Short Communication

Infection of *Aphanius sophiae* (Actinopterygii: Cyprinodontidae) with *Tetracotyle* sp.

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Received: 19 October 2010, Accepted: 20 February 2011

Abstract

During the study on the biology of *Aphanius sophiae*, a total of 20 fish specimens were collected in September 2007 using dip net at a depth of 30 - 50 cm in downstream of spring and preserved them in 10% formalin in spot until examination. The fish were dissected and the ear cavity was examined to determine the presence of parasites of which 4 specimens including 4 females were infected with *Tetracotyle* sp. metacercariae, a trematode which in its metacercarial stage may infect a wide range of freshwater fish species. These metacercariae were positioned into cavity of inner ear membrane of infected fish. Our data showed that the infection was higher in females than males also as the number of parasites increases in fish ear, the size of parasites decreases. The prevalence of the infection with *Tetracotyle* sp. was 20%, the parasite intensity was 2.5 and the parasite abundance was 0.5 in *A. sophiae*. Cercariae of *Tetracotyle* sp. infect fish by direct penetration. Two fish were infected with 3 parasites and other fish had 2 parasites in their ears, separately. This shows that even the inner ear is not exempted from the attacks of trematodes. This is the first report of *Aphanius sophiae* infection with *Tetracotyle* sp. metacercariae.

Key words: *Aphanius sophiae*; *Tetracotyle* sp., Metacercariae, Cheshmeh-Ali Damghan, Iran.

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Introduction

*Aphanius sophiae* (Heckel, 1849) is found in the endorheic basin of the Cheshmeh-Ali Damghan with freshwater flow which drains towards the Namak kavir (kavir = playa). This basin is ringed by mountains about 10 km North West of spring.¹ Population of *Aphanius* is the most northerly and easterly population of tooth-carp from Alborz Mountains in Iran. Males and females *A. sophiae* have distinct dimorphism. Trematodes are ubiquitous parasitic worms that infect a wide range of vertebrate and invertebrate animals. *Tetracotyle* belongs to platyhelminths, trematodes, strigeidae.² Cercariae of *Tetracotyle* sp. infect fish by direct penetration. Also, metacercariae of tetracotyloid type, are encysted (usually contained in cysts with thin or thick cover) in musculature, body cavity and on surface of various inner organs. Species of tetracotyloid larvae lead to disease and frequently death of hosts in mass infestations.³ Adults of these trematodes reach sexual maturity in the intestines of piscivorous birds and parasitize the intestines of various piscivorous and omnivorous birds. Identification at the metacercarial stage is more difficult due to the fact that they are sexually undifferentiated, showing no evidence of reproduction organs as an aid to their identification.⁴ Very few papers concerning the family strigeidae are available and don’t discuss the taxonomic relations. The purpose of this article is to report the infection of *A. sophiae* from its natural habitat with *Tetracotyle* sp., a species of strigeid metacercariae.

Materials and Methods

This survey was conducted in the Cheshmeh-Ali Damghan (36°17’N-54°05’E) in kavir-Namak basin, Damghan city, the Province of Semnan (north of Iran). The bottom of this spring is generally boulders, stones, gravel, sand and detritus. Much cover also is found in the bottom and floating and submerging plants and shrubs and trees in the region. The water is clear and running more fast in upstream than downstream. We collected a total of 20 fish (Fig. 1) from the water using dip net at a depth of 30-50 cm in downstream of spring about 500 m remote of headwater and preserved them in 10 % formalin in spot until examination. The fish were dissected and the ear cavity was examined to determine the presence of parasites. Total length, standard length and weight of the collected specimens were measured to the nearest 0.05 millimeter using a vernier caliper, weight of specimens to the nearest 0.001 gram and their sex were determined. Total length of parasites was measured by oculometer. The locations and number of parasites were recorded. Parasites were carefully removed using two sharp needles under a stereoscopic microscope and placed in petri dishes with physiological saline solution. They were fixed between 2 slide glasses with 70 % ethyalcohol and stained by Azo-carmine, washed in acid alcohol, dehydrated in alcohol series, cleared in xylol and mounted in Canada balsam, then used for morphological observations and measurements.⁵,⁶

Prevalence (%) was calculated according to the percent of infected fish divided by the total number of fish. Mean intensity was determined by dividing the total number of recovered parasites by the number of infected fish samples, while abundance was calculated by dividing the total number of recovered parasites by the number of (infected and uninfected) fish samples. A camera lucida was used to draw the metacercariae.

Results and Discussion

A total of 20 fish specimens (8 males and 12 females) were collected in September 2007 of which 4 specimens (20 %) including 4 females were infected with *Tetracotyle* sp. Total and standard length
of parasites ranged from 24.6-35.3 mm and 20.6-29.5 mm respectively and their weight ranged from 0.279-0.918 g. Two fish had 3 parasites and the other two had 2 parasites in their ears (Table 1). The morphology of the infected fish was normal. However, the parasite made a hole in inner ear membrane. Body of the metacercariae was short and stout, oval or pyriform, expanded anteriorly and concave ventrally (Fig. 2), its differentiation into sections was not always present. Sides of oral sucker with two lateral suckers of inconstant form usually ovaly elongated and surrounded by numerous glands. The prevalence of infection of *Tetracotyle* sp. was 20%, the parasite intensity was 2.5 and the parasite abundance was 0.5.

Maximum and minimum length of the parasite were 1.29 mm and 0.55 mm respectively (Table 1). Mean parasite length was 0.721 mm. With increasing the number of parasites in fish ear, the size of parasites decreases. The occurrence of a trematode in the inner ear cavity of a vertebrate is a unique phenomenon in many ways to the interest of all biologists. For one thing it shows that even the inner ear is not exempted from the attacks of trematodes. This is the first report of *tetracotyle* sp. in the inner ear cavity of fish yet found. The highest number of parasite counts in *Aphanius sophiae* in this study was 3 parasites. These metacercariae were positioned into cavity of inner ear membrane of the infected fish.

![Fig 1. Photographs of male (with flank bars) and female *Aphanius sophiae* in Cheshmeh-Ali Damghan, Semnan province, North of Iran.](image1)

![Fig 2. Photographs of parasite including microscopic and schematic shapes, respectively.](image2)

**Table 1.** Details of infected *A. sophiae* with *Tetracotyle* sp. metacercariae in Cheshmeh-Ali Damghan, Semnan Province, North of Iran

<table>
<thead>
<tr>
<th>Fish No.</th>
<th>Fish (sex)</th>
<th>Parasite T.L (mm) in Right ear</th>
<th>Parasite T.L (mm) in Left ear</th>
<th>Total No. of Parasites</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>0.63</td>
<td>0.8</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>0.74</td>
<td>0.57</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>0.55</td>
<td>0.61</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>1.29</td>
<td>0.66</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.76</td>
<td></td>
</tr>
</tbody>
</table>

*Tetracotyle* sp. larva was diagnosed from brain, connective tissue, eye, kidney, liver, muscle, spleen and vertebral column in *Cottus cognatus* and there were not significant differences in the prevalence, intensity and abundance of parasite between females and males. However, the current study reports the infection of...
Aphanius sophiae with Tetracotyle sp. that is located in the inner ear cavity. Parasites can interfere with host swimming behaviour in a variety of ways. The metacercariae of Diplostomum phoxini and Ornithodiplostomum ptychocheilus, which infect minnows in the UK and the USA, respectively, appear to achieve their effects via damage to the CNS. The metacercariae aggregate in lobes of the brain concerned with vision and motor control and heavy infections are associated with impaired optomotor responses and ‘conspicuous’ swimming behavior of host fish.8

Our data showed that the parasite infection was higher in females than males. This could be explained as males are less infected due to the reason that the spawning takes place on submerged leaves before and during spawning, the females are vulnerable to parasite attack, so the cercariae get more chance to penetrate. Moreover, the lymnaid snails are known to surface dweller, so chances of cercarial contact with females during the spawing are greater than the males.9 However, more research is required to elucidate the affinity of these parasites to females. Infection of Channa spp. with tetracotyle larvae that was also fairly common in muscles of Channa with prevalence of 44.44 % and mean intensity of 9 is reported.10 Prevalence, mean intensity and mean abundance of Tetracotyle sp. in 100 Cottus cognatus collected from six fathom bank, Lake Huron, attained 100, 155.8 and 155.8, respectively.7 In most cases, the prevalence varies from year to year and no statistically significant seasonal patterns can be recorded.11 Thus, further studies in the region on other fish species and their potential hazard for human infectivity is very important for a successful infection control strategies.

Acknowledgements

Authors wish to thank Mr Mohammad-Safa Golmohamadi for his help during fish collection.

References