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Short Communication

Histopathological survey of the mussel *Mytilus chilensis* (Mytilidae) and the clam *Gari solida* (Psammobiidae) from southern Chile

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ABSTRACT. A total of 175 specimens of mussels, *Mytilus chilensis* (Mytilidae), and 56 specimens of clams, *Gari solida* (Psammobiidae), were collected in natural beds and culture sites of southern Chile. Juvenile mussel specimens (3 cm of maximum length) were free of parasites and diseases, whilst the commercial sized populations was parasitized by intracellular inclusions of bacteria-like organisms in the digestive gland epithelium and in the gills, by ciliates in the gills, turbellarians similar to *Paravortex* (Rhabocoela) in the intestine lumen and copepods attached to the gills. In addition, the disseminated neoplasia disease was also present although in low prevalences. In the clam, *G. solida*, prokariotic inclusions were found in the digestive gland epithelium and bacteria-like organisms in the gills, often encapsulated by haemocytes; oocysts containing up to 8 sporozoites similar to *Nematopsis* (Apicomplexa) in the connective tissue, causing haemocytic infiltration when the intensity of infection was high; ciliates belonging to two different species (one of them similar to *Trichodina*) inhabiting the gills; and a turbellarian similar to *Paravortex* in the lumen of digestive system without apparent host reaction. The populations of the bivalve species here studied were devoid of serious pathogens.

Keywords: *Mytilus chilensis*, *Gari solida*, histophatology, parasites, southern Chile.

Estudio histopatológico del chorito *Mytilus chilensis* (Mytilidae) y del culengue *Gari solida* (Psammobiidae) en el sur de Chile

RESUMEN. Un total de 175 choritos, *Mytilus chilensis* (Mytilidae), y 56 almejas, *Gari solida* (Psammobiidae), fueron recolectados en bancos naturales y sitios de cultivo en el sur de Chile. Los choritos de hasta 3 cm de longitud máxima estaban libres de parásitos y enfermedades, mientras que los especímenes de tamaño comercial estaban parasitados por inclusiones intracelulares de organismos similares a bacterias en los epitelios de la glándula digestiva y de la branquia; ciliados en las branquias; turbelarios similares a *Paravortex* (Rhabocoela) en el lumen del intestino y copépodos anclados en las branquias. Además, la enfermedad neoplasia diseminada estuvo presente, aunque en bajas prevalencias. En *G. solida*, se encontraron organismos procariotas en el epitelio de la glándula digestiva y organismos similares a bacterias en la branquia, frecuentemente encapsulados por hemocitos; ooquistes conteniendo hasta 8 esporozoítos similares a *Nematopsis* (Apicomplexa) se hallaron en el tejido conectivo, causando infiltración hemocítica cuando la intensidad de infección fue alta; ciliados pertenecientes a dos especies diferentes (una de ellas similar a *Trichodina*) habitando las branquias y un turbelario similar a *Paravortex* en el lumen del sistema digestivo, sin causar reacción aparente en el hospedador. Las poblaciones de bivalvos estudiadas estaban libres de patógenos severos.

Palabras clave: *Mytilus chilensis*, *Gari solida*, histopatología, parásitos, sur de Chile.

Mollusc culture is an important economic and social activity in Chile. The Chilean mussel, *Mytilus chilensis* (Hupé, 1854) (Mytilidae), is the main species of commercial interest; since 2006, 100% of the mussel production comes from the culture activity (Uriarte, 2008). Nevertheless, culture conditions may stress the organisms, favouring the development of diseases, as well as providing better conditions for the transmission

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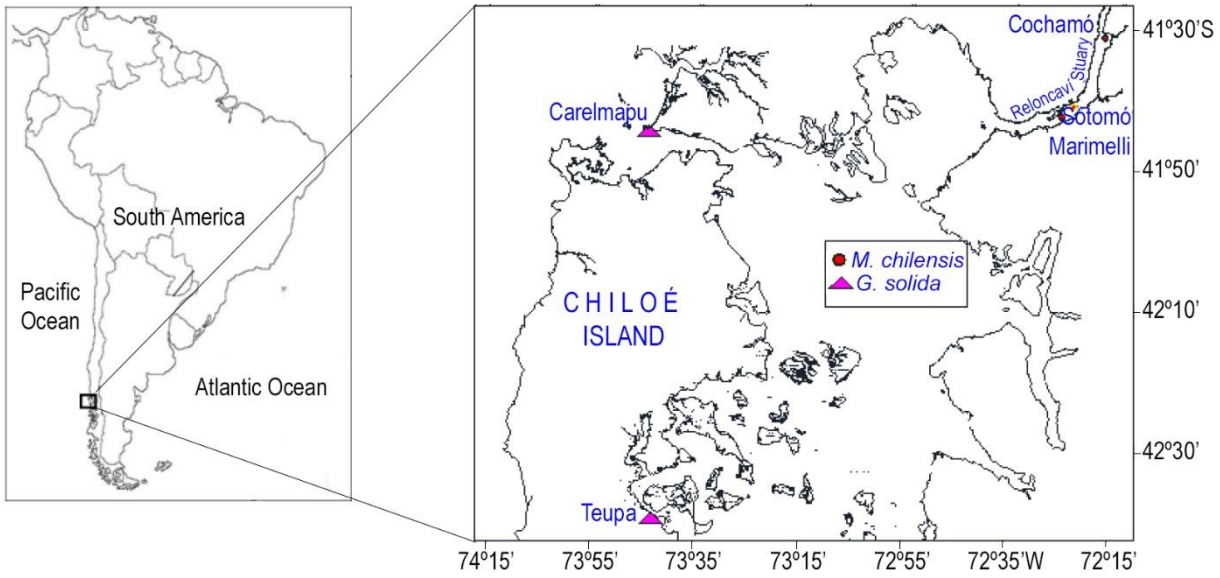

Figure 1. Sampling sites: Marimellí, Cochamó and Ralún in the Reloncaví Estuary, Teupa in Chiloé Island and Carlemapu, southern Chile.

of pathogens through crowding (Figueras & Novoa, 2011). The only studies about parasites and diseases on *M. chilensis* on the Chilean coast are those reporting an isopod inhabiting the pallial cavity and the disseminated neoplasia disease affecting digestive and mantle tissues (Jaramillo *et al.*, 1981; Campalans *et al.*, 1998). Disseminated neoplasia is a progressive disease that can result in death of the specimens, and at epizootic prevalences it has caused serious regional economic damage to the aquaculture industry (Barber, 2004; Carballal *et al.*, 2011).

In the Reloncaví Estuary, southern Chile, mussel seeds are collected by artisanal fishermen and sold to enterprises which grow the bivalve in the Chiloé Island; more than 6,000 ton was sold in 2011 in this area (unpublished data). Moreover, other species, such as the clam *Gari solida* (Gray, 1828) (Psammobiidae), fished in the same region, is starting to be cultured at a model scale (Uriarte, 2008). Besides of a tetraphyllidian cestode larva reported parasitizing this clam in northern Chile (Oliva, 1989), nothing is known about the health status of this fishing resource. Therefore, the aim of this study was to evaluate the health status of the mussel *M. chilensis* and the clam *G. solida* in southern Chile.

A total of 175 specimens of mussels, *M. chilensis*, were collected in southern Chile (Fig. 1). In November 2011, mussels were collected at Marimellí (41°42'S, 72°27'W) and Cochamó (41°30'S, 72°19'W) in the Reloncaví Estuary, at Teupa in Chiloé Island (42°39'S, 73°41'W) and in February 2012 at Ralún (41°23'S, 72°17'W) in the Reloncaví Estuary. A total of 56 specimens of clams *G. solida* (Psammobiidae) were collected in October and November 2011 at Carelmapu (41°42'S, 73°50'W) (Fig. 1). All bivalve specimens were collected and provided by artisanal fishermen and maintained in aerated aquaria until processed (up to 48 h). The soft parts of the specimens were carefully removed from their shells and fixed in Davidson's solution (Shaw & Battle, 1957) for 24 h. Oblique transverse sections, approximately 5 mm thick, including mantle, gills, gonad, digestive gland, nephridia and foot were taken from each specimen. Tissue samples were embedded in paraffin and 5 µm sections were stained with Harris' Hematoxylin and eosin. Histological sections were examined under a light microscopy for the presence of parasites and pathological alterations; measurements were taken with a digital camera (Leica DFC280) and its software (Leica Applications Suite version 4.1.0). The results of the histological examinations (prevalence and mean intensity of parasites and diseases) of *M. chilensis* and *G. solida* from all sampling localities are presented in the Tables 1 and 2 respectively. Prevalence was calculated as the total number of parasitized bivalves divided by the number of examined bivalves and intensity as the number of parasites per histological section divided by the number of parasitized bivalves.

Mytilus chilensis of 3 cm of maximum length were free of parasites and diseases. On the other hand, the commercial sized specimens were parasitized by intracellular inclusions of bacteria-like organisms, cilia-

Table 1. Sampling sites, number of specimens (n), length (in mm) and prevalence (P) and intensity [expressed as Mean (MI) Mean Intensity with range in parentheses] of infection of parasites and diseases of *Mytilus chilensis* from southern Chile. *Mussels from culture; the rest are from natural populations. The isopod is not included in the table because only one specimen was found.

Site	n	Mean length (range)	Disseminated neoplasia P (%)	Bacteria-like P (%) [MI (range)]	Ciliate P (%) [MI (range)]	Turbellaria P (%) [MI (range)]	Copepod P (%) [MI (range)]
Chiloé*	60	73 (56-89)	3.33	0	15 [4 (1-16)]	0	0
Marimellí	20	24 (22-30)	0	0	0	0	0
Marimellí	30	67 (57-81)	3.33	3.33 [1]	100 [58 (5-144)]	3.33 [1]	7 [1.5 (1-2)]
Cochamó	20	23 (20-27)	0	0	0	0	0
Cochamó	30	77 (62-92)	0	13 [20 (1-75)]	100 [30 (1-155)]	1.33 [1]	13 [2.33 (1-5)]
Ralún	15	74 (66-82)	0	7 [1-4]	100 [15 (1-75)]	0	0

Table 2. Sampling sites, number of specimens (n), length (in mm) and prevalence (P) and intensity (expressed as mean with range in parentheses) of infection of parasites and diseases of the clam *Gari solida* from southern Chile. *Clams were collected in October 2011 and maintained in aquaria until processed (November 2011).

Site	n	Mean length (range)	*Rickettsia*-like P (%) [MI (range)]	Bacteria-like P (%) [MI (range)]	*Nematopsis*-like P (%) [MI (range)]	Ciliate P (%) [MI (range)]	Turbellaria P (%) [MI (range)]
Carelmapu *	37	76 (59-85)	21 [8 (1-23)]	14 [2 (1-6)]	45 [10 (2-30)]	7 [1]	3.57 [1]
Carelmapu	19	83 (75-89)	53 [11 (2-43)]	0	82 [7 (1-39)]	12 [2 (1-3)]	5 [1.33 (1-2)]

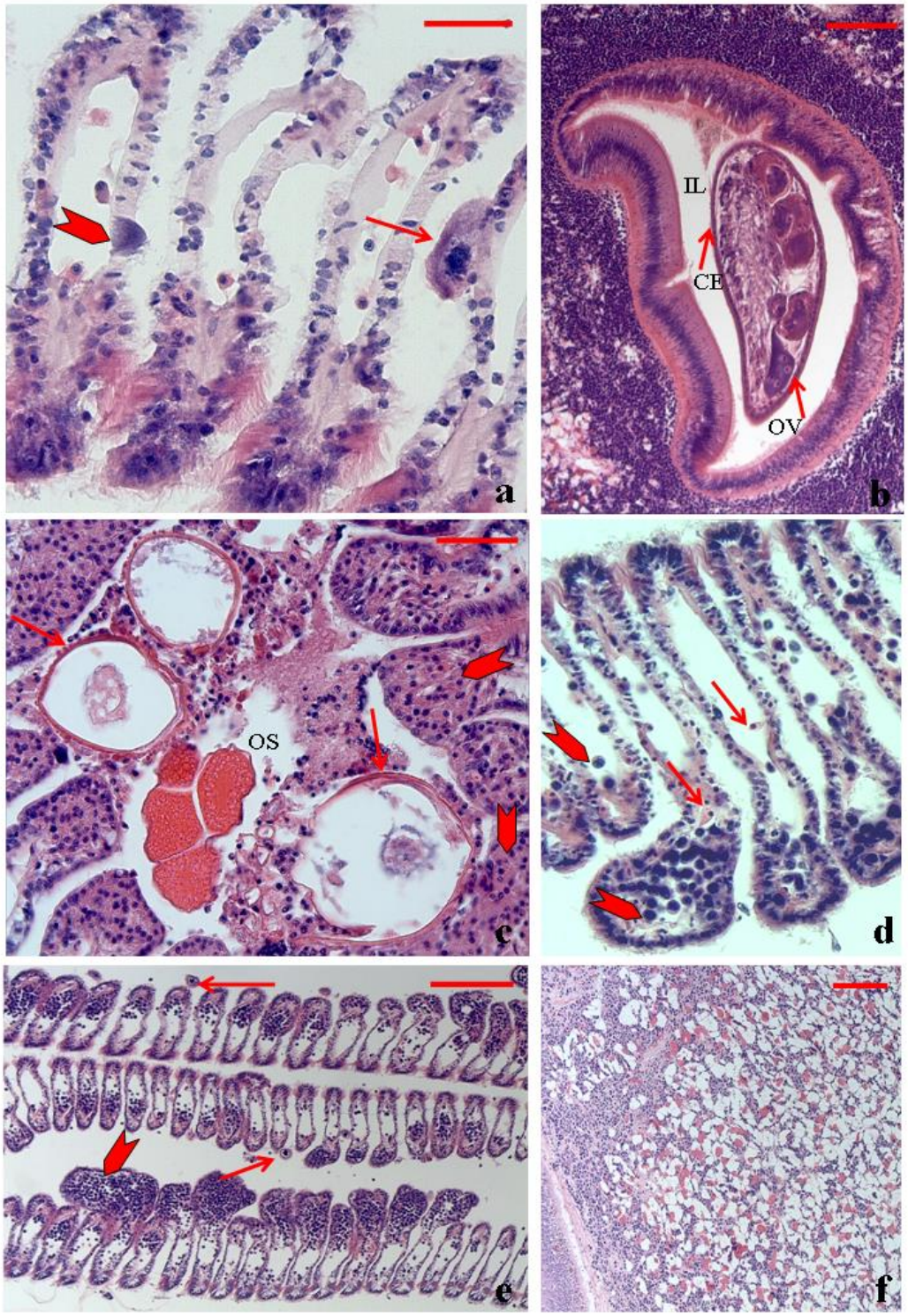

Figure 2. Histological sections (H&E) of *Mytilus chilensis* from southern Chile. a) Intracellular inclusions of bacteria-like organisms (arrow head) and ciliates (arrow) in the gill. Scale bar = 50 µm, b) *Paravortex*-like (Rhabochoela) turbellarian in the intestine lumen. Scale bar = 100 µm, c) haemocytic infiltration (arrow head) in transversal section of the gill where the copepod (arrow) is attached. Scale bar = 100 µm, d) detail of anaplastic cells (arrow head) and normal haemocytes (arrow). Scale bar = 100 µm, e) swollen parts of the gill by numerous anaplastic cells (arrow head) and ciliates (arrow). Scale bar = 100 µm, f) heavy levels of anaplastic cells infiltration showing an empty gonad. Scale bar = 100 µm. IL: intestine lumen, CE: ciliated epidermis, OS: ovigerous sacs, OV: ovocites.

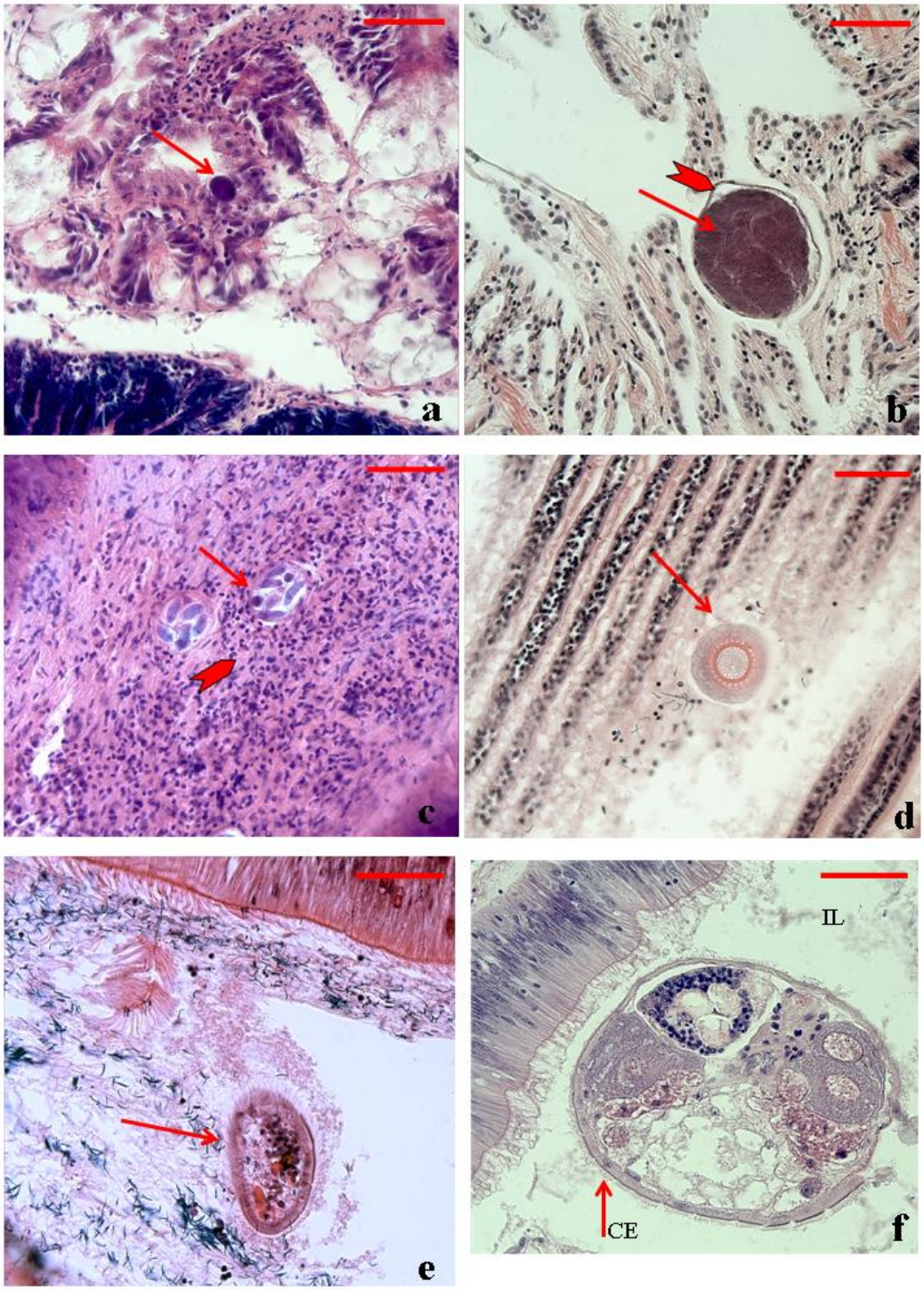

Figure 3. Histological sections (H&E) of the clam *Gari solida* from southern Chile. a) Prokaryotic inclusions (arrow) in digestive gland epithelium. Scale bar = 50 µm, b) bacteria-like organisms (arrow) encapsulated by haemocytes (arrow head) in the gill. Scale bar = 50 µm, c) ocysts similar to *Nematopsis* containing up to eight sporozoites (Apicomplexa) (arrow) causing haemocytic infiltration in the connective tissue (arrow head). Scale bar = 50 µm, d) Ciliate similar to *Trichodina* (arrow) in the gill. Scale bar = 50 µm, e) unidentified ciliate (arrow). Scale bar = 50 µm, f) turbellarian *Paravortex*-like specimen in the intestine lumen. Scale bar = 50 µm. IL: intestine lumen, CE: ciliated epidermis.

tes, turbellarians and copepods. Basophilic intracellular inclusions of bacteria-like colonies were found in the epithelium of the digestive gland and gills, without an apparent host reaction (Fig. 2a). Unidentified ciliates were recorded in gills, characterized by a big macronucleus and several micronuclei, with dense ciliature (Fig. 2a). Turbellarians, similar to *Paravortex* (Rhabdocoela), identified by their ciliated epidermis, ocelli, and muscular pharynx, were found in the intestinal lumen, without histopathological damage to the host tissues (Fig. 2b). Copepods were observed in the gills, which was heavily infiltrated by haemocytes all around the site where the copepod was attached (Fig. 2c). An unidentified isopod was found in the pallial cavity of one specimen from Cochamó site. A disseminated neoplasia (Figs. 2d-2e) was found in all studied sites excepting Cochamó. Diseased mussels showed heavy levels of anaplastic cells infiltration on the connective tissue, particularly in the subepithelial regions. Haemocytes of healthy mussels were typical granulocytes that average 5.78 ± 0.64 µm in length (SD; n = 5), with nuclei of 3.52 ± 0.2 µm in length, whilst the neoplastic cells of mussels with disseminated neoplasia were of 10.31 ± 1.2 µm in length (SD; n = 5) with pleomorphic nuclei of 7.27 ± 0.27 µm in length. One mussel with an advanced diseased stage showed empty gonad follicles (Fig. 2f), being most of the healthy mussels in mature gonad stage.

In the clam, *G. solida*, prokariotic inclusions were found in the epithelium of the digestive gland (Fig. 3a) and basophilic colonies of bacteria in the gills, where were often encapsulated by haemocytes (Fig. 3b). Oocysts containing up to eight sporozoites similar to *Nematopsis* (Apicomplexa) were observed in the connective tissue, causing haemocytic infiltration when the intensity of infection was high (Fig. 3c). *Trichodina*-like (Fig. 3d) and unidentified ciliates (Fig. 3e) were found inhabiting the gills. A turbellarian similar to *Paravortex* (Rhabdocoela) (Fig. 3f) was observed in the intestine lumen, without apparent host reaction.

This work presents the first histopathological survey of the mussel *M. chilensis* and the clam *G. solida* in the southeastern Pacific Ocean. Bacteria-like colonies, ciliates and disseminated neoplasia reported in the present study in the Chilean populations have been previously reported in cultured mussels from the Beagle Channel in Argentina by Cremonte *et al.* (2011). However, in the Beagle Channel mussel populations, a metacercaria parasitizing the foot and byssus gland was found, which was absent in the specimens here studied; conversely, turbellarians and copepods were absent in the Beagle Channel population (Cremonte *et al.,* 2011), but were present in the specimens here studied. Turbellarians similar to *Paravortex* are commonly found inhabiting the intestinal lumen of bivalves without apparent host damage (Brusa *et al.,* 2011; Cremonte, 2011). In only one mussel specimen, an isopod was found in the pallial cavity. Isopods identified as *Edotia magellanica* were reported in *M. chilensis* from the Magellan Strait, southern Chile (Jaramillo *et al.,* 1981).

Gills of *M. chilensis* were swollen due to the presence of anaplastic cells, infected with intracellular inclusions of bacteria-like organisms and by ciliates, and their structure seriously altered when copepods were found attached. The disseminated neoplasia disease here observed was firstly reported in South America in *Ostrea chilensis* by Mix & Breese (1980), from the Chiloé Island. Later, the disease was reported in *M. chilensis* from the same locality by Campalans *et al*. (1998) and from the Beagle Channel, southern Argentina by Cremonte *et al.* (2011). It is a progressive disease reported from about 15 bivalve species around the world and may result in mortalities of the affected populations (Barber, 2004; Carballal *et al.,* 2011). In the Beagle Channel, prevalence reached epizootic levels; however, in the populations here studied, prevalences found were low. In the previous report for *M. chilensis*, Campalans *et al.* (1998) found neoplastic cells mainly infiltrating the digestive gland, accompanied by the destruction of the digestive tubules; the authors did not mention a sign of gonadal atrophy as was found in this study.

Copepods known from histopathological surveys of commercially important bivalves are mainly reported parasitizing the intestinal tract (Cremonte, 2011). However, most copepods which use marine bivalves as hosts inhabit the pallial cavity and their body is modified to attach the gills (Kim & Sato, 2010). In Brazil, Da Suárez-Morales *et al*. (2010) reported *Monstrilla* in the brown mussel *Perna perna*. By other hand, *Ostrincola patagonianus* and another, unidentified, but closely related to *Tisbe celata* copepod, both inhabiting the pallial cavity of *Mytilus* sp., are known from South America (Humes, 1988; unpubl. data). In the present paper, an unidentified copepod was found attached to the gills of the mussels, usually in high intensities, causing severe haemocityc infiltration.

The clam *G. solida* was parasitized by prokaryotic inclusions in the digestive epithelium, intracellular inclusions of bacteria-like organisms and ciliates in the gills, gregarines similar to *Nematopsis* parasitizing exclusively the connective tissues of most organs, and a turbellarian similar to *Paravortex* in the intestine lumen. All the organisms found in *G. solida* are commonly infecting bivalves, without causing severe host damage (Lauckner, 1983; Bower *et al.,* 1994). In

the present study, *Nematopsis*-like oocysts were found causing a host response in form of haemocityc infiltration when the intensity of infection was high. This parasite is commonly found in bivalves, often using them as intermediate hosts and marine arthropods as final hosts (Lauckner, 1983).

The populations of the bivalve species here studied, *M. chilensis* and *G. solida*, were devoid of serious pathogens, being free of OIE notifiable diseases.

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