VERSITA

Paravortex panopea n. sp. (Platyhelminthes: Rhabdocoela) on clams from the northern Patagonian coast, Argentina: pathogeny and specificity

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Summary

During a histopathological survey of the geoduck, *Panopea abbreviata* (Hiatellidae), and the razor clam, *Ensis macha* (Pharidae), in northern Patagonian gulfs (Argentina), turbellarian worms were found in the intestine lumen. In this work, we described a new species of *Paravortex* (Platyhelminthes, Graffillidae). We collected the hosts by scuba diving and dissected alive for studying the turbellarians. In this new species, the pharynx continues in a short esophagus and the latter in a saccular intestine. Gravid specimens have from 2 to 8 twin embryos in the parenchyma. A short penis papilla arises from the seminal vesicle and the gonopore presents a strong sphincter. This species resembled *P. nicolli* Szidat, 1965, which was described paraziting *Mytilus edulis platensis* in Buenos Aires, Argentina. The new species can be differentiated from *P. nicolli* mainly by the presence of a penis papilla and a sphincter between genital atrium and gonopore. This turbellarian species was significantly more prevalent in geoducks (28 %, n = 210) than in razor clams (6 %, n = 480) (KW, p < 0.0001). For both host species, no evidence of direct physical damage caused by turbellarians was observed.

Keywords: Turbellaria; Graffillidae; mollusca; bivalvia; parasite; specificity

Introduction

Dalyellids "Turbellaria" (Platyhelminthes) of the genus *Paravortex* parasitize mainly marine bivalves, but some are associated with fishes (Lauckner, 1983; Cannon, 1986; Cannon & Lester, 1988). This knowledge is based mostly on the work done during the late nineteenth century until the middle of the twentieth century, by researchers in the Northern Hemisphere (i.e. Europe and USA) (Brusa *et al.*, 2006). These works were focused on bivalves of commercial interest and five species were described, while in South America only two species from two distinct host species; one from *Mytilus edulis platensis* from Buenos Aires, Argentina (Szidat, 1965), and the other from *Mesodesma mactroides* in northern Uruguay (Brusa *et al.*, 2006).

The aim of this work was to identify the turbellarian worms found in the intestine lumen during a health survey of the clams *Panopea abbreviata* and *Ensis macha* in northern Patagonian coast. The geoduck clam *P. abbreviata* (Valenciennes, 1839) (Hiatellidae) and the razor clam *E. macha* (Molina, 1782) (Pharidae) are infaunal clams inhabiting soft muddy sediments in northern Patagonia (Argentina). Both species represent a valuable fishery resource and have been exploited by artisanal fishing in the recent years (Barón *et al.*, 2004; Morsán & Ciocco, 2004). Moreover, we analyzed the eventual histopathological effects and the specificity of this new *Paravortex* species.

Material and methods

Thirty geoducks were sampled in October 2009 at Punta Conos (40º32´S, 64º02´W) and 19 razor clams in November 2010 at Punta Gales (42º25´S, 64º32´W) in San José Gulf. The bivalves were collected by scuba diving at approximately 15 meters depth with the help of a hidrojet to dislodge them from the sediment. Clams were maintained in an aquarium with aerated seawater at 13 °C until dissecting them under a stereomicroscope. Turbellarians were collected from the intestine, stained with neutral red and studied alive under a light microscope. Some specimens were fixed in 10 % seawater formaline for histological studies (sectioned sagittally at 4 µm thick, and then stained with Azan method) or for preparing in toto (stained with chlorhydric carmine and mounted in Canada balsam).

To evaluate their pathogenicity, 210 geoducks were sampled: 30 each in January, February and March of 2006 at Puerto Lobos (42°00′S, 65°05′W) in San Matías Gulf (n = 90); and 30 seasonally during 2007 at Punta Conos
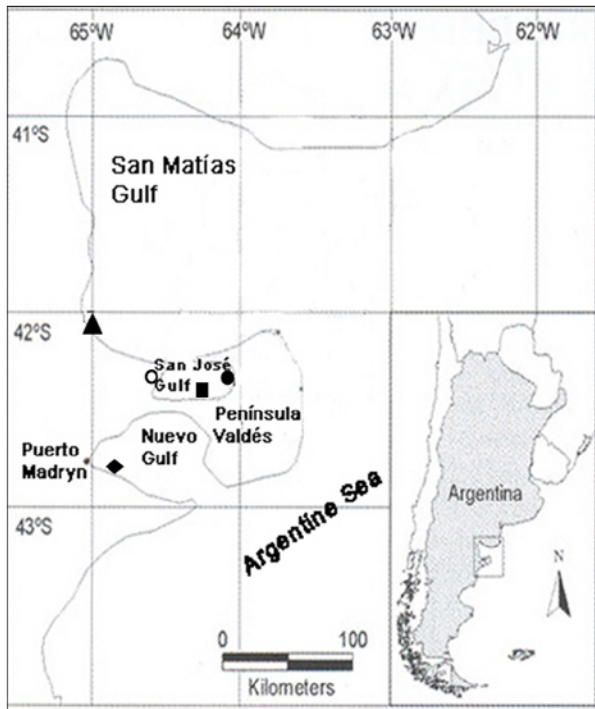
Fig. 1. Collection sites of clams *Panopea abbreviata* and *Ensis macha* in Argentine Sea.
References: ▲ = Puerto Lobos, ○ = La Tapera, ■ = Playa Fracasso, ● = Punta Conos, ♦ = Cerro Avanzado

(42º19´S, 64º02´W) (n = 120). Also, 480 razor clams were sampled: 60 seasonally during 2007 at Puerto Lobos (n= 240) and at Playa Fracasso (42º25'S, 64º07'W) in San José Gulf (n = 240) (Fig. 1). They were transported to the laboratory and maintained in aquaria with aerated seawater at 13 °C for 24 h until histological processing. Soft parts were fixed in Davidson's fixative (Shaw & Battle, 1957) for 24 h and an oblique transverse 5 mm thick section (one for each geoduck and two for each razor clam) containing gills, digestive gland, mantle, nephridia and gonad was obtained, placed in a biopsy cassette, dehydrated in an ethanol series and embedded in paraffin. Histological sections (5 μm thick) were stained with Harris' hematoxylin and eosin, and observed under a light microscope.

The type material was deposited at the Helminthological Collection of Museo de La Plata, Argentina (MLP) and voucher at the Parasitological Collection of Centro Nacional Patagónico (CNP-Pa), Puerto Madryn, Argentina.

Prevalence, range and mean intensities were calculated for turbellarian infection from fresh dissected clams (in this case only adults were counted) according to Bush *et al*. (1997), and from histological sections. Statistical analyses were conducted using non-parametric tests since the data indicated a not normal distribution. A Kruskal-Wallis (KW) test followed by post hoc t comparisons (Siegel & Castellan, 1998) was performed to analyze differences in the prevalence of the two parasitized clam species from histological sections.

Results

Paravortex panopea n. sp. (Figs. 2 – 14)

Holotype: One specimen in toto, MLP
Paratypes: One specimen in toto and one specimen sagittally sectioned, MLP
Aditional material: Several specimens, CNP-Pa and MLP
Type host: *Panopea abbreviata* (Valenciennes, 1839) (Hiatellidae).
Other host: *Ensis macha* (Molina, 1782) (Pharidae).
Type locality: Punta Conos (40º32'S, 64º02'W) in San José Gulf, Argentina.
Other localities: Playa Fracasso (42º25'S, 64º07'W) and La Tapera (42º33'S, 64º55'W) in San José Gulf; Puerto Lobos (42º00'S, 65º05'W) in San Matías Gulf, and Cerro Avanzado (42°49'S, 64°50'W) in Nuevo Gulf, Argentina.
Site of infection: Intestine lumen.
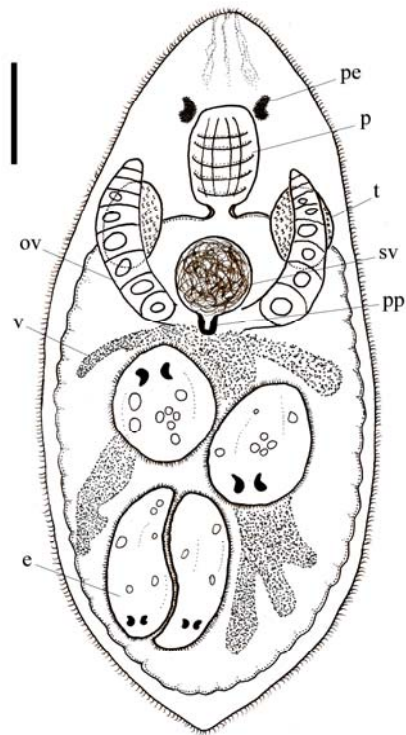Etymology: The species name refers to the generic name of the type host.

Fig. 2. Ventral view of *Paravortex panopea* n. sp.
References: e - embryo; ov - ovary; p - pharynx; pe - pigmented eyes; pp - penis papilla; sv - seminal vesicle; t - testis; v - vitelline glands. Scale bar: 100 μm.

Description

Live specimens are white, elongated oval, and actively swimming. The body of fixed gravid specimens is oval with a rounded anterior end and a tapered rear. The size of fixed specimens is 700 – 1000 μm long and 310 – 420 μm maximum width (n = 9) (Figs. 2, 3). They have a pair of
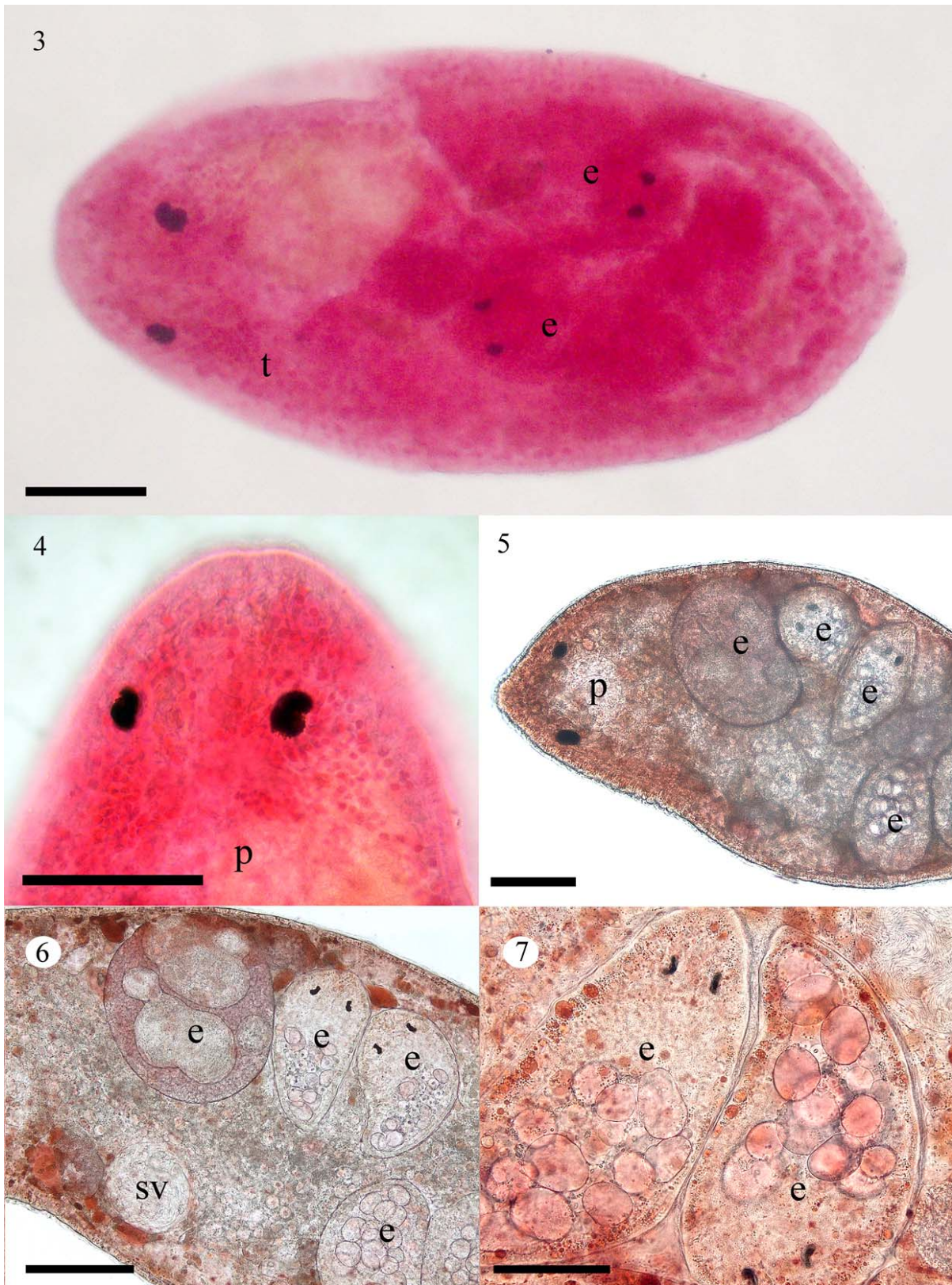
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Fig. 3. – 7. *Paravortex panopea* n. sp. from the intestinal lumen of the geoduck clam *Panopea abbreviata*. 3. – 4. - *In toto* specimen (stained with chlorhidric carmine). 5. – 7. - Alive specimen (stained with neutral red).
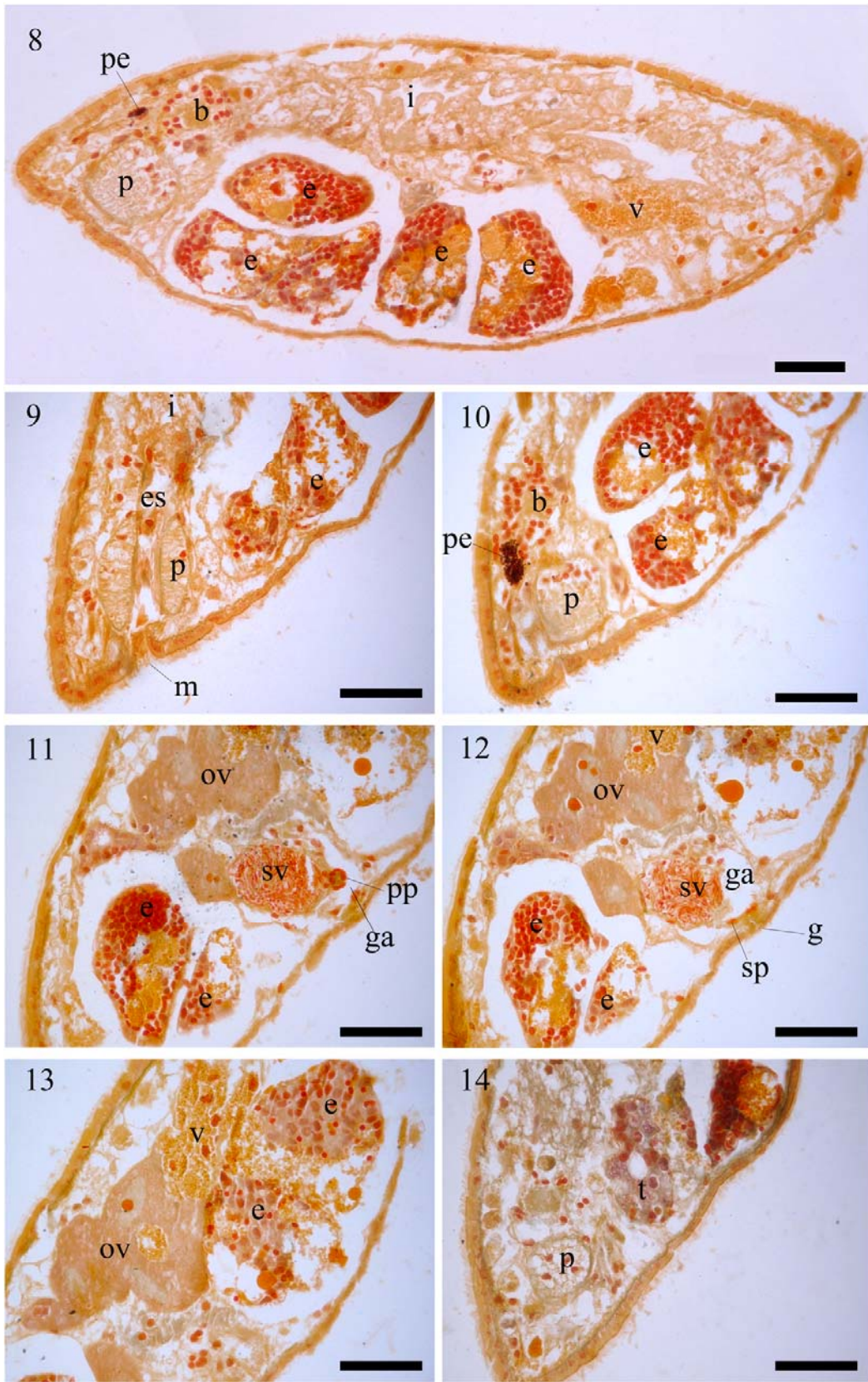References: e - embryo; p - pharynx; sv - seminal vesicle; t - testis. Scale bars 3-6: 100 µm, 7: 50 µm.

Fig. 8. – 14. Sagittaly sectioned adult specimen of *Paravortex panopea* n. sp. from the intestinal lumen of the geoduck clam *Panopea abbreviata*. References: b - brain; e - embryo; g - gonopore; ga - genital atrium; i - intestine; m - mouth; es - esophagus; ov - ovary; p - pharynx; pe - pigmented eyes; pp - penis papilla; sp - sphincter; sv - seminal vesicle; t - testis; v - vitelline glands. Scale bars: 50 µm.
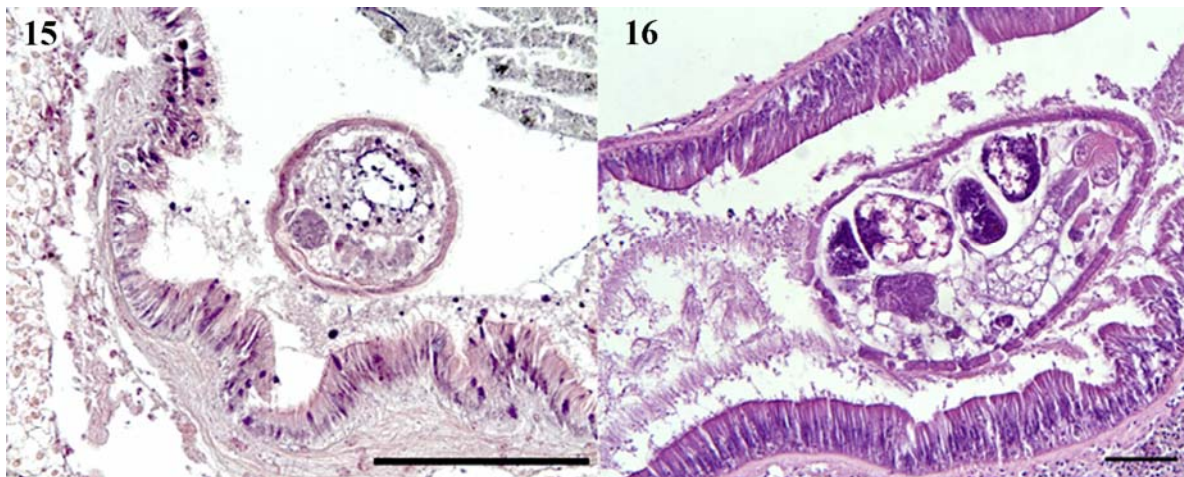
Fig. 15. – 16. Histological sections of the clams. 15. - Intestine of the geoduck *Panopea abbreviata* with a turbellarian worm inside. 16. - Gravid specimen of *Paravortex panopea* n. sp. in the intestine lumen of the razor clam *Ensis macha*. Scale bars: 200 µm.

large eyes of 25 µm long with numerous corpuscles of pigment (Fig. 10), kidney-shaped with the concave part towards the periphery of the body (Fig. 4). The eyes are located slightly ahead of the pharynx (Figs. 5, 10).

The mouth is antero-dorsal and the pharynx of fixed specimens is rounded, 75 – 80 µm in diameter (n = 4). A short glandular esophagus opens into a large saccular intestine with a highly developed vacuolar epithelium (Figs. 8, 9).

The antero-lateral testes are located posteriorly to the pharynx, running into a pair of vas deferens leading to a seminal vesicle, always full of sperm (Figs. 2, 3, 6, 11, 12). The seminal vesicle is rounded when is observed in vivo (Fig. 6), although it may adopt an oval shape in fixed specimens (Fig. 11). The seminal vesicle communicates with the atrium through a short penial papilla (Fig. 11). The atrium opens to the outside through a middle ventral gonopore with a strong sphincter (Fig. 12).

A pair of lateral ovaries develops from the anterior region of the body towards the middle, where they attach to the vitelline glands. Vitelline glands are more developed in the posterior region of the body. The ovaries, once their join the vitelline glands, empty into the genital atrium (Figs. 12, 13). Gravid specimens have few capsules (1 – 4) in the parenchyma, that measure 135 – 140 µm in length (n = 4) with twinned embryos (Figs. 2, 3, 5 – 8, 9 – 13). Embryos are in pairs in a single capsule. In the same individual, embryos at different stages of development coexist, from very early stages with only one blastocyst to embryos fully developed, with eyes and cilia that allow them to move (Figs. 5 – 7).

Remarks

At present, seven species of *Paravortex* parasitizing different species of bivalves are recognized; five of them from the Northern Hemisphere (Brusa *et al.* 2006). The species here described shows a greater resemblance to *Paravortex nicolli* Szidat, 1965, which was described parasitizing *Mytilus edulis platensis* (Bivalvia: Mytilidae) from Quequén, Buenos Aires, Argentina (Szidat, 1965). The validity of *P. nicolli* is not in doubt; however, the description is poor. The observation of the type material deposited at the Museo Argentino de Ciencias Naturales (MACN) of Buenos Aires city, Argentina, did not allow the observation of additional characters. The species here described has a similar morphology to *P. nicolli* in body size, eyes shape, pharynx, and embryo size; all these characters were seen in the specimens deposited at MACN. However, Szidat (1965) described *P. nicolli* as protandrous, finding no testes when embryos are present. In the case of *P. panopea* n. sp., testes were observed along with embryos (Figs. 3, 14); therefore, the development occurs simultaneously. In addition, a penis papilla and a strong sphincter between the genital atrium and gonopore are not present in *P. nicolli*. Other species of *Paravortex* such as *P. gemellipara* and *P. karlingi* that inhabit the North Atlantic Ocean

Table 1. Prevalence, range and mean intensity (MI) of the turbellarian *Paravortex panopea* n. sp. found by different examination methods in the clams *Panopea abbreviata* and *Ensis macha* in northern Patagonian gulfs (SMG= San Matías Gulf, SJG= San José Gulf).

Clam species (n)	Locality	Examination method	Prevalence (%)	Range and (MI)
P. abbreviata (90)	Puerto Lobos (SMG)	histological section	30.33	1 – 15 (3)
P. abbreviata (120)	Punta Conos (SJG)	histological section	23.96	1 – 14 (2.65)
P. abbreviata (30)	Punta Conos (SJG)	fresh clam dissected	66	1 – 19 (3)
E. macha (240)	Puerto Lobos (SMG)	histological section	4.16	1 (1)
E. macha (240)	Fracasso Beach (SJG)	histological section	7.08	1 – 4 (1.23)
E. macha (19)	Punta Gales (SJG)	fresh clam dissected	63.16	3.5 (1 – 10)

(USA, Canada and UK) also have penis papilla (Ball, 1916; Pike & Burt, 1981). However, these last mentioned species have a larger number of capsules with twinned embryos in parenchyma (about 20 embyos vs. 2 – 8 embryos) and they are smaller (<100 μm in length in *P. gemellipara* and *P. karlingi* vs. 135 – 140 μm in length).

Table 1 shows prevalences, range and mean intensities of infection in the different populations of the clam species studied from fresh dissected clams and from histological sections. The prevalence of turbellarians was significantly higher in geoducks than in razor clams (KW; *p* < 0.0001).

From the histological sections, no damage of the intestine epithelium in either clam species was observed (Figs. 15 – 16); however, occasionally, gravid specimens were occupying a great part of the intestine lumen.

Discussion

The new species of *Paravortex* is the third described from the Southern Hemisphere and the southernmost report of the genus.

The *Paravortex* species here described seems to exhibit some specificity for their bivalve hosts. *Paravortex panopea* n. sp. can be found also in *E. macha* with high prevalences. Whilst turbellaria have been found in low prevalences in the jingle-shell bivalve, *Pododesmus rudis* (4 % of 115 histological examined bivalves) and in scallops, *Aequipecten tehuelchus* (<5 % of 230 histological examined scallops) in other bivalves from the same study area we have examined, turbellaria were never found [i.e., the mytilids *Mytilus edulis*, *Aulacomya atra*, *Brachydontes rodriguezi* the oyster *Ostrea puelchana* and the clam *Protothaca antique,* Cremonte (1999); Cremonte *et al.* (2005); Cremonte F. and N. Vázquez (unpublished data)] . Fresh *P. rudis* and *A. tehuelchus* dissected should be examined for the presence of turbellarians. We analyzed the previous records of the *Paravortex* species and concluded that most of them exhibit a phylogenetic host specificity, since each turbellarian species parasites one bivalve family (*P. cardii* is found in Cardiidae, *P. gemellipara* in Mytilidae, *P. scrobiculariae* in Semelidae, and *P. karlingi*, *P. nicolli*, *P. tapetis* and *P. mesodesma* were registered in one host species). Nevertheless, it is uncertain if it is due to the specificity of the turbellarians or because other bivalves from the same area were not studied. In our case, *P. panopea* n. sp. seems to be present in clams living in sandy substrates.

Since the graffilids lack endogenous digestive enzymes, they use their host enzymes to feed on partially digested hosts food, and their food reserves consist mainly on glycogen (as it occurs in Digenea and Cestoda); these facts represent physiological adaptations to a parasite mode of life (Jennings, 1981; Jennings & Phillips, 1978; Jennings & Le Flore, 1979). This high degree of metabolic dependence may be responsible for the high specificity observed in the species of *Paravortex*.

Regarding the turbellarian pathogenicity, no evidence of direct physical damage was observed in our study, agreeing with previous reports on bivalves infected with *Paravortex* (Jennings & Phillips, 1978; Jennings & Le Flore, 1979; Jennings, 1981). Although it seems to be innocuous to the host, some gravid specimens occupying a great part of the intestine lumen were observed, therefore they may reduce the absorption of the nutrients by obstruction.

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