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# *Nematopsis* sp. (Apicomplexa: Porosporidae) infection in *Crassostrea madrasensis* and its associated histopathology

**G. Suja*, V. Kripa, K. S. Mohamed, P. Shamal and N. K. Sanil**
ICAR - Central Marine Fisheries Research Institute, PB No. 1603, Cochin - 682018, Kerala, India.

*Correspondence e-mail: suj1078@gmail.com

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Original Article

## Abstract

The present study forms the first report of *Nematopsis* sp. infection in the edible oyster, *Crassostrea madrasensis* from India. The study was carried out as part of a detailed pathological investigation of *C. madrasensis* along the southwest coast of India. Sporozoites of *Nematopsis* sp. were found in samples collected from two locations. Light microscopic observation revealed ellipsoidal oocysts measuring 16.63±2.40 $\mu$m in length and 11.11± 2.49 $\mu$m in width (n=30) in the connective tissues of gills, mantle, visceral mass and gonads. Prevalence of infection ranged from 11 to 27%. Apparent pathological changes included compression of adjacent digestive diverticulae in visceral connective tissue infections and presence of phagocytosed oocysts in water channels in the case of gill infections. With relevance to the expanding culture of *C. madrasensis*, monitoring potential pathogens of this species in its natural habitat is important for developing suitable health management packages.

***Keywords***: *Crassostrea madrasensis, Nematopsis sp.,* histopathology, Sporozoites.

## Introduction

*Crassostrea madrasensis*, the Indian backwater oyster is the most dominant oyster species, inhabiting the estuaries, bays and backwaters along the south-east and south-west coasts of India. *C. madrasensis* commands good market demand and has evolved to the status of a candidate bivalve species for culture following its successful hatchery breeding and seed production. Presently the species contributes to about 4000 tonnes to the fast growing bivalve farming sector in India. The rapidly expanding edible oyster farming and its management points to the importance of information on the parasites/pathogens of this species which is presently very limited (Samuel, 1978; Bijukumar, 2001; Sanil *et al.*, 2012; Suja *et al.*, 2014).

More than 30 species of *Nematopsis* have been reported so far from different geographic regions. *N. ostrearum* and *N. prytherchi* were reported from oyster growing areas along the Atlantic and Gulf coasts of USA (Winstead *et al.*, 2004; Aguirre-Macedo *et al.*, 2007) while *N. mytella* was reported from *Mytella falcate, Mytella guyanensis* and *Crassostrea rizophorae* from Brazil (Azevedo and Matos 1999; Padovan *et al.*, 2003). *Nematopsis* spp. was also reported in *Perna canaliculus* from New Zealand (Jones, 1975), *Cerastoderma edule* and *Ruditapes decussates* from

Portugal (Azevedo and Cachola, 1992), *Arcuatula arcuatula, Anadara granosa, Perna viridis* and *Paphia undulata* from Thailand (Tuntiwaranuruk *et al*., 2004) and *C. rhizophorae* from Brazil (Sabry *et al*., 2007). Though in most cases *Nematopsis* infections are not pathogenic enough to lead to mortalities, cockle mortalities associated with *Nematopsis* infections have been reported from Portugal (Azevedo and Cachola, 1992; Azevedo and Matos, 1999). Available reports on *Nematopsis* from India are limited to taxonomic identification of seven new species from various arthropods and no information exists on the occurrence and pathology of *Nematopsis* in bivalve hosts from India. Previous reports on *Nematopsis* infections from bivalves have been provided in Table 1.

Table 1. *Nematopsis* infections reported from bivalves

| Bivalve species | *Nematopsis* species reported | Location | Reference |
|---|---|---|---|
| *Crassostrea virginica* | *N. prytherchi* | Gulf of Mexico | Aguirre-Macedo *et al*., 2007 |
| *Crassostrea virginica* | *N. ostrearum* and *N. prytherchi* | Florida,USA | Winstead *et al*., 2004; |
| *Crassostrea corteziensis* | *Nematopsis* sp. | Pacificcoast of Mexico | Martinez *et al.,* 2010. |
| *Crassostrea rizophorae* | *Nematopsis* sp. | E coast of Brazil | Sabry *et al.,* 2007 |
| *Crassostrea iredalei* | *Nematopsis* sp. | Philippines | Pagador, 2010 |
| *Perna canaliculus* | *Nematopsis* sp. | New Zealand | Jones, 1975 |
| *Modiolus barbatus* | *Nematopsis* sp. | Croatia | Mladineo, I. 2008 |
| *Mytella falcate* and *Crassostrea rizophorae* | *N. mytella* | NE coast of Brazil | Padovan *et al*.,2003 |
| *Ruditapes decussatus, R. philippinarm, R. pullastra,R. rhomboideus,Venus verrucosa, Solen vagina, Mytilus galloprovincialis, Donax vittatus* | *Nematopsis* sp. | Ria De Vigo (Galicia, Spain). | Soto *et al.,* 1996 |
| *Cerastoderma edule* and *Ruditapes decussates* | *Nematopsis* sp. | Portugal | Azevedo and Cachola, 1992 |

Unlike most gregarine parasites, *Porospora* and *Nematopsis* exhibit host alteration between decapod crustaceans and marine pelycepods. Gymnospores released from crustaceans upon entering the gills and mantle of susceptible bivalves are engulfed by host phagocytes where they develop to naked sporozoites (*Porospora*) or monozoic oocyst having thick hyaline wall enclosing a vermiform sporozoite (*Nematopsis*). No multiplicative stage occurs in bivalves. Life-cycle is completed when these naked sporozoites or oocysts are ingested by decapod definitive hosts (Soto *et al*., 1996; Estevez *et al*., 1998). Oocyst stages of different species of *Nematopsis* usually elicit little or no host response in bivalve tissues. There exists conflicting reports on the pathology induced by *Nematopsis* and other gregarines (Canestri-Trotti *et al*., 2000; Estevez *et al*., 1998; Azevedo and Cachola, 1992). Increasing commercial importance of *C. madrasensis* warrants a detailed study on the pathogenicity of potential pathogens existing in its natural habitats. Thus, the present study attempts to understand the histopathology of *Nematopsis* sp., its prevalence and intensity in *C. madrasensis* from two different locations along the south west coast of India.

## Material and methods

### *Sampling and area of study*

Samples of *C. madrasensis* were collected during dry and rainy seasons from the oyster beds at Dalavapuram (Kollam) in 2011 and Sathar Island (Kochi) in 2012 along the south west coast of India. Details of the study area are provided in Table 2. Samples were brought to the laboratory and maintained until dissection.

Table 2. Site characteristics of study area.

| Location | Coast | Co-ordinates | Bottom character | Nature |
|---|---|---|---|---|
| Dalavapuram | south west | 8°56′ N 76°33′ E | muddy, silty | Estuary |
| Sathar Island | south west | 10°11′ N 76°11′ E | muddy, silty | Estuary |

### *Histopathology*

For histopathology, a transverse section of 5 mm (including gills, mantle and visceral mass) was made and fixed in Davidson's fixative (Shaw and Battle, 1957) for 24-48 hours and processed following standard histological procedures. Sections (5-6 $\mu$m thickness) were cut using a Leica Microtome (Leica, Wetzlar, Germany), stained with Harris Hematoxylin and Eosin (H&E) and photographs were taken using a Nikon Eclipse 80 i microscope.

Prevalence was calculated using the formula (Kim *et al*., 2006),

$$\text{Prevalence} = \frac{\text{Number of hosts with parasite or pathogen}}{\text{Number of hosts analyzed}}$$

Infection intensity was calculated using the formula (Kim *et al.*, 2006),

$$\text{Infection intensity} = \frac{\text{total number of occurrences of parasite or pathogen}}{\text{Number of hosts with parasite or pathogen}}$$

## Results

Histological examination of oyster samples collected from Kochi and Kollam revealed intra-haemocytic, ellipsoidal, monozoic oocysts with a thick hyaline wall, characteristic to *Nematopsis* sp. Oocyst (s) were observed within the parasitophorous vacuole inside the phagocyte, either in isolation or aggregated in cluster with more than two oocysts per hemocyte. Each oocyst contained a single, vermiform, sporozoite and measured 16.63±2.40 $\mu$m (range11.68- 20.01$\mu$m) in length and 11.11± 2.49 $\mu$m (range 7.39- 14.39 $\mu$m) in width (n=30). Oocysts were observed in the connective tissue of gills, mantle, visceral mass and gonads (Fig.1 A - D). Infection intensity was low and was limited to a maximum of eight oocysts per field. Hypertrophy of the infected cell was the most evident cellular pathology observed, and cell size increased progressively with oocysts numbers and reached a maximum of 96 $\mu$m in a single hemocyte with several oocysts (Fig.1A). Numerous oocysts were observed in infected digestive gland tissues and the pathological changes were limited to compressed digestive diverticulae. Compression of adjoining cells was also observed in the case of visceral/ connective tissue infections (Fig. 1 B). In the case of gill infections, phagocytes with oocysts were observed lodged in the water channels in the gill filaments (Fig.1 D). In all the above cases, specific host immune responses were totally absent in the infected tissues. Prevalence of *Nematopsis* infection in *C. madrasensis* at Kochi ranged from 11-21% while that at Kollam ranged from 15-25%. The intensity of infection along with morphometric details are provided in Table 3.

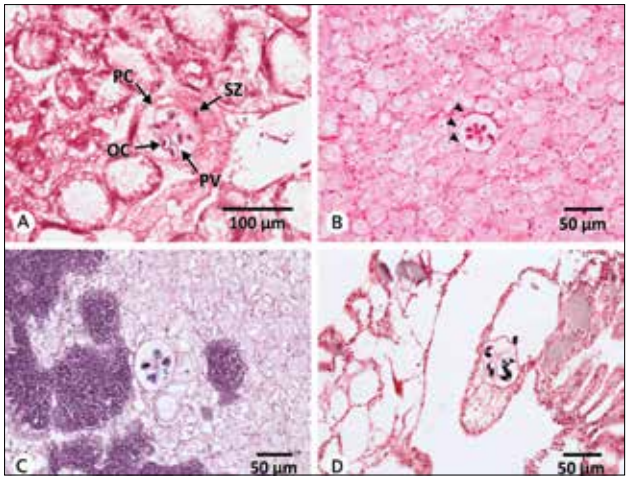


Fig.1. Histopathology of *Nematopsis* sp. infecting *C. madrasensis* (H&E stained). A. Phagocyte (PC) with *Nematopsis* sp. oocysts (OC) located in host connective tissue between digestive tubules. Oocysts are enclosed in parasitophorous vacuoles (PV), vermiform sporozoite (SZ) are seen inside the oocyst. B. Phagocyte within connective tissue of mantle; arrowheads indicate compression of cells. C. oocysts in male gonadal tissue D. An infected phagocyte within the gill lumen.

## Discussion

The present study forms the first report of *Nematopsis* sp. infection in *C. madrasensis.* Species-level identification of *Nematopsis* was not possible as the other life-cycle stages like trophozoites and sporadins occurring in decapod definitive host were not obtained.

A perusal of literature shows that different opinions exist on the prevalence and pathogenicity of *Nematopsis* sp. infections and related immune responses in bivalves. Usually, infected bivalves exhibit no host reaction and immune responses if any, are limited to focal, benign inflammation without any significant pathological changes (Sinderman, 1990; Bower *et al.*, 1994; Boehs *et al.*, 2010; Darriba *et al.*, 2010). However, Estevez *et al.* (1998) observed hypertrophy of infected cells and different degrees of tissue damage associated with high intensity *Nematopsis* infections leading to mechanical interference in host's physiological activities like food intake and gaseous exchange in bivalves from Spain. Similarly, Mladineo (2008) also observed tissue disruption, connective tissue cell hypertrophy with eccentrically displaced nuclei and light hemocytic infiltration in *Modiolus barbatus*. Azevedo and Cachola (1992) observed complete destruction of *Nematopsis* infected gill tissues in the cockle

Table 3. Number (N) and biometric details of host analysed per sample, Prevalence (P) and intensity (I) values of *Nematopsis* sp. infection in *C. madrasensis* collected during dry and wet seasons from two locations.

| location | Season | N | Mean shell L(mm)±SD | Mean tissue wet wt(g) ±SD | Mean CI±SD | P(%) | I |
|---|---|---|---|---|---|---|---|
| Kochi | Dry | 18 | 73±10.4 | 4.53±2.04 | 9.3±2.5 | 11 | 1.50 |
| | Wet | 28 | 79.9±7.2 | 5.36±1.20 | 10.5±2.8 | 21 | 2.00 |
| Kollam | Dry | 20 | 80.6±13.30 | 4.12±2.70 | 6.8±2.9 | 25 | 4.60 |
| | Wet | 20 | 64.7±6.6 | 4.97±1.54 | 16.4±3.6 | 15 | 2.30 |

*Cerastoderma edule* from Portugal and associated the mortalities with *Nematopsis* infection. Tutiwaranuruk *et al*., 2004 also observed heavy infections in gills of *Perna viridis* which resulted in complete occlusion of gill lumina with oocysts. In the present study, encysted *Nematopsis* sp. sporozoites were observed within the phagocytes of infected host tissues. Pathological indications observed in the present study are in agreement with the observations reported by Estevez *et al.* (1998), Tutiwaranuruk *et al.* (2004), Mladineo (2008) and Martinez *et al.* (2010). Condition index values of the infected samples exhibited no significant deviation from the normal values which indicates that at the present level of infection intensity, the physiology of the host is not compromised.

Further, in the present study, the intensity of infection and prevalence was low and apparent immune responses were totally absent and these observations are in accordance with the previous reports on *Nematopsis* sp. infections in oysters (Boehs *et al*., 2010; Darriba *et al*., 2010). Martinez *et al.* (2010) have also reported *Nematopsis* infections occurring in the pleasure oyster, with compression of adjacent cells and disruption of the connective tissue in heavy infections but with little immune response or pathology. It has also been reported that defense mechanisms of oyster can gradually eliminate *Nematopsis* form its tissues (Bower *et al*., 1994; Aguirre-Macedo *et al*., 2007).

Azevedo and Cachola (1992) observed very high prevalence (82%) of *Nematopsis* in cockle causing complete destruction of infected gill cells, while the clam, *Ruditapes decussatus* collected from same region and period showed very low prevalence (8%). Studies by Soto *et al.* (1996) also showed similar results with prevalence varying from 14-100% in different bivalve species from Spain. Thus it seems that there exists strong host specificity for *Nematopsis* and the associated pathology may vary with host type and location. Prevalence of *Nematopsis* infection in bivalves from any location depends on the abundance of definitive host in the habitat (Darriba *et al*., 2010; Boehs *et al*., 2010). Though various species of decapod crustaceans, the natural definitive hosts for *Nematopsis* sp. are present in the study area, the low prevalence and intensity recorded in the present study can be attributed to the lack of infected definitive hosts.

In view of the increasing culture potential of *C. madrasensis*, monitoring potential pathogens/parasites of this species in its natural habitat is important. The present study provides information on the histopathology and prevalence of *Nematopsis* sp. in *C. madrasensis*. Though low prevalence and intensity of infections with *Nematopsis* sp. may not affect the general health and physiology of the oysters, heavy infections may reduce the productivity in culture systems.

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## References

Aguirre-Macedo, M. L., R. A. Sima Alarez, M. K. Roman- Magana and J. I. Guemez-Ricalde. 2007. Parasite survey of the eastern oyster *Crassostrea virginica* in coastal lagoons of the southern Gulf of Mexico. *J. Aquat. Anim. Health,* 19:270–279.

Azevedo, C. and R. Cachola. 1992. Fine structure of the Apicomplexa oocyst of *Nematopsis* sp. of two marine bivalve mollusks. Dis. *Aquat. Org.,* 14: 69-73.

Azevedo, C. and E. Matos. 1999. Description of *Nematopsis mytella* sp. (Apicomplexa), parasite of the mussel *Mytella guyanensis* (Mytelidae) from the Amazon Estuary and description of its oocysts. *Eur. J. Protistol.,* 35:427–433.

Bijukumar, A. 2001. Foulers, borers and parasites associated with *Crassostrea madrasensis* (Preston) cultured in Ashtamudi Lake, Kerala, India. *Spl. Publ., Phuket mar. bioi. Centre,* 25(1): 59-66.

Boehs, G., A.Villalba, L. O. Ceuta and J. R. Luz. 2010. Parasites of three commercially exploited bivalve mollusc species of the estuarine region of the Cachoeira River (Ilhéus, Bahia, Brazil). *J. Invertebr. Pathol;* 103(1): 43-47.

Bower, S. M., S. E. McGladdery and I. M. Price. 1994. Synopsis of Infectious Diseases and Parasites of Commercially Exploited Shellfish. *Annu. Rev. Fish Dis.,* 4:1-199.

Canestri-Trotti, G., E. M. Baccarani, F. Paesanti and E. Turolla. 2000. Monitoring of infections by protozoa of the genera *Nematopsis*, *Perkinsus* and *Porospora* in the smooth venus clam *Callista chione* from the North-Western Adriatic Sea (Italy). *Dis. Aquat. Org.,* 42:157–161.

Darriba, S., D. Iglesias, M. Ruiz, R. Rodriguez and C. López. 2010. Histological survey of symbionts and other conditions in razor clam *Ensis arcuatus* (Jeffreys, 1865) (Pharidae) of the coast of Galicia (NW Spain). *J. Invertebr. Pathol.* 104: 23–30.

Estevez, J., M. Sot, H. Rodrigues and C. Arias. 1998. Histopathology of bivalves in the Ria de Vigo (Galicia, NW Spain) infected by *Nematopsis* sp. (Protozoa:Apicomplexa). *Res. Rev. in Parasitol.,* 58 (1): 63-65.

Jones, J. B. 1975. *Nematopsis* n. sp. (Sporozoa: Gregarinia) in *Perna canaliculus*. N. Z. *J. Mar. Freshwater. Res.,* 9: 567–568.

Kim, Y., E. N. Powell and K. A. Ashton-Alcox. 2006. Histopathology analysis. In: Y. Kim, K. A. Ashton-Alcox & E. N. Powell, editors. Histological techniques for marine bivalve molluscs: Update. NOAA Tech. Mem. NOS NCCOS. 27:19–52.

Martinez J. C., R. V. Yeomans and G. P. Lardizabal. 2010.Parasites of the Pleasure Oyster *Crassostrea corteziensis* Cultured in Nayarit, Mexico. *J. Aquat. Anim. Health*, 22:141–151.

Mladineo, I. 2008. Risk assessment of parasitic/symbiotic organisms of the commercially important my tilid *Modiolus barbatus* (Linnaeus, 1758). Aqua. Res., 39: 1705-1719.

Padovan, I. P., L. Corral, L. A. Tavares, P. A. Padovan and C. Azevedo. 2003. Fine structure of the oocyst of *Nematopsis mytella* (Apicomplexa, Porosporidae), a parasite of the mussel *Mytella falcata* and of the oyster *Crassostrea rizophorae* (Mollusca, Bivalvia) from the northeastern Atlantic coast of Brazil. *Braz. J. Morphol. Sci.,* 20:121–124.

Sabry, R. C., T. C. V. Gesteira, and G. Boehs. 2007. First record of parasites in the mangrove oyster *Crassostrea rhizophorae* (Bivalvia: Ostreidae) at Jaguaribe River estuary-Ceara´ Brazil. *Braz. J. Bio.,* 67:755–758.

Samuel, D. 1978. A digenetic trematode infection in the edible oyster *Crassostrea madrasensis* (Preston). *Indian J. Fish.,* 23(1&2): 153-159.

Sanil, N. K., G. Suja, J. Lijo and K. K. Vijayan. 2012. First report of *Perkinsus beihaiensis* in *Crassostrea madrasensis* from the Indian subcontinent. *Dis. Aquat. Org.,* 98: 209–220.

Shaw, B. L. and H. I. Battle. 1957. The gross and microscopic anatomy of the digestive tract of the oyster *Crassostrea virginica* (Gmelin). *Can. J. Zool.,* 35:325–347.

Sindermann, C. J. 1990. Principal diseases of marine fish and shellfish. Vol. 2, Diseases of marine shellfish. New York: Academic Press. 516 pp.

Soto, M., S. Pascual, H. Rodrigues, C. Gestal, E. Abollo, C. Arias and J. Estevez. 1996. *Nematopsis* spp. Schneider, 1892 (Apicomplexa:Gregarinida) in bivalve mollusc off Ria De Vigo (Galicia, NW Spain). *Bull.Eur.Ass.Fish Pathol.,* 16(5):157-160.

Suja, G., N. K. Sanil, S. Chinnadurai, and K. K. Vijayan. 2014. Reproductive dysfunction in the edible oyster, *Crassostrea madrasensis* due to larval bucephalid infection – a case study. *J. Mar. Biol. Ass. India,* 55(2): 5-10.

Tuntiwaranuruk, C., K. Chalermwat, E. S. Upathum, M. Kruatrachue and C. Azevedo. 2004. Investigation of *Nematopsis* spp. oocysts in 7 species of bivalves from Chonburi Province, Gulf of Thailand. *Dis. Aquat. Org.,* 58: 47–53.

Winstead, J. T., A. K. Volety and S. G. Tolley. 2004. Parasitic and symbiotic fauna in oyster (*Crassostrea virginica*) collected from the Caloosahatchee River and Estuary in Florida. *J. Shellfish Res.,* 23:831–840.