2007

New nemertean worms (Carcinonemertidae) on bythograeid crabs (Decapoda : Brachyura) from pacific hydrothermal vent sites

Jeffrey D. Shields
Virginia Institute of Marine Science

M Segonzac

JOURNAL OF CRUSTACEAN BIOLOGY, 27(4): 681–692, 2007

NEW NEMERTEAN WORMS (CARCINONEMERTIDAE) ON BYTHOGRAEID CRABS (DECAPODA: BRACHYURA) FROM PACIFIC HYDROTHERMAL VENT SITES

Jeffrey D. Shields and Michel Segonzac

(JDS) Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, Virginia 23062, U.S.A. (jeff@vims.edu);
(MS) Ifremer, Centre de Brest, Laboratoire Environnement profond-Centob, BP 70, 29280 Plouzané, France (segonzac@ifremer.fr);
(corresponding author (JDS): jeff@vims.edu, tel: (804) 684-7128, fax: (804) 684-7186)

A B S T R A C T

Several species of crabs from hydrothermal vent sites in the Pacific Ocean were found to be infested by small, symbiotic nemertean worms. Worms occurred on both male and female crabs, and were located in mucous sheaths adhering to the axillae between the limbs of males and females, the setae of the pleopods of females, and the sterna of infested male and female crabs. Only juvenile and regressed adult worms were observed, primarily because no ovigerous hosts were examined. Similar species of worms mature by eating eggs, then regress or die after host eclosion. Based on the size of the worms from the vent crabs, their habitus with their crustacean hosts, the presence of accessory stylet pouches, and the presence of a single stylet on a large basis (monostiliferous), we place the worms in the family Carcinonemertidae, within the genus *Ovicides*. Infestations were found on crabs from vent sites on the western Pacific back-arc basins, on the southern East Pacific Ridge, and on the Pacific-Antarctic Ridge, indicating a widespread distribution of the symbioses. This represents the first record of Carcinonemertidae from a deep-sea host, a new host family, Bythograeidae, for these symbionts, as well as the first record of parasitism on a deep-sea bythograeid crab.

Introduction

Nemerteans are members of a phylum of worms characterized by the presence of a rhynchocoelom, a body cavity that houses an eversible proboscis. Nemerteans are important, but often overlooked, predators that reside in sand and mud benthos. Free-living nemerteans have been reported from deep-sea pelagic habitats (Roe and Norenburg, 1999) as well as from several hydrothermal vent sites: North Pacific, Juan de Fuca (Rogers et al., 1996; Tunnicliffe et al., 1997), East Pacific Rise, 9°N (Bright, 2006), EPR-13°N and 17°S (M.S. personal observations) where they presumably prey upon a variety of invertebrates. Until now symbiotic nemerteans were unknown from deep sea fauna. However, a few genera of nemerteans are known symbionts, living in the mantle cavity of shallow-water bivalves, i.e., *Malacobdella* spp., on the eggs of crabs and lobsters, i.e., Carcinonemertidae, or in other rare associations, i.e., *Nemertoscolex parasiticus* Greeff, 1879, in the coelomic fluid of an echiuran (Berg and Gibson, 1996). During an investigation of bythograeid crabs from hydrothermal vent sites, one of us (M.S.) noted the presence of pink worms adhering to the axillae between the limbs of males and females, and on the pleopods and pleopodal setae of female crabs. These worms were different from the nematode *Chomadorita* sp. reported by Ramirez-Llodra and Segonzac (2006) on the eggs of *Alvinocaris muricola* Williams, 1988, in that they were nemerteans. Members of the family Carcinonemertidae Sumner, Osburn and Cole, 1913, are parasitic egg predators that live on shallow-water decapods where they feed upon the eggs of their crustacean hosts. Carcinonemertids are often overlooked because they frequently occur at low prevalences in host populations (see Wickham, 1986, for epidemic outbreaks), they live in cryptic locations on their hosts (limb axillae, sternum, pleopods, or gills), and they typically mature on or inhabit ovigerous hosts and, thus, have seasonal cycles in abundance that are often overlooked (Shields, 1993).

To date, only 15 species of Carcinonemertidae have been described, with 14 in the genus *Carcinonemertes* (Kölliker, 1845) and one in the genus *Ovicides* Shields, 2001. Most carcinonemertids occur on cancrid, portunid and xanthid crabs, but they are known to infest at least 58 species of crabs in 13 families and two species of palinurid lobsters (Humes, 1942; Wickham and Kuris, 1985; Campbell et al., 1989; Santos et al., 2006). Members of the family vary in their host specificity, with some found only on one host genus or species, e.g., *Ovicides juliaea* Shields, 2001; *C. errans*, Wickham, 1978, respectively; and others having general preferences, e.g., *C. epialti* Coe, 1902; *C. carcinophila* (Kölliker, 1845), reviewed in Kuris and Wickham (1987).

We describe three new species in the family Carcinonemertidae. The worms are clearly monostiliferous Hoplonemertea with typical carcinonemertid characters, and they share features with *Ovicides juliaea* that place them in the genus *Ovicides*. These worms represent the first record of Carcinonemertidae from a deep-sea host, a new host family for the nemerteans, as well as the first record of symbiosis or parasitism on several species of deep-sea hydrothermal Bythograeidae Williams, 1980. Some aspects of the ecology and biogeography of the symbionts are also presented.

Materials and Methods

The crab hosts, *Austinograea alayseae* Guinot, 1989; *Austinograea williamsi* Hessler and Martin, 1989; *Bythograea vrijenhoeki* Guinot and Hurtado, 2003; *B. laubieri* Guinot and Segonzac, 1997; and *Cyanagraea praedator* de Saint Laurent, 1984, were collected with baited traps or directly by automated grab using deep sea submersibles. Collections were

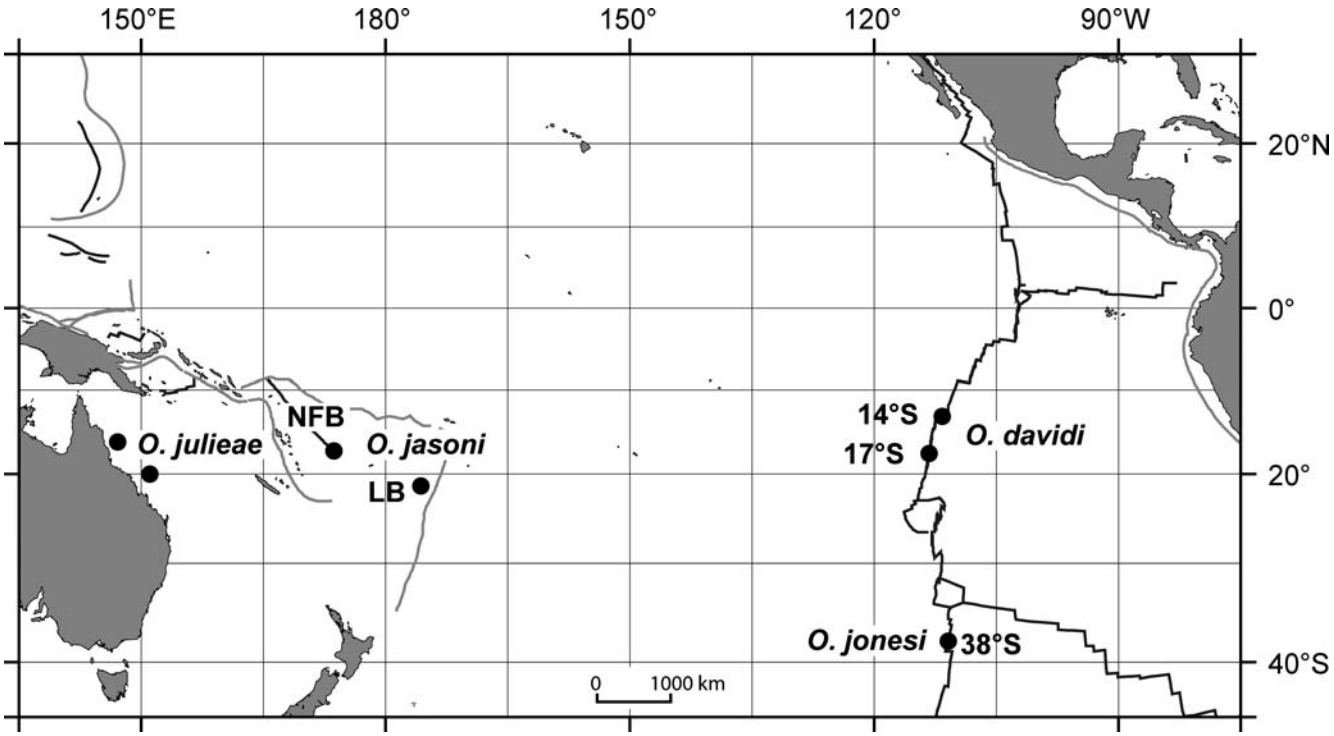

Fig. 1. Location of vent sites from which *Ovicides* spp. were found on bythograeid hosts. Biospeedo cruise (South East Pacific Rigde, 14°S and 17°S), PAR5 (South East Pacific Ridge: 31° and 32°S, and Pacific-Antarctic Ridge: 38°S), and TUIM06MV (NFB, North Fiji Basin; and LB, Lau Basin) sites. The type localities of *O. julieae* are included (Lizard and Heron Islands, Australia) for comparison.

undertaken during the French Biospeedo cruise (Chief scientist D. Jollivet, Roscoff, France) on the South East Pacific Rise, April, 2004, using the D/S *Nautile* supported by the R/V *L'Atalante*, and during two American cruises (Chief scientist R. Vrijenhoek, MBARI, USA): PAR 5 (Pacific-Antarctic Ridge, April, 2005), and TUIM06MV (N-Fiji and Lau Back-Arc Basins, May, 2005), using the D/S *Alvin* supported by the R/V *Atlantis*, and the ROV *Jason 2*, supported by the R/V *Melville*, respectively (Fig. 1). Crabs were examined immediately, or fixed entirely in 10% formalin, or frozen for later examination and genetic analysis. The carapace width (CW) and sex were recorded for infested *Cyanagraea praedator*, but not for the other hosts. Crabs were examined externally with a stereomicroscope for nemerteans (Fig. 2), then carefully washed under a light stream of water over a 25 μm sieve, which was furthered examined for worms. Worms were fixed in 10% formalin for histological analysis. Worms selected for histology were placed in micro-cassettes (Electron Microscopy Sciences, #62327-10), dehydrated in alcohol, embedded in paraffin and sectioned at 5-6 μm. This method was not suitable for the smallest specimens, which were embedded in 2% agar before being placed in cassettes. Measurements were made with an ocular micrometer on formalin-fixed and histologically-sectioned worms. All measurements are in micrometers unless otherwise stated. Where possible, means are given with the range in parentheses.

Collection Details

Host Crab *Austinograea alayseae* (American Cruise TUIM06MV).—No worms were found on *A. williamsi*.

Dive #142: 19 May 2005, Lau Basin, Cam Tow vent site, 20°19.07′S, 176°08.24′W, 2719 m, baited trap, 10 crabs examined.

Dive #143: 20 May 2005, Lau Basin, Tui Malila vent site, 21°59.34′S, 176°34.09′W, 1891 m, 5 crabs examined.

Dive #144: 21 May 2005, Lau Basin, Tui Malila vent site, 21°59.34′S, 176°34.09′W, 1891 m, 10 crabs examined.

Dive #151: 30 May 2005, North Fiji Basin, White Lady vent site, 16°59.45′S, 173°54.90′W, 1990 m, 7 crabs examined.

Dive #152: 31 May 2005, North Fiji Basin, White Lady vent site, 16°59.44′S, 173°54.90′W, 1990 m.

Host Crab *Cyanagraea praedator* (French Cruise Biospeedo).—PL 1588, Nasse F1, 29 April 2004, SEPR, Hobbs vent site, 17°35.20′S, 113°14.76′W, 2595 m. 1 male (CW = 92.5 mm); 1 female (CW = 106 mm).

PL 1592, Nasse F2, 4 May 2004, SEPR, Pagodes vent site, 13°58.96′S, 112°28.16′W, 2650 m. 1 female (CW = 95 mm).

Host Crabs *Bythograea vrijenhoeki* and *B. laubieri* (American Cruise PAR 5).—Dive #4089: 23 March 2005, PAR-38°S, Sebastian's Steamer vent site, 37°47.28′S, 110°54.51′W, 2204 m. Worms were separately obtained from each host species.

Dive #4093: 28 March 2005, EPR-32°S, Saguaro vent site, 31°51.88′S, 112°02.69′W, 2334 m; #4094: 29 March 2005, EPR-31°S, Fred's Fortress vent site, 31°09.26′S, 111°55.54′W, 2333 m.

Unfortunately the different host species were not identified prior to collection of the worms from these crabs, and several of the worms were lost in histological processing. They were identified as carcinonemertids, due to their habitus on the host and presence of a stylet, but they were not examined histologically. An ampharetid polychaete, *Amphisamytha galapagensis*, was also found in great number on these hosts.

Systematics

Ovicides jasoni new species
Fig. 3

Material.—Juveniles or regressed adults with observations from 10 fixed and sectioned specimens from *Austinograea alayseae* from dives #142, 143, 144, 151 and 152. Worms 1-3 mm long by 160-170 μm wide; found in mucous sheaths adhering to host crabs. Ocelli absent. Proboscis apparatus lateral to foregut. Anterior proboscis chamber pyriform, 15 (15-17) μm long × 15 μm in width at base. Basis robust, intensely eosinophilic, 25 (23-25) μm long by 8 (7-9) μm wide. Single dagger-like stylet on basis, 12 (10-13) μm long, with hub 4 μm wide. Stylet to basis ratio, 0.480 (0.480-

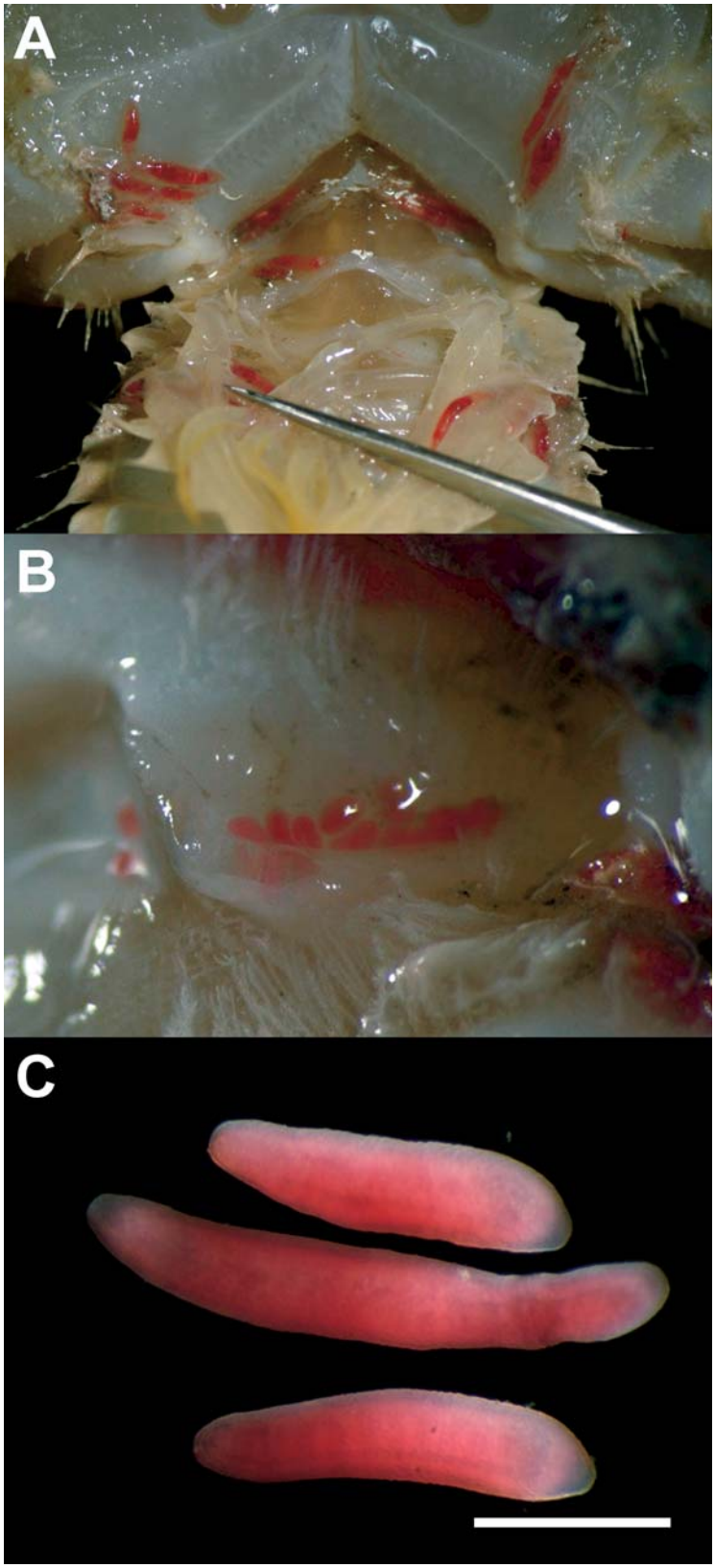

Fig. 2. A, Juvenile worms in situ on the sternum, pleon, and pleopods of *Austinograea alayseae*, Lau Basin, Tui Malila vent site, 21 May 2005, Dive #144. B, Worms ensheathed on the axilla of *A. alayaseae*. C, *Ovicides jasoni* from *A. alayseae*, formalin preserved specimens. The longest specimen is 3.2 mm in length, bar scale = 1.0 mm.

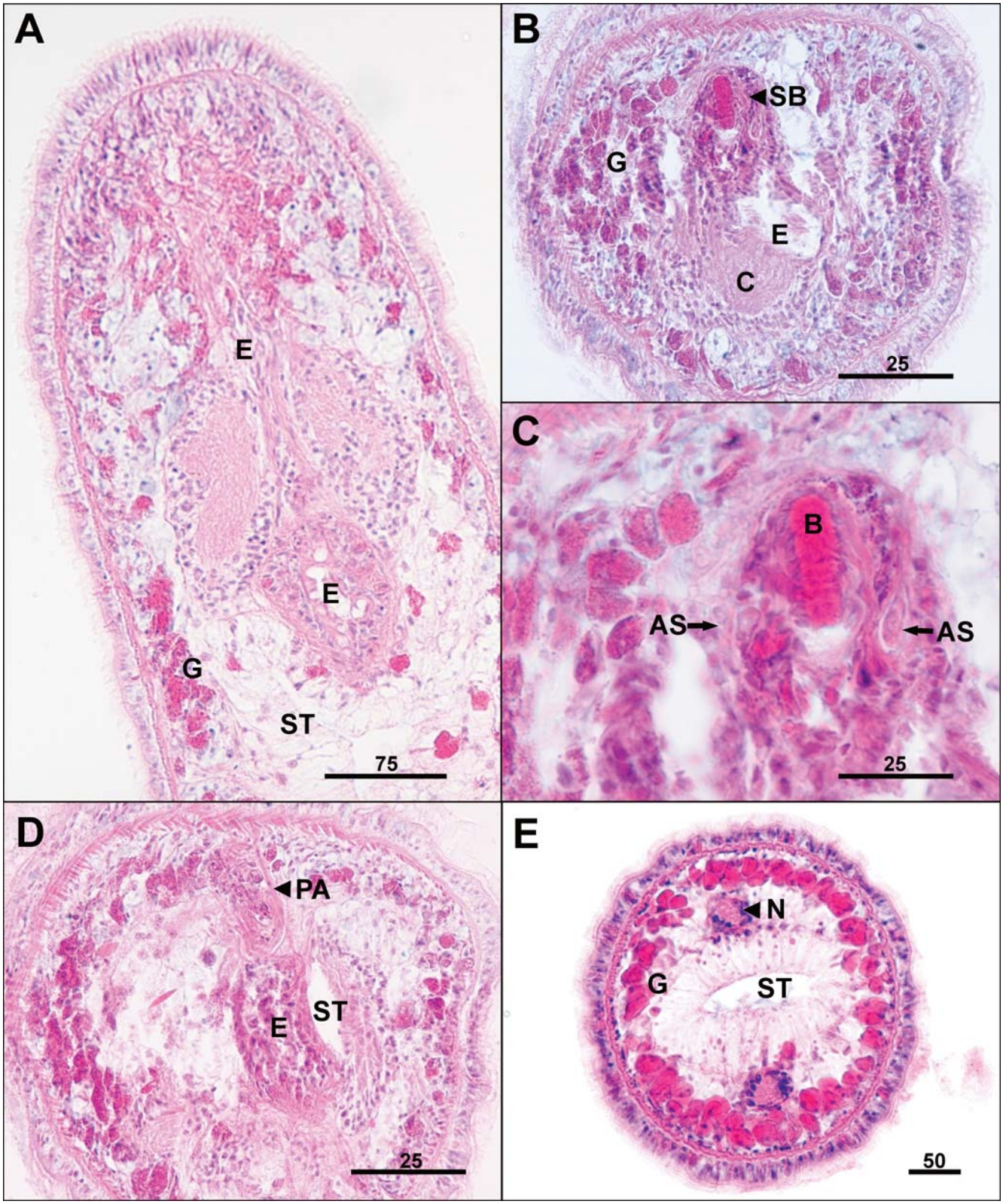

Fig. 3. Sections through *Ovicides jasoni* from *Austinograea alayseae*. A, Holotype: worm #144, frontal section. B, Paratype, worm #142, transverse section. C, Detail of stylet bulb region of holotype with the two accessory stylet pouches (AS) adjacent to the stylet bulb containing the basis. D, Paratype, worm #142, transverse section anterior to B, showing arrangement of proboscis armature and esophagus. E, Paratype, worm #151, transverse section with single row or band of submuscular glands in circumference around the worm.

Legend: AP = accessory stylet pouch, AS = accessory stylets, B = basis, BV = anterior loop of primary blood vessel, C = cerebrum, CG = cephalic glands, E = esophagus, EG = eosinophilic glands, F = frontal organ, G = submuscular glands, GL = glial cells of the cerebrum or lateral nerve chord, MPC = middle proboscis chamber, N = lateral nerve, O = ocellus, Ov = presumptive ovum, S = stylet, SG = stylet bulb, SH = sheath, ST = stomach. Numbers on scale bars are in microns.

Table 1. Morphological measurements (in microns) of the proboscis armature of species within Carcinonemertidae. SB = stylet bulb. * From Shields et al., 1989, ** From Shields and Kuris, 1990.

Species	Basis	Stylet	Stylet:basis ratio	Anterior proboscis chamber	Posterior proboscis chamber
Carcinonemertes					
C. australiensis Campbell et al., 1989	40	15-18	0.375-0.450	75	90 × 45
C. caissarum Santos et al., 2006	22	8	0.378	—	70 × 59
	20-25	5-10	0.250-0.500		
C. c. carcinophila (von Kollicker, 1845)	25	9.0	0.360	—	63 × 48
C. c. imminuta Humes, 1942	21	7.3	0.348	40-45	139 × 47
C. coei Humes, 1942	22.7	8.7	0.383	> 32	78 × 47
C. divae Santos et al., 2006	25	10	0.387	75 × 12	57 × 51
	22-30	8-12	0.250-0.500		
C. epialti Coe, 1902	30	13.5	0.450-0.465	61-66	63 × 41*
	31.2*	14.5*			
C. errans Wickham, 1978	35.2	11.0	0.313	> 46	100 × 50
C. humesi Gibson and Jones, 1990	30-32	7-8	0.219-0.267	30-35	
C. mitsukuri Takakura, 1910	27	8.0	0.296	30	86 × 28**
C. pinnotheridophila McDermott and Gibson, 1993	17.7	6.9	0.390	20 × 25	70 × 35
	14.3-20.5	5.5-8.0	0.313-0.538	SB 25 × 30	
C. regicides Shields et al., 1989	40.5	17.2	0.425	76	82 × 62
C. sebastianensis Santos et al., 2006	22	9	0.416	—	—
	20-25	8-10	0.375-0.444		
C. wickhami Shields and Kuris, 1990	40	20	0.500	> 79	> 125 × > 42
Ovicides					
Ovicides davidi	32	7-9	0.313-0.323	15	43-50 × 20
	30-32			SB 29 × 16-32	
Ovicides jasoni	25	11-12	0.480-0.520	15-17	
Ovicides jonesi	27	9-10	0.333-0.370	12	36-50 × 17-20
				SB 45 × 20	
Ovicides julieae Shields, 2001	20	13	0.650	14	25-33
	15-25	6-13	0.400-0.667	SB 40 × 32	

0.520). One stylet (9-12 μm) developing within each of two accessory stylet pouches anterolateral to stylet bulb. Accessory stylet pouches granular, eosinophilic. Middle proboscis chamber circular, 10-13 μm in diameter, granular or layered in appearance. Posterior proboscis chamber intensely eosinophilic, granular, 25 (20-26) μm long by 15-17 μm wide. Proboscis sheath weakly developed. Body musculature weakly developed with one band of circular muscles, one band of longitudinal muscles. Submuscular glands numerous, eosinophilic, 8-12 μm in diameter; as a field from anterior to near anus, fewer in number anterior to cerebrum. In cross section, submuscular glands arrayed as a single row around the body. Cephalic glands absent or reduced. Posterior nerve not observed at level of cloaca. Gonads undeveloped.

Type Host and Site of Infestation.—On the sterna, pleopods and axillae of *Austinograea alaysaea*.

Type Locality.—Dive #144: 21 May 2005, Lau Basin, Tui Malila vent site, 21°59.34′S, 176°34.09′W, 1891 m. Additional collections noted in Methods: Dives #142, 143, 151, 152.

Holotype.—Juvenile (Accession number USNM 1097948) on slide series 144 (dive site), worm #1 slides 11 through 13; deposited in the National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA. Other worms on slides 6 through 13 are paratypes (USNM 1097949).

Paratypes.—Juveniles on slide series 142, slides 4 through 6 (Accession number MNHN-NMRT 3) and a through c (MNHN-NMRT 4), and 152, slides 1 through 4 (MNHN-NMRT 5), Muséum National d'Histoire Naturelle, Paris.

Etymology.—The species is named after Jason Daniel Shields for his help with the French and English translation between the co-authors.

Remarks.—We place these worms in Carcinonemertidae, genus *Ovicides*, on the basis of their small size, their habitus on a crustacean host, the small relative size of the proboscis armature with the large stylet:basis ratio, and the presence of accessory stylet pouches. *Ovicides jasoni* possesses distinct carcinonemertid characters: reduced proboscis, short, poorly developed rhynchocoel, large numbers of submuscular glands, the absence or reduction of cephalic glands and the lack of a mid-dorsal vessel; all of these are features of the family (Shields et al., 1989; Gibson and Jones, 1990). Only one genus in Carcinonemertidae, *Ovicides juliaea*, is known to have accessory stylet pouches as an adult (Shields, 2001); therefore the new species fits within the genus *Ovicides*. Stylet pouches have been reported from at least one undescribed form from Alaska, which is presumably a species of *Carcinonemertes* (Wickham and Kuris, 1988), but at present, none of the described species of *Carcinonemertes* have an accessory stylet pouch. *Pseudocarcinonemertes homari* Fleming and Gibson, 1981 possesses two accessory stylet pouches, but it is probably a member of Tetrastemmatidae, and not a member of Carcinonemertidae

(Uhazy et al., 1985). *Ovicides jasoni* is distinct from *O. juliaea* on the basis of the smaller stylet:basis ratio (Table 1), the smaller anterior proboscis chamber, the presence of the single row of submuscular glands in cross section, and the lack of ocelli. The arrangement of the submuscular glands may not be a good character for the separation of the species because the appearance may vary depending upon the angle of the histological section. Nonetheless, we have included it as a possible character in the genus.

Ovicides davidi new species
Fig. 4

Material.—Juveniles or regressed adults with observations from 36 fixed and sectioned specimens from the bythograeid crab *Cyanagraea praedator* collected on the Biospeedo cruise at dive PL 1592, South EPR-14°S. Worms small, 1-10 mm long by 170-250 μm wide; found in mucous sheaths attached to host. Two cup-shaped ocelli dorsal, at anterior end. Proboscis apparatus ventral to cerebral ganglion. Anterior proboscis chamber pyriform, 15-18 μm long. Basis robust, 30-32 μm long by 8-10 μm wide. Single stylet on basis, 7-9 μm long. Stylet to basis ratio, 0.313-0.323. Two accessory stylet pouches anterolateral to stylet bulb, with one developing stylet (7-9 μm) in each. Middle proboscis chamber 20-23 μm in diameter, glandular in appearance. Posterior proboscis chamber glandular, 43-50 μm long by 18-25 μm wide. Proboscis sheath greatly reduced. Body musculature weakly developed with one band of circular muscles, one band of longitudinal muscles. Musculature with crossed myofibrils anterior to ocelli. Submuscular glands distinct, eosinophilic, 14 (11-19) μm long by 8 (8-9) μm wide; numerous; as a field from ocelli to near anus, thinning in number anterior to ocelli. In cross section, submuscular glands arrayed in three interspersed rows around the body, interior to muscles. Cephalic glands present; as a diffuse field, anterior to cephalic ganglia, dorsal to esophagus; comprised of enlarged, weakly basophilic submuscular cells, leading to a frontal organ. Cephalic glands sometimes possessing dense, darkly basophilic granules. Posterior nerve in one specimen ventral to cloaca. Gonads undeveloped.

Type Host and Site of Infestation.—On the sterna, pleopods and axillae of the pereiopods of *Cyanagraea praedator*.

Type Locality.—Dive PL 1592, Nasse F2, 4 May 2004, SEPR, Pagodes vent site, 13°58.96′S, 112°28.16W, 2650 m. Other localities: Dive PL 1588 (SEPR-17°S).

Holotype.—Juvenile on slide series PL 1592-F2-96 (worm 17, slides 1 through 5, Accession number: MNHN-NMRT 1, with other paratypes) deposited in the Muséum National d'Histoire Naturelle, Paris.

Paratypes.—Juveniles (Accession number USNM 1097950) on slide series PL 1592-F2-185, slides 1 through 5, in the National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA.

Etymology.—The species is named after David Parker Shields for his help in collecting and dissecting crustaceans on numerous field trips.

Remarks.—We place the worms from *C. praedator* into the genus *Ovicides* based on their habitus on their crustacean hosts, the small relative size of the proboscis armature, and the presence of the accessory stylet pouches. *Ovicides davidi* is distinct from other members of the genus by the presence of the cephalic glands and frontal organ, a different arrangement of the submuscular glands (three interspersed rows in cross section versus one row for *O. jasoni*), and by its smaller stylet:basis ratio (Table 1). *Ovicides davidi* also has a relatively larger posterior proboscis chamber compared to *O. jasoni* and *O. julieae*. As with other members of the genus, it is distinct from the genus *Carcinonemertes* due to the presence of the accessory stylet pouches. The frontal organ in *O. davidi* is unusual. Frontal organs are rare in Carcinonemertidae; only one other species, *Carcinonemertes australiensis* Campbell, Gibson and Evans, 1989, is known to possess one, and in that species it is well organized and extends to the level of the cerebral commissure (see also *O. jonesi* below). In *O. davidi*, the cephalic glands are not well organized and contain large basophilic granules, but also extend to the level of the cerebrum.

Ovicides jonesi new species
Figs. 5 and 6

Material.—Juveniles or regressed adults with observations on two fixed and sectioned specimens from *Bythograea vrijenhoeki* collected on the American cruise PAR 5-38°S, Dive 4089. On slide series 4089 Bv and series Carcino Bv (1). Worms small, 450-500 μm; found in mucous sheath on host. Ocelli not observed. Anterior proboscis chamber pyriform, 15 μm long. Basis eosinophilic, robust, oblique section, 9 μm wide. Single stylet on basis, at least 10 μm long, with basal hub of 5 μm. Stylet to basis ratio not calculated. Two accessory stylet pouches anterolateral to stylet bulb. Anterior proboscis chamber not measured. Middle proboscis chamber 15 μm in diameter, glandular in appearance. Posterior proboscis chamber glandular, 36-45 μm long by 17 μm wide, intensely basophilic, with weak fibrous coat. Proboscis sheath greatly reduced. Body musculature with one layer of outer circular muscles, one layer of inner longitudinal muscles. Submuscular glands eosinophilic; elongate, slender, 10-20 μm long by 4-5 μm wide; numerous. In section, submuscular glands as a single row around the body, not arrayed as in *O. davidi*; interior to muscles. Frontal glands present in esophageal region anterior to cerebrum; eosinophilic, as a diffuse field around esophagus. Gonads undeveloped.

Material.—Juveniles and regressed adults with observations on nine fixed and sectioned specimens from *Bythograea laubieri* collected on the American cruise PAR 5, Dive 4089. On slide series Carcino B.l. (1) (Accession number USNM 1097951). Worms small, 500-1000 μm long by 160-180 μm wide; found in mucous sheath on host. Ocelli absent. Basis eosinophilic, robust, 27 μm long by 8-10 μm wide. Single stylet on basis, 9-10 μm long, with basal hub of 3-5 μm wide. Stylet to basis ratio 0.333-0.370. Two accessory stylet pouches anterolateral to stylet bulb, 15 μm long by 11 μm wide, with developing stylets. Stylet bulb 45 μm long by 20 μm wide. Anterior proboscis chamber

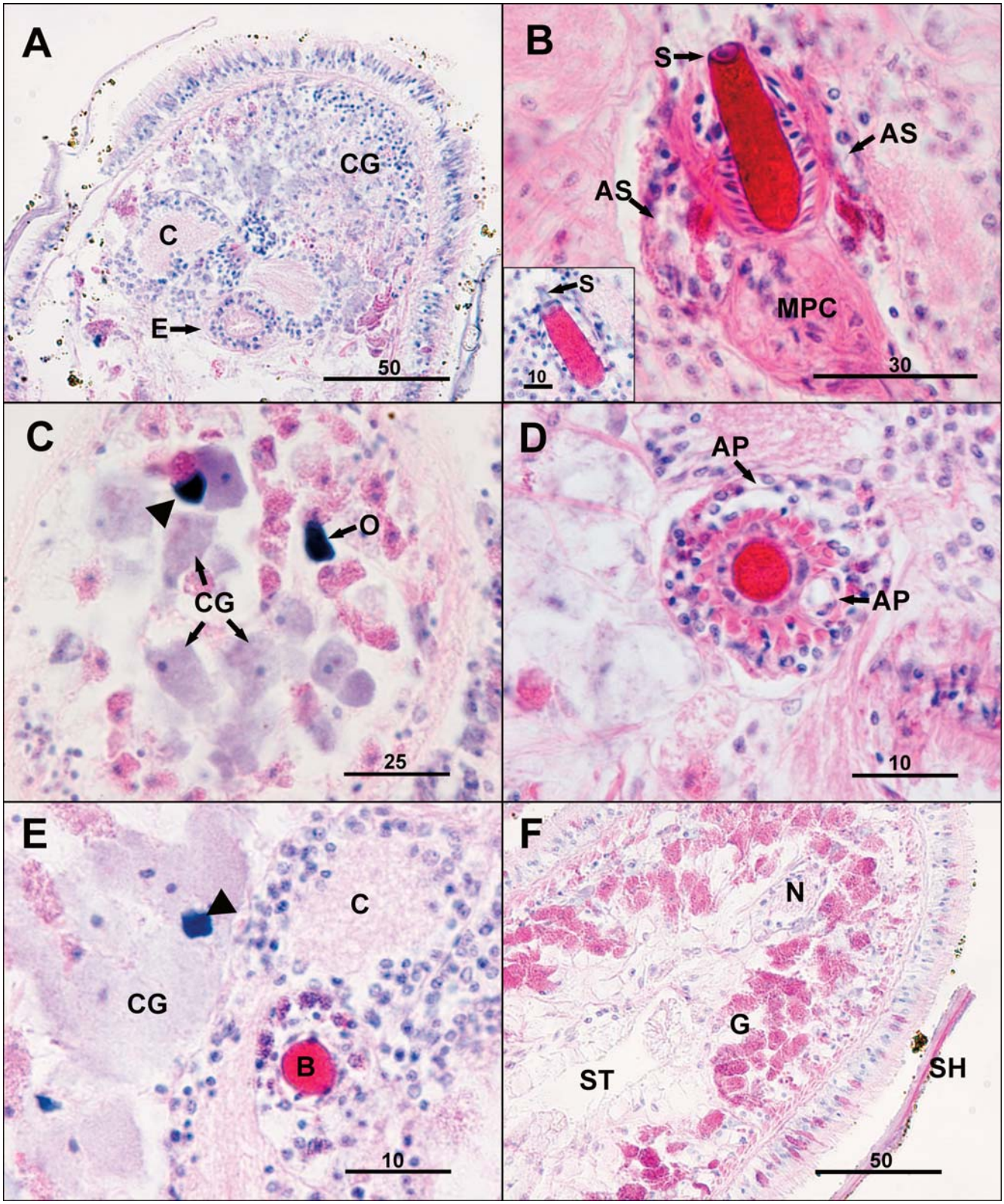

Fig. 4. Sections through *Ovicides davidi* from *Cyanagraea praedator*. A, Holotype (worm 1592-96-slide 2 - worm 17) with weakly developed cephalic glands (CG) anterior to the cerebrum; frontal section. B, Paratype (worm 1592-182-slide 1-worm 1) with eosinophilic basis, two accessory stylets (AS) and armed stylet possessing a hub (S); inset showing dagger-like stylet (1592-182 slide 1 worm 17). C, Paratype (worm 1592-182 worm 17) with weakly basophilic cephalic glands (CG) in the region of an ocellus. Note the presence of the darkly basophilic granule associated with the cephalic gland. D, Transverse section through the stylet bulb of paratype (worm 1592-185 worm 4b). Note the presence of the two accessory stylet pouches (AP) lateral to the basis. E, Paratype (worm 1592-182 worm 1b) with large, weakly basophilic cephalic glands (CG) with darkly basophilic granule (arrow) anterior to cerebrum. F, Slightly oblique transverse section through paratype (1592-185 worm 1 slide 2) showing the arrangement of the submuscular glands in interspersed rows around the circumference of the worm. Legend: AP = accessory stylet pouch, AS = accessory stylets, B = basis, BV = anterior loop of primary blood vessel, C = cerebrum, CG = cephalic glands, E = esophagus, EG = eosinophilic glands, F = frontal organ, G = submuscular glands, GL = glial cells of the cerebrum or lateral nerve chord, MPC = middle proboscis chamber, N = lateral nerve, O = ocellus, Ov = presumptive ovum, S = stylet, SG = stylet bulb, SH = sheath, ST = stomach. Numbers on scale bars are in microns.

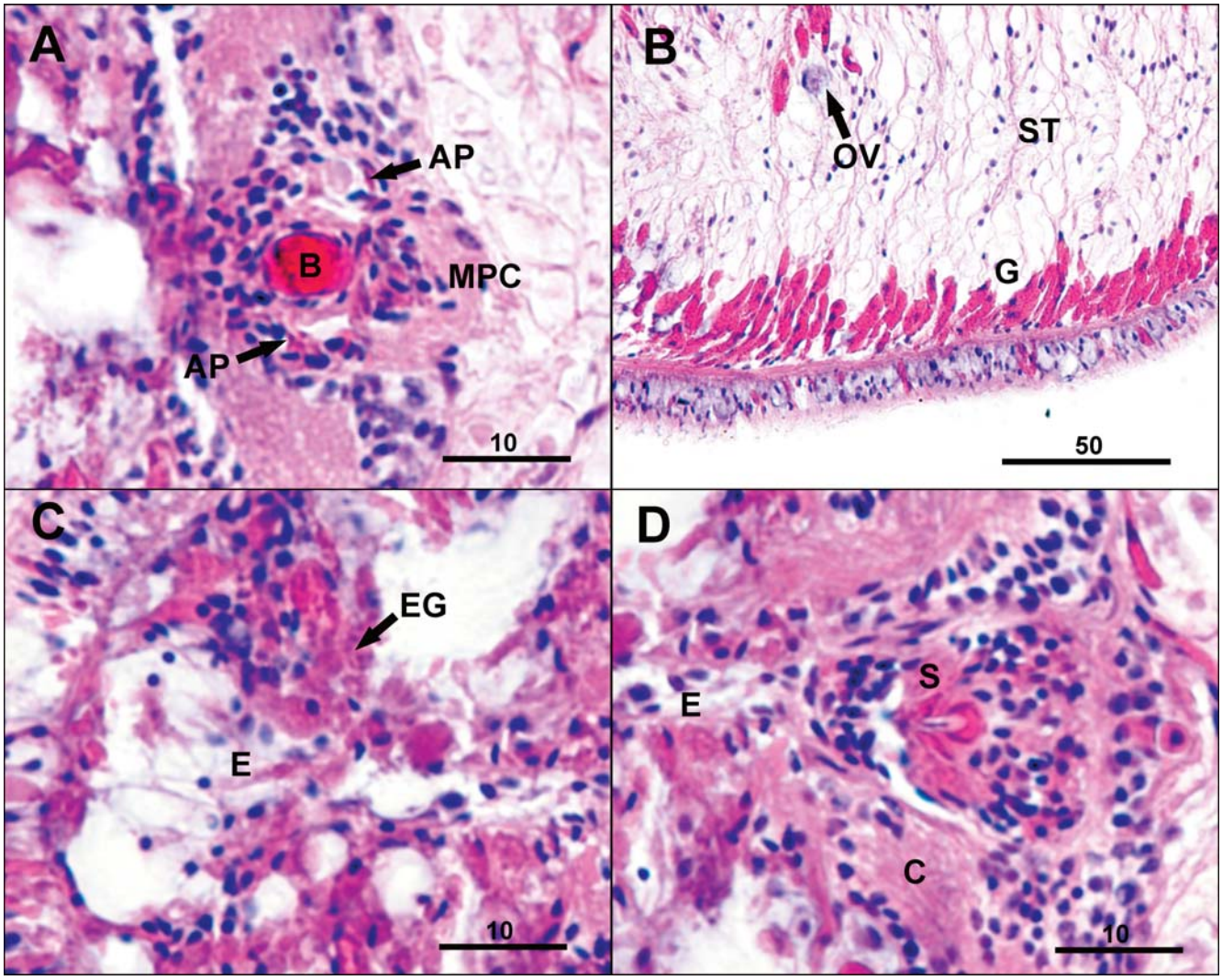

Fig. 5. Sections through *Ovicides jonesi* from *Bythograea vrijenhoeki*. A, Stylet bulb region of worm showing two accessory stylet pouches (AP) adjacent to the basis (B). B, Medial section of body with single row of submuscular glands (G) and a presumptive ovum undergoing resorption (Ov). C, Eosinophilic glands (EG) lining the anterior esophagus. D, Portion of the stylet in the anterior proboscis chamber showing the stylet hub, a portion of the esophagus (E). Legend: AP = accessory stylet pouch, AS = accessory stylets, B = basis, BV = anterior loop of primary blood vessel, C = cerebrum, CG = cephalic glands, E = esophagus, EG = eosinophilic glands, F = frontal organ, G = submuscular glands, GL = glial cells of the cerebrum or lateral nerve chord, MPC = middle proboscis chamber, N = lateral nerve, O = ocellus, Ov = presumptive ovum, S = stylet, SG = stylet bulb, SH = sheath, ST = stomach. Numbers on scale bars are in microns.

pyriform, 12 μm long. Middle proboscis chamber 18-25 μm in diameter, weakly eosinophilic, surrounded by muscles. Posterior proboscis chamber glandular, 40-50 μm long by 16-20 μm wide, intensely basophilic, with thin fibrous coat. Proboscis sheath greatly reduced. Body musculature with one layer of outer circular muscles, one layer of inner longitudinal muscles. Submuscular glands eosinophilic; elongate, slender, 8-12 μm long by 4-10 μm wide; numerous. In section, submuscular glands as a single row around the body, not arrayed as in *O. davidi*; interior to muscles. Cephalic glands in esophageal region anterior to cerebrum; developed as paired frontal organs; weakly basophilic in most worms, occasionally eosinophilic. Frontal organs in several worms weakly basophilic, with vesicular appearance. Gonads undeveloped. Two worms with regressed oöcytes.

Type Host and Site of Infestation.—On the sterna, pleopods and axillae of the pereiopods of *Bythograea laubieri* and *B. vrijenhoeki*.

Type Locality.—(American cruise PAR 5) Dive #4089: 23 March 2005, PAR-38°S, Sebastian's Steamer vent site, 37°47.28′S, 110°54.51′W, 2204 m.

Holotype.—Regressed adult (Accession number USNM 1097951) on slide series Carcino B.l. (1) (worm 1, slides 1 through 3) deposited in the National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA.

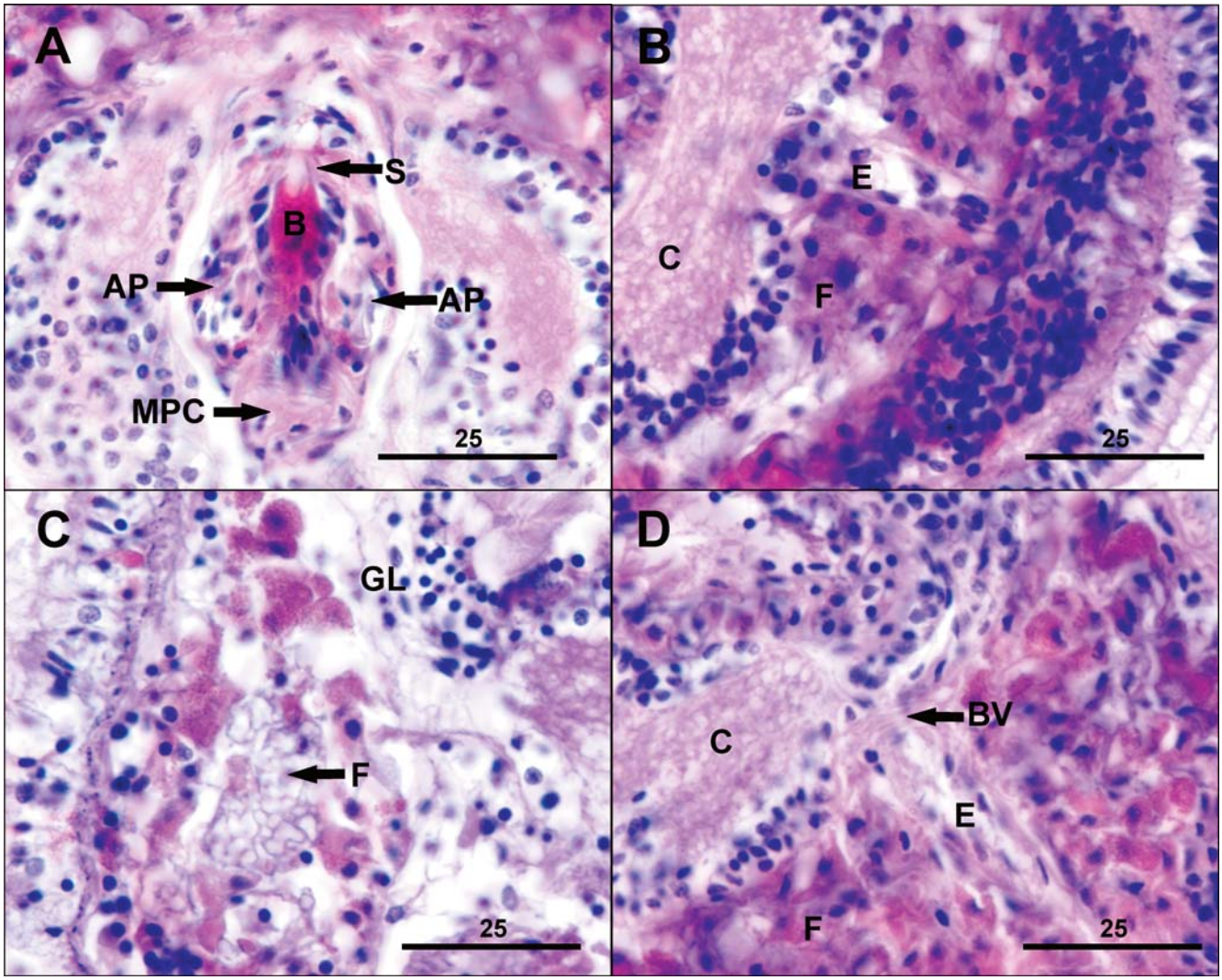

Fig. 6. Sections through *Ovicides jonesi* from *Bythograea laubieri*. A, Holotype (Carcino B.l. (1), slide 3, worm 1). Stylet bulb region of worm showing pyriform stylet (S) on the basis (B) with two accessory stylet pouches (AP) anterior to the middle proboscis chamber (MPC). B, Paratype (same slide, worm 5). Anterior of worm showing weakly basophilic frontal organ (F) developed as a field anterior to cerebrum (C) on both sides of the esophagus (E). C, Paratype (same slide, worm 8). The well-developed, vesiculated frontal organ (F) is anterior to the glial cells (GL) of the cerebrum. D, Paratype (slide 2, worm 9). Anterior loop of the primary blood vessel (BV) dorsal to the esophagus (E) and adjacent to the cerebrum (C). Note the large number of eosinophilic submuscular glands in the esophageal region.
Legend: AP = accessory stylet pouch, AS = accessory stylets, B = basis, BV = anterior loop of primary blood vessel, C = cerebrum, CG = cephalic glands, E = esophagus, EG = eosinophilic glands, F = frontal organ, G = submuscular glands, GL = glial cells of the cerebrum or lateral nerve chord, MPC = middle proboscis chamber, N = lateral nerve, O = ocellus, Ov = presumptive ovum, S = stylet, SG = stylet bulb, SH = sheath, ST = stomach. Numbers on scale bars are in microns.

Paratypes.—Juveniles and regressed adults (Accession number USNM 1097952) on slide series Carcino B.l. (1) (worms 2-9, slides 2 and 3) in the National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA. Juveniles or regressed adults (Accession number MNHN-NMRT 2) on slide series Carcino B.v. B.v. (2), slides 3 through 7, in the Muséum National d'Histoire Naturelle, Paris.

Etymology.—The species is named in honor of Dr. William "Joe" Jones of MBARI, whose hard work and careful attention to details made the oceanographic missions in the South Pacific successful and productive.

Remarks.—We place the worms from *B. vrijenhoeki* and *B. laubieri* into the genus *Ovicides* based on their carcinonemertid characters and the presence of the accessory stylet pouches. The worms from *B. vrijenhoeki* were smaller than those from *B. laubieri* and showed a few minor differences in morphology (somewhat larger submuscular glands, diffuse and eosinophilic frontal organs), but were otherwise quite similar. Therefore, we consider them to be the same species, *O. jonesi*. This worm has cephalic glands organized as a presumptive frontal organ in the esophageal region. In some of the worms, the organs are well organized and have a vesiculated appearance. These organs are better organized than the diffuse frontal organs present in *O.*

Table 2. Morphological differences between species of *Ovicides*.

Species	Sexuality	Eyes	Cephalic glands	Frontal organ	Submuscular glands
O. davidi	?	+	Diffuse	Diffuse	3 rows
O. jasoni	?	−	Absent	Absent	1 row, arrayed
O. jonesi	?	−	Robust	Paired	1 row, not arrayed
O. juliaea	Hermaphroditic	+	Not observed	Not observed	1 row, not arrayed

davidi. *Ovicides jonesi* shares features with both *O. jasoni* (blind, single band of submuscular glands, similar sized stylet) and *O. davidi* (large basis, large posterior proboscis chamber), but it can be separated from each by the opposite characters (Tables 1 and 2).

The frontal organ in *O. jonesi* is unusual in that it is well organized in some specimens, or only weakly developed in other specimens of the same species from the same host. This difference is difficult to explain, but it may be due to differences in maturity or metabolic state (juveniles vs. regressed adults that have fed previously). *Ovicides jonesi* is only the third species in the family known to possess a frontal organ, after *O. davidi* (see above) and *C. australiensis* (see Campbell et al., 1989). In *O. jonesi*, the frontal organ lies immediately anterior to the cerebrum and can be quite large, 15-30 μm in diameter. The frontal organ of *Carcinonemertes australiensis* is also large and well organized, whereas that of *O. davidi* is diffusely organized.

Discussion

Infestations of carcinonemertid worms were found on four species of bythograeid crabs from four vent sites in different basins within the Pacific Ocean (Fig. 1). This indicates a widespread distribution of the symbiosis on bythograeid crabs from hydrothermal vents. Given that different species of worms were found on different host species, it is likely that the worms are host specialists, and not generalists as occurs with some species of *Carcinonemertes* (Wickham and Kuris, 1985, 1988), or that we have not encountered other infested host species. The worms on the bythograeids appear to have a similar life history pattern as those found on shallow-water cancrid and grapsid crabs, in that immature worms are found on both male and female crabs, with those on males likely transmitted to female hosts during copulation as in *C. errans* (Wickham et al., 1984). Carcinonemertids have three general life history patterns depending on the reproductive cycle and life history of their hosts (Shields and Kuris, 1990). The embryogenesis of cancrid and grapsid crabs is of the 'intermediate' duration compared to that of other crustacean hosts (short for portunids, long for lithodids and palinurids) (Shields, 1991; Shields and Kuris, 1990; Kuris et al., 1991). Worms on cancrid and grapsid hosts also migrate out of the egg clutch and regress after host eclosion, a feature apparently shared by the worms on the bythograeid hosts. Therefore, based on the finding of infested male hosts, and the occurrence of juvenile and regressed adult worms on the axillae of the pereiopods and on the pleopods of the females, we speculate that embryogenesis is likely to be of an intermediate duration (30-90 d) for the bythograeid hosts.

The stylet:basis ratio has been used as a morphometric character for identifying species of Carcinonemertidae (McDermott and Gibson, 1993; see also Table 1). Worms with a large (> 35 μm), robust basis and large (> 15 μm) stylet feed on large host eggs with thick coats and whose embryos undergo long periods of embryogenesis (Shields et al., 1989; Shields and Kuris, 1990). Conversely, those with a small (< 30 μm) basis and stylet (< 10 μm) feed on smaller, thinly coated eggs, whose embryos typically undergo more rapid embryogenesis. The bases and stylets of *O. jasoni*, *O. davidi*, and *O. jonesi* are intermediate in size; which therefore adds further support for their hosts having moderate development times of a few months like those of cancrid and grapsid hosts.

The fact that only juvenile worms were observed is a common finding in infestations of carcinonemertids, which mature only after eating host eggs. Further, mature worms on cancrid and xanthid hosts regress and move out of the clutch area, or die after the host eggs hatch, which may explain the lack of mature worms even on post-ovigerous hosts (Wickham and Kuris, 1985; Shields and Kuris, 1990). However, this is not the case with *Carcinonemertes carcinophila*, which remains mature after migration out of the clutch (Hopkins, 1947). Worms on cancrid and xanthid hosts are also capable of migrating to the new instar during host molting (Wickham et al., 1984; Shields, 2001); and worms on bythograeids may also migrate thusly. Juvenile carcinonemertids, particularly those on cancrid hosts, are known to subsist on amino acids leaked from the lightly sclerotized arthrodial membranes of their hosts (Roe et al., 1981; Crowe et al., 1982); due to their habitus on the host and life history patterns, members of *Ovicides* appear to be no exception to this mode of nutrient uptake.

The bathymetric distribution (1900 m to 2700 m) and isolated nature of the vent communities raise some questions as to how the bythograeid hosts were originally colonized by carcinonemertid worms. Two scenarios seem possible: either the worms switched hosts from a deep-sea species to the bythograeids or they co-evolved with the lineage of hosts leading to the present day bythograeids. Given the host specificity of several of the present-day species, the first scenario would require a marked change in host preferences, from a host generalist, thereby allowing the switch to a new host, to that of a host specialist, as observed in this study. Infections could have been acquired from a shallow-water host genus with deep-water congeners. Such genera could include representatives of the families Lithodidae, Grapsidae, Xanthidae, and possibly the Cancridae, all of which are known to host carcinonemertid symbionts. For example, the lithodid crab *Paralithodes camtschaticus* hosts a diverse, but largely undescribed community of carcinonemertids (Wickham and Kuris, 1985; Shields et al., 1989; Kuris et al., 1991), and certain species of Lithodidae, notably species of *Neolithodes*, can be found at depths over 3000 m; however,

most species are restricted to depths of < 1000 m (reviewed by Zaklan, 2002). Nevertheless, no carcinonemertids were found on *Paralomis hirtella* Saint Laurent and MacPherson, 1997, The only representative of this family occurring on the Lau and N-Fiji back-arc basins (2000 m) (M. S. personal observation). Further, given the specific associations observed even for sympatric species (*Ovicides davidi* ex *Cyanagraea praedator* and *O. jonesi* ex *Bythograea laubieri* from the South EPR and that for *Ovicides julieae* on *Chlorodiella* spp.), it seems unlikely that host switches are common. As discussed above, some features of the bythograeid host-symbiont relationship resemble those of grapsid or xanthid hosts and their carcinonemertid symbionts, i.e., life history characteristics, stylet:basis ratios. Similarly, bythograeid crabs appear to be more closely related to Xanthidae (Tudge et al., 1998).

The second hypothesis, involving co-evolution of a host with a shallow-water lineage and its carcinonemertid symbiont, may be more likely than host switching. The two groups involved in the symbiosis most likely co-evolved from shallow water ancestors, and the association probably followed these lineages in their colonization of the deep sea. This hypothesis could be tested by both establishing a molecular phylogeny for the bythograeids and the carcinonemertids, and testing for evidence of co-evolution by comparing the tree topologies. In support of this hypothesis, several members of the endemic hydrothermal vent fauna (crabs, limpets, bivalves) have their closest phylogenetic relatives in warm, shallow waters; and shallow water seeps, methane pools and whale falls possibly could be used as stepping stones to deeper waters (Van Dover et al., 2002). Furthermore, brachyuran crabs rarely colonize deep-sea environments. Many are vagrants (Martin and Haney, 2005), and few are ever observed at cold seep sites (except for *Chaceon* spp. on the shallow cold seeps of the Gulf of Mexico) or at whale falls. Moreover, recent fossil evidence from hydrothermal vents suggests that vent fauna is derived from shallow water environments following the tectonic movements (Little and Vrijenhoek, 2003). Therefore, the symbiosis reported here may fit better with a more recent shallow-water origin involving co-evolution of the bythograeids and their nemertean worms.

Additional data on the occurrence of the carcinonemertids in the other deep-sea habitats and a careful examination of additional bythograeids from other localities may answer questions on the specificity of the association and provide insights into their evolution.

Acknowledgements

The D/S "Nautile" and D/S "Alvin" were supported by the R/V "L'Atalante" (Chief scientist D. Jollivet, Station biologique, Roscoff, France), and the R/V "Atlantis" (Chief scientist R. Vrijenhoek, MBARI, USA); and the ROV "Jason 2" was supported by the R/V "Melville" (Chief scientist R. Vrijenhoek). We thank Alex Rogers (National Oceanography Centre, Southampton, UK), Violaine Martin (Ifremer, Fig. 1), Greg Rouse (South Australian Museum, photographs in Fig. 2) and Kersten Wheeler (VIMS) for their help with the study. Jon Norenburg (Smithsonian Institution) and Christopher Tudge (American University, Washington) and Stéphane Hourdez (Station biologique, Roscoff) offered useful criticism. The PAR5 and TUIM6MV expeditions were funded by grants from the U.S. National Science Foundation to R. C. Vrijenhoek (OCE-0241613) and C. L. Van Dover (OCE-0350554). This is Contribution #2791 from the Virginia Institute of Marine Science.

References

Berg, G., and R. Gibson. 1996. A redescription of *Nemertoscolex parasiticus* Greeff, 1879, an apparently endoparasitic heteronemertean from the coelomic fluid of the echiuroid *Echiurus echiurus* (Pallas). Journal Natural History 30: 163-173.

Bright, M. 2006. *Thermonemertes valens* Rogers, Gibson and Tunnicliffe, 1996. Denisia 18: 183.

Campbell, A., R. Gibson, and L. A. Evans. 1989. A new species of *Carcinonemertes* (Nemertea: Carcinonemertidae) ectohabitant on *Panulirus cygnus* (Crustacea: Palinuridae) from Western Australia. Zoological Journal of the Linnean Society 95: 257-268.

Crowe, J. H., L. M. Crowe, P. Roe, and D. E. Wickham. 1982. Uptake of DOM by nemertean worms: association of worms with arthrodial membranes. American Zoologist 22: 671-682.

Gibson, R., and D. S. Jones. 1990. A new species of *Carcinonemertes* (Nemertea: Enopla: Carcinonemertidae) from the egg masses of *Naxia aurita* (Latreille) (Decapoda: Brachyura: Majidae) collected in the Albany region of Western Australia, pp. 195-202. In, F. E. Wells, D. I. Walker, H. Kirkman, and R. Lethbridge (eds.), Proceedings of the Third International Marine Biological Workshop: The Marine Flora and Fauna of Albany, Western Australia. Western Australian Museum, Perth. Vol. 1.

Hopkins, S. H. 1947. The nemertean *Carcinonemertes* as an indicator of the spawning history of the host, *Callinectes sapidus*. Journal of Parasitology 33: 146-150.

Humes, A. G. 1942. The morphology, taxonomy, and bionomics of the nemertean genus *Carcinonemertes*. Illinois Biological Monographs 18: 1-105.

Kuris, A. M., and D. E. Wickham. 1987. Effect of nemertean egg predators on crustaceans. Bulletin of Marine Science 41: 151-164.

———, S. F. Blau, A. J. Paul, J. D. Shields, and D. E. Wickham. 1991. Infestation by brood symbionts and their impact on egg mortality in the red king crab, *Paralithodes camtschatica*, in Alaska: geographic and temporal variation. Canadian Journal of Fisheries and Aquatic Sciences 48: 559-568.

Little, C. T. S., and R. C. Vrijenhoek. 2003. Are hydrothermal vent animals living fossils? Trends in Ecology and Evolution 18: 582-588.

Martin, J. W., and T. A. Haney. 2005. Decapod crustaceans from hydrothermal vents and cold seeps: a review through 2005. Zoological Journal of the Linnean Society 145: 445-522.

McDermott, J. J., and R. Gibson. 1993. *Carcinonemertes pinnotheridophila* sp. nov. (Nemertea, Enopla, Carcinonemertidae) from the branchial chambers of *Pinnixa chaetopterana* (Crustacea, Decapoda, Pinnotheridae): description, incidence and biological relationships with the host. Hydrobiologia 266: 57-80.

Ramirez-Llodra, E., and M. Segonzac. 2006. Reproductive biology of *Alvinocaris muricola* (Decapoda: Caridea: Alvinocarididea) from cold seeps in the Congo Basin. Journal of the Marine Biological Association of the United Kingdom 86: 1347-1356.

Roe, P., and J. L. Norenburg. 1999. Observations on depth distribution, diversity and abundance of pelagic nemerteans from the Pacific Ocean off California and Hawaii. Deep-Sea Research, Part I Oceanographic Research Papers 46: 1201-1220.

———, J. Crowe, L. Crowe, and D. E. Wickham. 1981. Uptake of amino acids by juveniles of *Carcinonemertes errans* (Nemertea). Comparative Biochemistry and Physiology 69A: 423-427.

Rogers, A. D., R. Gibson, and V. Tunnicliffe. 1996. A new genus and species of monostiliferous hoplonemertean colonizing an inchoated hydrothermal field on Juan de Fuca Ridge. Deep-Sea Research, Part I Oceanographic Research Papers 43: 1581-1599.

Santos, S., J. L. Norenburg, and S. L. S. Bueno. 2006. Three new species of *Carcinonemertes* (Nemertea, Carcinonemertidae) from the southeastern coast of Brazil. Proceedings of the 6th International Conference on Nemertean Biology. Journal of Natural History 40: 915-930.

Shields, J. D. 1991. Reproductive ecology and fecundity of *Cancer* crabs, pp. 193-213. In, A. Wenner and A. M. Kuris (eds.), Crustacean egg production, Crustacean Issues 7. Balkema, Rotterdam.

———. 1993. Infestation and dispersion patterns of *Carcinonemertes* spp. on their crab hosts. Hydrobiologia 266: 45-56.

———. 2001. *Ovicides julieae* n. gen., n. sp. (Nemertea: Carcinonemertidae) from a xanthid crab from the Great Barrier Reef, Australia. Journal Crustacean Biology 21: 304-312.

———, and A. M. Kuris. 1990. *Carcinonemertes wickhami* n. sp. (Nemertea), an egg predator on the California lobster, *Panulirus interruptus*. Fishery Bulletin (NOAA) 88: 279-287.

———, D. E. Wickham, and A. M. Kuris. 1989. *Carcinonemertes regicides* n. sp. (Nemertea), a symbiotic egg predator on the red king crab, *Paralithodes camtschatica*, from Alaska. Canadian Journal of Zoology 67: 923-930.

Tudge, C. C., B. G. M. Jamieson, M. Segonzac, and D. Guinot. 1998. Spermatozoal ultrastructure in three species of hydrothermal vent crab, in the genera *Bythograea*, *Austinograea* and *Segonzacia* (Decapoda, Brachyura, Bythograeidae). Invertebrate Reproduction and Development 34: 13-23.

Tunnicliffe, V., R. W. Embley, J. F. Holden, D. A. Butterfield, G. J. Massoth, and S. K. Juniper. 1997. Biological colonization of new hydrothermal vents following an eruption on Juan de Fuca Ridge. Deep-Sea Research, Part I Oceanographic Research Papers 44: 1627-1654.

Uhazy, L. S., D. E. Aiken, and A. Campbell. 1985. Morphology and systematics of the nemertean *Pseudocarcinonemertes homari* (Hoplonemertea: Monostilifera) from the American lobster, *Homarus americanus*. Canadian Journal of Fisheries and Aquatic Sciences 42: 342-350.

Van Dover, C. L., C. R. German, K. G. Speer, L. M. Parson, and R. C. Vrijenhoek. 2002. Evolution and biogeography of deep-sea vent and seep invertebrates. Science 295: 1253-1257.

Wickham, D. E. 1986. Epizootic infestations by nemertean brood parasites on commercially important crustaceans. Canadian Journal of Fisheries and Aquatic Sciences 43: 2295-2302.

———, and A. M. Kuris. 1985. The comparative ecology of nemertean egg predators. American Zoologist 25: 127-134.

———, and ———. 1988. Diversity among nemertean egg predators of decapod crustaceans. Hydrobiologia 156: 23-30.

———, P. Roe, and A. M. Kuris. 1984. Transfer of nemertean egg predators during host molting and copulation. Biological Bulletin 167: 331-338.

Zaklan, S. D. 2002. Review of the family Lithodidae (Crustacea: Anomura: Paguroidea): Distribution, Biology, and Fisheries, pp. 751-845. In, A. J. Paul, E. G. Dawe, R. Elner, G. S. Jamieson, G. H. Kruse, R. S. Otto, B. Sainte-Marie, T. C. Shirley, and D. Woodby (eds.), Crabs in Cold Water Regions: Management, and Economics; Alaska Sea Grant College Program. AK-SG-02-01.

RECEIVED: 31 October 2006.
ACCEPTED: 25 January 2007.