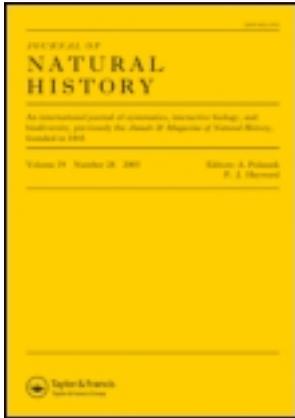


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Journal of Natural History

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/tnah20>

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Published online: 02 Dec 2010.

To cite this article: Ahmet Özer (2007) *Trichodina modesta* Lom, 1970 (Ciliophora: Peritrichia) infestations of an endemic Toothcarp *Aphanius danfordii* Boulenger, 1890 (Pisces: Cyprinodontidae) in Sinop, Turkey, *Journal of Natural History*, 41:41-44, 2543-2549, DOI: [10.1080/00222930701739724](https://doi.org/10.1080/00222930701739724)

To link to this article: <http://dx.doi.org/10.1080/00222930701739724>

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***Trichodina modesta* Lom, 1970 (Ciliophora: Peritrichia) infestations of an endemic Toothcarp *Aphanius danfordii* Boulenger, 1890 (Pisces: Cyprinodontidae) in Sinop, Turkey**

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(Accepted 8 October 2007)

Abstract

Toothcarp (*Aphanius danfordii*) were collected from a small stream connected to the Black Sea on the coast of Sinop, Turkey and examined for trichodinids. A total of 156 toothcarp was investigated. *Trichodina modesta* Lom, 1970 was the only trichodina species recovered. Overall infestation prevalence (%), mean intensity and abundance values were 97.4%, 182.7 ± 22.4 parasites per infested fish, and 178.0 ± 22.0 parasites per examined fish, respectively. Infestation prevalence, mean intensity and abundance values in relation to sampling months, fish size, and sex were also determined and discussed.

Keywords: *Aphanius danfordii*, *Ciliophora*, *Cyprinodontidae*, *Trichodina modesta*, *Turkey*

Introduction

Cyprinodont fishes are generally found in fresh or brackish water and sometimes in coastal marine environments. They are also found in tropical and warm temperate waters and some species are kept as aquarium pets. In many aquatic habitats, some naturally occurring predaceous fish species, such as *Gambusia affinis* Baird and Girard, 1853 and species of Poeciliidae and Cyprinodontidae, are most extensively used as biocontrol agents for mosquito control. For example, some *Aphanius* species, such as *Aphanius ibericus* Valenciennes, 1846 and *Aphanius dispar* Ruppel, 1829, are used as mosquito control agents (Fletcher et al. 1992; Huber 1996).

The genus *Aphanius* is currently composed of approximately 16 species (Blanco et al. 2006), eight of which are currently present in Turkey (Wildekamp et al. 1999). The toothcarp, *Aphanius danfordii* Boulenger, 1890, is a species endemic to the north-east of Turkey (Geldiay and Balik 1999). Studies on the parasites infecting species of genus *Aphanius* are very rare (Geldiay and Balik 1974; Kalantan et al. 1987) and there is no published study on the parasite fauna of *A. danfordii*.

Species of the genus *Trichodina* Ehrenberg, 1831 are among the most frequently encountered ciliates on both freshwater and marine fish. Trichodinids infest many fish species with varying intensities dictated by ecological conditions and host factors. *Trichodina modesta* Lom, 1970 is a species of *Trichodina* recorded from several host fishes including *Vimba vimba* L., 1758 and *Abramis brama* L., 1758 (Lom 1970), *Blicca bjoerkna* L., 1758 (Arthur and Lom 1984; Wierzbicka 1997), *Misgurnus anguillicaudatus* Cantor, 1842 and *Rhinogobius brunneus* Temminck and Schlegel, 1845 (Basson and Van As 1994).

The purpose of this study was to determine the existence of trichodinids on an unstudied fish species, which has a biocontrol potential, found in a small stream mouth connected with the Black Sea at Sinop, Turkey in relation to sampling month, and the length and sex of the host fish.

Materials and methods

Specimens of *A. danfordii* were collected by hand net and cast net from Sirakaraağaçlar stream which connects with the Black Sea on the coast of Sinop, Turkey (42°16'N, 25°55'E). Sirakaraağaçlar stream is characteristically slightly brackish during the late autumn and early spring months (October to March) when the water level rises and connects with the Black Sea. In summer and early autumn, however, the water level drops, the connection is broken and the stream turns to fresh water.

Sampling was carried out on a monthly basis from May to August 2000 when fish were present in the sampling area. For parasitological examinations, fish were transported alive in local water directly to the Sinop Fisheries Faculty Laboratory. A total of 156 toothcarp were investigated. Fish were measured to the nearest millimetre and their sex determined post-mortem. The total number of trichodina was determined by screening all body surfaces including skin, fins, and gills using a light microscope at $\times 200$ magnification. Dry smears of trichodinids taken from each fish specimen were made in accordance with Klein's silver nitrate (AgNO_3) method (Lom and Dykova 1992). All biometric data are in micrometres and based on 35 trichodinid specimens in June.

The prevalence, mean intensity, and abundance values of parasites were determined according to Bush et al. (1997). The Kruskal–Wallis test (non-parametric ANOVA) was performed to test for significant differences in the mean intensity values of *T. modesta* for the length classes of fish as well as for the months in which this study was conducted. Arithmetic mean values are followed by the standard error. The comparison between parasite loading on males and females was tested by the Mann–Whitney *U* test. All *P* values < 0.05 were considered to be significant. Statistical analyses were performed using the statistical program StatGraph 3.0.

Results

The current study is the first to report a trichodinid from a toothcarp captured from its natural environment on the Black Sea coast at Sinop, Turkey. *Trichodina modesta* was the only trichodinid recovered, mainly from gills but rarely on skin and fins of *Aphanius danfordii*.

Trichodina modesta (Figure 1) is a medium-sized species with cell diameter 36–41 μm (38.8 ± 0.3). In lateral view living cells are flattened cylindrical-shaped, and circular in oral and aboral view. In silver impregnation, the adhesive disc 29–35 μm (31.9 ± 0.3) reveals a

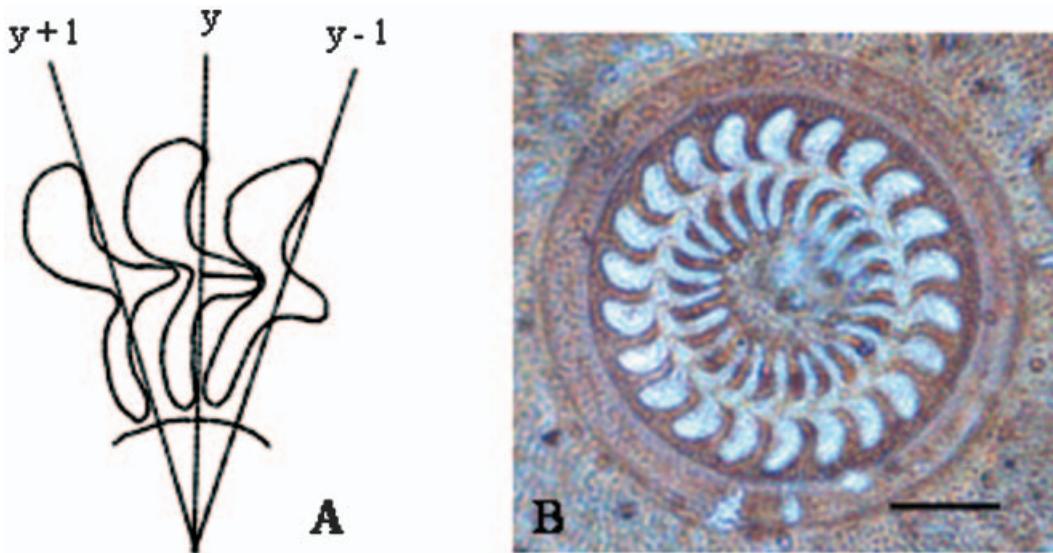


Figure 1. (A) Diagrammatic drawings of the denticles of *Trichodina modesta*; (B) a silver nitrate-stained specimen of *T. modesta*. Scale bar: 10 μm .

dark-stained centre. Adhesive disc surrounded by a border membrane of 3.1–4.4 μm (3.80 ± 0.07) width. Diameter of denticulate ring 17.5–21.5 μm (19.4 ± 0.2); number of denticles 21–25 (22); six to seven radial pins per denticle. Blades 3.7–4.8 μm (4.16 ± 0.05) nearly sickle-shaped, the distal margin of blade is away from the border membrane, rounded, and slants away from the border membrane. The tangent point is flat. The anterior margin of the blade curves sharply down. The apex of blade is round, not touching the $y + 1$ axis. Blade apophysis is not visible. Posterior blade margin is parallel to anterior blade margin. Blade connection thin. A central part in front of thorns present. Short central part 1.2–2.4 μm (1.69 ± 0.06) opens posteriorly of transition of blade. The central part of denticle narrow, pointed and extending to slightly more than halfway towards the $y - 1$ axis. Length of thorn 3.1–4.6 μm (3.90 ± 0.07); span of denticle 8.5–10.9 μm (9.46 ± 0.12), length of denticle 4–6 μm (4.71 ± 0.11). Ray connection short and thin. Rays slightly curved in posterior direction with tips extending slightly beyond y -axes. Section of denticle above x -axis to denticle below similar, ratio one.

The overall infestation prevalence (%), mean intensity, and abundance values from a total of 156 fish specimens were 97.4%, 182.7 ± 22.4 parasites per infested fish, and 178.9 ± 22.0 parasites per examined fish, respectively. Infection prevalence, mean intensity, and abundance values were also determined in relation to infestation months, the sex and the size classes of the toothcarp (Table I). Statistically significant differences were determined between months as well as between sex of fish and are shown in Table I ($P < 0.05$). On the other hand, despite consistent decrease in the infestation prevalence, mean intensity, and abundance values as the fish become larger, no statistically significant difference was determined between different length classes of fish ($P > 0.05$; Table I).

Discussion

Most species of killifish are strong candidates as biological control agents, having an affinity for mosquito larvae as well as being very eurythermal and euryhaline (Whitehead et al.

Table I. Infestation prevalence (%), mean intensity, and abundance levels of *Trichodina modesta* over *Aphanius danfordii* and the results of the statistical tests performed.

	Length of fish infested ± SE (mm)	Prevalence (%)	Abundance ± SE	Mean intensity ± SE	Possible comparison	Statistical test used	Significance
Months							
May (n=40)	36.75 ± 0.5 ^a	90	82.8 ± 16.9	92.0 ± 18.1 ^a	(May) vs (June) vs (July) vs (August)	Kruskal–Wallis	P < 0.0001
June (n=44)	36.59 ± 0.5 ^a	100	168.9 ± 24.8	168.9 ± 24.8 ^a	(May) vs (June)	Dunn's	P > 0.05
July (n=50)	43.80 ± 0.5 ^b	100	128.2 ± 14.8	128.2 ± 14.8 ^a	(May) vs (July)	Dunn's	P > 0.05
August (n=22)	38.40 ± 1.3 ^a	100	482.4 ± 123.1	482.4 ± 123.1 ^b	(May) vs (August)	Dunn's	P < 0.001
					(June) vs (July)	Dunn's	P > 0.05
					(June) vs (August)	Dunn's	P < 0.01
					(July) vs (August)	Dunn's	P < 0.001
Sex							
Female (n=106)	40.50 ± 0.4 ^b	100	137.9 ± 14.6	137.9 ± 14.6 ^a	(Female) vs (Male)	Mann–Whitney U	P = 0.01
Male (n=50)	36.44 ± 0.5 ^a	92	263.1 ± 59.8	286.0 ± 64.0 ^b			
Length classes (mm)							
≤ 35 (n=43)		100	234.9 ± 70.4	234.9 ± 70.4 ^a	(≤ 35) vs (36–43) vs (≥ 44)	Kruskal–Wallis	P = 0.5940
36–43 (n=70)		97.1	158.7 ± 18.0	163.4 ± 18.2 ^a	(≤ 35) vs (36–43)	Dunn's	P > 0.05
≥ 44 (n=43)		95.3	152.3 ± 23.1	160.4 ± 23.7 ^a	(≤ 35) vs (≥ 44)	Dunn's	P > 0.05
					(36–43) vs (≥ 44)	Dunn's	P > 0.05
Overall	39.2 ± 0.4	97.4	178.0 ± 22.0	182.7 ± 22.4			

^{a,b}Values with the same superscript letters are not significantly different ($P \geq 0.05$).

1986). The toothcarp, *Aphanius danfordii*, is a fish species endemic to the north-east of Turkey (Geldiay and Balik 1999). *Aphanius danfordii* is omnivorous and mosquito larvae could be included in its diet. The introduction of this fish into different areas for biocontrol is possible and the determination of the existent parasite fauna might also be important before the introduction of this fish to new areas.

Peritrich ciliophorans, especially trichodinids, are well-documented parasites. Their importance is reflected in the volume of literature dealing with the varying aspects of the biology of these parasites, i.e. behaviour, distribution, the impact of environmental factors, concomitant infections and their relative pathogenicity (Lom 1970, 1973; Das and Pal 1987; Van As and Basson 1987; Sanmartin Duran et al. 1991; Özer and Erdem 1998, 1999; Özer 2000, 2003a, 2003b). Trichodinids, namely *Trichodina tenuidens* Faure-Fremiet, 1944 on three-spined stickleback, *Gasterosteus aculeatus* L., 1758 and *Trichodina domerguei* Wallengren, 1897 on the round goby, *Neogobius melanostomus* Pallas, 1811 and three-spined stickleback, *G. aculeatus*, *T. puytoraci* Lom, 1962 and *T. lepsi* Lom, 1962 on *Mugil cephalus* L., 1758 and *Liza aurata* Risso, 1810 have been reported from the same sampling area (Özer 2003a, 2003b; Özer and Öztürk 2004). It must be noted that none of the above-mentioned trichodina species were recorded on *A. danfordii*. *Trichodina acuta* Lom, 1961, *T. nigra* Lom, 1960 and *T. mutabilis* Kazubski and Migala, 1968 have also been reported to be present on the common carp *Cyprinus carpio* L., 1758 in Sinop, Turkey (Özer and Erdem 1998, 1999).

The morphological data concerning the species *T. modesta* fall within the size ranges reported by other authors (Lom 1970; Arthur and Lom 1984; Wierzbicka 1997). Specimens of *T. modesta* are somewhat smaller in their overall size and denticle dimensions, possibly because of different species of host fish, but conform in shape and the number of denticles to *T. modesta* found by Basson and Van As (1994). The data concerning the precise microhabitat of *T. modesta* agree in part with certain findings of those authors mentioned above. While Lom (1970), Arthur and Lom (1984), and Wierzbicka (1997) reported this species to be gills specific, Basson and Van As (1994) found this species on the gills, skin, and fins as in the present study.

Trichodinids often show seasonal changes in prevalence and intensity of infestation, and the occurrence of trichodinids is generally related to rise in water temperature. Some authors reported peak levels of trichodinid infestation in spring and early summer (Özer and Erdem 1998; Özer 2000, 2003a, 2003b). Statistically significant differences in the mean intensity levels between months were determined ($P < 0.05$), parallel to a general trend for increase from May to August. This obvious increase could be a result of the increase in temperature as the protozoan infestations in fish are strongly dependent on ecological conditions such as temperature. High organic loads and deterioration of water quality caused by the blockage between the sampling area and the Black Sea, due to the low level of water present, especially in August, could be another factor associated with heavy, debilitating *Trichodina* infestations.

The high infestation values with regard to sex of *A. danfordii* are evidence that both ecological relationships of fish (occupation of habitat and diet) are similar among males and females. The number of studies on the existence of trichodinid parasites on both female and male fish is rare (Özer 2003a, 2003b). While Özer (2003a) determined a statistically significant difference between combined intensity values of *Trichodina domerguei* Wallengren, 1897 and *Trichodina tenuidens* Faure-Fremiet, 1944 on female over male three-spined stickleback, *G. aculeatus*, Özer (2003b) did not determine a statistically significant difference between intensity values of *T. domerguei* on female and male round

goby, *N. melanostomus* (in both studies female fish had higher infestation values than male fish). In contrast to those results, in the present study male toothcarp had a higher intensity value than females, though, and was statistically significant. Pickering (1977), Pickering and Christie (1980), and Urawa (1992) attributed infestation differences between male and female fish to several factors such as rhythmical changes in the epidermis of host fish, a decrease in number of AB-positive mucus cells, and an increase in PAS-positive mucus cells.

Fish size may affect parasite prevalence and abundance. It is known that for the increase or decrease of infection rate, besides ecological factors, size of host fish is also an important factor, because, as a result of increase in size, an immune system develops against parasites. No statistically significant differences were determined between the mean intensity values of different fish length classes. Despite the decreasing number of parasites as the size of fish increases, the size of toothcarp was not a factor affecting the number of trichodinids here. Some authors noted an increased tendency in the mean intensity of *Trichodina* spp. in relation to the length of fish (Özer and Erdem 1998; Özer 2003a, 2003b) as a result of the differences in host species, which also differed in size. In addition, it is known that as the fish gets longer, the space for parasite settlement increases. However, in the present study there was a decrease in the number of *T. modesta* as the length classes of fish increased. Thus, it is thought that this might be a result of longer fish having a more developed immunological response to infestation.

In summary, this research has determined the identity of one trichodinid species infesting a potential biocontrol toothcarp present in a small stream connected to the Black Sea when the water turned to fresh water from slightly brackish water. Levels of prevalence, mean intensity, and abundance were quantified. It was also shown that male fish are more susceptible to infestation and that smaller fish are more heavily parasitized than larger fish.

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