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Assemblages of symbionts in tropical shallow-water crinoids and assessment of symbionts' host-specificity

Dimitri D. Deheyn[1], Sergey Lyskin[2], and Igor Eeckhaut[3*]

[1]Marine Biology Research Division, Scripps Institution of Oceanography, University of California, San Diego,
9500 Gilman Drive, La Jolla, CA 92093-0202, USA, Email. ddeheyn@ucsd.edu;
[2]Laboratory of Ecology and Morphology of Marine Invertebrates, Institute of Ecology and Evolution of RAS,
Leninsky prospect, 33, 119071 Moscow, Russia;
[3]Marine Biology Laboratory, Natural Sciences Building, Av. Champ de Mars, 6, University of Mons-Hainaut,
7000 Mons, Belgium, Email. Igor.Eeckhaut@umh.ac.be

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Abstract

This paper characterizes symbiotic assemblages living on shallow-water crinoids in Papua New Guinea. A total of 1064 specimens of symbionts (47 species) were isolated from 141 crinoids (25 species). Amongst the symbionts, myzostomids were the most abundant taxon, followed by shrimps and polychaetes, and to a lower extent by crabs, galatheids, gastropods, nematodes, ophiuroids and fishes. Data analyses showed that (i) composition of symbiont assemblages remained similar within a geographical area (i.e., symbiotic fauna on a given species of crinoid remained similar among sampling sites), (ii) the co-occurrence of symbiotic species was not lower than if expected only by chance (i.e., there was no negative interactions between the symbionts), (iii) the number of shrimps on a crinoid was correlated with the size of the crinoid, (iv) symbionts are composed of species-specific, selective and opportunistic species, and (v) host-specificity of myzostomids was greater than for shrimps or polychaetes.

Keywords: Symbiosis, crinoids, host-specificity

1. Introduction

It is long known that tropical shallow-water comatulid crinoids can shelter a diversity of marine organisms (Potts, 1915; Clark, 1921). Crinoids therefore can be considered a living substratum sustaining a microcosm ruled by crinoid-symbiont and symbiont-symbiont interactions. The literature on this topic remains non-extensive and mostly descriptive, with little information available on host-specificity.

Crinoid symbiotic fauna was first investigated in the Great Barrier Reef providing data on coloration, distribution and behavior of symbionts (Potts, 1915), which was followed by an exhaustive list of symbiont species (Clark, 1921). Colour similarity between symbionts and crinoid hosts was observed in the Bay of Bengal (Rao and Sowbhagyavathi, 1972) while feeding strategies of symbionts was assessed from crinoids in the Red Sea (Fishelson, 1974). Symbionts were listed from crinoids of the Marshall and Maldive Islands (Zmarzly, 1984 and Tchesunov et al., 1989 respectively) as well as from Hong Kong (Morton and Mladenov, 1992). Only then was the distinction made between true symbionts and casual associates among crinoid symbiotic fauna. Ecological parameters were assessed for occurrence of symbionts on crinoid hosts in the Great Barrier Reef (Fabricius and Dale, 1993) and in southern Taiwan (Huang et al., 2005a). Recently chemotaxis process of attraction of crabs (Eeckhaut et al., 2000) and shrimps (Vandenspiegel et al., 1998; Huang et al., 2005b) by crinoids was investigated.

This paper characterizes the symbiotic assemblages living on shallow-water crinoids from Papua New Guinea. It investigates possible environmental/ecological factors that assign a symbiont to a specific species of crinoid when multiple species are available.

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[*] *The author to whom correspondence should be sent.*

2. Materials and Methods

Echinoderm symbionts were surveyed in Hansa Bay (Madang Province, Papua New Guinea) between 1992 and 1996 with emphasis on associates of crinoids. Crinoids were hand-collected by SCUBA diving in June and July 1994, at five sites of Hansa Bay: on the East, South-West and North-West reef slopes of Laing Island, on the East reef slope of Durangit reef, and on the East of Sushimaru shipwreck located closer to the mainland shoreline (Fig. 1). Collection was always similar: crinoids were gently pulled away from their support using a blade placed under the calyx, immediately placed in individual Zip-lock® plastic bags, and transported separated for analysis in the laboratory. For each specimen, length of the longest arm was measured using a graduated caliper, and the number of arm counted before sacrifice in 70% ethanol. Symbiotic organisms falling off the crinoid during transport in Zip-lock® bag or during arm length measurement were added back to the corresponding host in ethanol. Diversity of symbiotic fauna was assessed by analysis of randomly collected crinoid specimens for a given dive. Change of symbiotic fauna with geographical location was assessed from twenty specimens of *Capillaster multiradiata* (a small-sized, lightly infested crinoid) and five specimens of *Phanogenia multifida* (a large-sized, heavily infested crinoid) collected at each site. In the laboratory, crinoids were each processed separately and independently from each other.

Ectosymbiotic fauna was "extracted" by vigorous shaking for several minutes of the crinoid in fixative solution. The crinoid sample was then analyzed under binocular microscope to check for (i) any additional ectosymbionts, (ii) external sign of endosymbiosis then followed by removal of the endosymbiont by dissection, (iii) endosymbionts in the calyx by dissection, even if showing no external evidence of endosymbiosis. Crinoids were then fixed in 10% formaldehyde-seawater solution for several hours before being air-dried preserved. With the exception of copepods, all naked-eye or binocular visible symbiotic organisms were preserved in 70% ethanol for further analysis and taxonomic identification; all identifications were completed by experts in the appropriate taxa of symbiont (see acknowledgement section).

Symbiotic infestation was analyzed with respect to the crinoid (i) species, (ii) size, and (iii) collection site. This last factor was assessed by comparing the structure of symbiotic assemblages at the five collecting sites for two crinoid species, *Capillaster multiradiata* and *Phanogenia multifida*, using Kendall's coefficient of concordance (Zar, 1984). Crinoid relative size (i.e., the product between arm length and arms number) and the corresponding number of symbionts were analyzed for correlation using linear model. Pairs of symbiotic organisms occurring on same hosts were tested for normal versus non-normal co-occurrence (infestation rate lower or higher than expected by chance) using Fisher's Exact Test (Zar, 1984).

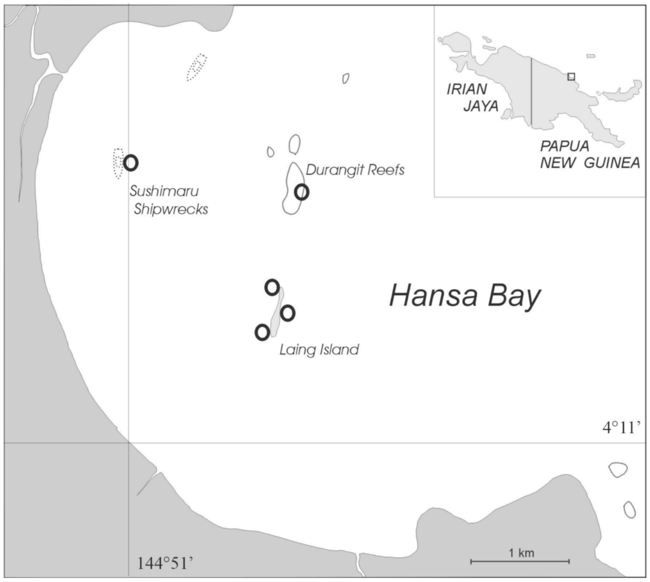

Figure 1. Location of sampling sites (circles) in Hansa Bay, Papua New Guinea.

This study also assessed host-specificity defined as the degree of intimacy of a symbiotic taxon (species or supraspecies) for a potential host. A symbiotic taxon infesting only one host species was qualified species-specific while a taxon infesting a wide range of hosts of various taxonomic groups opportunistic. Host-specificity of the most abundant taxa (in term of individuals) was calculated using the Rohde's index (Rohde, 1980):

$$S_i = \frac{\sum_j \frac{x_{ij}}{n_{ij}h_{ij}}}{\sum_j \frac{x_{ij}}{n_{ij}}}$$

where x_{ij} = number of "j" host crinoid species infected with "i" symbiont species, n_{ij} = total number of "j" host crinoid species examined for "i" symbiont species, h_{ij} = rank of "j" host species based on frequency of infestation for "i" symbiont species (rank is 1 for species with highest frequency), $\frac{x_{ij}}{n_{ij}}$ = mean frequency of infestation of "j" crinoid species for "i" symbiont species. Index varies from 0 to 1; the closer to 1, the higher the degree of host specificity.

3. Results

Characteristics of infestation

The collection effort provided a total of 141 specimens

Table 1. Crinoid species collected in Hansa Bay (Papua New Guinea) presented by family and according to the maximal number of symbionts per crinoid individual (Nmax). N: Number of crinoid individuals; F(%): Frequency of infestation (portion of crinoid individuals that is infested); R: rate of infestation (number of symbiont individual per infested crinoid); Myzo: Myzostomida; Nata: Natantia; Brac: Brachyura; Gala: Galatheidea; Gast: Gastropoda; Poly: Polychaeta; Nema: Nematoda; Ophi: Ophiuroidea; Pisc: Pisces

Crinoids species (N)	Myzo	Nata	Brac	Gala	Gast	Poly	Nema	Ophi	Pisc	N Max	F(%)/R	Total (average)
Comasteridae												
Alloeocomatella pectinifera (2)	1	0	0	0	0	0	0	0	0	1	50.0/1.0	**1**(0.5)
Comanthus gisleni (2)	0	0	1	0	0	1	0	0	0	1	100.0/1.0	**2**(1.0)
Comanthus wahlbergi (1)	0	0	0	0	0	1	0	1	0	2	100.0/2.0	**2**(2.0)
Comanthina audax (1)	0	3	0	0	0	1	0	0	0	4	100.0/4.0	**4**(4.0)
Comanthus mirabilis (2)	1	2	0	0	0	3	0	0	0	4	100.0/3.0	**6**(3.0)
Clarkcomanthus littoralis (4)	1	3	1	1	0	1	0	0	0	4	75.0/2.3	**7**(1.8)
Capillaster multiradiatus (19)	5	19	0	4	2	14	0	0	0	5	78.9/2.9	**44**(2.3)
Comanthus suavius (1)	3	2	0	0	0	2	0	0	0	7	100.0/7.0	**7**(7.0)
Clarkcomanthus albinotus (13)	22	14	0	0	0	1	0	0	0	8	92.3/3.1	**37**(2.8)
Comanthus parvicirrus (8)	12	20	0	6	0	6	0	0	0	13	100.0/5.5	**44**(5.5)
Phanogenia gracilis (4)	16	3	0	2	0	6	0	1	0	13	100.0/7.0	**28**(7.0)
Comanthina schlegelii (1)	15	1	0	0	1	1	0	0	0	18	100.0/18.0	**18**(18.0)
Comanthus alternans (7)	10	25	0	0	0	17	0	0	0	19	85.7/8.7	**52**(7.4)
Phanogenia multibrachiata (2)	1	20	0	3	0	2	0	0	0	21	100.0/13.0	**26**(13.0)
Phanogenia multifida (9)	53	37	2	3	0	10	0	1	0	26	88.9/13.2	**106**(11.8)
Comatella stelligera (8)	137	3	0	1	0	4	0	0	0	53	100.0/18.1	**145**(18.1)
Oxycomanthus bennetti (20)	340	22	1	18	2	21	2	1	1	53	100.0/20.4	**408**(20.4)
Colobometridae												
Cenometra bella (5)	0	9	0	2	0	7	0	2	0	7	100.0/4.0	**20**(4.0)
Colobometra perspinosa (2)	0	8	0	1	0	4	0	0	0	7	100.0/6.5	**13**(6.5)
Himenometridae												
Heterometra savignii (1)	0	0	0	2	0	1	0	0	0	3	100.0/3.0	**3**(3.0)
Himerometra robustipinna (6)	1	16	1	10	2	8	0	0	0	8	100.0/6.3	**38**(6.3)
Mariametridae												
Lamprometra palmata (2)	1	0	0	0	0	2	0	0	0	2	100.0/1.5	**3**(1.5)
Stephanometra spicata (3)	0	1	2	1	0	5	0	0	0	4	100.0/3.0	**9**(3.0)
Dichrometra flagellata (13)	15	4	2	5	1	16	1	0	0	5	92.3/3.7	**44**(3.4)
Stephanometra oxyacantha (5)	5	6	1	1	0	4	0	0	0	11	80.0/4.2	**17**(4.2)

of crinoids belonging to 25 species of 4 families (Table 1). Seventeen species belonged to Comasteridae, with the two most abundant species being *Oxycomanthus bennetti* and *Capillaster multiradiatus*, while two species belonged to Colobometridae, two to Himerometridae and four to Mariametridae. Out of the 25 species, all except two, *Alloeocomatella pectinifera* and *Dichrometra flagellata*, were collected during the day. *A pectinifera* and *D. flagellata* were hidden during the day and visible only at night. Of all the crinoids collected, 130 (92.2%) were found infested, with an average number of symbionts per host of 8.18.

Out of the 13 crinoid species for which more than 3 individuals were sampled, 6 were always infested (100% frequency of infestation), while the 7 others had a frequency of infestation ranging from 75 to 92.3%, the lower being for *Clarkcomanthus littoralis* (Table 1). Rate of infestation ranged from 2.3 in *C. littoralis* to 20.4 in *O. bennetti* (Table 1). The maximum number of symbionts was observed for *O. bennetti* and *Comatella stelligera*, with 53 organisms infesting a single individual host. Overall the average rate of infestation was greater for Comasteridae (7.6) and lower for Colobometridae (5.25), followed by Himerometridae (4.65), and Mariametridae (3.1).

A total of 1064 specimens of symbionts belonging to 47 species were found from crinoids (Table 2). Five species (three myzostomids and two gatropods) were parasites, all the others appearing commensals. Except for the polychaete *Hololepidella millari* (Britayev et al., 1999) that was also found in association with asteroids, all symbionts were specifically associated with crinoids. Considering both number of species and number of specimens, myzostomids and shrimps were the most abundant (Fig. 2). Myzostomids (18 species) represented 58.9% of all symbiont specimens (Fig. 3), shrimps (16 species) 20.1%, and polychaetes (3 species) 12.7%. Crabs, galatheids, gastropods, polychaetes, nematodes, ophiuroids and fishes were also found on crinoids but in lower number (Figs. 2 and 3).

Table 2. Total number of individuals of symbiont species collected in association with crinoids from Hansa Bay (Papua New Guinea). No distinction of species when counting polychaetes and galatheans.

Polychaeta	
Polynoidae	
Hololepidella laingensis	
Hololepidella millari	138
Paradyte crinoidicola	
Nematoda	
(Unidentified nematodes)	3
Myzostoma	
Contramyzostoma sphaera	128
Endomyzostoma sp.	1
Hypomyzostoma crosslandi	2
Myzostoma ambiguum	346
Myzostoma capitocutis	23
Myzostoma cuniculus	15
Myzostoma fissum	13
Myzostoma laingense	5
Myzostoma longitergum	15
Myzostoma mortenseni	3
Myzostoma nigromaculatum	43
Myzostoma polycyclus	8
Myzostoma sp. *A*	6
Myzostoma sp. *B*	1
Myzostoma sp. *C*	1
Myzostoma sp. *D*	2
Myzostoma stochoeides	5
Notopharyngoides aruensis	2
Crustacea	
Natantia	
Alpheidae	
Athanas ornithorynchus	1
Synalpheus comatularum	2
Synalpheus demani	13
Synalpheus sp. *A*	21
Synalpheus sp. *B*	16
Synalpheus stimpsoni	5
Stenopodidae	
Microprosthema sp.	2
Palaemonidae (Pontoniinae)	
Palaemonella pottsi	29
Periclimenes sp.	21
Periclimenes attenuatus	7
Periclimenes tenuis	7
Periclimenes amboinensis	2
Periclimenes aff. cornutus	4
Periclimenes ceratophthalmus	7
Periclimenes sp. *aff. commensalis*	9
Pontoniopsis comanthi	30
(*Pontoniinae gen.* sp.)	16
(Unidentified shrimps)	26
Anomura	
Galatheidae	
Galathea inflata	61
Allogalathea elegans	
Brachyura	
Eumedonidae	
Harrovia longipes	8
Permanotus purpureus	1
Ceratocarcinus longimanus	1
(Unidentified crabs)	1
Gastropoda	
Eulemidae	
Annulobalcis sp.	2
Curveulima sp.	2
(Unidentified gastropods)	3
Ophiuroidea	
Ophiotrichidae	
Ophiolophus novarae	6
Pisces	
Gobiesocidae	
Discotrema crinophila	1

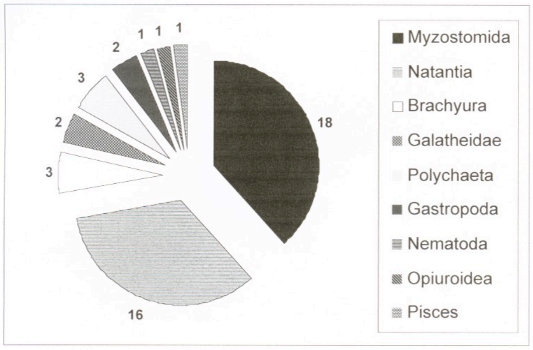

Figure 2. Diagram representing the number of symbiont species of the various symbiotic taxa found in association with crinoids in Hansa Bay, PNG.

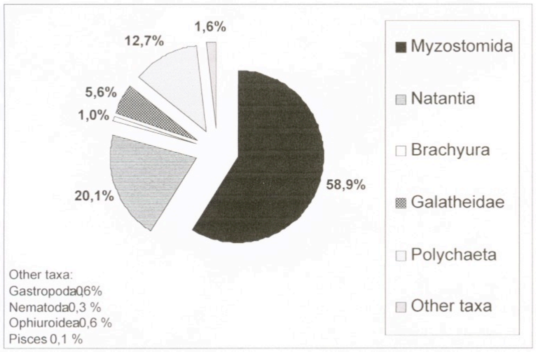

Figure 3. Diagram representing the fraction (expressed in percents) of symbiont individuals from various symbiotic taxa found in association with crinoids in Hansa Bay, PNG.

Polychaetes, myzostomids or shrimps infested each >50% of crinoid individuals. Rate of infestation of crinoids by myzostomids was the highest (x=8.3) while it was much lower for the other symbiotic taxa, with an average of 1 or 2 individuals per host. The exception came from shrimps with up to 16 individuals infesting one crinoid individual.

Myzostomids were abundant on *C. stelligera, O. bennetti* and *P. multifida*, with usually more than 10 symbionts per host (Table 1). Fifteen species of myzostomids were ectocommensals and moved freely at the surface of their hosts. They were never observed (neither under laboratory or field condition) leaving the hosts to displace on surrounding substrates. They tended to prefer different anatomical areas of the host: *Hypomyzostoma crosslandi* was more often observed on the aboral side of the arms, while *M. fissum, M. laingense* and *M. longitergum* were mainly found on pinnules. The other ectocommensals (*M. ambiguum, M. capitocutis, M. cuniculus, M. mortenseni, M. nigromaculatum, M. stochoeides* and *Myzostoma* sp. A, B, C and D) were observed spread anywhere on the external parts of the crinoid. Two myzostomids, *Contramyzostoma sphaera* and *Endomyzostoma* sp., were endoparasitic and infested the integument of the arm. This infestation triggered a cyst that was always located close to the arm ambulacral grooves from which food particles were observed to be diverted into the cyst, the later always containing one myzostomid specimen only. Another endoparasitic myzostomid was *Notopharyngoides aruense* that always occurred by pair in the forepart of the crinoid digestive system.

Natantia were abundant on *P. multifida* and *C. alternans*, two large crinoids with numerous arms (Table 1). Synalpheids were most often observed single or by pairs on *P. multifida* at the level of the calyx, or the centrodorsals between cirri. The other shrimp species often occurred in larger number on Comasteridae, being found on either arms, pinnules or calyx of the host. Crabs and galatheids occurred single or by pairs and stayed mainly on centrodorsals. Crabs were not observed on Colobometridae while galatheids were present on various species in the four crinoid families (Table 1). Polychaetes regrouped into three species with similar behavior mainly found on the calyx of crinoids but moving to any other part of the host once disturbed. Polychaetes were observed on all crinoid species, except *A. pectinifera* (Table 1). The ophiuroid *Ophiolophus novarae* was observed on Comasteridae and Colobometridae, always found wrapped around the calyx of the host, with the mouth facing the crinoid's mouth. The only symbiotic fish collected was *Discotrema crinoidicola*, always found single on the calyx of *O. bennetti* (Table 1) even though observed several times *in situ* in mating pair. Gastropods were always located on pinnules or arms of various crinoids, emitting their proboscis into the host integument. Unidentified nematods were often observed in the digestive system of *O. bennetti* and *D. flagellata*.

Effect of crinoid size, collection sites and host-specificity

The number of myzostomids on a specific host was not correlated with the crinoid size (p=0.33) while the correlation was significant considering the number of symbionts (all confounded; p<0.01) or the number of shrimps (p<0.01). The five sampling sites showed concordance of symbiont taxa for the two analyzed crinoid, *P. multifida* (Kendall coefficient W=0.846; p<0.001; Table 3) and *C. multiradiata* (W=0.716; p<0.01; Table 4). In other words, the pattern of symbiont assemblages was not significantly different between the two crinoid species throughout Hansa Bay. *P. multifida* always hosted two species of myzostomids (*M. nigromaculatum* and *M. capitocutis*), the alpheid *Synalpheus stimpsoni*, pontoniids, polychaetes, galatheids, crabs and ophiuroids, in relatively similar proportion amongst sampling sites (Table 3). *C. multiradiata* always hosted galatheids, polychaetes and shrimps while myzostomids and crabs were only occasional (Table 4).

Overall, frequency of symbiont co-occurrence for supra-specific taxa was not significantly different than expected by chance, except for the shrimp-galatheid pair (Fisher's Exact test, p=0.05). From analysis considering couples of species, the co-occurrence of symbiotic pairs was not different than if expected by chance except for *M. capitocutis-M. nigromaculatum*, *M. capitocutis-Synalpheus* sp. *3, M. nigromaculatum-Synalpheus* sp. *3, M. nigromaculatum-Periclimenes attenuatus* and *M. ambiguum-Periclimenes tenuis* that co-occurred with higher frequency than expected (p≤0.05).

Table 3. Number of individuals of symbiont taxa collected from *Phanogenia multifida* sampled at different sites in Hansa Bay. SW, NW, E: Southwest, northwest and east of Lang Island, respectively (see Fig. 1 for map location).

Symbiont taxa	SW	NW	E	Sushimaru	Durangit
Myzostoma nigromaculatum	31	74	36	48	46
Myzostoma capitocutis	24	26	10	2	2
Synalpheus stimpsoni	9	4	9	10	7
Pontoniids	16	5	2	12	5
Polychaetes	3	4	1	2	3
Galatheids	1	1	0	0	1
Crabs	1	0	0	0	2
Ophiuroids	0	0	1	0	0

Table 4. Number of individuals of symbiont taxa collected from *Cappilaster multiradiata* sampled at different sites in Hansa Bay. SW, NW, E: Southwest, northwest and east of Lang Island, respectively (see Fig. 1 for map location).

Symbiont taxa	SW	NW	E	Sushimaru	Durangit
Myzostomids	0	2	1	1	1
Galatheids	9	8	3	2	7
Polychaetes	4	4	6	2	6
Shrimps	1	3	1	19	5
Crabs	0	0	2	1	3

Table 5. Rohde's index values for myzostomids and shrimps found in association with crinoids (when ≥ 4 individuals collected) in Hansa Bay, Papua New Guinea.

Symbiont taxon	Number of specimens	Number of species of infested crinoids	Number of families of infested crinoids	Rohde's index for prevalence
Myzostomida				
Contramyzostoma sphaera	129	1	1	1,00
Myzostoma ambiguum	348	4	2	0,76
Myzostoma capitocutis	17	3	1	0,82
Myzostoma cuniculus	15	1	1	1,00
Myzostoma fissum	13	1	1	1,00
Myzostoma laingense	5	1	1	1,00
Myzostoma nigromaculatum	34	1	1	1,00
Crustacea (Natantia)				
Palaemonella pottsi	29	7	4	0,61
Pereclimenes attenuatus	7	2	1	0,91
Pereclimenes ceratophtalmus	7	2	2	0,93
Pereclimenes sp. *aff. commensalis*	9	1	1	1,00
Pereclimenes sp.	21	6	3	0,54
Pereclimenes tenuis	7	2	2	0,76
Pontoniopsis comanthi	26	7	4	0,57
Synalpheus demani	8	4	3	0,59
Synalpheus sp. *A*	17	3	1	0,68
Synalpheus sp. *B*	16	2	1	0,77
Synalpheus stimpsoni	5	2	1	0,78

In general myzostomids had greater Rohde's index (x=0.94) than shrimps (x=0.74) indicating greater host specificity (Table 5). Accordingly, five myzostomid species (*C. sphaera, M. cuniculus, M. fissum, M. laingense, M. nigromaculatum*) infested only one single species of crinoid, the most opportunistic myzostomid species *M. ambiguum* infesting four crinoids species. In contrast, only one shrimp (*Periclimenes* sp. *aff. commensalis*) infested only one single species of crinoid while the case of opportunism was more extensive with the shrimp *Pontoniopsis comanthi* that infested seven crinoid species.

4. Discussion

Crinoid infestation by symbiotic organisms was important in Hansa Bay, with about 92% of crinoids infested at a rate of about 8 symbionts per crinoid, with a maximum of 52 symbionts (all taxa confounded) on a single host. This level of infestation is greater than for the Great Barrier Reef where about 56% of crinoids are infested at a rate of infestation of about 3 and with a maximum of 30 symbionts from various taxa on a single host (Fabricius and Dale, 1993).

Amongst echinoderms, crinoids are certainly the group that is the most infested by ectosymbiotic metazoans in tropical shallow water. Their success as hospitable substratum is probably due to their complex external morphology that protects symbiotic organisms from possible predators by providing diversity of efficient shelters like mobile arms around the calyx, stiff pinnules and cirri (Fabricius and Dale, 1993). The occurrence of stiff proximal pinnules could explain why synalpheids, that were the largest symbiotic shrimps observed on crinoids in this study were absent from Mariametridae and Himerometridae. Indeed, in these families of crinoids, such pinnules protect from predation and infestation by symbiotic organisms of larger size (Meyer et al., 1984).

A second characteristic of crinoids making them hospitable substrate is probably related to their feeding mechanisms. Crinoids are filter feeders and they use ambulacral grooves running along each arm to transport to the mouth food particules normally spread in the water column. Such concentration and transport process into the grooves makes food easily accessible to a variety of symbionts. The ambulacral grooves can be closed by lappets except in Comasteridae, which might explain why this family is often heavily infested (Fabricius and Dale, 1993). Symbionts then have access to the strings of mucus and plankton carried along the crinoid arms that is otherwise not easily accessible in other crinoids.

A third characteristic of crinoids playing possible role in their hospitability to symbiosis is that they do not secrete saponins such as other echinoderms do as chemical defensive strategy (Burnell and Apsimon, 1983). In asteroids and holothuroids, saponins cause deleterious effects on free-living animals and specific physiological adaptations towards these substances are required for symbionts that live associated with these hosts (Patterson et al., 1978).

Density and variety of symbionts allocated to a certain crinoid did not change with sampling site in Hansa Bay. Presumably, geographical location has lower influence than other factors such as depth of collection (the deeper the host the least symbiont abundance; Zmarzly, 1984), and/or host density (the denser the host population the denser the symbiont population; Fabricius and Dale, 1993). Symbionts on crinoids should normally have to compete for space and food (Fishelson, 1974); however this study suggests low level of competition among symbionts, which was also observed for Great Barrier Reef symbionts (Fabricius and Dale, 1993). One commonly assumes that crinoids with relatively big size and complex body shape most likely provide symbionts with enough ecological micro-niches to co-exist together. This study shows that five pairs of symbiotic species co-occur together more favorably than with others, which probably does not reflect any advantage of the co-occurrence itself but rather independent and convergent preferences to shelter on the same hosts. Yet, Huang et al. (2005a) reported that symbionts located on oral disk of *Comanthina schlegeli*, mainly *Synalpheus* shrimps and clingfishes, are mutually exclusive. We also never found shrimps and clingfishes occurring together on oral disks of crinoids but these symbionts were commonly observed with polychaetes and/or myzostomids on disks.

One of the main features that regulate symbiont distribution across crinoid hosts is the size of crinoids, the larger being more likely infested (Fabricius and Dale, 1993). Thus intuitively one expects significant correlation between the number of symbiont specimens and the crinoid size. In this study, however, this was not the generality amongst symbionts. It was observed mainly for shrimps but not for myzostomids, thus indicating that abundance of symbionts is not always dependent on crinoid size.

Multispecies assemblages on crinoids are believed to be highly non-specific symbioses: all common symbiont species would be found on more than one host species (Fabricius and Dale, 1993). This study considers three types of symbionts based on host-specificity: species-specific symbionts, selective symbionts, and opportunistic symbionts. Species-specific symbionts had high Rohde's index value and were associated with only one host-species; they included most myzostomid species, and a shrimp species close to *Periclimines commensalis*. The relatively high host-specificity shown by myzostomids is probably related to their long time association with crinoids in term of evolution. Indeed, some myzostomid galls and pits deformation have been identified from fossilized Carboniferan and Ordovician crinoids (Warn, 1974; Meyer and Ausich, 1983; Eeckhaut and Lanterbecq, 2005). Due to their long history as host-specific symbionts, myzostomids have acquired a unique, highly derived anatomy, totally devoted to their life on echinoderms, especially crinoids (Lanterbecq et al., 2006). In contrast to shrimps and most other symbiotic organisms, myzostomids cannot move on substrates surrounding crinoids and the only period during which they are not associated with their host is a short free larval stage (Eeckhaut and Jangoux, 1993). In this case, host-identity recognition at the time of settlement and metamorphosis is probably the main factor that regulates the distribution of these species-specific symbionts.

Selective symbionts have high Rohde's index yet are associated with more than one host having non-homogeneous distribution. In this study, they included some myzostomids and shrimps for which distribution certainly relied on various ecological/environmental parameters, one of which being the ability of the selected crinoids to offer adequate shelters. In general, large shrimps sheltered on large-sized crinoids, as the case for synalpheids that were most often found on or underneath the host's calyx. Synalpheids seemed to shelter preferably on large crinoids that were partially hidden during daytime and had numerous arms but no large cirri. Synalpheids were thus mainly found on large Comasteridae, but not on those that had large cirri and stiff proximal pinnules such as *O. bennetti*. This underlies the fact that the occurrence of selective symbionts with various alternative hosts and some preferred hosts depends on habitat restrictions during settlement process (Baeza and Stotz, 2001) and/or competitions among symbionts for the preferred hosts (Huang et al., 2005a). Mutually exclusive competition for a given crinoid host species has been observed between synalpheid snapping shrimps and clingfishes (Huang et al., 2005a). Selection of hosts could occur either at the larval (Baeza and Stotz, 1998) or adult stage (Vandenspiegel et al., 1998). However, larval stages of symbionts have not been observed on *Comanthina schlegeli* in the study of Huang et al. (2005a, b) nor in the present study, suggesting that the second hypothesis would be a general rule in symbionts of crinoids. Host selection by adult symbionts would therefore rely on visual (Vandenspiegel et al., 1998; Huang et al., 2005a) and/or chemical cues (Vandenspiegel et al., 1998; Eeckhaut et al., 2000; Vaïtiligon et al., 2004).

Opportunistic symbionts have low Rohde's index and are observed on numerous crinoid species. In this study they included some shrimps, the myzostomid *Myzostoma polycyclus,* the crab *Harrovia longipes*, the ophiuroid *Ophiolophus novarae*, and most likely the polychaetes *Paradyte crinoidicola and Hololepidella millari*. Opportunistic symbionts would probably infest large number of crinoid species if their populations were large enough. They appear less selective than the two first categories yet are probably able to select between various potential hosts, as shown for the crab *H. longipes* that can distinguishes natural hosts from other crinoids by chemodetection (Eeckhaut et al., 2000).

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