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Presence and histopathological effects of the *Parvatrema* sp. (Digenea, Gymnophallidae) in the stout razor clam *Tagelus plebeius* (Bivalvia, Psammobiidae)

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ABSTRACT

The stout razor clam *Tagelus plebeius* (Bivalvia, Psammobiidae) has a wide geographic distribution range, including the Brazilian coasts from the northeast (Alagoas) to the south (Santa Catarina). In March 2008, an episode of mass *T. plebeius* mortality (70%) occurred in an intertidal bed at The Pontal da Daniela, State of Santa Catarina, Brazil. We report here high prevalences (to 100%) of the trematode parasite *Parvatrema* sp. Cable, 1953 (Digenea, Gymnophallidae) infecting *T. plebeius* at high intensities. We describe the gymnophalid, echinostomatid and unidentified metacercariae parasites infecting the clam and the host reactions elicited by them. The use of special diagnostic techniques such as Ray's fluid thioglycollate medium (RFTM) and PCR assays to detect *Perkinsus* sp. pathogens, hemolymph cytology, and histopathological examinations did not show *Perkinsus* sp. infections, microcell infections, or neoplastic conditions. However, neither infections or pathology caused by trematode parasites; nor any other pathological condition could be uniquely correlated with the mortality event. A coincident flash flood might have contributed to cause the mortality episode. This is the first report of the *Parvatrema* sp. metacercariae larvae infecting the stout razor clam *T. plebeius* from Brazil.

1. Introduction

In March 2008, a mortality episode among stout razor clams *Tagelus plebeius* occurred in an intertidal bed at The Pontal da Daniela Preserve Area, State of Santa Catarina, southern Brazil. Although a fishery for *T. plebeius* does not exist in any region of the coast of Santa Catarina state, we worked to discover the cause of the mortality event that occurred in the natural bed of *T. plebeius* by applying recommended techniques for detection of *Perkinsus* spp., and histopathological assays for other parasites and pathologies present during the mortality episode. Here we report results of various efforts to determine the cause of this mortality.

Despite the wide distribution of *T. plebeius* razor clams along western Atlantic coasts from the USA (42°N) to Argentina (42°S) (Rios, 1994), their populations have hardly been studied, and there is no report of a pathological study on *T. plebeius* from Brazil. In South America, most of the studies undertaken on *T. plebeius* pathologies are from Argentina, where a small-scale *T. plebeius* fishery exists. The diseases currently diagnosed in *T. plebeius* from Argentinean waters are mainly caused by digenean parasites from the Gymnophallidae family (Cremonte, 1999; Lomovasky et al., 2005; Vázquez et al., 2006; Ituarte et al., 2009). The latter reports concur in concluding that pathologies elicited by metacercariae inhabiting bivalves have never been associated with mass mortalities.

Pathologies caused by Gymnophallidae metacercariae are manifest at the cellular, tissue and shell levels, and include hyperplasia and metaplasia at the outer mantle epithelium, and abnormal calcification process in the bivalves *Gaimardia trapesina* (Ituarte et al., 2001) and *Darina solenoides* (Cremonte and Ituarte, 2003). The same shell alterations elicited by digenean larvae from the genus *Bartolius* were also observed in other modern families (Nuculanidae, Cyamiidae and Neoleptonidade), as well as in Holocene bivalves (Ituarte et al., 2005). Brownish pigmentation of the inner shell surface concurrent with irregular carbonate deposition was another evidence of the presence of Gymnophallidae metacercariae infecting *T. plebeius* (Cremonte, 1999; Lomovasky et al., 2005; Vázquez et al., 2006; Ituarte et al., 2009).

Perkinsus sp. infections in *T. plebeius* were first reported among clams from Chesapeake Bay, USA on the northwestern Atlantic

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coast, with prevalences of 30–100% of frequently high-intensity infections (Dungan et al., 2002). That pathogen was subsequently confirmed to be *Perkinsus chesapeaki* (Burreson et al., 2005), which infected *T. plebeius* clams with light infection intensities in nearby Delaware Bay, USA (Bushek et al., 2008). Among South American bivalves, only *Perkinsus olseni* infections are reported to date at prevalences of 22–27% among *Pitar rostrata* venerid clams from the coast of Uruguay (Cremonte et al., 2005). Thus far, no direct effort to diagnose *Perkinsus* sp. infections has ever been made on *T. plebeius* from populations in either Argentina or Brazil. Therefore, in the present work we tested for the presence of this OIE (World Organisation for Animal Health) notifiable parasite that has never been reported in Brazil.

2. Materials and methods

2.1. Sampling and site description

An episode of stout razor clam *T. plebeius* (Bivalvia, Psammobiidae) mortality was observed in March 2008 in a natural bed of Pontal da Daniela, a Protected Area on Santa Catarina Island (Southern Brazil) during a routine sampling of a surveillance program for sanitary control of bivalves. The population of *T. plebeius* was found in a small intertidal sand-mud habitat extending over approximately 1800 m^2, located in an estuarine area at the mouth of the Ratones River beside a mangrove forest. No influence of direct domestic or industry effluent exists, but a small stream flows into the clam bed.

Mortality was estimated in 1 m^2 quadrats that were haphazardly selected. Live and dead clams (empty but articulated valves) were counted from the surface to 50 cm deep. Samples of live ($N = 25$) and moribund ($N = 8$) stout razor clams were collected at low tide on the 4th March. All clams were measured (shell length), examined for gross abnormalities, and analysed for evidence of potential pathogenic organisms and conditions with the techniques described below.

In 9th June 2008, the natural bed was visited again to analyse the recovery of the population, and to collect more samples of clams ($N = 15$) in order to compare the presence of pathologies among animals from the two periods toward discovering differences indicating the probable causes of the mortality event.

In 17th September 2008, the natural bed was visited again to collect clams ($N = 30$) for dissections and metacercariae isolation for macroscopic diagnosis, as well as to check for the presence of empty shells indicative of recent local mortalities on the sediment surface.

2.2. Clam handling

Moribund clams were processed immediately upon arrival at the laboratory, and live clams were maintained in tanks with aerated, filtered (1 μm) seawater until analyses were done (in a maximum of five days).

2.3. Haemolymph cell monolayers

To test for the presence of microcell parasites infecting haemocytes, and potential neoplastic disorders, haemolymph was collected (da Silva and Villalba, 2004). Stout razor clam shells were slightly opened and haemolymph was withdrawn from adductor muscles with 26-gauge needles attached to 1-ml syringes.

Haemolymph cell monolayers (HCM) through active cell adhesion were prepared by setting cells (100 μl of haemolymph) onto a glass slide for 20 min in a humidified chamber. Cell monolayers were then fixed with methanol and stained with Giemsa. The slides were analysed for morphological cell alterations or neoplastic conditions, and for the presence of microcell parasites.

2.4. Histological sections

Each stout razor clam was shucked and a transverse section of 5 mm (including visceral mass, gills and foot) or the whole animal body was excised and fixed in Davidson's solution (Shaw and Battle, 1957). Fixed tissues were embedded in paraffin, sectioned at 5 μm thickness, and stained with Harris' hematoxylin and eosin (Howard et al., 2004).

2.5. Tissue imprints

Small fragments of digestive gland-gonad and gills were excised, blotted on a paper towel to remove excess moisture, and slightly squeezed onto a slide. The slides were fixed with methanol and stained with Giemsa.

2.6. Identification, prevalence and intensity of digenean parasites

Digenean parasites were detected by histology and by the presence of macroscopically brownish pigmentation of the inner shell and/or sacs at the clam dorsal region (Fig. 1). Metacercariae' sacs were removed from the host tissues and incubated at bird physiological temperature (40 °C) during 24–48 h for parasite isolation and characterisation. The prevalence of digenean parasites was estimated as the percentage of infected clams in each monthly sample. The intensity of metacercariae larvae (Gymnophalidae) in sacs was only accurately estimated in September as the number of metacercariae larvae per animal. On March and June, parasite (Gymnophallidae, Echinostomatidae and reabsorbing unidentified metacercariae) burden was estimated as the number of parasites present in one histological section.

2.7. Detection of Perkinsus sp. organisms

The presence of parasites from the genus *Perkinsus* was investigated. Each stout razor clam was shucked, and the two branchial lamellae and labial palps from one side of the body were excised ($N = 10$ per sampling). In March (4th), the body burden assay was also applied ($N = 4$ moribunds) according to Choi et al. (1989). Tissues were incubated in Ray's fluid thioglycollate medium (RFTM, Ray, 1966) for 7 days in the dark at room temperature. The whole body assay was prepared by subsequent digestion of clam tissues with 2 N NaOH and successive centrifugations, and rinsing to obtain a final volume of 1 ml of cell suspension (Choi et al., 1989). In contrast, the gills/labial palps were prepared by chopping the tissues with a scalpel. Finally, both preparations were flooded with Lugol's iodine solution and examined by light microscopy for dark-stained spheres of *Perkinsus* hypnospores.

2.8. Polymerase chain reaction (PCR)

Small samples of all organs from every animal were preserved in 95% ethanol for molecular detection of parasite DNAs. After studying HCM, tissue imprints, histological sections, and RFTM samples, clams from the mortality period (March 2008) suspected of containing unidentified protozoan parasites were selected for DNA extractions. DNA extractions were performed using DNAzol reagent® (Invitrogen Life Technologies™) according to the manufacturer's instructions. Digestive gland and gill tissues were used ($N = 10$ from March and $N = 5$ from June). The set of primers used to detect parasites from the genus *Perkinsus* were those of Casas et al. (2002) that target the ITS region of the rRNA gene complex (ITS85/ITS750) of all *Perkinsus* species except *Perkinsus qugwadi*.

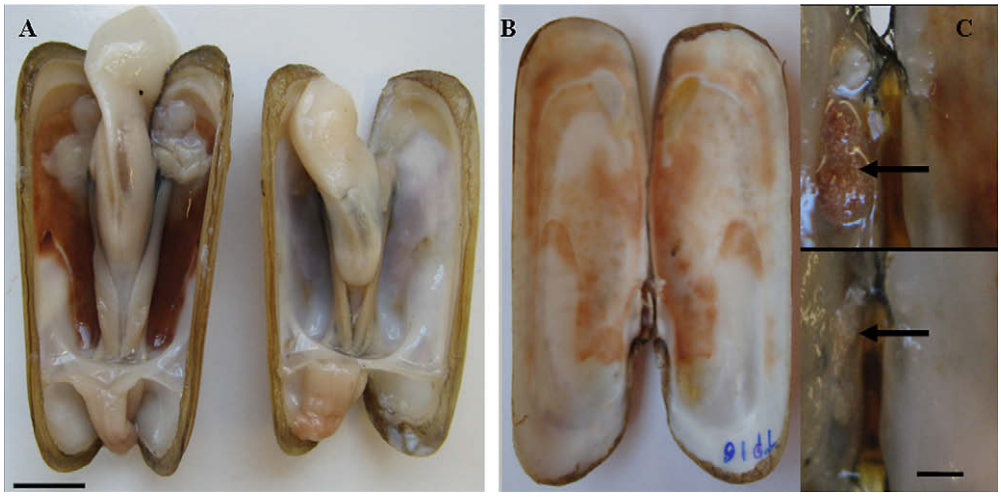

Fig. 1. Macroscopic view of *Tagelus plebeius* infected with metacercariae of *Parvatrema* sp. (A) Strong orange/brownish shell pigmentation (left) and normal shell pigmentation (right). (B) Shell pigmentation pattern from a heavily infected clam. Bar = 10 mm (for A and B). (C) Dorsal body region under the hinge (ligament) showing the grouping of parasite's sacs in heavy (upper) and low (lower) infected individuals. Bar = 5 mm.

Since there are no reported positive controls of *Perkinsus* sp.-infected bivalves from Brazil, positive controls were preserved *P. olseni in vitro* isolate cells from Galicia, Spain (provided by Dr. Antonio Villalba). PCR reactions were performed in total volumes of 25 μl containing 1 μl (50 ng) of genomic sample DNA, PCR buffer at 1x concentration, $MgCl_2$ at 1.5 mM, nucleotides at 0.2 mM each, primers at 0.4 μM, and 1 unit of Taq DNA polymerase. The cycling protocol included template DNA denaturation at 94 °C for 10 min, 35 amplification cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min, followed by a 72 °C final extension for 10 min.

PCR products were separated on a 1.5% agarose gel that was prepared and bathed in 1× Tris–acetate EDTA buffer (TAE), and were stained with ethidium bromide.

2.9. Statistical analysis

One way ANOVA was performed to analyse for differences in lengths of clams between the three months of sampling.

3. Results

3.1. Estimation of mortality

The present work reports on an episode of stout razor clam *T. plebeius* mortality that occurred on March 2008 in an intertidal bed of Santa Catarina Island, Brazil. The episode was characterised by the presence of a large number of empty articulated clam valves either on the surface's sediment and buried, and moribund clams (partially opened) located near the sediment surface. Counts of dead and live clams revealed a recent mortality of 70%. After 3 months (June), the mortality estimated was 62%, only 11.4% less than that observed during March; probably because several empty shells from the previous mortality episode were still there. Those shells were found mainly buried and few on the surface's sediment. However, in contrast to March, no moribund clams were observed in June. In September, neither empty shells nor moribund clams were observed on the sediment surface.

Clams collected during the mortality episode were significantly smaller ($P = 0.000$; length: 51.1 ± 1.23 cm, $N = 33$; mean ± SD) than clams collected after the mortality event, in June (length: 57.4 ± 1.18 cm, $N = 15$; mean ± SD) and in September ($P = 0.000$; length: 61.6 ± 1.18 cm, $N = 15$; mean ± SD).

3.2. Haemolymph cell monolayer (HCM) and tissue imprints

To investigate the possible causes of *T. plebeius* mortality, several techniques were employed. The analysis of haemolymph cells did not show any abnormality or parasite inhabiting the cytoplasm of the haemocytes. Haemocytes were morphologically normal; no micronuclei, neoplasia, or signs of apoptosis were observed. Two populations of haemocytes could be distinguished, granulocytes with granules inside the cytoplasm and hyalinocytes without cytoplasmic granules.

The tissue imprints did not reveal any pathological condition of cells from the several tested organs.

3.3. Detection of Perkinsus spp.

To check for *Perkinsus* spp. two techniques were applied: RFTM assays of gills/labial palps or the whole body, and rDNA-ITS region PCR. The results of these two techniques confirmed the absence of *Perkinsus* sp. cells or DNAs in the samples analysed. The analyses of the four post-hydrolyses particle aliquots obtained from the whole bodies of *T. plebeius* incubated in RFTM and prepared according to Choi et al. (1989) showed the presence of some spherical bodies or cells (measuring around 300 μM) that were reddish with Lugol's iodine staining, and green without.

Performance of the primers targeting *Perkinsus* sp. parasite DNAs was verified under the PCR conditions used, with positive control *P. olseni* isolate DNA, and produced the expected approximate 700 bp band in all cases. The PCR assays performed with *T. plebeius* samples did not show any positive results, either with March samples collected during the mortality event, or with June samples collected after the mortality event.

3.4. Pathological analysis

In contrast to all other techniques, only histological sections and macroscopic observations of the inner shell surface revealed the presence of digenean parasites. Two families, Gymnophallidae and Echinostomatidae, as well as unidentified encapsulated larvae infecting different host organs, were detected.

In samples from March and June, prevalences of these organisms was estimated only by histology (Table 1), which should be considered as minimum prevalence estimates since subsequent analysis of the inner shells of the sampled clams showed the presence of abnormal orange to brownish pigmentation (Fig. 1) that is

Table 1
Prevalence (P, %) and intensity (I) (larvae per animal) of Gymnophallidae, Echinostomatidae, and unidentified encapsulated metacercariae in *Tagelus plebeius*. GT: gymnophallid metacercariae in tissues; GS: gymonophallid metacercariae in dorsal mantle sacs; ET: echinostomatid metacercariae in tissues; RM: reabsorbing unidentified metacercariae; n.h.: no histology available, n.d.: not done. *N* = number of analysed animals.

Sampling date, *N*	P/I	GT	GS	ET	RM
4th March, 4 moribund clams	P	0	25	0	50
	Minimum (I)	0	n.d.	0	3
	Maximum (I)	0	n.d.	0	6
	Mean (I)	0	n.d.	0	4.5
4th March, 25 live clams	P	8	20	12	20
	Minimum (I)	1	n.d.	1	1
	Maximum (I)	1	n.d.	1	6
	Mean (I)	1	n.d.	1	2
9th June, 15 live clams	P	13.3	13.3	26.7	60
	Minimum (I)	1	n.d.	1	1
	Maximum (I)	1	n.d.	2	6
	Mean (I)	1	n.d.	1	3
17th September, 30 live clams	P	n.h.	100	n.h.	n.h.
	Minimum (I)	n.h.	85	n.h.	n.h.
	Maximum (I)	n.h.	734	n.h.	n.h.
	Mean (I)	n.h.	361	n.h.	n.h.

diagnostic for Gymnophallidae larvae infestations in *T. plebeius* (Cremonte, 1999). Unfortunately, shells were attentively observed for the presence of shell pigmentation only for clams in the September sample, and infection intensities could be precisely estimated only for that sample (Table 1). The area extent of the shell pigmentation varied among clams, sometimes being limited to the dorsal area near the umbo (low intensity) or extended to larger areas covering almost the whole valve surface (heavy intensity) in other clams (Fig. 1). The pigmentation resulted from the presence of metacercariae (Gymnophallidae) sacs at the clam dorsal region.

Metacercariae isolated from sacs after incubation at 40 °C were diagnosed as belonging to the *Parvatrema* genus Cable, 1953 (Fig. 2A) because of their pre-testicular ovary, the absence of a pars prostatic, and the presence of a wide and oval genital pore located at a certain distance anterior to the ventral sucker.

The majority of gymnophallid metacercariae larvae were found inhabiting sacs at the dorsal region of *T. plebeius* mantles, inducing tissue hyperplasia (Fig. 3). The prevalences of these sacs among moribund clams, and clams collected during March, June, and September, were 25%, 10%, 13.3% and 100%, respectively (Table 1). Curiously, histological results from the September sample showed that clams without abnormal shell pigmentation (*N* = 7; 23%) were all infected (had metacercarial sacs), as were all clams with orange shell pigmentation (*N* = 23; 77%) (Fig. 1). The prevalence of metacercariae larvae in clams sampled during September was therefore 100%, and prevalences estimated histopathologically from March and June samples almost certainly underestimated actual prevalences.

In addition, gymnophallid metacercariae were observed infecting other clam tissues, such as the connective tissue of mantle and labial palps, at low intensities (1 larva per section) in both March and June samples (Table 1, Fig. 4A). In the September sample, the intensity of gymnophallid infections expressed as the number of metacercariae larvae inside the sacs was 361 per animal on average (Table 1).

Only one animal had gymnophallid sporocysts, containing cercariae at different developmental stages, which were distributed throughout the connective tissues without eliciting an inflammatory reaction (Fig. 5).

Encysted Echinostomatidae larvae showing a typical ring head collar spines (Fig. 2B) were detected among clams in the March and June samples, except among moribund animals, at low intensities (1 larva per section) and prevalences of 12% and 26.7%, respectively (Table 1). Echinostomatid metacercariae infected mainly the gonad connective tissues (Fig. 6A) or follicles (Fig. 6B) of clams.

Some unidentified larvae, supposed to be Echinostomatidae, caused haemocytic infiltration which often resulted in parasite encapsulation and destruction that was highlighted by the presence of residual acidophilic material surrounded by haemocytes in connective tissue of mantle, labial palps, gonad, and muscle (Fig. 4B).

4. Discussion

The present work studied the cause of a mortality episode, which occurred in summer 2008 (March) on an intertidal bed of stout razor clams *T. plebeius* in Pontal da Daniela, State of Santa Catarina, southern Brazil. For the first time in Brazil, we report here the presence of Gymnophallidae metacercariae of the genus *Parvatrema*, and Echinostomatidae metacercariae infecting *T. plebeius*. We describe the prevalence and intensity of these parasites during and subsequent to a mortality episode, and the host reactions elicited by the parasites.

The high number of empty shells distributed throughout the sediment surface, and the presence of moribund animals unable

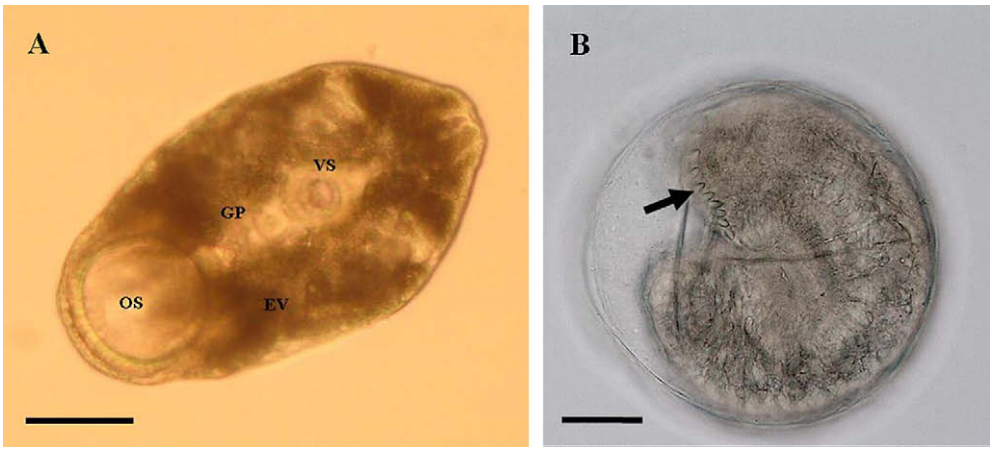

Fig. 2. Trematodes infecting *Tagelus plebeius*. (A) Gymnophallidae *Parvatrema* sp. metacercariae larvae isolated from *Tagelus plebeius*. References: OS = oral sucker, VS = ventral sucker, GP = genital pore, EV = excretory vesicle. Bar = 50 μm. (B) Encysted Echinostomatidae metacercariae larvae showing a head collar spines (arrow). Bar = 100 μm.

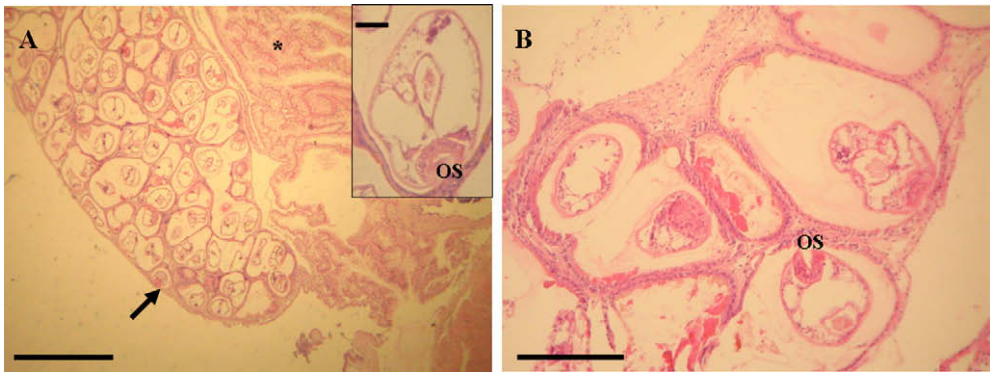

Fig. 3. Histological sections of *Tagelus plebeius*. (A) Metacercariae larvae of *Parvatrema* sp. enclosed in a large segmented sac at the dorsal region, near the kidneys (∗). Bar = 600 μm. Inset shows details of metacercariae larvae. Bar = 30 μm. (B) Magnification of image A showing metacercariae larvae individually distributed inside a large sac. Os = oral sucker. Bar = 150 μm.

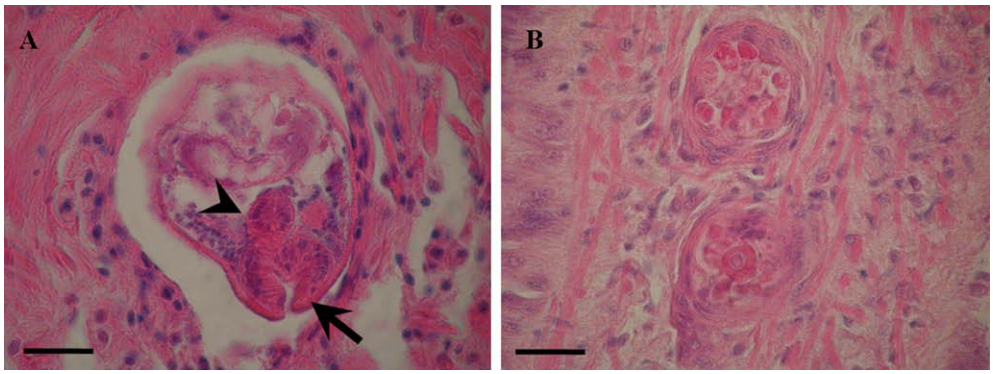

Fig. 4. Histological sections of *Tagelus plebeius*. (A) Magnification of a Gymnophallidae metacercariae larvae surrounded by haemocytes in the connective tissue. Observe the oral sucker (arrow) and pharynx (arrowhead). Bar = 20 μm. (B) Host reaction in the connective tissue of the mantle; encapsulation and destruction of a putative metacercariae larvae highlighted by acidophilic debris. Bar = 20 μm.

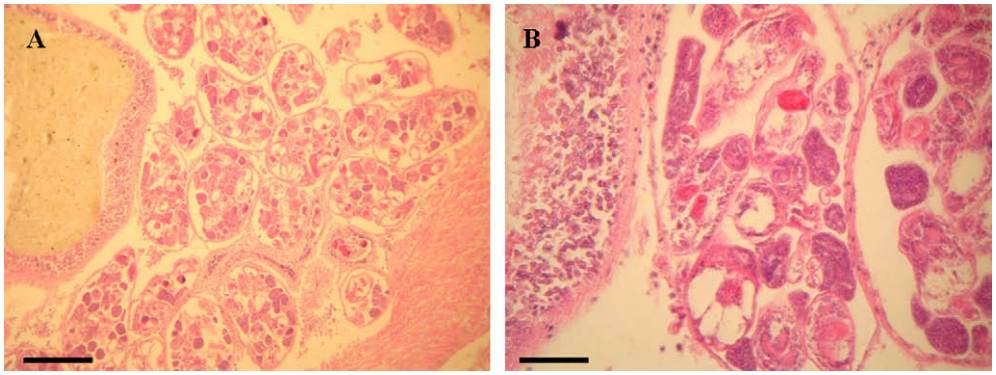

Fig. 5. Histological sections of *Tagelus plebeius*. (A) Sporocysts of *Parvatrema* sp. distributed throughout the connective tissue without host reaction. Bar = 200 μm. (B) Magnification of image A showing sporocyts full of cercariae larvae. Bar = 50 μm.

to burrow deep in the sediment, characterised the mortality event. In contrast, in September 2008 neither empty shells nor moribund clams were observed.

The arduous attempts to find some parasitic or pathological conditions that could explain the *T. plebeius* mortality failed. Several techniques were employed in an attempt to identify potential pathogen groups, including those as small as the protozoan microcell *Bonamia* spp. using haemolymph cell monolayers (da Silva and Villalba, 2004), and the very hazardous *Perkinsus* spp. using the OIE recommended techniques, including the incubation of tissues in RFTM (Choi et al., 1989; Villalba et al., 2004), but no parasites from either of those genera were detected. *Perkinsus* sp. was blamed for *T. plebeius* mortalities in the USA (Dungan et al., 2002) and parasites of this genus infect several bivalve species worldwide (see review of Villalba et al., 2004; Dungan and Reece, 2006; Dungan et al., 2007; Sheppard and Phillips, 2008). *Perkinsus* sp. was never been found in molluscs from Brazil, although only one study applying the recommended method (RFTM) for *Perkinsus* spp. diagnosis was conducted, in which the authors studied the occurrence of pathologies of *Crassostrea rhizophorae* in Bahia, northeast Brazil (Nascimento et al., 1986).

Special efforts to find protozoan parasites that could cause the mortality but be missed by histology were made. Cells remaining after the whole body preparation by RFTM probably belong to

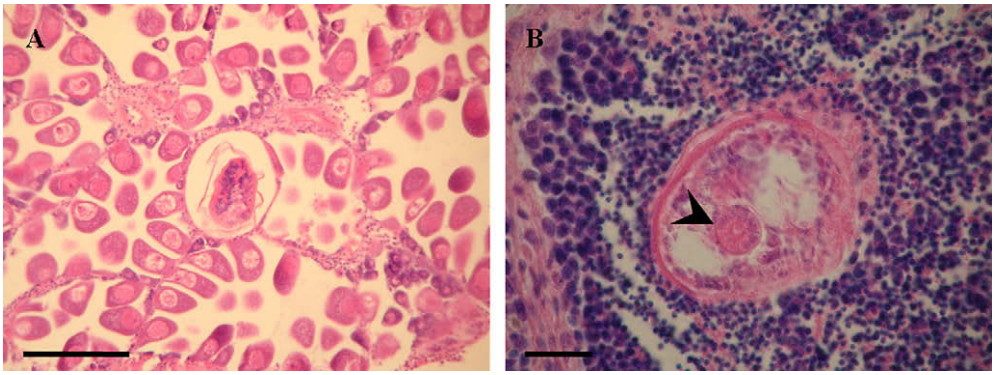

Fig. 6. Histological sections of *Tagelus plebeius*. (A) Echinostomatidae metacercariae infected female gonadal follicle. Bar = 150 μm. (B) Infected male follicle. Observe the ventral sucker (arrowhead). Bar = 20 μm.

some microalgae group present as cysts that persist after NaOH digestion, and may come from the intestinal tract of clams used for the whole body assay.

Histological sections and macroscopic observations were the two techniques showing the presence of a parasite, the *Parvatrema* sp. and unidentified Echinostomatidae metacercariae. In addition, the characteristic shell pigmentation (Cremonte, 1999; Lomovasky et al., 2005; Vázquez et al., 2006; Ituarte et al., 2009) produced by these larvae helped us verifying that the prevalence of the parasite was much higher than first estimated histologically. Moreover, after discovering that metacercariae could be easily visualised macroscopically as a small sac on the dorsal region of the clam, we realised that some histological sections did not cover that region and therefore may have given false–negative results. Thus, we emphasise that the prevalences recorded during March and June should probably be considered to be as high as the one observed in September (100%). Such high prevalences were also previously documented in *T. plebeius* from more southern localities (Vázquez et al., 2006; Ituarte et al., 2009). Intensity of infection estimated from September suggests that clams undergo heavy parasite's burden.

Even with the high prevalence of sacs inhabited by high number of metarcercariae of *Parvatrema* sp. in clams collected in the three sampling months, the animals seemed to be in a good state of health; tissues observed by histology or haemolymph cell monolayer did not shown profound abnormalities but mantle hyperplasia and haemocytic infiltration. This conclusion is corroborated by the fact that when gymnophalid/echinostomatid metacercariae were observed infecting other tissues, which occurred at very low intensity, a host reaction was elicited. The presence of individually intact metacercariae or acidophilic debris surrounded by a haemocytic infiltration, evidenced the capacity of the host to first efficiently isolate and then destroy the larvae when they were located outside of the habitual site of infection (i.e., below the hinge). In contrast, sporocysts of *Parvatrema* sp. seem to be more dangerous to the host, since no inflammatory reaction occurred. It seems that the absence of immune recognition and destruction by the host haemocytes is a rule for infections caused by trematode larvae that undergo asexual reproduction on molluscs (Cheng and Rifkin, 1970; Perkins, 1993). The sporocysts of *Bucephalus* sp. have been shown to infect the *Perna perna* mussel from Brazil without host reaction. The parasite invades the mantle tissue, where gonads develop, causing host castration by preventing normal gametogenesis and reproduction (da Silva et al., 2002). Cheng et al. (1966) studied the susceptibility of different species of marine bivalves to an echinostomatid metacercariae. The authors found that six from eight species were infected with different degrees of responses; metacercariae were found in several tissues mainly enveloped by a non-cellular, parasite-secreted inner cyst wall or comprised by a host haemocyic layer and fibres. Considering the present work, it seems that the *T. plebeius* response to the metacercariae was stronger than those described by Cheng et al. (1966). Therefore, suggesting that *T. plebeius* is not a totally compatible host. Similar reabsorption processes have been described for *Tylocephalum* metacestodes in the clam *Tapes semidecussata* by (Cheng and Rifkin, 1968).

The main parasite found in *T. plebeius* from Brazil, the gymnophallid *Parvatrema* sp., is also present in other localities of the clam's distribution range, such as Argentina (Vázquez et al., 2006; Ituarte et al., 2009) at the extreme southern limit of that range. Other parasites such as digenean (Fellodistomidae) and nematode larvae, which were reported in Argentina clams (Vázquez et al., 2006), have not yet been observed in Brazilian samples. By contrast, Echinostomatidae were found in Brazil but not in Argentina clams. However, because fellodistomid larvae were previously reported from bivalves of the Gulf of Mexico, USA (Wardle, 1983), they could also be present in *T. plebeius* populations from Brazil; larger samples are required to assess their presence since they occur at very low prevalences (Lauckner, 1983). Similarly, *Perkinsus* sp. and cestode larvae reported from USA samples (Holland and Dean, 1977; Dungan et al., 2002) seem to be present only in northern localities. The result of this study, performed in *T. plebeius* from a geographically intermediate range of distribution, is in accordance with the hypothesis that most parasite species have restricted, continental, geographical distributions, even though their hosts may have wider distributions (Carney and Dick, 2000). No common parasite is reported throughout the entire geographical distribution of *T. plebeius*.

Considering that the prevalence of *Parvatrema* sp., echinostomatid as well unidentified metacercariae infecting the stout razor clam was high in samples from all (3) months included in the current investigation, we concluded that theses parasites were not the direct cause of the mortality episode. Nevertheless, it is true that heavy parasitism as observed here can compromise fitness and favour morbidity and/or mortality of bivalves. In addition, the size of clams recorded from March was significantly smaller than the clams collected from June and September suggesting that bigger animals died. As stated by Lauckner (1983) bigger (older) mollusks are heavier infected by trematode parasites than smaller. Moreover, it is important to report the phenomenon occurred during the last days of February and beginning of March 2008 at Santa Catarina Island, the flash flood consisted of 187.8 mm in five days which results in increasing freshwater at the bed. Based on precipitation data from 1911 to 2006 for Florianópolis, the expected monthly precipitation for February and March was 186.7 and 170.0 mm, respectively (EPAGRI/CIRAM). Thus, the flash flood

could have caused a decline in salinity which in synergism with parasitism and other unknown factors might have contributed to the mortality found herein. Elston et al. (2003) reported the detrimental effects, including mortality, of exposure clams *Ruditapes philippinarum* to low salinity (<15 ppt.) during few days. Therefore, we recommend a surveillance program for diseases and mortalities at that estuary.

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References

Burreson, E.M., Reece, K.S., Dungan, C.F., 2005. Molecular, morphological, and experimental evidence support the synonymy of *Perkinsus chesapeaki* and *Perkinsus andrewsi*. J. Eukaryot. Microbiol. 52, 258–270.

Bushek, D., Landau, B., Scarpa, E., 2008. *Perkinsus chesapeaki* in stout razor clams *Tagelus plebeius* from Delaware Bay. Dis. Aquat. Org. 78, 243–247.

Carney, J.P., Dick, T.A., 2000. Parasite biogeography: a review of the origins and ideas with specific examples from Holartic fishes. Vie Milleu 50, 221–243.

Casas, S.M., Villalba, A., Reece, K.S., 2002. Study of perkinsosis in the carpet shell clam *Tapes decussatus* in Galicia (NW Spain). I. Identification of the aetiological agent and in vitro modulation of zoosporulation by temperature and salinity. Dis. Aquat. Org. 50, 51–65.

Cheng, T.C., Rifkin, E., 1968. The occurrence and resorption of *Tylocephalum metacestodes* in the clam *Tapes semidecussata*. J. Invertebr. Pathol. 10, 65–69.

Cheng, T.C., Rifkin, E. 1970. Cellular reactions in marine molluscs in response to helmint parasitism. In: Snieszko, S.F. (Ed.), Symposium on Diseases of Fishes and Shellfishes. American Fisheries Society, pp. 443–496.

Cheng, T.C., Shuster, C.N.J., Anderson, A.H., 1966. A comparative study of the susceptibility and response of eight species of marine pelecypods to the trematode *Himasthla quissetensis*. Trans. Am. Microsc. Soc. 85, 284–295.

Choi, K.S., Wilson, E.A., Lewis, D.H., Powell, E.N., Ray, S.M., 1989. The energetic costs of *Perkinsus marinus* parasitism in oysters: quantification of the thioglycollate method. J. Shellfish Res. 8, 125–131.

Cremonte, F., 1999. Estudio parasitológico de bivalvos que habitan ambientes marinos y mixohalinos en Argentina. Thesis Dissertation, Universidad Nacional de La Plata, La Plata, Argentina.

Cremonte, F., Ituarte, C., 2003. Pathologies elicited by the gymnophallid metacercariae of *Bartolius pierrei* in the clam *Darina solenoides*. J. Mar. Biol. Ass. UK 83, 311–318.

Cremonte, F., Balseiro, P., Figueras, A., 2005. Occurrence of *Perkinsus olseni* (Protozoa: Apicomplexa) and other parasites in the venerid commercial clam *Pitar rostrata* from Uruguay, southwestern Atlantic coast. Dis. Aquat. Org. 64, 85–90.

da Silva, P.M., Villalba, A., 2004. Comparison of light miscroscopic techniques for the diagnosis of the infection of the European flat oyster *Ostrea edulis* by protozoan *Bonamia ostreae*. J. Invertebr. Pathol. 85, 97–104.

da Silva, P.M., Magalhães, A.R.M., Barracco, M.A., 2002. Effects of *Bucephalus* sp. (Trematoda: Bucephalidae) on *Perna perna* mussels from a culture station in Ratones Grande Island, Brazil. J. Invertebr. Pathol. 79, 154–162.

Dungan, C.F., Reece, K.S., 2006. In vitro propagation of two *Perkinsus* spp. parasites from Japanese manila clams *Venerupis philippinarum* and description of *Perkinsus honshuensis* n. sp.. J. Eukaryot. Microbiol. 53, 316–326.

Dungan, C.F., Hamilton, R.M., Hudson, K.L., McCollough, C.B., Reece, K.S., 2002. Two epizootic diseases in Chesapeake Bay commercial clams, *Mya arenaria* and *Tagelus plebeius*. Dis. Aquat. Org. 50, 67–78.

Dungan, C.F., Reece, K.S., Moss, J.A., Hamilton, R.M., Diggles, B.K., 2007. *Perkinsus olseni* in vitro isolates from the New Zealand clam *Austrovenus stutchburyi*. J. Eukaryot. Microbiol. 54, 263–270.

Elston, R.A., Cheney, D.P., Macdonald, B.F., Suhrbier, A.D., 2003. Tolerance and response of Manila clams, *Venerupis philippinarum* (A. Adams and Reeve, 1850) to slow salinity. J. Shellfish Res. 22, 667–674.

Holland, A.F., Dean, J.M., 1977. The biology of the stout razor clam *Tagelus plebeius*: II. Some aspects of the population dynamics. Chesapeake Sci. 18, 188–196.

Howard, D.W., Lewis, E.J., Keller, B.J., Smith, C.S., 2004. Histological Techniques for Marine Bivalve Mollusks and Crustaceans. NOAA Technical Memorandum NOS NCCOS5, Oxford, MD.

Ituarte, C.F., Cremonte, F., Deferrari, G., 2001. Mantle-shell complex reactions elicited by digenean metacercariae in *Gaimardia trapesina* (Bivalvia: Gaimardiidae) from the Southwestern Atlantic Ocean and Magellan Strait. Dis. Aquat. Org. 48, 47–56.

Ituarte, C., Cremonte, F., Zelaya, D.G., 2005. Parasite-mediated shell alterations in Recent and Holocene sub-Antarctic bivalves: the parasite as modeller of host reaction. Invertebr. Biol. 124, 220–229.

Ituarte, C., Cremonte, F., Scarano, A., 2009. Tissue reaction of *Tagelus plebeius* (Bivalvia: Psammobiidae) against larval digeneans in mixohaline habitats connected to the south-western Atlantic. J. Mar. Biol. Ass. UK 89, 569–577.

Lauckner, G., 1983. Diseases of Mollusca: Bivalvia. In: Kinne, O. (Ed.), Diseases of Marine Animals, Introduction Bivalvia to Scaphopoda, Biologische Anstalt Helgoland, Hamburg, pp. 477–961.

Lomovasky, B.J., Gutiérrez, J.L., Iribarne, O.O., 2005. Identifying repaired shell damage and abnormal calcification in the stout razor clam *Tagelus plebeius* as a tool to investigate its ecological interactions. J. Sea Res. 42, 163–175.

Nascimento, I.A., Smith, D.H., Kern, F., Pereira, S.A., 1986. Pathological findings in *Crassostrea rhizophorae* from Todos os Santos Bay, Bahia, Brazil. J. Invertebr. Pathol. 47, 340–349.

Perkins, F.O., 1993. Infectious diseases of molluscs. In: Couch, J.A., Fournie, J.W. (Eds.), Pathobiology of Marine and Estuarine Organisms, vol. 10. CRC Press, London, Boca Raton, pp. 255–287.

Ray, S.M., 1966. A review of the culture method of detecting *Dermocystidium marinum* with suggested modifications and precautions. Proc. Natl. Shellfish Assoc. 54, 55–69.

Rios, E.C., 1994. Seashells of Brazil, second ed. Museu Oceanográfico da Fundação Universidade de Rio Grande, Rio Grande.

Shaw, B.L., Battle, H., 1957. The gross and microscopic anatomy of the digestive tract of the oyster *Crassostrea virginica* (Gmelin). Can. J. Zool. 35, 325–347.

Sheppard, B.J., Phillips, A.C., 2008. *Perkinsus olseni* detected in Vietnamese aquacultured reef clams *Tridacna crocea* imported to the USA, following a mortality event. Dis. Aquat. Org. 79, 229–235.

Vázquez, N.N., Ituarte, C., Navone, G.T., Cremonte, F., 2006. Parasites of the stout razor clam *Tagelus plebeius* (Psammobiidae) from the Southwestern Atlantic Ocean. J. Shellfish Res. 25, 877–886.

Villalba, A., Reece, K.S., Ordás, M.C., Casas, S.M., Figueras, A., 2004. Perkinsosis in molluscs: a review. Aquat. Living Resour. 17, 411–432.

Wardle, W.J., 1983. Two new non-ocellate *Trichocercous cercariae* (Digenea: Fellodistomidae) from estuarine bivalve molluscs in Galveston Bay, Texas. Contrib. Mar. Sci. 26, 15–22.