Ceratomyxa bohari sp. n. (Myxozoa: Ceratomyxidae) from the gall bladder of Lutjanus bohar Forsskål from the Red Sea coast off Saudi Arabia: morphology, seasonality and SSU rDNA sequence

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Abstract: A new myxozoan, Ceratomyxa bohari sp. n., infecting the gall bladder of two-spot red snapper, Lutjanus bohar Forsskål, in the Red Sea off Saudi Arabia, is described using light microscopy and characterised genetically. The infection was recorded as mature spores floating free in the bile. The overall prevalence of infection of the type host was 19% (67 fish infected of 360 examined), with the highest prevalence in autumn (31%; 28/90) and the lowest in winter at 12% (11/90). Mature spores are slender and slightly crescent-shaped in the frontal view, with anterior and posterior margins tapered gradually to rounded valvular tips. Spore valves are unequal with a prominent suture line. The spore dimensions are 3–4 μm (mean 3.5 μm) in length and 16–19 μm (mean 17 μm) in thickness. Two polar capsules are spherical, equal in size, 1.5 μm in diameter. Coils of the polar filament are indiscernible. The sporoplasm is bimucellate and fills nearly one third of the extracapsular space restricted to the area below the capsules. The molecular analysis based on the SSU rDNA sequence revealed a close relationship with majority of species of Ceratomyxa Thélohan, 1892 and phylogenetic clustering with species from different geographical location. Thus, the shorter spore of the present Ceratomyxa species and the divergence of the SSU rDNA sequences are the distinctive features that separate it from all previously described species and identified this parasite as a new species of Ceratomyxa.

Keywords: Myxosporea, fish parasites, Bivalvulida, spore, coelozoic infection, phylogeny, Lutjanidae

Members of the myxozoan genus Ceratomyxa Thélohan, 1892 are predominantly parasites of the gall bladder of teleosts and elasmobranchs (Gunter et al. 2009). Species of Ceratomyxa have elongated, generally crescent-shaped or arcuate, sometimes subshperical or ovoid, spores. Shell valves are frequently conical or subhemispherical and exceed significantly in length one half of the axial diameter of the spore (Gunter et al. 2010). Since the establishment of Ceratonova Atkinson, Footh et Bartholomew, 2014, which includes C. shasta (Noble, 1950) (syn. Ceratomyxa shasta Noble, 1950) and Ceratonova gasterostea Atkinson, Footh et Bartholomew, 2014 (see Atkinson et al. 2014), all species accommodated in Ceratomyxa are coelozoic and occur in the gall bladder of marine teleost fish.

All Ceratomyxa species in a single large clade within the marine myxosporean lineage (Gunter et al. 2009, Fiala et al. 2015). Although this clade contains more than 70 species of Ceratomyxa, the presence of Palliatus indecorus Shulman, Kovaleva et Dubina, 1979 and Myxodavisia bulani Fiala, Hlavníčková, Kodádková, Freeman, Bartošová-Sojková et Atkinson, 2015 caused the paraphyletic character of the Ceratomyxa clade (Fiala et al. 2015, Rocha et al. 2015).

The fish genus Lutjanus Bloch contains 70 species and a review of the available literature revealed that this genus is known to be infected with seven myxozoan species belonging to four genera. These species are Kudoa hypoepicardialis Blaylock, Bullard et Whippes, 2004; Kudoa lutjanus Wang, Huang, Tsai, Cheng, Tsai, Chen, Chen, Chiu, Liaw, Chang et Chen, 2005; Kudoa lemmiscati Miller et Adlard, 2012; Unicapsula andersenae Miller et Adlard, 2013; Sphaerospora motemarini Holzer, Pecková, Patra, Brennan, Yanes-Roca et Main, 2013 and Henneguya jocu Azevedo, Rocha, Matos, Matos, Oliveira, Al-Quraishy et Casal, 2014 (see Miller and Adlard 2013, Azevedo et al. 2014, Holzer et al. 2013). To our knowledge, however, only one Ceratomyxa species has been reported from fish of this genus, namely C. milleri Gunter, Whippes et Adlard,
2009 described from the gall bladder of Lutjanus fulviflamma Forsskål collected in the Helena Island, Moreton Bay, Queensland, Australia (Gunter et al. 2009).

Although there is considerably exhaustive information on the ceratomyxan parasites from different parts of the world, little is known about the distribution and diversity of species of this genus in the Red Sea fishes. Research on these parasites is restricted to light microscopic description of just five species from the entire extent of the Red Sea coastline, all of which having been described from Egypt: Ceratomyxa ghaffari Ali, Abdel-Baki et Sakran, 2006 from Tylosurus choram Rüppell, C. bassoni Abdel-Ghaffar, Ali, Al Quraishy, Al Rasheid, Al Farraj, Abdel-Baki et Bashtar, 2008 from Plectorinchus gaterinus Forsskål, C. entzerothi Abdel-Ghaffar, Ali, Al Quraishy, Al Rasheid, Al Farraj, Abdel-Baki et Bashtar, 2008 from Valamugil seheli Forsskål, C. hurghadensis Abdel-Ghaffar, Ali, Al Quraishy, Al Rasheid, Al Farraj, Abdel-Baki et Bashtar, 2008 from Fistularia commersonii Rüppell, and C. swasi Abdel-Ghaffar, Ali, Al Quraishy, Al Rasheid, Al Farraj, Abdel-Baki et Bashtar, 2008 from Saurida undosquamis Richardson (see Abdel-Ghaffar et al. 2008, Ali et al. 2008).

Here, we describe a new species of Ceratomyxa from the gall bladder of the two-spot red snapper, Lutjanus bohar Forsskål, based on morphological and molecular data. We ascertain the phylogenetic position of this species among congeneric taxa and describe the seasonal variation in prevalence of its infection.

MATERIALS AND METHODS

A total of 360 specimens of fresh two-spot red snapper, Lutjanus bohar (Teleostei: Lutjanidae), were bought from the fishermen at the boat landing sites of the Red Sea coastline, all of which having been described from Egypt: Ceratomyxa ghaffari Ali, Abdel-Baki et Sakran, 2006 from Tylosurus choram Rüppell, C. bassoni Abdel-Ghaffar, Ali, Al Quraishy, Al Rasheid, Al Farraj, Abdel-Baki et Bashtar, 2008 from Plectorinchus gaterinus Forsskål, C. entzerothi Abdel-Ghaffar, Ali, Al Quraishy, Al Rasheid, Al Farraj, Abdel-Baki et Bashtar, 2008 from Valamugil seheli Forsskål, C. hurghadensis Abdel-Ghaffar, Ali, Al Quraishy, Al Rasheid, Al Farraj, Abdel-Baki et Bashtar, 2008 from Fistularia commersonii Rüppell, and C. swasi Abdel-Ghaffar, Ali, Al Quraishy, Al Rasheid, Al Farraj, Abdel-Baki et Bashtar, 2008 from Saurida undosquamis Richardson (see Abdel-Ghaffar et al. 2008, Ali et al. 2008).

For MP, tree was obtained using the Subtree-Pruning-Regrafting algorithm with search level 4 in which the initial trees were obtained by the random addition of sequences (4 replicates). Bayesian analyses were conducted by MrBayes v3.2.5 (Ronquist and Huelsenbeck 2003) under the GTR + I + F model, selected by Modeltest v. 3.7 (Posada 2008) with parameters setting to 4000000 generations (ngen = 4000000) with 2 runs each containing 4 simultaneous Markov Chain Monte Carlo chains (nchains = 4) and every 100th tree saved (samplefreq = 100). Each run was considered to have reached a stationary distribution based on split frequencies reported in MrBayes and by plotting the log likelihood values (the value 0.009 of the split frequency was reached after 450 000 generations). A total of 36667 trees per run were generated. The first 25% of sampled trees of each Bayesian run were discarded as burnin, the remaining trees in each analysis were used to calculate the posterior probabilities and the final 95% of trees were used to produce a majority rule consensus tree. Fisher’s exact test was carried out to compare prevalences using Quantitative Parasitology web software Version 1.0.9 (Reiczigel et al. 2014). The results were considered significant at p < 0.05.
RESULTS

*Ceratomyxa bohari* sp. n.  
Figs. 1–3


Vegetative stages

Vegetative stages were not observed. The infection was detected as free mature spores floating in the bile.

Mature spores

Mature spores slender and slightly crescent-shaped in frontal view, with convex anterior end and slightly bent posterior one (Fig. 1). Anterior and posterior margins tapered gradually terminating in rounded valvar tips (Fig. 1). Spore valves unequal. Sutural line prominent and clearly seen passing between two polar capsules. Spores 3–4 (3.5) long and 16–19 (17) thick. Two polar capsules equal in size, spherical in shape and 1–2 (1.6) in diameter. Polar filament coils indiscernible. Sporoplasm binucleate and nearly filling one third of extracapsular space restricted to area beneath two polar capsules (Fig. 2).

Prevalence and seasonal variation

Of the 360 examined specimens of *Lutjanus bohar*, 67 (19%) have gall bladders infected with *C. bohari*. The highest prevalence was observed in autumn, with 31% fish (28 infected of 90 examined) but the prevalence then declined sharply to 17% (15/90) in spring followed by another decline to 14% (13/90) in summer, with the lowest level of 12% (11/90) being recorded in the winter. The data revealed a highly significant seasonal pattern of prevalence ($p = 0.0079$) fundamentally due to the significant differences between autumn and spring ($p = 0.0352$), autumn and summer ($p = 0.0123$) and autumn and winter ($p = 0.0034$) samples; no other significant differences in prevalence were found (all $p > 0.05$).

Molecular analysis

Three partial SSU rRNA gene sequences were obtained from myxozoans in three gall bladders. These sequences were identical and the consensus nucleotide sequence of the 12% (11/90) being recorded in the winter. The data revealed a highly significant seasonal pattern of prevalence ($p = 0.0079$) fundamentally due to the significant differences between autumn and spring ($p = 0.0352$), autumn and summer ($p = 0.0123$) and autumn and winter ($p = 0.0034$) samples; no other significant differences in prevalence were found (all $p > 0.05$).

Molecular analysis

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Fig. 1A–D. Fresh spores of *Ceratomyxa bohari* sp. n. during their movement from the gall bladder of *Lutjanus bohar* Forsskål.

Fig. 2. Schematic drawing of a mature spore of *Ceratomyxa bohari* sp. n. from the gall bladder of *Lutjanus bohar* Forsskål.

Fig. 3. Phylogenetic tree resulting from Bayesian analysis inferred from the SSU rRNA dataset. Support values at branching nodes are listed as: bootstrap values from maximum likelihood/ bootstrap values from parsimony analyses/Bayesian posterior probabilities from Bayesian analysis. *Tetracapsuloides bryosalmonae* Canning, Curry, Feist, Longshaw et Okamura, 1999 was used as outgroup. Values below 50% or not supported by the analysis are indicated by dashes. Scale bar is probability of nucleotide change.
Table 1. Comparative data for Ceratomyxa bohari sp. n. and morphologically similar species (measurements in micrometres).

<table>
<thead>
<tr>
<th>Species</th>
<th>Host</th>
<th>Locality</th>
<th>Spore size</th>
<th>PC size</th>
<th>Spore shape</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. anko</td>
<td>Freeman, Yokoyama et(Jordan)</td>
<td>Lophius litulon (Pacific Ocean)</td>
<td>9.7–11.9 × 36.9–47.2</td>
<td>4.1–5.3 (4.6) x in diameter</td>
<td>SP: arcuate to crescent V: equal with rounded ends PC: spherical</td>
<td>Freeman et al. 2008</td>
</tr>
<tr>
<td>C. arabica</td>
<td>Al-Qahtani, Mansour, Al-Quraishy et Abdel-Baki, 2015</td>
<td>Acanthopagrus bifasciatus (Forskál)</td>
<td>7.9–10.14 × 8 (8 × 12)</td>
<td>2.5–3.5 &lt; 1.5–2.5 (3 × 2)</td>
<td>SP: stubby-shaped V: unequal PC: subpherical, unequal</td>
<td>Al-Qahtani et al. 2015</td>
</tr>
<tr>
<td>C. choerodonae</td>
<td>Heiniger, Gunter et Adlard, 2008</td>
<td>Cheirodon cyanodus (Richardson)</td>
<td>4.2–5.8 × 15.8–26.7</td>
<td>1.7–2.8 &lt; 1.4–2.5 (2.2 × 2)</td>
<td>SP: crescent-shaped V: equal</td>
<td>Heiniger et al. 2008</td>
</tr>
<tr>
<td>C. choleospora</td>
<td>Sandsberg, 1993</td>
<td>Centropomus undecimalis (Bloch)</td>
<td>4.5–15.23 (4.5 × 18.3)</td>
<td>2 x in diameter</td>
<td>V: equal with rounded ends PC: circular</td>
<td>Landsberg 1993</td>
</tr>
<tr>
<td>C. dissotici</td>
<td>Brickle, Kalavati et MacKenzie, 2001</td>
<td>Dissostichus eleginoides Smitt</td>
<td>3.2–4.5 × 15.4–22.8 (3.8 × 17.8)</td>
<td>2.3–3.6 (2.6–1.8)</td>
<td>SP: crescent-shaped V: equal with rounded ends PC: pyriform</td>
<td>Brickle et al. 2001</td>
</tr>
<tr>
<td>C. hamour</td>
<td>Mansour, Al-Qahtani et Abdel-Baki, 2015</td>
<td>Epinephelus fuscoguttatus (Laelle)</td>
<td>6.8–15.18 × 7 (6–16.5)</td>
<td>2–4 × 3.5 (4 × 3)</td>
<td>SP: crescent shaped V: equal PC: pyriform</td>
<td>Mansour et al. 2015</td>
</tr>
<tr>
<td>C. heinigeriae</td>
<td>Gunter, Whippes et Adlard, 2009</td>
<td>Choerodon cephalaotes (Castelnau)</td>
<td>167–32.3 × 4.8–6.3 (5.6 × 24)</td>
<td>1.8–2.4 (2) diameter</td>
<td>SP: crescent-shaped V: unequal</td>
<td>Gunter et al. 2009</td>
</tr>
<tr>
<td>C. husseini</td>
<td>Abdel-Baki, Mansour, Al-Quraishy et Al-Quraishy, 2015</td>
<td>Cephalopholis holotomus (Rüppell)</td>
<td>8–10 × 14–18 (9 × 16)</td>
<td>4.0–5.0 (4.5) diameter</td>
<td>V: equal, with rounded ends PC: spherical</td>
<td>Abdel-Baki et al. 2015</td>
</tr>
<tr>
<td>C. intessa</td>
<td>Mehlitz, 1960</td>
<td>Jordania solandri Cuvier</td>
<td>3.4–5.4 × 9.3–20.1 (4.4 × 15.4)</td>
<td>1.2–2.2 (1.8) diameter</td>
<td>SP: slightly curved V: equal PC: spherical</td>
<td>Eiras 2006</td>
</tr>
<tr>
<td>C. koiace</td>
<td>Gunter, Burger et Adlard, 2010</td>
<td>Sphyraena forsteri Cuvier</td>
<td>4.96–7.65 × 28.6–41.2 (5.5 × 36.7)</td>
<td>2.5–2.9 &lt; 2.3–2.9 (2.8 × 2.6)</td>
<td>SP: crescent-shaped V: equal</td>
<td>Gunter et al. 2010</td>
</tr>
<tr>
<td>C. milleri</td>
<td>Gunter, Whippes et Adlard, 2009</td>
<td>Lutjanus fulviflamma (Forskál)</td>
<td>4.1–5.4 × 11.4–20.9 (4.7 × 16.4)</td>
<td>1.3–2.0 (1.6 × 1.5)</td>
<td>SP: slightly crescent V: unequal</td>
<td>Gunter et al. 2009</td>
</tr>
<tr>
<td>C. pantherini</td>
<td>Gunter, Burger et Adlard, 2010</td>
<td>Bothus pantherinus (Rüppell)</td>
<td>7.3–9.1 × 17.9–24.6 (8.1 × 21.6)</td>
<td>2.3 × 2.2 (1.8–2.6 × 1.7–2.6)</td>
<td>SP: crescent-shaped V: unequal</td>
<td>Gunter et al. 2010</td>
</tr>
<tr>
<td>C. spreenti</td>
<td>Moser, Kent et Dennis, 1989</td>
<td>Chaetodon aurifasciatus (Macleay)</td>
<td>4–8 × 14–23 (5.5 × 16.3)</td>
<td>2–3 (2.4) diameter</td>
<td>SP: stubby, equal, slightly tapered with rounded ends V: equal</td>
<td>Eiras 2006</td>
</tr>
<tr>
<td>C. subtilis</td>
<td>Mehlitz, 1960</td>
<td>Coelacanthus australis (Richardson)</td>
<td>3.4–4.5 × 15.7–26 (3.9 × 21.5)</td>
<td>1.5–2 (1.8) diameter</td>
<td>SP: slender V: unequal or somewhat unequal</td>
<td>Eiras 2006</td>
</tr>
<tr>
<td>Ceratomyxa bohari sp. n.</td>
<td>Lutjanus bohar (Red Sea)</td>
<td>Lutjanus bohar (Forskál)</td>
<td>3–4 × 16–19 (3.5 × 17)</td>
<td>1–2 (1.6) diameter</td>
<td>SP: slender shape V: unequal PC: spherical</td>
<td>Present study</td>
</tr>
</tbody>
</table>

SP – spores; PC – polar capsules; V – valves.

1 562 bp was deposited in GenBank database under the accession number KP893567. BLAST search using the obtained SSU rRNA sequence revealed no identical myxosporean sequence deposited in GenBank.

The maximum level of similarity was obtained with myxosporidia belonging to the genus Ceratomyxa. Comparison of the 50 selected sequences of species of Ceratomyxa revealed the percentages of identity varying between 86.2% with C. heinigeriae Gunter, Whippes et Adlard, 2009 and 76.9% with C. aegyptica Yenmen, Marton, Eszterbauer et Bahri, 2012. The sequence differs from the aligned sequences of Ceratomyxa spp. at 158–310 bp over 1 240 nucleotide alignment.

Bayesian inference and maximum parsimony methods yielded trees with similar topology. Ceratomyxa bohari sp. n. was placed at the base of the clade grouping C. choerodonae Heiniger, Gunter et Adlard, 2008, C. koiace Gunter, Burger et Adlard, 2010, C. heinigeriae and C. milleri (Fig. 3).

**Type host:** Lutjanus bohar Forskál (Perciformes: Lutjanidae), two-spot red snapper.

**Type locality:** Red Sea off Jizan city (16°53’21’’N; 42°32’3’’E), Saudi Arabia.

**Type material:** Syntype spores in 80% ethanol are deposited in the parasitological collection of the Hungarian Natural History Museum under the number HNHM-70639.

**Site of infection:** The infection was detected as large numbers of free floating spores in the bile solution. Vegetative stages were not observed.

**Prevalence:** 19% (overall prevalence; in 67 out of 360 fish examined).

**Etymology:** The specific name is given after the Arabic common name of the fish host ‘bohar’.

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DISCUSSION

The present species exhibits some morphometric similarities with other members of the genus Ceratomyxa that parasitise fish from different families and different geographical regions (Table 1). Despite the similarity of these species, they differ from Ceratomyxa bohari sp. n. in several characteristics. Ceratomyxa choleaspora Landsberg, 1993 differs in having more crescentic and slightly larger spores (4.5 μm × 15–23 μm vs 3–4 μm × 16–19 μm), equal shell valves and a sporoplasm filling the entire extracapsular space. Ceratomyxa dissostichi Brickle, Kalavati et MacKenzie, 2001 is distinguished from the present species by having equal valves, triangular intercapsular thickening and a larger elliptoidal polar capsule, compared to the rounded ones in our species (2.3–3.6 μm vs 1–2 μm). Spores of Ceratomyxa intexxa Meglitsch, 1960 can be readily distinguished from the present species by its equal valves that terminate in narrow tips with straight posterior margins and a wide range of spore thicknesses (9.3–20.1 μm vs 16–19 μm).

Similarly, Ceratomyxa sprenti Moser, Kent et Dennis, 1989 has quite long spores (4–8 μm vs 3–4 μm) with straight anterior and posterior margins and equal shell valves, whereas Ceratomyxa subitis Meglitsch, 1960 has thicker spores [21.5 μm (15.7–26 μm) vs 17 μm (16–19 μm)] with equal valves. Ceratomyxa anko Freeman, Yokoyama et Ogawa, 2008 is quite different to our species in all its body dimensions [(11 μm (10–12 μm) × 42 μm (37–47 μm) vs 3.5 μm (3–4 μm) × 17 μm (16–19 μm))]. Also, C. choerodonae differ in having lager spores with equal valves (5 μm × 21 μm vs 3.5 μm × 17 μm).

Ceratomyxa heingiera can be differentiated by their longer and thicker spores (5.6 μm × 24.0 μm vs 3.5 μm × 17 μm). Ceratomyxa milleri differs in having thicker spores (4.7 μm vs 3.5 μm) with straight posterior margins and subspherical polar capsules. Ceratomyxa koieae differs in having quite larger spores with equal valves (5.5 μm × 36.7 μm vs 3.5 μm × 17.0 μm). Ceratomyxa pantherini Gunter, Burger et Adlard, 2010 has rather longer and thicker spores (7.3–9.1 μm × 17.9–24.6 μm vs 3–4 μm × 16–19 μm).

It is worth mentioning that, recently, our research group described three ceratomyxan species from fishes in the Arabian Gulf off Saudi Arabia. These species are Ceratomyxa arabica Al-Qtahani, Mansour, Al-Quraishy and Abdel-Baki, 2015, Ceratomyxa hamour Mansour, Al-Qtahani et al., 2015 and Ceratomyxa husseini Abdel-Baki, Mansour, Al-Qtahani, Al-Omar et Al-Quraishy, 2015, but all of these differ from this new species in having longer spores (7–9 μm, 6–8 μm, 8–10 μm, respectively vs 3–4 μm) (see Abdel-Baki et al. 2015, Al-Qtahani et al. 2015, Mansour et al. 2015).

Ceratomyxa bohari was present all year round with a maximum prevalence in autumn (31%) and a minimum prevalence in winter (12%). In general, many factors are involved in seasonal fluctuations of myxosporeans, including temperature, endogenous cycles of the parasites and the availability of susceptible hosts (Foott and Hedrick 1987, Alvarez-Pellitero and Sitjá-Bobadilla 1993, Alvarez-Pellitero et al. 1995, Yokoyama and Fukuda 2001). Additionally, the seasonal variation in prevalence of ceratomyxan parasites could be attributable to the variable condition of bile secretion at each sampling period (Yokoyama and Fukuda 2001).

Phylogenetic analysis based on maximum likelihood, maximum parsimony and Bayesian inference methods clusters C. bohari within the clade grouping the majority of species of Ceratomyxa within the marine lineage. Recently, a large phylogenetic reconstruction of Ceratomyxa resulted in distinction of five subclades (Fiala et al. 2015). The newly identified species clusters within subclade E of Fiala et al. (2015), which contains the highest number of species analysed.

The new species appears at the base of a well-supported clade grouping C. choerodonae, C. koieae, C. heinigerae and C. milleri Gunter, Whippes et Adlard, 2009. All these species of Ceratomyxa were reported from fishes off Australia. Of them, C. milleri was described in the gall bladder of a dory snapper, Lutjanus fulviflamma (Forsskål) Lutjanidae.

The obtained sequence has at least 8% divergence from other deposited sequences of identified species. The least sequence divergence was 7.9% with C. heinigerae from Choerodon cephalotes (Castelnau) (Pericormorpha: Labridae) in Australia (Gunter et al. 2009). Sequence divergence with the three recently identified species of Ceratomyxa in the Arabian Gulf, C. arabica, C. hamour and C. husseini, was 8.5%, 15.4% and 9.6%, respectively. These data support validity of the new species from L. bohar in the Red Sea.

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