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Infestation by *Carcinonemertes divae* (Nemertea: Carcinonemertidae) in *Libinia spinosa* (Decapoda: Pisidae) from São Sebastião Island, SP, Brazil

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Abstract

Nemerteans of the genus *Carcinonemertes* are obligate symbiotic egg predators of many decapod crustaceans. This study presents the prevalence and intensity of infestation by *Carcinonemertes divae* in the crab, *Libinia spinosa*, from São Sebastião Island, São Paulo State, Brazil. Overall prevalence of infestation was 69.2% and mean intensity of infestation was 20.8 ± 3.4 (range: 1–148). Significant differences in prevalence and mean intensity of infestation were observed between males and females and between ovigerous and non-ovigerous adult females of *Libinia spinosa*. Prevalence and mean intensity of infestation did not differ significantly between juvenile and adult crabs or among ovigerous crabs with eggs in different stages of embryogenesis. Carapace width of male crabs was negatively correlated with prevalence of infestation by *Carcinonemertes divae*. Sexual contact appeared to be a source of transmission of worms between males and females of *Libinia spinosa*.

Keywords: *Infestation, symbionts,* Carcinonemertes divae, Libinia spinosa, *Brazil*

Introduction

Nemerteans of the genus *Carcinonemertes* (Kölliker) are obligate 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 since they kill individual embryos (Kuris 1993; Torchin et al. 1996). Epidemic outbreaks have been recorded from several places in North America, which were responsible for substantial mortality of crab eggs, for example, in *Paralithodes camtschaticus* (Tilesius) from Alaska, *Cancer magister* Dana and *Hemigrapsus oregonensis* (Dana) from California (Wickham and Kuris 1985; Wickham 1986; Shields and Kuris 1988; Shields et al. 1990; Kuris 1993).

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The first record of *Carcinonemertes* for Brazil was made by Humes (1942), who found worms that he attributed to *Carcinonemertes carcinophila imminuta* Humes associated with *Callinectes danae* Smith from Rio de Janeiro. Later, *C. c. imminuta* was recorded from *C. danae* and *C. ornatus* Ordway, in São Sebastião, São Paulo State (Santos and Bueno 2001). *Aranaeus cribrarius* (Lamarck) and *Libinia ferreirae* Brito Capello were recorded by Mantelatto et al. (2003) as hosts for *C. c. imminuta* in a study conducted in the Ubatuba region, São Paulo State. However, the identity of the carcinonemertid species associated with these last two hosts should be re-evaluated or confirmed since all crabs were previously frozen before they were checked for worms. A careful observation of live specimens can be critical for a correct identification of nemerteans in this group characterized by major morphological similarity among species.

This study presents the prevalence and intensity of infestation by *Carcinonemertes divae* Santos, Norenburg & Bueno on the crab *Libinia spinosa* H. Milne Edwards.

Materials and methods

Crabs were collected with the aid of a double-rigged trawl at Praia do Poço, São Sebastião Island, Brazil, during the months of September 2002, January, February, July and September 2003, and were transported to the nearby laboratory facilities at the Centro de Biologia Marinha (CEBIMar–USP) where they were kept alive in tanks with a flow-through seawater system.

The identification of crabs followed Melo (1996). Determination of sex and maturity of crabs were based on abdomen shape. Carapace width was measured at its widest dimension with a Vernier calipers to the nearest 0.1 mm.

Dissection of live juvenile and adult crabs of both sexes was performed on specimens previously anesthetized by cold temperature (10 min at -18°C). The exoskeleton surface and arthrodial membranes of crabs were examined for nemerteans. The dorsal carapace of crabs was removed to expose the branchial chambers. Gills were removed with the aid of forceps and inspected for worms. Ovigerous female crabs were examined as above and their egg-bearing pleopods removed and also inspected for worms. Crabs were examined for nemerteans with the aid of a dissecting microscope, which was also used to count the worms.

The term "symbiosis" is used here as a general term for all intimate interspecific interactions that include commensalism, mutualism and parasitism (Torchin et al. 1996). Prevalence is defined as the percentage of infested crabs and mean intensity as the average number of worms per infested crab (Margolis et al. 1982; Bush et al. 1997).

Parametric tests were used when sampled populations were normal and had equal variances. When those assumptions were not met, non-parametric tests were applied (Zar 1996). Tests are two-tailed.

Fisher's exact test (Zar 1996) was used to verify the null hypothesis that male and female, juvenile and adult, and ovigerous and non-ovigerous adult female crabs had similar prevalences of infestation by *C. divae*.

The null hypothesis that mean intensity of infestation was similar between juvenile and adult, male and female, ovigerous and non-ovigerous adult female crabs was tested with a Mann–Whitney test (Zar 1996). The null hypothesis that mean intensity of infestation was similar among the different stages of egg development was tested with a Kruskal–Wallis test (Zar 1996).

The null hypothesis that size (carapace width) was similar between infested and uninfested hosts and infested and uninfested female crabs was tested with a Mann–Whitney test (Zar 1996). The null hypothesis that size (carapace width) was similar between infested and uninfested male crabs was tested with an unpaired *t*-test (Zar 1996).

The relationship between the carapace width of *L. spinosa* and percentage of infestation by *C. divae* was tested with a Spearman correlation test (Zar 1996). Statistical analyses were conducted using GraphPad InStat version 3.0b for Macintosh (GraphPad Software, San Diego, CA, USA). Results were considered significant at $P<0.05$.

Results

Infestation site

Adults of *C. divae* were found in the egg mass of the hosts. Immature worms were found on the abdomen of juvenile male crabs; on the abdomen and at the arthrodial 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. No worms were found in the branchial chambers or between the gill lamellae of the hosts.

Prevalence

The overall prevalence of infestation by *C. divae* on *L. spinosa* was 69.2% (63 of 91). Significantly more females (47 of 54 or 87.0%) than males (16 of 37 or 43.2%) were found to be infested with carcinonemertids (Fisher's exact test; $P<0.0001$). Worms infested significantly more ovigerous females (43 of 43 or 100%) than non-ovigerous adult females (two of six or 33.3%) (Fisher's exact test; $P<0.0001$).

The prevalence did not differ significantly between juvenile (three of six or 50.0%) and adult crabs (60 of 85 or 70.6%) (Fisher's exact test; $P=0.37$). Prevalence of infestation did not differ among adult female crabs with eggs in initial (28 of 28 or 100%), intermediate (13 of 13 or 100%) and final stages (two of two or 100%) of development.

Intensity of infestation

The mean intensity of infestation was 20.8 ± 3.4 (range: 1–148). Female crabs (median=18.0; mean=26.0; s=29.8; $n=47$) had significantly more worms than males (median=4.0; mean=5.2; s=5.0; $n=16$) (Mann–Whitney, $U=638$; $P<0.0005$). Ovigerous female crabs (median=18.0; mean=27.8; s=30.5; $n=43$) had significantly more worms than non-ovigerous females (median=3.0; mean=3.0; s=0; $n=2$). Mean intensity of infestation did not differ significantly between juvenile (median=8.0; mean=9.3; s=9.1; $n=3$) and adult crabs (median=12.0; mean=21.3; s=27.9; $n=60$) (Mann–Whitney, $U=116.0$; $P=0.41$) nor among ovigerous crabs with embryos in different stages of development (initial: median=20.5; mean=26.4; s=23.4; $n=28$; intermediate: median=16.0; mean=34.7; s=43.2; $n=13$; final: median=4.0; mean=4.0; s=1.4; $n=2$) (Kruskal–Wallis, KW=4.63; $P=0.10$). There were only two ovigerous crabs with eggs in the final stage of development. Therefore, a comparison on mean intensity of infestation between ovigerous crabs with eggs in initial stage of development (median=20.5; mean=26.4; s=23.4; n=28) and those with eggs in intermediate stage of development (median=16.0; mean=34.7; s=43.2; n=13) was made. The mean intensity did not differ between these ovigerous female crabs (Mann–Whitney, U=196, P=0.70).

Infestation and host size

Carapace width was measured for 86 of the 91 crabs examined for worms. Infested hosts (median=49.1 mm; mean=48.0 mm; s=9.4; n=61; range=23.4–71.7 mm) were significantly smaller than uninfested crabs (median=55.5 mm; mean=53.8 mm; s=16.0; n=25; range=26.4–77.8 mm) (Mann–Whitney, U=544.5; P=0.04). Infested males (median=49.6 mm; mean=49.9 mm; s=12.9; n=16; range=27.2–71.7 mm) were significantly smaller than uninfested males (median=59.4 mm; mean=60.5 mm; s=12.0; n=18; range=37.9–77.8 mm) (unpaired t-test, t=2.47; d.f.=32; P=0.02). Infested females (median=49.1 mm; mean=47.3 mm; s=7.8; n=45; range=23.4–58.5 mm) were significantly larger than uninfested females (median=29.5 mm; mean=36.6 mm; s=11.3; n=7; range=26.4–53.4 mm) (Mann–Whitney, U=239; P=0.03). There was no correlation between the carapace width of female crabs and the prevalence of infestation (Spearman correlation, r_s=0.22; P=0.12; n=52) (Table I), whereas a negative correlation was observed in male crabs ($r_s=-0.42$; P=0.01; n=34) (Table II). There was no correlation between the carapace width of female crabs and the intensity of infestation (r_s=0.21; $P \leqslant 0.18$; n=45) or between the carapace width of male crabs and the intensity of infestation (r_s=0.38; $P \leqslant 1$; n=16).

Discussion

The higher prevalence and mean intensity of infestation observed in females, especially in ovigerous females, can be explained by the critical role played by ovigerous female crabs in

Table I. Prevalence of infestation by *Carcinonemertes divae*, according to size-class categories of females (n=52), of *Libinia spinosa* from Poço Beach, São Sebastião Island, Brazil.

Size-class: carapace width (mm)	Number of crabs	Number of worms	Prevalence (%)
23.0–25.0	1	1	100.0
25.0–27.0	1	0	0.0
27.0–29.0	3	19	33.3
29.0–31.0	1	0	0.0
31.0–33.0	0	–	–
33.0–35.0	0	–	–
35.0–37.0	3	97	100.0
37.0–39.0	1	27	100.0
39.0–41.0	2	113	100.0
41.0–43.0	4	95	100.0
43.0–45.0	6	125	83.3
45.0–47.0	3	16	100.0
47.0–49.0	3	10	66.7
49.0–51.0	7	202	100.0
51.0–53.0	5	114	100.0
53.0–55.0	3	25	66.7
55.0–57.0	5	147	100.0
57.0–59.0	4	76	100.0

Table II. Prevalence of infestation by *Carcinonemertes divae*, according to size-class categories of males (n=34), of *Libinia spinosa* from Poço Beach, São Sebastião Island, Brazil.

Size-class: carapace width (mm)	Number of crabs	Number of worms	Prevalence (%)
27.0–31.0	1	8	100.0
31.0–35.0	2	9	100.0
35.0–39.0	1	0	0.0
39.0–43.0	2	6	50.0
43.0–47.0	3	23	66.7
47.0–51.0	3	11	100.0
51.0–55.0	3	1	33.3
55.0–59.0	6	8	50.0
59.0–63.0	5	13	20.0
63.0–67.0	1	0	0.0
67.0–71.0	1	0	0.0
71.0–75.0	2	5	100.0
75.0–79.0	4	0	0.0

the life cycle of carcinonemertids. The worms attain maturity only after migrating to the host's eggs, which are consumed as food, and where the nemerteans reproduce and deposit their own eggs (Humes 1942; Shields et al. 1988; Santos and Bueno 2001).

Intimate contact between males and females of *Libinia emarginata* Leach has been reported by Hinsch (1968) and by Laufer and Ahl (1995). When an ovigerous female, whose fully developed zoea larvae are about to be released from the eggs, is encountered by a male, the latter seizes her and holds her beneath him; this behavior is known as 'obstetrical' (Hinsch 1968). After mating, the male uses his last pair of walking legs to carry the female behind him and guards her from being taken-over by another male until she releases the next clutch of fertilized eggs (Laufer and Ahl 1995). This mating behavior may enable juvenile worms to be transmitted by contact between male and female crabs and may also allow veneral transmission. Transmission of juvenile worms during host copulation was demonstrated in experiments conducted with *Carcinonemertes errans* from *Cancer magister*. In these experiments, approximately 90% of the worms from the male were transferred to the female (Wickham et al. 1984). According to Kuris (1978), both juvenile transfer and larval settlement are regarded as important factors of a transmission model accounting for the distribution of nemertean on host crabs. Gregarious behavior has been recorded during the mating season (De Goursey and Auster 1992) and during the molting period (De Goursey and Stewart 1985) in *L. emarginata*. If gregarious behavior also occurs in *L. spinosa* crabs, they might be found more easily by carcinonemertid larvae. Since male crabs are significantly larger (mean carapace width: 55.5 ± 13.4 mm) than females (mean carapace width: 45.8 ± 9.0 mm) (Mann–Whitney, U=1859; P=0.0008), nemertean larvae might find a greater surface area to settle in comparison to females, which could explain the remarkable prevalence of infestation (43.2%) by juvenile *C. divae* on male crabs.

According to Shields and Kuris (1990), carcinonemertid larvae settle on male and female crab hosts with moderate duration of embryogenesis, as demonstrated for *Carcinonemertes errans* and *Carcinonemertes epialti*. The remarkable prevalence of infestation by juvenile worms on male crabs of *L. spinosa* might indicate that recruitment of juveniles is not linked to a moderate period between broods, as suggested by Kuris (1993), since *L. spinosa* probably has short periods between broods, as in *L. emarginata*, which extrudes a new egg mass in the brood pouch just a short time after zoeae from a previous batch are released (Hinsch 1968).

The fact that no significant differences in mean intensity were observed among ovigerous females with eggs in different stages of embryogenesis may be explained by the small number of crabs with eggs in the final stage of development ($n=2$) or by the fact that in *L. spinosa* juvenile worms are found in the egg mass of the hosts and not in their branchial chambers. In the portunid crabs, *C. danae* and *C. ornatus*, juvenile worms are found between the gill lamellae of hosts. Worms take some time to migrate from the branchial chamber to the egg mass of the hosts. Therefore, in *Callinectes* Stimpson species, we find a difference in prevalence of infestation by *C. c. imminuta* among crabs with eggs in different stages of development; worms being more prevalent in ovigerous females with eggs in the intermediate and final stages of development than in the initial stage (Santos and Bueno 2001). An increase in prevalence of infestation by carcinonemertids during embryogenesis of the host was also recorded by Kuris (1978), who found some juveniles of *C. epialti* in the branchial chamber of *Hemigrapsus oregonensis*.

The small sample of juvenile crabs ($n=6$) might explain why no significant differences were observed in prevalence and mean intensity of infestation by *C. divae* between juveniles and adults of *L. spinosa*.

Infested female crabs were larger than uninfested ones. Male crabs of the genus *Libinia* Leach differ in length of claws relative to length of carapace, and in condition of the epicuticle covering their exoskeleton (abraded and unabraded) (Laufer and Ahl 1995). Large-bodied, abraded males with long claws act as primary reproductive males (Homola et al. 1991) and are more successful in maintaining intimate contact with females during mating and obstetrical behavior (Hinsch 1968), favoring the transmission of juvenile worms from male to female crab. This may explain the negative correlation between prevalence of infestation and carapace width in males, with uninfested male crabs being larger than infested ones.

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