

Occurrence and histopathological effects of *Monstrilla* sp. (Copepoda: Monstrilloida) and other parasites in the brown mussel *Perna perna* from Brazil

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During a parasitological survey of the brown mussel Perna perna from highly productive culture fields in the southern sector of Brazil, monstrilloid copepods were discovered in the mantle of this mytilid bivalve. Numerous specimens of endoparasitic copepods were found within nodules in the mantle of the host; they belong to an undetermined species of Monstrilla. Monstrilloid copepods were known to be endoparasitic in polychaetes and in one species of prosobranch mollusc only; their occurrence as parasites of bivalve molluscs has not been previously documented. This is also the first record of these crustaceans infecting commercial molluscs. The prevalence of Monstrilla sp. infecting the brown mussel was 25.6%. At the histological level, it was observed that the larvae induced a strong haemocytic infiltration resulting from the complete larval encapsulation within the host mantle. The effect of this monstrilloid in the cultured populations of P. perna deserves further study.

Keywords: mussel, bivalve molluscs, *Perna perna*, parasitic copepods, crustacean parasites, *Monstrilla*

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INTRODUCTION

The state of Santa Catarina (SC), in southern Brazil is the major Brazilian producer of the brown mussel *Perna perna* Linnaeus, 1758 and of the Pacific oyster *Crassostrea gigas* Thunberg, 1795; 10,600 and 3150 tons were produced during 2007, respectively. Particularly, the districts of Governador Celso Ramos and Penha contribute with most of the production, with 4900 and 2000 tons, respectively, in 2007. An episode of mortality of the mussel *P. perna* (almost 20%) was observed by the farmers from the district of Penha during August 2008. This event was notified to the Santa Catarina Institution for Agricultural Research and Rural Extension Services (EPAGRI) and then to the Santa Catarina Federal University (UFSC) in order to study the causes of this event.

Monstrilloids are protelean parasitic copepods infecting benthic polychaetes and prosobranch molluscs; recently, they have been recorded also in sponges (Huys *et al.*, 2007). The free-living adult stage is present in the water column and mature individuals are collected frequently by plankton nets in coastal waters from all latitudes (Davis, 1984; Suárez-Morales, 2001). Very little is known about the development of these endoparasite crustaceans. The early works by Giard (1895, 1896), Malaquin (1901), Caullery & Mesnil

(1914) and Pelseneer (1914) were among the most relevant in describing the general patterns of larval development in Monstrilloida. There are some recent studies on the morphology of the free-swimming early naupliar stages (see Grygier & Ohtsuka, 1995). Most of their life cycle takes place in the body of the host after the last naupliar stage, which burrows into the host to complete its development. Shortly after the infection, a protective sheath is produced around the larva, which feeds from the host through a pair of ventral antenna-like processes (Davis, 1984). The development takes place within the sheath until a pre-adult stage that emerges from the host as a free-living, non-feeding copepod. The free-living form rapidly becomes a reproductive adult in the water column.

In this paper we report the occurrence of different parasites in the brown mussel *Perna perna* collected from southern Brazil culture fields, particularly in reference to the finding of the endoparasitic monstrilloid copepods. We evaluate the intensity and prevalence of the infection by these crustacean parasites and their histopathological effects on the mantle tissues of the host.

MATERIALS AND METHODS

Specimens of the brown mussel *P. perna* (N = 90) were collected from a culture area at Penha, Santa Catarina State in August 2008 during a local episode of mussel mortality

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(almost 20%); the specimens were kept alive and analysed upon arrival at the laboratory. All animals were examined for external body and shell gross abnormalities. Ten specimens were randomly selected to determine the occurrence and extent of pathological conditions by histological examination, as follows: mussels were shucked and a sagittal (5 mm thick) section containing gills and visceral mass was excised, fixed in Davidson's solution, and embedded in a paraffin block. Serial tissue sections (5 µm) were stained with Harris haematoxylin and eosin. A piece of mantle with nodules from five animals was also submitted to histological examination as described above.

The mantle from 5 other mussels bearing abnormal nodules was also excised. Some nodules were dissected under the stereoscope to extract the copepod larvae. Isolated copepod larvae and entire nodules were immediately fixed in 70% alcohol for further taxonomic and parasitological examination. In order to facilitate the observation process the copepods were transferred to a freshly prepared solution of glycerol (70%) in ethanol, and then into pure glycerine. Most of the juvenile specimens examined were strongly bent inwards; these were observed from different angles and illustrations were made from undissected specimens.

Prevalence was estimated as the percentage of infected mussels in the sample. The intensity of pathogenic organisms was scored as low, moderate, or heavy according to the macroscopic observations.

RESULTS

The mean values (\pm SD) for whole weight and shell length among the examined mussels were 30 ± 7.9 g and 72 ± 5.8 cm, respectively; these measurements are within the commercial size-range for this species. The prevalences of pathological conditions observed in *P. perna* mussels from the surveyed area are shown in Table 1.

Macroscopic observations of the specimens examined showed the presence of the colonial tunicate *Didemnum psammathodes* Sluiter, 1895 covering the shell of 20% of the examined mussels. In some cases (9%) the tunicate covered more than half of the outer surface of the shell, which was considered as a heavy infestation. Ciliated turbellarian *Urastoma cyprinae* Graff, 1882 (Platyhelminthes) were observed moving freely throughout the gill lamellae in 100% of the mussels examined; this infestation occurred always at low intensity (few individuals). The polychaete *Polydora websteri* Hartman, 1943 occurred with 20% prevalence. The intensity was low; infested mussels had 1 or 2 small conchioline-covered tubes on the inner surface of the shells, but no

observable damage was associated with the adjacent mantle. The trematode *Bucephalus margaritae* Ozaki & Ishibashi, 1934 (= *varicus*) (Bucephalidae) was detected macroscopically in one mussel (1.1%) (see Table 1). The infection by *B. margaritae* produced an orange pigmentation and a filamentous aspect of the mantle as a result of an intense parasite burden. The heavy intensity of infection probably caused host castration since the mantle was extremely thin with no apparent gonadal material, typically of red (female) or white (male) colour.

From all mussels examined, 23 had rose-coloured nodules near the border of the mantle, with sizes ranging from 1 to 4 mm (Table 1; Figure 1A); up to 10 nodules were detected on the mantle of the most infected animals. The nodules were opened under the stereomicroscope and the larval monstrellid copepods recovered (Figure 2). The monstrellid copepods were identified as belonging to the genus *Monstrilla* by the lack of eyes and by the presence of five caudal setae; all specimens examined were juvenile females, with 4-segmented antennules and a pair of structures on the genital somite that will become the ovigerous spines. At least two different developmental stages were observed in the specimens examined (see Figure 2).

At the histological level, it was observed that the copepod larvae induced a strong alteration of the mantle's architecture. The copepod larvae were observed: (1) surrounded by a space; probably filled with the sheath (before histological procedure) produced by the larva after the infection; (2) encapsulated by a thick layer of infiltrated haemocytes resulting from the host response to the invasive process of larval development within the host tissue; and (3) invaded by those haemocytes which destroyed the parasite's wall and penetrated into their internal tissues (see Figure 1).

DISCUSSION

The brown mussel *P. perna* cultured at Penha, Santa Catarina State, was sampled after a mortality episode; the population was studied herein to determine possible links with parasitic organisms. Different commensal and parasitic organisms were recorded; most of them occurred at low levels of prevalence and intensity. A similar mortality event occurred in the summer of 2007 but no parasitological survey was carried out because the economic loss was relatively unimportant.

The ascidian *Didemnum psammathodes* was observed covering partially the mussels' outer shell surfaces, except for 8 individuals that had almost the whole shell inhabited by *D. psammathodes*; this condition might have compromised to some extent the bivalves' filtering process. The ascidians *D. psammathodes* and *Didemnum perlucidum* Monniot, 1983 are frequent fouling species inhabiting bivalve shells and aquaculture equipment, such as lantern nets or long-lines at Santa Catarina Island and Penha (A.R.M. Magalhães, Brazil, personal communication; Kremer, 2008). *Didemnum* sp. was reported as one of the most common fouling species covering the shells of pearl oysters in Australia (Guenther & De Nys, 2006).

The turbellarian *Urastoma cyprinae* has long been considered as a commensal to different species of bivalve molluscs, including mussels; however, in some cases it can become a true parasite, causing some damage to the host, as reported for the oyster *Crassostrea virginica* (Brun *et al.*, 1999) and the

Table 1. Prevalence (%) of organisms or parasites on brown mussel *Perna perna* from the culture area of Penha, Santa Catarina State.

Parasite or pathological condition	Prevalence	Intensity	N
<i>Didemnum psammathodes</i>	20	10 (M), 8 (H)	90
<i>Urastoma cyprinae</i>	100	90 (L)	90
<i>Polydora websteri</i>	20	90 (L)	90
<i>Bucephalus margaritae</i>	1.1	1 (H)	90
<i>Monstrilla</i> nodules in mantle	25.6	nd	90

N, number of analysed animals; L, low; M, moderate; H, heavy; nd, not determined.

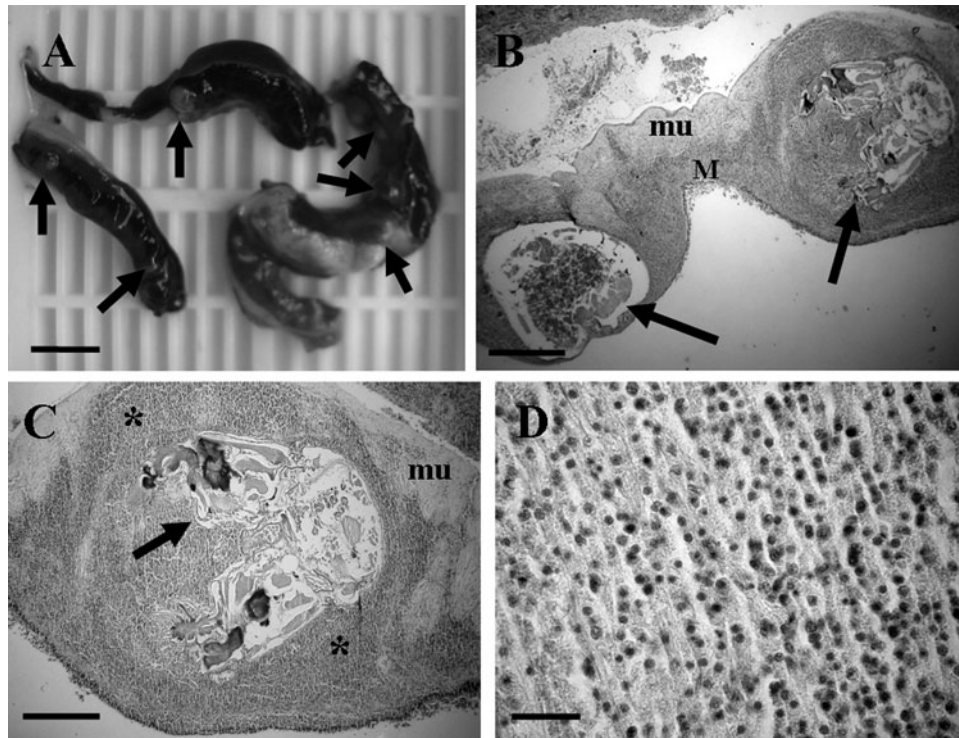


Fig. 1. *Monstrilla* sp. infecting the brown mussel *Perna perna* from Brazil. (A) Infected mussel's mantle with several nodules (arrows): scale bars = 10 mm; (B) general view of the mantle's border (M) showing two nodules containing larvae of copepods (arrows), and alteration of the architecture of mantle's tissue: scale bars = 100 μ m. mu, muscle fibers of mantle's border; (C) copepod larvae induced-nodules formation and intense haemocytic infiltration (*): scale bars = 200 μ m; (D) magnification of a connective tissue heavily infiltrated by haemocytes: scale bars = 20 μ m.

mussel *Mytilus galloprovincialis* (Robledo *et al.*, 1994). Cáceres-Martínez *et al.* (1998) reported prevalences of *U. cyprinae* varying from 57 to 100% in the mussel *M. galloprovincialis* from a culture area in Baja California, Mexico and observed haemocytic infiltration around the turbellarian parasite. Although the occurrence of *U. cyprinae* in the examined mussels was widespread, it was present as a free-living organism moving around the gills. Our histopathological analysis did not reveal any lesions or host response to the turbellarian on the mussel's gills. Hence, there is no evidence that this turbellarian causes morbidity or is parasitic to the populations of *P. perna* from the surveyed area.

Burrowing forms of the polychaete genus *Polydora* are common parasites infesting the inner shell's surface of edible molluscs (Lauckner, 1983). There are several culture areas at Santa Catarina Island with high prevalences (up to 100%) of *Polydora websteri* Hartman, 1943 infesting the oysters *Crassostrea gigas* (Sabry & Magalhães, 2005); however, prevalences and intensities are low in the brown mussel *P. perna* (Magalhães & Ferreira, 2006). *Polydora* can cause significant damage to the oyster *C. gigas*, including weakness of the shell and lesions to the adductor muscle and mantle (Sabry & Magalhães, 2005). However, the main problem related to this parasite concerns the repulsive aspect of the shells resulting from the presence of abundant blisters and detrital tubes of *Polydora*; the infected specimens lose their commercial value and are rejected by potential consumers (Magalhães & Ferreira, 2006).

Bucephalus margaritae is the foremost parasite infecting the brown mussel *P. perna* at Brazil and the Santa Catarina coast (Magalhães & Ferreira, 2006; Cochôa & Magalhães, 2008). *Bucephalus* sp. has been blamed for host (*P. perna*)

castration and induction of haemocytic infiltration at connective tissue and consequent reduction in the number of circulating haemocytes (da Silva *et al.*, 2002). During this work, the local prevalence of this trematode (1.1%) was very low in comparison to culture and wild areas at Santa Catarina Island such as Ribeirão da Ilha (south) and Ponta das Canas (north) reaching 32.1 and 63.6%, respectively (da Costa, 2007), or even the same area at Penha with previously recorded prevalences higher than 20% (de Mesquita *et al.*, 2008).

The copepod *Monstrilla* sp. was found infecting the mantle of *P. perna* mussel with a considerable prevalence in this cultured population. The work by Pelseneer (1914) includes the only previous record of monstrilloid copepods in a mollusc host; he found a low prevalence (2%, 4 out of 200) of these parasites in a population of the prosobranch gastropod *Odostomia rissoides* (now considered as a junior synonym of *O. scalaris*) from the English Channel. These specimens were reared to adulthood and identified as *Monstrilla helgolandica* Claus, 1863. Hartman (1964) reported a 1% prevalence of *M. capitellicola* Hartman, 1964 in the polychaete *Capitella capitata oculata* Hartman, 1961 from California. This species was described based on immature females only, as Hartman (1964) indicates that they were still enclosed in the capsular sheath. Because of this, the species is not currently recognized as valid and its taxonomic status remains uncertain. Unfortunately, no adults of *Monstrilla* were collected from the cultures of *P. perna* in this survey, but the occurrence of the genus could be confirmed by the analysis of the morphology of the juveniles. The prevalence in the cultured mussels during our survey in Brazil is higher than that observed by Pelseneer (1914) in a natural population of the

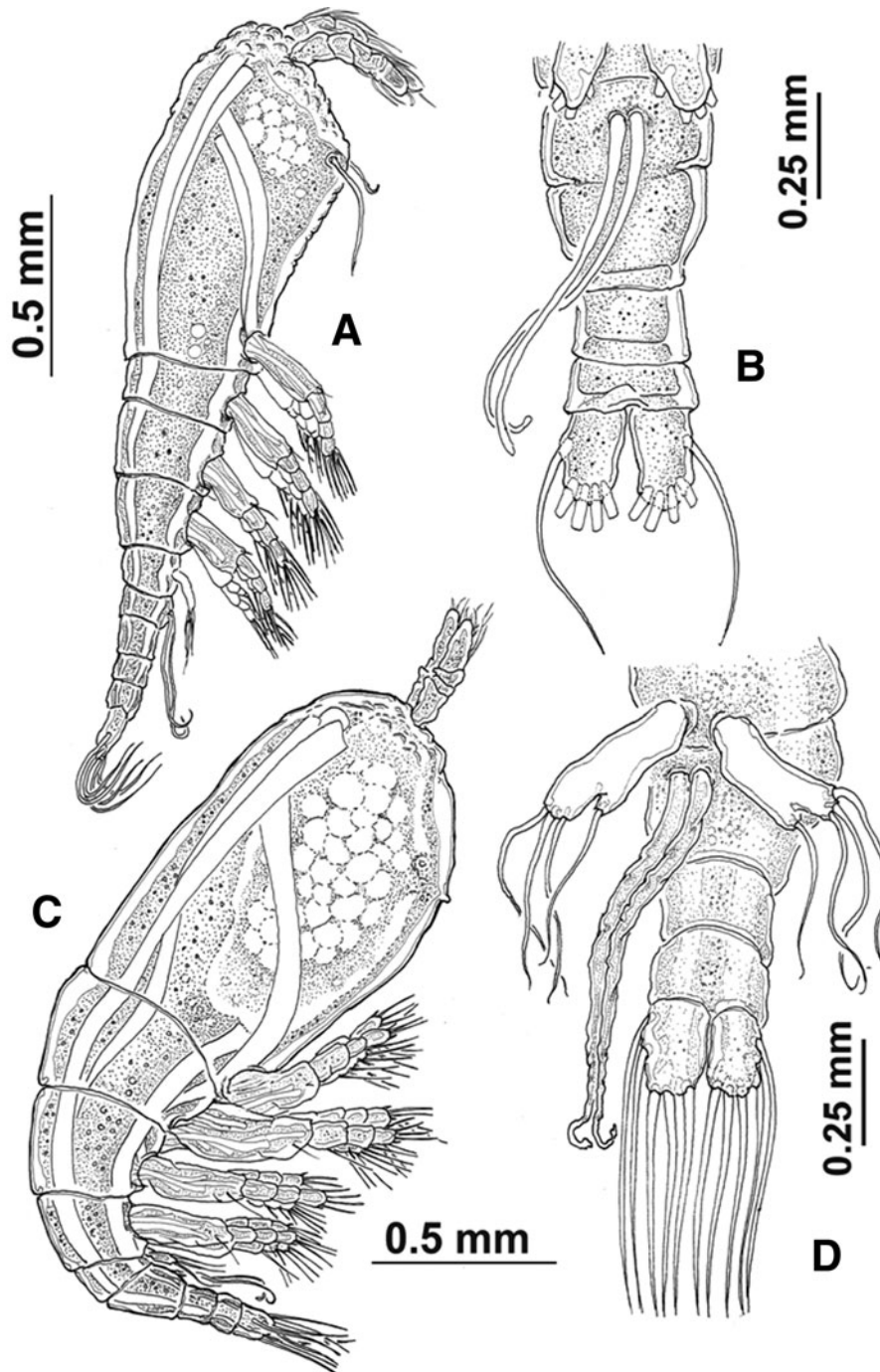


Fig. 2. General morphology of two juvenile specimens of *Monstrilla* sp. at different developmental stages recovered from the brown mussel *Perna perna* in Santa Catarina Island (southern Brazil). (A) Habitus of early female copepodite, lateral view; note the feeding tubes on the ventral surface, the genital somite is still 2-segmented; (B) urosome, ventral view showing genital spines and outer juvenile and inner cuticle of next stage; (C) habitus of juvenile late copepodite nearing its final moult, lateral view; (D) urosome, ventral view. Note that the total size of both specimens is equal, the early copepodite has bent caudal rami and antennules, and the late copepodite has the urosome bent inwards to fit in the chamber.

prosobranch *O. scalaris*. The moderate prevalence (25.6%) of *Monstrilla* in the brown mussels from the surveyed area is very likely a result of the high culture density of potential hosts for larval monstrilloids. The availability of hosts is an essential factor in determining the adult populations of monstrilloids in the water column, as suggested by Suárez-Morales (2001) who recorded a dense aggregation of monstrilloids in a reef environment, where high densities of polychaete hosts were expected.

The specimens examined were identified as juvenile females belonging to the genus *Monstrilla* by the presence of two urosomites posterior to the genital somite (Figure 2); however, this character alone does not differentiate *Monstrilla* from *Monstrillopsis*. The specimens obtained from *P. perna* have five caudal setae; *Monstrilla* is the only genus in which five caudal setae are found in females (Grygier, 1994; Suárez-Morales & Gasca, 2004). The setal armament of the caudal rami remains unchanged during the

juvenile and pre-adult stages of Mostrilloidea; for instance, Caullery & Mesnil (1914) described the juveniles of both *Cymbasoma germanicus* Timm, 1893 and *C. malaquini* Caullery & Mesnil, 1914 from polychaetes and found three caudal setae, a genus-related character, at different stages. The stability of this character through the ontogenetic development of the copepodid phase in the Mostrilloidea is also confirmed by Huys *et al.* (2007). Another characteristic present in the juvenile specimens examined was the absence of eyes even in those specimens nearing their final moult; this is another feature exclusive to the genus *Monstrilla* (Grygier, 1994). In these specimens there were also remains of the feeding tubules connecting them to the host; these appendages remain almost entirely within the host after the pre-adult emerges (Caullery & Mesnil, 1914; Davis, 1984). The armature and structure of the fifth leg is an important taxonomic characteristic within the group and it appears to remain constant during the last developmental stage as well as in the adult; the specimens examined clearly show a fifth leg with an inner lobe armed with a single seta and an outer lobe with three setae (Figure 2). Two species in the region are known to present such features: *Monstrilla pustulata* Suárez-Morales & Dias, 2001 and *M. brasiliensis* Suárez-Morales & Dias, 2000, both from Brazil. The taxonomy of the Mostrilloidea is largely based on the morphology of the adult; hence, it is not possible to provide a reliable identification of the immature specimens until adult individuals can be recovered from the area or reared in laboratory. A complete morphological and taxonomic analysis of these specimens is still due.

The present work is the first study on the histopathological effects of the endoparasitic stages of the Mostrilloidea on its hosts. We demonstrated the damage caused by the copepod larvae to the host at histological level, nodules containing copepods were already macroscopically evident on the mantle's border; some of them were large enough to potentially cause a deficiency in the open-close shell mechanism; however, this aspect needs further evaluation. Nevertheless, the presence of copepod larvae infecting the mantle of the mussel *P. perna* caused a severe alteration of the histological structure of the mantle. Accordingly, an important inflammatory response consisting of haemocyte migration into the copepod larvae tissue was observed, indicating that haemocytes were able to recognize the copepod larvae as a foreign invasive organism. In some cases the haemocytes were able to disrupt the copepod wall enabling tissue destruction. The present work provided interesting data to encourage further research on lesions and host response caused by a *Monstrilla* copepod larva.

After an anecdotal mention of a monstrilloid present in a bivalve in Hong Kong (Boxshall & Halsey, 2004), the finding of *Monstrilla* in *P. perna* is the first documented record of such crustacean parasites in bivalve molluscs; furthermore, there was no previous report of monstrilloids as parasites of commercial, edible molluscs.

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