Carcinonemertes regicides n.sp. (Nemertea), a symbiotic egg predator from the red king crab, Paralithodes camtschatica (Decapoda: Anomura), in Alaska

JEFFREY D. SHIELDS

Marine Science Institute and Department of Biological Sciences, University of California, Santa Barbara, CA 93106, U.S.A.

DANIEL E. WICKHAM

Bodega Marine Laboratory, University of California, Bodega Bay, CA 94923, U.S.A.

AND

ARMAND M. KURIS

Marine Science Institute and Department of Biological Sciences, University of California, Santa Barbara, CA 93106, U.S.A.

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A symbiotic nemerteans worm, Carcinonemertes regicides n.sp., found on red king crabs, Paralithodes camtschatica, is described and compared with other members of the genus Carcinonemertes. The family Carcinonemertidae Humes, 1942 is emended to include this species and to include known but as yet undescribed members. The following familial characters are considered diagnostic: (i) symbiotic relationship with a decapod crustacean, (ii) short proboscis, (iii) absence of cerebral organs, (iv) presence of Takakura’s duct, and (v) a hoplonemertean larva. The new species of Carcinonemertes has a relatively large basic and stylet, a large anterior proboscis chamber, and a newly described flame cell pattern of 2[(1+1+1+1)]. In addition, at maturity it is the smallest member of the genus (typical adult size is 2.0 mm in length), and female worms bear ovarian pores before oviposition. The above characters distinguish this species from others in the genus.


On trouvera ici la description d’une némathe de genre Carcinonemertes, Carcinonemertes regicides sp.nov., symbiote de crabes Paralithodes camtschatica ; l’espèce est comparée aux autres espèces du genre. La famille des Carcinonemertidae Humes, 1942 est révisée de façon à inclure la nouvelle espèce ainsi que d’autres espèces connues mais encore inédites. Les caractéristiques suivantes sont distinctives de la famille : (i) symbiose avec un crustacé décapode, (ii) proboscis court, (iii) absence d’organes cérébraux, (iv) présence du canal de Takakura, (v) larve hoplonémétique. La nouvelle espèce de Carcinonemertes a un stylet et une base de tailles relativement grandes, un proboscis à cavité antérieure importante et une répartition de cellules à flamme encore jamais rencontrée 2[(1+1+1+1)]. De plus, à maturité, c’est la plus petite espèce du genre (la taille typique d’un adulte est de 2,0 mm de longueur) et les femelles portent des pores ovariens avant la ponction. Ces caractéristiques distinguent la nouvelle espèce des autres espèces du genre.

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Introduction

Nemerteans of the genus Carcinonemertes (von Kolliker, 1845) are specialized symbionts of decapod crustaceans. Several species of Carcinonemertes have been recognized as predators on the eggs of their crab hosts (Humes 1942; Kuris 1971; Wickham 1978; Roe 1984); this trophic habit appears to be widespread in the genus (Wickham and Kuris 1988). Research initiated in 1983 by the Alaska Department of Fish and Game in cooperation with the University of California and the University of Alaska documented high intensity infestations of a previously undescribed carincarneantid from the red king crab, Paralithodes camtschatica (Tilesius, 1815). The occurrence of these nemerteans worms in the clutches of female crabs has resulted in partial or complete brood loss for many females in local populations (Wickham et al. 1985; Blau 1986; Wickham 1986; A. M. Kuris, S. F. Blau, A. J. Paul, J. D. Shields, and D. E. Wickham, manuscript in preparation). Two species, Carcinonemertes epialti Coe, 1902 and Carcinonemertes errans Wickham, 1978, have previously been described from the west coast of North America. Nemerteans from the red king crab were found to be quite different from those species in both morphology and life history.

Here we describe the egg predator Carcinonemertes regicides n.sp. from the broods of the red king crab, P. camtschatica, from Alaska. In addition, the distinctive morphological characters of the species of Carcinonemertes are discussed, and aspects of the life history of the new species are presented.

Materials and methods

Red king crabs, Paralithodes camtschatica, were collected by pot traps or trawls in Kachemak Bay, Alaska. The pleopods of infested female crabs were excised and immediately placed in ambient seawater. Pleopods were examined at the University of Alaska, Seward Marine Laboratory, or packed in ice and shipped via express mail to the University of California, Santa Barbara, for further observation. Nemerteans were examined in situ on the pleopod, or were gently manipulated onto slides for measuring and photomicrography. Lengths and widths of worms were measured with an ocular micrometer in a dissecting microscope; the worms were then covered with a cover slip, and various measurements were taken with an ocular micrometer in a compound microscope. Measurements are in

1Present address: Department of Parasitology, University of Brisbane, Queensland 4067, Australia.
Affixative, Van Cleave’s hematoxylin, Heidenhain’s hematoxylin, compared with standard protozoological techniques.

Worms were fixed in hot AFA (alcohol – formalin – acetic acid, Humason 1979) or 5% formalin – seawater. Representative specimens were sectioned at 10 μm and stained with hematoxylin and eosin. Additional specimens were prepared as whole mounts after staining with Van Cleave’s hematoxylin and decolorizing with potassium permanganate and oxalic acid (Humason 1979, p. 503).

Carcinonemertes regicides n.sp
(Figs. 1–16)

Description
Female (10 living specimens)

Body ciliated, color dull pink to dull orange with numerous refractile white spots beneath the musculature (submuscular glands). Shape cylindrical, slender with rounded anterior end, blunt posterior end; sometimes found in fragile sheaths, often free; adhesive when live, adhering readily to implements and glassware. Length 2.1 mm (1.2–4.0), and up to 6.0 mm when fully extended. Average maximum width 236 (148–370). Two ocelli anterior to prominent bilobed cerebral ganglia. Ocelli cup shaped, small, inconspicuous; 27 (17–35) long by 16 (12–23) wide, located 131 (73–197) from anterior end. Distance between ocelli 149 (75–212). Rhynchodaeum and esophagus ventral to cerebral commissure. Proboscis with three chambers, lateral to posterior part of esophagus. Anterior proboscis chamber 76 (64–96) long by 73 (55–93) in diameter, with basis and stylet. Basis large, 40.5 (37.7–43.5) long by 13.2 (8.7–17.4) posterior diameter, tapering to 6.1 (5.8–8.7) anterior diameter. A single, large stylet, 17.2 (14.5–18.8) long. Stylet appears dagger- or stiletto-shaped, with posterior knob-like proximal piece. Middle proboscis chamber granular in appearance, 43 (35–52) in diameter. Posterior proboscis chamber large, 82 (58–116) long by 62 (44–87) in diameter, glandular, with lumen. Foregut (anterior portion of esophagus) joins rhynchodaeum at or just anterior to cephalic ganglia. Foregut everted during feeding, peristalsis evident. Posterior part of esophagus large, 158 (116–261) long, muscular, with ciliated lumen. Intestine immediately posterior to posterior proboscis chamber and posterior portion of esophagus. Intestine with numerous paired, unbranched diverticula. Anus terminal. Ovaries numerous, regularly distributed between intestinal diverticula. Each ovary with lateral ovarian pore. Ovarian pore oblong, 64 (44–81) long by 57 (26–81) wide. Pore size 16 (12–23) long. Ovarian pores apparent before ovulation. Egg strands variable in size (1.9–2.9 mm long by 418–580 μm in diameter), with approximately 150 (90–200) eggs. Eggs 84 (75–93) in diameter. Eggs deposited in mucous sheath secreted by female. Sheaths fragile, without lapilli, grainy in texture, slightly longer than accompanying worm.

Male (10 living specimens)

Similar in gross morphology to female; differences include color of the posterior region with reddish tinge, posterior fourth of worm somewhat inflated, length 1.6 mm (1.0–2.7 mm), up to 3.0 mm when fully extended. Flame cell pattern on immature males, 2(1+1+1+1), difficult to observe on adult nemerteans. Flame cells approximately 14.5 long by

Fig. 1. Composite drawing of female (left) and male (right) Carcinonemertes regicides from Paralithodes camtschatica. The foregut of the male is everted for comparison. Note that the reproductive system of the male is represented on the right, while the digestive system is figured on the left. APC, anterior proboscis chamber; CE, cerebrum; CL, cloaca; FO, foregut—esophagus; INT, intestine; LN, lateral nerve cord; MPC, middle proboscis chamber; OC, ocelli; OP, ovarian pore; OV, ovary; PA, parenchyma; PPC, posterior proboscis chamber; PS, proboscis sheath; RH, rhynchodaeum; ST, stomach; SV, seminal vesicle; TE, testis; VD, vas deferens.

Fig. 2. Anterior end of a female C. regicides with the foregut—esophagus retracted (F). Note the prominent dorsal ganglia (G). Scale bar = 100 μm. Fig. 3. Detail of the feeding armature of C. regicides; F, posterior of foregut—esophagus; A, anterior proboscis chamber; arrow, middle proboscis chamber; P, posterior proboscis chamber. Scale bar = 100 μm. Fig. 4. Dorsoventral aspect of the stylet (dagger-like) in the anterior proboscis chamber of C. regicides. Scale bar = 25 μm. Fig. 5. Lateral aspect of the stylet (stiletto-like) in the anterior proboscis chamber. Scale bar = 25 μm. Fig. 6. Lateral aspect of female C. regicides showing ovarian pore (P). Scale bar = 100 μm. Fig. 7. Detail of ovarian pore of C. regicides. Scale bar = 25 μm. Fig. 8. Posterior aspect of male C. regicides; S, seminal vesicle; N, posterior nerve ganglia; C, cloaca. Note the blunt posterior form. Scale bar = 100 μm. Fig. 9. Detail of posterior aspect of male C. regicides; S, seminal vesicle; arrows, scale bar = 25 μm.
duct) extending from antero-most testes to gonopore. A single, large seminal vesicle near posterior end, 151 (81–203) long by 89 (38–116) in diameter, difficult to observe when not inflated with spermatozoa. Sperm heads thread-like, approximately 23 long. Testicular field terminates 174 (102–260) from cloaca. Anus and gonoduct empty into cloaca.

Larvae (based on living and fixed specimens)

Body completely ciliated, white in color. Larva spherical or ovoid, length approximately 106, width approximately 60. Anterior and posterior cirri or tufts (20–23). Two ocelli (7–8.4). Hoplonemertean larva in form.

TYPE HOST: Red king crab, Paralithodes camtschatica (Tilesius).

ADDITIONAL HOST: Tanner crab, Chionoecetes bairdi Rathbun.

SITE OF INFestation: Egg-bearing pleopods of female crabs.

HOLOTYPE LOCALITY: Alaska, Kachemak Bay, near the city of Homer.

RANGE: Southeastern Alaska to Dutch Harbor, Alaska (see Wickham et al. 1985; Wickham 1986).


PARATYPE: USNM Helm. Coll. No. 80265, 1 microslide: female worm in serial section; 1 vial: 5 male worms (Kache-


ETYMOLOGY: Named for its habit of killing king crab embryos.

Definition

Hoplonemertea, Monostilifera, Carcinonemertidae. Small, cylindrical nemertans, less than 6.0 mm long by less than 400 μm wide, with small, white submuscular spots (submuscular glands). Anterior proboscis chamber large, 76 (64–95) long by 73 (55–93) in diameter. Basis stout, 40.5 (37.7–43.5) long by 13.2 (8.7–17.4) basal diameter, tapering to 6.1 (5.8–8.7) anterior diameter. Stylet large, with posterior hub, 17.2 (14.5–18.8) long. Female with lateral ovarian pores which are prominent before oviposition. Males with subterminal cloaca. Flame cell pattern (2[1+1+1+1]). Hoplonemertean larva.

Diagnosis

Carcinonemertes regicides most closely resembles C. coei, C. epialti, and C. errans. These four species have relatively small adult forms. Carcinonemertes coei can be distinguished from the other carcinonemertids by the presence of 4 rows of ovaries rather than 2 rows (Humes 1942). Carcinonemertes regicides can be distinguished from C. epialti and C. errans by its larger basis and stylet, a large anterior proboscis chamber, lateral ovarian pores in females, and the newly described flame cell pattern. Behavioral traits and color comparisons of these three species are given in Table 1.

Remarks and discussion

The family (Humes 1942) appears to be too narrowly defined (Wickham and Kuris 1988). We view the following as diagnostic familial characters: (i) symbiotic relationship with a decapod crustacean, (ii) short proboscis, (iii) absence of cere-
Changes in the family definition are warranted by the presence of an excretory system in at least *Carcinonemertes regicides* and *C. epialti* (J. D. Shields, personal observation) and the large anterior proboscis chamber of *C. regicides*. Further, Wickham and Kuris (1988) noted the presence of Takakura's duct in several carcinonemertid forms from *Paralithodes camtschatica*. These forms have accessory stylet pouches and stylets, but otherwise appear related to the type genus, *Carcinonemertes* (von Kolliker, 1845). Thus, we remove the following characters from the family diagnosis: one central stylet, no accessory stylet pouches or stylets, anterior proboscis chamber small and nonglandular, and excretory apparatus absent. These characters may, however, be useful for generic diagnosis.

Humes (1942) and Wickham and Kuris (1988) have reviewed the genus *Carcinonemertes*. Six species are presently recognized, one of which contains two subspecies, *C. carcinophila carcinophila*, and *C. c. imminuta* (Table 2). *Carcinonemertes regicides* is the first species described from the red king crab and the Tanner crab, and the third species described from the eastern Pacific. It was discussed as form No. 1 of Wickham and Kuris (1988).

At maturity, *Carcinonemertes regicides* is the smallest species of the genus. Mature individuals measuring as small as 1.0 mm in length have been found. In contrast, mature individuals of *C. epialti* and *C. errans* are typically over 3.0 mm in length. Although adult *C. carcinophila* of less than 1.0 mm have been reported, the typical size of mature individuals is well over 10.0 mm in length (Humes 1942).
Table 1. Comparison of color and behavioral traits of Carcinonemertes epialti, C. errans, and C. regicides

<table>
<thead>
<tr>
<th>Trait</th>
<th>C. epialti</th>
<th>C. errans</th>
<th>C. regicides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Pink, no spots</td>
<td>Pink, with white epidermal</td>
<td>Pink, with white submuscular spots</td>
</tr>
<tr>
<td>Muscular strength and agility</td>
<td>Relatively fragile, often damaged when handled, less muscular agility, movement more often ciliary</td>
<td>Sturdy, readily handled without damage, agile due to contraction of longitudinal musculature</td>
<td>Sturdy, readily handled without damage, agile due to contraction of longitudinal musculature</td>
</tr>
<tr>
<td>Adhesion</td>
<td>Less adhesive</td>
<td>Strongly adhesive</td>
<td>Strongly adhesive</td>
</tr>
<tr>
<td>Sheath structure</td>
<td>Sturdy, usually encases female</td>
<td>No sheath</td>
<td>Flimsy, many females not ensheathed</td>
</tr>
<tr>
<td>Sheath location</td>
<td>Along host pleopods or egg-bearing setae</td>
<td>—</td>
<td>Along funiculi of host eggs or on surface of eggs</td>
</tr>
<tr>
<td>Juveniles</td>
<td>Common on exoskeleton of nonovigerous host, infrequent in egg mass</td>
<td>Common on exoskeleton of nonovigerous host, infrequent in egg mass</td>
<td>Uncommon on exoskeleton of nonovigerous host, common in egg mass</td>
</tr>
</tbody>
</table>

An excretory system is reported for the first time for this genus. Flame cells and the flame cell pattern were observed on several occasions. The pattern was most evident in juvenile worms, although individual flame cells were occasionally observed in adults. The flame cell pattern of C. regicides was 2[(1+1+1+1)], whereas that of C. epialti from Cancer antennarius was 2[(2+2+2)] (J. D. Shields, personal observation). Thus, these patterns may be of taxonomic value in the genus. Flame cell patterns are used as diagnostic characters in other taxa (i.e., Trematoda, Cestoda). A description of flame cell patterns may be found in Schell (1985). Briefly, the number outside the bracket refers to the two sides of the body; numbers within parentheses refer to groups of flame cells occurring together; groups found in anterior of posterior positions along the body are denoted by separate parentheses within the bracket, e.g., for plagioclid trematodes the pattern is 2[(3+3+3)+(3+3+3)], which denotes anterior and posterior sets of flame cells in three groups of three along each side of the body.

On several occasions, we observed the suctorial feeding of C. regicides on the eggs of P. camtschatica. Feeding was similar to that of other carcinonemertids (Roe 1984). Briefly, the basis and stylet move anteriorly to the rhynchodaeum and puncture the outer coat of the crab egg. The initial penetration of the egg coat usually occurs near the junction of the funiculus and the egg. The basis and stylet withdraw and the foregut—esophagus of the worm is everted into the crab egg. Peristaltic contractions and ciliary action aid in the ingestion process. Rarely are the contents of the egg consumed in their entirety. The empty or partially eaten eggs remain attached, via the fun-
the thick outer coat of the egg (Kuris and Wickham 1987).

Adult C. regicides are often aligned along the tangled webbing of funicular strands near the base of the eggs. Their position may account for the observation that the puncture in the host egg during feeding is usually near the funicular attachment. The location of the worm, along with its small size (scarce-ly greater than the diameter of the eggs of the host) makes it less readily observed in the egg mass than other species of Carcinonemertes. Sometimes smaller C. regicides were ensheathed on the surface of the eggs of the crab, and in heavy infestations, worms were frequently found inside empty or partially empty eggs.

Given its small size, C. regicides has the largest anterior proboscis chamber and the largest basis and stylet complex yet described for this genus. The eggs of P. camtschatica are quite large (approx. 1.0 mm) with respect to other crustaceans known to be infested by nemerteans. The large basis and stylet of the worm may be adaptations allowing penetration of the thick outer coat of the egg (Kuris and Wickham 1987; Wickham and Kuris 1988).

The stylet of C. regicides has one form, but may appear to have two forms. Lying flat, the stylet appears as a broad, flat dagger-like blade. When turned dorsoventrally, the stylet appears as a thin, conical stiletto-like blade.

Male C. regicides possess a distinctive cloaca, consisting of an anus and a gonoduct. Sperm are released via the gonoduct into the cloaca. The posterior terminus of the male is concave, thus giving the posterior a blunt appearance.

Female worms lack a cloaca and do not have a blunt posterior. The large ovarian pores on female C. regicides are unique to this species. Ovarian development and an ovarian gonoduct have been reported for C. epialti (Stricker 1986), but distinct ovarian pores are not found in that species (Stricker 1986; J. D. Shields, personal observation). The pores on C. regicides were observed before ovulation, but were inapparent before oviposition. Large, distinct pores were not observed on gravid C. regicides. Their function remains unknown, but we suggest that ovarian pores aid in the entry and storage of sperm in maturing females. Sperm may be stored in connective tissue surrounding the ovary, or in the cytoplasm of somatic epithelial cells (Stricker 1986). Eggs are fertilized in the ovary and embryos are extruded through the gonoduct in C. epialti (Stricker 1986). While internal fertilization occurs in many species of Carcinonemertes, it was not observed in C. regicides. However, recently oviposited embryos were observed in the many-cell stage of egg development, indicating that fertilization may occur internally.

The larval morphology of the carcinonemertids is of the typical rhabdocoel-like hoplonemertean form (see Gibson 1972). These larvae most likely undergo direct development into the juvenile stage (see Gibson 1972; Stricker and Reed 1981). The larval morphology of C. regicides is similar to that of C. carcinophila and C. epialti. Differences in the sizes of the larvae are apparent, however. The larva of C. carcinophila is larger than the larvae of either C. epialti or C. regicides (127 × 75 μm vs. 110 × 60 μm and 106 × 60 μm, respectively; Humes 1942; Stricker and Reed 1981; present study).

When present, juvenile worms could be recovered from the limb axillae, and under the abdominal flap of female hosts. They were most commonly collected ensheathed on the unciliated surface of the abdomen of the host. No nemerteans were recovered from the branchiae.

Mature C. regicides were only found on ovigerous hosts. Unlike other carcinonemertids, reproduction in C. regicides may not be tightly synchronized with host embryogenesis (Shields 1987). Paralithodes camtschatica has an extended embryogenic period of 11–12 months (Blau 1986), and worms may be found in large numbers (>103) in the brood only during the summer and fall, i.e., in the middle of the development period (Wickham et al. 1985). Egg strands of C. regicides have been found in the broods of P. camtschatica from July to November, and host eclosion follows in the spring, March–April. Reproduction in other species of Carcinonemertes appears to be closely linked to host embryogenesis (Kuris 1978; Roe 1979; Wickham 1980; Shields 1987). That is, worm oviposition and egg hatching coincide with the development and eclosion of the host embryos. Thus, reproductive timing of C. regicides is distinctly different from that of other carcinonemertids.

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