First record of protozoan parasites, *Tetrahymena rostrata* and *Callimastix equi* from the edible oyster in Sundarbans region of West Bengal, India

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Abstract Several protozoan parasites have been found infecting the edible oysters, hence deteriorating the meat quality. Protozoan parasites such as, *Tetrahymena rostrata* and *Callimastix equi* infested the edible oyster in Sundarbans region, West Bengal, India, are first record from this region. Due to filter feeding habit of the organisms, oysters provides excellent ecological services in regard to efficient cleaning of infectious agents from surrounding water as a potential measure to improve water quality. However, these environmental benefits are associated with public health risks from contaminated oysters intended for human consumption.

Keywords *Tetrahymena rostrata* · *Callimastix equi* · Protozoan parasite · Edible oyster · West Bengal

Introduction

Sundarbans region of West Bengal coast is highly diverse and ecologically important as it supports large number of protozoans, annelids, molluscs, crabs and shrimps, fin fishes, reptiles and mammals. These habitats play an important role in the socio-economical interest of the coastal people as they can provide jobs in rural areas in agriculture, forestry, fisheries and the tourism industry. The edible oysters are capable of tolerating a wide range of salinity and heavy metal pollution (Biswas et al. 2013). They contribute a great tonnage of animal protein in the food market (Korringa 1976). Its tender flesh forms a

cheaper, nutritious and easily digestible food source. The oysters are known to show large variations in their meat quality depending on their physiological conditions and associated environmental factors (Durve 1964). Several protozoan parasites have been found infecting the edible oyster, hence deteriorating the meat quality. Parasites have evolved numerous ways to adapt to the dramatic changes in environment experienced through out their life cycle. According to Kinne (1983), the main biologic agents causing diseases in marine bivalve molluscs involve viruses, bacteria, fungi, protists, digenean trematodes, polychaetes and copepods. But the present study concentrated on the identification of protozoan parasites infecting edible oyster such as *Crassostrea gryphoides* and *Saccostrea cucullata* in Sundarbans region, West Bengal, India.

Materials and methods

A systematic monitoring practice was undertaken for a period of 3 years during 2009–2012. Samples of oyster were collected randomly during low tide from the two selected study sites namely, Frasergunj and Kaikhali of Sundarbans region during the study period. Stainless steel hammer and rod were used to separate oysters from their surrounding cliffs. Both *C. gryphoides* and *S. cucullata* were collected from Frasergunj, *C. gryphoides* only were collected from Kaikhali.

In the laboratory, the shell of the oyster were opened with a fine knife. One of the first stages of examination was to observe the size and colour of the organs such as the adductor muscle, digestive gland, gill, mantle, labial palps, contents of stomach and intestines minutely. The smear materials were scrapped out from adductor muscle, digestive gland, gill, mantle, labial palps and other visceral

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tissues from oysters and placed on glass slides in 0.6 % saline and observed under the microscope for examination of parasites. Thin and uniform smears were drawn on slide without allowing them to dry. Semidried smears containing the protozoans were stained with Giemsa.

Measurements of the parasites were taken with the aid of a calibrated ocular micrometer. All measurements are presented in μm . Drawings were made on stained material with the aid of a mirror type camera lucida. Photographs were taken with Olympus phase contrast microscope fitted with Olympus digital camera.

Results and discussion

Molluscan shellfish such as oysters, mussels, cockles and clams can concentrate pathogenic microorganisms as a result of filtering large volumes of water (Trollope 1984). Incidences of various infectious protozoan parasites have been observed and identified by their characteristic features from edible oyster during the course of study. Six types of protozoan parasites such as *Cristigera crassostrae*, *C. susmai*, *Tetrahymena rostrata*, *Cryptosporidium* sp., *Peneroplis* sp. and *Callimastix equi* infection (Biswas and Bandyopadhyay 2013a, b; Biswas et al. 2011) were discovered from which two types of protozoan parasites *T. rostrata* and *C. equi* have been recorded for the first time from *C. gryphoides* and *S. cucullata* from Kaikhali and Frasergunj of South 24 Parganas of West Bengal, India.

Tetrahymena rostrata

Site of infection

Tetrahymena rostrata are isolated from the mantle, gill and labial palp of *C. gryphoides* and *S. cucullata*.

Symptoms

Infections of *T. rostrata* are accompanied by yellow discoloration and extensive lesions in the gills and mantle. Gills of infested oysters are covered with cysts of different sizes and colours. A brown scar often occurs on the shell, adjacent to abscess on the mantle surface. Heavy lesions appear as a swollen mantle edge or nodules on the mantle though these lesions are not unique to *T. rostrata* infection.

Systematic position

Kingdm	Animalia
Phylum	Ciliophora
Class	Ciliata

Subclass	Rhabdophorina
Order	Hymenostomatida
Suborder	Tetrahymenina
Family	Tetrahymenidae
Genus	<i>Tetrahymena</i>
Species	<i>rostrata</i>

Morphology

Relatively slender body, narrowing anteriorly with slightly curved pointed end. Body contains food vacuoles, terminal contractile vacuole, spherical macronucleus placed more or less centrally and ovoid micronucleus (Fig. 1a, b). Measurements are given in Table 1.

Callimastix equi

Site of infection

Callimastix equi are isolated from the mantle and labial palp of the oysters *C. gryphoides* and *S. cucullata*.

Symptoms

The gross signs of include the visceral tissues losing their pigmentation and become pale yellow. In some cases the mantle becomes translucent and shell growth may cease. The mantle and labial palps become brownish to blackish in colour that can lead to ulcerations in such areas.

Systematic position

Kingdom	Animalia
Phylum	Plasmodroma/protozoa
Class	Mastigophora
Subclass	Zoomastigina
Order	Polymastigina
Suborder	Monomonadina
Family	Callimastigidae
Genus	<i>Callimastix</i>

Species *equi*

Morphology

Callimastix equi is morphologically distinct from the other flagellate protozoa, with characteristic movements. It is ovoid with compact nucleus placed at central or anterior of the body. A tuft of long flagella are present near anterior

Fig. 1 **a** Phase contrast microscopic photograph of *Tetrahymena rostrata* **b** Camera lucida drawing of *Tetrahymena rostrata* **c** Phase contrast microscopic photograph of *Callimastix equi* **d** Camera lucida drawing of *Callimastix equi*

Table 1 Morphometric parameters of *Tetrahymena rostrata* found from edible oyster

Parameters	Normal range (μm)	Mean ± SD (μm)	CV %	SE
Length of the body	39.78–26.52	33.677 ± 4.223	12.541	1.336
Width of the body	19.89–8.16	12.376 ± 5.237	42.317	1.656
Length of the macronucleus	6.63–4.42	5.372 ± 1.021	18.999	0.323
Width of the macronucleus	6.63–2.21	5.049 ± 1.372	27.171	0.434
Diameter of the micronucleus	4.80–2.21	3.429 ± 1.292	37.691	0.409
Distance of the macronucleus from the anterior end	13.26–7.14	10.540 ± 2.742	26.018	0.867

end and vibrate in unison (Fig. 1c, d). Body measurements are given in Table 2.

In Protozoa, comparatively highly complicated organisms are represented by numerous ciliates which are a diverse and successful group of protozoan parasites. *Ciliates*, *T. rostrata* have been isolated and identified during present study. The parasite was first described as *Paraglaucoma rostrata* by Kahl (1926). Corliss (1952) transferred this species to the genus *Tetrahymena* as *T. rostrata* on the basis of its capacity to form both reproductive and resting cysts, to exhibit edaphic and parasitic forms and to possess histophagous or parasitic habits, Corliss (1952) established it as the ‘type’ of the rostrata complex. Hauschka and Doll (1944) observed *Paraglaucoma* present in

the gastro-vascular cavities of *Hydra americana* and its complete immunity to *Hydra* digestive enzymes strongly suggested its facultative parasitism. In culture, the form of *T. rostrata* from *Deroceras reticulatum* appears to be morphologically identical with a strain (NZ-4) recovered from soil of New Zealand (Kozloff 1957). Brooks (1968) demonstrated that *T. rostrata* enters into the slug host through dorsal integumentary pouch.

Tetrahymena (*Paraglaucoma*) *rostrata* (Kahl 1926; Corliss 1952) occurs commonly in litter and occasionally in soil. It has previously been recorded from moss. In nature it feeds on cytolyzed or moribund tissue but in presence of peptone it feeds on bacteria and flagellates. The ciliate is an obligate histophage and a facultative parasite

Table 2 Morphometric parameters of *Callimastix equi* found from edible oyster

Parameters	Normal range (μm)	Mean ± SD (μm)	CV %	SE
Length of the body	10.20–8.16	8.874 ± 0.840	9.463	0.266
Width of the body	6.12–4.08	4.794 ± 0.840	17.516	0.266
Diameter of the nucleus	2.04–1.02	1.224 ± 0.430	35.136	0.136
Length of flagella	8.16–6.12	6.732 ± 0.713	10.594	0.226

of enchytraeid worms which it infects through degenerate setal follicles (Stout 1954). Ciliate infections in certain other invertebrates are also characterized by rapid pathogenicity (Stout 1954). It may also infect accidentally injured worms. From the point of view of food, it depends on the turnover of microbial life and the frequent incidence of dead worms, rotifers, nematodes, tardigrades or even dead ciliates, as much as the incidence of living enchytraeids which in nature are probably only occasionally parasitized (Stout 1958). The ciliates are attracted by histolysis and by peptone. Resistant cysts are formed regularly in absence of food and encystment appears to be accelerated by crowding. Excystment is readily obtained with hypotonic salt solution. Theront, trophont, tomont and tomita stages are recognizable in the life cycle (Stout 1954).

Callimastix cyclopis, was originally described as a protozoan a polyflagellate parasite of Cyclops (Weissenberg 1912). This organism had also been associated with Blastocladales (Weissenberg 1950; Vavra and Joyon 1966). Whisler et al. (1972) demonstrated that *Callimastix*-like organisms were an alternate stage of the mosquito parasite *Coelomomyces psorophorae* Couch, a member of the Blastocladales. Subsequently, Vavra and Joyon (1966) concluded that *C. cyclopis* was likely a fungus related to the chytrids (Phylum Chytridiomycota). Earle et al. (1950) found rumen protozoa, *Callimastix frontalis* in Florida dairy cattle.

C. equi was reported from the large intestine of the horse (Hsiung 1929). The length of this flagellate ranged from 12 to 18 μ, (mean 14.4 μ), width from 7 to 10 μ, (mean 8.16 μ), length of the flagella from 25 to 30 μ. Its nucleus was centrally located and had a large karyosome.

During the present study, heterogeneity in *T. rostrata* and *C. equi* has been observed as they are present in the edible oyster, *C. gryphoides* and *S. cucullata* collected from Kaikhali and Frasergunj of South 24 Parganas. Marine organisms serve as hosts for a diversity of parasites and pathogens. Dogiel (1964) suggested that ‘more or less a definitive number of species is characteristic of different animal host groups differing perhaps from one part of their distribution range to another’ in explaining patterns and processes influencing the number of parasite species a host can support. It is especially undesirable that any species

should suffer from the regions of diseases. Parasites that alter the phenotype of their host can have several indirect effects on the whole community. When a host species constitutes an important component of habitat structure, manipulative parasites altering the characteristics of host populations could have a variety of indirect effects on other species. However, ecological consequences of phenotypic alterations induced by parasites remain largely unexplored. Humans can be intermediate, paratenic or accidental hosts. While the transmissive stages of some of these zoonoses can be transmitted directly or through contaminated water and foods, parasitic diseases can pose major threats to animal populations and have serious economic impacts on host health, therefore, research into host parasitology ecology is essential and critical (Cleaveland et al. 2002; Galvani 2003). Diseases of bivalve molluscs are noteworthy not only for their impact on population dynamics, especially of key commercial species, but also for characteristics that separate them from typical diseases affecting mammals and fish. Most described bivalve diseases are of protozoan origin (Bower et al. 1994) and transmission is commonly via the water column since most adult hosts are immobile. Transmission occurs over large distances and can be rapid under favorable climatic conditions, resulting in rapid parasite proliferation and elevated adult mortalities (Andrews and Wood 1967; Lafferty and Kuris 1993; Bureson and Ragone-Calvo 1996; Ford and Tripp 1996). Throughout the region in which the disease organism can exist, most hosts are exposed to the disease organism and probably become infected, even though infections may be undetected (Bushek et al. 1994; Stokes et al. 1995; Culloty et al. 2003). The spread and intensification of infections can result in epizootics, which are disease epidemics in nonhuman animal populations. Parasite-induced alterations in host phenotype have been frequently reported in a wide range of protozoan and metazoan parasites with complex life cycles (Combes 1991; Poulin 1998). Unfortunately, the aetiology of diseases is poorly understood and warrants a deeper study.

Although most of the organisms living in intertidal habitats are hosts for parasites (Thomas et al. 1997), ecologists in general paid little attention to the possible influence of parasites in structuring intertidal communities. The study of parasites and diseases affecting molluscs with

economic interest is important both for the management of natural stock and for aquaculture. It could even be helpful for the sanitary evaluation for human consumption. However the knowledge on how host and parasites interact in natural systems remains limited and consequently, international bodies like the World Health Organization have advocated an increased study of host-parasite ecology in wild host populations (Real 1996; Schall and Pearson 2000).

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