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Scientific Note

Urastoma cyprinae IN MUSSEL *Perna perna* CULTIVATED IN SOUTHERN BRAZIL*

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ABSTRACT

Urastoma cyprinae (Platyhelminthes: Urastomidae) was collected among the gill filaments of cultured mussel *Perna perna* from three localities of Santa Catarina state in the south of Brazil. Samples of *P. perna* were taken monthly during July 2010 to June 2011. The diagnostic of *U. cyprinae* in *P. perna* showed to be more efficient by stereoscopic microscope that histopathology, where less prevalence and infestation rate were registered. Seawater temperature and salinity during the survey period did not affect *U. cyprinae* prevalence in *P. perna*. The analysis on the stereoscopic microscope showed highest prevalence rates (54.2%) of *U. cyprinae* in the mussels from the northern locality studied (Penha - 26º46'S). In addition, the mussels from the southern locality (Palhoça - 27º45'S) had the highest infestation rate (7.4). Despite showing high prevalence and infestation rate during the study period, there is no evidence that this flatworm is causing histopathological damage to its host. Even being treated as a parasite of bivalve molluscs, in this study it was observed that the relationship between *U. cyprinae* and *P. perna* would not parasitism *sensu stricto*.

Key words: parasitism; Urastomidae; mussel culture; mariculture.

Urastoma cyprinae, NO MEXILHÃO *Perna perna* CULTIVADO NO SUL DO BRASIL

RESUMO

Urastoma cyprinae (Platyhelminthes: Urastomidae) foi coletado entre os filamentos branquiais do mexilhão cultivado *Perna perna* de três localidades do estado de Santa Catarina no sul do Brasil. Amostras de *P. perna* foram coletadas mensalmente de julho de 2010 a junho de 2011. O diagnóstico de *U. cyprinae* em *P. perna* mostrou-se mais eficiente por observações ao microscópio estereoscópico do que por histopatologia, onde menor prevalência e taxa de infestação foram registradas. A temperatura do mar e a salinidade durante o período de pesquisa não afetaram a prevalência de *U. cyprinae* em *P. perna*. A análise no microscópio estereoscópico apresentou maior prevalência (54,2%) de *U. cyprinae* nos mexilhões da localidade do norte estudada (Penha - 26º46'S). Além disso, os mexilhões da localidade do sul (Palhoça - 27º45'S) apresentaram a maior taxa de infestação (7,4). Apesar de apresentar alta prevalência e taxa de infestação durante o período de estudo, não há evidências de que este turbelário esteja causando danos histopatológicos ao hospedeiro. Mesmo sendo tratado como um parasita de moluscos bivalves, neste estudo observou-se que a relação entre *U. cyprinae* e *P. perna* não é de parasitismo *sensu stricto*.

Palavras-chave: parasitismo; Urastomidae; cultivo de mexilhões; maricultura.

INTRODUCTION

Between free-living Platyhelminthes, known as turbellarians, most species are found colonizing freshwater and marine environments, being able to live associated with vertebrates, but mainly invertebrates (ROHDE, 2001). According to JENNINGS (1971) several species of turbellarians living as commensals or parasites, mainly in invertebrates. The turbellarians (Rhabdocoela) of the genus *Paravortex* and *Graffilla*, belonging to the family Graffillidae are intestinal parasites of molluscs (CANNON and LESTER, 1988). *Paravortex* species live in the digestive gland of bivalves and gastropods (LINTON, 1910; BALL, 1916; FLEMING *et al*., 1981; BRUSA *et al*., 2006, 2011), and *Graffilla* species mainly live in gastropods hosts.

The flatworm *Urastoma cyprinae* (Fecampiida: Urastomidae) was regarded as commensal in pallial cavity of bivalve *Arctica islandica* in Germany (WESTBLAD, 1955), which recorded a phase of its life on seaweed. MARCUS (1951) mentions *U. cyprinae* in the coast of São Paulo (Brazil) living between algae. MURINA and SOLONCHENKO (1991) consider it as commensal and can influence the reproduction and growth of *Mytilus galloprovincialis*. ROBLEDO *et al*. (1994a) reported that this flatworm could cause pathological reactions and reduced mussel power capacity due to disruption of gill filaments. ZEIDAN *et al*. (2012) nominate *U. cyprinae* as an ectocomensal without causing apparent damage in *Crassostrea rhizophorae*, *Mytella guyanensis* and *Lucina pectinata*.

Associated to the aquaculture of bivalve molluscs, research has been conducted focusing on parasites. *Urastoma cyprinae* was reported in *M. galloprovincialis, Mytilus edulis*, *Mytilus californianus* mainly in the north hemisphere and in *Perna perna* from south hemisphere (Table 1).

In previous study (CARNEIRO-SCHAEFER *et al*., 2017) registered the occurrence of the genus *Urastoma* in cultured mussels *Perna perna* from Santa Catarina coast, Brazil. The present study identified the species using different methods.

In Brazil, turbellarians of the genus *Urastoma* also were recorded in the gills of *Crassostrea gigas* and *Crassostrea rhizophorae* (SABRY *et al*., 2011; SILVA *et al*., 2012) and of *Anomalocardia brasiliana* (SILVA *et al*., 2012), in the south region. In the northeast coast of the country (Bahia State), similar turbellarians were observed in the gills and mantle cavity of *C. rhizophorae* and *Mytella guyanensis* (ZEIDAN *et al*., 2012).

METHODS

Location sampled

During the period from July 2010 to June 2011, 240 mussels were monthly sampled in four localities on the Santa Catarina coast in southern Brazil. Mussel samples (n=30) were taken in commercial farms from three municipalities, Palhoça (27º45'23"S, 48º37'07"W), Governador Celso Ramos (27º22'16"S, 48º33'43"W) and Penha

Table 1. Prevalence and tissue or organ parasitized by *Urastoma cyprinae* in wild and cultured mytilids.

Species of bivalve	Origen From	Locality	Tissue or organ parasitized (prevalence in %)	Reference
Mytilus galloprovincialis (Lamarck, 1819)	Wild	Bulgaria	Gills (40.5)	MURINA and SOLONCHENKO (1991)
		Crimeia Caucasus		
	Culture and wild	Spain	Gills (0 to 90)	ROBLEDO *et al*. (1994b)
	Culture	Spain	Gills (3.3 to 13.3)	VILLALBA *et al*. (1997)
	Culture	Italy France Spain	Mantle cavity and gills (0 to 86.3)	CANESTRI TROTTI *et al*. (1998)
	Culture and wild	Mexico	Mantle cavity (57 to 100)	CÁCERES-MARTÍNEZ *et al*. (1998)
	Culture	Greece	Gills (31.1 to 57.9)	RAYYAN *et al*. (2004)
	Wild	Spain	Gills (82.7 to 99.4 and 48.3 to 93.6)	CRESPO-GONZÁLEZ *et al*. (2010)
	Culture and wild	Morocco	Gills (3 to 23)	BHABY *et al*. (2013)
Mytilus edulis (Linnaeus, 1758)	Culture	Portugal	Free live and mantle cavity (0 to 70)	SANTOS and COIMBRA (1995)
Mytilus californianus (Conrad, 1837)	Culture and wild	Mexico	Mantle cavity (10 to 87)	CÁCERES-MARTÍNEZ *et al*. (1998)
Perna perna (Linnaeus, 1758)	Culture	Brazil	Gills (100)	SUÁREZ-MORALES *et al*. (2010)
		Brazil	Gills and mantle cavity	Present study

(26º46'59"S, 48º36'17"W) and in the experimental culture area of Universidade Federal de Santa Catarina (UFSC), Florianópolis (27º29'27"S, 48º32'23"W), totalling 2,880 specimens. Of this total, 1,440 mussels were analyzed using a stereomicroscope to observe the presence or absence of flatworms in the mantle cavity. The other 1,440 mussels were submitted to a histopathology protocol. The salinity and the temperature were recorded along the sampling period.

The mussels were collected from five strings in each commercial farm (one in each extremity and one in center), stored in thermic boxes and then transported to the Laboratório de Malacologia Experimental (LAMEX) at the Núcleo de Estudos em Patologia Aquícola (NEPAQ/UFSC.

Analysis at stereomicroscope

The shell was opened by adductor muscle dissection and subsequent exposition of mantle. The remaining part of the animal was divided in two parts transversely, attached at the valve. The presence of flatworms from the mantle cavity was registered. Tissue anomaly were observed and registered.

The flatworms were observed in vivo, histology and scanning electron microscopy (SEM).

In vivo observations

The flatworms kept alive were stained with neutral red vital dye, placed between slide and covered with slip with a seawater drop (NOREÑA *et al*., 2015), and observed and photographed at light microscope.

Histology

The flatworms were dehydrated in an ascendant ethanol series and butyl alcohol, embedded in Paraplast, sliced at 5 µm thickness in sagittal plane, stained with trichrome staining orange G, ponceau xylidine and acid fuchsin, a change from Mallory stain and Masson dye (ROMEIS, 1968), observed and photographed at light microscope.

Scanning Electron Microscopy (SEM)

The flatworms 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

Mussel tissues were sectioned into 2 mm slices (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 observed and photographed at light microscope.

Statistical analysis

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

RESULTS

White flatworms measuring about 1.2 mm each, with rapid movement were observed in the soft parts from mollusc mainly on the mantle and between the gills.

In vivo analysis of flatworms showed cilia throughout the body, a pair of eyes in anterior region and detail of the copulatory apparatus. Pharynx and copulatory apparatus were located in the posterior region of the body (Figures 1, 2 and 3).

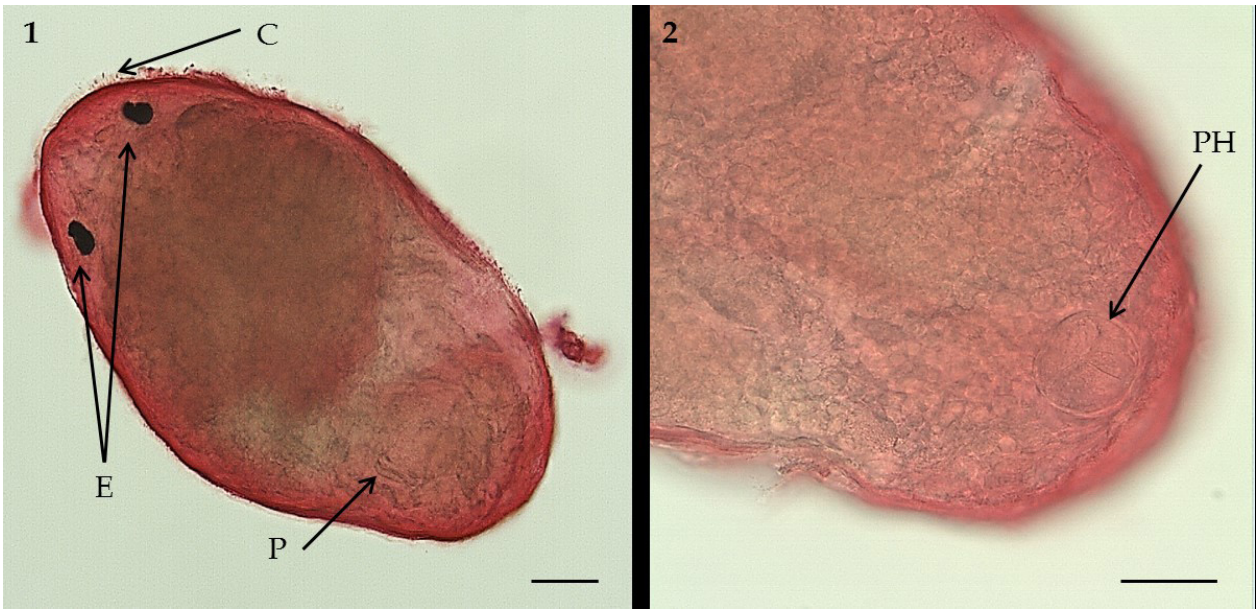
Series sections of turbellarians collected in mussels *P. perna*, showed internal structures (Figure 3), composed by similar structures recorded by MARCUS (1951) and WESTBLAD (1955): epidermis with abundant cilias**;** frontal glands and trilobates eyes in anterior region; testicles and oocytes in the middle region, and pharynx, pharyngeal glands, penis, seminal vesicle, male genital tract and atrium in the posterior region. In the parenchyma there are some cutaneous glands and a diffuse intestine without a membrane that separates from the rest of the body.

In histology serial section, detail of internal structures and its location in the posterior region of the body were observed (Figure 4). With this detail was possible to visualize the atrium, the secretory columns of the penis, penis papilla, seminal vesicle, vesicle granulosa and diffuse intestine.

Scanning Electron Microscopy of *U. cyprinae* showed that the animal's body is completely covered by cilia that are responsible for their rapid movements (Figures 5 and 6).

The highest amount of parasites observed in the mussels from Penha in November and December was 143 and 162, respectively; from Governador Celso Ramos in December was 240 and from Palhoça in June was 827. Histopathology analysis not showed the presence of disorders in gill filaments, hemocyte infiltration or necrosis of tissue mussels (Figures 7 and 8).

Lengths of the mussels were significantly ($p<0.05$) lower for animals from Governador Celso Ramos mussels in relation to Palhoça and Florianópolis. Analyzing the length of the mussels by seasons, animals from summer were significantly ($p<0.05$) highest compared to autumn and winter (Table 2).



Figures 1-2. *Urastoma cyprinae* collected in *Perna perna* stained with neutral red vital dye. Fig. 1 – Details of cilia (C), eye (E) and penis (P) (bar: 100 µm); Fig. 2 – Details of pharynx (PH). (bar: 40 µm).

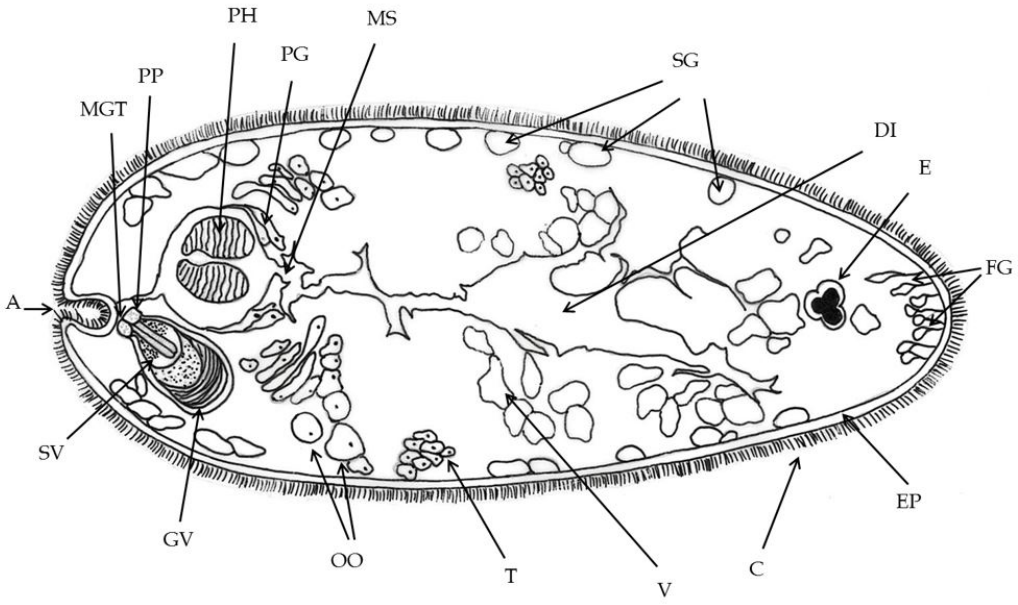


Figure 3. Scheme of the internal structures that characterize *Urastoma cyprinae*, based on serial histological sections of flatworms collected in *Perna perna*. A - atrium; C - cilia; DI - diffuse intestine; E - eyes; EP - epidermis; FG - frontal glands; GV - granular vesicle; MGT - male genital tract; MS - muscular septum; OO - oocytes; PG - pharyngeal glands; PH - pharynx; PP - penis papilla; SG - skin glands; SV - seminal vesicle; T - testicles; V – vitellarium.

Prevalence and infestation of *Urastoma cyprinae* during the studied period were low in general and no significant difference between sites and seasons were observed (Table 2).

In analyzes by stereomicroscopy no changes were observed in the tissues and organs of mussels.

Temperature and salinity showed no statistical difference between the study sites and no variation during the collection period (Table 2).

Sex of all analysed mussels by stereomicroscope and histopathology showed no significant difference in the rate between males and females.

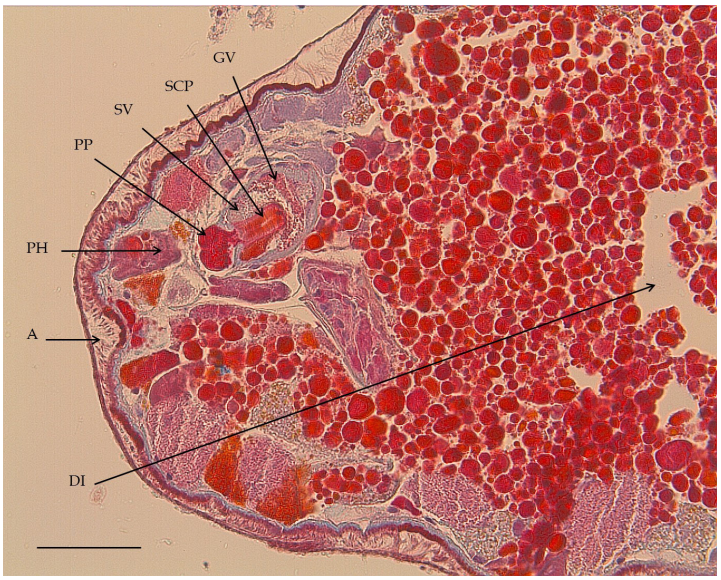
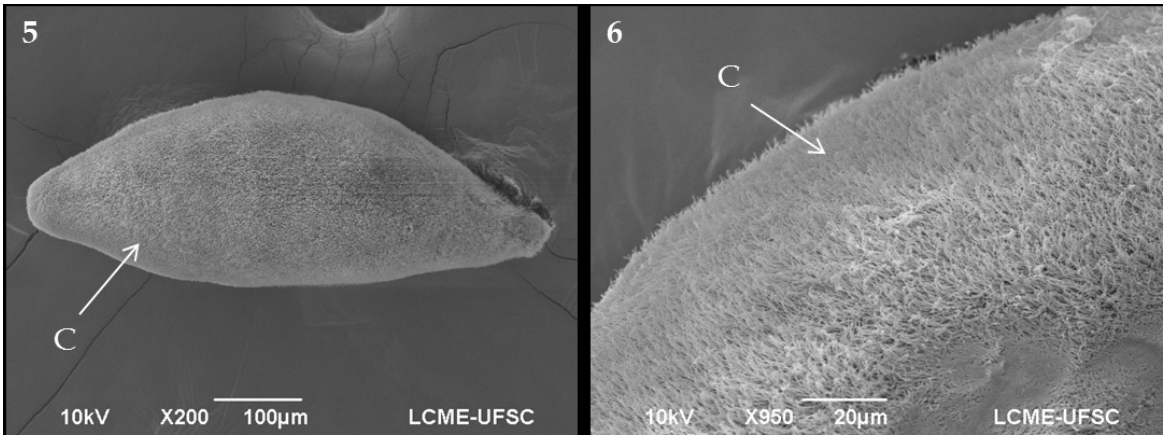
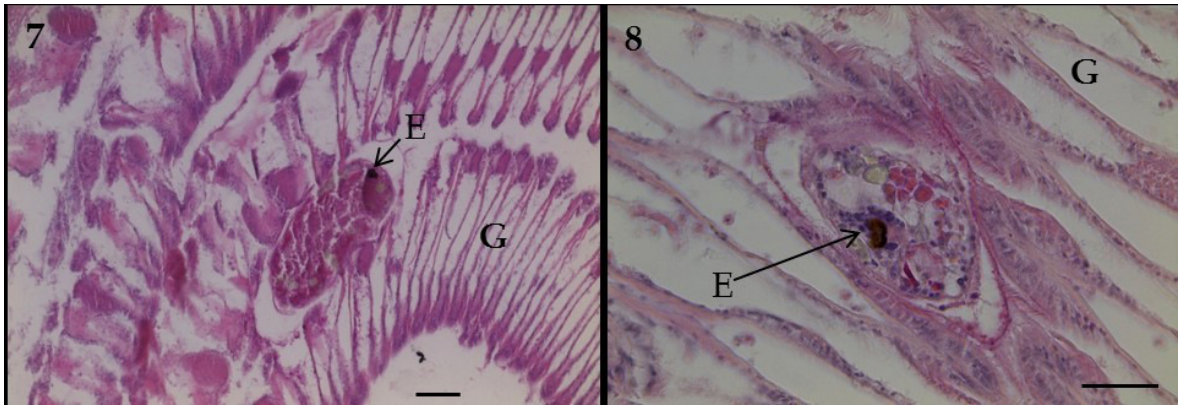


Figure 4. Histological section of *Urastoma cyprinae* collected in *Perna perna*. A - atrium; DI - diffuse intestine; GV - granular vesicle; PH - pharynx; PP - penis papilla; SCP - secreting columns of the penis; SV - seminal vesicle. (bar: 40µm).



Figures 5-6. *Urastoma cyprinae* prepared by Scanning Electron Microscopy of overview protocol. Fig. 5 – body covered by cilia (C); Fig. 6 – location of the cilia (C) that covers the body of the animal.



Figures 7-8. Histological sections showing the presence of *Urastoma cyprinae* in the mussel *Perna perna* tissues. Fig. 7 – Longitudinal section of *U. cyprinae* on gill tissue; Fig. 8 – previous cross section of *U. cyprinae* between the lamellae of the gill tissue. Where: E = eye; G = gill. (bars: 40µm).

Table 2. Mean (± standard deviation) of length of cultured mussels *Perna perna* sampled, temperature and salinity by study site and season, prevalence(P), infestation (I) rate per site and season of flatworm *Urastoma cyprinae* by stereomicroscopy (S) and histology (H). Where GCR = Governador Celso Ramos.

Study site/ Season	*Perna perna*	Flatworm				Temperature (ºC)	Salinity (‰)
		P (%)		I			
	Lengths (mm)	S	H	S	H		
Palhoça	85.69 ± 11.74	38.05	0.56	7.44	1.0	20.62 ± 3.39	32.25 ± 2.52
Florianópolis	88.88 ± 13.96	15.55	0.56	1.45	1.0	20.45 ± 3.44	32.66 ± 2.70
GCR	76.29 ± 18.54	37.77	0.83	4.01	1.0	20.62 ± 4.21	33.08 ± 4.44
Penha	80.06 ± 49.68	54.16	2.50	3.17	1.0	21.25 ± 3.78	32.66 ± 2.87
Winter	76.55 ± 11.63	38.89	1.39	2.36	1.0	17.33 ± 2.23	34.00 ± 1.34
Spring	84.37 ± 11.71	51.94	1.39	4.02	1.0	20.95 ± 2.09	32.50 ± 1.67
Summer	91.98 ± 51.50	29.72	0.28	2.15	1.0	24.75 ± 1.95	29.75 ± 4.20
Autumn	78.48 ± 13.20	25.00	1.39	10.59	1.2	19.91 ± 3.42	34.41 ± 2.27

DISCUSSION

Results from observations *in vivo*, serial sections, scanning electron microscopy and histopathology analysis let us to say that turbellarians collected in the mussel *Perna perna* is *Urastoma cyprinae*. This species was registered in the gill of *P. perna*. Morphologically, the flatworm showed uniformly body covered by cilia, 2 dark trilobated eyes at the front end, pharynx at the back, common pore which flow into the mouth, common genital duct, diffuse gut, default location of the testes and ovaries. This morphological description corroborates the observation of MARCUS (1951) in the coast of the São Sebastião Island, São Paulo, Brazil and CANESTRI TROTTI *et al*. (1998), in *M. galloprovincialis* from Italy.

According to ROHDE (2001), *U. cyprinae* is the only valid species in Urastomidae and the most widely recorded in the gills of several species of bivalve molluscs. MARCUS (1951) reports that *U. cyprinae* is a free living flatworm found in muddy bottoms and also inhabits the mantle cavity and gills of bivalve molluscs. VILLALBA *et al*. (1997) observed the presence of white spots between the gill lamellae *M. galloprovincialis* from Spain. In Mexico, CÁCERES-MARTÍNEZ *et al*. (1998) recorded turbellarians in the gill filaments and mantle cavity of *M. galloprovincialis* and *M. californianus*.

The diagnostic of *U. cyprinae* in *P. perna* showed to be more efficient by stereoscopic microscope that histopathology, where less prevalence and infestation rate were registered. According to BRUN *et al*. (1999) the adults flatworm have a negative phototactic behaviour, that could hinder the sampling process. CRESPO-GONZÁLEZ *et al*. (2010) suggest that some models may raise doubts about the accuracy of some results, reporting that ROBLEDO *et al*. (1994b) surprised to have more favorable results by histology than by observations of dissected gills and observed under the stereomicroscope when histological techniques are rarely used to estimate the prevalence and intensity of helminth infections.

The present study suggests that the sex of the mussels did not affect prevalence of *U. cyprinae*, that corroborates to SANTOS and COIMBRA (1995), who also observed no relation of mussels *M. edulis* sex and prevalence of this flatworm in Portugal.

Seawater temperature and salinity during the survey period did not affect *U. cyprinae* prevalence in *P. perna.* MURINA and SOLONCHENKO (1991) reported that salinity has no effect on the occurrence of this parasite in *M. galloprovincialis* in the Black Sea.

Similarly, season did not affect *U. cyprinae* prevalence in *P. perna* during the studied period. However for *M. galloprovincialis* from Caucasus, MURINA and SOLONCHENKO (1991) and from Greece (RAYYAN *et al*., 2004; KARAGIANNIS (Δ. ΚΑΡΑΓΙΑΝΝΗΣ) *et al*., 2013), observed highest prevalence of the flatworm in winter. Yet, in mussels cultivated in Spain (ROBLEDO *et al*., 1994b; CRESPO-GONZÁLEZ *et al*., 2010), high prevalence was recorded in summer and autumn and less in winter.

Size range of studied mussel *P. perna* did not showed relation to *U. cyprinae* prevalence. High *U. cyprinae* infestation rate in *M. galloprovincialis* were observed in Spain, in mussels larger than 60 mm length (CRESPO-GONZÁLEZ *et al*., 2010), in Greece, in mussels with 71-80 mm length (RAYYAN *et al*., 2004) and in the Black Sea, in mussels in 50-70 mm length (MURINA and SOLONCHENKO, 1991).

In the present study, mussels parasitized by *U. cyprinae* were collected in the first meter deep of culture, distant from the seabed, approximated 4-8 meters deep. Previously, MURINA and SOLONCHENKO (1991), recorded high prevalence of turbellarians in mussels that were closer to the sand than the rocky bottom and even less in mussel cultured. KARAGIANNIS (Δ. ΚΑΡΑΓΙΑΝΝΗΣ) *et al*. (2013) reported an increase in the prevalence of infection by turbellarians in cultured mussels closer to the bottom, which could encourage the involvement of the parasite to its host. RAYYAN *et al*., (2004) recorded an increase in prevalence and intensity of *U. cyprinae* in mussels located in lower parts of the ropes. These data can be related those observed by CRESPO-GONZÁLEZ *et al*. (2005), that found *U. cyprinae*

in sexual maturity stage in the *M. gallaprovincialis* gills and the reproductive period in the substrate, with the production of cocoons, performs posture, with subsequent hatching and looking for a new host.

According to CÁCERES-MARTÍNEZ *et al*. (1998), prevalence of *U. cyprinae* may depend on environmental conditions where most polluted sites would have less turbellarians. Also, CÁCERES-MARTÍNEZ *et al*., (1998) suggested that the exposure of mussels to air, during low tides, would reduce the chances of infection due to the closing of valves.

Infestation rates registered in the present study showed no risk for the mussels cultured in the studied sites. BOWER *et al*. (1994) observed similar results in Europe for different bivalves, were *U. cyprinae* is reported as an opportunistic mantle inhabitant. ROBLEDO *et al*. (1994a), in Spain, and VILLALBA *et al*. (1997) in Galicia mention that the flatworm could be considered a potential threat to the mussel *M. galloprovincialis* culture or classified as pathogenic imperceptible.

Even being the flatworm treated as a parasite of bivalve molluscs as reports by other authors, in the present study the relationship between *U. cyprinae* and *P. perna* showed to be not parasitism *sensu stricto*.

CONCLUSIONS

The turbellarians registered in the mussel *Perna perna* is *Urastoma cyprinae*. This parasite showed no damage to the host during the survey period and the terms of prevalence and recorded infestation rates. Observation by stereomicroscope is more effective for ectoparasite detection than histopathological procedures.

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