Journal of Sustainability Science and Management
Volume 12 *Number* 2, December 2017: 161-166
ISSN: 1823-8556

FIRST RECORD OF *Ceratomyxa* (THÉLOHAN, 1892) FROM THE GALL BLADDER OF ORANGE SPOTTED GROUPER, *Epinephelus coioides* (PERCIFORMES: SERRANIDAE) FROM SETIU WETLANDS, TERENGGANU

NUR FATIN SHAHAR[1], MUHAMMAD ARIF SHAMSURI[1], FAIZAH SHAHAROM[2] AND MUHAMMAD HAFIZ BORKHANUDDIN[1]*

[1]*School of Marine and Environmental Sciences,* [2]*Kenyir Research Institute, Universiti Malaysia Terengganu, 21030, Kuala Nerus, Terengganu, Malaysia.*

**Corresponding author: hborhan@umt.edu.my*

Abstract: Three cultured orange-spotted grouper, *Epinephelus coioides* sizes 22.1 to 24.5-cm-long from the Setiu Wetland were examined for fish parasites, in November 2015. One individual from 3 adult *E. coioides* harboured a myxosporean infection in its gall bladder. In this study, *Ceratomyxa* sp. specimens were studied under the phase contrast of light compound microscope. The myxosporean is here identified as *Ceratomyxa* Thélohan, 1892, diagnosed based on the elongated spores from valvular view whereas crescent-shaped from sutural view. Dimensions of spores were found to be 4.1 × 18.2 µm. The spores have two equal-sized pyriform polar capsules 1.7 × 1.4 µm, the polar filaments turns not detected. This is the first record of *Ceratomyxa* Thélohan, 1892 from the gall bladder of orange spotted grouper, *Epinephelus coioides* from Setiu Wetlands, Terengganu.

Keywords: Myxozoan, *Ceratomyxa*, estuarine, Setiu Wetlands, fish, grouper.

Introduction

The orange-spotted grouper, *Epinephelus coioides* belongs to family Serranidae (Mansour *et al*., 2015) and represents an important mariculture species in Asia. It has been used in commercial rearing in China and Southeast Asian countries such as Thailand, Malaysia, Singapore and Indonesia (Tudkaew *et al*., 2008) for its excellent biological characteristics such as fast growth, disease resistance, popular taste and high economic value (Yeh *et al*., 2003; Sun *et al*., 2009). Presence of parasites in grouper from tropical marine water have been of special interest in recent years (Kleinertz & Palm, 2015; Rimmer *et al*., 2004).

Myxosporean parasites have a worldwide distribution, mostly in fish hosts (Mansour *et al*., 2015). Myxosporean parasites increasing in grouper culture indicates the important pathogens for the cage-cultured grouper (China *et al*., 2013). Xu *et al*. (2014) emphasized the significance of monitoring and investigating myxosporidiosis in groupers from South China Sea. In China, rapid development of grouper aquaculture industry was linked to myxosporidiosis causing mass mortality on commercially important fish and has been considered a priority in China (Yuan *et al*., 2015). A similar incident in China also reported renal sphaerosphorosis in cage-cultured *E. coioides* in a fish farm in China caused by *Sphaerospora epinepheli which* highly infected the kidney and liver of the fish and caused fish to exhibit anaemia, anorexia, emaciation and occasionally skin ulcer (Xu *et al*., 2014).

The family Serranidae hosts a diverse range of ceratomyxid parasites (Gunter & Adlard, 2009). *Ceratomyxa* Thélohan, 1982 is the largest myxozoan genus with 280 species described globally (Gunter *et al*., 2010). Members of the genus *Ceratomyxa* are mostly coelozoic, parasiting the gall bladder of marine fishes, and distinguished by spores of elongated shape with shell valves exceeding in extent the axial diameter of the spore (Lom & Dykova, 2006).

Ceratomyxa occur in the gall bladder of marine teleost fish (Mansour *et al*., 2016). Example of host infected include *Bothus pantherinus* Rüppell, *Chaetodon vagabundus* Linnaeus, *Hyporhampus dussumieri* Valenciennes, *Sphyraena forsteri* Cuvier and *Zubrasoma veliferum* Bloch from Australia

(Gunter *et al*., 2010), *Paralichthys olivaceus* from Southern Korea (Cho *et al*., 2004), *Lutjanus bohar* Forsskål (Mansour *et al*., 2016), *Epinephelus coioides* (Mansour *et al*., 2015) and *Cephalopholis hemistiktos* (Abdel-Baki *et al*., 2015) off Saudi Arabia. Reports of serranid fishes infected by *Ceratomyxa* sp. include *Plectropomus leopardus, Epinephelus quoyanus*, *Cephalopholic boenak* and *Epinephelus maculatus* (Gunter *et al*., 2010).

To the best of our knowledge, this is the first record *of Ceratomyxa* species collected from the gall bladder of the estuarine fish *E. coioides* from Setiu Wetlands, Terengganu. Herein, we present the morphological and morphometrical description of this myxosporean.

Materials and Methods

A total number of 3 fishes from cage culture were collected in Setiu Wetland (5°40'52.4"N, 102°42'43.0"E) on the 3rd of November 2015. All fishes were transported back to AKUATROP Hatchery, UMT where they were maintained in an aquarium tank supplied with aeration. Fresh fish were pithed, dissected and examined within two to three days. External measurements of the fish host were taken after the fishes were excised. Examinations for the presence of myxozoans were carried out using Leica DM582 dissecting microscope and Leica CM01 compound microscope at different magnifications appropriate for the analysis of myxozoan. Infected organ with myxozoan plasmodia were carefully removed from the host body and dissected on a slide. Further images of the spores were observed and captured by using Leica DMLB2 advance microscope. Line drawings of the spore were made with the aid of camera Lucida from photos. Measurements of the morphological characteristics were taken from 11 fresh spores. Morphometric measurement and distinct diagnosis follow (Lom & Arthur, 1989).

Results

Only one of three *E. coioides* specimens was infected by *Ceratomyxa* sp. spores, found in the gall bladder. No other myxozoans were found in the other tissues of the orange spotted grouper. Known *Ceratomyxa* sp. from *Epinephelus* spp. differs morphometrically and are presented in Table 1.

Ceratomyxa sp. spores description

Type host Orange-spotted grouper *E. coioides*, Family Serranidae.

Type locality Setiu Wetlands, Terengganu, Malaysia (5°40'52.4"N, 102°42'43.0"E).

Site of infection Gall bladder.

Prevalence 1/3 of 22.1 to 24.5-cm-long fish

Intensity of infection 11 spores observed within one gall bladder

Diagnosis Spores were typical of the genus *Ceratomyxa* sp. (Figure 1). Spores slightly crescent-shaped, 4.1 (2.7-5.3) µm in length and 18.2 (14.7-23.3) µm in width (n = 11). Valves almost equal and smoothly ovoid in lateral view. Straight sutural lines visible between valves. Polar capsule spherical, 1.7 (0.7-2.7) µm in length and 1.4 (0.7-2.7) µm in width (n = 11).

Remarks

We have compared our specimens with five *Ceratomyxa* sp. descriptions from *Epinephelus* sp. hosts that include *Ceratomyxa hamour* (Mansour, Abdel-Baki, Tamihi & Al-Quraishy, 2015) from *Epinephelus coioides, Ceratomyxa cutmorei* (Gunter & Adlard, 2009) from *Epinephelus fasciatus, Ceratomyxa hooperi* (Gunter & Adlard, 2009) from *Epinephelus quoyanus*, *Ceratomyxa nolani* (Gunter & Adlard, 2009) from *Epinephelus quoyanus and Ceratomyxa yokoyamai* (Gunter & Adlard, 2009) from *Epinephelus maculatus*. Spore morphometric of our specimen do not correlate with other known *Ceratomyxa* spp. from *Epinephelus* including the shorter length of the spore body (4.1 *vs* 4.7–7.0 µm), bigger spore body width (18.2 *vs* 12.9 – 16.5 µm in the case of *C. hooperi*, *C. cutmorei* and *C.hamour*). The length of spore body for our specimen proved to be the shortest as compared to the other *Ceratomyxa* spp. described from *Epinephelus*

hosts. However, the dimensions of polar capsules (1.7 × 1.4 µm) were closest to *Ceratomyxa nolani* (1.6 × 1.6 µm) and *Ceratomyxa hooperi* (1.5 x 1.4 µm).

Some similarities in morphometric dimensions were found between our specimens to the following species, *Ceratomyxa americana* (Wierzbicka, 1987) from *Scomber scombrus* in Atlantic Ocean, *Ceratomyxa arripica* (Su & White, 1994) from *Arripis trutta* in Indian Ocean, *Ceratomyxa intexua* (Meglitsch, 1960) from *Jordanidia solandri* and *Plagiogenion rubiginosus* and *Ceratomyxa subtilis* (Meglitsch, 1960) from *Coelorhynchus australis* in Pacific Ocean (Eiras, 2006). However, our specimen differed substantially in at least one of the dimension. The polar capsule of all these similar species were spherical as our *Ceratomyxa* sp. specimens. However, it can be distinguished on the basis of the spore size and polar capsule length (Table 2). Our specimens has smaller spore size (4.1 x 18.2 µm) than *C. intexua* (4.4 x 15.4 µm) and *C. nolani* (5.1 x 19.0 µm). *C. americana* (4.8 x 9.8 µm) has longer spore length but less thickness of spore. *Ceratomyxa* sp. 1.7 (0.7–2.7 µm) has shorter polar capsule length compared to *C. arripica* 1.9 (1.8–2.0 µm) and *C. subtilis* 1.8 (1.5–2.0 µm).

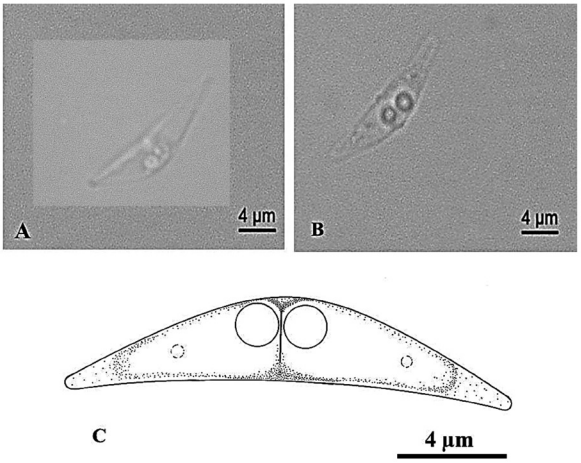

Figure 1: A–B. Fresh spores of *Ceratomyxa* from gall-bladder of *E. coioides.* C. Schematic drawing of the *Ceratomyxa* spore in frontal view

Table 1: Mean spore dimensions in µm including range of *Ceratomyxa* spp. from *Epinephelus* hosts

Species	Host	SL	ST	LPC	WPC
Ceratomyxa hamour n. sp. (Mansour *et al*. 2015)	*Epinephelus coioides*	7 (6–8)	16.5 (15–18)	4 (3–5)	3 (2–4)
Ceratomyxa cutmorei (Gunter and Adlard, 2009)	*Epinephelus fasciatus*	7.0 (5.0–8.5)	16.1 (12.0–21.5)	2.4 (1.5–3.0)	2.3 (1.5–3.0)
Ceratomyxa hooperi (Gunter and Adlard, 2009)	*Epinephelus quoyanus*	4.7 (4.0–5.5)	12.9 (10.0–15.5)	1.5 (1.0–2.0)	1.4 (1.0–2.0)
Ceratomyxa nolani (Gunter and Adlard, 2009)	*Epinephelus quoyanus*	5.1 (3.5–7.0)	19.0 (12.5–29.5)	1.6 (1.0–2.0)	1.6 (1.0–2.0)
Ceratomyxa yokoyamai (Gunter and Adlard, 2009)	*Epinephelus maculatus*	5.4 (4.5–6.5)	25.2 (20.5–31.0)	2.2 (2.0–2.5)	2.2 (2.0–2.5)
Ceratomyxa sp. (present study)	*Epinephelus coioides*	4.1 (2.7–5.3)	18.2 (14.7–23.3)	1.7 (0.7–2.7)	1.4 (0.7–2.7)

(SL) Body spore length, (ST) body spore thickness, (LPC) length of the polar capsules, (WPC) width of the polar capsules.

Table 2: Features of species of *Ceratomyxa* (Thélohan, 1892) from their original hosts

Species	Host	Spore Length (µm)	ST	Polar Capsule Length (µm)	Spore Shape	Location
Ceratomyxa americana Wierzbicka, 1987	*Scomber scombrus*	4.8 (4.4–5.2)	9.8 (8.8–11.1)	1.7 (1.6–1.8)	Almost spherical	USA (Atlantic Ocean)
Ceratomyxa arripica Su and White, 1994	*Arripis trutta*	4.9 (4.8–5.1)	10.2 (9.3–11.3)	1.9 (1.8–2.0)	Spherical	Australia (Indian Ocean)
Ceratomyxa intexua Meglitsch, 1960	*Jordanidia solandri* and *Plagiogenion rubiginosus*	4.4 (3.4–5.4)	15.4 (9.3–20.1)	1.8 (1.2–2.2)	Spherical	New Zealand (Pacific Ocean)
Ceratomyxa subtilis Meglitsch, 1960	*Coelorhynchus australis*	3.9 (3.4–4.5)	21.5 (15.7–26)	1.8 (1.5–2.0)	Spherical	New Zealand (Pacific Ocean)
Ceratomyxa sp. (present study)	*Epinephelus coioides*	4.1 (2.7–5.3)	18.2 (14.7–23.3)	1.7 (0.7–2.7)	Elongated	Malaysia (Setiu Wetlands)

Discussion

Ceratomyxa Thélohan, 1892, have a world-wide distribution, usually infecting the gall bladder of host and few infect other organs including urinary bladder and renal tubules (Eiras, 2006). More than 280 *Ceratomyxa* spp. has been described in fishes (Heiniger & Adlard, 2013) and because of its host specific characteristic, more species will be expected to be discovered. Ceratomyxids can be restricted to a host species but a single host can harbour more than one species of ceratomyxids (Gunter & Adlard, 2009). However, because of its lack of morphological character and spore plasticity make it difficult to determine whether closely related host harboured same or closely related *Ceratomyxa* spp. (Gunter *et al*., 2009).

To date, nine species of *Ceratomyxa* was described from family Serranidae (Mansour *et al*., 2015; Gunter &d Adlard, 2009; Abdel-Baki *et al*., 2015) and five from *Epinephelus* hosts (Table 1). Record on *Ceratomyxa* infecting *Epinephelus coioides* has been documented in (Mansour *et al*., 2015) but the size of polar capsule of our specimen is smaller and distinguishable in a sense of morphology. Based on morphological and measurement comparison from other *Ceratomyxa* spores, only few of them were close to our *Ceratomyxa* sp. collection. For instance, *C. intexua* and *C. nolani* are bigger size of spore than the present *Ceratomyxa* although the spore is spherical. *C. americana* has longer spore length but less thickness of spore. *C. arripica* and *C. subtilis* have longer polar capsule length. *C. americana* has narrowed endings. *C. intexua* and *C. arripica* have similar crescent shape with the posterior straight or slightly concave and polar capsule is spherical but bigger in size while *C. nolani* has smaller polar capsule. *C. subtilis* spore slightly arched. However, only one known species that infect Serranid fish was described which is *C. nolani*. *C. nolani* (5.1 x 1.9µm) has close spore length measurement compared with present *Ceratomyxa* sp. (4.1 x 18.2µm) but the polar capsule of *C. nolani* is shorter.

Morphological and morphometrical characterisation alone is not enough to compare and describe myxosporean without molecular genetic analysis due to the plasticity character of myxosporean. Hence, the combination of

morphometric, morphology and genetic analysis will provide a rigid data in describing, naming and distributing the position of *Ceratomyxa* species. Even though, the value of molecular analyses has been questioned in placing the position of myxosporean taxonomy, with an appropriate model, taxa, data sampling and in-depth data exploration, it will give a stable interpretation and result (Evans *et al*., 2010).

Conclusion

Based on morphometric and morphological data alone, we are yet unable to establish the novelty of the coelozoic myxosporean in the gall bladder of *Epinephelus coioides* from Setiu Wetlands. Further taxonomic description from molecular data is required to reveal the status of this *Ceratomyxa* among other species. Nevertheless, this preliminary finding provides an insight to the presence of parasitic myxozoan among other cultured estuarine fishes of Malaysia and as such might be an emerging parasite likely to impact the survival of this fish. It is highly recommended that future investigation would monitor and further study the transmission of grouper myxosporidiosis.

Acknowledgements

The research was supported by the Niche Research Grant Scheme NRGS/2015/53131/33 of the Malaysian Ministry of Higher Education (MOHE) granted to MHB. We thank the staff of the AKUATROP Hatchery and Biodiversity laboratory, PPSMS, UMT for their assistants.

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