한국어병학회지 제36권 제2호 (2023)
J. Fish Pathol., 36(2) : 395~401

www.ksfp.org
pISSN 1226-0819, eISSN 2233-5412
http://dx.doi.org/10.7847/jfp.2023.36.2.395

Note

First report of gill thelohanellosis from common carp (*Cyprinus carpio*) fingerling in Korea

Mariem BESSAID and Ki Hong Kim*

Department of Aquatic Life Medicine, Pukyong National University, Busan 48513, South Korea

Myxosporeans are widespread cnidarian endoparasites in marine and freshwater ecosystems and several species were reported to be a threat to cultured fish causing serious diseases with mass mortality. In the present study, we found a myxosporean species in the genus *Thelohanellus* from the gills of the cultured common carp (*Cyprinus carpio*) fingerling for the first time in Korea. The morphological observation showed 500 μm ~ 1 mm size, oval to circular shaped plasmodia containing spores which are pyriform at the anterior end and round at the posterior end (average size 20.1 μm × 9.1 μm), with 5 to 6 turns of a single polar filament located in the polar capsule with an average size of 10 μm × 4.6 μm. The 18S rRNA sequence was closest to the sequence of *T. wangi* among *Thelohanellus* species infecting gills but was not completely identical. Based on the morphological characteristics and molecular analysis results, we classified the present myxosporean parasite as *Thelohanellus* sp., temporarily. The prevalence and infection intensity of *Thelohanellus* sp. in the common carp fingerling were very high, which was thought to be the main cause of high mortality.

Key words: Gill Thelohanellosis, *Cyprinus carpio, Thelohanellus* sp.

Introduction

Myxosporean parasites have been reported to be the causative agents of several diseases that can lead to high mortality and severe morbidity in both wild and cultured fish worldwide (Kent et al.,2001; Paul et al., 2020). The damage caused by myxosporean infection ranges from no symptoms to fatal, according to infection intensity and infection sites (Sanaullah and Ahmed, 1980; Sanyal et al., 2018; Markiw and Wolf, 1983). Myxosporean gill diseases often lead to the death of infected fish due to the induction of respiratory difficulty. Several myxosporean genera, such as *Myxobolus*, *Henneguya*, *Kudoa*, *Thelohanellus*, etc., are reported to cause gill myxoboliasis in fish (Sanyal et al., 2018; Qadri, 1962; Kaur, 2014).

Common carp (*Cyprinus carpio*) is one of the main cultured fish species in the world, especially in Europe and Asia (Kloskowski, 2011; Rahman, 2015; Karnai and Szucs, 2018). Although the aquaculture production of carp in Korea has decreased due to the prohibition of cage culture in lakes and reservoirs since the late 1990s, common carp is still one of the major cultured freshwater fish species in Korea.

Recently, we purchased common carp fingerlings from a local farm and experienced more than 90% mortality within one week. To know whether the death was caused by infectious diseases, we examined the presence of viruses, bacteria, and parasites. No trace of viruses or bacteria has been found in fish samples, but multiple species of parasites were observed from different parts of the fish. Almost all fish had large numbers of cysts containing myxosporeans

†Corresponding author: Ki Hong Kim
Tel: +82-51-629-5943, Fax: +82-51-629-5938
E-mail: khkim@pknu.ac.kr

belonging to the genus *Thelohanellus* in the gill filaments. *Thelohanellus* species are considered one of the most common myxosporeans infecting freshwater fish, and the majority of the described species infect cyprinid fishes, especially in India and China (Zhai et al., 2016; Zhang et al., 2013).

In Korea, as there is no record of the gill thelohanellosis in carp, we conducted identification of the present gill myxosporean based on the mature spore morphology and 18S ribosomal RNA gene sequence, through which we classified the present myxosporean parasite as *Thelohanellus* sp., temporarily. Besides *Thelohanellus* sp., most fish were infected with a ciliate parasite, *Ichthyophthirius multifiliis*, a monogenean parasite, *Dactylogyrus* sp., and a cestode parasite, *Bothriocephalus acheilognathi*.

Material and methods

Fish and sampling

During the period of June 2023, common carp fingerlings (average weight: 7.85 g) were obtained from a local aquaculture farm located in Andong, South Korea. After verifying free from viral (spring viremia carp virus and koi herpes virus) and bacterial infections, thirty fish were randomly sampled and checked for the presence of parasites. Different parts of the body including skin, fins, gills, and internal organs were dissected and examined under the microscope. The isolated parasites such as ciliates, monogeneans, and cestodes were immediately mounted on glass slides for identification. In the case of gill myxosporeans, spores were mounted on glass slips for observation or fixed in absolute ethanol and stored at -20°C for molecular identification.

Morphological investigation and identification

The morphological identification of the myxosporean parasite and other parasites was carried out using an Olympus Bx51 light microscope and the measurements were done with DP2-BSW software (Olympus).

The identification of species of *Thelohanellus* was done based on the guidelines of Lom and Arthur (1989). Plasmodia were extracted from the gill filaments and exploded by a syringe needle on a glass slip to liberate the spores. Fresh spores were smeared and directly observed under the microscope or permanently mounted via staining with Giemsa solution. Briefly, fresh extracted plasmodia from gills were smeared on a glass slide, air-dried then fixed with methanol. The staining was done with a 10% Giemsa solution (diluted in PBS) for 40 min, then rinsed with distilled water, air-dried, then mounted in a drop of malinol (Sigma).

For the identification of ciliates and monogeneans, fresh smears of infected gills were made on a glass slip or stained with Giemsa's solution, then observed by microscope.

Molecular identification of species of *Thelohanellus*

Thelohanellus plasmodia preserved in absolute ethanol were briefly centrifuged to remove the ethanol and washed with phosphate-buffered saline (PBS) to remove the excess of the conservative. The genomic DNA (gDNA) was extracted from the pelleted spores using Exgene Clinic SV mini-Kit (GeneAll) according to the manufacturer's instructions. The extracted gDNA was used as the template for PCR using specific primers set to amplify the 18S rRNA gene (Table 1). After analysis of the PCR product on an electrophoresis gel, the amplicon was purified, cloned into pGEM-T Easy plasmid (Promega), then sequenced with sequencing primers (Székely, 2015) (Table 1). The obtained sequence was analyzed using the basic local alignment tool (blast) in NCBI GenBank.

Results and Discussion

In the present study, all the examined fish had plasmodia of the myxosporean belonging to the genus *Thelohanellus*. The plasmodia were whitish in color,

Table 1. Primers used for PCR and sequencing

Primers	Sequence
For PCR	
Thw-18S-F (Present study)	CTGGTTGATTCTGCCAGTCAATCAAG
Thw-18S-R (Present study)	TCTACGGAAACCTTGTTACGACTTTTACTTC
For sequencing	
Thw-18S-F (Present study)	CTGGTTGATTCTGCCAGTCAATCAAG
CR1F (Székely, 2015)	CGAAGACGATCAGATACCGTCCTAG

oval to round, and measured around 500 to 1 μm in size. A large number of plasmodia were found in all the gill filaments of each gill arch and were the intra-filamental-cartilage type (Type II) (Molnár, 2002) (Fig. 1a). The mature spores were pyriform or oval, elongated in the upper part and round at the inferior part, with a length ranging from 17.6 to 24.0 μm (average 20.1 μm) and width from 8.1 to 10.0 μm (average 9.1 μm). The polar capsule of the mature spore was almond-shaped, occupying almost half the size of the spore, with a length between 9.2 to 11.2 μm (average 10.0 μm) and a width between 4.1 to 5.8 μm (average 4.6 μm) with a single polar filament with 5 to 6 spirals (Fig. 1b), that were spotted extruded on several occasion (Fig. 1d). The two sporoplasmic nuclei were positioned lateral to the iodino-

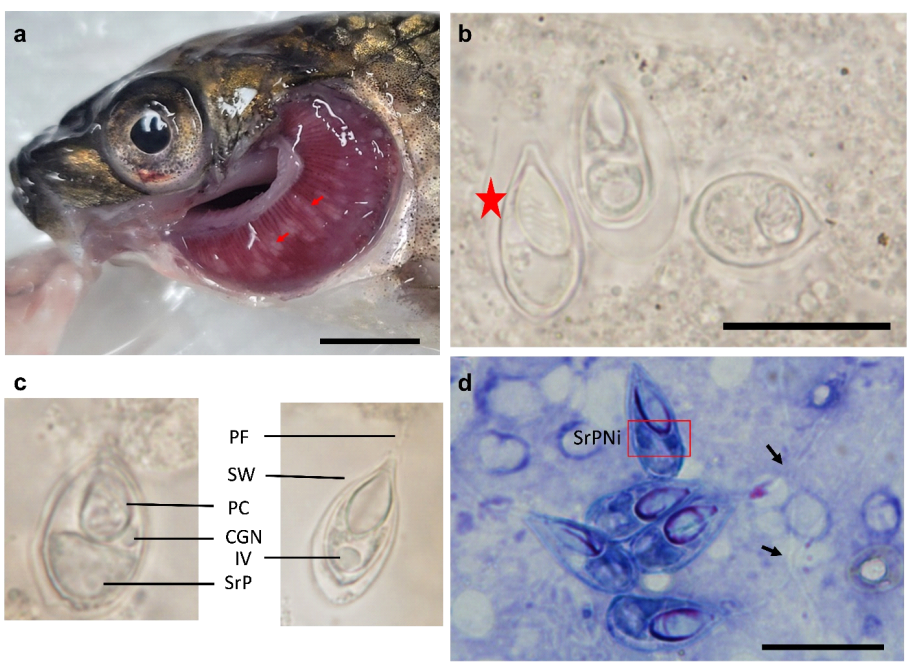

Fig. 1. *Thelohanellus* sp. plasmodia and spores from the gill filaments of *Cyprinus carpio* (a) *Cyprinus carpio* gills with the intra-filamentary plasmodia (red arrows); scale bar: 500 mm. (b) fresh spores showing the coiled polar filament (the spore labeled with the red star); scale bar: 20 μm. (c) fresh spore: detailed description; PF: polar filament, SW: spore wall, PC: polar capsule, CGN: capsulogenous nucleus, IV: iodinophilous vacuole, SrP: sporoplasm. (d) Spores stained with Giemsa showing extruded polar filament (black arrows) and the sporoplasmic nuclei (SrPNi); scale bar: 20 μm.

philous vacuole in the sporoplasm, and the capsulogenous nucleus was observed near the lateral posterior end of the polar capsule (Fig. 1c).

The morphological characteristics of the present *Thelohanellus* compared with other *Thelohanellus* species infecting fish gills showed overlapped morphometrics of mature spores (Table 2). The sequence of the 18S rRNA gene was closest to that of *Thelohanellus wangi* which was previously reported from the gill of gibel carp (*Carassius auratus gibelio*) in China, but the identity was 92.27% (Fig. 2). Based on these results, we classified the present myxosporean parasite as *Thelohanellus* sp., temporarily.

In addition, the microscopic observation of the gill smear showed the presence of a monogenean species identified as *Dactylogyrus* sp. and trophonts of *I. multifiliis*. In the intestine, cestodes identified as *Bothriocephalus acheilognathi* were isolated. However, the prevalence of those three parasites was not high (Table 3).

Considering the life cycle of myxosporeans, organisms that can produce actinosporeans of *Thelohanel-*

Table 2. Comparison of morp\hological parameters of *Thelohanellus* sp. with other *Thelohanellus* species infecting gills of cyprinid fish

Species	Host	localization	Plasmodium and spore
Thelohanellus sp. present study	*Cyprinus carpio*	South Korea	P: 0.5-1 SP: 17.6-24 x 8.1-10.03 PC: 9.23-11.21 x 4.06-5.8
T. anilae (Hemananda, 2010)	*Labeo rohita*	India	SP: 12-13.5 x 6.8 PC: 6.8-7.6 x 2.5-3.4
T. bifurcata (Basu & Haldar, 1999) (Ref, Kaur, 2014)	*Labeo rohita*	India	P: 1.3-1.4 x 0.7-0.8 SP: 33.1-34.1 x 8.4-9.4 PC: 18.0-18.2 x 5.3-6.3
T. callisporis (Ky, 1971)	*Cyprinus carpio carpio*	Vietnam	SP: 23.4-25.3 x 12.6-16.2 PC: 10.8 X 7.2-8.1
T. fili (Kaur *et al.*, 2014)	*Labeo rohita*	India	SP: 27.08 x 10.56 PC: 16.63 x 8.25
T. kynom (Ky, 1971)	*Cyprinus carpio carpio*	Vietnam	SP: 19.8-21.6 X 7.2-8.1 PC: 10.8-14.4 x 4.5-5.4
T. pyriformis (Thelohan, 1892) (Ref, Kudo, 1933)	*Tinca tinca*, *Misgurnus fossilis* and *Rutilus rutilus*	France Russia	SP: 14.9-22 x 6.1-9.7 PC: 5.1-10.6 x 2.3-4.5
T. toyamai (Kudo, 1915) (Griffin and Goodwin, 2011)	*Cyprinus carpio carpio*	USA	P: 0.2 SP: 14.7-16.8 x 5.4-6 PC: 5.8-7.2
T. wangi n. sp. (Yuan *et al.*, 2015)	*Carassius auratus gibelio*	China	P: 0.5-1.5 SP: 16.5-22.3 x 9.1-10.8 PC: 8.4-11.2 x 6.1-7
T. wuhanensis (Xiao & Chen, 1993) (Ref, Abdul-Ameer and Obaid, 2021)	*Carassius auratus*	Iraq	P: 0.2-1.7 SP: 21.9-26.9 x 11.4-15.5 PC: 9.6-12.8 x 8.1-10.3 [same measurements as mentioned in Xiao & Chen, 1993]

P, plasmodium (mm); SP, spore (μm); PC, polar capsule (μm), Ref, Reference

Fig. 2. Alignment of 18S rRNA sequence of the present *Thelohanellus* sp. with other *Thelohanellus* species parasitizing fish gills. *Thelohanellus* sp, present study; *Thelohanellus wangi* isolate JZ-2012 (GenBank, JX458816.1); *Thelohanellus bifurcata* isolate R1 (GenBank, ON820188.1); *Thelohanellus filli* ranjit sagar wetland isolate (GenBank, KR340464.1); *Thelohanellus toyamai* (GenBank, HQ338729.1); *Thelohanellus wuhanensis* isolate 6-1 (GenBank, JQ690370.1).

lus sp. might exist in the hatchery. As effective therapeutic measures are not developed against myxosporean diseases, endeavors to find and remove vector organisms in and around hatcheries and farms are required. Furthermore, the simultaneous occurrence of intestinal cestode, *B. acheilognathi*, which needs in-

Table 3. Prevalence of parasites isolated in the present study

Parasite	Prevalence (%)
Thelohanellus wangi	100% (30/30)
Ichthyophtirius multifiliis	16.7% (6/30)
Dactylogyrus sp.	50% (15/30)
Bothriocephalus acheilognathi	36.7% (11/30)

termediate hosts for the completion of the life cycle, also indicates that environmental control measures to stop the life cycle should be taken.

In conclusion, in this study, we first report gill thelohanellosis from common carp in Korea, and the high prevalence with the high infection intensity of *Thelohanellus* sp. was thought to be the main cause of the high mortality. Concurrent infection with other protozoan and metazoan parasites would also be involved in the cause of the high mortality, and effective control measures against multiple parasitic species infection are urgently needed.

Acknowledgments

This work was supported by a Research Grant of Pukyong National University (2023).

References

Abdul-Amir, K. N. and Obaid, A. S.: First record of *Thelohanellus wuhanensis* Xiao & Chen, 1993 (Myxozoa: Myxosporea) in Iraq on the gills of *Carassius auratus* (Linnaeus, 1758). J Phys: Conf Ser., 1879:022052, 2021. DOI:10.1088/1742-6596/1879/2/022052.

Griffin, M. J. and Goodwin, A.E.: *Thelohanellus toyamai* (Syn. *Myxobolus toyamai*) Infecting the Gills of Koi *Cyprinus carpio* in the Eastern United States. J Parasitol., 97:493–502, 2011. DOI: 10.1645/GE-2674.1.

Hemananda, T., Bandyopadhyay, P., Mohilal, N. and Mitra, A. K.: On the occurrence of a myxozoan parasite, *Thelohanellus anilae* sp. N. From a freshwater fish of India. Anim Biol., 60:329-336, 2010. DOI:10.1163/157075610X516538.

Karnai, L. and Szucs, I.: Outlooks and perspectives of the common carp production. Ann. Pol. Assoc. Agric. Agribus. Econ., XX:64-72, 2018. DOI:10.5604/01.3001.0011.7230.

Kaur, H.: Gill Disease Caused by *Thelohanellus bifurcata* Basu and Haldar, 1999 a Pathogenic Myxozoan Parasite in Cultured Indian Carp, *Labeo rohita* (Hamilton, 1822) in Punjab, India. J. Anim. Heal. Prod., 2:19-24, 2014. DOI:10.14737/journal.jahp/2014/2.2.19.24.

Kaur, H., Katoch, A. and Gupta, M.: *Thelohanellus filli* sp.n., a pathogenic myxosporean infecting gills of cultured carp, *Labeo rohita* (Hamilton 1822) in Punjab, India. Species, 10:31-38, 2014.

Kent, M.L., Andree, K. B., Bartholomew, J. L., El-Matbouli, M., Desser, S. S. and Devlin, R. H.: Recent Advances in Our Knowledge of Myxozoa. J. Eukaryot. Microbiol., 48:395-413, 2001. DOI: 10.1111/j.1550-7408.2001.tb00173.x.

Kloskowski, J.: Impact of common carp *Cyprinus carpio* on aquatic communities: direct trophic effects versus habitat deterioration. Fundam. Appl. Limnol. Arch. Für. Hydrobiol., 178:245-255, 2011. DOI:10.1127/1863-9135/2011/0178-0245.

Kudo, R. R.: A taxonomic consideration of Myxosporidia. Trans. Am. Micros. Soc. 52: 195-216, 1933. DOI:10.2307/3222254.

Ky, H.: Some myxosporidians of freshwater fishes of the North Vietnam. Acta Protozool., 8:283-298, 1971.

Markiw, M. E. and Wolf, K.: *Myxosoma cerebralis* (Myxozoa: Myxosporea) Etiologic Agent of Salmonid Whirling Disease Requires Tubificid Worm (Annelida: Oligochaeta) in its Life Cycle. J. Protozool., 30:561-564, 1983. DOI:10.1111/j.1550-7408.1983.tb01422.x.

Molnár, K.: Site preference of fish myxosporeans in the gill. Dis. Aquat. Org., 48:197-207, 2002. DOI: 10.3354/dao048197.

Paul, A., Pattanayak, S., Sahoo, M. K., Kumar, P. R., Kumar, R. and Sahoo, P. K.: Co-infection of bacterial and parasitic pathogens including the brain myxosporean *Zschokkella auratis* in farmed striped murrel *Channa striata* (Bloch, 1793) causing large scale mortality. Indian. J. Fish., 67, 105-115, 2020. DOI:10.21077/ijf.2020.67.4.94943-13.

Pavlásková, M. and Strelkov, O. N.: First record of *Sphaerospora renicola* Dyková et Lom, 1982 and *Myxobolus encephalicus* Mulsow, 1911, pathogenic protozoans of carp from the U.S.S.R. Folia. Parasitol., 34:285-286, 1987.

Qadri, S. S.: New myxosporidia from indian freshwater fish, *Labeo fimbriatus*. Z. für. Parasitenkd., 21:517–520, 1962. DOI:10.1007/BF00260255.

Rahman, M.: Effects of co-cultured common carp on nutrients and food web dynamics in rohu aquaculture ponds. Aquac. Environ. Interact., 6:223-232, 2015. DOI:10.3354/aei00127.

Sanaullah, M. and Ahmed. A. T. A.: Gill myxoboliasis of major carps in Bangladesh. J. Fish. Dis., 3:349-354,1980. DOI:10.1111/j.1365-2761.1980.tb00404.x.

Sanyal, K. B., Mukherjee, D. and Dash, G.: Isolation and identification of different parasites from Indian major carps and exotic carps from South 24-Parganas, West Bengal. Indian. J. Anim. Sci., 88:979-984, 2018. DOI:10.56093/ijans.v88i8.82970.

Székely, C., Molnár, K. and Cech, G.: Description of *Myxobolus balatonicus n. Sp.* (Myxozoa: Myxobolidae) from the common carp *Cyprinus carpio* L. In Lake Balaton. Syst. Parasitol., 91:71-79, 2015. DOI:10.1007/s11230-015-9560-5.

Yuan, S., Xi, B. W., Wang, J. G., Xie, J. and Zhang, J. Y.: *Thelohanellus wangi n. Sp.* (Myxozoa, Myxosporea), a new gill parasite of allogynogenetic gibel carp (*Carassius auratus gibelio* Bloch) in China, causing severe gill myxosporidiosis. Parasitol. Res., 114:37-45, 2015. DOI:10.1007/s00436-014-4157-5.

Zhai, Y., Gu, Z., Guo, Q., Wu, Z., Wang, H. and Liu, Y.: New type of pathogenicity of *Thelohanellus kitauei* Egusa & Nakajima, 1981 infecting the skin of common carp *Cyprinus carpio* L. Parasitol. Int., 65:78-82, 2016. DOI:10.1016/j.parint.2015.10.010.

Zhang, J. Y., Gu, Z. M., Kalavati, C., Eiras, J. C., Liu, Y. and Guo, Q. Y.: Synopsis of the species of *Thelohanellus* Kudo, 1933 (Myxozoa: Myxosporea: Bivalvulida). Syst. Parasitol., 86:235-256, 2013. DOI:10.1007/s11230-013-9449-0.

Manuscript Received : Sep 04 2023
Revised : Sep 22 2023
Accepted : Sep 26 2023