Parasite Inspection in Five Commercially Important Oyster Species (*Mollusca: Bivalvia*) of Pakistan

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Abstract: A total of five native oyster species namely; *Crassostrea gryphoides, C. madrasensis, C. glomerata, Ostrea nomades* and *Saccostrea cucullata* were collected from three sites: Buleji of Sindh coast; Hub and Jiwani of Balochistan coast, Pakistan. Parasitic inclusion was present only in histological sections of *O. nomades* and *S. cucullata* at Buleji. Individuals of *O. nomades* found to be infected with protozoan parasite *Nematopsis* species and *Proctoeces* sp. trematode parasite. Additionally, unknown parasitic cyst was observed at outer epithelium in a male gonadal section of *S. cucullata*. There is no previous record in literature of pathogens in oyster species at Buleji.

Keywords: *Nematopsis*, Northern Arabian Sea, *Ostrea*, *Saccostrea*, *Crassostrea*, Trematode, parasite.

INTRODUCTION

Over the past 60 years number of pathogens, related diseases and mortalities have been reported in bivalves across the globe [1-7]. The main biological disease-causing agents in marine bivalves are viruses, bacteria, fungi, protists, digenean trematodes, polychaetes and copepods [8]. Parasitic diseases pose a serious threat to the world's oyster aquaculture industry and to oyster fisheries. Historically, some diseases are of regulatory significance [9, 10] have been associated with *Ostrea* spp. and *Crassostrea* spp. and account for major commercial losses and limit expansion of oyster culture. Pakistan has a sizeable diversity of native species of oysters [11] which can provide a potential resource for the development of mariculture in Pakistan [5]. However, although they are not exploited commercially, oyster stocks in Pakistan have become depleted and their natural spat fall is very low [12]. Like elsewhere in the world the study of parasites and disease distressing oysters is important due to their potential economic impacts on the management of natural stocks and aquaculture [5-6]. Siddiqui *et al.,* [5] reported the *Nematopsis* spp. in *O. nomades* and *C. belcheri* from Keamari Sea wall area adjacent to Manora Channel along the Sindh coast of Pakistan. The aim of the present study was to broaden this previous study to other species and sites as the diseases of regulatory significance not found in native oyster species which make them exclusively important from the mariculture point of view for commercial exploitation.

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METHODS

Samples were procured, between January 2012 and March 2012 at low tides (Table **1**). Five oyster species, *Crassostrea gryphoides* (Schlotheim, 1813), *C. madrasensis* (Preston, 1916), *C. glomerata* (Gould, 1850), *Ostrea nomads* (Iredale, 1939) and *Saccostrea cucullata* (Born, 1778) were sampled from the intertidal zones of three sites: Buleji (24° 50' N, 66° 48' E), Hub (24° 54' N, 66° 43' E) and Jiwani (25° 07.630' N, 61° 45.662' E) (Figure **1**). Measurements were taken before histological preparations. The animals were brought live to laboratory and the length along their longest axis and shell height of specimens were measured to the nearest millimeter (0.1 mm) by the method described by Galtsoff [13] and Siddiqui and Ahmed [14]. Wet tissue weight (g) was also recorded prior to further examination. Seven (7) to twenty (20) individuals of each species were histologically examined. A portion (5 mm) of soft tissue was excised with a razor blade along a line extending from the lower corner of the labial palps across the stomach to the posterior end of the body. This yielded a transverse section through the stomach, digestive diverticulum, intestine, connective tissue, demibranchs of both gills, and the mantle lobes; tissue samples were then fixed in Davidson's solution [15] for 48 hrs and preserved in 70% ethanol. Prior to sectioning the tissues were dehydrated in an ethanol series, embedded in paraffin wax, sectioned (7µm) and stained with haematoxylin and eosin. Histologically prepared slides of each species were examined for parasitic inclusion following the World Organization for Animal Health guidelines [9, 10]. Gonadal developmental stages were also studies in prepared slides. Gonadal developmental stages

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Table 1: Sampling Sites, Species, Sample Size and Date of Collection

Site	Species	N	Date of Collection
Hub	*Crassostrea glomerata*	10	8/3/2012
"	*Crassostrea madransis*	7	24/1/2012 & 14/2/2012
"	*Crassostrea gryphoides*	20	14/2/2012
Jiwani and Buleji	*Saccostrea cucullata*	20	2/2/2012 & 21/2/2012
Buleji	*Ostrea nomads*	7	25/2/2012

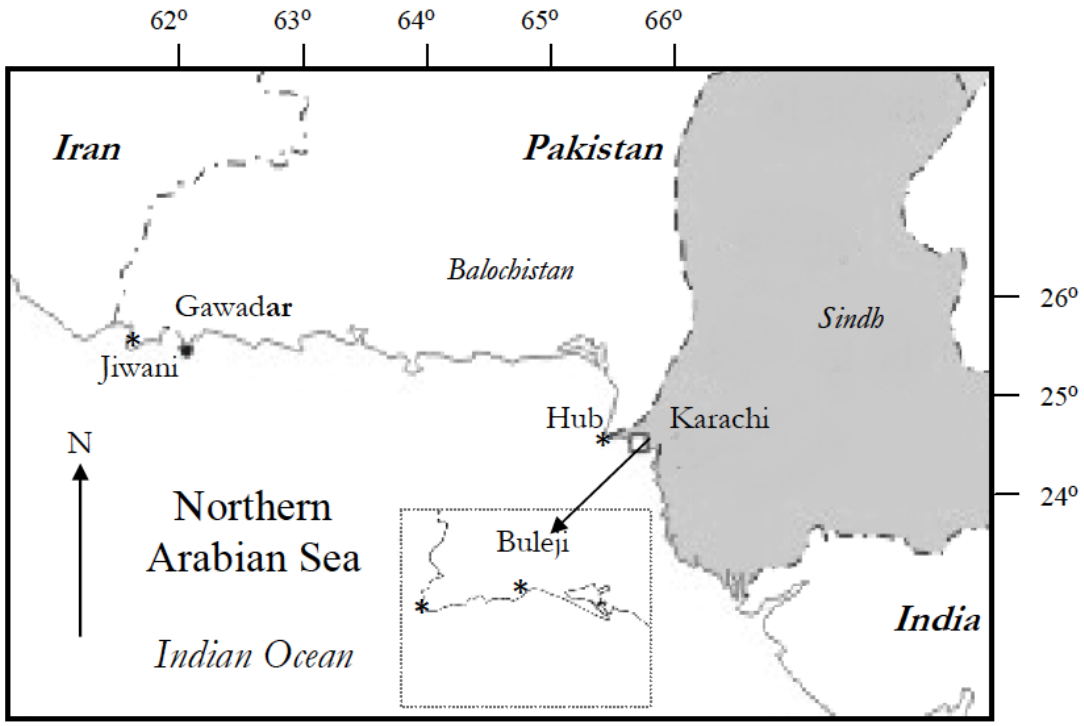


Figure 1: Map is showing collection sites*.

were determined by assigning the numeric ranks 0-4 for each gametogenic stage (0=spent, 1=early developing, 2=nearly ripe, 3=ripe, 4=spawning). Scoring scheme was used to assess gonadal condition of different oyster species at different sites to map gonadal variation among species.

RESULTS

Protozoan *Nematopsis* sp., *Proctoeces* sp. (Trematoda) and some unknown parasites were recorded in *O. nomades* and *S. cucullata* only at Buleji. Calculated biometrics have shown highest shell length (mm) 86.60±7.61 for *C.madransis* followed by 75.60±3.77 *C. gryphoides,* 55.29±0.79 *O. nomads,* 41.60±0.35 *C. glomerata* and collectively 41.05±2.35 for *S. cucullata* obtained from Buleji and Jiwani (Figure **2**). Meanwhile highest wet weight (g) 10.6±3.8 was calculated for *C. gryphoides* followed by 10.2±7.6 *C. madrasensis* and 5.3±2.3 for *S. cucullata* communally (Figure **2**).

Histological inspection showed specimens of *C. gryphoides* were set up to be almost in resting state. 95.0% individuals were found spent (0) besides one individual was observed with early developmental (1) stage. Whereas *C. madrasensis* was found with 42.9% spent (0) 14.3% early developing (1) and 42.9% in ripe (3) condition. *C. glomerata* showed 10.0% spent (0) 80% nearly ripe (2) and 10% ripe (3) gonads (Figure **2**).

In recent studies individuals of *O. nomades* from Buleji found in ripe (3) state with female gametes and developing (2) male follicles in hermaphroditic individuals. A total of six (6) hermaphroditic and one (1)

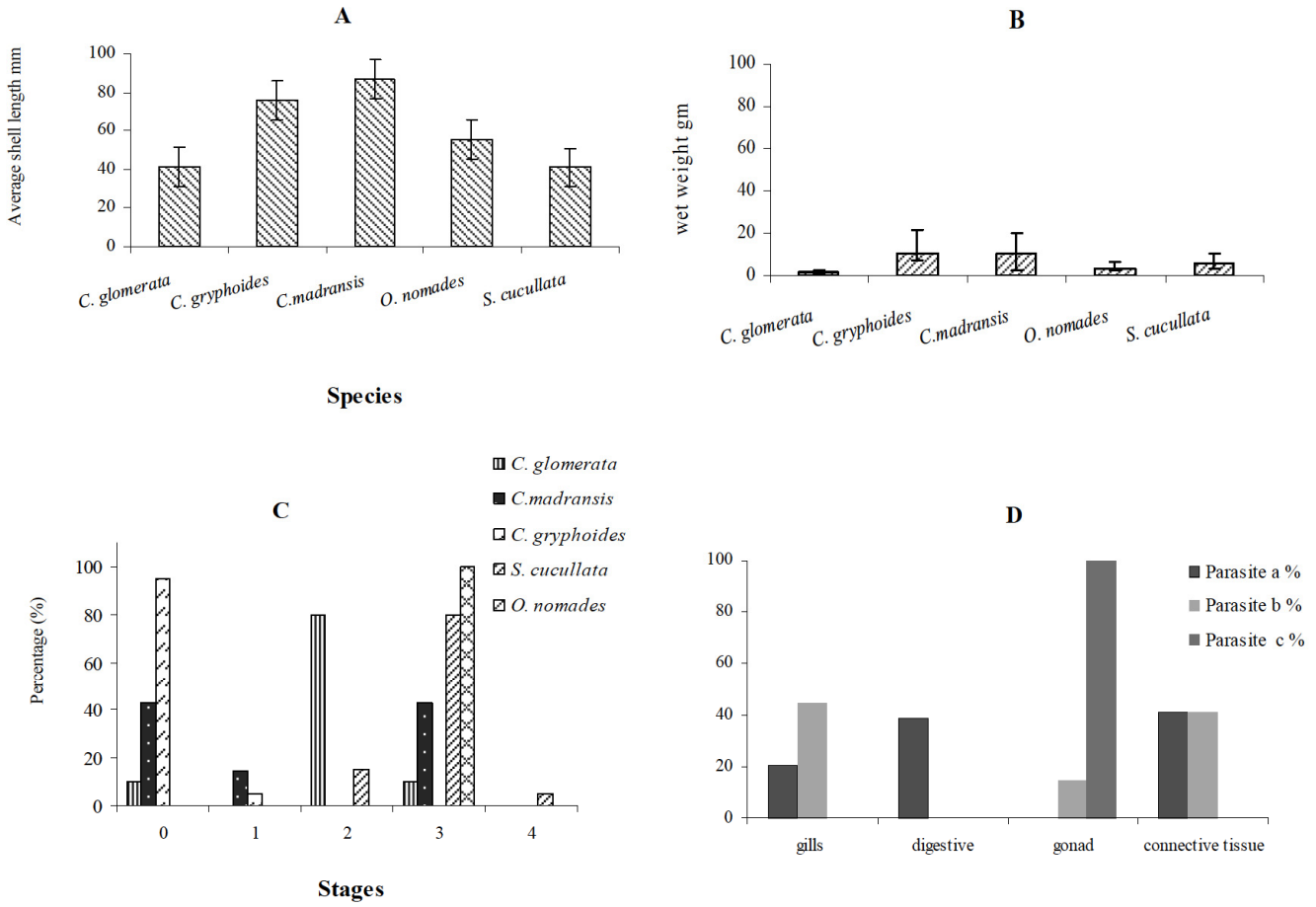


Figure 2: Showing average shell length (**A**); average wet tissue weight (**B**); gonadal developmental stages of oyster species (**C**) and parasitic load in different tissues (**D**). Parasite a-Nematopsis sp.; parasite b-Proctoeces sp. Trematode; parasite c-unknown.

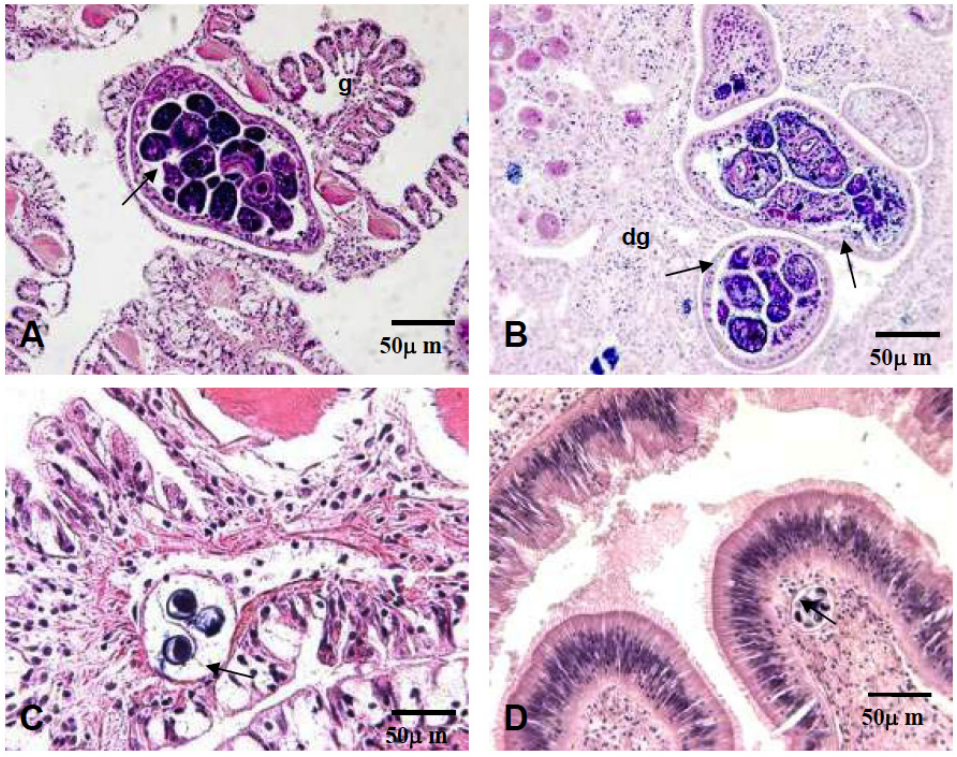
Stages:0=spent; 1=early developing; 2=nearly ripe; 3=ripe;1=spawning.

pure female were encountered during histological investigations. Whereas 15% individuals of *S. cucullata* found developing or nearly ripe (2), 80% ripe (3) and 5.0% spawning (4) observed.

Histological examination has revealed that both of the candidate species were in the verge of the sexually mature state at Buleji and some of these sexually mature individuals have been found with parasitic burden which shows that mature individuals in the wild population have more chances to acquire parasitic burden as during the present study protozoan *Nematopsis* sp., *Proctoeces* sp. *Trematode* parasite and one unknown parasitic cyst were observed in *O. nomades* and *S. cucullata* specimens obtained from Buleji. Seven examined slides of *O. nomades* (one female and six hermaphrodites) were found with protozoan *Nematopsis* species; among them one hermaphroditic individual was found heavily parasitized with trematode *Proctoeces* sp. in addition to *Nematopsis* sp. (Figure **3a, b**).

Highest prevalence of infestation were recorded in *O. nomades* at Buleji where *Nematopsis* sp. and *Proctoeces* sp. were found respectively in 100% and 14.8% of individuals, respectively. Furthermore 8% of *S. cucullata* at Buleji were infested with an unknown parasite. Parasitic loads differed between different tissues; *Nematopsis* sp. was found in the gills, digestive and connective tissues in 20.4%, 38.8% and 40.8% respectively of individuals from Buleji. The parasitic load of *Proctoeces* sp. was 44.3% in gills, 14.8% in gonadal portion and 41.0% in connective tissues from the same site (Figure **2**).

A total of thirteen (13) specimens of *S. cucullata* examined for parasitic inclusion from Buleiji and seven (7) individuals from Jiwani were free of any parasitic infection, except for one male specimen that was infected with an unknown parasite, was procured from Buleji. A spherical thraustochytrid-like cyst with honeycomb cellular structures and endospores was observed at the outer periphery of gonadal tissue



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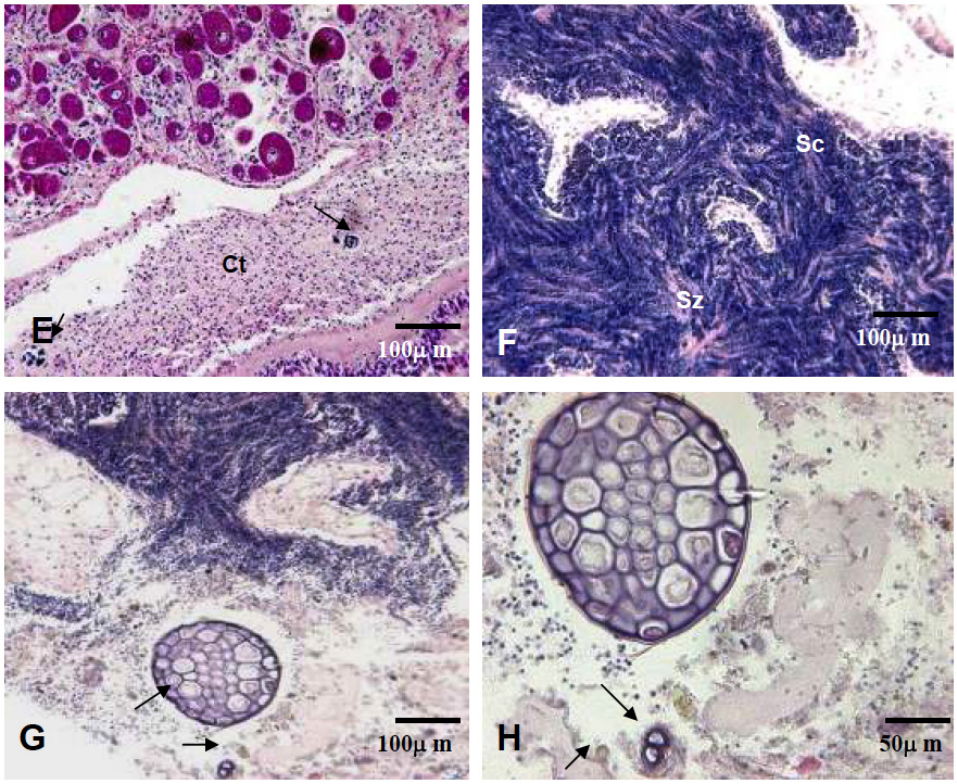

b

Figure 3: a. Histological sections of *Ostrea nomades*: *A-B* showing sporocysts of *Proctoeces* sp. in the gill filament and the female gonad (mantle), respectively; *C-D* showing Nematopsis sp. in connective tissue and lumen of digestive diverticulum. Infection (black arrows) in gills, gonad, connective tissue and lumen tissues.

b. Photomicrograph showing infectious gonadal tissue of *O. nomades* (*E*) and *Saccostrea cucullata* (F-H) male tissue. Arrow points to the *Nematopsis sp.* (*E*) and an unknown (thraustochytrid-like) parasitic cyst (*G*, *H*). Ct=connective tissue; Sc=spermatocytes; Sz=spermatozoa.

(Figure **3b**). In specimens of *C. gryphoides, C. madransis* and *C. glomerata* there was no parasitic inclusion in any specimen examined.

DISCUSSION

Earlier researchers [14] documented the maximum 241-280 mm size for *C. gryphoides* at the same site (Hub Delta), the largest size among edible oysters of the world during past decades,. whereas same time the populations of *C. gryphoides* and *C. madrasensis* were mostly between 41-200 mm size ranges at Hub Delta. Additionally, larger sizes (40-70 mm) for *C. glomerata* from Karachi, 32-82 mm for *S. cucullata* from Paradise Point and 26-97 mm for *O. nomads* from Buleji and Keamari Sea wall have been recorded formerly [16]. Recent studies have shown the considerable size decrease in existing wild populations when compared to previous data. Although oyster populations at Hub have been considerably depleted during the last decade, any contributing reason behind this is not known so far, but a possible reason which has never been thoroughly investigated in past was the presence of a nuclear power plant at Hub. Nevertheless these species could be useful potential resource for commercial exploitation being acquiring considerable wet tissue mass. Wet tissue weight data for respective species is not available in previous records. However onset of gametogenesis in *C. gryphoides* in intermediate and large size classes was the same [14]. Further it was indicated that the percentage of females remains lower in the colder months i. e. from November to January and also stated that in winter months more females remain indifferent and undergo resorption. Siddiqui and Ahmed [11] examined the larviparous oyster *O. nomads* from Buleji and Keamari area and histologically individuals were found to be hermaphroditic, pure male, pure female and indifferent at two sites throughout the year with increasing activity in the winter months followed by immediate ripening and later spawning and resorption around the year.

There is no previous record available pertaining report of trematode parasitic inclusion in Pakistani oyster species. Earlier workers observed 48% to 92% protozoan parasite *Nematopsis* sp. prevalence with an average of 67% in *O. nomads* [5]. Moreover parasitophorous vacuoles containing oocysts were investigated in the digestive gland, palps, mantle, gonads, gills and kidney of the oysters. According to previous report [5] inclusion of *Nematopsis* sp. in *O. nomades* and *C. belcheri* was profound from Keamari Sea Wall constructed near Karachi Port. The presence of *Nematopsis* sp. in Buleji population could be due to the fact that Buleji rocky ledge is rich in fauna and flora and number of arthropod species including brachyuran crabs are quite abundant in the area [17]. Oysters are the intermediate hosts of many species of *Nematopsis*, and life cycle of these parasites is completed in intestine of marine arthropods, moreover species of Xanthidae, Portunidae, Grapsidae, Ocypodidae have been designated by authors which could be the possible final host for pathogenic prevalence [5, 6, 17-19]. Similarly several species of marine brachyuran crabs from Manora Island have been reported [17], which lies adjacent to Keamari sea wall and Buleji. Xanthid crab *Leptodious exaratus* found to be principal species at both sites. *Thalamita prymna, Charybdis species, Eriphia laevimana, Pilumnus vespertilio, Pseudozius caystrus, Grapsus strigosus* and *Atergatis roseus* were also turned out dominant species at both of the sites. These crab species possibly serve as final host for pathogenic infestation.

Identification of observed parasites to species level was not attempted. *Nematopsis* sp. oocysts and metacercaria of the trematode, *Proctoeces* sp. were observed in gills, digestive and connective tissues of *O. nomads* from Buleji. *Nematopsis* has been reported in many bivalve and gastropod mollusks [6, 20, 21]. Boehs *et al.* [6] stated that infestation by sporocysts, which are structures of digenetic trematodes could be a threat for host bivalve because of its potential to reduce host fecundity.

Digenean trematodes have complex life cycles in which bivalve mollusks are intermediate hosts harboring larval stages of the digenean. Some finfish species are known ultimate hosts for bucephalid digenean trematodes [22]. By contrast, Couch [23] observed the un-encysted metacercariae of *Proctoeces* sp. in the gonadal follicles and ducts of oysters from three Gulf of Mexico estuaries and suggested that the presence of this trematode to its oyster host is in fact not harmful. In the present study metacercariae and mature adults of *Proctoeces* sp. were observed in gills, digestive and connective tissues of gonadal sections in *O. nomades*. Trematode metacercariae usually cause no apparent harm to host bivalve molluscs [24-26], but infection with *Proctoeces* sp. may cause serious lesions and inhibit gametogenesis [27].

CONCLUSION

During the present study protozoan *Nematopsis* sp., trematode *Proctoeces* sp. and an unknown (thraustochytrid-like) parasites have been recorded in

O. nomades and *S. cucullata* at Buleji of Sindh coast. On the contrary, Balochistan coast, Pakistan was found to be free of pathogenic infestation during the present oyster watch. There is no previous record of trematode parasites in Pakistani oyster species in literature. In this paucity it is recommended that investigative endeavor ought to be expanded in sustainable deployment perspective.

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