



Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Morphometric criteria and partial sequence of the 18S rRNA gene of *Ceratomyxa sultani* n. sp. from the gallbladder of *Upeneus margarethae* in the Arabian Gulf, with a note on its seasonal prevalence

Abdel-Azeem S. Abdel-Baki^{a,b,*}, Hussain A. Al-Qahtani^a, Esam Almalki^c, Saleh Al-Quraishy^a, Ali Al Ghamdi^d, Lamjed Mansour^{a,e}

^a Zoology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

^b Zoology Department, Faculty of Science, Beni-Suef University, Egypt

^c Department of Medical Laboratory, College of Applied Medical Sciences, Majmaah University, Saudi Arabia

^d Biology Department, College of Science, Al Baha University, Al Baha, Saudi Arabia

^e Unité de Recherche de Biologie intégrative et Écologie évolutive et Fonctionnelle des Milieux Aquatiques, Département de Biologie, Faculté des Sciences de Tunis, Université De Tunis El Manar, Tunisia

ARTICLE INFO

Article history:

Received 30 October 2017

Revised 22 November 2017

Accepted 3 December 2017

Available online 5 December 2017

Keywords:

Myxosporea

Coelezoic

Ceratomyxa

Phylogeny

Seasonal variation

ABSTRACT

This paper describes a new coelozoic myxosporean parasite named *Ceratomyxa sultani* n. sp. isolated from the gallbladder of *Upeneus margarethae* sourced from the Arabian Gulf off Saudi Arabia. Of 104 *U. margarethae* specimens examined, 27 (26%) were infected, with the highest prevalence in winter and lowest in autumn. The pseudoplasmodia were disporous and irregularly elliptical in shape, with an average size of $22 \times 17 \mu\text{m}$. Mature spores were mostly elliptical with symmetrical valves and equal spherical polar capsules. Spores were $9 \mu\text{m}$ in length and $25 \mu\text{m}$ in thickness, while polar capsules were $4 \mu\text{m}$ wide with four filament coils. The paper further provides a morphological comparison with closely related *Ceratomyxa* spp. together with phylogenetic analysis based on the partial 18S rRNA sequence, which revealed that *C. sultani* n. sp. clustered within a robust clade of *Ceratomyxa* species from the Arabian Gulf and Red Sea or nearby geographic regions.

© 2017 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Myxozoans are typical and occasionally highly suspicious parasites of fish that have been known since the 1800s and which have a very convoluted biphasic life cycle, epitomised by the formation of multicellular spores (Okamura et al., 2015). It has progressively become clear that myxozoans are widespread, with more than 2400 species in 62 genera now known, an incredible level of species diversity representing about 18% of currently known cnidarian

species (Lom and Dyková, 2006; Okamura et al., 2015; Laamiri, 2017; Liu et al., 2017). The identification of some of these species has been predominantly based on the shape and structure of their spores, however; which is a generally inadequate taxonomic approach, sometimes making identification very problematic (Lom and Dyková, 2006; Gunter and Adlard, 2010). Nowadays, molecular techniques based on sequence variations of the 18S rRNA gene have become an extremely useful complementary tool for differentiating closely related myxosporeans, especially cryptic species (Heiniger and Adlard, 2014; Abdel-Baki et al., 2017). The combination of spore morphometry with the greatly expanded use of molecular-genetic methods therefore provides a powerful tool for ascertaining the taxonomy of recently described species, and also for the clarification of the taxonomy and phylogeny of the myxozoan genera (Heiniger and Adlard, 2014; Zhang et al., 2017). The Arabian Gulf is home to rich and diversified fish fauna, with nearly 500 species of bony and cartilaginous fish having been reported from its various coasts (Krupp and Muller, 1994). Until recently, little attention has been paid to the myxosporean

* Corresponding author at: Zoology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia.

E-mail addresses: aabdelbaki@ksu.edu.sa, azema1@yahoo.com (A.-A.S. Abdel-Baki).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

parasites of these Arabian Gulf fish, and thus very little is known about these parasites. Most of the previous studies that have done on Arabian Gulf fish have concentrated mainly on the helminthes (Kardousha, 2016), and although sporadic work has been carried out on myxosporean parasites (Kardousha and El-Tantawy, 2002; Mansour et al., 2014, 2015a,b; Zhang et al., 2014; Abdel-Baki et al., 2015, 2017; Al-Qahtani et al., 2015), there is clearly a need for more extensive work to get a better idea of the species infecting fish in the Arabian Gulf in general and those off Saudi Arabia in particular. Here we present a minor contribution to this assemblage of

work by describing a new species of *Ceratomyxa* from the gallbladder of *Upeneus* based on the morphometric criteria of its spores and the partial sequence of the 18S rRNA gene.

2. Materials and methods

During a survey of myxosporean parasites in fishes collected from the Arabian Gulf off Dammam city (26° 26' 0"N, 50° 6' 0"E) in Saudi Arabia, 104 specimens of *Upeneus margarethae*, Uiblein and Heemstra, were collected during monthly visits between March 2014 and March 2015. Immediately after collection, the fish were dissected and their organs and body fluids were examined for the presence of myxosporean infection. Fresh spores were examined and photographed with the aid of an Olympus BX51 microscope equipped with an Olympus DP71 digital camera (Olympus, Japan). Parasite identification and measurements were taken from 30 randomly selected fresh spores according to Lom and Arthur (1989). Measurements are in micrometres (μm) and data are expressed as range (mean \pm SD). Gallbladders that were heavily infected with spores were preserved in 85% ethanol for molecular analysis.

3. Phylogeny

Ethanol-preserved gallbladders were washed three times with saline buffer in order to remove alcohol. Then, DNA was extracted using a DNeasy[®] Blood & Tissue Kit (QIAGEN Inc., Valencia, CA) according to the manufacturer's recommendations. Two commonly recommended primers were used for the amplification of the partial 18S rRNA gene of the myxozoan parasite: MyxospecF 5' TTCTGCCGTATC AACTWGTG 3' (Fiala, 2006) and reverse 18R 5'CTACGGAAACCTTGTTACG3' (Whipps et al., 2004). The PCR amplifications were conducted in 30 μl of final volume following the same protocol reported by Mansour et al. (2015a). Briefly, 50–100 ng of DNA template was mixed with 0.5 μM of each primer, 2 mM dNTPs (0.5 mM each), and 0.5 U of iProof[™] High-Fidelity DNA polymerase, purchased from Bio-Rad (Hercules, CA, USA), 1X iProof[™] HF buffer, and 1.5 mM MgCl₂. Amplifications were performed in a Techne TC-Plus Satellites personal thermocycler apparatus (Staffordshire, UK) following the program reported in Mansour et al. (2015a). Sequencing of the extracted fragments was carried out by MacroGen Inc. (Seoul, South Korea), using the same primers as for SSU rDNA amplifications. The sequences were visualized, assembled and edited using BioEdit 7.2.5.0 (Hall, 1999).

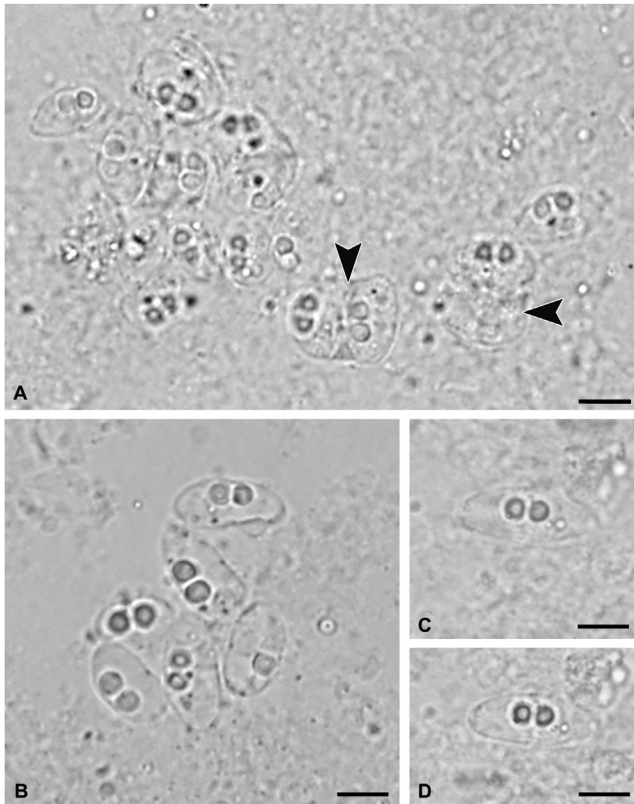


Fig. 1. Fresh spores of *Ceratomyxa sultani* n. sp. from the gall bladder of *Upeneus margarethae*. Arrowheads referring to the disporic pseudoplasmodia. Scale-bars = 10 μm .

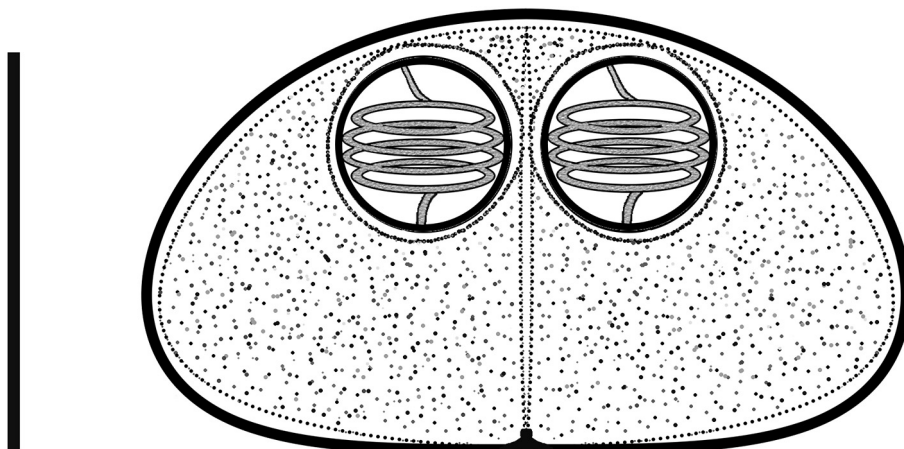


Fig. 2. Line drawing of a mature spore of *Ceratomyxa sultani* n. sp. from the gall bladder of *Upeneus margarethae*. Scale-bars = 10 μm .

A consensus sequence was obtained and then used to extract the most closely related sequences among *Ceratomyxa* species using the BLASTn search (Altschul et al., 1997). Alignments were produced for phylogenetic analyses using ClustalX 2.1.0.12 software (Larkin et al., 2007) with default parameters. Phylogenetic trees were constructed with maximum likelihood (ML) and Neighbour joining (NJ) methods using MEGA software, version 7 (Kumar et al., 2016). For ML, we used the General Time Reversible with invariant sites and gamma distributed rates approach (GTR + I +

G) as the model for nucleotide substitution. The NJ method was based on the Kimura 2-parameter model (Kimura, 1980). Both trees were constructed based on 1000 replicates.

3.1. Statistical analysis

Statistical analyses were performed using the One-way ANOVA and the Holm-Sidak methods within the Sigma Plot statistical

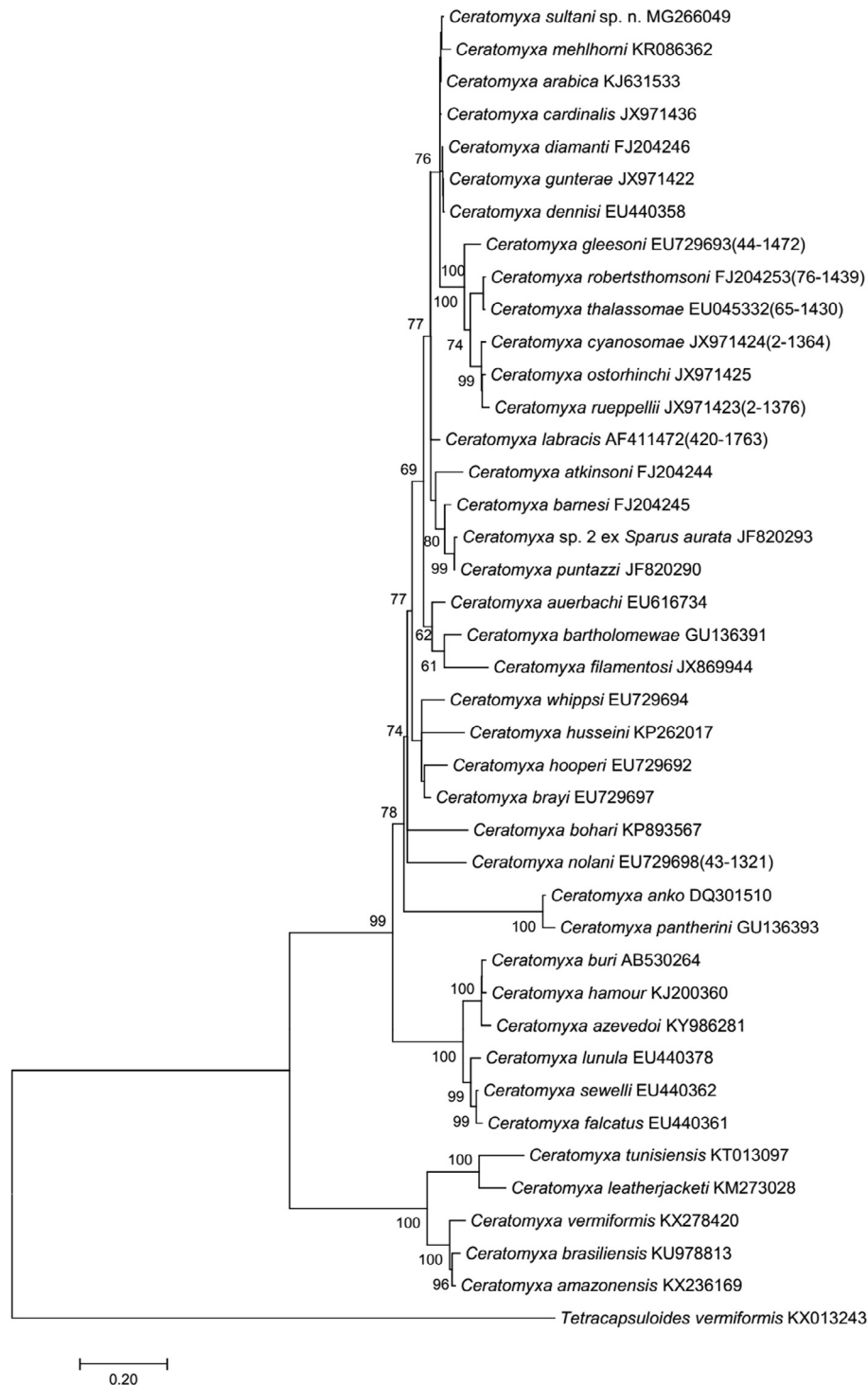


Fig. 3. The maximum likelihood tree based on the analysis of SSU rDNA data showing the phylogenetic position of *Ceratomyxa sultani* n. sp. Nodal supports are indicated for ML (for 1000 replicates) and (posterior probabilities) BI. Species names are given with their GenBank accession numbers. The number of changes per site is given by scale bar. *Tetracapsuloides vermiformis* was used as outgroups.

package, version 11.0. All *p* values are two-tailed and results were judged to be statistically significant difference when $P \leq 0.001$.

4. Results

4.1. Vegetative stages

The infection was reported as pseudoplasmodia mixed with a large number of free spores. The pseudoplasmodia were disporous, free floating in the bile, and irregularly elliptical in shape (Fig. 1A), with an average length of 19–24 (22 ± 0.8) μm and width of 15–19 (17 ± 0.7) μm .

4.2. Spore description

Mature spores were almost elliptical with a slightly convex anterior margin and a usually flat posterior one. Spores were 7–11 (9 ± 0.2) μm in length and 22–27 (25 ± 0.3) μm in thickness ($n = 30$) (Fig. 1). The spore valves were symmetrical and smooth with a slightly curved suture (Fig. 2). Polar capsules were spherical, equal in size and 3–5 (4 ± 0.2) μm wide ($n = 30$). Polar filaments had four turns, perpendicular to the longitudinal axis of the capsule. The sporoplasm was binucleated, filling most of the extracapsular space (Figs. 1 and 2).

4.3. Phylogeny

A consensus sequence of 985 bp was produced and submitted to GenBank under the accession number (MG266049). The GC content of this sequence was 51.7%. Pairwise alignment with sequences in the database shows a maximum similarity (96.8%) with *C. mehlhorni* (Mansour et al., 2015a) (31 substitutions and 7 gaps) followed by *C. dennisi* (Gunter and Adlard, 2008) with 96.6%. and *C. arabica* (Al-Qahtani et al., 2015) with 95.8% (14 substitutions and 1 gap). The lowest similarity was observed with *C. tunisiensis* (62.8%) (Thabet et al., 2016). *Ceratomyxa arabica* and *C. mehlhorni* have both been reported from fish in the Arabian Gulf (Mansour et al., 2015a,b; Al-Qahtani et al., 2015). Forty SSU rRNA *Ceratomyxa* spp. were selected for the construction of the phylogenetic tree based on having the highest BLAST scores and identity, and similar geographic distribution. The sequence of *Tetracapsuloides vermiformis* was used as an outgroup. The trees generated by ML and NJ had similar topologies with slight differences in the nodal support values in favour of the ML tree. As shown in Fig. 3, the phylogenetic analysis reveals clustering of *C. sultani* with *C. mehlhorni*, *C. arabica*, *C. cardinalis*, *C. diamanti*, *C. gunterae* and *C. dennisi* with a high level of bootstrap support.

4.4. Prevalence and seasonality of infection

The overall prevalence was 26% (27/104), with the highest infection rate in winter (42.3%, 11/26) followed by spring (30.8%, 8/26) and summer (23.1%, 6/26), and lowest infection rate in autumn (7.7%, 2/26) (Table 1; Fig. 4). Analysis of this data revealed a significant difference between winter and autumn ($P < .001$), winter and summer ($P < .001$), spring and autumn ($P < .001$), summer and autumn ($P = .001$), winter and spring ($P = .007$) and spring and summer ($P = .050$).

4.5. Taxonomic summary

Type host: *Upeneus margarethae* Uiblein and Heemstra, 2010 (Teleostei, Perciformes, Mullidae).

Type locality: Saudi Arabian coast off the Arabian Gulf.

Site of infection: Gallbladder.

Table 1

The relationship between the seasons and the prevalence of *Ceratomyxa sultani* n. sp. infecting the gallbladder of *Upeneus margarethae* from the Arabian Gulf.

Seasons	No. examined fish	No. infected fish	Percent of infection
Spring	26	8	30.8%
Summer	26	6	23.1%
Autumn	26	2	7.7%
Winter	26	11	42.3%
Total	104	27	26%

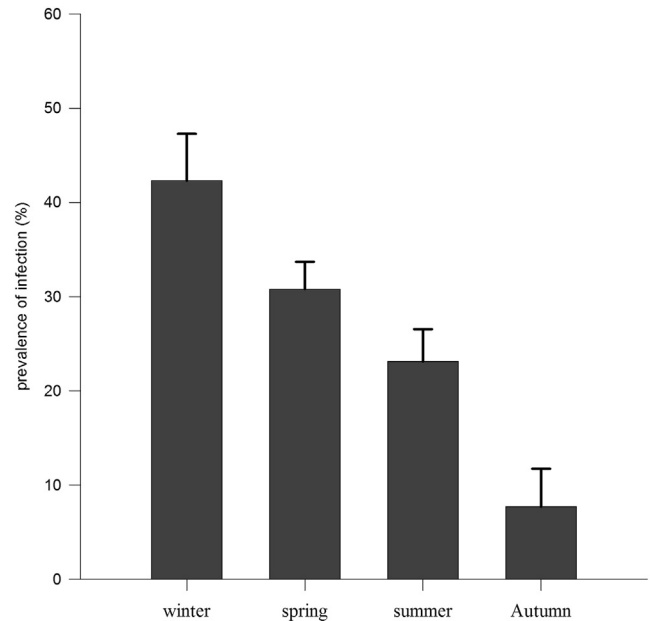


Fig. 4. The relationship between the seasons and the prevalence of *Ceratomyxa sultani* n. sp. infecting the gall bladder of *Upeneus margarethae*.

Prevalence: 26% (27/104).

Type-material: Gallbladder in 70% ethanol was deposited in the parasitological collection of the Zoology Department Museum, College of Science, King Saud University, Saudi Arabia, with number (C/11/2017). SSU rDNA sequence was deposited in the GenBank database with the accession number MG266049.

Etymology: The specific epithet is given after the common name of the fish host “Sultan Ibraheem”.

4.6. Remarks

Although no species of the genus *Ceratomyxa* Thelohan, 1892 have yet been described from members of the family Mullidae, some species can be compared with the present form either due to some similarities in their spores or due to their geographical location and habitat (Table 2). Species with apparently close similarities are: *C. laxa* (Meglitsch, 1960), *C. chromis* (Lubat et al., 1989), *C. peculiaria* (Yurakhno, 1991), *C. syacii* (Kpatcha et al., 1996), *C. azonusi* (Aseeva, 2003) and *C. moseri* (Gunter and Adlard, 2008). *Ceratomyxa laxa* differs in having shorter and more bent spores with a higher number of polar filament coils (5–6 vs. 4). In the same way, *C. chromis* differs in having crescent-shaped and shorter spores with tapered extremities and small polar capsules. Also, *C. peculiaria* could be differentiated by its shorter spores and small pyriform polar capsules. The shorter spores with unequal valves and smaller polar capsules of *C. syacii* easily differentiate it from *Ceratomyxa sultani* n. sp. Similarly, *C. azonusi* could be differentiated by its shorter and arch-like spores with pyriform polar

Table 2
Comparative data for *Ceratomyxa sultani* sp. n. and morphologically similar species.

Species	Host	Locality	Spore size	PC size	Spore shape	References
<i>Ceratomyxa sultani</i> n. sp. (Present study)	<i>Upeneus margarethae</i> Uiblein & Heemstra	Saudi Arabia (Arabian Gulf)	9 (7–11) × 24.5 (22–27)	4 (3–5)	SP: elongated elliptical Valves: equal PC: spherical with 4PF	Present study
<i>C. arabica</i> Al-Qahtani, Mansour, Al-Quraishy, Abdel-Baki, 2015	<i>Acanthopagrus</i> <i>bifasciatus</i> Forsskål	Saudi Arabia (Arabian Gulf)	7–9 × 10–14 (8 × 12)	2.5–3.5 × 1.5– 2.5 (3 × 2)	SP: stubby-shaped V: unequal PC: subspherical, unequal	Al-Qahtani et al. (2015)
<i>C. azevedoi</i> Abdel-Baki, Al-Qahtani, Al-Quraishy, Mansour, 2017	<i>Lutjanus ehrenbergii</i> Peters	Saudi Arabia (Arabian Gulf)	5–7 × 10–14 (6 × 12)	2–3 (2.5)	SP: crescent-shaped Valves: equal PC: spherical	Abdel-Baki et al. (2017)
<i>C. azonusi</i> Aseeva, 2003	<i>Pleurogrammus azonus</i> Jordan and Metz	Russia (Sea of Japan)	7–9 × 24–30	3 × 1.8–2.5	SP: arch-like Valves: equal with rounded ends PC: pyriform with 5PF.	Aseeva (2003)
<i>C. chromis</i> Lubat, Radujkovic, Marques & Bouix, 1989	<i>Chromis chromis</i> (Linnaeus)	Montenegro (Adriatic Sea)	7.5 (7–8.3) × 25 (20–27)	2.5	SP: crescent-shaped Valves: equal with tapered extremities PC: spherical	Lubat et al. (1989)
<i>C. hamour</i> Mansour, Al-Qahtani, Al-Quraishy, Abdel-Baki, 2015	<i>Epinephelus coioides</i> Hamilton	Saudi Arabia (Arabian Gulf)	6–8 × 15–18 (7 × 16.5)	2–4 × 3–5 (4 × 3)	SP: crescent shaped V: equal PC: pyriform	Mansour et al. (2015a)
<i>C. husseini</i> Abdel-Baki, Mansour, Al-Qahtani, Al Omar, Al-Quraishy, 2015	<i>Cephalopholis</i> <i>hemistiktos</i> (Rüppell)	Saudi Arabia (Arabian Gulf)	9 (8–10) × 16 (14–18)	4.5 (4–5)	SP: arched Valves: equal with rounded ends PC: spherical	Abdel-Baki et al. (2015)
<i>C. laxa</i> Meglitsch, 1960	<i>Caulopsetta scapha</i> (Forster)	New Zealand (Pacific Ocean)	8.7 (7.8–9.8) × m 25.6 (20.8–30.3)	3.4 (2.9–3.9)	SP: bent and flattened horizontally Valves: equal, very rounded tips PC: spherical with 5–6 PF	Meglitsch (1960)
<i>C. mehlhorni</i> Mansour, Abdel-Baki, Al-Qahtani, Al-Quraishy, 2015	<i>Gnathanodon speciosus</i> (Forsskål)	Saudi Arabia (Arabian Gulf)	8 (7–9) × 12 (10–14)	3 (2–4)	SP: hat or helm-like Valves: unequal PC: spherical	Mansour et al. (2015b)
<i>C. moseri</i> Gunter and Adlard, 2008	<i>Pomacentrus wardi</i> (Whitley)	Australia (Great Barrier Reef)	4.5 (3.5–6) × 11.9 (9–14.3)	1.6 (1.3–2.2) × 1.5 (1.2–1.9)	SP: crescent-shaped Valves: equal PC: pyriform	Gunter and Adlard (2008)
<i>C. peculiaris</i> Yurakhno, 1991	<i>Spicara flexuosa</i> Rafinesque	Ukraine (Black Sea)	6.5–8.5 × 21–29.3	2.4–2.7 × 1.9– 2.4	SP: slightly convex Valves: equal with rounded ends PC: Pyriform with 3PF.	Yurakhno (1991)
<i>C. syacii</i> Kpatcha, Diebakate, Faye, Toguebaye, 1996	<i>Syacum micrurum</i> Ranzani	Senegal (Great Coast)	9.3 (9–9.5) × 23.6 (22.5–25)	1.9 (1.5–2.0)	SP: straight Valves: unequal PC: spherical	Kpatcha et al. (1996)

Abbreviations: SP, spores; PC, polar capsules; PF, polar filament.

capsules. *Ceratomyxa moseri*, meanwhile, has shorter and thinner spores with rather smaller pyriform polar capsules. It is worth mentioning that our team recently reported five *Ceratomyxa* spp. in the Arabian Gulf. These species are *C. arabica* (Al-Qahtani et al., 2015), *C. hamour* (Mansour et al., 2015a), *C. husseini* (Abdel-Baki et al., 2015), *C. mehlhorni* (Mansour et al., 2015b) and *C. azevedoi* (Abdel-Baki et al., 2017), all of which have shorter and thinner spores. In addition, *C. arabica* and *C. mehlhorni* have unequal valves. Moreover, *C. arabica* and *C. hamour* have pyriform polar capsules.

5. Discussion

Ceratomyxa Thélohan, 1892, is the second largest genus among the myxozoan groups, comprising 300 known species, which is about 8% of the myxozoan diversity (Fiala et al., 2015; Abdel-Baki et al., 2017; Zatti et al., 2017). Members of the genus *Ceratomyxa* are mainly coelozoic, inhabiting the gallbladders of their fish hosts, although a few species have been reported from other organs, including the digestive tract, urinary bladder and kidney tubules (Eiras, 2006). The majority of ceratomyxan species have

been reported from marine fish hosts, with a few species being described from freshwater teleosts (Zatti et al., 2017). The morphometric features of mature spores in fish hosts are commonly used for the identification and differentiation of *Ceratomyxa* spp., as is common for all the myxosporean genera. This is due three reasons: (i) this is the most readily accessible stage in field studies; (ii) the lack of uniform characterization of vegetative stages (when available) and; (iii) because differences may exist in the other parasite life cycle stages, which are usually unknown and/or potentially inaccessible (Gunter et al., 2009). Sometimes, however, the dimensions of the spore components overlap between species and may even vary within a single species due to the spore plasticity at the light microscope level (Heiniger and Adlard, 2013). Also, life cycles for marine *Ceratomyxa* spp. are poorly resolved, with only one having been described, for *Ceratomyxa auerbachii* (Kodádková et al., 2014). Consequently, in order to arrive at more reliable identifications, it is always worthwhile to take into account as many additional criteria as possible, such as the partial sequence of the SSU rDNA gene, host specificity and the geographical location (Fiala, 2006; Heiniger and Adlard, 2013; Kalatzis et al., 2013). In order to establish species novelty, therefore, it is now considered

to be best practice to use SSU rDNA data in combination with morphological and/or biological data (Heiniger and Adlard, 2013; Rocha et al., 2016). Following this perspective, we have here described a new species of *Ceratomyxa* using a combination of spore morphometric characters and 18S rDNA sequence data. Additionally, Heiniger and Adlard (2013) confirmed that marine *Ceratomyxa* are highly host specific and typically confined to a single host species. The fact, therefore, that no *Ceratomyxa* spp. have previously been described from the family Mullidae, gives an initial indication of the novelty of the present form. We confirmed this using the molecular sequence of the SSU rDNA gene. Despite the fairly high percentage of similarity with *C. arabica* (98.5%), and *C. mehlhorni* (96.8%) the difference in sequences can be considered quite enough to separate the present new species from these related species, when combined with the morphological differences we have identified. In other studies, similarity values as high as 99% and 98.7% have been associated with species discrimination due to morphological differences (Gunter et al., 2009; Heiniger and Adlard, 2013). This indicates that there is no specified benchmark for inter-specific variation. Thus, we believe that although molecular data are essential in the differentiation of morphologically closely related species, especially cryptic ones, the SSU rDNA sequence is not in itself enough to distinguish all closely related *Ceratomyxa* spp. In order to overcome this problem, Heiniger and Adlard (2013) suggested the use of some alternative genetic markers, such as the LSU rDNA, which is 2–3 times longer than the SSU. The LSU rDNA actually gives a similar level of interspecific variation, however, and thus a similar degree of taxonomic information (Bartošová-Sojtková et al., 2014). Accordingly, several other genes are now being investigated for their potential to distinguish myxosporeans at various levels, and over the next 5–10 years further markers for species differentiation are likely to be identified (Atkinson et al., 2015).

Generally, myxosporeans exhibit seasonal variations in prevalence which may be due to many biological and environmental factors. Environmental factors, including the water temperature and the water flow have been shown to influence the prevalence of several myxosporean species (Milanin et al., 2010). Furthermore, the myxosporean life cycle itself represents the most important biological factor (Guitang et al., 2003). Since myxosporeans have an alternate actinospore stage in the oligochaete host (El-Matbouli et al., 1999; Kent et al., 2001), the seasonal variation of myxosporean is often synchronized with the rate of actinospore release from these oligochaete hosts (Cone, 1994; Guitang et al., 2003). In the present study, the infection showed an overall prevalence of 26% (27/104) but there were significant seasonal variations between winter and autumn ($P < .001$), winter and summer ($P < .001$), spring and autumn ($P < .001$) and summer and autumn ($P = .001$) winter and spring ($P = .007$) and spring and summer ($P = .050$). A similar pattern has been reported for several *Ceratomyxa* spp. e.g. *Ceratomyxa husseini* (Abdel-Baki et al., 2015), *Ceratomyxa hamour* (Mansour et al., 2015a,b) and *Ceratomyxa azevedoi* (Abdel-Baki et al., 2017). In addition to the biological and environmental factors, Yokoyama and Fukuda (2001) attributed the variation in seasonal prevalence of *Ceratomyxa* spp. to the fact that the condition of the bile secretion varies according to the sampling time.

In conclusion, the few studies that have been performed on myxosporean parasites in the fish of the Arabian Gulf have not yet been able to take full account of the diversity of fish in this environment. The results of this study, along with the previous work of our team (Mansour et al., 2014, 2015a,b; Zhang et al., 2014; Abdel-Baki et al., 2015, 2017; Al-Qahtani et al., 2015) provides an initial indication of the potential importance of the Arabian Gulf as a source of new information on this field of science.

Acknowledgement

We extend our appreciation to the Dean of Scientific Research, King Saud University, for funding the work through the research group project number RGP-004.

References

- Abdel-Baki, A.S., Al-Qahtani, H.A., Al-Quraishy, S., Mansour, L., 2017. *Ceratomyxa azevedoi* n. sp. (Myxozoa: Myxosporae) parasitizing the gallbladder of *Lutjanus ehrenbergii* in the Arabian Gulf. *Parasitol. Res.* 116, 2757–2763.
- Abdel-Baki, A.S., Mansour, L., Al-Qahtani, H.A., Al Omar, S.Y., Al-Quraishy, S., 2015. Morphology, seasonality and phylogenetic relationships of *Ceratomyxa husseini* n. sp. from the gall-bladder of *Cephalopholis hemistiktos* (Rüppell) (Perciformes: Serranidae) in the Arabian Gulf off Saudi Arabia. *Syst. Parasitol.* 91, 91–99.
- Al-Qahtani, H.A., Mansour, L., Al-Quraishy, S., Abdel-Baki, A.S., 2015. Morphology, phylogeny and seasonal prevalence of *Ceratomyxa arabica* n. sp. (Myxozoa: Myxosporae) infecting the gallbladder of *Acanthopagrus bifasciatus* (Pisces: Sparidae) from the Arabian Gulf, Saudi Arabia. *Parasitol. Res.* 114, 465–471.
- Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucl. Acids Res.* 25, 3389–3402.
- Aseeva, N.L., 2003. New species of myxosporean (Myxozoa: Myxosporae) parasites of *Ceratomyxa* from fishes of Peter the Great Bay (Japan Sea). *J. Parasitol.* 89, 1172–1180.
- Atkinson, S., Bartošová, P., Whipps, C.M., Bartholomew, J.L., 2015. Approaches for characterizing myxozoan species. In: Okamura, B., Gruhl, A., Bartholomew, J.L. (Eds.), *Myxozoan Evolution, Ecology and Development*. Springer International Publishing, Cham, pp. 111–123.
- Bartošová-Sojtková, P., Hrabcová, M., Pecková, H., Patra, S., Kodádková, A., Jurajda, P., Tým, T., Holzer, A.S., 2014. Hidden diversity and evolutionary trends in malacosporean parasites (Cnidaria: Myxozoa) identified using molecular phylogenetics. *Int. J. Parasitol.* 44, 565–577.
- Cone, D.K., 1994. Annual cycle of *Henneguya dooi* (Myxosporae) parasitizing yellow perch (*Perca flavescens*). *J. Parasitol.* 80, 900–904.
- El-Matbouli, M., Hoffmann, R.W., Schoel, H., McDowell, T.S., Hedrick, R.P., 1999. Whirling disease: host specificity and interaction between the actinosporean stage of *Myxobolus cerebralis* and rainbow trout *Oncorhynchus mykiss*. *Dis. Aquat. Org.* 35, 1–12.
- Eiras, J.C., 2006. Synopsis of the species of *Ceratomyxa* Thélohan, 1892 (Myxozoa: Myxosporae: Ceratomyxidae). *Syst. Parasitol.* 65, 49–71.
- Fiala, I., 2006. The phylogeny of Myxosporae (Myxozoa) based on small subunit ribosomal RNA gene analysis. *Int. J. Parasitol.* 36, 1521–1534.
- Fiala, I., Hlavničková, M., Kodádková, A., Freeman, M.A., Bartošová-Sojtková, P., Atkinson, S.D., 2015. Evolutionary origin of *Ceratonyxa* shasta and phylogeny of the marine myxosporean lineage. *Mol. Phylogenet. Evol.* 86, 75–89.
- Guitang, W., Weijian, Y., Xiaoning, G., Jianguo, W., Pin, N., 2003. Seasonal fluctuation of *Myxobolus gibelioi* (Myxosporae) plasmodia in the gills of the farmed allogynogenetic gibel carp in China. *Chinese J. Oceanol. Limnol.* 21, 149–153.
- Gunter, N.L., Adlard, R.D., 2008. Bivalvulid (Myxozoa: Myxosporae) parasites of damselfishes with description of twelve novel species from Australia's Great Barrier Reef. *Parasitology* 135, 1165–1178.
- Gunter, N.L., Adlard, R.D., 2010. The demise of *Leptotheca* Thélohan, 1895 (Myxozoa: Myxosporae: Ceratomyxidae) and assignment of its species to *Ceratomyxa* Thélohan, 1892 (Myxosporae: Ceratomyxidae), *Ellipsomyxa* Køie, 2003 (Myxosporae: Ceratomyxidae), *Myxobolus* Bütschli, 1882 and *Sphaerospora* Thélohan, 1892 (Myxosporae: Sphaerosporidae). *Syst. Parasitol.* 75, 81–104.
- Gunter, N.L., Whipps, C.M., Adlard, R.D., 2009. *Ceratomyxa* (Myxozoa: Bivalvulida): robust taxon or genus of convenience? *Int. J. Parasitol.* 39, 1395–1405.
- Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.* 41, 95–98.
- Heiniger, H., Adlard, R.D., 2013. Molecular identification of cryptic species of *Ceratomyxa* Thélohan, 1892 (Myxosporae: Bivalvulida) including the description of eight novel species from apogonid fishes (Perciformes: Apogonidae) from Australian waters. *Acta Parasitol.* 58, 342–360.
- Heiniger, H., Adlard, R.D., 2014. Relatedness of novel species of *Myxidium* Bütschli, 1882, *Zschokkella* Auerbach, 1910 and *Ellipsomyxa* Køie, 2003 (Myxosporae: Bivalvulida) from the gall bladders of marine fishes (Teleostei) from Australian waters. *Syst. Parasitol.* 87, 47–72.
- Kalatzis, P.G., Kokkari, C., Katharios, P., 2013. Description and relationships of two novel species of *Ceratomyxa* Thélohan, 1892 infecting the gallbladders of Aulopiformes: Atlantic lizardfish *Synodus saurus* Linnaeus, 1758 and royal flagfin *Aulopus filamentosus* Bloch, 1792 from Cretan Sea, Greece. *Parasitol. Res.* 112, 2055–2061.
- Kardousha, M.M., 2016. Fish parasites of Qatari waters of the Arabian Gulf: current status with an annotated checklist. *Int. J. Recent Sci. Res.* 7, 12388–12393.
- Kardousha, M.M., El-Tantawy, S.A., 2002. First record of microsporeans and myxosporeans (Protozoa) infecting some Arabian Gulf fishes off the coasts of the Emirates and Qatar with a description of *Myxobolus arabicus* n. sp. *Arab Gulf J. Scient. Res.* 20, 18–24.
- Kent, M.L., Andree, K.B., Bartholomew, J.L., El-Matbouli, M., et al., 2001. Recent advances in our knowledge of the Myxozoa. *J. Eukaryot. Microbiol.* 48, 395–413.

- Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16, 111–120.
- Kodádková, A., Dyková, I., Tým, T., Ditrich, O., Fiala, I., 2014. Myxozoa in high Arctic: Survey on the central part of Svalbard archipelago. *Int. J. Parasitol. Parasites Wildl.* 3, 41–56.
- Kpatcha, T.K., Diebakate, C., Faye, N., Toguebaye, B.S., 1996. Quelques nouvelles espèces de myxosporidies, du genre *Ceratomyxa* Thélohan, 1895 parasites des poissons marins du Sénégal, Afrique de l'Ouest. *Parasite* 3, 223–228.
- Krupp, F., Muller, T., 1994. The status of fish populations in the northern Arabian Gulf two years after the 1991 Gulf War oil spill. *Cour. Forsch.-Inst. Senckenberg* 166, 67–75.
- Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* 33, 1870–1874.
- Laamiri, S., 2017. Myxosporea (Cnidaria: Myxozoa) infecting the saddled seabream *Oblada melanura* (L. 1758) (Teleostei: Sparidae) and the painted comber *Serranus scriba* (L. 1758) (Teleostei: Serranidae) in Tunisia. *Zootaxa* 4269, 61–100.
- Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23, 2947–2948.
- Liu, X., Hua, C., Zhang, Q., Zhao, Y., Zhang, D., Zhang, J., 2017. *Myxobolus taibaiensis* sp. n. (Myxozoa: Myxosporea) infecting the intestinal wall of common carp *Cyprinus carpio* Linnaeus in China. *Folia Parasitol.* 64, pii: 2017.001.
- Lom, J., Arthur, J.R., 1989. A guideline for the preparation of species descriptions in Myxosporea. *J. Fish Dis.* 12, 151–156.
- Lom, J., Dykova, I., 2006. Myxozoan genera: definition and notes on taxonomy, life cycle terminology and pathogenic species. *Folia Parasitol.* 53, 1–36.
- Lubat, J., Radujkovic, B., Marques, A., Bouix, G., 1989. Parasites des poissons marins du Montenegro. *Acta Adriat.* 30, 31–50.
- Mansour, L., Abdel-Baki, A.S., Al-Qahtani, H.A., Al-Quraishy, S., 2015a. Morphological and molecular aspects of *Ceratomyxa mehlhorni* n. sp., a parasite of the golden trevally *Gnathanodon speciosus* in the Arabian Gulf off the Saudi Arabian coast, with data on its seasonal prevalence. *Parasitol. Res.* 114, 3783–3789.
- Mansour, L., Al-Qahtani, H.A., Al-Quraishy, S., Abdel-Baki, A.S., 2015b. Molecular and morphometric characteristics of *Ceratomyxa hamour* n. sp. (Myxosporea: Bivalvulida) infecting the gallbladder of the orange-spotted grouper *Epinephelus coioides* from the Arabian Gulf, Saudi Arabia. *J. Eukaryot. Microbiol.* 62, 95–101.
- Mansour, L., Harrath, A.H., Abd-Elkader, O.H., Alwasel, S., Abdel-Baki, A.S., Al Omar, S.Y., 2014. Structural and molecular characterization of *Kudoa quraishii* n. sp. from the trunk muscle of the Indian mackerel *Rastrelliger kanagurta* (Perciforme, Scombridae) in Saudi Arabia coasts. *Parasitol. Res.* 113, 1361–1370.
- Meglitsch, P.A., 1960. Some coelozoic myxosporidia from New Zealand fishes. I. General and family Ceratomyxidae. *Trans. Roy. Soc. New Zealand* 88, 265–365.
- Milanin, T., Eiras, J.C., Arana, S., Maia, A.A., Alves, A.L., Silva, M.R., Carriero, M.M., Ceccarelli, P.S., Adriano, E.A., 2010. Phylogeny, ultrastructure, histopathology and prevalence of *Myxobolus oliveirai* sp. nov., a parasite of *Brycon hilarii* (Characidae) in the Pantanal wetland, Brazil. *Mem. Inst. Oswaldo Cruz.* 105, 762–769.
- Okamura, B., Gruhl, A., Bartholomew, J.L., 2015. Myxozoan Evolution, Ecology and Development. Springer International Publishing, Cham, p. 413.
- Rocha, S., Rangel, L.F., Castro, R., Severino, R., Azevedo, C., Santos, M.J., Casal, G., 2016. Ultrastructure and phylogeny of *Ceratomyxa diplodae* (Myxosporea: Ceratomyxidae), from gall bladder of European seabass *Dicentrarchus labrax*. *Dis. Aquat. Organ.* 121, 117–128.
- Thabet, A., Mansour, L., Al Omar, S.Y., Tlig-Zouari, S., 2016. *Ceratomyxa tunisiensis* n. sp. (Myxosporea: Bivalvulida) from the Gallbladders of two carangid fish caught off the Coast of Tunisia. *J. Eukaryot. Microbiol.* 63, 86–92.
- Whipps, C.M., El-Matbouli, M., Hedrick, R.P., Blazer, V., Kent, M.L., 2004. *Myxobolus cerebralis* internal transcribed spacer 1 (ITS-1) sequences support recent spread of the parasite to North America and within Europe. *Dis. Aquat. Organ.* 60, 105–108.
- Yokoyama, H., Fukuda, Y., 2001. *Ceratomyxa seriola* n. sp. and *C. buri* n. sp. (Myxozoa: Myxosporea) from the gall-bladder of cultured yellowtail *Seriola quinqueradiata*. *Syst. Parasitol.* 48, 125–130.
- Yurakhno, V.M., 1991. New species of myxosporidia from fishes of the Black Sea. *Parazitologiya* 25, 1054–1109.
- Zatti, S.A., Atkinson, S.D., Bartholomew, J.L., Maia, A.A.M., Adriano, E.A., 2017. Amazonian waters harbour an ancient freshwater *Ceratomyxa* lineage (Cnidaria: Myxosporea). *Acta Trop.* 169, 100–106.
- Zhang, B., Zhai, Y., Liu, Y., Gu, Z., 2017. *Myxobolus pseudowulii* sp. n. (Myxozoa: Myxosporea), a new skin parasite of yellow catfish *Tachysurus fulvidraco* (Richardson) and redescription of *Myxobolus voremkhai* (Akhmerov, 1960). *Folia Parasitol.* 64, pii: 2017.030.
- Zhang, J.Y., Al-Quraishy, S., Abdel-Baki, A.S., 2014. The morphological and molecular characterization of *Myxobolus khaliji* n. sp. (Myxozoa: Myxosporea) from the double bar seabream *Acanthopagrus bifasciatus* (Forsskål, 1775) in the Arabian Gulf, Saudi Arabia. *Parasitol. Res.* 113, 2177–2183.