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FIRST RECORDS OF THE EPIZOIC BARNACLE *OCTOLASMIS BULLATA* (CIRRIPEDIA: THORACICA: POECILASMATIDAE) ON THE SWIMMING CRAB *PORTUNUS SANGUINOLENTUS* (DECAPODA: PORTUNIDAE)

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ABSTRACT

This is the first report of the usually lobster-inhabiting epizoic barnacle *Octolasmis bullata* (Aurivillius, 1894) from the branchial chambers of a crab, *Portunus sanguinolentus* (Herbst, 1783). This association was noted in Hailing Bay, P.R. China, where infestation rates were as high as 34.8% with a mean intensity of 25.44 ± 17.89 (mean ± S.D.) barnacles per infested host. The maximum number was 102 individuals of *O. bullata* on a single female crab. Prevalence and mean intensity of infestation did not differ between crab sexes, but did differ significantly between immature and mature crabs and among crabs in different molt stages. Larger crabs showed a higher prevalence and mean intensity of barnacle infestation, as has been reported in previous studies of *Octolasmis*. More than 80% of the barnacles were distributed on the inner surfaces of the gills. The largest number appeared on gill 6, but barnacles were completely absent from gill 1. This species appeared to be more highly host specific than certain other species of *Octolasmis*, but its spatial distribution on *P. sanguinolentus* was similar to the patterns shown by some of its crab-inhabiting congeners.

KEY WORDS: barnacle, branchial chamber, epibionts, *Octolasmis bullata*, *Portunus sanguinolentus*, P.R. China

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INTRODUCTION

Most barnacles of the genus *Octolasmis* Gray, 1825 are well known as epibionts of other organisms, especially decapod crustaceans (Jeffries et al., 1982; Jeffries and Voris, 1996). These barnacles may colonize the outer surfaces of the carapace and appendages, or live inside the branchial chambers of their decapod hosts. Once attachment by the cyprid larvae occur, *Octolasmis* will permanently stay with its host unless the latter either undergoes ecdysis, whereby the barnacle is discarded with the old exoskeleton and cannot reattach, or dies (Jeffries and Voris, 1996). *Octolasmis bullata* (Aurivilius, 1894) is one such species that inhabits the branchial chambers of commercially important lobsters such as *Panulirus polyphagus* (Herbst, 1793) and *Panulirus stimpsoni* Holthuis, 1963 in the Indo-West Pacific (Jeffries et al., 1982; Jones et al., 2000). Adaptations to an epibiotic mode of life include reduction of the calcareous plates of the capitulum, and the tergal plates may even be absent. *Octolasmis bullata* was at first considered as a form of *Octolasmis angulata* (Aurivillius, 1894), but later it was recognized as a distinct species because it lacks the tergal plate that *O. angulata* displays (Liu and Ren, 2007).

The swimming crab *Portunus sanguinolentus* (Herbst, 1783) is a commercially and ecologically important species in Asian regions (Yang et al., 2011). The widespread natural populations of *P. sanguinolentus* provide an ideal substrate for colonization by epibionts such as *Octolasmis*. To date, at least four species of *Octolasmis* have been reported on this host, including *O. angulata*, *O. tridens* (Aurivillius, 1894), *O. warwickii* Gray, 1825, and *O. cor* (Aurivillius, 1892) (Jeffries et al., 1982; Kumaravel et al., 2009). Although *O. bullata* has not previously been recorded from any crabs, during a field survey in 2010 we found a large number of *P. sanguinolentus* bearing *O. bullata* in Hailing Bay, P.R. China. The present study was undertaken in order to make the first quantitative observations of the occurrence of *O. bullata* on the different sexes, states of maturity, and molt stages of this commercially important crab. In addition, the patterns of infestation were compared with those of other species of *Octolasmis*, to further elucidate the relationship between these epibiotic barnacles and their hosts.

MATERIALS AND METHODS

Sample Collection and Treatment

We collected *P. sanguinolentus* by a trawl with four nets in Hailing Bay (21°30′-21°39′N, 111°42′-111°50′E, average water depth ca. 12 m), Guangdong, P.R. China. The trawls were made once a week during 18 October to 15 November 2010 in the same water areas. Specimens from each trawl were held on ice and transported to the nearby laboratory within one hour after collection. In the laboratory, all crabs were sexed, measured (carapace width, CW, between the tips of the epibranchial spines, ±0.1 mm), and scored for molt stage (premolt = new cuticle formed beneath carapace, intermolt = branchiostegite and sternites inflexible, and postmolt = branchiostegite and sternites flexible, no new cuticle beneath

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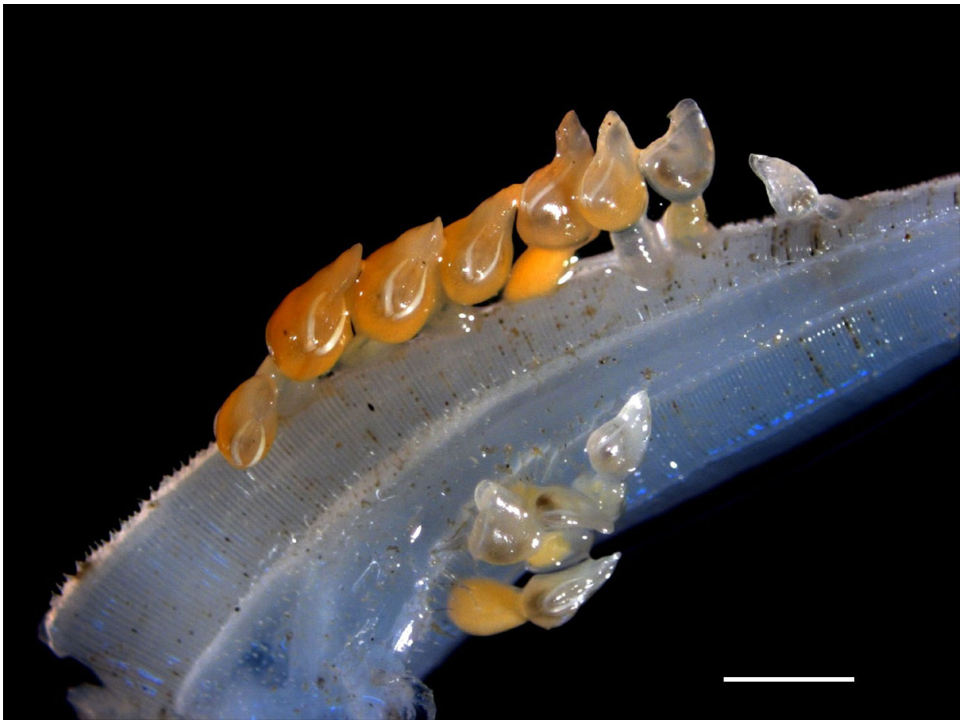

Fig. 1. *Octolasmis bullata* attached on both sides of the vessel of the inner surface of gill 4, in the branchial chamber of *Portunus sanguinolentus* from Hailing Bay, P.R. China. Scale bars = 200 μm.

carapace; Sumpton et al., 1989) and state of sexual maturity (based on the development stage of the gonads and the morphology of the pleon: Yang et al., 2011).

All barnacles from the branchial chambers were then carefully examined and identified, and confirmed as *O. bullata* (Liu and Ren, 2007; Fig. 1). Individual numbers, growth stages, and exact locations of *O. bullata* were observed and recorded under a stereomicroscope. The attachment sites for inspection included the left and right branchial chambers, the inner and outer surfaces of gills 1 to 8, the chamber wall beneath the gills, and the anterior cuticle of the chamber walls. The prevalence of infestation was defined as the proportion of hosts bearing any developmental stage of *O. bullata* among the total number of crab specimens. The mean intensity of infestation was calculated as the mean number of *O. bullata* individuals per infested crab. Growth stage of *O. bullata* was ascertained using the methods of Voris et al. (2000): individuals were categorized as cyprid larvae (newly settled), juveniles (without ovigerous lamellae in the capitulum), and adults (with distinct ovigerous lamellae and eggs in the capitulum).

Statistical Analyses

Differences in the prevalence of infestation were analyzed with Chi-square tests for males and females, immature and mature crabs, and different molt stages of the hosts. Differences in mean intensity of infestation were compared with Mann-Whitney tests for males and females, immature and mature crabs, left and right branchial chambers, and the inner and outer surface of gills. Kruskal-Wallis tests were used to test for variation in mean intensity of infestation among different molt stages of crabs, and different gills (1-8) of the hosts. The relationships between both the prevalence and mean intensity of infestation and host size were appraised using Spearman's correlation test. Differences in all tests were considered significant at $P < 0.05$.

Results

Prevalence of Infestation

A total of 765 individuals of *P. sanguinolentus*, including 356 males and 409 females, were sampled. Of these, 267 crabs (119 males and 148 females) were hosts of *O. bullata* (34.8% prevalence of infestation). Female crabs seemed to be susceptibly infested, but the prevalence of infestation was not significant between male and female crabs (Chi-square test, df = 1, $P > 0.05$; Table 1). Small individuals of less than 60 mm CW were free of infestation, and the smallest host was a 64.42 mm CW male. A positive correlation was

Table 1. The prevalence and mean intensity of infestation for different stages of the epizoic barnacle *Octolasmis bullata* in male and female *Portunus sanguinolentus* from Hailing Bay, P.R. China. Values with the same superscript letter are not significantly different from each other ($P > 0.05$, Chi-square tests for differences in prevalence and Mann-Whitney tests for differences in mean intensity).

Sex of crabs	Prevalence of infestation			Mean intensity of infestation			
	Total crabs (N)	Infested crabs (N)	Prevalence (%)	Total barnacles (mean ± S.D.)	Cyprid larvae (mean ± S.D.)	Juveniles (mean ± S.D.)	Adults (mean ± S.D.)
Male	356	119	33.4^{A}	23.89 ± 16.79^{B}	3.92 ± 6.41^{C}	6.88 ± 7.46^{D}	13.08 ± 16.17^{E}
Female	409	148	36.2^{A}	26.68 ± 18.69^{B}	3.65 ± 6.45^{C}	6.96 ± 8.07^{D}	16.07 ± 17.77^{E}
Total	765	267	34.8	25.44 ± 17.89	3.77 ± 6.42	6.93 ± 7.79	14.74 ± 17.11

Table 2. The prevalence and mean intensity of infection for different stages of the epizoic barnacle *Octolasmis bullata* in various-sized (carapace width, CW) *Portunus sanguinolentus* from Hailing Bay, China. Asterisks indicate that standard deviation is not shown because sample size is < 3.

Size of crabs (mm)	Prevalence of infestation			Mean intensity of infestation			
	Total crabs (*N*)	Infested crabs (*N*)	Prevalence (%)	Total barnacles (mean ± S.D.)	Cyprid larvae (mean ± S.D.)	Juveniles (mean ± S.D.)	Adults (mean ± S.D.)
30-40	1	0	0	0	0	0	0
40-50	10	0	0	0	0	0	0
50-60	8	0	0	0	0	0	0
60-70	17	1	6.0	1*	1*	0	0
70-80	36	2	5.6	1.50 ± 0.71	1.50 ± 0.71	0	0
80-90	77	16	20.8	7.49 ± 6.93	6.25 ± 4.86	1.50 ± 2.58	0.19 ± 0.54
90-100	157	49	31.2	19.86 ± 11.09	4.02 ± 5.89	7.00 ± 7.58	8.84 ± 10.86
100-110	197	68	34.5	24.46 ± 16.70	3.76 ± 7.60	7.29 ± 9.01	13.40 ± 15.41
110-120	139	57	41.0	24.37 ± 15.54	3.61 ± 6.74	6.44 ± 6.68	14.32 ± 17.04
120-130	78	42	53.8	30.98 ± 18.86	3.24 ± 5.93	8.07 ± 7.92	19.67 ± 19.25
130-140	22	15	68.2	37.93 ± 23.33	3.40 ± 6.08	8.93 ± 7.79	25.60 ± 20.35
140-150	17	13	76.5	43.31 ± 18.11	2.08 ± 3.38	8.92 ± 8.77	32.31 ± 17.02
150-160	4	2	50.0	59.00 ± 32.53	6.00 ± 8.49	13.00 ± 7.07	40.00 ± 16.97
160-170	2	2	100	28*	0	0	28*
Total	765	267	34.8	25.44 ± 17.89	3.77 ± 6.42	6.93 ± 7.79	14.74 ± 17.11

found between the prevalence of infestation and size (CW) of crabs (Spearman correlation test, $r_s = 0.965$, $N = 14$, $P < 0.01$; Table 2). Male and female crabs attained sexual maturity at 88.11 mm and 90.34 mm, respectively. The prevalence of infestation in immature crabs was 11.9%, significantly lower than the 40.6% recorded in mature hosts (Chi-square test, df = 1, $P < 0.001$; Table 3). Infestation by *O. bullata* varied significantly among crabs of different molt stages (Chi-square test, df = 2, $P < 0.001$; Table 4). The highest prevalence (40.7%) was recorded in crabs in the intermolt stage, followed by 26.0% in the postmolt stage, and then 12.0% in the premolt stage.

Intensity of Infestation

In total, 6792 *O. bullata* occurred in the branchial chambers of the 267 infested host crabs, corresponding to a mean intensity of infestation of 25.44 ± 17.89 (mean ± S.D.). The mean intensity did not differ significantly between male and female hosts (Mann-Whitney test, $U = 8069.5$, $P > 0.05$; Table 1), but was significantly different between immature and mature hosts (Mann-Whitney test, $U = 576$, $P < 0.001$; Table 3). Differences were not found, however, in mean intensity of infestation for hosts of different molt stages (Kruskal-Wallis test, $P > 0.05$; Table 4), although the mean intensity was lower in postmolt crabs than in premolt and intermolt crabs. Mean intensity of infestation by *O. bullata* was strongly correlated positively with host size (Spearman correlation test, $r_s = 0.978$, $N = 14$, $P < 0.01$, Table 2). The maximum number of attached barnacles was 102 on a single female crab.

Infestation as Related to Life Stage

Among the total, 1007 individuals (accounting for 14.8%) of *O. bullata* were cyprid larvae, 1849 (27.2%) were juveniles and 3936 (58.0%) were adults. No differences were recorded in the mean intensity of infestation by cyprid larvae (Mann-Whitney test, $U = 8479.5$, $P > 0.05$; Table 1), juveniles (Mann-Whitney test, $U = 8607.5$, $P > 0.05$; Table 1), or adults (Mann-Whitney test, $U = 27\,748.5$, $P > 0.05$; Table 1) for both sexes of hosts. Immature crabs only bore cyprid larvae and juvenile barnacles whereas mature crabs could be infested with cyprid larvae, juveniles, and adults. The mean intensities of infestation for both cyprid larvae (Mann-Whitney test, $U = 576$, $P < 0.05$; Table 3) and juveniles (Mann-Whitney test, $U = 120.5$, $P < 0.05$; Table 3) were significantly different between immature and mature hosts. Different stages of *O. bullata* (cyprid larvae, juveniles, and adults) were found together with greatly

Table 3. The prevalence and mean intensity of infection by different stages of the epizoic barnacle *Octolasmis bullata* in immature and mature *Portunus sanguinolentus* from Hailing Bay, P.R. China. Values with the same superscript letter are significantly different ($P < 0.05$, Chi-square test for differences in prevalence and Mann-Whitney tests for differences in mean intensity). An asterisk indicates that no statistical analysis was conducted because no immature crabs bearing adult barnacles were found.

Maturity of crabs	Prevalence of infestation			Mean intensity of infestation			
	Total crabs (*N*)	Infested crabs (*N*)	Prevalence (%)	Total barnacles (mean ± S.D.)	Cyprid larvae (mean ± S.D.)	Juveniles (mean ± S.D.)	Adults (mean ± S.D.)
Immature	151	18	11.9^a	6.56 ± 6.70^b	5.44 ± 4.83^c	1.11 ± 2.40^d	0
Mature	614	249	40.6^a	26.80 ± 17.67^b	3.65 ± 6.51^c	7.35 ± 7.88^d	15.81 ± 17.23*
Total	765	267	34.8	25.44 ± 17.89	3.77 ± 6.42	6.93 ± 7.79	14.74 ± 17.11

Table 4. The prevalence and mean intensity of infection by different stages of the barnacle *Octolasmis bullata* in different moult stages of *Portunus sanguinolentus* from Hailing Bay, P.R. China. Values with the same capital superscript letter are not significantly different ($P > 0.05$), while those with the same lower case superscript letter are significantly different ($P < 0.05$, Chi-square test for differences in prevalence and Kruskal-Wallis tests for differences in mean intensity).

Moult stage of crabs	Prevalence of infestation			Mean intensity of infestation			
	Total crabs (N)	Infested crabs (N)	Prevalence (%)	Total barnacles (mean ± S.D.)	Cyprid larvae (mean ± S.D.)	Juveniles (mean ± S.D.)	Adults (mean ± S.D.)
Posmoult	73	19	26.0^{a}	16.84 ± 8.71^{B}	16.84 ± 8.71^{c}	0	0
Premoult	117	14	12.0^{a}	27.00 ± 13.14^{B}	0	0.29 ± 0.83^{d}	26.71 ± 12.84^{e}
Intermoult	575	234	40.7^{a}	26.04 ± 18.53^{B}	2.94 ± 5.07^{c}	7.88 ± 7.86^{d}	15.23 ± 17.28^{e}
Total	765	267	34.8	25.44 ± 17.89	3.77 ± 6.42	6.93 ± 7.79	14.74 ± 17.11

different mean intensity of infestation on hosts in different molt stages (Table 4). Postmolt *P. sanguinolentus* had only cyprid larvae, while premolt crabs supported juvenile and adult barnacles, and intermolt crabs could host larvae, juveniles, and adults. There were strong positive correlations between host size and the mean intensity of infestation for both juvenile (Spearman correlation test, $r_s = 0.905$, $N = 8$, $P < 0.01$) and adult (Spearman correlation test, $r_s = 0.950$, $N = 9$, $P < 0.01$) *O. bullata*, but not for the cyprid larvae (Spearman correlation test, $r_s = 0.248$, $N = 10$, $P > 0.05$).

Distribution of Infestation

Although each crab has two branchial chambers, *O. bullata* only occurred in 79.6% (425 of 534) of the chambers of the 267 infested crabs. Despite this, there was no significant difference in mean intensity of infestation between left (10.48 ± 12.16) and right chambers (11.01 ± 13.04, Mann-Whitney test, $U = 4235$, $P > 0.05$). As for spatial distribution, 5736 barnacles (84.5% of the total) occurred on the surface of the gills, 891 (13.1%) on the anterior cuticle of the chamber walls, and 165 (2.4%) on the chamber wall beneath the gills (Kruskal-Wallis test, $P < 0.001$). The gills were evidently the preferred site for attachment. The numbers of barnacles on the gills differed significantly from gill 2 to gill 8 (Kruskal-Wallis test, $P < 0.001$, Table 5), and between the inner and outer surfaces of the gills (Mann-Whitney test, $U = 394$, $P < 0.001$). The surface of gill 6 was found to harbor the largest number of barnacles (1976 individuals, 26.4% of the total, Table 5), but no barnacles were recorded on gill 1. The inner surfaces of the gills bore 83.8% (5692 individuals, Table 5) of the total number of barnacles whereas the outer surfaces only bore 16.2% of the total (42 individuals), these only being found when the number of barnacles on a host exceeded 65 individuals.

Discussion

Octolasmis bullata was recorded on *P. sanguinolentus*, for the first time in this study, and this suggests that it can exploit other host taxa than the previously recorded lobster species (Jeffries et al., 1982; Jones et al., 2000). Other portunid crabs such as *Portunus pelagicus* (Linnaeus, 1758) and *Charybdis feriatus* (Linnaeus, 1758) in the same waters were not found to be infested by *O. bullata* in the present survey, which suggests a degree of crab host specificity on the part of *O. bullata*. Host specificity by the species was also found in the seas adjacent to Singapore, where *O. bullata* was only observed on the lobster *Panulirus polyphagus* of 56 examined decapod species (Jeffries et al., 1982). However, some common epizoic barnacle species, such as *O. warwickii* and *O. angulata*, are not very host specific and have been frequently encountered on a variety of decapod hosts (Jeffries et al., 1982, 1989, 2005; Voris et al., 1994; Walker, 2001; Xue et al., 2003; Yan et al., 2004; Kumaravel et al., 2009; Mushtaq and Mustaquim, 2009). For most species of *Octolasmis*, a particular set of species serves as hosts, and only a subset of the host population depending on factors such as age, sex, and molt stage (Jeffries et al., 1992; Voris et al., 1994).

Table 5. The spatial distribution of individual *Octolasmis bullata* barnacles (including cypris larvae) on the gills of *Portunus sanguinolentus* from Hailing Bay, P.R. China.

Gill number	Left chamber			Right chamber			Left + Right
	Inner	Outer	Inner + Outer	Inner	Outer	Inner + Outer	
1	0	0	0	0	0	0	0
2	6	0	6	23	0	23	29
3	106	1	107	190	0	190	297
4	655	1	656	417	2	419	1075
5	571	6	577	619	9	628	1205
6	922	11	933	1030	13	1043	1976
7	368	1	369	529	0	529	898
8	149	0	149	107	0	107	256
Total	2777	20	2797	2915	24	2939	5736

Portunus sanguinolentus has been recorded as bearing *O. angulata*, *O. tridens*, and *O. warwickii*, but not *O. bullata*, in Singapore (Jeffries et al., 1982). Along the coast of India, the same crab species harbors multiple species of barnacles in its branchial chambers, including *O. angulata*, *O. tridens*, and *O. cor* (Kumaravel et al., 2009). In the present study, *P. sanguinolentus* was found with *O. bullata*. This all suggests that *P. sanguinolentus* is an ideal host for the attachment of *Octolasmis*.

Infestation of *O. bullata* was confirmed only within the branchial chambers of *P. sanguinolentus*. A previous study (Voris and Jeffries, 1997) found that the spatial distribution of *Octolasmis* is most often related to the degree of calcification of the capitular plates. Those of *O. bullata* are severely degenerated with only small areas of calcification; the barnacles are thus subtranslucent and very delicate, well adapted to the sheltered environment of the branchial chamber, in which food and oxygen are provided by the respiratory currents. This is very different from well-calcified species such as *O. warwickii* and *O. tridens*, which have large capitular plates, are attached to the outer surface of their hosts, and are subject to flowing water, abrasion, and predation (Voris and Jeffries, 1997).

In the branchial chamber, the spatial distribution of *O. bullata* was nonrandom, which is consistent with patterns reported for other species of *Octolasmis*, such as *O. angulata* on *Charybdis feriatus* (Yan et al., 2004), *O. muelleri* on *Callinectes sapidus* Rathbun, 1896 (Walker, 1974; Jeffries and Voris, 1983), and *O. cor* and *O. angulata* on *Scylla serrata* (Forskål, 1775) (Voris et al., 1994), all of which showed heavy colonization of the inner surface of the gills. This distribution patterns is considered to be related more to the crab's respiratory currents than to the available space in the chamber, because the current flow governs the provision of both food and oxygen to the permanently cemented barnacles, and thus might affect the settlement decisions of the cyprid larvae (Gannon and Wheatly, 1992; Voris et al., 1994; Voris and Jeffries, 2001; Gaddes and Sumpton, 2004). The normal circulation of water into the branchial chambers via openings at the bases of the thoracic appendages, whereby the flow first reaches the inner surfaces of the gills, may also explain why cyprid larvae are more prone to settle on the inner than on the outer surface of the gills (Voris et al., 1994). Reversal of the current occurs in some buried crabs, though, and this might intermittently cause the cyprid larvae to use the chamber wall and outer surface of the gills as settlement sites (Voris and Jeffries, 2001; Walker, 2001).

The prevalence of infestation recorded in the present study was low compared to 48% reported in the lobster *Panulirus polyphagus* from Singapore (Jeffries et al., 1982). For a given species of barnacle, this parameter is often found to vary greatly among host species and localities. For example, *O. angulata* showed a high prevalence (85.7%) on *C. feriatus* from China (Yan et al., 2004), but a lower prevalence (63.7%) on *Charybdis callianassa* (Herbst, 1789) from Australia (Walker, 2001). Similarly, 85% of *Scylla tranquebarica* (Fabricius, 1798) were infested with *O. cor* and *O. angulata* in Pakistan (Mushtaq and Mustaquim, 2009), but only 30.4% of *S. serrata* infested in Thailand (Voris et al., 1994). In Brazil, the prevalence of infestation by *Octolasmis lowei* (Darwin, 1851) on *Libinia spinosa* H. Milne Edwards, 1834 was 63% (Cordeiro and Costa, 2010), much higher than the 22.4% recorded on *Callinectes danae* Smith, 1869 and 12.1% on *Callinectes ornatus* Ordway, 1863 (Santos and Bueno, 2002). Differences in the prevalence of infestation might be due to a variety of biotic and abiotic factors, e.g., the abundance and host specificity of the barnacles, the habits and body condition of the hosts, and the microhabitat and macrohabitat of the infestation (Jeffries et al., 1992).

In our study, a positive correlation was observed between mean intensity of infestation and host size. Many studies have confirmed that the infestation pattern of *Octolasmis* in small hosts is significantly different than that in large hosts (Jeffries et al., 1992; Voris et al., 1994; Santos and Bueno, 2002; Gaddes and Sumpton, 2004; Yan et al., 2004; Cordeiro and Costa, 2010). One explanation for this may be that the duration of the intermolt phase is longer in mature crabs than in immature individuals (Jeffries et al., 1992). An insufficient interval between host molts will prevent the barnacles from completing their life cycle from settlement and metamorphosis through maturity and reproduction (Jeffries et al., 1992, 1995). In fact, a high intensity of infestation was noted in older crabs following their terminal molts and on ovigerous females that cease molting during the reproductive period (Cordeiro and Costa, 2010). Massive infestations could severely reduce the space available for ventilatory currents and impede crab respiration, leading to an increase in host mortality (Gannon and Wheatly, 1992). The high prevalence and high intensity of infestation found on large specimens of *P. sanguinolentus* in the present study deserve attention, especially in light of the commercial and ecological importance of this host crab along the coast of Guangdong Province, P.R. China.

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