mm) collected in North Carolina 1965 (A); ventral view of larva (B); left antennule (top) and antenna (bottom) (C); left pereopod 1 (D); right pereopod 4 (E); left pereopod 6 (F); right telson (G). Scale bars: A, B = 50  $\mu$ m, C–G = 25  $\mu$ m.

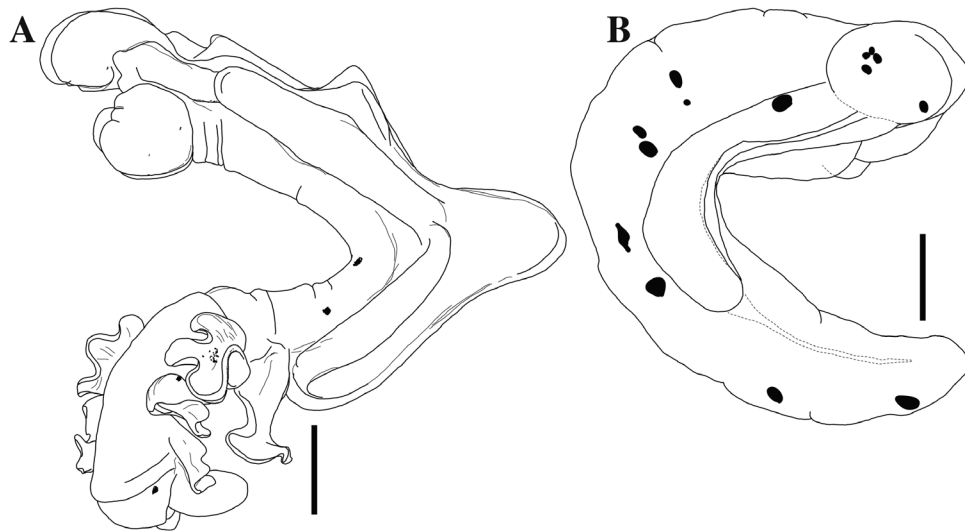
(see Kuris *et al.*, 1980); this also occurred in some males in crabs without female parasites.

Usually only one female entoniscid became mature in any one crab, but occasionally there were other developing females present in the same host. For example, in a 16.8 mm non-ovigerous female crab, two female entoniscids were present. One was larger than the other; its marsupium filled with epicaridium larvae ready to emerge, whereas the other female was distinctly stunted with only small numbers of developed larvae present. The larger parasite had an external connection in the wall of the left branchial chamber. The external opening of the stunted female (if present) was not located, and the parasite was not oriented in the crab's hemocoel in the usual manner. Two males and an immature female were also present in this same crab. This host was not ovigerous and appeared to be castrated. A similar infestation was seen in a

crab from NJ, but there were two mature females of approximately the same size, one of which had released its larvae (Fig. 3).

A 15.5 mm ovigerous female crab, was parasitized by a mature female exhibiting the typical flexed body with the posterior end in contact with the right branchial chamber, through which epicaridium larvae were being released. Two males, one smaller than the other, were inside this female's marsupium. Another relatively large (8 mm long) immature unflexed female, with considerable development of the brood pouches, was also in the hemocoel forward of the mature female, and its posterior was also to the right. In this case, the right side of the host's carapace was distinctly swollen. Another crab harboring only one mature female parasite also exhibited carapace swelling.

In addition to detection of liberated epicaridium larvae, infestation of a crab with a mature female parasite may be detected



**Figure 8.** *Pinnixion sexdecennia* n. gen., n. sp. Immature female, lateral view of right side as it appeared in host *Zaops ostreus* collected in Florida and preserved on 31 July 1967 (A); immature living female entoniscids from hemocoel of *Zaops ostreus* collected in Florida and preserved on 16 August 1965 (B). Scale bars: A = 500  $\mu$ m; B = 100  $\mu$ m.

by carefully lifting the pleon and exposing the transparent, membranous area between the crab's sternum and pleon. The female brood pouches of entoniscids, especially if filled with actively moving epicaridium larvae, may be visible with a dissecting microscope. This maneuver caused damage to the less flexible male pleon. Of thirteen female crabs collected from NC, eight were ovigerous and harbored mature female entoniscids. Seven of the total female entoniscids liberated epicaridium larvae. Female entoniscids in three other crabs were dead and in the process of being encapsulated by the host. Encapsulation was also observed in other life-history stages (i.e., cryptoniscid larvae), undifferentiated stages (very early developing females and males), and males. Many different combinations of life-history stages occurred in individual crabs, e.g., one host had five males, two of which were dead, and five undifferentiated stages; another had one encapsulated mature female, four dead males (one in an encapsulated female), one apparent immature male, and six immature females. Cryptoniscid larvae and molted exoskeletons were also found attached to the gills and mouthparts of infested crabs.

The overall prevalence of *P. sexdecennia* n. gen., n. sp. in 95 fifth-stage females of *Zaops ostreus* from NC was 57.9% (Supplementary material Table S7) and a higher percentage of parasites occurred among the larger individuals (Fig. 9C). Eight crabs were infested with mature female entoniscids (with developing or mature epicaridium larvae) and one crab had two mature female parasites. There was evidence of gonadal atrophy in female hosts infested with mature parasites. The number of entoniscid developmental stages in individual crabs ranged from one to five except for two crabs that harbored 17 and 24 parasites, respectively. Male entoniscids occurred in 17 of the 63 infested crabs, with a maximum of three per crab. Males were usually found with developing or mature females (six of eight of the latter). Males were  $3.23 \pm 0.50$  mm in length ( $N = 4$ ). Parasites were enclosed with a sheath produced by the host except for an opening at the terminal end, which connected to a pore in the branchial wall leading to the branchial cavity. Mature female entoniscids represented 9–25% of the wet weight of hosts (Supplementary material Table S8).

**Florida:** Ninety-seven of 101 specimens of *Pinnixia chaetoptera* (50 males, 47 females) from the Miami area were examined for entoniscid infestations. Crab hosts were smaller than those collected in NJ and NC (males CW 1.9–9.1 mm, females CW 1.7–9.8 mm), and females were significantly larger than males

(see McDermott, 2005). Eight of the 47 female hosts examined ( $8.3 \pm 1.5$  mm CW, 6.9–9.8 mm) were ovigerous.

Infestations were limited mainly to the larger crabs of each sex, i.e., mature crabs larger than 6.0 mm. Overall prevalence of *P. sexdecennia* n. gen., n. sp. in 97 *P. chaetoptera* from FL was 41.2%; 11 of 50 (22.0%) male hosts were infested and 29 of 47 (61.7%) female hosts were infested. Female hosts were found more often to be parasitized than predicted based on expected equal distribution of parasites among female and male hosts ( $\chi^2 = 15.76$ ,  $N = 97$ ,  $P < 0.0001$ ); prevalence in females was nearly three times that in males (Supplementary material Table S9). Mean intensity in female crabs was approximately twice that in males (3.5 versus 1.8, respectively).

Four of 47 female crabs (8.7%) were infested with mature females of *P. sexdecennia* n. gen., n. sp., all of which liberated epicaridium larvae in the laboratory. One of these crabs became ovigerous a second time while in the laboratory between two periods of epicaridium larvae liberation. The brood of this crab developed and first zoeae were eventually liberated. Male entoniscids were the dominant life history stage infesting these crabs. Male crabs did not harbor mature female parasites. One female crab was infested with 15 parasites, seven males (two dead), seven undifferentiated, and a dead cryptoniscid larva. The carapaces were visibly swollen in two of the crabs infested with mature female parasites.

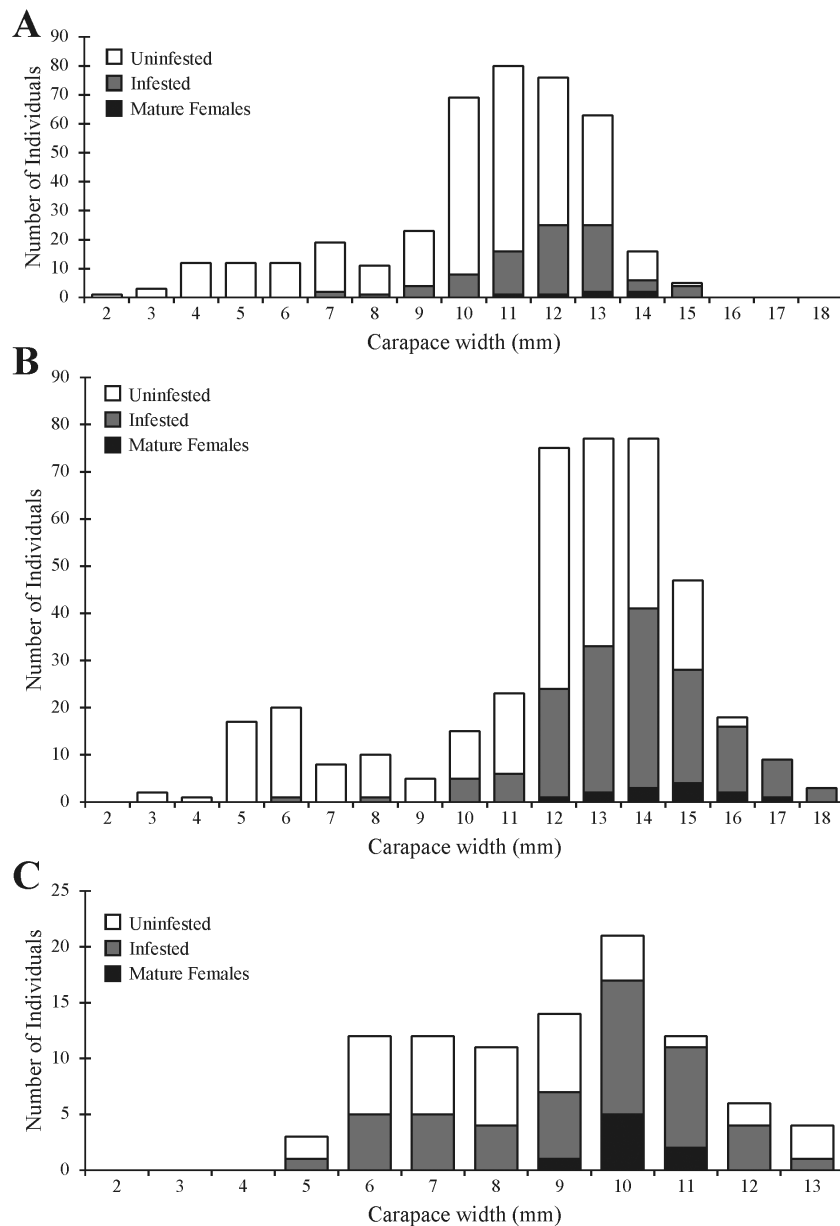
*Pinnixion sexdecennia* n. gen., n. sp. also parasitized *Austinxia gorei* in FL, where overall prevalence in 91 host crabs was 6.6%. One of 39 male hosts (2.6%) was infested and 5 of 52 (9.6%) female hosts were infested; no significant difference was found from expected equal distribution of parasites among female and male hosts was found ( $\chi^2 = 1.8$ ,  $N = 91$ ,  $P < 0.18$ ). One parasitized female crab (8.5 mm CW) was ovigerous.

**Nomenclatural statement:** A life science identifier (LSID) number was obtained for the new species: urn:lsid:zoobank.org:pub:13A66D63-63CB-4F96-A9D8-195DEDD25282.

## DISCUSSION

### Prevalence

*Pinnixion sexdecennia* n. gen., n. sp. ranged in prevalence from ~5–50% across hosts and localities. *Pinnixia chaetoptera* from



**Figure 9.** Prevalence of *Pinnixion sexdecennia* n. gen., n. sp. infestations. Prevalence in male *Pinnixa chaetoptera* examined from North Carolina (1965–1967) (A); prevalence in female *P. chaetoptera* examined from North Carolina (1965–1967) (B); prevalence in fifth-stage female *Zeaps ostreus* examined from North Carolina (1965–1967) (C). Unshaded, uninfested host crabs; grey, host crabs infested with immature entoniscids; black, host crabs infested with mature female entoniscids.

NC and FL exhibited the highest prevalence, with over 40% of crabs infested in some collections. In contrast, *P. chaetoptera* hosts from NJ had only ~5% infestation rate. *Zeaps ostreus* from NC had the highest levels of the parasite (> 57% prevalence), but only fifth-stage female hosts (mature females; see Christensen & McDermott, 1958) were examined, therefore, the prevalence is higher than what would be found across all stages and both sexes of hosts. A total of 6.6% of *Austinia gori* from FL were infested but the sample size was relatively small. It should be noted that the prevalence of *Pinnixion sexdecennia* n. gen., n. sp. in the large collections of *Pinnixa chaetoptera* from NC was based on the presence of all stages of the parasite's development. Typically in past studies, only mature females have been used to quantify prevalence of entoniscids because examination for males and immature stages in the hosts were incomplete.

Female *Pinnixa chaetoptera* from FL, NC, and NJ were more often found parasitized by *Pinnixion sexdecennia* n. gen., n. sp. than male hosts. As in our findings, the pea crab *Pinnotherea pisum* removed from blue mussels *Mytilus edulis* from the Camel Estuary, Cornwall, U.K. were commonly found with the entoniscid *Pinnothereion vermiforme* (overall prevalence 27.7%) and significantly more female crabs harbored the parasites (Atkins, 1933). Kuris *et al.* (1980) studied the entoniscid *Portunium conformis* Muscatine, 1956 and the sympatric grapsoid crab hosts *Hemigrapsus nudus* (Dana, 1951) and *H. oregonensis* (Dana, 1951) (Varunidae) from California, USA to Vancouver, BC, Canada. Kuris *et al.* (1980) examined large numbers of crabs, recorded all developmental stages of parasites, and found prevalences as high and higher than those in our study. Their data, however, are not strictly comparable with ours, because they emphasized collecting mature female crabs, and crabs less than 8.0 mm CW were not examined.

Most other reports do not contain details about the methods of examination for parasites and if stages other than fully mature females were searched for; these studies typically show relatively low prevalence. Examples include Choy (1986), who found 2.4% of 329 *Liocarcinus holsatus* (Fabricius, 1798) from South Wales, U.K. infested with *Priapion fraissiei* Giard & Bonnier, 1888, as well as Shields & Early (1993) who found 13.9% of *Thalamita sima* H. Milne Edwards, 1834, from Queensland, Australia to be infested with *Cancrion australiensis* Shields & Early, 1993. Shields & Ward (1998) reported *Tiarinia* spp. (possibly a mixed species assemblage) on the Great Barrier Reef, Australia being infested with *Tiarinon texopallium* Shields & Ward, 1998 at rate of 3.2%. The entoniscid *Paguritherium alatum* Reinhard, 1945, a parasite of the hermit crab *Pagurus longicarpus* Say, 1817 along the Atlantic coast of USA, showed a prevalence of less than 3.0% in surveys that involved thousands of crabs (Reinhard, 1945; Adkison & Heard, 1978; McDermott, 1998b). Mushtaq et al. (2016) found a prevalence of 4.8% for *Charybdis feriata* (Linnaeus, 1758) infested with *Micippion asymmetricus* Shiino, 1942, from Karachi, Pakistan, and reported no difference in prevalence between male and female hosts. Prevalences of entoniscid species appear to be quite variable and skewed distributions (more often found in female than male hosts) have been reported in some but not all hosts; future studies should address the factors that could account for these differences in experimentally amenable host/parasite systems.

Female crabs often harbor both *P. sexdecennia* **n. gen., n. sp.** and the hoplonemertean *Carcinonemertes pinotheridophila* McDermott & Gibson, 1993 (McDermott, 2005, 2006, 2009). Simultaneous occurrences involved immature parasites of both species, and most were found in immature crabs. Miyashita (1941) reported simultaneous infestations of the entoniscid *Entionella fluviatilis* Miyashita, 1941 and the rhizocephalan *Polyascus gregaria* (Okada & Miyashita, 1935) in the brachyuran *Eriocheir japonica* (De Haan, 1835), and claimed that externa development of the rhizocephalan was inhibited by presence of the entoniscid. Choy (1986) found that all *L. holsatus* parasitized by *Priapion fraissiei* were also parasitized by *Sacculini carcini* Thompson, 1836, and that half of these hosts bore mature rhizocephalan externae.

### Effects of entoniscid infestations on hosts

Entoniscid isopods may adversely affect molting, growth, reproduction, and development of secondary sex characteristics in decapod hosts (carideans, anomurans, brachyurans). Adverse effects are poorly documented in most of the host-parasite relationships studied (Reinhard, 1956; O'Brien & van Wyk, 1985). Castration occurs in females of *Pinnotheres pisum* (Giard & Bonnier, 1889; Atkins, 1933), and secondary sex characters (pleopods) are modified in male hosts (Mercier & Poisson, 1929). Infestation of *Pinnixa chaetoptera* females with mature females of *Pinnixion sexdecennia* **n. gen., n. sp.** does not inhibit breeding, which indicates that castration does not normally occur. This relationship involves iteroparity in both hosts and parasites. Impacts on gonads and secondary sex characters of infested male hosts were not investigated.

Relationships between free-living, non-symbiotic brachyuran hosts and their entoniscid parasites are somewhat better studied than those involving pinnotheroids. *Portunio conformis* castrates female *Hemigrapsus nudus* and *H. oregonensis* and induces feminization of secondary sex characters in males (Kuris et al., 1980). Mature females of an unidentified species of *Portunio* caused castration in infested females of three species of grapsoids and one species of macrophthalmid from New Zealand, but male hosts showed no apparent effects of parasitism (Brockhoff, 2004). Miyashita (1941) found that females of *Eriocheir japonica* were castrated by adult females of *Entionella fluviatilis*, and that the pleon of these hosts exhibited an immature morphology; males were not noticeably affected. Male green crabs, *Carcinus maenas* Linnaeus, 1758,

infested with *Portunio maenadis* (Giard, 1886) in Denmark become feminized as evidence by their swollen carapaces, smaller chelipeds, and broader pleons (Rasmussen, 1973). Two infested females had a hyperfeminized carapace ("extraordinarily strong bulging of the dorsal part...") but neither host sex exhibited any pleonal changes (Rasmussen, 1973: 164). Hartnoll (1960) observed feminization (shorter chelae and broader pleons) in males of the spider crab *Euryome aspera* (Pennant, 1777) infested with *Entionella monensis* Hartnoll, 1960, and Hartnoll (1966) found immature gonads in *Microphrys bicornutus* (Latreille, 1825) and *Stenorhynchus seticornis* (Herbst, 1788) from Jamaica that were parasitized with *Achelion occidentalis* Hartnoll, 1966.

Hermit crab-entoniscid relationships have received little attention except for the work of Reinhard (1945) and Reinhard & Buckeridge (1950), who showed that *Paguritherium alatum* castrated female *Pagurus longicarpus* in Massachusetts, USA and disrupted the normal development of their secondary sex characters (pleopods). Gonads of males atrophied but there was normal pleopod development. The adverse effects in parasitized females were also observed in the same host-parasite relationship in NJ (McDermott, 1998b).

There is generally little information on the effect of entoniscid parasitism on host molting (O'Brien & Van Wyk, 1985). Rasmussen (1973: 164) indicated that ecdysis was "evidently retarded" in *C. maenas* but most other reports suggest it is not affected. Brockhoff (2004) showed that molting is not inhibited by an undescribed species of *Portunio* in at least two species of its varunid hosts. A.M. Kuris (in O'Brien & Van Wyk, 1985) found no adverse effects on molting in *H. nudus* and *H. oregonensis*, hosts of *Portunio conformis*. Infestations with mature *Paguritherium alatum* females do not prevent molting of the hermit crab *Pagurus longicarpus* (McDermott, 1998b). Atkins (1933), however, was uncertain of the effect of *Pinnotherion vermiforme* on molting in *Pinnotheres pisum*. Our findings show that infestation with *Pinnixion sexdecennia* **n. gen., n. sp.** does not interfere with molting and growth in *Pinnixa chaetoptera*, and this is likely also the case for *A. gorei* and *Z. ostreus*, although more data is needed regarding the effects on the latter two species.

### Host response to infestation

Encapsulation of males and immature developmental stages in melanized tissue was observed in *Pinnixa chaetoptera*. Atkins (1933) found dead males of *Pinnotherion vermiforme* in different regions of the hemocoel of *Pinnotheres pisum*; their bodies were usually flexed at the pereonal-pleonal junction and encapsulated within host tissue. Kuris et al. (1980) described a similar response by *H. oregonensis* to infestations with *Portunio conformis*. Dead male and female parasites within the hemocoel of crabs were covered with host-produced, thick, melanized sheaths. Kuris et al. (1980) suggested that mortality of the parasites might be due to some host immune defensive process because melanization essentially mummifies the parasites. Re-infestation of hosts was not prevented by the presence of dead parasites, thus, there appeared to be no acquired immunity. Castrated female hosts recovered their reproductive potential following the death and mummification of mature, reproducing female parasites.

### External manifestations of internal parasitism

Mature females of *Pinnotherion vermiforme* infesting the poorly calcified pea crab *Pinnotheres pisum* can be seen through the crab's semitransparent carapace; the same is true for the female host's gonad (Atkins, 1933). Although this was not possible to observe in the highly calcified *Pinnixa chaetoptera*, a subtle but distinct asymmetric swelling of the carapace was observed in some crabs from NC and FL, which identified them as infested with mature female parasites. Such external manifestations of mature entoniscid

infestations are not unprecedented. Miyashita (1933, 1941) found similar asymmetry in *Eriocheir japonica* infested with *Entionella fluvialilis*. Among 60 individuals parasitized with mature females, 26 had carapaces swollen on the right side, 29 on the left, and 5 with both sides swollen, all of which indicated the presence of mature parasites (Miyashita, 1933, 1941). A slightly swollen left branchial region of the carapace of one specimen of *H. nudus* infested with a mature female of *Portunium conformis* was reported by Shields & Kuris (1985). This condition, however, was due to an unusual positioning of the parasite in the branchial hemocoel. Muscatine (1956) found no external signs of infestation with *P. conformis* in *H. oregonensis*, a species sympatric with *H. nudus*. Rasmussen (1973) observed carapace swelling in male *C. maenas* infested with *Portunium maenadis*. Although we detected carapace swelling in only three specimens of infested *Pinnixa chaetoptera*, this condition may have been overlooked in other infested hosts.

The transparent membranous ventral junction between the sternum and pleon is poorly calcified in females of *Pinnixa chaetoptera*. Brood chambers of mature entoniscids filled with developing embryos or epicaridium larvae may be observed microscopically through this membranous area. Detecting the mature female parasite at the pleonal-sternal junction, as well as detecting emerging larvae from isolated crabs, provides a source of specimens for studies on this host-parasite relationship. This source of larvae should be particularly useful for exploring relations with copepod intermediate hosts, and eventually recovering cryptoniscus larvae for morphological and host specificity studies. The entoniscid *Entoniscoides okadai* Miyashita, 1940 from the brachyuran *Cycloxanthops truncatus* (De Haan, 1837) (Xanthidae), which has direct development from egg to the cryptoniscus larval stage, is a species ideal for experimental studies on host choice (Miyashita, 1940).

#### Host specificity and future studies

*Pinnixion sexdecennia* n. gen., n. sp. parasitizes three pinnotherid species across two subfamilies: Pinnotherelinae (*A. gorei* and *Pinnixa chaetoptera*) and Pinnotherinae (*Z. ostreus*). Although most species of entoniscids are presently known to infest only one species of definitive host, specificity is poorly known in the group and has not been experimentally tested for most species. If more than one definitive host is used, typically the hosts are often from the same family, e.g., *Portunium conformis* parasitizes the varunid crabs *H. nudus* and *H. oregonensis*. Brockerhoff (2004) found that an unidentified species of *Portunium* from New Zealand infested three varunid crabs: *Austrohelice crassa* (Dana, 1851), *Cyclograpsus lavauxi* H. Milne Edwards, 1853, and *Hemigrapsus crenulatus* (H. Milne Edwards, 1837), but not *Hemigrapsus sexdentatus* (H. Milne Edwards, 1837). A.M. Brockerhoff (unpublished data), however, also recorded *Hemiplax hirtipes* Heller, 1865 as host of another entoniscid belonging to *Portunium*. Identification of the *Portunium* sp. (or spp.) parasitizing these hosts is necessary for further clarification of the above relationships. Seven identified species of *Portunium* infest eight species of hosts belonging to five brachyuran families, three of which are closely related (Grapsidae, Plagusidae, and Varunidae), but also in Epialtidae and Portunidae (Boyko et al., 2008 onwards). The majoids *Microphrys bicornutus* and *Stenorhynchus seticornis* are hosts for *Achelion occidentalis* (Hartnoll, 1966). The monotypic *Paguritherium alatum* parasitizes the closely related hermit crabs *Pagurus annulipes* Stimpson 1860 and *P. longicarpus* (Reinhard, 1945; Adkison & Heard, 1978; McDermott, 1998b). In contrast, each of five different host species in Porcellanidae are infested with a different species of *Entoniscus*.

The European pea crab *Pinnotheres pisum* is the only pinnotherid previously known to harbor an entoniscid species, *Pinnotherion vermiforme*; however, Shiino (1942) described another species in this genus (*Pinnotheres setoensis*) and it parasitizes the western Pacific spider crab *Camposcia retusa* (Latreille, 1829) (Majoidea). *Pinnixion*

*sexdecennia* n. gen., n. sp. is thus only the second entoniscid species described from pinnotherid hosts. It is possible that *P. sexdecennia* n. gen., n. sp. as recorded from the three pinnotherid hosts represents a species complex (i.e., they may be more host specific than presently known) but addressing that question would require fresh samples for molecular work. Conspecificity across samples from all three hosts, however, seems likely considering that the morphology of the adults and epicaridium larvae (with their unique sixth pair of pereopods) are indistinguishable across all hosts. The geographic range and host use of *P. sexdecennia* n. gen., n. sp. should be explored in more detail, particularly in *Z. ostreus* and *Pinnixa chaetoptera*, both of which range from Massachusetts to Brazil (McDermott, 2005, 2009). The lower prevalence of the parasite in NJ and lack of reports on this species from northern localities suggests *P. sexdecennia* n. gen., n. sp. may occupy a subset of its hosts' ranges, a phenomenon seen in many other species of parasites (Pielou, 1974), but further sampling is needed. In addition to these ecological studies, entoniscids are in need of much taxonomic work as many species remain to be described (Adkison, 1990; JDW and CBB pers. obs.).

#### SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Crustacean Biology* online.

S1 Table. Names used for species described or subsequently placed in *Entione* Kossmann, 1881.

S2 Supplementary material paratype list.

S3 Table. Mean carapace widths of *Pinnixa chaetoptera* examined for *Pinnixion sexdecennia* n. gen., n. sp. in NC.

S4 Table. Prevalence of *Pinnixion sexdecennia* n. gen., n. sp. in NC.

S5 Table. Intensities of *Pinnixion sexdecennia* n. gen., n. sp. in NC.

S6 Table. Number and mean intensity of developmental stages of *Pinnixion sexdecennia* n. gen., n. sp. in NC.

S7 Table. Prevalence of *Pinnixion sexdecennia* n. gen., n. sp. in fifth-stage females of *Zoops ostreus* in NC.

S8 Table. Damp weights of *Pinnixion sexdecennia* n. gen., n. sp. in NC.

S9 Table. Prevalence of *Pinnixion sexdecennia* n. gen., n. sp. in Miami, FL.

#### ACKNOWLEDGEMENTS

We would like to thank the efforts of associate editor Niel Bruce (Queensland Museum), the anonymous reviewers, and the Editor-in-Chief for their reviews of the manuscript. JJM appreciated the help of many individuals during several years of field studies along the Atlantic coast, H.H. Haskin (Rutgers University), C.G. Bookhout, J.D. Costlow, and F.J. Vernberg (Duke University), and A.A. Myrberg (University of Miami) for research facilities; W.R. Belzer, J.A. Cole, J.J. McDermott III, T.M. McDermott, M.H. Taylor, and F.R. Weis for assistance in the field; I.E. Gray (Duke University) for sharing unpublished data; K.R. Boas, B.K. Davis, J. Kaufman, E.V. Lonsdorf, K. Miller, L. Norbeck, J.T. Thompson, and S. Yu (Franklin and Marshall College) for computer, statistical, and microscopic assistance. Thanks to A. Simms and Z. Haque (Hofstra University) for help in SEM work on male entoniscids. Much of this research was partially supported by funds to JJM from National Science Foundation grants (G11448, G22068 and B959) and post-doctoral research grants from Duke University Marine Laboratory and Franklin and Marshall College. This research was also supported, in part, by a grant to JDW from the National Science Foundation (DBI-1337525).

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