OCCURRENCE OF *Monstrilla* sp. IN *Perna perna* GROWN IN BRAZIL

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ABSTRACT

Copepodids of the genus *Monstrilla* has been registered in the mussels culture *Perna perna* on the coast of Santa Catarina state, southern Brazil. Between July 2010 and June 2011, 2,880 specimens of *P. perna* were collected from the main mussel-farming municipalities for analysis under stereomicroscope, differential contrast microscopy (DIC), scanning electron microscopy (SEM) and histopathology. The presence of copepodites and pre-adult females was observed in nodules only in the connective tissue of mussels mantle border. Solely Palhoça presented infestation (prevalence of 43.33%) during June 2010, without mortality. There was no infiltration of hemocytes in the infested tissue.

Key words: copepod; Monstrilloida; mussel culture; parasitism.

OCORRÊNCIA DE *Monstrilla* sp. EM *Perna perna* CULTIVADO NO BRASIL

RESUMO

Copepoditos do gênero *Monstrilla* foram registrados em *Perna perna* cultivados no litoral do estado de Santa Catarina, Brasil. Entre julho de 2010 a junho de 2011, foram coletados 2.880 indivíduos de *P. perna* nos principais municípios produtores do Estado para análises ao estereomicroscópio, microscopia de contraste diferencial (DIC), microscopia eletrônica de varredura (SEM) e histopatologia. Foi registrada a presença de copepoditos e fêmeas pré-adultas em nódulos apenas no tecido conjuntivo da borda do manto dos animais, com prevalência de 43,33% nos mexilhões de Palhoça em junho de 2010, sem casos de mortalidade. Não foi observada infiltração de hemócitos no tecido infestado.

Palavras-chave: copépode; Monstrilloida; mitilicultura; parasitismo.

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INTRODUCTION

Monstrilloida (SARS, 1901) species are copepods parasites that infect benthic polychaetes, bivalve molluscs and sponges (HUYS *et al.*, 2007; SUÁREZ-MORALES, 2011. The development of the parasitic stage of this crustacean is not well known (SUÁREZ-MORALES *et al.*, 2010). Monstrilloida copepodids have an infectious naupliar stage which penetrates in the host tissue (GRYGIER and OHTSUKA , 1995). After infection a protective sheath and a food tube are present (DAVIS, 1984; SUÁREZ-MORALES, 2011).The free adult life stage is present in the water column where mature individuals can be collected regularly by plankton nets (DAVIS, 1984; SUÁREZ-MORALES, 2001). According to SUÁREZ-MORALES (2011) 45% of the total registration of Monstrilloida order is in Northeast Atlantic, followed by the Northwest Atlantic with 17% and the South hemisphere with less than 3%. Parasitic monstrilloids occurrences in molluscs or polychaetes are presented in Table 1.

Table 1. Synthesis of research with copepodids Monstrilloida.

Host or origin	Site	Reference
Molluscs	Penha/SC, Brazil	SUÁREZ-MORALES *et al.* (2010)
	Palhoça/SC, Brazil	Present study
Polychaetes	Hawai, USA	SUÁREZ-MORALES *et al.* (2014)
Plankton	Yucatan Peninsula, Mexico	SUÁREZ-MORALES and ISLAS-LANDEROS (1993); SUÁREZ-MORALES (1994) SUÁREZ-MORALES and GASCA (1998)
	Rio de Janeiro, Brazil	DIAS (1996)
	Santa Catarina, Brazil	DUARTE (1999)
	Espírito Santo, Rio de Janeiro, Bahia, Pernambuco, Brazil	SUÁREZ-MORALES and DIAS (2000)
	Rio de Janeiro, Brazil	SUÁREZ-MORALES and DIAS (2001a)
	Espírito Santo, Rio de Janeiro and Bahia, Brazil	SUÁREZ-MORALES and DIAS (2001b)
	Santa Catarina, Paraná, São Paulo, Alagoas, Rio Grande do Norte, Brazil. Coast of Argentina.	DIAS and BONECKER (2007)
	Mexican Caribbean	SUÁREZ-MORALES (2001)
	Mexican Pacific	SUÁREZ-MORALES and ALVAREZ-SILVA (2001)
	Mexican Caribbean	SUÁREZ-MORALES (2003)
	Valparaíso, Chile	SUÁREZ-MORALES *et al.* (2006)
	Beagle Channel, Chile	SUÁREZ-MORALES *et al.* (2008)
Museum	Aegean Sea, Greece	SUÁREZ-MORALES and ALVAREZ-SILVA (2001)
	Mindanao, Philippines	SUÁREZ-MORALES (2000)
	Florida, EUA	SUÁREZ-MORALES and GASCA (2004)
	Moluccas Sea, Indonesia	SUÁREZ-MORALES (2007)
	Sulu Bay, Philippines	
	Norway Sea	SUÁREZ-MORALES (2010)

The objective of this study was to evaluate the occurrence of the copepodid genus *Monstrillia* in mussels *Perna perna*, cultured in Palhoça, Governador Celso Ramos, Penha and Florianópolis, Santa Catarina, Brazil.

METHODS

Local sampling

During one year (July 2010 to June 2011), 240 mussels were sampled monthly in four localities on the Santa Catarina coast in southern Brazil. 2.800 mussel specimens were sampled from commercial farms in four municipalities, Palhoça (27º45'23"S, 48º37'07"W), Governador Celso Ramos (27º22'16"S, 48º33'43"W), Penha (26º46'59"S, 48º36'17"W) and at the experimental culture area of the Universidade Federal de Santa Catarina (UFSC), Florianópolis (27º29'27"S, 48º32'23"W). From this total, 1.440 mussels were analyzed under a stereomicroscope to

observe the presence of copepods. The 1.440 remaining specimens were submitted to a histopathology protocol. The local salinity and temperatures was recorded along the sampling period. Collected mussels were stored in thermic boxes and then transported to the laboratory at the Center for Aquatic Pathology Studies/UFSC

Analysis at stereoscope

The shell was opened by adductor muscle dissection and subsequent exposition of mantle and the soft parts was divided in two transversely, attached at the valve. The presence of copepods in the gill, mantle and the external part of the gonadic tissue was registered.

Tissue anomaly were observed and registered. Collected copepods were submitted to a protocol for differential phase contrast microscopy (DIC) and scanning electron microscopy (SEM).

Differential phase contrast microscopy (DIC) photography

For DIC, live copepods were fixed in 70% ethanol, clarified using lactic acid for a few minutes, and conditioned over a slide for analysis (HUMES and GOODING, 1964).

Scanning Electron Microscopy (SEM)

For SEM, live copepods were fixed in glutaraldehyde, washed with sodium cacodylate, dehydrated in an ascendant ethanol series, covered with gold and photographed under a scanning electron microscope (JEOL JSM-6390LV), (Dawes, 1971; Hayat, 1972; Dykstra, 1993), at the Electron Microscopy Center Laboratory (LCME) of UFSC

Histopathology

The mussels were sectioned transversely into 2 mm thick slices containing the mantle, gill, gonad and digestive gland tissues (HOWARD and SMITH, 1983; HOWARD *et al*., 2004), fixed in Davidson solution (BELL and LIGHTNER, 1988), dehydrated in an ascendant ethanol series and embedded in paraffin (PAULETE-VANRELL, 1967). Sections of 5 μm thickness were cut in a microtome (LUPE, MRP-03) using disposable razors, mounted on slides, and stained with hematoxylin-eosin (HOWARD and SMITH, 1983).

Coverslips were added, mounted with Erv-Mount and the sections were analyzed and photographed using an optical microscope.

Statistical analysis

The prevalence and infestation rate of copepod in the mussels was analysed by site and season. The prevalence was calculated by the number of pathogens/the number of hosts ratio (BUSH *et al*., 1997). Considering that the data of prevalence and infestation were nonparametric (non normal distribution), datas were analyzed using a t-test with permutation using proc multitest in SAS® (WESTFALL *et al*., 1999).

RESULTS

The presence of the *Monstrilla* sp. copepodids only at the mantle edge of mussels *Perna perna* was recorded only from Palhoça (Figures 1 and 2; Table 2) in June (autumn) of 2011. The temperature and salinity showed no statistical differences (Table 2).

Mussels length from Governador Celso Ramos was significantly lower ($p<0.05$) than in Florianópolis, Palhoça and Penha. Also, mussels length in the summer was significantly higher ($p<0.05$) than in autumn and winter (Table 2).

Monstrilla sp. *analyzed under the Microscope Stereoscope*

Microscope stereoscope analysis showed the presence of copepodids (n=39) only at the mantles edges of mussels *Perna perna* (Figures 1 and 2) and the presence of nodules in this mussel tissue. Differential interference contrast and scanning electron microscopy photography and histology showed that the copepodids present in the mantle of mussels are females and belong to the genus *Monstrilla*.

Monstrilla sp. *copepodids analyzed under the Differential Phase Contrast Microscopy (DIC)*

Photography at DIC showed the copepodids in two stages, probably copepodid III (Figure 3) and copepodid IV (Figure 4). Details of feeding tubes in younger copepodid (Figure 3), copepodid with sharper segmentation (Figures 4 to 6), copepodid with the body divided into cephalosome with the presence of front antennules, metasomal and urosome (Figure 4), four pairs of swimming appendages and double genital somite with ovigerous spines (Figure 5), and fifth legs and caudal rami (Figure 6) were observed. In Figure 7 the 1st, 2nd, 3rd, 4th and 5th pairs of swimming appendages characteristics fit with *Monstrilla* diagnosis (SUÁREZ-MORALES and DIAS, 2000). The females are characterized by a pair of ovigerous spines in urossome. The 4th of swimming appendages are located in the metasomal consisting of endopodite and exopodite with adornments of spines and setae at different numbers (Table 3). Copepodids

photographed at DIC showed mean length of 1.21 ± 0.12 mm (1.50 mm of higher length and 1.03 mm of lower).

Monstrilla sp. *photographed under a Scanning Electron Microscopy (SEM)*

SEM photography showed the pair of feeding tubes and frontal absorption processes (Figures 8 and 9), the membrane that surrounds the copepodid when it is inside mantle edge of mussels (Figure 10), the targets of the copepodid body (Figure 11), the region between the cephalosome and metasomal with pairs of filaments that copepodids use to connect to the host's body and feed your fluids (Figure 12), and the oral sealed tube and antennules details in front of the cephalosome (Figure 13). Morphological structure observed at SEM contribute to identify the genus.

Histopathology

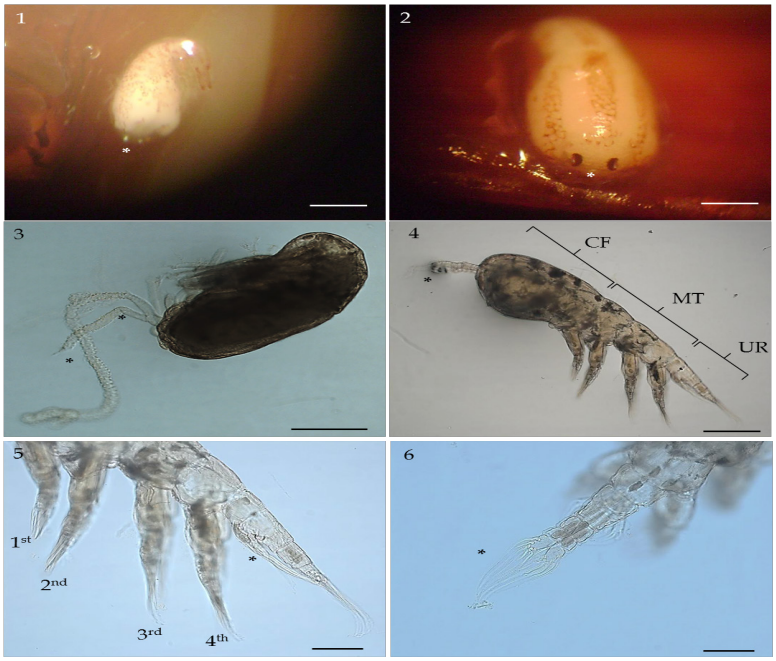
In the histological sections of the connective tissue of mussels mantle edge was observed copepodids *Monstrilla* sp. (Figures 14 to 17). No haemocite infiltrations were observed in the mussels tissue.

Prevalence and infestation

Occurrence of the genus copepodid *Monstrilla* was

Table 2. Mean (± standard desviation) of length of cultured mussels *Perna perna* sampled, prevalence (%) and infestation rate of *Monstrilla* sp. copepodids in the mussels and mean (± standard desviation) of temperature and salinity by site and season.

Study site / Season	Mussels lengths (mm)	Prevalence (%)	Infestation	Temperature (ºC)	Salinity (‰)
Palhoça	85.69 ± 11.74	43.3	3	20.62 ± 3.39	32.25 ± 2.52
Florianópolis	88.88 ± 13.96	-	-	20.45 ± 3.44	32.66 ± 2.70
Gov. Celso Ramos	76.29 ± 18.54	-	-	20.62 ± 4.21	33.08 ± 4.44
Penha	80.06 ± 49.68	-	-	21.25 ± 3.78	32.66 ± 2.87
Winter	76.55 ± 11.63	-	-	17.33 ± 2.23	34.00 ± 1.34
Spring	84.37 ± 11.71	-	-	20.95 ± 2.09	32.50 ± 1.67
Summer	91.98 ± 51.50	-	-	24.75 ± 1.95	29.75 ± 4.20
Autumn	78.48 ± 13.20	43,3	3	19.91 ± 3.42	34.41 ± 2.27



Figures 1 to 6. Specimens of copepodid of genus *Monstrilla* (female) from *Perna perna* culture at Palhoça, Santa Catarina, Brazil. Figs. 1 and 2 - Copepodid of *Monstrilla* sp., in views side-dorsal and dorsal, at stereomicroscope. Details of the front, large and pigmented ocelli (white asterisks). Figs. 3 to 6 - Copepodid of *Monstrilla* sp. at DIC. Fig. 3 - Copepodid side view. Detail of the front feeding tubes (black asterisks). Fig. 4 - Copepodid side view. Detail of the front antennules pair (black asterisk). Fig. 5 - Side view of the metasoma and urosome copepodid. Detail of four pairs of swimming appendages (1st, 2nd, 3rd and 4th) and double genital somite with the pair of ovigerous spines (black asterisk). Fig. 6 - Dorsal view of the urosome copepodid. Details of 5th legs (black arrows) and caudal rami with 6 setaes (black asterisk). Bars: 40 µm (Figs. 5 and 6) and 200 µm (Figs. 1 to 4). CF: cephalosome; MT: metasomal; UR: urosome.

Table 3. Representation of endo and exopodite pairs of swimming appendages copepodid of genus *Monstrilla*. Roman numerals indicate the number of spines and the Arabic number of setae according SUAREZ-MORALES and DIAS (2000).

Legs	Basipodite	Endopodite	Exopodite
Leg 1	1-1	0-1; 0-1; 1,2,2	I-1; 0-1; I,1,3
Legs 2 – 4	1-1	0-1; 0-1; 1,2,2	I-1; 0-1; I,1,2,2

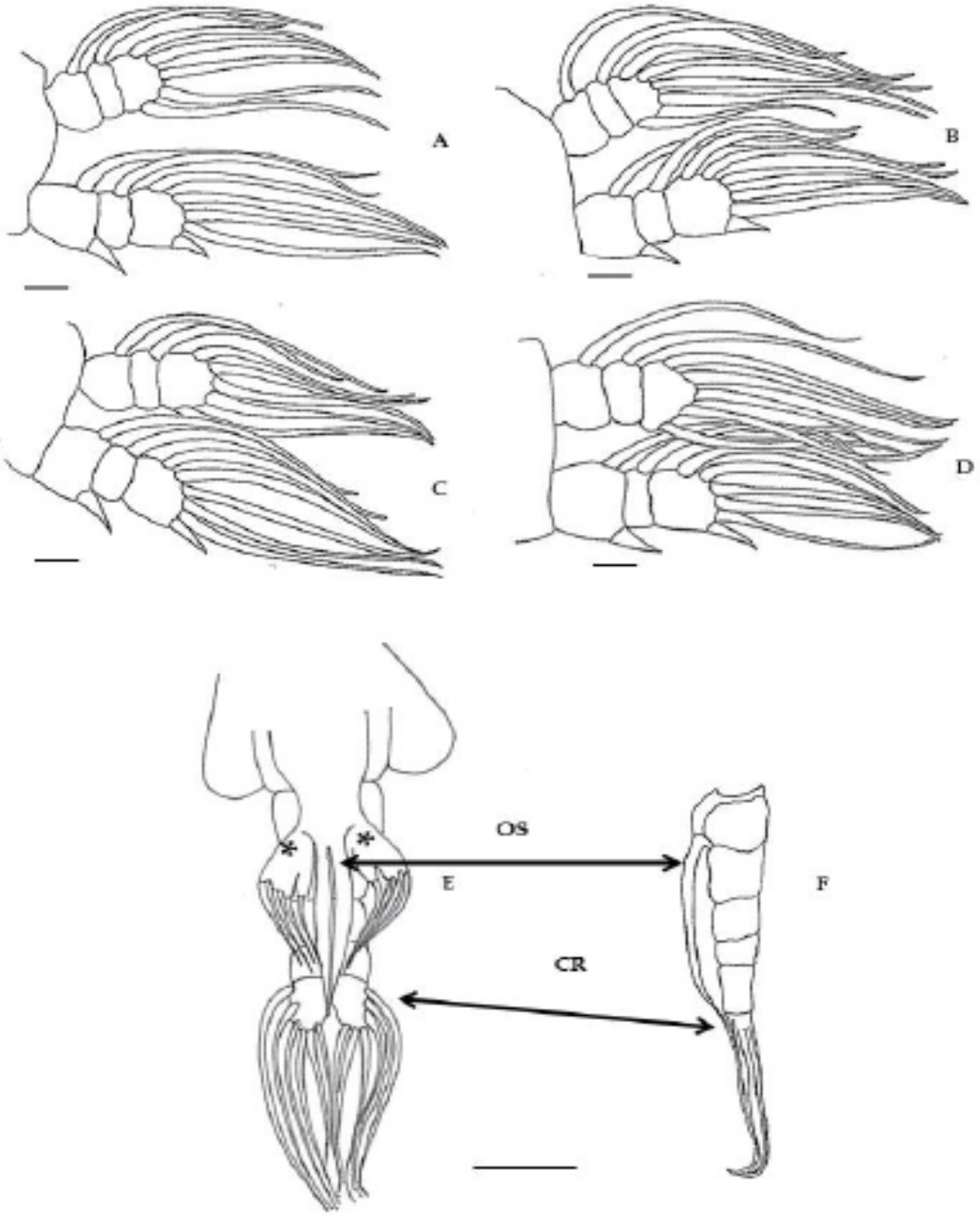
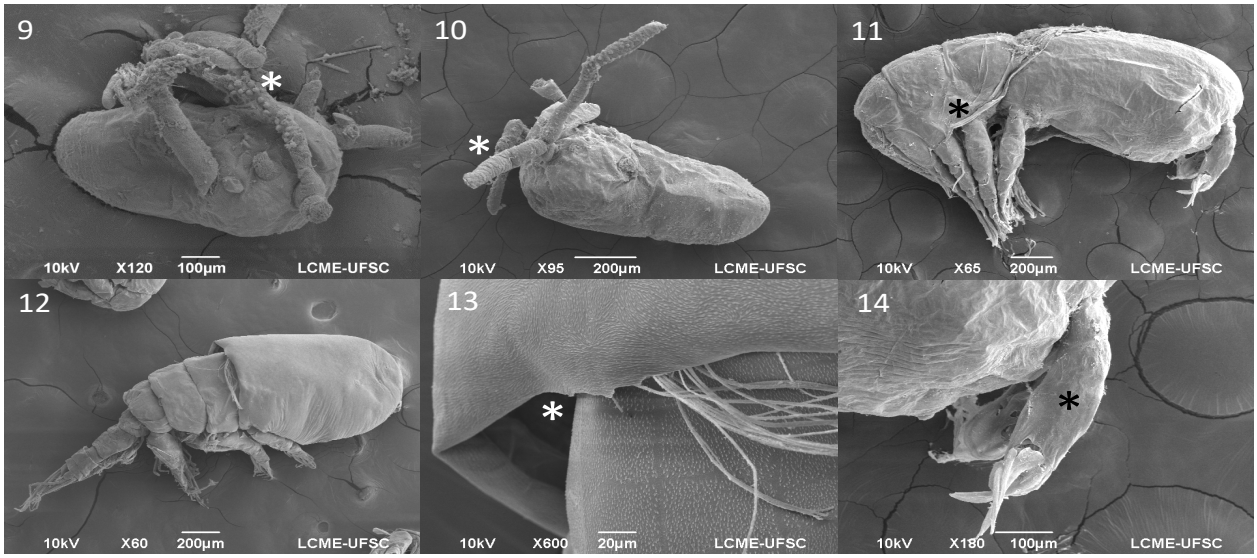
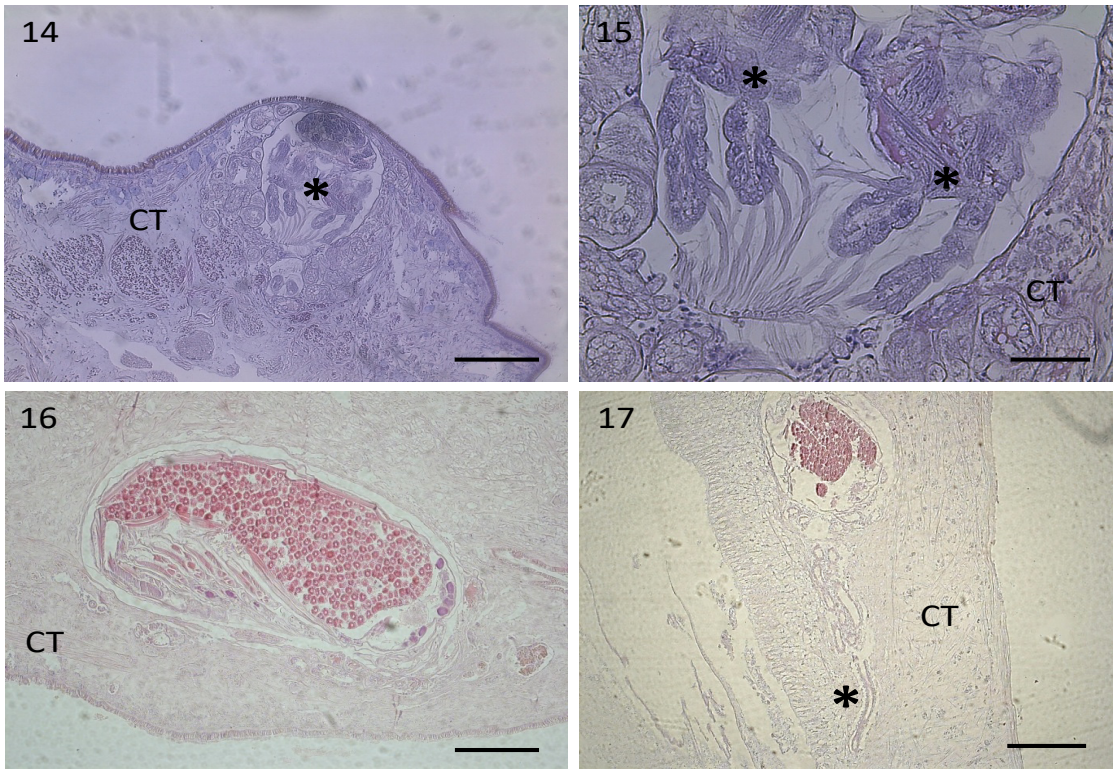


Figure 7. Schematic representation of swimming appendages pairs and urosome of copepodid *Monstrilla* sp.: (A) 1st pair of swimming appendages; (B) 2nd pair of swimming appendages; (C) 3rd pair of swimming appendages; (D) 4th pair of swimming appendages; (E) ventral view of urosome indicating the 5th pair of legs (black asterisks), the pair of ovigerous spine (OS) and the caudal rami (CR); (F) side view of urosome. Bars: 40 µm (A, B, C and D); 100 µm (E and F).



Figures 8 to 13. SEM photographs of specimens of copepodid of genus *Monstrilla* (female) collected in mussels *Perna perna* culture at Palhoça, Santa Catarina, Brazil at different stages. Figs. 8 and 9 - ventral view of copepodids with pairs of feeding tubes and frontal absorption processes (white asterisks). Fig. 10 - side view of copepodid; part of the membrane that surrounds within the host (black asterisk). Fig. 11 - lateral-dorsal view copepodid. Fig. 12 - dorsal view of copepodid in detail the region between cephalosome and metasomal showing pairs of filaments used for food. Fig. 13 - side view of copepodid in detail the closed oral tube and antennules in front of the cephalosome (black arrow).



Figures 14 to 17. Specimens of copepodid of genus Monstrilla collected in mussels *Perna perna* culture at Palhoça, Santa Catarina, Brazil. Fig. 14 - cross section of copepodid (black asterisks) in the connective tissue (CT) of the mantle edge. Fig. 15 - detail of copepodid appendages (black asterisks) in cross section. Fig. 16 - longitudinal section of copepodid in the connective tissue of the mantle edge. Fig. 17 - longitudinal section of copepodid indicating the feed tube (black asterisks) in the connective tissue of the mantle edge. Bars: 40 µm (Figs. 15 and 16); 200 µm (Figs. 14 and 17).

DISCUSSION

The discovery of the copepodids order Monstrilloida date 1841, being represented by over 120 species, grouped into five valid genera: *Monstrilla* Dana, *Monstrillopsis* Sars, *Cymbasoma* Thompson, *Maemonstrilla* Grygier and Ohtsuka and *Australomonstrillopsis* (SUÁREZ-MORALES, 2011; SUÁREZ-MORALES and MCKINNON, 2014). Most of these studies dealt with specimens in mature stages, from plankton samples or biological material deposited in scientific collections and descriptions of parasitic stages are rare. The taxonomic studies using material collected in hosts such were performed by SUÁREZ-MORALES *et al*. (2010) and SUÁREZ-MORALES *et al*. (2014).

The occurrence of copepodid genus *Monstrilla* in commercial crops of *Perna perna*, was first reported by SUÁREZ-MORALES *et al*. (2010) in Penha/SC, Brazil, with a prevalence of 25.6% causing hemocyte infiltration in the connective tissue of the mantle edge of these bivalve molluscs. Previous occurrence was reported in prosobranch gastropods a prevalence of 2% (PELSENEER, 1914) and polychaete annelids, with a prevalence of 1% (HARTMAN, 1961).

In the current study the presence of this copepodid was recorded a prevalence of 43.33% in a mussels culture in the municipality of Palhoça, distant 124 km south of Penha, without causing hemocyte infiltration in their hosts. This fact can be attributed to the mussels have no response to the parasite infestation.

The adult and naupliar stages of Mosntrilloida are planktonic free-living when they do not feed (ISAAC, 1975). In their endoparasite phase they have a food tube (Figures 3, 8, 9 and 17) in its endoparasites phase its host (Figures 1 and 2).

According SUÁREZ-MORALES *et al*. (2010), who collected copepodids females in the connective tissue of the edge of the mantle of mussels *Perna perna*, the Monstrilloida taxonomy is made exclusively from studies of adult specimens. The identification of copepodids females was possible by the presence of the double genital somite, the ovigerous spines (Figures 5, 7E and 7F) and 6 setae in the caudal rami (Figures 6 and 7E), which remain,), unchanged during their juvenile stages and pre-adult unlike the same characters in the male (SUÁREZ-MORALES and GASCA, 2004; SUÁREZ MORALES, 2011). As recorded by SUÁREZ-MORALES et al. (2014), in the examples analyzed stereomicroscope after the opening of nodules was observed the presence of front eyespot (Figures 1 and 2).

Morphologically we can say that the copepodid collected tissue from the edge of the mantle of mussels *Perna perna* of this study, it is a young female belonging to *Monstrilla* genus.

The specimens analyzed in this study show similarities (number setae in 5th pair of leg and existing somites after genital segment) with those found by SUÁREZ-MORALES *et al*. (2010) in Penha/SC. During this study no specimens were found in Penha (SC), in order to make a more accurate comparison. DUARTE (1999) reports the presence *Monstrilla rugosa*, *Monstrilla* sp., *Thaumaleus longispinosum* and *Thaumaleus* sp. in Penha/SC. SUÁREZ-MORALES and DIAS (2000) mentions the presence *M. brasiliensis* and *M. careli* and SUÁREZ-MORALES and DIAS (2001a) the presence *M. pustulata*, both on the Brazilian coast. However, it is not possible to provide a reliable identification from immature individuals.

CONCLUSIONS

The copepodids removed from the nodules located in the mantle edges of mussels *Perna perna* grown in Palhoça, are juvenile females belonging to the genus *Monstrilla*.

The analysis of histological sections showed no hemocyte infiltration in tissues mantle edges, indicating that the presence of these endoparasites did not cause the host response to the parasitic infestation.

Additional studies are needed in the main producing municipalities of mussels in Santa Catarina, with the purpose of collecting adults copepods to characterize species, and make a comparison with specimens found in other state locations.

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