Parasitology International 65 (2016) 303–307

Contents lists available at ScienceDirect

Parasitology International

journal homepage: www.elsevier.com/locate/parint

Morphological and molecular characterization of *Thelohanellus macrovacuolaris* n. sp. (Myxosporea: Bivalvulida) infecting the palate in the mouth of common carp *Cyprinus carpio* L. in China

Yang Liu, Yanhua Zhai, Zemao Gu *

[a] Department of Aquatic Animal Medicine, College of Fisheries, Huazhong Agricultural University, Wuhan 430070, People's Republic of China
[b] Key Lab of Freshwater Animal Breeding, Ministry of Agriculture, Wuhan 430070, People's Republic of China
[c] Freshwater Aquaculture Collaborative Innovation Center of Hubei Province, Wuhan 430070, People's Republic of China

ARTICLE INFO

Article history:
Received 15 December 2015
Received in revised form 23 February 2016
Accepted 25 February 2016
Available online 3 March 2016

Keywords:
Thelohanellus macrovacuolaris n. sp.
Myxosporean
Cyprinus carpio
Mouth
Morphology
ssrRNA

ABSTRACT

Thelohanellus macrovacuolaris n. sp. is described during a survey on myxozoan diversity of common carp *Cyprinus carpio* L. in China. It is characterized by the presence of round or ellipsoidal plasmodium in the palate in the mouth of host. Mature spores were pyriform in frontal view, lemon shaped in lateral view, measuring 21.6 ± 0.9 (19.3–23.8) long, 12.5 ± 0.7 (10.3–13.6) wide, and 10.2 ± 0.4 (9.8–11.8) thick. Most spores were surrounded by the membrane sheath. Single polar capsule was round with an apophysis at its top end presented close to apex of spore, measuring 9.1 ± 0.6 (8.0–10.0) in length, 8.6 ± 0.5 (7.8–9.6) in width. Polar filaments coiled, with 7 to 8 turns. A large, round iodinophilous vacuole was present, with 5.8–7.5 in diameter. The present species is morphologically distinct from all other *Thelohanellus* species. The BLAST search indicated that the newly obtained small subunit ribosomal RNA (ssrRNA) gene sequence of *T. macrovacuolaris* n. sp. did not match any available sequences in GenBank. Phylogenetically, *T. macrovacuolaris* n. sp. was placed sister to *Thelohanellus wangi* in the *Thelohanellus* clade. Both morphology and ssrRNA gene sequence data revealed that the present parasite is a new species of genus *Thelohanellus*.

1. Introduction

Myxosporeans are an economically important group of metazoan and spore-forming endoparasites that mainly infect fish with a few representatives infecting amphibians, reptiles, birds and mammals [1]. Throughout the world, approximately 2200 myxosporean species have been reported [1]. Owing to the technical limitations of the past, most of species were identified solely based on spore morphology [2]. Given the incredible diversity coupled with the simplicity of the diagnostic stage of these species, it is often difficult to determine the validity of morphologically similar species using spore morphology alone [3]. As such, a large number of misidentified and cryptic species exist in the reported morphologically identified species and the present species diversity of myxosporeans is not reliable. Fortunately, with the increasing knowledge of myxosporeans, molecular and histologic characteristics (host, organ and tissue specificity) become a sharp blade in myxosporean classification and a number of misidentification and confusions are resolved [4]. At present, parasitologists from various countries are carrying out a large range of myxosporean identification to improve myxosporean classification relying on the crucial gauge including morphology, histologic, and molecular data [5–6].

Common carp *Cyprinus carpio* L. is an economically important fish in China. It has been cultured for more than 4000 years, and its annual product has increased to more than 300 million kg in China in 2013 [7]. Given that this fish is demersal and omnivorous, it is likely to occupy habitats similar to where the alternate host is found and therefore increased likelihood of contact with the actinospore stage [8]. Until now, approximately 83 myxosporeans have been found from different tissues and organs of common carp in China [2]. Although most of the species do not cause severe diseases, some species have been reported as pathogenic species resulting in mass mortality or loss of economic value of common carp [6,9]. Minimizing the severe negative impact of the myxosporidiosis on this commercially important species has been considered a priority in China.

In order to generate baseline data on the myxosporean diversity in common carp, a survey of these parasites in China is conducted. Here, a novel *Thelohanellus* species infecting the palate in the mouth of common carp was described, and data were presented on spore morphology and molecular characteristics.

* Corresponding author at: Department of Aquatic Animal Medicine, College of Fisheries, Huazhong Agricultural University, Wuhan 430070, People's Republic of China.
E-mail address: guzemao@mail.hzau.edu.cn (Z. Gu).

http://dx.doi.org/10.1016/j.parint.2016.02.013
1383-5769/

2. Materials and methods

2.1. Fish samples

Twenty specimens of common carp with 13.6 ± 0.7 (12.5–15.0) cm in total length were harvested by a fine-meshed seine from the aquaculture facility in College of Fisheries, Huazhong Agricultural University, Wuhan City, Hubei Province, China in July 2013. Fish were transported to the Laboratory of Fish Diseases in College of Fisheries, Huazhong Agricultural University, Wuhan City, Hubei Province and held in aquaria, where they were euthanized with 0.2 mg/ml tricaine methanesulfonate (MS-222, Sigma) prior to dissection.

2.2. Morphological methods

Gross microscopic examinations of all organs for myxosporean infections were conducted according to Lom and Dyková [10] within 24 h after transportation. A plasmodium containing myxospores consistent with those of the genus *Thelohanellus* were collected from the palate of common carp. Fresh spores were measured according to Lom and Arthur [11]. Measurements of spores were performed using an Olympus BH2 microscope equipped with an ocular micrometer. Mean and standard deviations of each spore dimension were obtained from fresh mature spores (n = 30). Digitized images were obtained from the fresh wet mounts by a Nikon Eclipse 80i microscope. Line drawings were made based on the digitized images. All measurements are given in micrometers (μm) unless otherwise indicated.

2.3. DNA isolation and sequencing

Genomic DNA was extracted using BioTeke™ Genomic DNA extraction kit (BioTeke Co., Ltd., Beijing) according to the manufacturer's protocol. PCR amplification of the small subunit ribosomal RNA (ssrRNA) gene was performed with primers SphF (ACTCGTTGGTAAGGTAGTGG CT)/SphR (GTTACCATTGTAGCGCGCGT) [12] in a 50 μl volume, which contained approximate 200 ng of extracted genomic DNA, 1 × *Taq* Buffer (MBI Fermentas, Vilnius, Lithuania), 2.5 mM $MgCl_2$, 0.2 mM dNTPs (MBI Fermentas), 2 μM each primer, and 2.5 U of Taq DNA polymerase (MBI Fermentas) in MilliQ purified water. An EDC-810 DNA Engine (EastWin Bio., Co., Ltd., Beijing) was used to control the cycling conditions: 94 °C for 50 s, 55 °C for 50 s, and 72 °C for 60 s for 35 cycles, with an initial denaturation at 94 °C for 7 min, and a terminal extension at 72 °C for 10 min. The PCR products were purified using the AxyPrep™ DNA Gel Extraction Kit (AxyGen Bio., Co., Ltd., Hangzhou) and sequenced directly using the primers described above for PCR amplification in both directions on the ABI PRISM® 3730XL DNA sequencer (Applied Biosystems Inc., Foster City, CA, USA). Forward and reverse sequence segments were aligned and a contiguous sequence was deposited in GenBank. A standard nucleotide-nucleotide BLAST search was conducted to query posted sequences.

2.4. Phylogenetic analysis

Sequences were assembled in BioEdit [13], and verified as myxozoan by GenBank BLAST search. To evaluate the relationship of the current species to existing myxobolids, 51 sequences were aligned with Clustal X version 1.8 [14]. The alignment consisted of the top BLAST search matches and representatives of neighboring clades based on earlier analyses of the myxobolids [15–16]. *Ceratonova shasta* were designated as outgroup taxa. Phylogenetic analyses were carried out on this 1349 character alignment as follows. Optimal evolutionary models for maximum likelihood (ML) and Bayesian analysis were determined using jModeltest [17] which identified the optimal evolutionary model using the Akaike information criteria, as the general time reversible model (GTR + I + G). Nucleotide frequencies were estimated from the data (A = 0.2409, C = 0.1863, G = 0.2908, T = 0.2820), six rates of nucleotide substitution were [AC] = 1.0759, [AG] = 3.3720, [AT] = 1.7214, [CG] = 0.6472, [CT] = 6.2484, [GT] = 1.0000; proportion of invariable sites = 0.1274; gamma distribution = 0.3581 estimated with six rate categories. ML analysis was performed using PhyML 3.0 online (http://www.atgc-montpellier.fr/phyml/) [18]. Bootstrap confidence values were calculated with 100 replicates. Bayesian analyses were conducted in Mr. Bayes [19] using the evolutionary model as above, with 10^6 generations, tree sampling every 100 generations, with a burn-in of 2500 trees. Trees were initially examined in TreeView X [20] and edited and annotated in Adobe Illustrator (Adobe Systems Inc. San Jose, CA).

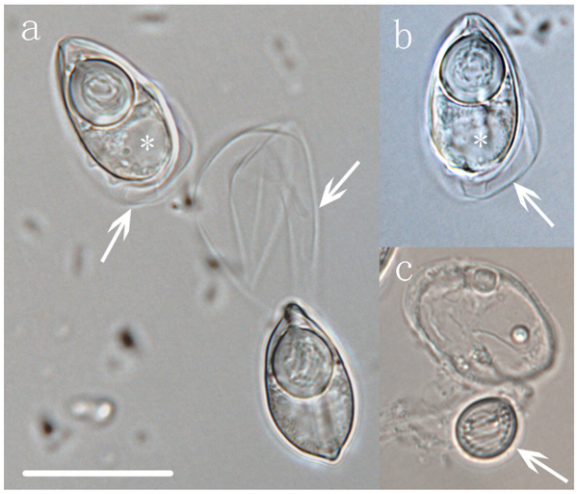

Fig. 1. Photomicrograph of fresh spores of *Thelohanellus macrovacuolaris* n. sp. (a, b), c showing the shedding polar capsule (arrow), arrows in a, b showing the membrane sheath. Scale bar 20 μm.

3. Results

3.1. Description

Thelohanellus macrovacuolaris n. sp.: Plasmodium round or ellipsoidal, 2.9 mm in diameter, histozoic in the palate. Myxospores

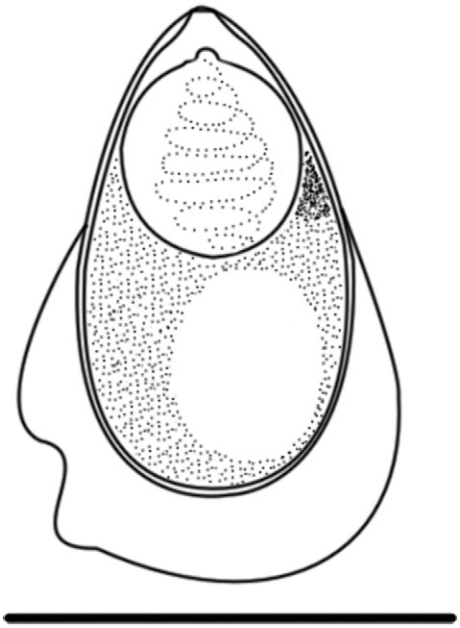

Fig. 2. Line drawing of fresh spores of *Thelohanellus macrovacuolaris* n. sp. Scale bar 20 μm.