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FULL PAPER

Ong-ard Lawhavinit · Kanit Chukanhom · Kishio Hatai

Effect of *Tetrahymena* on the occurrence of achlyosis in the guppy

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Abstract *Tetrahymena* infection has become the most problematic parasitic disease of the guppy *Poecilia reticulata* in Southeast Asia. *Tetrahymena corlissi* was isolated from guppies with a fungal infection in Thailand, and the fungus was identified as *Achlya bisexualis*. Male and female guppies were artificially infected with both organisms. The results showed that guppies could easily be artificially infected with a culture of *Tetrahymena corlissi* and that female guppies were more sensitive than male guppies. *Achlya bisexualis* infection was shown to be a secondary infection after the *Tetrahymena* infection.

Key words *Achlya bisexualis* · Ciliate · Guppy · *Tetrahymena*

Introduction

Tetrahymena infection has become the most problematic parasitic disease of the guppy *Poecilia reticulata* (Peters) in Southeast Asia. Many exported guppies die of *Tetrahymena corlissi*. infection either during transportation or soon after they arrive at port. Imai et al. (2000) identified the ciliated parasite isolated from imported Singapore guppies as *Tetrahymena corlissi* Thompson, the same pathogen that infects guppies in North America (Nigrelli et al. 1956; Hoffman et al. 1975). In Thailand, parasitic diseases have also caused great losses at ornamental fish farms because the intensive production of fishes is conducive to the rapid spread of these diseases. Typical lesions were characterized by protrusion of scales, swelling, ulcerative wounds on the skin, blindness, and protrusion of eyes (Johnson 1978). Guppies collected from a farm in Ratchaburi province, Thailand, were killed by parasitic ciliates that were identified as *Tetrahymena corlissi* (Hatai et al., 2001). At that time, an infectious fungus was isolated from fish infected with *Tetrahymena corlissi.* Therefore, the goal of the present study was to determine the effect of *Tetrahymena* on the occurrence of achlyosis in the guppy.

O.-a. Lawhavinit
Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand

K. Chukanhom · K. Hatai (✉)
Division of Fish Disease, Department of Veterinary Science, Nippon Veterinary and Animal Science University, Tokyo 180-8602, Japan
Tel. +81-422-31-4151; Fax +81-422-31-6796
e-mail: hatai@scan-net.ne.jp

K. Chukanhom
Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, Thailand

Materials and methods

Isolation of parasites and fungi

Dying guppies were collected from a fish farm in Ratchaburi province, near Bangkok, Thailand, on December 22, 1999. A lesion was removed with tweezers and the material was transferred to PPYG medium (0.3% proteose peptone, 0.2% yeast extract, and 1% glucose at pH 7.0). A few grains of ampicillin and streptomycin were added to the medium to inhibit bacterial growth. For fungi, the hyphae from the lesion were inoculated on GY agar (1% glucose, 0.25% yeast extract, 1.5% agar) (Hatai and Egusa 1979), and a few grains of ampicillin and streptomycin were added. The morphological characteristics of the isolated fungus were compared with those of *Achlya bisexualis* Coker & Couch (Johnson 1956).

Experimental design

Four experiments were conducted (Table 1) as described in detail in the following sections. In each experiment, healthy guppies (0.2–0.4 g males and 0.3–1.0 g females) were placed in 1000-ml beakers containing 500 ml of dechlorinated tap water at 25°C. The number and sex of the fish in each beaker and the number of beakers for each experiment are shown in Table 1. The fish were exposed to high and low doses of a 3-day-old culture of *Tetrahymena corlissi* and zoospores after

Table 1. Design of four inoculation experiments in the guppy

Experiment		No. fish in each group (m, f)	No. of groups
Agent	Group		
Tetrahymena	Uninfected	3 (1, 2)	5 (2 intact, 2 injured, 1 control)
Achlya bisexualis NJM 9905	Uninfected	3 (1, 2)	5 (2 intact, 2 injured, 1 control)
	Artificially infected with *Tetrahymena*	6 (0, 6)	4 (3 inoculated, 1 control)
	Naturally infected with *Tetrahymena*	3 (0, 3)	4 (3 inoculated, 1 control)

m, male; f, female

18-h culture of *Achlya bisexualis* NJM 9905. The fish were fed on alternate days with a commercial guppy food (Tetra Guppy; Tetra, Warner-Lambert, Melle, Germany). Some of the fish used in the first two experiments (see Table 1) were artificially injured by removing 2–3 scales from each fish.

Pathogenicity of *Tetrahymena corlissi* and *Achlya bisexualis*

The pathogenicity of *Tetrahymena corlissi* and the isolated fungus to normal guppies was determined. One male uninjured fish, one male injured fish, two female uninjured fish, and two female injured fish were used for artificial infection. For *Tetrahymena corlissi*, 2.4×10^4 cells/ml and 2.4×10^2 cells/ml were used as high and low doses, respectively. For the isolated fungus, 4.4×10^3 spores/ml and 4.4×10^2 spores/ml were used as high and low doses. The mortality of the fish was recorded 5 days after the inoculation.

Pathogenicity of *Achyla bisexualis* to guppies artificially infected with *Tetrahymena corlissi*

Two days after guppies were artificially infected with *Tetrahymena corlissi*, they were exposed to *A. bisexualis*. Six female guppies, 0.3–0.9 g, were injured by removing 2–3 scales and used in each of the four groups (a total of 24 injured females). The inoculated dose of *Tetrahymena corlissi* was 1.2×10^3 cells/ml. The fish were exposed to high and low doses of the isolated fungus, 2.2×10^3 spores/ml and 4.4×10^2 spores/ml, respectively.

Pathogenicity of *Achlya bisexualis* to guppies that were naturally infected with *Tetrahymena corlissi*

Three females naturally infected with *Tetrahymena corlissi* were used for artificial infection. For the isolated fungus, 4.4×10^3 spores/ml and 4.4×10^2 spores/ml were used as the high and low doses, respectively.

Results

Isolation and identification of parasites and fungi

Hatai et al. (2001) succeeded in isolating two isolates, strains T1 and T2, of *Tetrahymena corlissi* and three isolates of fungi, strains NJM 9904, 9905, and 9906, from guppies collected in Thailand in 1999. *Tetrahymena corlissi* strain T2 and the isolated fungus strain NJM 9905 were used in the present experiments.

Table 2. Comparison of morphological characteristics of isolate NJM 9905 and *Achlya bisexualis*

Morphological characteristic	*A. bisexualis* Coker & Couch, 1927	NJM9905
Zoosporangia:		
Number	Abundant	Abundant
Shape	Fusiform	Fusiform
Size:		
Width	25–45 μm in diameter	22–43 μm in diameter
Length	110–400 μm	100–310 μm
Zoospore discharged	Achlyoid	Achlyoid
Oogonium:		
Number	Moderate	Moderate
Shape	Spherical or pyriform	Spherical dominant
Size (diameter)	35–130 μm, predominately 60–75 μm	42–56 μm; average, 48 μm
Wall	Smooth, pitted	Smooth, pitted
Oospore:		
Number	2–28, generally 5–10	3–14, generally 4–6
Type	Eccentric, not filled	Eccentric, not filled
Size (diameter)	16–40 μm, predominately 24–26 μm	22–26 μm; average, 24 μm
Antheridium:		
Type	Tubular curved	Tubular curved
Attachment site	Lateral projection	Lateral projection

Source: Johnson (1956)

The isolated fungus was identified as *Achlya bisexualis* NJM 9905. Morphological characteristics of the fungus were whitish, separated colonies on GY agar (Fig. 1), stout hyphae, and zoospores accumulated at the tip of the sporangium (achlyoid type) (Fig. 2). These characteristics indicated that the fungus belonged to the genus *Achlya*. In the sexual stage, oogonia were found at the top of the principal vegetative thalli after incubation on hemp seeds for 7–9 days at 20°C. The origin of the antheridial branches was single or of the sparingly branched diclinous type (Fig. 3). The oogonia were mainly spherical in shape, averaging 48 μm in diameter, with pitted walls and slightly curved or almost straight stalks. The oogonia were not filled with mature oospores, containing 3–14 oospores, but usually 4–6 oospores (Fig. 4). The mature spores were mainly spherical in shape, averaging 24 μm in diameter, and the antheridial cells were laterally appressed or attached by projection. These data show that the strain NJM 9905 belongs to the species *A. bisexualis*. Its morphological characteristics are compared with those of *A. bisexualis* in Table 2.

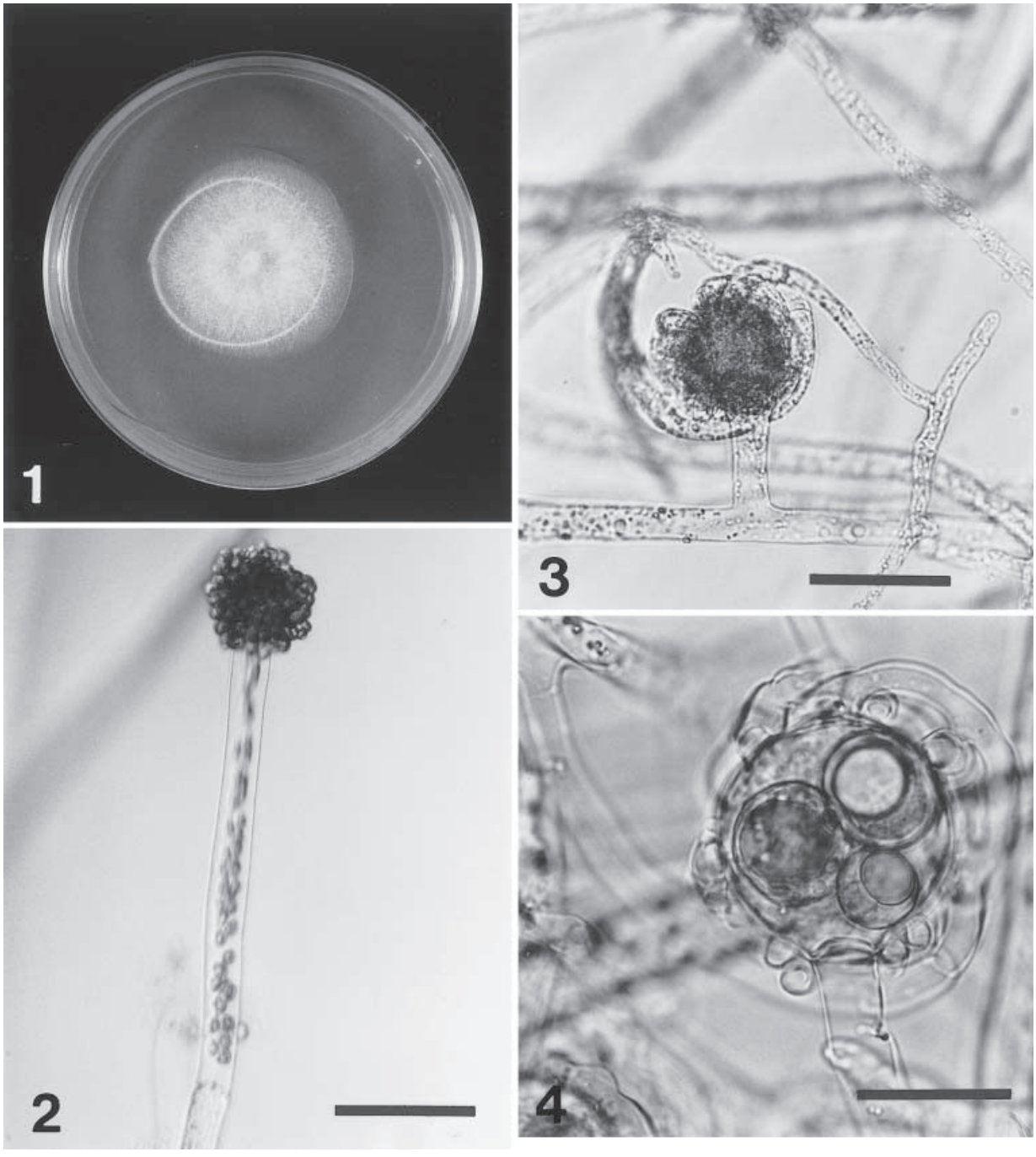

Fig. 1. A whitish, separated colony of *Achlya bisexualis* NJM 9905 grown on GY (glucose and yeast) agar at 20°C, 3 days after inoculation
Fig. 2. Zoospores accumulated at the tip of a sporangium. *Bar* 100 μm
Fig. 3. Oogonium of *Achlya bisexualis* NJM 9905. The shape is mainly spherical, averaging 48 μm in diameter. Note that the origin of antheridial branch of the isolate is of the diclinous type. *Bar* 50 μm
Fig. 4. Mature oospores. Oospores did not fill the oogonium. *Bar* 30 μm

The pathogenicity of *Tetrahymena corlissi* and *Achlya bisexualis*

The pathogenicity of *Tetrahymena corlissi* to guppy is shown in Table 3. All fish of groups 1, 3, and 4 died within 5 days after inoculation, but there was no mortality in groups 2 and 5. Some dead fish showed severe *Tetrahymena corlissi* infection. The pathogenicity of *A. bisexualis* NJM 9905 to guppy is shown in Table 4. One fish from each of groups 1 and 4 died, but no fungal infections were observed. In the other groups, all fish were alive until 5 days after inoculation.

Table 3. Pathogenicity of *Tetrahymena corlissi* in the guppy

Group	Conditions	Inoculated dose of the parasite (cells/ml)[a]	No. of dead fish Days after inoculation				Total
			2	3	4	5	
1	Intact	2.4×10^3			1	2[b]	3
2	Intact	2.4×10^2					0
3	Injured[c]	2.4×10^3				1 + 2[b]	3
4	Injured	2.4×10^2			1	2[d]	3
5	Control	0					0

Three guppies, 1 male and 2 females, were used in each group
[a] Each experiment was done in a 1-l beaker containing 500 ml of dechlorinated tap water at 25°C
[b] One of two showed severe *Tetrahymena* infection
[c] Fish were injured by scale removal
[d] Both fish showed severe *Tetrahymena* infection

Table 4. Pathogenicity of *Achlya bisexualis* NJM 9905 in the guppy

Group	Conditions	Inoculated dose of the fungus (spores/ml)[a]	No. of dead fish Days after inoculation				Total
			2	3	4	5	
1	Intact	4.4×10^3				1[b]	1
2	Intact	4.4×10^2					0
3	Injured[c]	4.4×10^3					0
4	Injured	4.4×10^2		1[b]			1
5	Control	0					0

Three guppies, 1 male and 2 females, were used in each group
[a] Each experiment was done in a 1-l beaker containing 500 ml of dechlorinated tap water at 25°C
[b] No fungal infection was observed
[c] Fish were injured by scale removal

Table 5. Pathogenicity of *Achlya bisexualis* NJM 9905 in guppies artificially infected with *Tetrahymena corlissi*

Group[a]	Inoculated dose of the parasite (cells/ml)[b]	No. of fish infected with parasite after 2 days of inoculation	Inoculated dose of the fungus (spores/ml)[b]	No. of dead fish Days after inoculation					Total
				1	2	3	4	5	
1	1.2×10^3	6	2.2×10^3				2	1	3
2	1.2×10^3	4	4.4×10^2	1[c]		2	1	1	5
3	1.2×10^3	4	0		2			2	4
4	Control	0	0						0

[a] Six females guppies injured by removal of 2–3 scales were used in each group
[b] Each experiment was done in a 1-l beaker containing 500 ml of dechlorinated tap water at 25°C
[c] Fish showed fungal infection

Pathogenicity of *Achlya bisexualis* to guppies after being artificially infected with *Tetrahymena corlissi*

The pathogenicity of a combination of *Tetrahymena corlissi* and *A. bisexualis* NJM 9905 to guppy is shown in Table 5. Two days after being inoculated with *Tetrahymena corlissi*, 14 fish from groups 1, 2, and 3 were infected with *Tetrahymena corlissi* and no fungal infections were found. The control fish in group 4 survived until the end of the experiment. In the 5-day period after inoculation with the isolated fungus, 3 fish died in group 1 and 5 fish died in group 2. In group 3, 4 fish died in the 7-day period following inoculation with *Tetrahymena corlissi.*

Pathogenicity of *Achlya bisexualis* to guppies naturally infected with *Tetrahymena corlissi*

The pathogenicity of *A. bisexualis* NJM 9905 to guppy naturally infected with *Tetrahymena corlissi* is shown in Table 6. In the 5-day period after inoculation with the isolated fungus, two fish died in group 1, two fish died in group 2, and three fish died in group 3. However, in the 2 days following inoculation with the fungus, only one fish died in group 1.

Table 6. Pathogenicity of *Achlya bisexualis* NJM 9905 in guppies naturally infected with *Tetrahymena corlissi*

Group[a]	Inoculated dose of the fungus (spores/ml)[b]	No. of dead fish Days after inoculation					Total
		1	2	3	4	5	
1	4.4×10^3		1[c]	1			2
2	4.4×10^2	1			1		2
3	control	1		2			3

[a]Three female guppies naturally infected with *Tetrahymena corlissi* were used in each group
[b]Each experiment was done in a 1-l beaker containing 500 ml dechlorinated tap water at 25°C
[c]Fish showed fungal infection

Discussion

The morphological characteristics of the fungus NJM 9905 isolated from guppies with *Tetrahymena corlissi* were the same as those of *A. bisexualis*, which was described by Johnson (1956). In particular, the isolated fungus forms whitish, separated colonies on GY agar and accumulates zoospores at the tip of the sporangium. These characteristics indicate that it belongs to the genus *Achlya*. In addition, cells involved in sexual reproduction such as oogonia and antheridia (see Table 2) show the morphological characteristics of *A. bisexualis*. The guppies infected with *Tetrahymena corlissi* showed obvious scale loss and ulceration with fungus infections. Hatai et al. (2001) reported the biological characteristics of *Tetrahymena corlissi* that was isolated from Thai guppies and cultured under laboratory conditions in 1999. This isolate of *Tetrahymena corlissi* was used in the present study.

The data in Table 2 show that guppies could be easily infected with cultured *Tetrahymena corlissi.* However, the guppies could not be artificially infected with *A. bisexualis* NJM 9905 (see Table 4). Female guppies are more vulnerable than male guppies to infection with *Tetrahymena corlissi.* These results are similar to those reported by Hoffman et al. (1975), in which female guppies died in 1 week but male guppies died in 1–3 weeks. Moreover, fewer males than females died after being infected with *Tetrahymena corlissi*. In this study, only one fish in group 1, in which guppies were naturally infected with *Tetrahymena corlissi* and artificially infected with *A. bisexualis* NJM 9905, was observed to have a fungal infection. *Achlya bisexualis* could be isolated only from this fish (see Tables 5, 6). This result showed that *Achlya bisexualis* infection was a secondary infection after *Tetrahymena* infection and was not a cause of mortality in the guppy.

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