Parasitology Research (2019) 118:1647–1651
https://doi.org/10.1007/s00436-019-06217-w

FISH PARASITOLOGY - SHORT COMMUNICATION

Morphological and molecular characterization of *Ceratomyxa batam* n. sp. (Myxozoa: Ceratomyxidae) infecting the gallbladder of the cultured *Trachinotus ovatus* (Perciformes: Carangidae) in Batam Island, Indonesia

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Received: 5 September 2017 / Accepted: 17 January 2019 / Published online: 23 March 2019

Abstract

A new coelozoic myxozoan species, *Ceratomyxa batam* n. sp., was identified in cultured carangid fish, *Trachinotus ovatus* (Perciformes: Carangidae), in waters off Batam Island of Indonesia. The bi- and trivalved spores were observed in the gallbladder of *T. ovatus*. Mature bivalved spores of *C. batam* n. sp. were transversely elongated and narrowly crescent in shape, 3.8 ± 0.36 (2.7–4.6) μm long and 19.2 ± 1.75 (16.2–22.0) μm thick. Two sub-spherical polar capsules were 2.3 ± 0.18 (2.0–2.8) μm long and 2.6 ± 0.16 (2.3–2.9) μm wide. Prevalence was 72.2% in 72 examined *T. ovatus* according to evaluations dating from November 2016. The maximum likelihood phylogenetic tree based on small subunit rDNA sequence showed similarity with *Ceratomyxa robertsthomsoni* and *Ceratomyxa thalassomae* found in Australia. This is the first report of *Ceratomyxa* species identified in a seawater fish at Batam Island, Indonesia.

Keywords *Ceratomyxa Batam* n. sp. · Characterization · Parasite · Gallbladder · *Trachinotus ovatus*

Introduction

The Carangid fish ovate pompano (*Trachinotus ovatus*) is the most successfully cultured marine fish in the world. Nevertheless, in recent years, a range of fish-associated outbreaks was caused by bacteria, virus or parasites (Dan et al. 2006; Su et al. 2015; Wang et al. 2010). *Trachinotus ovatus* is considered as a susceptible host for many marine parasites such as *Cryptocaryon irritans* (Prorodontida: Cryptocaryonidae) (Dan et al. 2006), *Paradeontacylix mcintosh* (Trematoda: Sanguinicolidae), *Benedenia diesing* (Monogenea: Capsalidae), and *Trichodibna ehrenberg* (Ciliophora: Peritrichida) (Xiong et al. 2015). Myxosporeans (Cnidaria: Myxozoa) are entozoic parasites that infect vertebrates (fish, amphibians, rarely reptiles, birds, and mammals) and invertebrates (polychaete and oligochaete worms) worldwide. The genus *Ceratomyxa* Thelohan, 1892 is among the more diverse myxosporean genera and includes more than 300 reported species (Lom and Dyková 2006). Species belonging to the genus *Ceratomyxa* are characterized by mature spores with the following morphological structures: crescent or arcuate shaped and with elongate shell valves that are generally thicker than the length (Lom and Dyková 2006). Members of the Ceratomyxidae Doflein, 1899 within the order Myxosporea are mainly considered parasites of marine teleosts and are rarely reported in freshwater fish (Lom and Dyková 2006). Pathogenic potentials of *Ceratomyxa* species are very rarely documented (Feist and Longshaw 2005). Prior to being reassigned as *Ceratonova shasta*, *Ceratomyxa shasta* was one of the best known pathogenic *Ceratomyxa* species which caused significant losses to salmonid aquaculture in

Section Editor: Christopher Whipps

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North America (Bartholomew et al. 1989; Bower and Margolis 2002). In addition, Maita et al. (1997) suspected a myxosporean may have been associated with the green liver symptom in *Seriola quinqueradiata*. A subsequent survey by Yokoyama and Fukuda (2001) identified two *Ceratomyxa* species, but pathological changes were not apparent (Maita et al. 1997; Yokoyama and Fukuda 2001). During the processing stage of cultured *T. ovatus* in Batam Island, Indonesia, we identified a new myxosporean in the gallbladder of *T. ovatus*, and we described in detail its morphology and phylogenetic relationships.

Materials and methods

Host fish and parasite sampling

In November 2016, 72 cultured *T. ovatus* were collected from the aquaculture area off Batam Island, Indonesia (0° 38' 19" N 104° 15' 23" E). The gallbladders of *T. ovatus* were removed, and the bile was examined for myxosporean infections using light microscopy. Fresh spores were photographed and measured using a Nikon Eclipse Ci microscope. The description and measurements of spores ($n = 30$) were made following the guidelines of Lom and Arthur (1989) and Heiniger et al. (2008) (Fig. 1). For Giemsa stain preparation, the spores were fixed in absolute methanol for 10 min, air dried, and then stained using Giemsa stain solution (Solarbio, G4640), following the manufacturer's instruction. For DNA extraction, positive gallbladders ($n = 5$) were preserved in absolute ethanol and were then stored in − 20 °C.

Small subunit ribosomal DNA amplification

When myxosporea species were detected, 100 μl of ethanol-preserved bile was pelleted and washed three times in ddH_2O. DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, CA) following the manufacturer's instructions. The extracted DNA was then stored at − 20 °C.

The small subunit (SSU) rDNA was amplified via PCR using forward primer MyxospecF (5′-TTC TGC CGT ATC AAC TWG TTG-3′) (Fiala 2006) and reverse primer18R (5′-CTA CGG AAA CCT TGT TAC G-3′) (Whipps et al. 2003). PCR reactions were performed in a total reaction volume of 25 μl, containing 15 μl of PCR-grade ddH_2O, 2.5 μl of 10× PCR buffer, 0.1 mM dNTP mix (2.5 mM), 0.4 μM each primer (10 mM), 1.5 U Taq polymerase (Takara), and 0.5 μg of DNA. PCR amplification parameters were as follows: 1 cycle of 95 °C for 5 min, 30 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 60 s, followed by the final extension at 72 °C for 7 min. The PCR products were separated by electrophoresis on a 1% agarose gel, visualized on a UV transillumination system (Tanon-3500R), and fragments excised and purified using EasyPure Quick Gel Extraction Kit (TRANSGEN BIOTECH). PCR products were sequenced at Shanghai Sangon Company (Shanghai, China) using a pair of internal primers, MyxF (5′-GAC TCA ACA CGG GAA AAC TTA-3′) and MyxR (5′-TGG CCG TTC TTA GTT CGT GGA GTG AT-3′) (Mansour et al. 2013).

Phylogenetic analysis

For phylogenetic analysis, the obtained SSU rDNA sequence was queried at National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) using the Basic Local Alignment Search Tool. Taxa with similar DNA sequence and similar morphology were selected for phylogenetic analysis. The multiple sequence alignment was performed using ClustalX2 with other homologous sequences. The maximum likelihood (ML) phylogenetic tree was generated using online PhyML 3.0 (http://www.atgc-montpellier.fr/phyml/) with Smart Model Selection (Guindon et al. 2010). An Akaike Information Criterion (AIC) model was selected, and subtree pruning and regrafting (SPR) was selected as the type of tree improvement. The number of bootstrap replicates was 100.

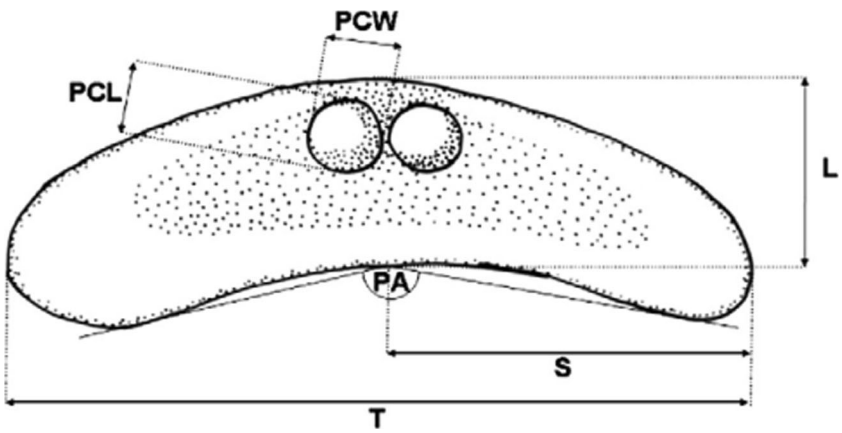

Fig. 1 Schematic diagram of spore measurements (Heiniger et al. 2008). L, length; PA, posterior angle, PCL, polar capsule length; PCW, polar capsule width; S, sutural position; T, thickness

Results

Ceratomyxa batam n. sp.

Morphological description of spores

Bi- and trivalved spores were observed in the bile of cultured *T. ovatus* (Perciformes: Carangidae) from Batam Island, Indonesia. Mature spores of *C. batam* n. sp. were typical of the genus *Ceratomyxa* with a transversely elongated and narrowly crescent shape. Mature spores were separated into two unequal valves by the straight sutural line, forming a slightly convex anterior end and a concave posterior end. Both spore valves had one polar capsule with equal size near the sutural line (Fig. 2a). Mature spores were 3.8 ± 0.36 (2.7–4.6) μm long and 19.2 ± 1.75 (16.2–22.0) μm thick. The two subspherical polar capsules were equal in size (2.3 ± 0.18 (2.0–2.8) μm long and 2.6 ± 0.16 (2.3–2.9) μm wide). Posterior angle was 162.3° ± 5.39° (155.4–175.2°) (Table 1). "Y" shaped trivalved spores (Fig. 2b) were also observed in small amounts in the bile of the cultured *T. ovatus*. The prevalence of *C. batam* n. sp. in November 2016 was 72.2% in the 72 examined *T. ovatus.*

Taxonomic summary Type host: Ovate pompano, *Trachinotus ovatus* (Perciformes: Carangidae) (Linnaeus, 1758).

Type host-locality: Batam Island, Indonesia (0° 38' 1" N 104° 15' 23"E).

Site of infection: Within gallbladder.

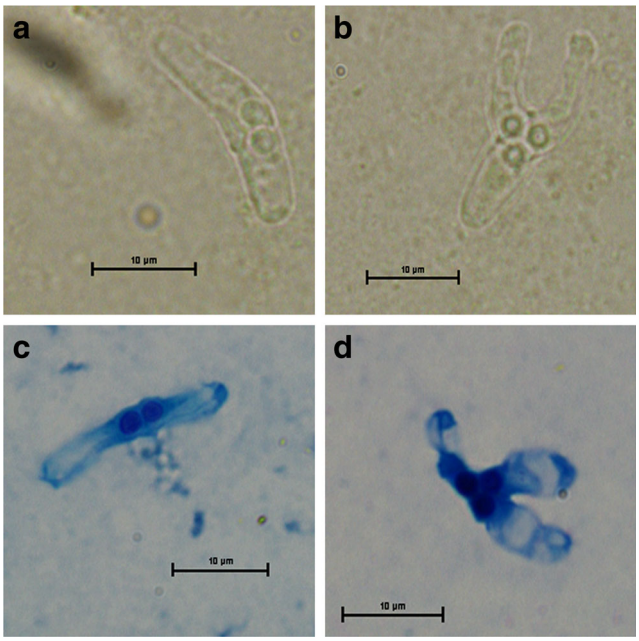

Fig. 2 The fresh spores of *Ceratomyxa batam* n. sp. from the gallbladder of cultured *T. ovatus*. **a** Fresh spore of *C. batam* n. sp. **b** Fresh "Y"-shaped trivalved spore of *C. batam* n. sp. **c** Giemsa-stained free spores. **d** Giemsa-stained trivalved spore of *C. batam* n. sp. Scale bar = 10 μm

Table 1 Comparative morphological description of *Ceratomyxa batam* n. sp. with similar species (measurements in μm)

Name (*Ceratomyxa* spp.)	Hosts	Locality	L	T	PCL	PCW	PA	References
C. batam n. sp.	*Trachinotus ovatus*	Indonesia	3.8 ± 0.36 (2.7–4.6)	19.2 ± 1.75 (16.2–22.0)	2.3 ± 0.18 (2.0–2.8)	2.6 ± 0.16 (2.3–2.9)	162.3 ± 5.39 (155.4–175.2)	Present research
C. tunisiensis	*Caranx rhonchus*, *Trachurus trachurus*	Gulf of Gabes, Tunisia	6 ± 0.26 (5–8)	23 ± 0.27 (20–25)	N	N	N	Thabet et al. (2016)
C. seriolae	*Seriola quinqueradiata*	Japan	6.5 (6.0–7.5)	33.7 (28.0–41.5)	1.9 (1.5–2.0)	N	N	Yokoyama and Fukuda (2001)
C. buri	*Seriola quinqueradiata*	Japan	6.5 (5.5–7.5)	14.3 (11.0–16.5)	2.4 (2.0–3.0)	N	N	
C. thalassomae	*Thalassoma lunare*	Queensland, Australia	5.0 (3.3–6.4)	18.9 (16.4–22.2)	2.9 (2.2–3.3)	2.8 (2.2–3.0)	173.1 ± 7.7 (158–189)	Heiniger et al. (2008)
C. robertsthomsoni	*Liza vaigiensis*	Queensland, Australia	4.72 (4.02–5.86)	17.25 (12.21–23.98)	2.09 (1.72–2.72)	2.0 (1.46–2.87)	161.6 (109–180)	Gunter et al. (2009)
C. hallettae	*Lethrinus harak*	Queensland, Australia	4.93 (3.66–5.93)	20.46 (13.89–26.49)	2.07 (1.16–2.99)	1.93 (1.19–2.51)	176.1 (150–190)	
C. gleesoni	*Plectropomus leopardus*	Queensland, Australia	6.1 ± 0.5 (5.0–7.0)	19.9 ± 1.6 (16.0–22.0)	2.3 ± 0.2 (1.5–3.0)	2.2 ± 0.2 (1.5–2.5)	156.9 (135–180)	Gunter and Adlard (2009)

L spore length, *T* spore thickness, *PCL* polar capsule length, *PCW* polar capsule width, *PA* posterior angle (dimension is in °), *N* no record

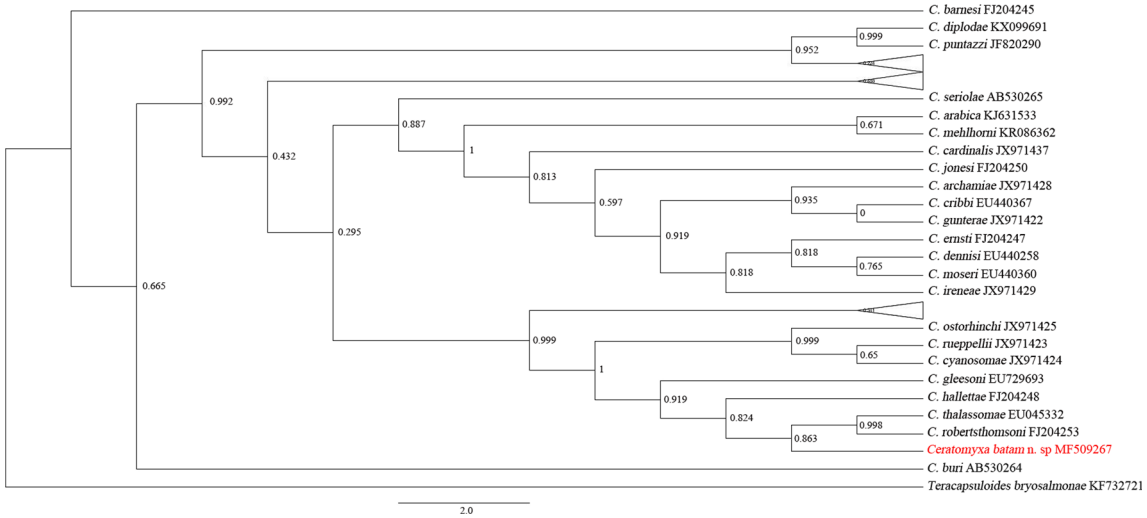

Fig. 3 Phylogenetic tree of *C. batam* n. sp. and related species based on the SSU rDNA sequences using maximum likelihood analysis. *Ceratomyxa batam* n. sp. is solid red. *Tetracapsuloides bryosalmonae* was used as outgroup

Prevalence: 72.2% in November 2016 (52/72).

Type material deposited: Giemsa stained slide, infected gallbladder and fixed spores in 95% ethanol were deposited in Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, Shandong Province, P. R. China. The SSU rDNA sequence was deposited in GenBank (accession number, MF509267).

ZooBank registration: Following the regulation of the international code of zoological nomenclature (ICZN), details of the new species have been submitted to ZooBank. Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:2F11F3DC-95AC-470E-A53B-A9C368A6C163. LSID for the new name *Ceratomyxa batam* n. sp. is urn:lsid:zoobank.org:act:94EEBA4F-D7A3-43E1-8F0E-5A0A22F5D2A1.

Etymology: The species name refers to the locality where the *Ceratomyxa* species were recovered, Batam Island, Indonesia.

Molecular identification and phylogenetic analysis

A sequence of 1567 bp of the SSU rDNA was obtained from *C. batam* n. sp. (GenBank accession number: MF509267) which was also used to identify similar sequences in GenBank using a BLAST search. The BLAST search revealed no identical sequences in the GenBank database. The percentage of similarities with other *Ceratomyxa* species included in this study ranged between 51.7 and 89%. The highest percent identity was 89% observed with *Ceratomyxa robertsthomsoni* reported from the gallbladder of *Liza vaigiensis* (Quoy and Gaimard, 1825), from Lizard Island, Great Barrier Reef, Queensland (Gunter et al. 2009). Maximum likelihood analysis revealed that *C. batam* n. sp. was most closely related to *C. robertsthomsoni* and *Ceratomyxa thalassomae* with the three species clustering within the same clade (Fig. 3). *Ceratomyxa batam* n. sp. showed a close phylogenetic relationship with *C. thalassomae* and *C. robertsthompsoni* and formed a larger clade with these two species as well as *Ceratomyxa gleesoni* and *Ceratomyxa hallettae.*

Discussion

In the present study, we identified a new species of *C. batam* n. sp. in the gallbladder of the pompano fish, *T. ovatus*. More than 300 species of *Ceratomyxa* had been identified from marine fish and subsequently described (Gunter et al. 2009). *Ceratomyxa* species have shown the ability to infect a range of different marine fish, but only three other species of *Ceratomyxa* have been previously reported from carangid hosts.

The bi- and trivalved spores were observed in the bile of the cultured *T. ovatus* while *C. tunisiensis* was identified in the gallbladders of two carangid fish, *Caranx rhonchus* and *Trachurus trachurus*, found in Gulf of Gabes, southern coast of Tunisia. Essentially, *C. tunisiensis* showed no morphologic similarity to *C. batam* n. sp.. *C. batam* n. sp. is shorter (3.8 ± 0.36/6 ± 0.26 μm) and thinner (19.2 ± 1.75/23 ± 0.27 μm) compared to *C. tunisiensis* (Table 1). In addition, *C. tunisiensis* presented the lowest similarity of the SSU rDNA (51.7%) among all the *Ceratomyxa* species. *C. seriolae* and *C. buri* were also identified in carangid fish *Seriola quinqueradiata* in Oita Prefecture, Japan. Compared to *C. batam* n. sp., the spores of *C. seriolae* are significantly longer (6.5/3.8 ± 0.36 μm) and thicker (33.7/19.2 ± 1.75 μm) but with a shorter polar capsule length (1.9/2.3 ± 0.18 μm). The spore of *C. buri* is longer (6.5/3.8 ± 0.36 μm) and thinner (14.3/19.2 ± 1.75 μm) compared to *C. batam* n. sp. (Table 1).

The observed trivalved spores were morphologically similar to those of *C. huanghainesis* (Zhao and Song 2003) which were defined as abnormal forms. Nonetheless, similar aberrant

spores with three valves are also present in some other *Ceratomyxa* species such as *C. bassoni* (Abdel-Ghaffar et al. 2008), *C. puntazzi* (Alama-Bermejo et al. 2011) and *C. gurnardi* (Sobecka et al. 2013).

Phylogenetic analysis results show that *C. batam* n. sp. is clustered with *C. robertsthomsoni* and *C. thalassomae* in one clade. *Ceratomyxa robertsthomsoni* was identified in the bile of *Liza vaigiensis* in Lizard Island, Great Barrier Reef, Queensland, while *C. thalassomae* was found in the bile of *Thalassoma lunare*, derived from Heron Island, Great Barrier Reef, Queensland, Australia (Gunter et al. 2009). *Ceratomyxa batam* n. sp., *C. robertsthomsoni*, and *C. thalassomae* are morphologically similar to each other and share the most similarity with the SSU rDNA. *Ceratomyxa hallettae* and *C. gleesoni* were also discovered in Queensland, Australia, and have shown close phylogenetic relationships with *C. batam* n. sp. Nevertheless, the spore length of *C. gleesoni* was much longer than that of *C. batam* n. sp. ($6.1 \pm 0.5/3.8 \pm 0.36$ μm) (Gunter and Adlard 2009).

In the present study, we observed and identified a parasitic *Ceratomyxa* species in Batam Island, Indonesia. As such, we propose the new species of *C. batam* n. sp. according to the fish host locality, the unique morphological characteristics compared with other species, and the results of phylogenetic analysis. Because of the importance of *T. ovatus* in aquaculture, for the imperfection of morphological characteristics in many records, more appropriate molecular barcodes should be exploited for the support of future taxonomic studies. Moreover, the pathogenic potential of *C. batam* n. sp. and its possible relationship with the host should be further studied.

Acknowledgments This work was supported by the China ASEAN Maritime Cooperation Fund Project (2016–2018). We would also like to thank PT. CAHAYA TERANG SEJATI, Indonesia, for facilitating the research process.

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