Saudi Journal of Biological Sciences xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Morphometeric 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

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ARTICLE INFO

Article history:
Received 30 October 2017
Revised 22 November 2017
Accepted 3 December 2017
Available online xxxx

Keywords:
Myxosporea
Coelozoic
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 × 17 μm. Mature spores were mostly elliptical with symmetrical valves and equal spherical polar capsules. Spores were 9 μm in length and 25 μm in thickness, while polar capsules were 4 μm 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.

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

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2017.12.001
1319-562X/

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.

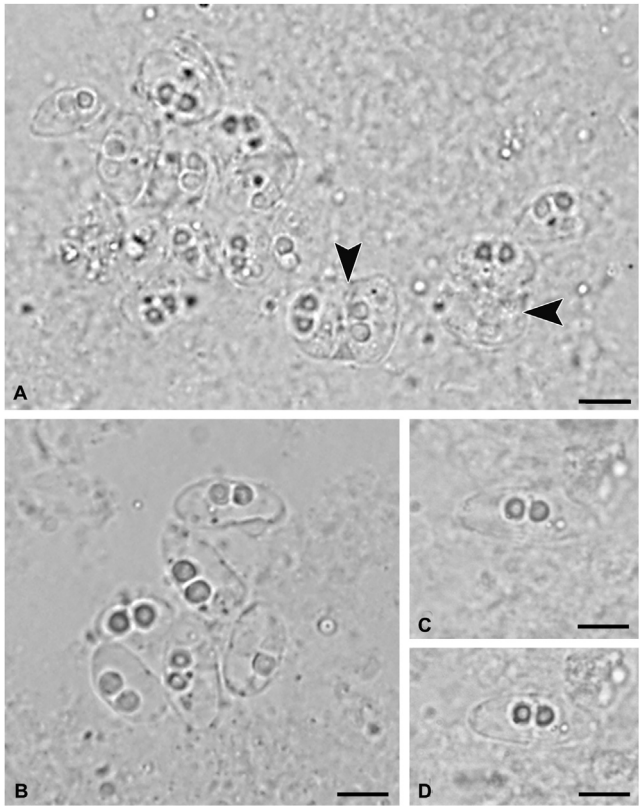

Fig. 1. Fresh spores of *Ceratomyxa sultani* n. sp. from the gall bladder of *Upeneus margarethae*. Arrowheads referring to the disporic pseudoplasmodia. Scale-bars = 10 μm.

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 ± 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 AACTWGTTG 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).

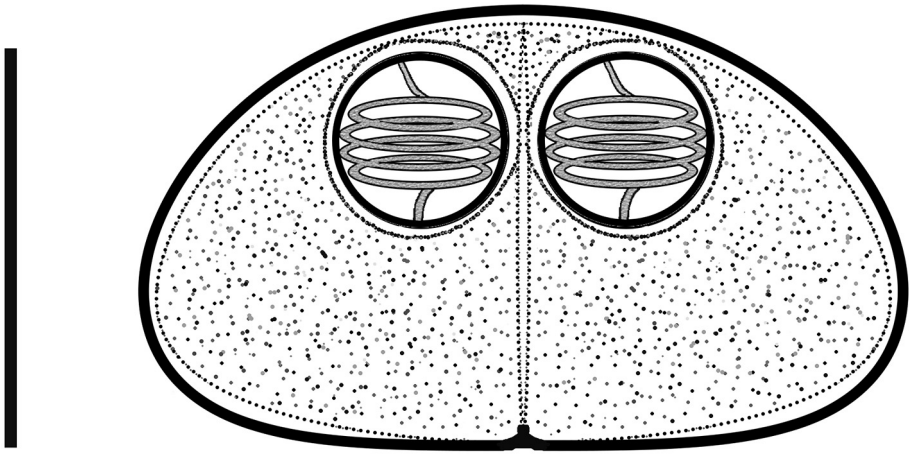

Fig. 2. Line drawing of a mature spore of *Ceratomyxa sultani* n. sp. from the gall bladder of *Upeneus margarethae*. Scale-bars = 10 μm.

Please cite this article in press as: Abdel-Baki, A.-A.S., et al. Morphometeric 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 prevalenceCeratomyxa sultani n. sp. –>. Saudi Journal of Biological Sciences (2017), https://doi.org/10.1016/j.sjbs.2017.12.001