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## Three new species of *Carcinonemertes* (Nemertea, Carcinonemertidae) from the southeastern coast of Brazil

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### Abstract

Three new species of *Carcinonemertes* from the southeastern coast of Brazil are described: *Carcinonemertes divae* new species, *Carcinonemertes caissarum* new species and *Carcinonemertes sebastianensis* new species. They were found, respectively, associated with the crabs *Libinia spinosa*, *Hepatus pudibundus* and *Menippe nodifrons*, each a newly recorded host for *Carcinonemertes*. Characters not previously used to describe members of the family Carcinonemertidae, such as distance from ovaries to tip of head, distance from brain to tip of head and distance from stylet to tip of head are included in the descriptions of the new species and are discussed. The locations of these new carcinonemertid worms in their respective hosts are presented in detail and a novelty regarding the infestation site is registered.

**Keywords:** *Carcinonemertidae*, *decapod crustacean*, *symbiosis*, *predation*, *Carcinonemertes divae*, *Carcinonemertes caissarum*, *Carcinonemertes sebastianensis*, *Brazil*

### Introduction

Nemertean worms of the family Carcinonemertidae are symbiotic egg predators of many decapod crustaceans. Owing to their life cycle, intimacy and use of chemically mediated cues from their hosts, their biology is effectively akin to parasitism; their ecological impact, however, is that of a predator because they kill individual embryos (Kuris 1993; Torchin et al. 1996).

The family Carcinonemertidae comprises two genera, *Carcinonemertes* (Kölliker 1845), which lacks accessory stylets, and *Ovicides* Shields, 2001, the latter with a single species, *O. juliae* Shields, 2001, which has accessory stylets. Ten species of *Carcinonemertes* have been described so far: *C. carcinophila* (Kölliker 1845), with two recognized subspecies, *C. c. carcinophila* (Kölliker 1845) and *C. c. imminuta* Humes, 1942; *C. epialti* Coe, 1902; *C.*

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*mitsukurii* Takakura, 1910; *C. coei* Humes, 1942; *C. errans* Wickham, 1978; *C. regicides* Shields, Wickham & Kuris, 1989; *C. australiensis* Campbell, Gibson & Evans, 1989; *C. humesi* Gibson & Jones, 1990; *C. wickhami* Shields & Kuris, 1990, and *C. pinnotheridophila* McDermott & Gibson, 1993 (Shields 2001).

To date only *Carcinonemertes carcinophila imminuta* has been reported for Brazil, in the portunid crabs *Callinectes danae* Smith and *Callinectes ornatus* Ordway (Santos and Bueno 2001). In the present paper, three new species of the genus *Carcinonemertes* are described, obtained from the crabs *Libinia spinosa* H. Milne Edwards (Decapoda, Pisidae), *Hepatus pudibundus* (Herbst) (Decapoda, Hepatidae) and *Menippe nodifrons* Stimpson (Decapoda, Menippidae) collected on the southeastern coast of Brazil. The locations of these new worms in their respective hosts are presented in detail. The distinctive morphological characters of the new species are compared to the previously described ones; new characters are included in the descriptions and discussed.

### Material and methods

Decapod crustaceans were collected by trawling on the southeastern coast of Brazil along the following beaches: Anchovas Beach, São Sebastião Island, (23°55'S, 45°19'W); Enseada Beach, São Sebastião, (23°43'S, 45°25'W), and Poço Beach, São Sebastião Island, (23°45'S, 45°16'W). Crabs were also collected by hand at Figueira Beach, São Sebastião (23°44'56"S, 45°24'34"W).

Crabs were transported to the nearby laboratory facilities at the Centro de Biologia Marinha, Universidade de São Paulo, São Sebastião, where they were kept alive in tanks with a flow-through seawater system until dissection. Identification of crabs followed Melo (1996).

The exoskeleton surface and the arthrodial membranes of the crabs were macroscopically examined for nemertean. The dorsal carapace was removed to expose the branchial chambers. Gills and pleopods were removed with the aid of forceps and were examined under a dissecting microscope.

The nemerteans were collected from crabs and placed in Petri dishes filled with seawater until the moment of taking measurements, pictures and notes on characters. Nemerteans were relaxed in a 1:1 solution of 7.5% MgCl<sub>2</sub> (prepared with bottled drinking water) and seawater for 15–30 min, after which, length and width of body were determined with the aid of an ocular micrometer in a dissecting microscope. Measurements of internal features were made with the aid of an ocular micrometer in a compound microscope after covering the worms with a coverslip. Photomicrographs were made with a Canon PowerShot A10 digital camera. Holotype and paratypes of each new species are deposited at the Museu de Zoologia da Universidade de São Paulo, Brazil (Abbreviation: MZUSP).

Some mucus sheaths produced by *Carcinonemertes sebastianensis* n. sp were prepared for scanning electron microscopy to obtain more details on their morphology. The mucus sheaths were cleaned in a 1:1 solution of 7.5% MgCl<sub>2</sub> (prepared with bottled drinking water) and seawater and were fixed in a 10% seawater formalin solution. Mucus sheaths were dehydrated in a series of graded ethanol (50, 70, 95, 100 and 100% for 10 min each), dried by the critical point method with CO<sub>2</sub> and coated with gold, then examined with a JEOL 6400 Visions scanning electron microscope.

**Systematics****Family Carcinonemertidae** Sumner, Osburn & Cole, 1913*Diagnosis*

Modified by Shields et al. (1989) from Humes (1942): Monostiliferous hoplonemerteans living as symbionts (egg predators) on the gills, under the abdomen, on the apodemes, and axillae, and in or on the egg masses of decapod crustaceans. Short proboscis, reaching scarcely beyond the posterior end of the muscular portion of the esophagus. Lateral nerves lie internal to the well-developed submuscular glands. Cephalic glands well developed, with cephalic muscle fibers present. Cerebral organs lacking. Takakura's duct system present in males. Internal fertilization and oviparity occur commonly. In most species, adult worms occupy, at least temporarily, mucus sheaths secreted and attached to the setae on the pleopods and hairs of endopodites of ovigerous decapods. Embryos hatch as hoplonemertean larvae.

**Genus Carcinonemertes** (Kölliker, 1845)*Diagnosis*

From Coe (1902) and amended here (*italic*): Nemerteans living *as symbionts (egg predators)* on various species of Crustacea. Proboscis but little developed, very small in size, and extremely short, without lateral pouches of reserve stylets, but armed with central stylet and basis only; anterior proboscis very short, without distinct muscular layers, without distinct nerves, and without a thickened glandular epithelium. Cerebral organs lacking. Two ocelli. Cephalic glands massively developed. Usually oviparous, though fertilization often takes place internally.

***Carcinonemertes divae* new species**

(Figure 1A–F)

*Diagnosis*

Body color varies from translucent white to orange. Two eyes, black. Anterior end of body rounded or pointed; posterior end pointed. Worms 1.3–4.3 mm long. Accessory stylets absent. Ovaries arranged in one row on each side of intestine. Takakura's duct present. Ornamented and filiform mucus sheath attached to pleopods of hosts may be present in adult worms.

*Material examined*

Fourteen females, 16 males and one larva were examined. Holotype: male, from the egg mass of *Libinia spinosa*; type locality: Poço Beach, São Sebastião Island, Brazil, (23°45'S, 45°16'W), 18 Jul 2003; Coll. Cynthia Santos; MZUSP No. 001. Paratype: female, from the egg mass of *L. spinosa*; type locality: Poço Beach, São Sebastião Island, Brazil (23°45'S, 45°16'W); 18 Jul 2003; Coll. Cynthia Santos; MZUSP No. 002.

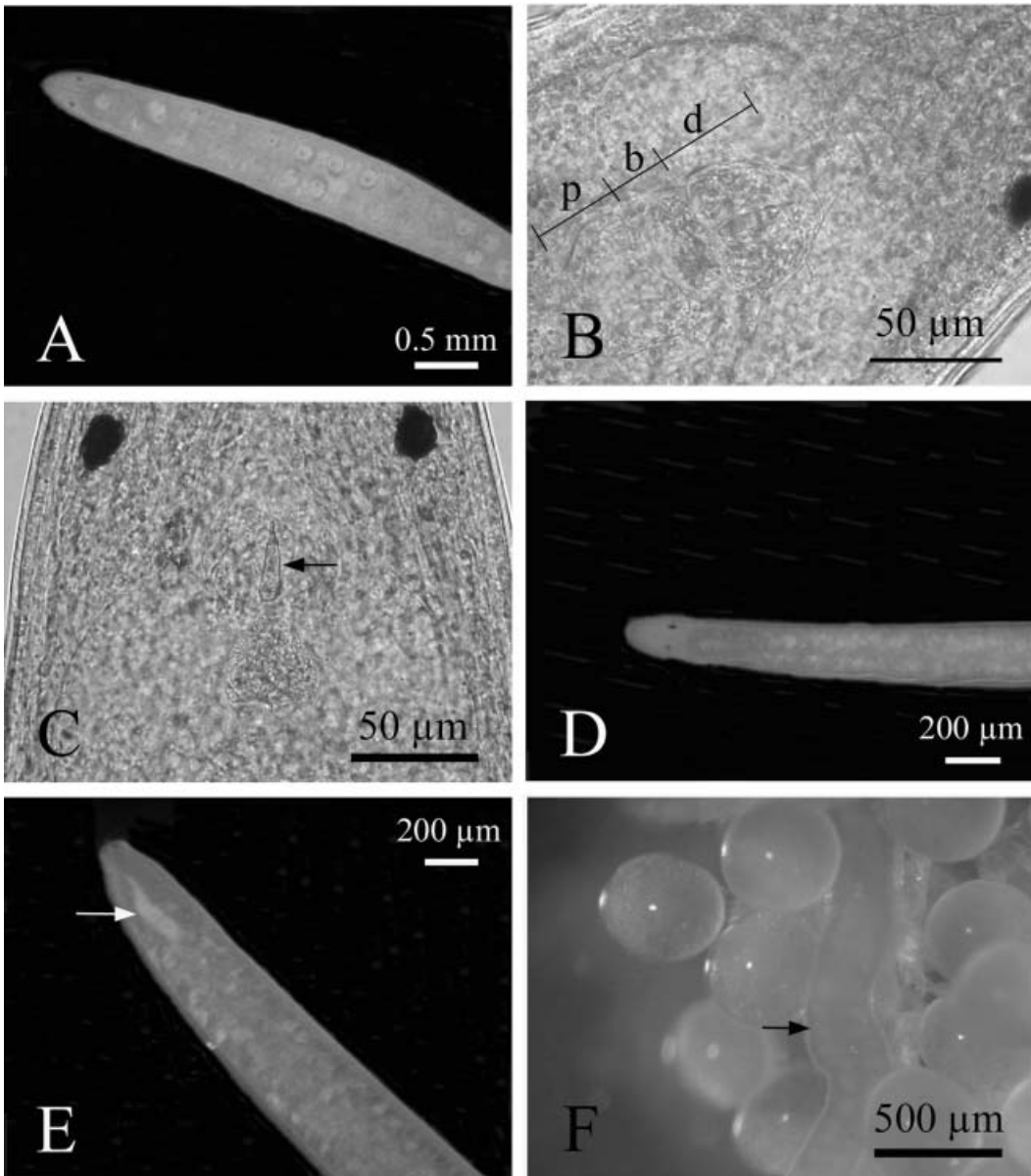


Figure 1. *Carcinonemertes divae* n. sp. (A) Anterior end of female, (B) proboscis aligned in a straight line, (C) stylet and basis (arrow) in a female worm, (D) male with rounded anterior end, (E) pointed posterior end of male, with seminal vesicle, (F) adult female (arrow) on *Libinia spinosa* eggs. Abbreviations: b, proboscis bulb; d, diaphragm; p, posterior proboscis chamber.

### Etymology

The species name is a noun in the genitive singular and honors Dr Diva D. Corrêa, a Brazilian nemertean specialist, formerly from the Universidade de São Paulo, who dedicated almost 40 years of her life to the study of nemerteans and greatly contributed to the knowledge of this phylum.

*Description*

The description is based on living adults and one larva. The latter was obtained from crabs collected at Anchovas Beach. Measurements are given as mean  $\pm$  SE (range, number of specimens observed).

*Female.* Body color varied from translucent white to orange; gut orange; gonads translucent white. Two eyes, black, irregular, circular or elliptical; elliptical eyes the most common shape. Found free among egg mass of host or in a filiform, ornamented mucus sheath attached to the pleopods. Lapilli homogeneously distributed on mucus sheath and  $4 \pm 1 \mu\text{m}$  (3–5  $\mu\text{m}$ ;  $n=2$ ) in height. Anterior end of body rounded or pointed (Figure 1A); posterior end pointed. Dimensions of relaxed worms  $2.6 \pm 0.1 \text{ mm}$  (1.3–3.2 mm;  $n=14$ ) long and  $332 \pm 17 \mu\text{m}$  (200–480  $\mu\text{m}$ ;  $n=14$ ) wide. Eye  $26 \pm 1 \mu\text{m}$  (20–36  $\mu\text{m}$ ;  $n=13$ ) long and  $16 \pm 1 \mu\text{m}$  (10–20  $\mu\text{m}$ ;  $n=13$ ) wide. Distance between eyes  $103 \pm 4 \mu\text{m}$  (80–130  $\mu\text{m}$ ;  $n=12$ ). Distance from eyes to tip of head  $143 \pm 9.5 \mu\text{m}$  (100–204  $\mu\text{m}$ ;  $n=12$ ). Brain  $117 \pm 9 \mu\text{m}$  (92–150  $\mu\text{m}$ ;  $n=7$ ) long and  $57 \pm 3 \mu\text{m}$  (45–70  $\mu\text{m}$ ;  $n=7$ ) wide. Distance from brain to tip of head  $171 \pm 7 \mu\text{m}$  (140–200  $\mu\text{m}$ ;  $n=8$ ). Proboscis aligned in a straight line (Figure 1B). Anterior proboscis chamber  $75 \mu\text{m}$  ( $n=1$ ) long and  $12 \mu\text{m}$  ( $n=1$ ) wide. Diaphragm  $53 \pm 2 \mu\text{m}$  (42–80  $\mu\text{m}$ ;  $n=14$ ) long and  $54 \pm 3 \mu\text{m}$  (45–78  $\mu\text{m}$ ;  $n=14$ ) wide. Single stylet on basis,  $10 \pm 1 \mu\text{m}$  (8–12  $\mu\text{m}$ ;  $n=13$ ) long. Stylet basis  $25 \pm 1 \mu\text{m}$  (22–30  $\mu\text{m}$ ;  $n=14$ ) long and  $7 \pm 0 \mu\text{m}$  (5–8  $\mu\text{m}$ ;  $n=14$ ) wide (Figure 1C). Stylet:basis ratio 0.387 (0.250–0.500;  $n=13$ ). Distance from central stylet to tip of head  $221 \pm 20 \mu\text{m}$  (130–400  $\mu\text{m}$ ;  $n=12$ ). Accessory stylets absent. Proboscis bulb  $36 \pm 2 \mu\text{m}$  (22–48  $\mu\text{m}$ ;  $n=12$ ) long and  $45 \pm 2 \mu\text{m}$  (38–56  $\mu\text{m}$ ;  $n=14$ ) wide. Posterior proboscis  $57 \pm 6 \mu\text{m}$  (30–72  $\mu\text{m}$ ;  $n=6$ ) long and  $51 \pm 5 \mu\text{m}$  (35–68  $\mu\text{m}$ ;  $n=7$ ) wide. Ovaries arranged in one row on each side of the intestinal diverticula. Number of ovaries  $31 \pm 4$  (13–53;  $n=11$ ). Distance from first gonad to tip of head  $428 \pm 17 \mu\text{m}$  (348–540  $\mu\text{m}$ ;  $n=12$ ).

*Male.* Body color cream; gut orange; gonads translucent white. Two eyes, black, irregular, circular or elliptical; elliptical eyes the most common shape. Found free among egg mass of host or in a filiform, ornamented mucus sheath attached to the pleopods. Lapilli homogeneously distributed on mucus sheath and  $4 \mu\text{m}$  ( $n=1$ ) in height. Anterior end of body rounded or pointed (Figure 1D), posterior end pointed (Figure 1E). Dimensions of relaxed worms  $2.6 \pm 0.2 \text{ mm}$  (1.3–4.3 mm;  $n=14$ ) long and  $316 \pm 32 \mu\text{m}$  (170–720  $\mu\text{m}$ ;  $n=16$ ) wide. Eye  $29 \pm 2 \mu\text{m}$  (22–48  $\mu\text{m}$ ;  $n=16$ ) long and  $17 \pm 1 \mu\text{m}$  (10–25  $\mu\text{m}$ ;  $n=16$ ) wide. Distance between eyes  $96 \pm 3 \mu\text{m}$  (75–112  $\mu\text{m}$ ;  $n=15$ ). Distance from eyes to tip of head  $160 \pm 10 \mu\text{m}$  (110–230  $\mu\text{m}$ ;  $n=16$ ). Brain  $118 \pm 9 \mu\text{m}$  (90–175  $\mu\text{m}$ ;  $n=9$ ) long and  $52 \pm 3 \mu\text{m}$  (45–70  $\mu\text{m}$ ;  $n=7$ ) wide. Distance from brain to tip of head  $212 \pm 5 \mu\text{m}$  (200–260  $\mu\text{m}$ ;  $n=13$ ). Proboscis aligned in a straight line. Anterior proboscis chamber  $46 \pm 6 \mu\text{m}$  (40–52  $\mu\text{m}$ ;  $n=2$ ) long and  $18 \mu\text{m}$  ( $n=2$ ) wide. Diaphragm  $48 \pm 1 \mu\text{m}$  (45–55  $\mu\text{m}$ ;  $n=14$ ) long and  $50 \pm 2 \mu\text{m}$  (38–60  $\mu\text{m}$ ;  $n=14$ ) wide. Single stylet on basis  $10 \pm 0 \mu\text{m}$  (8–12  $\mu\text{m}$ ;  $n=13$ ) long. Stylet basis  $28 \pm 1 \mu\text{m}$  (22–30  $\mu\text{m}$ ;  $n=15$ ) long and  $7 \pm 0 \mu\text{m}$  (5–10  $\mu\text{m}$ ;  $n=15$ ) wide. Stylet:basis ratio 0.371 (0.250–0.500;  $n=13$ ). Distance from central stylet to tip of head  $231 \pm 14 \mu\text{m}$  (160–310  $\mu\text{m}$ ;  $n=13$ ). Accessory stylets absent. Proboscis bulb  $35 \pm 2 \mu\text{m}$  (25–48  $\mu\text{m}$ ;  $n=13$ ) long and  $39 \pm 2 \mu\text{m}$  (25–48  $\mu\text{m}$ ;  $n=13$ ) wide. Posterior proboscis  $72 \pm 11 \mu\text{m}$  (40–100  $\mu\text{m}$ ;  $n=5$ ) long and  $48 \pm 1 \mu\text{m}$  (25–50  $\mu\text{m}$ ;  $n=6$ ) wide. Distance from first gonad to tip of head  $475 \pm 9 \mu\text{m}$  (370–500  $\mu\text{m}$ ;  $n=12$ ). Seminal vesicle (Figure 1E) easily visible under stereomicroscope in the majority of adult male worms.

*Larva.* Body ciliated with anterior and posterior ciliary tuft. Body shape spherical or ovoid. Two eyes. Length 110  $\mu\text{m}$  ( $n=1$ ). Width 70  $\mu\text{m}$  ( $n=1$ ). Anterior tuft 20  $\mu\text{m}$  ( $n=1$ ) in length. Posterior tuft 24  $\mu\text{m}$  ( $n=1$ ) in length. Eye 12  $\mu\text{m}$  ( $n=1$ ) long and 6  $\mu\text{m}$  ( $n=1$ ) wide. Distance between eyes 22  $\mu\text{m}$  ( $n=1$ ). Distance from eyes to tip of head 33  $\mu\text{m}$  ( $n=1$ ).

#### *Infestation site*

Adult worms were found in the host's egg mass (Figure 1F) (ovigerous females with eggs in initial, intermediate and final stages of development). Immature worms were found on the abdomen of juvenile male crabs; on the abdomen and at the arthrodistal membrane of pereopods of juvenile female crabs; at the base of pereopods, on the ventral and dorsal sides of the abdomen of adult male crabs; on the ventral and dorsal sides of the abdomen of non-ovigerous adult female crabs; at the base of pleopods, on the abdomen and on the eggs of ovigerous females; at the base of pleopods and on the ventral side of the abdomen of post-ovigerous females.

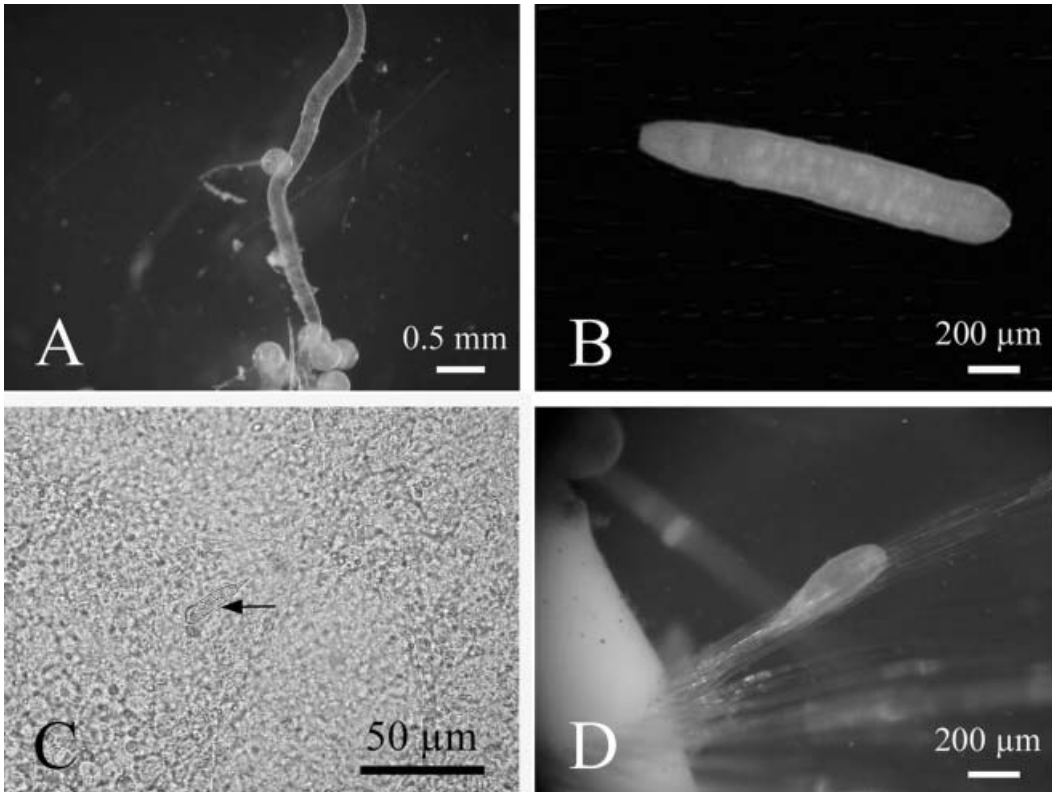


Figure 2. *Carcinonemertes caissarum* n. sp. (A) Filiform mucus sheath of adult worm; (B) male with rounded posterior end; (C) stilet and basis (arrow) in a male worm; (D) juvenile worm encysted on pleopod setae of *Hepatus pudibundus*.



***Carcinonemertes caissarum* new species**

(Figure 2A–D)

*Diagnosis*

Body color varies from translucent white to cream; male with a red spot at posterior end. Two eyes, black. Anterior end of body rounded, posterior end rounded or truncated (males). Worms 1.2–11.0 mm long. Accessory stylets absent. Ovaries arranged in one row on each side of intestine. Takakura's duct present. Ornamented and filiform mucus sheath may be present in adult worms.

*Material examined*

Nine females and eleven males were examined. Holotype: female, from the egg mass of *Hepatus pudibundus*; type locality: Poço Beach, São Sebastião Island, Brazil, (23°45'S, 45°16'W), 16 September 2002; Coll. Cynthia Santos; MZUSP No. 003. Paratype: female, from the egg mass of *H. pudibundus*; type locality Poço Beach, São Sebastião Island, Brazil, (23°45'S, 45°16'W), 16 September 2002; Coll. Cynthia Santos; MZUSP No. 004.

*Etymology*

The species name is a noun in the genitive plural and is in recognition of the human communities known as “caiçaras” (a tupi-guarani word), which are found in the localities where the crabs were collected. The people of these communities depend mainly on fisheries; therefore, this name honors the fishermen who helped us collect, Rogério dos Santos Júnior and his father, Rogério dos Santos.

*Description*

The description is based on living adults. Measurements are given in mean  $\pm$  SE (range, number of specimens observed).

*Female.* Body color varied from translucent white to cream; gut varied from orange to brownish; gonads translucent white. Two eyes, black, round or cup-shaped; round eyes the most common shape. Found free among egg mass of host or in a filiform, ornamented mucus sheath (Figure 2A). Lapilli  $7 \pm 0 \mu\text{m}$  (7–8  $\mu\text{m}$ ;  $n=2$ ) in height, larger at center of sheath and smaller and scarce at extremities. Anterior and posterior end of body rounded. Dimensions of relaxed worms  $5.5 \pm 1.0 \text{ mm}$  (2.6–11.0 mm;  $n=7$ ) long and  $282 \pm 20 \mu\text{m}$  (210–376  $\mu\text{m}$ ;  $n=8$ ) wide. Eye  $11 \pm 9 \mu\text{m}$  (8–15  $\mu\text{m}$ ;  $n=9$ ) long and  $9 \pm 1 \mu\text{m}$  (5–12  $\mu\text{m}$ ;  $n=9$ ) wide. Distance between eyes  $112 \pm 11 \mu\text{m}$  (75–160  $\mu\text{m}$ ;  $n=7$ ). Distance from eyes to tip of head  $115 \pm 6 \mu\text{m}$  (100–155  $\mu\text{m}$ ;  $n=9$ ). Brain  $97 \pm 6 \mu\text{m}$  (88–120  $\mu\text{m}$ ;  $n=5$ ) long and  $46 \pm 2 \mu\text{m}$  (40–52  $\mu\text{m}$ ;  $n=7$ ) wide. Distance from brain to tip of head  $132 \pm 6 \mu\text{m}$  (120–160  $\mu\text{m}$ ;  $n=6$ ). Anterior proboscis chamber could not be distinguished. Diaphragm  $38 \pm 2 \mu\text{m}$  (30–48  $\mu\text{m}$ ;  $n=8$ ) long and  $46 \pm 2 \mu\text{m}$  (38–55  $\mu\text{m}$ ;  $n=7$ ) wide. Single stylet on basis  $8 \pm 1 \mu\text{m}$  (5–10  $\mu\text{m}$ ;  $n=8$ ) long. Stylet basis  $22 \pm 1 \mu\text{m}$  (20–25  $\mu\text{m}$ ;  $n=9$ ) long and  $7 \pm 0 \mu\text{m}$  (5–8  $\mu\text{m}$ ;  $n=9$ ) wide. Stylet:basis ratio 0.378 (0.250–0.500;  $n=8$ ). Distance from central stylet to tip of head  $160 \pm 8 \mu\text{m}$  (122–190  $\mu\text{m}$ ;  $n=8$ ). Accessory stylets absent. Proboscis bulb  $26 \pm 2 \mu\text{m}$  (20–38  $\mu\text{m}$ ;  $n=8$ ) long and  $30 \pm 2 \mu\text{m}$  (22–38  $\mu\text{m}$ ;  $n=8$ ) wide.

Posterior proboscis  $70 \pm 4 \mu\text{m}$  ( $55\text{--}88 \mu\text{m}$ ;  $n=4$ ) long and  $59 \pm 4 \mu\text{m}$  ( $52\text{--}68 \mu\text{m}$ ;  $n=4$ ) wide. Ovaries arranged in one row on each side of the intestinal diverticula. Number of ovaries  $158 \pm 30$  ( $76\text{--}252$ ;  $n=5$ ). Distance from first gonad to tip of head  $568 \pm 56 \mu\text{m}$  ( $430\text{--}900 \mu\text{m}$ ;  $n=8$ ).

*Male.* Body color cream with a red spot at the posterior end; gut varied from orange to brownish; gonads translucent white. Two eyes, black, irregular, circular or cup-shaped. Found free among egg mass of host or in a filiform, ornamented mucus sheath. Lapilli  $4 \mu\text{m}$  ( $n=1$ ) in height. Anterior end of body rounded, posterior end rounded (Figure 2B) or truncated. Dimensions of relaxed worms  $2.0 \pm 0.3 \text{ mm}$  ( $1.2\text{--}4.0 \text{ mm}$ ;  $n=9$ ) long and  $400 \pm 25 \mu\text{m}$  ( $230\text{--}520 \mu\text{m}$ ;  $n=11$ ) wide. Eye  $11 \pm 1 \mu\text{m}$  ( $8\text{--}15 \mu\text{m}$ ;  $n=10$ ) long and  $10 \pm 0 \mu\text{m}$  ( $8\text{--}12 \mu\text{m}$ ;  $n=10$ ) wide. Distance between eyes  $107 \pm 9 \mu\text{m}$  ( $62\text{--}150 \mu\text{m}$ ;  $n=8$ ). Distance from eyes to tip of head  $106 \pm 7 \mu\text{m}$  ( $60\text{--}130 \mu\text{m}$ ;  $n=10$ ). Brain  $110 \pm 6 \mu\text{m}$  ( $86\text{--}125 \mu\text{m}$ ;  $n=8$ ) long and  $54 \pm 4 \mu\text{m}$  ( $38\text{--}75 \mu\text{m}$ ;  $n=8$ ) wide. Distance from brain to tip of head  $135 \pm 9 \mu\text{m}$  ( $80\text{--}170 \mu\text{m}$ ;  $n=8$ ). Anterior proboscis chamber could not be distinguished. Diaphragm  $42 \pm 4 \mu\text{m}$  ( $30\text{--}60 \mu\text{m}$ ;  $n=7$ ) long and  $42 \pm 2 \mu\text{m}$  ( $32\text{--}50 \mu\text{m}$ ;  $n=7$ ) wide. Single stylet on basis  $8 \pm 1 \mu\text{m}$  ( $5\text{--}10 \mu\text{m}$ ;  $n=9$ ) long. Stylet basis  $21 \pm 1 \mu\text{m}$  ( $18\text{--}25 \mu\text{m}$ ;  $n=10$ ) long and  $7 \pm 0 \mu\text{m}$  ( $5\text{--}8 \mu\text{m}$ ;  $n=9$ ) wide (Figure 2C). Stylet:basis ratio  $0.372$  ( $0.250\text{--}0.571$ ;  $n=9$ ). Distance from central stylet to tip of head  $172 \pm 13 \mu\text{m}$  ( $110\text{--}216 \mu\text{m}$ ;  $n=8$ ). Accessory stylets absent. Proboscis bulb  $22 \pm 2 \mu\text{m}$  ( $20\text{--}25 \mu\text{m}$ ;  $n=2$ ) long and  $30 \mu\text{m}$  ( $n=1$ ) wide. Posterior proboscis could not be observed. Distance from first gonad to tip of head  $325 \pm 46 \mu\text{m}$  ( $230\text{--}510 \mu\text{m}$ ;  $n=5$ ). Seminal vesicle easily visible under stereomicroscope in the majority of adult male worms.

#### *Infestation site*

Adult worms were found in the host's egg mass (ovigerous females with eggs in initial, intermediate and final stages of development). Immature worms were found on the ventral side of the abdomen of adult male crabs; on the abdomen, at the gonopores, at the base of pleopods and encysted on the setae of pleopods (Figure 2D) of non-ovigerous adult female crabs; encysted on the setae of pleopods of ovigerous females; on the abdomen, at the gonopores, on the arthrodistal membrane of pleopods, and encysted on the setae of pleopods of post-ovigerous females.

### ***Carcinonemertes sebastianensis* new species**

(Figures 3, 4A–E and 5A, B)

#### *Diagnosis*

Body color translucent white. Two eyes, brown. Anterior and posterior end of body rounded. Worms  $2.0\text{--}11.0 \text{ mm}$  long. Accessory stylets absent. Ovaries arranged in one row on each side of intestine. Distance from first ovary to tip of head  $1250\text{--}2000 \mu\text{m}$ . Takakura's duct present. Ornamented and filiform mucus sheath may be present in adult worms.

#### *Material examined*

Four females, six males, 12 eggs and one larva were examined. Holotype: male, from the egg mass of *Menippe nodifrons*; type locality: Figueira Beach, São Sebastião, Brazil,

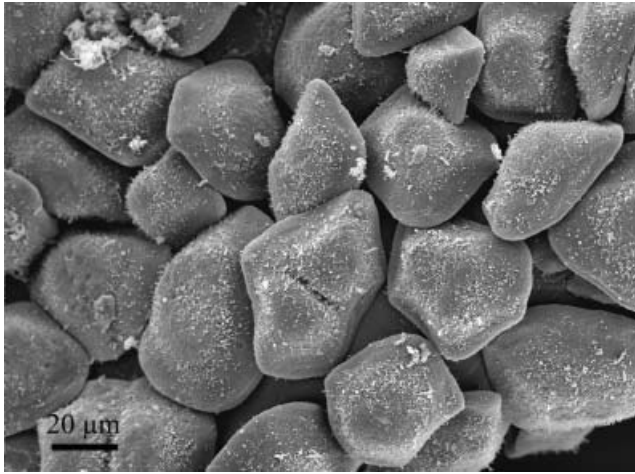


Figure 3. Scanning electron micrograph of the lapilli of the mucus sheath produced by the adults of *Carcinonemertes sebastianensis* n. sp.

(23°44'56"S, 45°24'34"W); 22 January 2004; Coll. Cynthia Santos; MZUSP No. 005. Paratype: female, from the egg mass of *M. nodifrons*; type locality: Figueira Beach, São Sebastião, Brazil, (23°44'56"S, 45°24'34"W); 22 January 2004; Coll. Cynthia Santos; MZUSP No. 006.

#### *Etymology*

The species name is an adjective in feminine singular and is in recognition of the locality where the host crab was found, São Sebastião.

#### *Description*

The description is based on living adults, eggs and larva. Measurements are given in mean  $\pm$  SE (range, number of specimens observed).

*Female.* Body color translucent white; gut varied from orange to brownish; gonads translucent white. Two eyes, brown, circular or elliptical. Found free among egg mass of host or in a filiform, ornamented mucus sheath. Irregular lapilli on the mucus sheath (Figure 3); lapilli with concentric lines (Figure 4A). Anterior (Figure 4B) and posterior end of body rounded. Dimensions of relaxed worms were  $7.0 \pm 4.0$  mm (3.0–11.0 mm;  $n=2$ ) long and  $405 \pm 62$   $\mu$ m (230–500  $\mu$ m;  $n=4$ ) wide. Eye  $14 \pm 2$   $\mu$ m (10–18  $\mu$ m;  $n=4$ ) long and  $9 \pm 1$   $\mu$ m (8–12  $\mu$ m;  $n=4$ ) wide. Distance between eyes  $131 \pm 14$   $\mu$ m (102–160  $\mu$ m;  $n=4$ ). Distance from eyes to tip of head  $110 \pm 18$   $\mu$ m (70–150  $\mu$ m;  $n=4$ ). Brain  $99 \pm 6$   $\mu$ m (88–110  $\mu$ m;  $n=3$ ) long and  $64 \pm 4$   $\mu$ m (58–72  $\mu$ m;  $n=3$ ) wide. Distance from brain to tip of head  $150 \pm 23$   $\mu$ m (100–210  $\mu$ m;  $n=4$ ). Proboscis bent on itself. Anterior proboscis chamber could not be distinguished. Diaphragm  $51 \pm 2$   $\mu$ m (48–58  $\mu$ m;  $n=4$ ) long and  $56 \pm 3$   $\mu$ m (50–62  $\mu$ m;  $n=4$ ) wide. Single stylet on basis  $9 \pm 1$   $\mu$ m (8–10  $\mu$ m;  $n=4$ ) long. Stylet basis  $22 \pm 1$   $\mu$ m (20–25  $\mu$ m;  $n=4$ ) long and  $8 \pm 1$   $\mu$ m (8–10  $\mu$ m;  $n=4$ ) wide (Figure 4C). Stylet:basis ratio 0.416 (0.375–0.444;  $n=4$ ). Distance from central stylet to tip of head  $208 \pm 28$   $\mu$ m (170–290  $\mu$ m;  $n=4$ ). Accessory stylets absent. Proboscis bulb

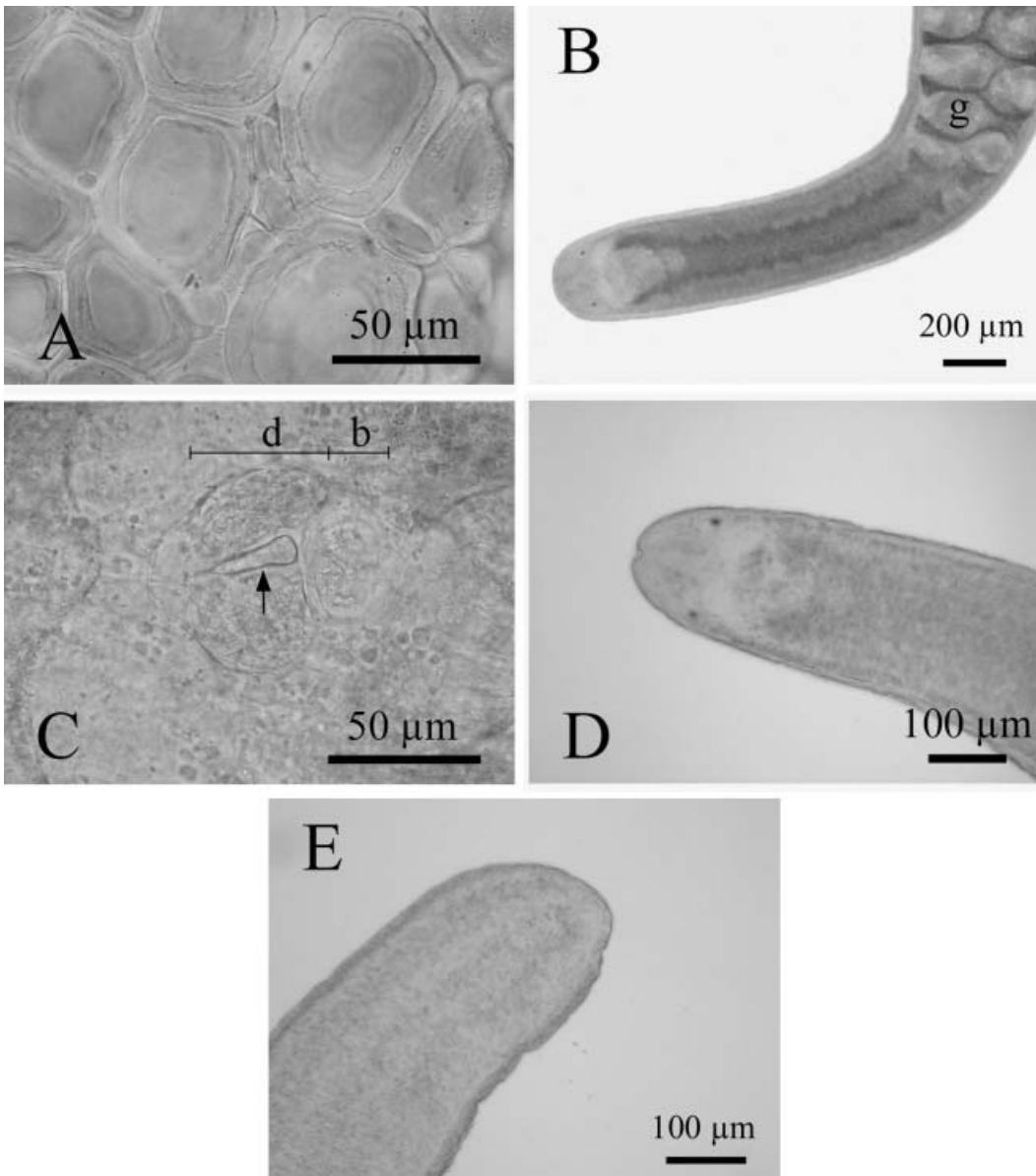


Figure 4. *Carcinonemertes sebastianensis* n. sp. (A) Detail of lapilli of adult mucus sheath. (B) Rounded anterior end of female, showing the great distance from first gonad to tip of head. (C) Stylet and basis (arrow) in a female worm. (D) Anterior end of male. (E) Posterior end of male. Abbreviations: b, proboscis bulb; d, diaphragm; g, gonad.

$34 \pm 4 \mu\text{m}$  (28–42  $\mu\text{m}$ ;  $n=4$ ) long and  $38 \pm 4 \mu\text{m}$  (30–42  $\mu\text{m}$ ;  $n=4$ ) wide. Posterior proboscis could not be visualized. Ovaries arranged in one row on each side of the intestinal diverticula. Distance from first gonad to tip of head  $1700 \pm 167 \mu\text{m}$  (1250–2000  $\mu\text{m}$ ;  $n=4$ ) (Figure 4B).

*Male.* Body color translucent white; gut yellow; gonads translucent white. Two eyes, brown, circular or elliptical; elliptical eyes the most common shape. Found free among

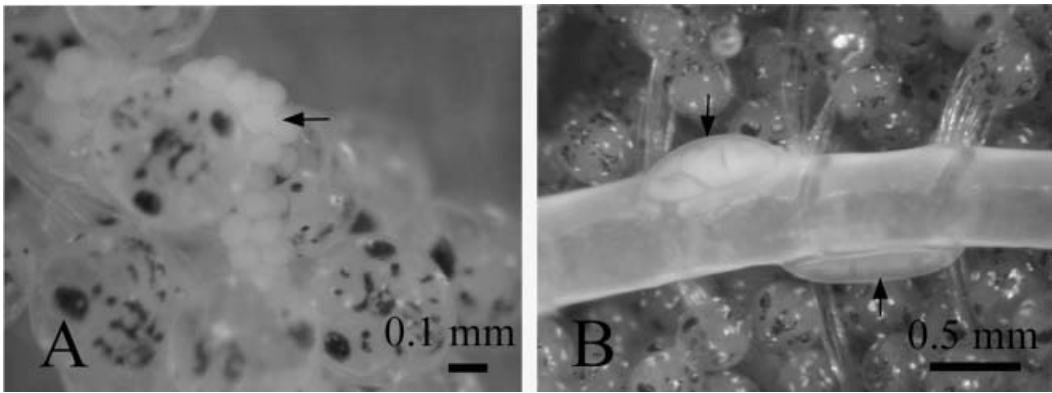


Figure 5. *Carcinonemertes sebastianensis* n. sp. (A) Egg strand (arrow) on *Menippe nodifrons* eggs. (B) Juvenile worms encysted (arrows) on the axis of the pleopod of *Menippe nodifrons*.

egg mass of host or in a filiform, ornamented mucus sheath. Irregular lapilli on mucus sheath (Figure 3); lapilli with concentric lines (Figure 4A). Anterior and posterior end of body rounded (Figure 4D, E). Dimensions of relaxed worms  $6.2 \pm 1.4$  mm (2.0–11.0 mm;  $n=6$ ) long and  $362 \pm 28$   $\mu$ m (280–480  $\mu$ m;  $n=6$ ) wide. Eye  $15 \pm 1$   $\mu$ m (12–18  $\mu$ m;  $n=6$ ) long and  $10 \pm 1$   $\mu$ m (5–12  $\mu$ m;  $n=6$ ) wide. Distance between eyes  $110 \pm 5$   $\mu$ m (90–120  $\mu$ m;  $n=6$ ). Distance from eyes to tip of head  $112 \pm 11$   $\mu$ m (80–150  $\mu$ m;  $n=6$ ). Brain  $101 \pm 10$   $\mu$ m (75–125  $\mu$ m;  $n=5$ ) long and  $52 \pm 3$   $\mu$ m (42–62  $\mu$ m;  $n=5$ ) wide. Distance from brain to tip of head  $150 \pm 13$   $\mu$ m (112–200  $\mu$ m;  $n=6$ ). Proboscis bent in on itself. Anterior proboscis chamber could not be distinguished. Diaphragm  $46 \pm 2$   $\mu$ m (40–50  $\mu$ m;  $n=6$ ) long and  $48 \pm 1$   $\mu$ m (45–50  $\mu$ m;  $n=6$ ) wide. Single stylet on basis  $11 \pm 0$   $\mu$ m (10–12  $\mu$ m;  $n=6$ ) long. Stylet basis  $25 \pm 1$   $\mu$ m (22–30  $\mu$ m;  $n=6$ ) long and  $10 \pm 0$   $\mu$ m (8–10  $\mu$ m;  $n=6$ ) wide (Figure 4C). Stylet:basis ratio 0.431 (0.333–0.556;  $n=6$ ). Distance from central stylet to tip of head  $206 \pm 24$   $\mu$ m (150–280  $\mu$ m;  $n=5$ ). Accessory stylets absent. Proboscis bulb  $30 \pm 2$   $\mu$ m (25–38  $\mu$ m;  $n=6$ ) long and  $36 \pm 2$   $\mu$ m (32–40  $\mu$ m;  $n=6$ ) wide. Posterior proboscis  $128 \pm 18$   $\mu$ m (100–160  $\mu$ m;  $n=3$ ) long and  $43 \pm 9$   $\mu$ m (25–55  $\mu$ m;  $n=3$ ) wide. Seminal vesicle not visible under stereomicroscope, but could be seen under compound microscope in some specimens.

*Egg.* Color white. Egg strings (Figure 5A) found in a firm and transparent sheath. Developing eggs  $86 \pm 2$   $\mu$ m in diameter (75–100  $\mu$ m;  $n=12$ ). Egg strings  $232 \pm 14$   $\mu$ m (180–260  $\mu$ m;  $n=5$ ) wide. Number of eggs per egg string  $386 \pm 66$  (320–451;  $n=2$ ). Number of rows of eggs per egg string  $3 \pm 0$  ( $n=5$ ).

*Larva.* Body ciliated with anterior and posterior ciliary tufts. Body shape ovoid. Two eyes. Body length 92  $\mu$ m ( $n=1$ ). Body width 62  $\mu$ m ( $n=1$ ).

#### *Infestation site*

Adult worms were found in the host's egg mass (ovigerous females with eggs in initial, intermediate and final stages of development). All immature worms were found encysted in a mucus sheath without ornamentation in the following sites: on the ventral side of the abdomen, on the setae that follow the border of the abdomen, on the central axis of

pleopods (Figure 5B), and on the setae of pleopods of non-ovigerous adult female crabs; on the ventral side of the abdomen, on the central axis of pleopods, and on the setae of pleopods of ovigerous females; at the ventral side of the abdomen, on the central axis of pleopods, and on the setae of pleopods of post-ovigerous females. No immature worms were found on male crabs.

## Discussion

The three species described here are gonochoric, do not present accessory stylets and, thereby, conform to the diagnosis of Carcinonemertidae provided by Humes (1942) and modified by Shields et al. (1989) and to the diagnosis of *Carcinonemertes* provided by Coe (1902). The genus *Ovicides* is characterized by being hermaphroditic and by having two accessory stylets (Shields 2001).

*Carcinonemertes divae* n. sp., *C. caissarum* n. sp. and *C. sebastianensis* n. sp. can be distinguished from each other by color of the body, shape of the posterior region of body, eye length, and by distance between the first ovary and tip of head (Table I).

*Carcinonemertes pinnotheridophila* is found on the floor and wall of branchial chambers as well as on eggs of its hosts (McDermott and Gibson 1993). Juvenile worms of *C. c. carcinophila*, *C. c. imminuta* and *C. mitsukurii* are found between the gills of their hosts (Humes 1942; Shields 1992; Santos and Bueno 2001; Table II). None of the three species described here was found in the branchial chamber or between the gills of their hosts.

The three species described here also can be distinguished from *C. pinnotheridophila* by the presence of eyes in the adult worms, shape of the egg mucus sheath and by the number of rows of ovaries on each side of the intestine (McDermott and Gibson 1993) (Table II). *Carcinonemertes coei* also has two rows of ovaries on each side of the intestine (Humes 1942; Table III).

*Carcinonemertes divae* n. sp., *C. caissarum* n. sp. and *C. sebastianensis* n. sp. can be distinguished from *C. c. carcinophila* by body length (Humes 1942), and from *C. c. imminuta* by body length and by eye width (Humes 1942; Table II).

Adult worms of *C. errans* do not have a mucus sheath (Wickham 1978), whereas adult worms of *C. regicides* have a mucus sheath with no lapilli (Shields et al. 1989). The adult worms of the three species described here have a mucus sheath with lapilli, which are, according to Humes (1942), irregularly shaped, raised concretions, present on the surface of the sheath. In addition, *C. errans* can be distinguished from *C. divae* n. sp., *C. caissarum* n. sp. and *C. sebastianensis* n. sp. by diaphragm length and by stylet basis length (personal

Table I. Morphological and ecological differences that distinguish the species *Carcinonemertes divae* n. sp., *Carcinonemertes caissarum* n. sp. and *Carcinonemertes sebastianensis* n. sp. (measurements in  $\mu\text{m}$ ).

Characters	<i>C. divae</i> n. sp.	<i>C. caissarum</i> n. sp.	<i>C. sebastianensis</i> n. sp.
Body color	White to orange	White to cream, red spot (male)	White
Shape of posterior end of body	Pointed	Truncated on males	Rounded
Eye length	20–48	8–15	10–18
Distance 1st ovary–tip of head	348–540	430–900	1250–2000
Infestation site	e, ab, per, pl	e, ab, gon, pl, sepl	e, ab, seab, axpl, sepl

Abbreviations for infestation site: ab, abdomen; axpl, axis of pleopod; bc, branchial chamber; e, eggs; gl, gill lamellae; gon, gonopores; per, pereopods; pl, pleopods; seab, setae of abdomen; sepl, setae of pleopods.

Table II. Morphological and ecological differences that distinguish the species *Carcinonemertes carcinophila*, *Carcinonemertes carcinophila imminuta*, *Carcinonemertes mitsukurii* and *Carcinonemertes pinnotheridophila* from the species described here (body length in mm; other measurements in  $\mu\text{m}$ ).

Characters	<i>C.</i>						
	<i>C. c. carcinophila</i>	<i>C. c. imminuta</i>	<i>C. mitsukurii</i>	<i>C. pinnotheridophila</i>	<i>C. divae</i> n. sp.	<i>C. caissarum</i> n. sp.	<i>C. sebastianensis</i> n. sp.
Body length	20.0–70.0	4.0–15.0	1.0–16.0	0.9–15.0	1.3–4.3	1.2–11.0	2.0–11.0
Eye width	?	20–70	?	eyeless	10–25	5–12	5–12
Number of rows of ovaries	1	1	1	2	1	1	1
Shape of egg mucus sheath	Filiform	Filiform	Filiform	Oval	Filiform	Filiform	Filiform
Infestation site	e, gl	e, gl	e, gl	e, bc	e, ab, per, pl	e, ab, gon, pl, sepl	e, ab, seab, axpl, sepl

Abbreviations for infestation site: ab, abdomen; axpl, axis of pleopod; bc, branchial chamber; e, eggs; gl, gill lamellae; gon, gonopores; per, pereopods; pl, pleopods; seab, setae of abdomen; sepl, setae of pleopods.

observation; Table III). The stylet basis in *C. regicides* is 38–44  $\mu\text{m}$  long (Shields et al. 1989; Table III).

*Carcinonemertes australiensis* can be distinguished from *C. divae* n. sp., *C. caissarum* n. sp. and *C. sebastianensis* n. sp. by stylet basis length, stylet basis width and by stylet length (Campbell et al. 1989; Table III).

The stylet basis is 10–12  $\mu\text{m}$  wide (Gibson and Jones 1990) in *C. humesi* and 14  $\mu\text{m}$  (Shields and Kuris 1990) in *C. wickhami*. The latter also differs from *C. divae* n. sp., *C. caissarum* n. sp. and *C. sebastianensis* n. sp. in diaphragm length, stylet basis length, stylet length and in stylet:basis ratio (Shields and Kuris 1990; Table IV). Stylet:basis ratio in *C. humesi* varies from 0.219 to 0.267 (Gibson and Jones 1990).

*Carcinonemertes divae* n. sp., *C. caissarum* n. sp. and *C. sebastianensis* n. sp. can be distinguished from *C. epialti* by diaphragm width (personal observation; Table IV). *Carcinonemertes epialti* differs from *C. divae* new species in the alignment of proboscis and in

Table III. Morphological and ecological differences that distinguish the species *Carcinonemertes coei*, *Carcinonemertes errans*, *Carcinonemertes regicides* and *Carcinonemertes australiensis* from the species described here (measurements in  $\mu\text{m}$ ).

Characters	<i>C.</i>						
	<i>C. coei</i>	<i>C. errans</i>	<i>C. regicides</i>	<i>C. australiensis</i>	<i>C. divae</i> n. sp.	<i>C. caissarum</i> n. sp.	<i>C. sebastianensis</i> n. sp.
Presence of mucus sheath	Present	Absent	Present	?	Present	Present	Present
Mucus sheath ornamentation	?	N/A	No lapilli	?	Ornamented	Ornamented	Ornamented
Diaphragm length	?	62–125	64–96	75	42–80	30–60	40–58
Stylet basis length	21–23	30–40	38–44	40	22–30	18–25	20–30
Stylet basis width	6–8	10–13	9–17	15–16	5–10	5–8	8–10
Stylet length	8–9	12 (no variation)	14–19	15–18	8–12	5–10	8–12
Number of rows of ovaries	2	1	1	1	1	1	1

Table IV. Morphological and ecological differences that distinguish the species *Carcinonemertes wickhami*, *Carcinonemertes humesi* and *Carcinonemertes epialti* from the species described here (measurements in  $\mu\text{m}$ ).

Characters	<i>C. wickhami</i>	<i>C. humesi</i>	<i>C. epialti</i>	<i>C. divae</i> n. sp.	<i>C. caissarum</i> n. sp. <i>C. sebastianensis</i> n. sp.	
					<i>C. caissarum</i> n. sp.	<i>C. sebastianensis</i> n. sp.
Eye length	34–39	?	15–28	20–48	8–15	10–18
Distance eyes–tip of head	126–196	?	136–200	100–230	60–155	70–150
Proboscis alignment	?	Curved	Curved	Straight line	?	?
Diaphragm length	70–112	?	50–93	42–80	30–60	40–58
Diaphragm width	56–87	?	62–112	38–78	32–55	45–62
Stylet basis length	36–42	30–32	21–23	22–30	18–25	20–30
Stylet basis width	14	10–12	5–8	5–10	5–8	8–10
Stylet length	19–20	7–8	10–15	8–12	5–10	8–12
Style:basis ratio	0.476–0.528	0.219–0.267	0.333–0.500	0.250–0.500	0.250–0.571	0.333–0.556

the eye length (Coe 1902; Table IV). The eye is 8–15  $\mu\text{m}$  long in *C. caissarum* n. sp. and 10–18  $\mu\text{m}$  long in *C. sebastianensis* n. sp. *Carcinonemertes epialti* also can be distinguished from *C. caissarum* n. sp. by stylet length (personal observation) and from *C. sebastianensis* new species by the distance between the eyes and tip of head (personal observation; Table IV).

Juvenile worms of some nemerteans of the genus *Carcinonemertes* were previously found on the pleopods of their hosts (at the base or on the arthroal membranes). A novelty regarding the infestation site by nemerteans of the genus *Carcinonemertes* is registered in the present study for *C. sebastianensis* n. sp., whose juveniles can be found encysted on setae of pleopods (a site also used by juveniles of *C. caissarum* n. sp.) or on the axis of pleopods, or even on setae that follow the border of the abdomen of the hosts.

In addition to the diagnostics mentioned above, the three new species differ from other carcinonemertids in that they infest, respectively, *L. spinosa*, *H. pudibundus* and *M. nodifrons*, all registered here for the first time as hosts for nemerteans of the genus *Carcinonemertes*.

Specimens of *H. epheliticus* and *M. mercenaria* collected at Grand Isle, Louisiana, USA, were registered by Humes (1942) as hosts for *C. c. imminuta*. Humes (1942) found juvenile worms on gills of *H. epheliticus*, which makes us believe he could be right about the identification of these specimens of *Carcinonemertes*. Nevertheless, the following facts suggest that there is a chance the worms found in *H. epheliticus* and *M. mercenaria* were misidentified as *C. c. imminuta*: (1) only one worm was found on the gill of *H. epheliticus* from Louisiana (Humes 1942); this infestation could be casual, not a regular pattern; (2) an adult male of *H. epheliticus* was collected in Florida and none of the 90 juvenile worms infesting the crab was found on gills (personal observation); (3) Humes (1942) did not find any worms on the gills of *M. mercenaria* and stated that “in the non-portunid species infested at Grand Isle, the worms were all minute, though were sexually mature”; it seems that the worms found in *H. epheliticus* and in *M. mercenaria* by Humes (1942) were smaller than *C. c. imminuta*.

There are some factors that can lead to misidentifications among the species belonging to the family Carcinonemertidae: (1) the small size of worms, (2) morphological simplification apparently resulting from their parasite-like life style, and (3) the consequent morphological ambiguity of structures and morphological similarity between species. For example, the structure referenced as anterior proboscis chamber in some publications (Shields et al. 1989; Shields and Kuris 1990) appears to us to be the proboscis diaphragm and that is how



we treat it. Similarly, the region designated as the stylet bulb, appears to include the diaphragm in a number of publications (Gibson 1972; Campbell et al. 1989; Gibson and Jones 1990; McDermott and Gibson 1993); we distinguish between the two.

The stifling morphological homogeneity of *Carcinonemertes* species led us to search for other, relatively practical characters, such as distance from first gonad to tip of head, distance from brain to tip of head and distance from stylet to tip of head. Distance from first gonad to tip of head enabled us to distinguish among the three species described here. We believe that information, such as infestation site and infestation according to sex and maturity of host, may also provide valuable diagnostic and systematic data. With this information in hand from previously described species it may be possible to conduct phylogenetic analyses with useful results that will lead to understanding of the diversification and host relationships of this potentially damaging group of nemerteans.

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