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First record of *Stephanostomum sp.* Looss, 1899 (Digenea: Acanthocolpidae) metacercariae parasitising the pleasure oyster *Crassostrea corteziensis* (Hertlein) from the Mexican Pacific coast

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Summary

The aim of this investigation was to identify the parasites present in the largely understudied pleasure oyster *Crassostrea corteziensis* in Sinaloa state in the northwestern Mexican Pacific coast. Inspection of twenty-eight oysters collected on "Ceuta" lagoon revealed the presence of the digenean *Stephanostomum sp*. (Digenea: Acanthocolpidae) cysts. Metacercariae were found encapsulated and embedded in the digestive gland and mantle tissue of oysters. The prevalence of infection revealed that 84.6 % were infected, the abundance was 13.62, with a mean intensity of 16.09 per host. The members of this genus are characterized by a double crown of spines in the cephalic region surrounding the buccal opening of the worm. Significantly, we report the first incidence of the digenean *Stephanostomum* sp of the family Acanthocolpidae parasitizing *Crassostrea corteziensis*. Further we report that this bivalve is now considered a new intermediate host, and the northwestern Mexican Pacific coast is a new geographical distribution area for this digenean. The findings contribute to our understanding of the biology, biodiversity and host preference of these parasites, with implications for health risks posed by human consumption of the pleasure oyster.
Keywords: bivalve; digenean; trematode; parasites; helminth; oysters; Mexico

Introduction

There are some species of digenens that are known to employ mollusks and use them as definitive or final, intermediate or paratenic hosts for unidentified reasons. Digeneans are one of the most common groups of helminths found parasitising vertebrates. In Mexico, around 503 species have been reported in nearly 440 of the 4,697 species of vertebrates known and a total of 153 digenean species are endemic within Mexican territory (Perez-Ponce de Leon, 2001). Digeneans have a complex life cycle, involving one or two, but rarely more, intermediate hosts prior to infecting the definitive host. Many invertebrates may function as intermediate hosts for a wide variety of parasites. Bivalve mollusks play a key role as hosts for larval stages of digeneans, mainly trematodes (Lasiak, 1992; Ukong, 2007; Thieltges *et al.,* 2006). Mollusk infection may occur through ingestion of the eggs or penetration by free-swimming larvae called miriacidia. Inside the first intermediate host, several different larval stages (i.e., sporocyst, redia, and cercaria) are formed by asexual reproduction. After emerging from the animal tissue, the larvae of trematodes (cercariae) may find a suitable secondary intermediate host i.e., a crustacean by transmission thru a trophic link (metacercariae-cyst) (Gomez del Prado *et al.,* 2003; Grano-Maldonado & Alvarez-Cadena, 2010) or a definitive host by active penetration until it reaches a vertebrate host. The mollusk-trematode model system which has been extensively studied is the host-parasite *Biomphalaria-Schistosoma mansoni.*

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According to Lasiak (1992), the larvae of the bucephalid trematode sporocysts are found between the mantle tissue and visceral mass of intertidal mytilid bivalves from Chile. In Brazil, Ceuta & Boehs (2012) reported that some parasites found in the mangrove mussel *Mytella guyanensis* (Lamarck, 1819) showed *Rickettsia*-like organisms, *Nematopsis* sp. (Apicomplexa), including a turbellarian, sporocysts and metacercariae of *Bucephalus* sp., and metacestodes of *Tylocephalum* sp. in the gills, mantle, and digestive gland. In Mexico, in a coastal lagoon of the southern Gulf of Mexico, Aguirre-Macedo *et al.,* (2007) performed a parasite survey of the oyster *Crassostrea virginica* Gmelin, 1791. These authors found two protozoan (*Nematopsis prytherchi* and *Perkinsus marinus*), and 4 helminth species (*Urastoma cyprinae, Proctoeces maculatus, Bucephalus sp., Tylocephalum sp.*). However, in the Mexican Pacific coast, information about the parasite fauna of the pleasure oyster is almost absent.

The study of parasites in intermediate hosts is important for human health. For example, the host-parasite *Biomphalaria-Schistosoma mansoni* is responsible for human schistosomiasis (Hung *et al.,* 2013; Hotez *et al.,* 2014; Keiser & Utzinger, 2009). Metacercarie *Clinostomum complanatum* has been mainly found in fish *P. pardalis* in the southwest of Mexico (Rodriguez-Santiago *et al.,* 2016), and members of this species have been found attached to the human larynx and pharynx after eating raw seafood (Witenberg, 1944; García *et al*., 2007; Park *et al.,* 2009). Thus, the consumption of raw fish and bivalves such as oysters potentially poses a health risk and should be explored further, motivating this study of parasites in the much understudied mollusk: the pleasure oyster *Crassostrea corteziensis* (Hertlein, 1951).

In the Sinaloa coast in northwest Mexico, the culture of the pleasure oyster is emerging as an important commercial alternative to the Pacific oyster *Crassostrea gigas* for oyster consumers. This mollusk is actively being cultured by local coastal communities for local consumption and commercial market (Chávez-Villalba *et al.,* 2010; Chavez-Villalba 2014; Chávez-Villalba & Aragón-Noriega, 2015). Research of oysters in the Pacific coast has focused on histopathological surveys revealing the presence of hypertrophied gametes, rickettsiales-like prokaryotes, the protozoan *Perkinsus marinus*, *Nematopsis* sp., *Ancistrocoma*, *Sphenophrya*-like ciliates, a turbellarian *Urastoma* sp., hyperparasite of the rickettsiales-like and copepods *Pseudomyicola spinosus and Modiolicola gracilis* (Cruz Flores & Cáceres- Martínez, 2016; Caceres-Martinez *et al.,* 1996, 1998, 1999, 2005, 2010, 2012, 2015, 2016; Costa *et al.,* 2013; Da Silva *et al.,* 2012, 2016; Dantas-Neto *et al.,* 2015; Pinho *et al.,* 2013).

The aim of this investigation was to identify for the first time the helminth parasites present in the pleasure oyster *Crassostrea corteziensis* in Sinaloa state in the northwestern Mexican Pacific coast to increase the current parasite life cycle and biological knowledge. Further research should consider the potential transmission to humans through consumption of this bivalve.

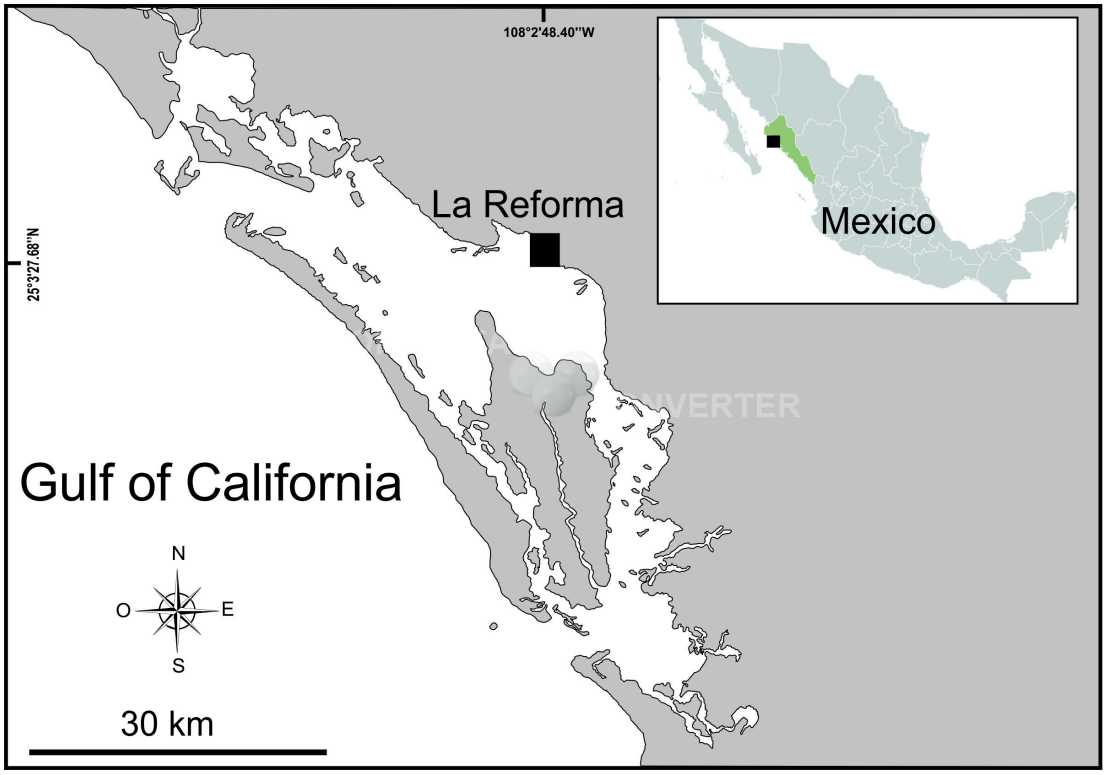

Fig. 1. Location of the collecting area of the pleasure oyster *Crassostrea corteziensis* on coast of Sinaloa, Mexico.

Materials and Methods

Source of parasites

Twenty-eight pleasure oysters *Crassostrea corteziensis* (7.4 ± 1.05 cm average length) were collected by hand from intertidal mudflats close to the mangrove roots from a local fish cooperative in 'Ceuta coastal lagoon, la Reforma' in the State of Sinaloa on the northwest Pacific coast of Mexico (25° 3'27.68"N 108° 2'48.40"W) (Fig. 1). The oysters were transported live to the Microalgae laboratory facilities at the Marine Science Faculty, Autonomous University of Sinaloa. Upon arrival, the organisms were place individually in 2L aquaria supplied with filtered flow-through seawater and constant aeration. The oysters were fed daily with the microalgae *Thalassiosira weissflogii* (Grunow) around 241, 300 cel/ml for five days when all were sacrificed. Animal care and handling were carried out in accordance with Mexican laws (NOM-033-ZOO-1995). The oysters were dissected and organs were carefully separated on individual petri dishes with clean and filtered seawater then reviewed by compression between two 10 cm^2 glass slides under a stereomicroscope (LEICA MZ 9.5, Wetzlar, Germany). Cysts were found in the mantle and digestive gland. The metacercariae were extracted from the cysts using small needles. The parasites were then mounted with a coverslip to flatten the specimens. AFA (a mixture of 85 ml of ethanol, 25 ml of formaldehyde, and 5 ml of acetic acid) was added drop by drop to the edge of the coverslip to fix the parasites, and then they were made transparent with glycerin and mounted. Further observations were done with an optic microscope (LEICA DMLB 10, Wetzlar, Germany) for better resolution. In order to perform morphological descriptions, the parasites were observed under an optical microscope (100 x/oil immersion magnification).

The preparations were made for each parasite for their identification to the lowest taxonomical level. The taxonomic identification of the metacercariae found in this study was based on the work of previous studies (Bray & Cribb, 2003; 2004; 2006; 2008; Bray *et al.,* 2007).

Ethical approval and/or Informed Consent

Animal care and handling were carried out in accordance with institutional guidelines according to Mexican laws (NOM-033-ZOO-1995).

Results

A total of 354 metacercariae cysts recovered from the 28 oysters host (Figs. 1 – 3). The parasite was identified as a *Stephanostomum* Looss, 1899 (Digenea:Acanthocolpidae) by several charac-

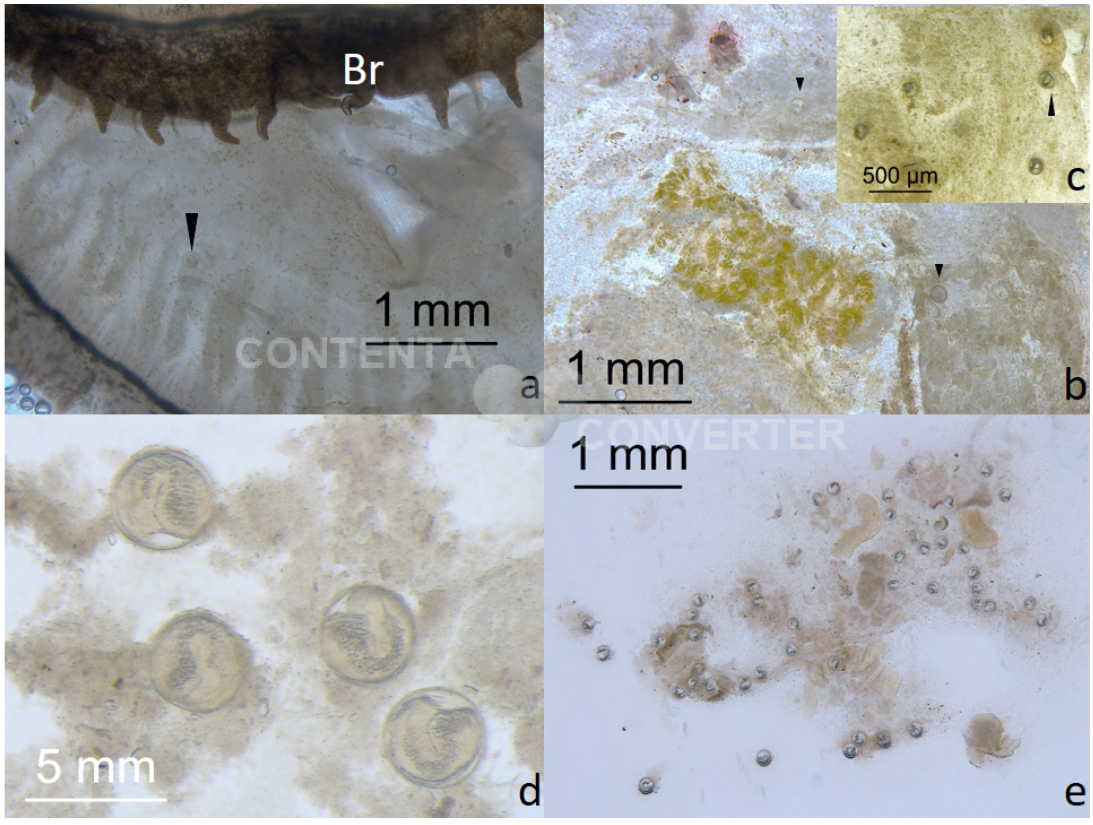

Fig. 2. *Stephanostomum* spp. (Digenea) from the pleasure oyster *Crassostrea corteziensis*. a-b) Cyst in the digestive gland. d-e) Cyst in the mantle.

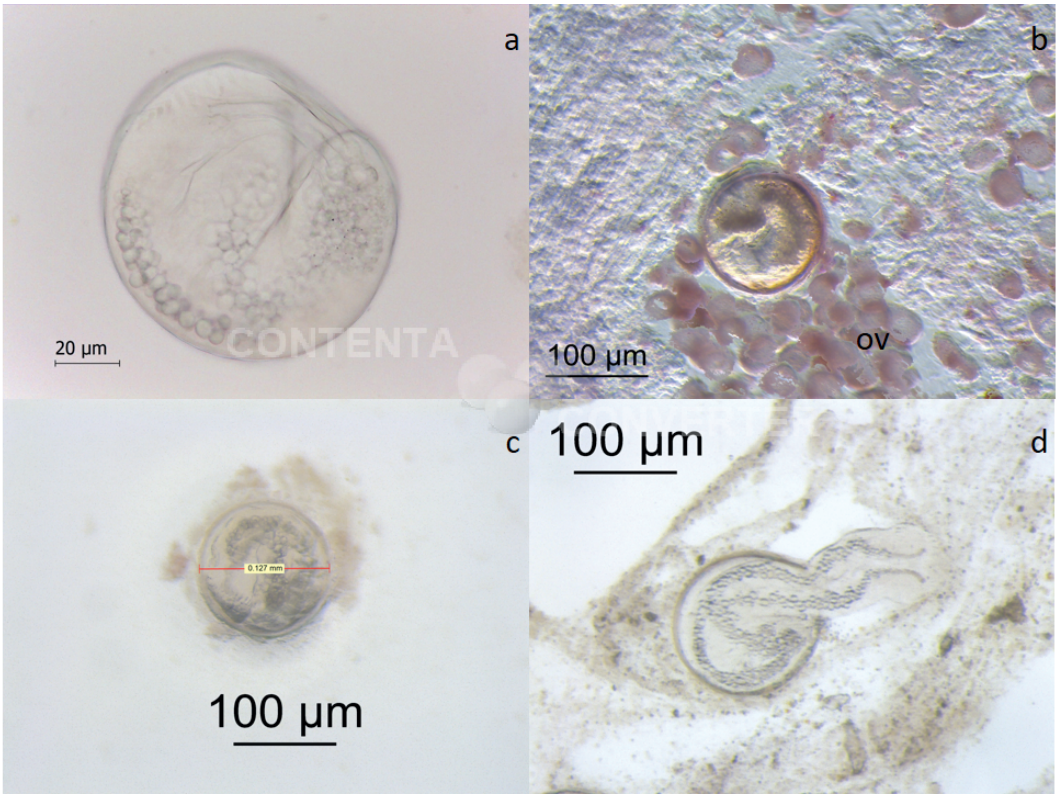

Fig. 3. Metacercariae *Stephanostomum* spp. (Digenea) were found encapsulated and embedded in the digestive gland and mantle tissue of oysters *Crassostrea corteziensis* a-d) individual cysts located in the mantle. b) cyst located close to the gonad, d) opened cyst to reveal the metacercariae.

teristics, particularly the double crown of spines in the cephalic region surrounding the buccal opening of the parasite (Fig. 3). Exemplars were registered (catalogue number: 10630) in the CNHE-IBUNAM. The prevalence was 84 %, and the abundance was 13. The mean intensity was16 per host and the number of parasites per host varied from 1 to 56.

Taxonomic Remarks

Parasite: *Stephanostomum* sp. Looss, 1899 (Digenea: Acanthocolpidae)
Type-host: the pleasure oyster *Crassostrea corteziensis* (Hertlein) (Molluska: Bivalvia)
Habitat: Intertidal mudflats
Site of infection: mantle and digestive gland
Type-locality: Ceuta coastal lagoon Sinaloa, México 25°3'27.68"N 108° 2'48.40"W
Date collection: November 2017
Deposition of specimens: CNHE-IBUNAM- 10630

Recent and extensive studies (Bartoli & Bray, 2001; Saoud *et al.,* 2002, Bray & Cribb, 2003; Bartoli & Bray, 2004) point out that *Stephanostomum* sp. Looss, 1899 is an extremely large genus. It is composed of 112 species nominally distributed around the world that infect a considerable number of teleost fishes, particularly those of temperate waters (Bartoli & Bray, 2001). Cribb *et al.,* (2001) revised the overall pattern of specificity of trematodes in fishes and first intermediate hosts. The acanthocolpid fauna of fishes from Australian and South Pacific waters is very well known. Cribb *et al.,* (2001) inferred that this family Acanthocolpidae is consistent with the overall mollusk pattern. Agreeing with Barnet et al., (2010) who performed and extensive review, the success of this genus is marked by the extensive range of fishes (over 70 families) that are infected as adults. Species of *Stephanostomum* reported from eastern Australian waters include *S. adlardi*, *S. aaravi, S. bicoronatum, S. carangi, S. cobia, S. ditrematis, S. hawaiiense, S. lamothei, S. madhaviae, S. nyoomwa, S. pagrosomi, S. petimba, S. talakitok, S. tantabiddii, S. tupatupa,* and *S. cf. uku*. Barnet *et al.* (2010) reported that there are nine families of trematodes that routinely infect bivalves as first intermediate hosts and a few others (e.g. Lepocreadiidae, Hemiuridae) that infect them exceptionally. However, far more host-parasite records may need to be accumulated for better understanding of the relationships in the Acanthocolpidae group. Our results agree with Barnet *et al*., (2010) and Cribb *et al.,* (2002) who reported that i) *Stephanostomum* was the second largest genus of trematodes of fishes, ii) the genus also appears to have low specificity for first intermediate hosts, and iii) given the current size of the genus there are clearly many more intermediate hosts to be found within and beyond the mollusk. In the present study, *Stephanostomum* was found in the pleasure oyster *Crassostrea corteziensis* in the northwest of the Pacific coast of Mexico. In Mexico, the species of this type of digenan is widely

distributed in marine fish, according with Lamothe-Argumedo *et al.,* (1997) where eight species that parasitize fish of different families have been recorded (*S. californicum* Manter and Van Cleave, 1951 in the intestine of *Genyonemus Californucum* California, USA; *S. casum* was collected from the intestine of *Microlepidotus brevipinnis* in Chamela Bay, Jalisco, *S. dentatum* (Linton, 1910) Linton, 1940 of the intestine of *Balistes polylepis* and *Paralicthys californicus* of Baja California; *S. ditrematis* collected in the stomach of *Seriola dorsalis* captured on the Partida Island; Baja California Sur; *S. hispidum* (Yamaguti, 1934) of *Caranx hippos* in Manzanillo, Colima; *S. megacephalum* Manter, 1940 collected in the intestine of *Caranx hippos* in the Bay of Chamela, Jalisco; *S. tenue* Linton 1898 collected from the *Selar crumenophthalmus* fish from Puerto Vallarta, Jalisco in members of the Lutjanidae family (Garcia-Vargas, 2010).

The members of this genus are characterized by a double crown of spines in the cephalic region surrounding the buccal opening of the worm. Bray *et al.,* (2005) pointed out that the characteristic with the highest taxonomic load to separate the species of this genus is the number of spines that surround the mouth, but this is not a regular pattern. The identification for species level could not be carried out as the parasites in our study displayed a greater number of circumoral spines 63 (58-64, arranged in oral 28-32 and 30 – 32 suboral). Additionally, molecular identification of these parasites merits further investigation to better determine accurate taxonomic status.

We found that the pleasure oyster acts as an intermediate host having infective stages of *Stephanostomum* sp. Looss, 1899 (Digenea:Acanthocolpidae) parasites. Members of the genus *Stephanostomum* have been studied comprehensively in Australia (Bray & Cribb, 2003; 2004, 2004a; 2005; 2008; Bray *et al.,* 2007) where adult stages were present in fishes of the family Carangidae and Scombrids. Based on our contribution in which we identified the parasitic larval stages metacercarie existing in *Crassostrea cortezensis,* further research should consider the potential transmission to humans through consumption of this bivalve.

Discussion

This study is the first report identifying the metacercariae of *Stephanostomum* species, a digenean of the family Acanthocolpidae, parasitising the pleasure oyster *Crassostrea corteziensis* (Hertlein) from the Mexican Pacific Coast. According to Caceres-Martinez *et al.,* (2016) other pathogens like *Perkinsus marinus* seems to represent a more significant risk for the health of pleasure oysters than do other parasites, and surveillance and control of these parasites are needed for the development of pleasure oyster culture. However, in our study the majority of parasitised oysters appeared

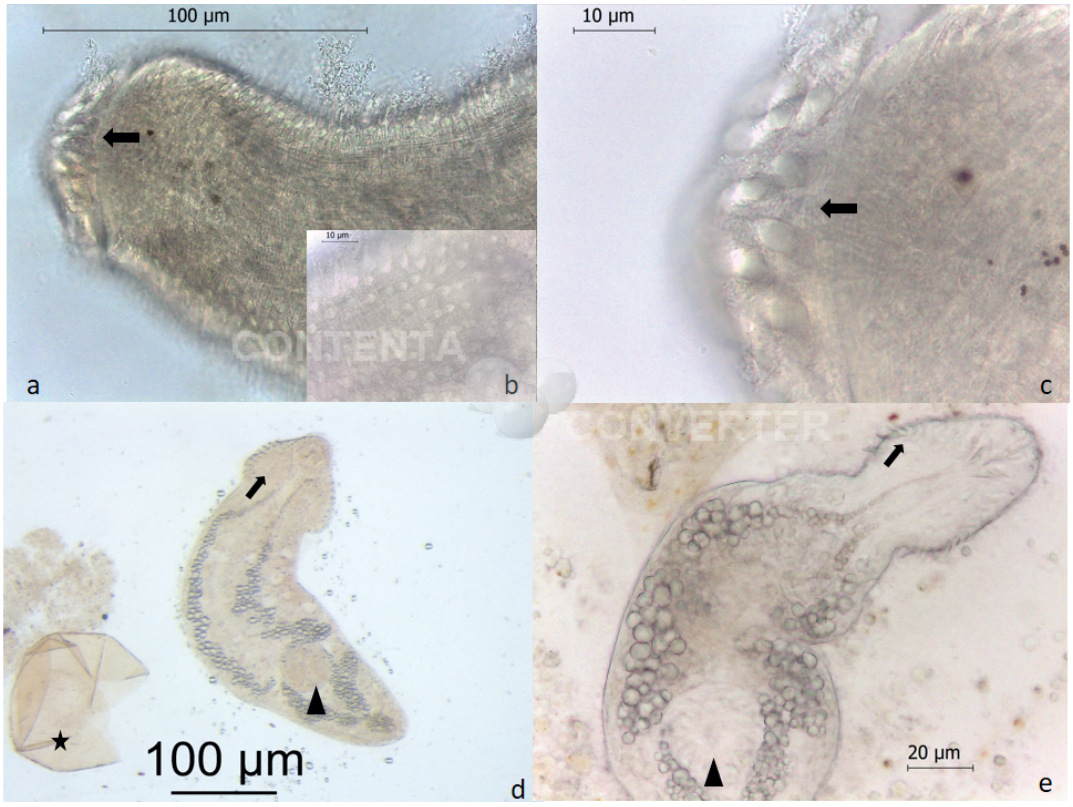

Fig. 4. Microphotography using an optical microscope of cyst released metacercariae of *Stephanostomum* spp. (Digenea). a-d) noticed the a double crown of spines in the cephalic region (arrow) surrounding the buccal opening of the worm; d-e) acetabulum (arrow head), empty cyst (star).

to be free of infection and no apparent destruction of the host's gonadal tissue, digestive or branchial damage was observed. In the parasitological study performed by Aguirre-Macedo et al. (2007) the prevalence and mean abundances for the protozoa and helminths varied widely between locations but were generally below 50 %. *Nematopsis prytherchi* and the *Tylocephalum sp*. were the most prevalent species (>60 %). *Perkinsus marinus* was present in oysters of eight coastal lagoons and had low prevalence (<30 %) in almost all samples. All identified protozoa and helminths are widely distributed in the Gulf of Mexico and are common oyster parasites. Only *P. marinus* and the *Bucephalus sp*. were associated with damage to host tissues. In our study the prevalence of *Stephanostomum* species infection was 84.6 %, the abundance was 13.62 and the mean intensity of 16.09 per host. These results revealed a high percentage of parasitism in the zone, this is alarming since the typical consumption of this animal is uncooked. It is important to mention that most human-parasite disease transmission is mainly associated with the consumption of raw seafood dishes i.e., sushi, sashimi, ceviche (Kuchta *et al.,* 2005ab). Most of the species potentially pathogenic to human are pathogenic mostly in larval stage, i.e., L1 and L3 in nematodes, plerocercoid in cestodes and metacercariae in digenan trematodes (Rodriguez-Santiago *et al.,* 2016). Consequently, the ingestion of bivalves such as oysters as a source of raw food may have a health risk factor and should be explored further.

Barnet *et al.,* (2010) reported in the case of other *Stephanostomum* spp. cercariae from mollusks *(*Gastropoda*:* Nassariidae*)* in Australia. Their results suggest that parasite location in the host was similar to our findings (Digestive gland, gonads) and the habitat of bivalve collection of intertidal mudflats was similar with a prevalence of emergence: 0.57 % (10 of 1766 *Nassarius dorsatus*), 0.21 % (4 of 1908 *Nassarius olivaceus*). In our study, the parasite *Stephanostomum sp.*was also present in a costal lagoon in Sinaloa state in quantities greater than 84.6 %. With respect to the digenetic trematodes, the presence of metacercariae observed in our study reveals that bivalves may serve as potentially primary and secondary intermediate hosts at the same time.

A limitation of this study is that molecular techniques could not be used because the samples were preserved on formalin. Future comparison of the 18S ribosomal RNA gene (or other conserved genes) sequence of the parasite collected from pleasure oysters should be conducted. There is no doubt, nonetheless, that infections by parasites have major consequences for species of host in natural conditions and must consequently be considered as a fundamental factor within any system of aquaculture.

Conflicts of Interest

Authors have no potential conflict of interest pertaining to this submission to *Helminthologia*.

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