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ORIGINAL PAPER

Morphology, phylogeny and seasonal prevalence of *Ceratomyxa arabica* n. sp. (Myxozoa: Myxosporea) infecting the gallbladder of *Acanthopagrus bifasciatus* (Pisces: Sparidae) from the Arabian Gulf, Saudi Arabia

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Abstract A new myxozoan species was recovered from the gallbladder of *Acanthopagrus bifasciatus* from the Arabian Gulf in Saudi Arabia. The overall prevalence of infection was 28.6 % (32/112), with the highest prevalence 42.9 % (12/28) in winter and 10.7 % (3/28) as the lowest in autumn. The new species is described using its morphological characteristics and small subunit (SSU) rDNA. Spores of *Ceratomyxa arabica* n. sp. are stubby-shaped with unequal shell valves, 8 (7–9) μm in length × 12 (10–14) μm in thickness. Polar capsules are sub-spherical, unequal, 3 (2.5–3.5) × 2 (1.5–2.5) μm. The polar filament has three turns and is slightly slanted towards the longitudinal axis of the capsules. The small subunit rDNA (SSU rDNA) sequence confirms that the present species is a member of the genus *Ceratomyxa*, being most closely related to *Ceratomyxa cardinalis* with a sequence similarity of 97.77 %.

Keywords *Ceratomyxa arabica* · *Acanthopagrus bifasciatus* · Myxozoa

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Introduction

The family Sparidae, often called sea breams, inhabit both tropical and temperate coastal waters (Grandcourt et al. 2004). Most sea breams are excellent food fish and are of great importance to both commercial and recreational fisheries all through their range (Smale and Buxton 1985; Sommer et al. 1996). Of the various species of sea bream, the Doublebar sea bream, *Acanthopagrus bifasciatus* (Forsskål, 1775), is distributed in the western Indian Ocean from the Red Sea and Arabian Gulf to Natal in South Africa. It is found in association with reefs in shallow coastal waters, estuaries and bays (Sommer et al. 1996).

Myxosporeans are a major group of fish parasites whose impact on both wild and cultured fish is significant (Kent et al. 2001; Azevedo et al. 2011; Abdel-Baki et al. 2014). Species of the genus *Ceratomyxa* are important Myxozoa, parasitizing a large range of fish hosts across a wide geographical distribution (Eiras 2006). Although there are 14 valid species of the genus *Acanthopagrus* (Iwatsuki et al. 2010), only three Myxozoa species have so far been described from this genus. These species are *Kudoa iwatai*, *Henneguya ogawai* and *Henneguya yokoyamai*; all of whom have been described from *Acanthopagrus schlegelii* (Matsukane et al. 2011; Li et al. 2012). In the present study, we describe a new myxozoan species, *Ceratomyxa arabica* n. sp., isolated from the gallbladder of *A. bifasciatus*, collected from the Arabian Gulf, Saudi Arabia.

Materials and methods

A total of 112 Doublebar sea bream fish *A. bifasciatus* were collected throughout a period from December 2012 until

Table 1 The relationship between the seasons and the prevalence of *C. arabica* n. sp. infecting the gallbladder of Doublebar *Acanthopagrus bifasciatus* from the Arabian Gulf

Seasons	No. examined fish	No. infected fish	Percent of infection
Spring	28	10	35.7
Summer	28	7	25
Autumn	28	3	10.7
Winter	28	12	42.9
Total	112	32	28.6

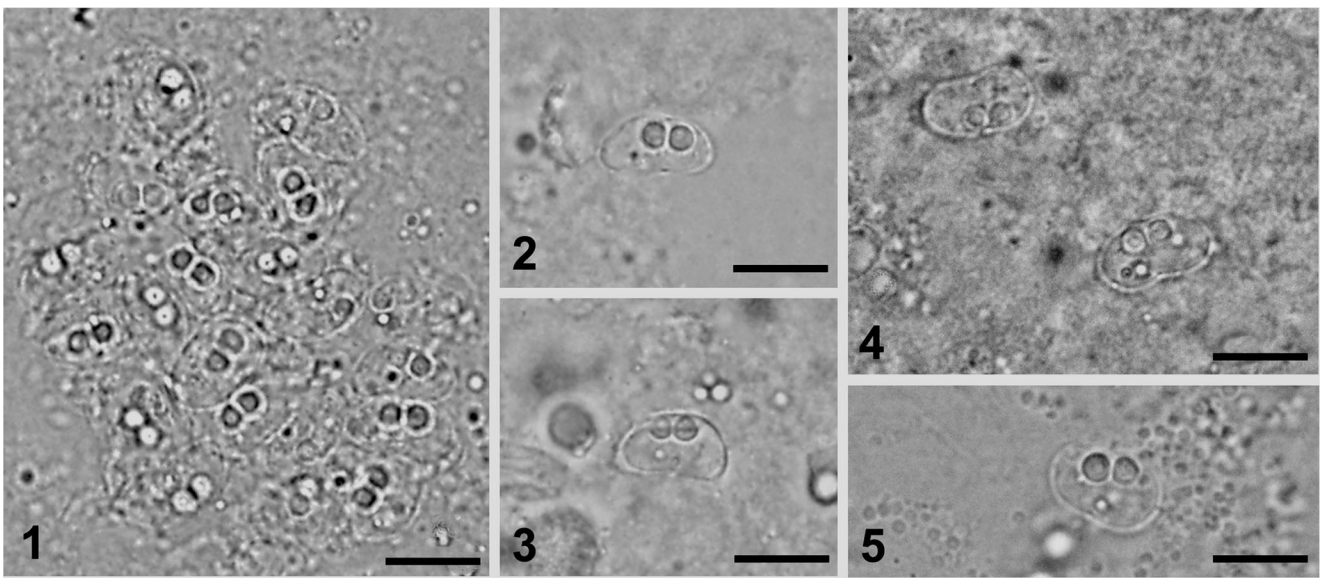
December 2013, from a boat landing site at Dammam on the Arabian Gulf coast of Saudi Arabia (26° 35′ 360″ N, 50° 12′ 816″ E). The fish were necropsied, and the gallbladders were examined microscopically to detect the presence of myxosporean infection. Fresh spores were measured and photographed using an Olympus microscope fitted with a digital camera. Measurements (in micrometres) were taken for 40 fresh spores following the guidelines of Lom and Arthur (1989). The measurements are presented here as mean±SD (range). Some positive gallbladders were maintained in 85 % ethanol for subsequent molecular studies.

DNA extraction

DNA extraction was carried out from 150 μl of the infected bile, maintained in ethanol, using the QIAGEN DNeasy kit (QIAGEN Inc., Valencia, California). The small subunit ribosomal DNA (SSU rDNA) was amplified by the PCR technique, using the two primers MyxospecF and 18R (Fiala 2006; Whipps et al. 2004). PCR reactions were conducted with a final volume of 30 μl with the PCR mixture containing 1x Taq DNA polymerase buffer (MBI, Fermentas), 0.2 mmol of mixed dNTP, 1.5 mmol of $MgCl_2$, 0.2 pmol of each primer, 1 U of Taq DNA polymerase and 50–100 ng of DNA and ultra-pure (MilliQ). The PCR conditions were as follows: initial denaturation for 5 min at 95 °C, 30 cycles of 30 s at 95 °C, 30 s at 52 °C and 2 min at 72 °C, followed by a final extension step for 5 min at 72 °C. Five microlitres of the PCR products were electrophoresed in a 1 % agarose gel and then visualized under a UV trans-illuminator. Purification and sequencing of the PCR product were carried out by Macrogen Inc. (Seoul, South Korea), using the same forward and reverse primers as in PCR amplification together with two additional internal primers, MyxF1338 and MyxR1437 (Mansour et al. 2013), allowing the production of four overlapping sequences of at least 700 bp each. For this study, two PCR products from two different gallbladders were sequenced. The sequences were assembled and edited by visual inspection. A consensus sequence of 1556 bp was submitted to GenBank (accession number KJ631533).

Phylogenetic analysis

Twenty-nine similar SSU rDNA sequences were extracted from GenBank (Table 1) and used for multiple alignments with the ClustalX 2.1.0.12 software (Larkin et al. 2007). The phylogenetic analysis was conducted using version 6 of the MEGA software (Tamura et al. 2013). Relationships between selected sequences were determined using maximum likelihood (ML) and neighbour joining (NJ) methods for a total of 1211 sites. For ML analysis, the parameters used were a GTR model, gamma distribution with invariant sites (G+I), ML heuristic model with nearest-neighbour interchange (NNI), number discrete rates of 5, complete deletion and Kimura 2-parameter model for substitution. The NJ and ML analyses



Figs. 1–5 Fresh spores of *Ceratomyxa arabica* n. sp. from gallbladder of the *Acanthopagrus bifasciatus. Scale bar*=10 μm

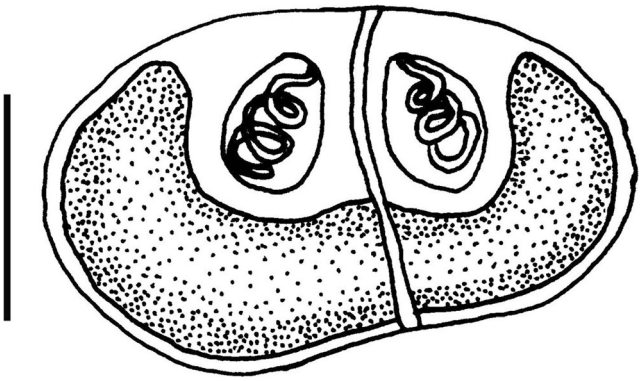


Fig. 6 Schematic drawing of a mature spore of *Ceratomyxa arabica* n. sp. *Scale bar*=5 μm

were performed with 1000 replications. For genetic distance estimation, the matrix was calculated using the Kimura 2-parameter model distance for transition and transversion.

Statistical analysis

One-way ANOVA was carried out, and the statistical comparisons among the seasons were performed with Holm-Sidak method using a statistical package program (Sigma Plot version 11.0). All *P* values are two-tailed, and $P=<0.001$ was considered as significant for all statistical analyses in this study.

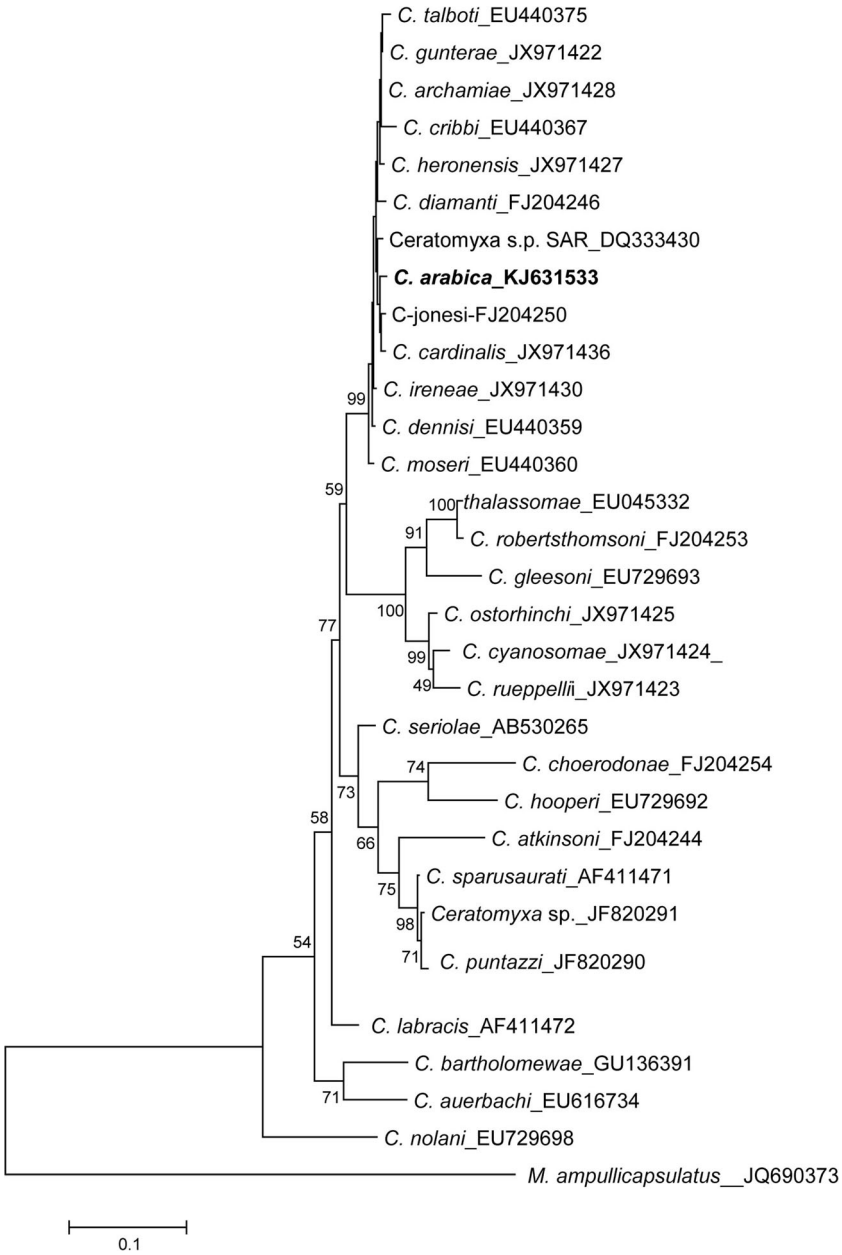


Fig. 7 Maximum likelihood phylogenetic tree obtained from SSU rDNA aligned *Ceratomyxa* sequences showing the position of *Ceratomyxa arabica* n. sp. (in bold). The tree was obtained with the highest log likelihood (−6364.98). Bootstrap values from maximum likelihood analysis are indicated at each node. Accession numbers of each species are shown. *Myxobolus ampullicapsulatus* was used as outgroup. The *scale bar* shows the number of changes per site

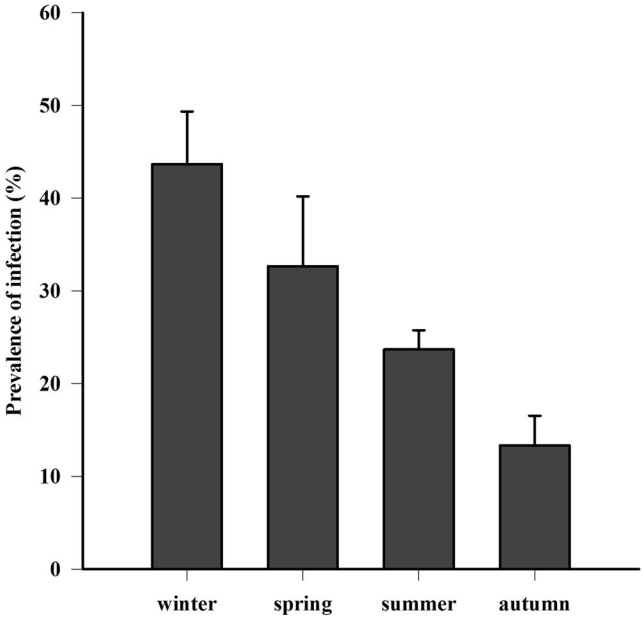


Fig. 8 The relationship between the seasons and the prevalence of *Ceratomyxa arabica* n. sp. infecting *Acanthopagrus bifasciatus* from the Arabian Gulf

Results

The infection was observed as huge number of free-floating spores in the bile (Fig. 1).

Spore description

Mature spores are typical for the genus *Ceratomyxa*: spores are stubby-shaped; valves are unequal, smoothly ovoid in lateral view and convex in the frontal view; the suture line is slightly curved and the sporoplasm almost fills the entire spore cavity (Figs. 1–5 and 6). The spores measure 8±0.4 (7–9) in length and 12±0.4 (10–14) in thickness. The polar capsules are unequal in size, sub-spherical in shape and measure 3±0.3 (2.5–3.5) in length and 2±0.2 (1.5–2.5) in width. The polar filament comprises three turns and is slightly slanted towards the longitudinal axis of the capsules (Fig. 6).

Phylogenetic analysis

A BLASTN search based on the obtained 1506 bp sequence revealed that *C. arabica* has a close association with 11 *Ceratomyxa* species reported in Australia; *Ceratomyxa dennisi* (Gunter & Adlard 2008), *Ceratomyxa ireneae* (Heiniger & Adlard 2013), *Ceratomyxa jonesi* (Gunter et al. 2009), *Ceratomyxa cardinalis* (Heiniger & Adlard 2013), *Ceratomyxa moseri* (Gunter & Adlard 2008), *Ceratomyxa gunterae* (Heiniger & Adlard 2013), *Ceratomyxa archamiae* (Heiniger & Adlard 2013), *Ceratomyxa diamanti* (Gunter et al. 2009), *Ceratomyxa heronensis* (Heiniger & Adlard 2013), *Ceratomyxa talboti* (Gunter & Adlard 2008), *Ceratomyxa cribbi* (Gunter & Adlard 2008) and an unidentified species from the Israeli coast, *Ceratomyxa* sp. SAR. The percentages of similarity between *C. arabica* and these 12 species vary between 97.75 (with *C. cardinalis*) and 96.30 (with *C. cribbi*). In the phylogenetic tree based on the SSU rDNA gene sequence, *C. arabica* clusters with the abovementioned *Ceratomyxa* species in the same clade with a bootstrap support of 99 (Fig. 7)

Prevalence and seasonality

The overall prevalence of infection was 28.6 % (32/112). The highest prevalence was reported in winter, at 42.9 % (12/28), followed by spring, at 35.7 % (10/28), and summer, at 25 % (7/28), while the lowest prevalence was reported in autumn at 10.7 % (3/28) (Table 1, Fig. 8). There was a significant difference in the prevalence of infection between the various seasons ($P<0.001$).

Taxonomic summary

Type host: Doublebar sea bream, *Acanthopagrus bifasciatus* (Forsskål, 1775)

Type locality: Saudi Arabian coast of the Arabian Gulf

Site of infection: Gallbladder

Prevalence: 28.6 % (32/112)

Type-material: Small subunit ribosomal DNA sequence was deposited in GenBank (accession number KJ631533)

Etymology: The specific name refers to the locality Saudi Arabia and the Arab Gulf

Discussion

According to the accessible publications, nine species are within the morphometrical range of the present species (Table 2). These are *C. cardinalis* Heiniger & Adlard 2013 from *Nectamia fucata* in Australia; *Ceratomyxa castigata* Meglitsch, 1960 from *Congiopodus leucopaecilis* in New Zealand; *Ceratomyxa castigastoides* Meglitsch, 1960 from *Pseudolabrus coccineus* in New Zealand; *Ceratomyxa faba* Meglitsch, 1960 from *Caulopsetta scapha* in New Zealand; *C. ireneae* Heiniger & Adlard 2013 in *Archamia fucata* in Australia; *Ceratomyxa minuta* Meglitsch, 1960 from *Thyrsites atun*, *Jordanidia solandri* in New Zealand; *Ceratomyxa obesa* Jameson, 1929 from *Clinocottus analis* in USA; *Ceratomyxa recta* Meglitsch, 1960 from *Genypterus blacodes* in New Zealand and *Ceratomyxa vepallida* Meglitsch, 1960 from *C. scapha* in New Zealand (see Eiras 2006; Heiniger and Adlard 2013) (Table 2).

C. arabica n. sp. can be distinguished from *C. cardinalis*, *C. castigata*, *C. castigastoides*, *C. faba* and *C. obesa* by its

Table 2 Comparative description of *Ceratomyxa arabica* n. sp. with morphologically similar species (measurements in micrometres)

Species	Host	Locality	Spore size	PC size	Spore shape	References
C. cardinalis Heiniger & Adlard 2013	*Nectamia fucata*	Australia (Lizard Island)	5.5 (4.3–7.3)×12.2 (10–14.4)	2.4 (1.9–3.3)×1.9 (1.4–2.6)	SP: slightly crescent shaped Valves: unequal PC: sub-spherical to spherical	Heiniger and Adlard 2013
C. castigata Meglitsch, 1960	*Congiopodus leucopaecilis*	New Zealand (Pacific Ocean)	5.9 (5.1–6.9)×13.1 (9.2–15.3)	2.2 (1.6–3.3)×2 (1.6–2.5)	SP: slightly curved Valves: unequal PC: unequal	Eiras 2006
C. castigastoides Meglitsch, 1960	*Pseudolabrus coccineus*	New Zealand (Pacific Ocean)	5.9 (5.1–7.3)×14.7 (9.8–17.8)	2 (1.8–2.6)	SP: slightly curved PC: equal spherical	Eiras 2006
C. faba Meglitsch, 1960	*Caulopsetta scapha*	New Zealand (Pacific Ocean)	6.2 (5.6–6.7)×12.7 (10.7–14.1)	2.4 (2.0–3.1)	SP: stubby Valves: equal PC: spherical	Eiras 2006
C. ireneae Heiniger & Adlard, 2013	*Zoramia viridiventer*	Australia (Lizard Island)	6.1 (5.2–8.3)×15 (11.4–17.8)	2.7 (2.1–3.5)×2.2 (1.7–3)	SP: slightly crescent Valves: equal PC: sub-spherical to pyriform	Heiniger and Adlard 2013
C. minuta Meglitsch, 1960	*Thyrsites atun*, *Jordanidia solandri*	New Zealand (Pacific Ocean)	5.8 (5–7.9)×11.8 (9.6–14.2)	2.7 (2.4–3.4)×2.4 (2–3.4)	SP: stubby Valves: two equal or unequal PC: oval	Eiras 2006
C. obesa Jameson, 1929	*Clinocottus analis*	USA (Pacific Ocean)	4.5–5.9×12.4–14.8	–	SP: stumpy and sausage-like slightly arched Valves: equal	Eiras 2006
C. recta Meglitsch, 1960	*Genypterus blacodes*	New Zealand (Pacific Ocean)	7.8 (6.8–8.8)×15.6 (14.7–16.7)	2.6 (2–3.4)	SP: oval elongate Valves: equal PC: spherical	Eiras 2006
C. vepallida Meglitsch, 1960	*Caulopsetta scapha*	New Zealand (Pacific Ocean)	8.6 (7.8–9.6)×18.7 (16–21.4)	3.2 (2.9–3.6)×2.9 (2.5–3.2)	SP: stubby Valves: equal to unequal	Eiras 2006
Ceratomyxa arabica n. sp. (present study)	*Acanthopagrus bifasciatus*	Saudi Arabia (Arabian Gulf)	8 (7–9)×12 (10–14)	3 (2.5–3.5)×2 (1.5–2.5)	SP: stubby Valves: unequal PC: sub-spherical to spherical	Present study

PC polar capsule, *SP* spore, *PC* polar capsule

longer spore (7–9 vs 4.3–7.3, 5.1–6.9, 5.1–7.3, 5.6–6.7, 4.5–5.9, respectively). In addition, *C. castigata* has unequal polar capsules, while *C. castigastoides* has smaller polar capsules (1.8–2.6 vs 2.5–3.5) and both *C. faba* and *C. obesa* have equal valves. *C. ireneae* has shorter and thicker spores (5.2–8.3 × 11.4–17.8 vs 7–9 × 10–14) with curved equal valves and pyriform polar capsules. The spores of *C. minuta*, meanwhile, were shorter (5–7.9 vs 7–9), with equal valves and unequal oval polar capsules. *C. recta* and *C. vepallida* differ in having thicker spores with equal valves (14.7–16.7, 16–21.4 vs 10–14, respectively).

Molecular analysis based on the small subunit shows that there is no identical sequence in GenBank to that of this *Ceratomyxa*, infecting the gallbladder of *A. bifasciatus* from the Arabian Gulf. The highest percentage of similarity was 97.77 %, with *C. cardinalis*, which has been reported infecting more than 15 apogonid species belonging to five genera in the Great Barrier Reef in Queensland, Australia. Ten SSU rDNA sequences of *C. cardinalis* from these different host species have been submitted to GenBank. The percentages of differences between these sequences vary between 99.5 and 99.9 %, and the numbers of different nucleotides between these isolates were 1–7 bp. In a pairwise comparison between the SSU rDNA sequence of *C. arabica* and all the submitted sequences of the different *C. cardinalis* isolates, the number of nucleotide differences varied by between 48 and 49 bp. The number of different nucleotides between *C. Arabica* and *C. cardinalis* was, therefore, much higher than between sequences of the same species. The second very similar *Ceratomyxa* SSU rDNA sequence corresponds to the one deposited by Diamant and Lipshitz (direct submission, accession number DQ333430) of an unidentified species of *Ceratomyxa* infecting the rabbitfish *Siganus rivulatus*. Pairwise sequence comparison between *C.* sp. SAR and *C. arabica* shows the existence of 48 different nucleotides and seven gaps and a percentage of similarity of 97.58 %.

Taking into account the morphological and molecular differences with the related species, the present myxozoan is therefore considered to be a new species and the name *C. arabica* is proposed after its locality.

Prevalence and seasonal variation

The infection was encountered throughout the year, with the highest prevalence occurring in winter and the lowest prevalence in autumn. Generally, *Ceratomyxa* spp. are also most prevalent in winter and least prevalent in autumn, including *Ceratomyxa shasta* (Henderickson et al. 1989), *Ceratomyxa buri* and *Ceratomyxa seriolae* (Yokoyama & Fukuda 2001), *Ceratomyxa puntazzi* (Alama-Bermejo et al. 2013) and *Ceratomyxa hamour* (Mansour et al. 2014). Some myxozoans belonging to other genera also follow the same pattern of prevalence, for example, *Henneguya ghaffari* (Abdel-Baki et al. 2014). Conversely, however, other myxozoan species have demonstrated a higher prevalence in summer and a lower prevalence during other seasons (Yokoyama et al. 2005; Abdel-Baki et al. 2011; Yemmen et al. 2013).

Authors have variously suggested that the seasonal and annual patterns of myxosporean infection may be due to the endogenous cycles of the parasites, the availability of susceptible hosts or the effects of environmental factors (Foott and Hedrick 1987; Yokoyama and Fukuda 2001). In the case of *Ceratomyxa*, the seasonal fluctuation in prevalence may be due to the variable condition of bile secretion at each sampling period (Yokoyama and Fukuda 2001).

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