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The Occurrence of *Trichodina domerguei* Wallengren, 1897 and *Trichodina tenuidens* Fauré-Fremiet, 1944 (Peritrichia) on Three-spined Stickleback, *Gasterosteus aculeatus* L., 1758 Found in a Brackish and Freshwater Environment

Ahmet ÖZER

Ondokuzmay s Üniversity, Sinop Fisheries Faculty, Sinop, Turkey

Summary. *Trichodina domerguei* Wallengren, 1897 and *Trichodina tenuidens* Fauré-Fremiet, 1944 infestations on three-spined stickleback (*Gasterosteus aculeatus* L., 1758) were studied in the period from January to May 2000 in the Black Sea coasts of Sinop, Turkey. The overall infestation prevalence and mean intensity level were 60.9% and 109.1 ± 21.5 trichodinids per fish, respectively. Statistically significant differences existed for the trichodinid species among sites on the fish, among length classes and between the sexes of fish hosts. While *T. tenuidens* itself represent a new parasite record, *Gasterosteus aculeatus* is a new host record for *T. domerguei* in Turkey. Many structures of the parasites were smaller in March, when the habitat was brackish, compared to May, when waters were fresh.

Key words: fish ectoparasite, *Gasterosteus aculeatus, Trichodina domerguei, T. tenuidens.*

INTRODUCTION

Trichodinids are a widely dispersed group of ectoparasites. Many species are morphologically variable and show low host specificity which make their determination difficult (Lom and Dykova 1992). Host specificity in trichodinids appears highly variable, some species (i.e. *Trichodina domerguei* Wallengren, 1897; *T. acuta* Lom, 1961 and *T. nigra* Lom, 1961) infesting a large number of host species, while others (i.e. *T. intermedia* Lom, 1961 and *T. tenuidens* Fauré-Fremiet, 1944) infest only one or two host species (Lom 1970 a, b). *Trichodina domerguei* and *T. tenuidens* are both commonly found parasitising three-spined (*Gasterosteus aculeatus* L., 1758) and nine-spined sticklebacks (*Pungitius pungitius* L., 1758). Along with the fact that many trichodinids have several hosts, the site of infestation of trichodinids on the host seems to be variable (Van As and Basson 1987). While *T. domerguei* primarily infects the skin and rarely the gills, for *T. tenuidens* it is the reverse with the gills being the principle site of infection (Lom and Stein 1966, Calenius 1980, Gaze 1995).

In this study, the existence of *T. domerguei* and *T. tenuidens* in relation to different length classes and

Address for correspondence: Ahmet Özer, Ondokuzmay s Üniversitesi, Sinop Su Ürünleri Fakültesi, 57000 Sinop, Turkey; Fax: (+90) 368 2715530; E-mail: ahmetozer100@hotmail.com

the sex of three-spined sticklebacks as well as environmental conditions are investigated. While *T. tenuidens* itself represents a new parasite record in Turkey, *Gasterosteus aculeatus* is a new host record for *T. domerguei* in Turkey. This study is also the first that both trichodinid species are observed in a brackish and freshwater environment.

MATERIALS AND METHODS

Specimens of *Gasterosteus aculeatus* were collected by gill net and cast net from S rak rka açlar stream which connects with the Black Sea on the coast of Sinop. S rak rka açlar 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 freshwater.

Sampling was carried out on a monthly basis. Following the May collection however, as the water levels dropped fish became harder to collect as they moved upstream and into deeper waters. For parasitological examinations, fish were transported alive in local water directly to the Sinop Fisheries Faculty Laboratory. A total of 151 fish were investigated over the period January to May 2000. Sticklebacks were weighed, the total length measured and their sex determined at post-mortem. The total number of trichodinids were determined by screening all body surfaces including the fins and gills using a light microscope at x200 magnification. For species identification and determination of infestation site, following total counts, samples of *Trichodina* were taken from each fish specimen and dry smears were made in accordance with Klein's silver nitrate ($AgNO_3$) method (Lom and Dykova 1992).

The prevalence and mean intensity levels of the trichodinids were determined according to Bush *et al.* (1997). The prevalence and mean intensity values of *T. domerguei* and *T. tenuidens* were given for pooled data rather than by species.

Kruskal-Wallis test (Nonparametric ANOVA) was performed to find out the significant differences in the mean intensity values of trichodinids for infestation sites, length classes of fish as well as for the months in which this study was conducted. The difference between parasite loading on male and female sticklebacks as well as the differences in the morphometric dimensions measured in March and May were tested by the Mann-Whitney U-test. All the statistical tests performed at the significance level of 5% and were given in Table 1 along with the results of the possible comparisons.

RESULTS

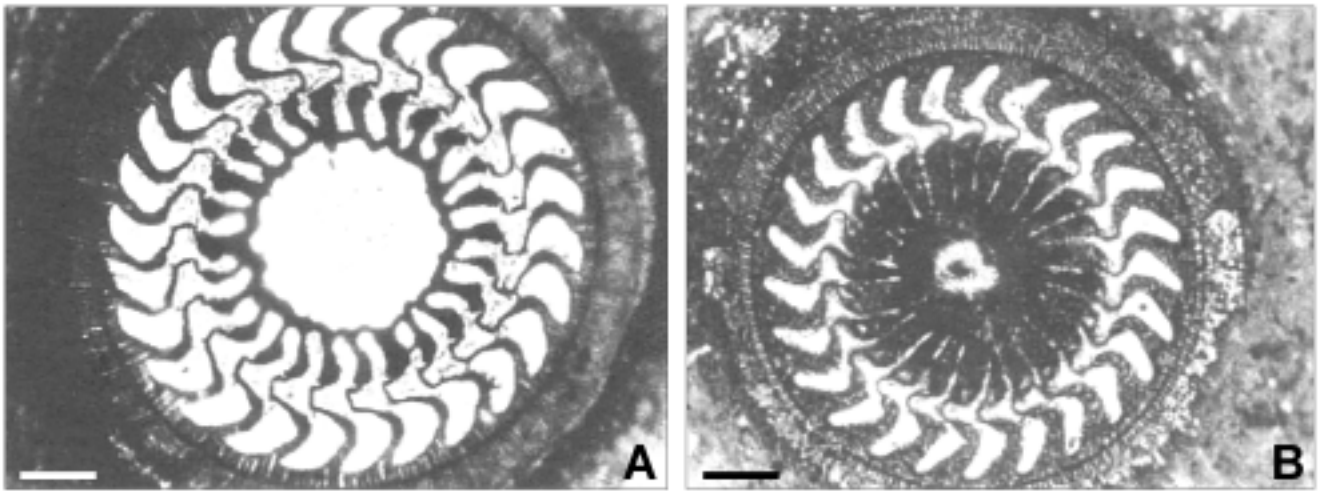
Throughout the investigation period, *T. domerguei* and *T. tenuidens* were the only species identified (Figs 1A, B). Both trichodinid species were found to have slightly smaller dimensions in brackish water in March than those recorded in May when waters became fresh (Table 2). Some particular parts of the trichodinids also had statistically significant differences when compared between March and May (Table 2). The overall infestation prevalence (%) and mean intensity levels recorded from a total of 151 fish specimens were 60.9% and 109.1 ± 21.5 trichodinids per infested fish, respectively (Table 1). Both levels were also recorded for all body parts as well as for the sex and length classes of *Gasterosteus aculeatus* (Table 1). Statistically significant differences were determined in relation to the sex and length classes of fish and are shown in Table 1. However, it must be noted that no statistically significant differences were determined between the infestation values of each of the three length classes of fish in all months, thus the data were pooled and analysed for three length classes regardless of sampling months (Table 1).

Monthly occurrences of both species were recorded. No trichodinids were observed in January but, an increase in both the infestation prevalence and mean intensity levels were recorded throughout the investigation period as can be seen in Fig. 2. Statistically significant differences were determined in the mean intensity values of trichodinids in February and March versus May ($P<0.01$). In addition, proportions of 1/8 *T. domerguei /T. tenuidens* in February and 1/10 *T. domerguei/ T. tenuidens* in March and April and 1/1 *T. domerguei /T. tenuidens* in May were observed in stained slides of each month.

DISCUSSION

Trichodinids are geographically a widely dispersed group of ectoparasites in freshwater, marine and euryhaline environments. About 70 species were identified in marine fishes (Kinne 1984) and more than 112 from freshwater fishes worldwide (Lom and Dykova 1992). Some trichodinids including *T. domerguei* and *T. tenuidens* parasitising *Gasterosteus aculeatus* and *Pungitius pungitius* have been recorded in euryhaline waters (Lom and Stein 1966, Calenius 1980).

The morphological data of the species *T. domerguei* and *T. tenuidens* fall within the size ranges given by other authors (Lom and Stein 1966, Calenius 1980, Gaze 1995). The morphological variations of denticle form and appearance of central circle in *T. tenuidens* observed in this study are also in agreement with the statement of Lom and Stein (1966) that this species was found to be among the most variable of trichodinid species. During



Figs 1A, B. A - *Trichodina domerguei* Wallengren, 1897; **B** - *Trichodina tenuidens* Fauré-Fremiet, 1944. Specimens stained by silver-nitrate. Scale bar 10 µm.

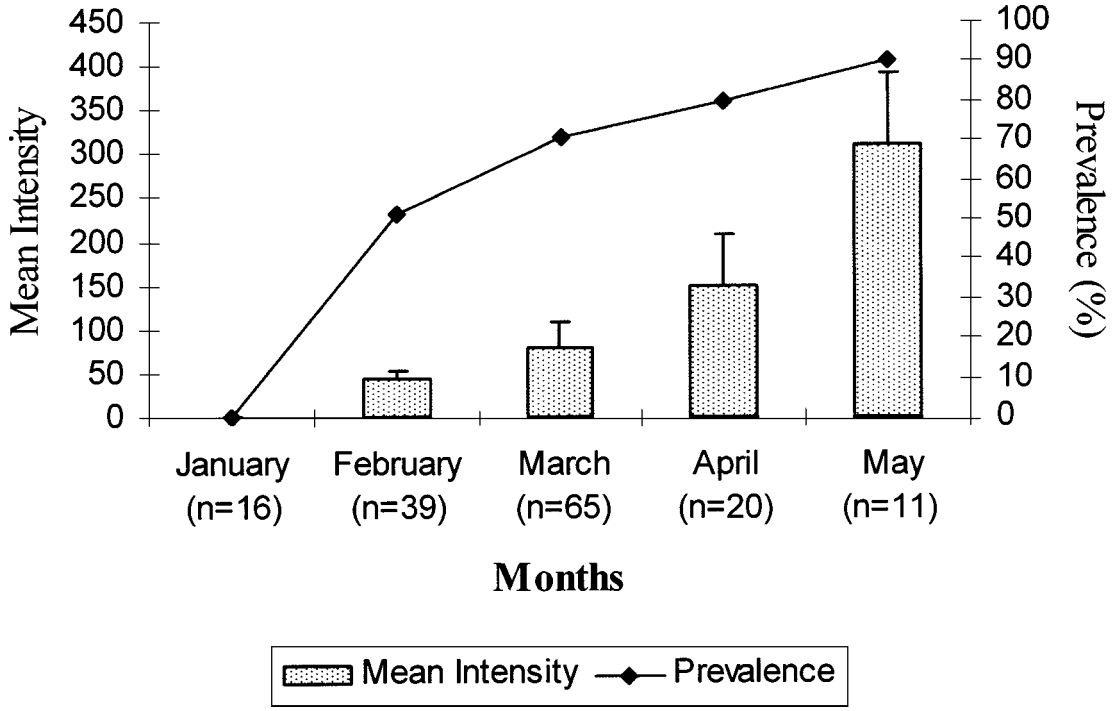


Fig. 2. Monthly infestation prevalence (%) and mean intensity levels of *Trichodina domerguei* and *T. tenuidens* on *Gasterosteus aculeatus*. n - represents the number of fish examined in each month.

Table 1. Infestation prevalence (%) and mean intensity levels of *Trichodina domerguei* and *T. tenuidens* on *Gasterosteus aculeatus* and the results of statistical tests performed.

	Infestation prevalence (%)	Mean intensity ± S.E	Possible comparisons	Statistial test used	Significance
Infestation site					
			(gills) *vs.* (skin) *vs.* (fins)	Kruskal-Wallis	P = 0.0001
Gills (n = 85)	60.9	87.9 ± 19.8			
			(gills) *vs.* (skin)	Dunn's	P < 0.001
Skin (n = 65)	56.3	32.9 ± 14.1			
			(gills) *vs.* (fins)	Dunn's	P < 0.001
Fins (n = 60)	43.1	7.4 ± 1.1			
			(skin) *vs.* (fins)	Dunn's	P > 0.05
Sex of fish					
Female (n = 116)	56.9	125.1 ± 28.9	(female) *vs.* (male)	Mann-Whitney U	P < 0.05
Male (n = 34)	70.6	65.9 ± 16.8			
Length classes of fish (mm)					
			(<60) *vs.* (61-69) *vs.* (70>)	Kruskal-Wallis	P = 0.0076
≤ 60 (n = 28)	35.7	11.1 ± 1.1			
			(<60) *vs.* (61-69)	Dunn's	P < 0.01
61 - 69 (n = 83)	72.4	122.3 ± 30.9			
			(<60) *vs.* (70>)	Dunn's	P < 0.05
70 ≥ (n = 40)	72.3	114.4 ± 27.8			
			(61 - 69) *vs.* (70>)	Dunn's	P > 0.05
Overall	60.9	109.1 ± 21.5			

Table 2. Biometric data of *Trichodina domerguei* and *T. tenuidens.*

	Trichodina domerguei (measured in March) (range; mean ± S.E)	*Trichodina domerguei* (measured in May) (range; mean ± S.E)	*Trichodina tenuidens* (measured in March) (range; mean ± S.E)	*Trichodina tenuidens* (measured in May) (range; mean ± S.E)
Host	*Gasterosteus aculeatus*	*Gasterosteus aculeatus*	*Gasterosteus aculeatus*	*Gasterosteus aculeatus*
Location	skin, fins, gills	skin, fins, gills	gills, skin	gills, skin
Adhesive disc diameter	48-56 (52 ± 0.53)	50-57.5 (53.5 ± 0.55)	40-57 (44.9 ± 1.3)	43-60 (47.5 ± 1.1)
Border membrane width	5-6.2 (5.4 ± 0.06)	5-6.3 (5.5 ± 0.07)	4.7-5.8 (5.4 ± 0.06)	4.9-5.9 (5.5 ± 0.06)
Denticle ring diameter	29-38.1 (33.8 ± 0.62)	30-38.6 (34.7 ± 0.53)	30.2-32 (31.0 ± 0.13)	30.9-32.4 (31.3 ± 0.16)
Denticle number	24-28 (26)	24-29 (26)	24-29 (25)	24-30 (25)
Number of radial pins per denticle	8-11 (9)	8-11 (9)	8-10 (9)	8-11 (9)
Denticle length	11-12.1 (12.5 ± 0.07)*	12-13.9 (13.3 ± 0.08)*	6.6-8.2 (7.1 ± 0.11)	6.6-8.5 (7.3 ± 0.15)
Blade length	5.0-6.1 (5.6 ± 0.06)	5.2-6.4 (5.8 ± 0.08)	4.8-6.6 (5.3 ± 0.11)	4.9 - 6.7 (5.5 ± 0.14)
Thorn length	4.4-5.4 (4.9 ± 0.07)*	4.9-5.8 (5.1 ± 0.05)*	5.6-7.4 (6.4 ± 0.17)	5.8-7.5 (6.5 ± 0.15)
Central part width	2-3 (2.2)	2-3 (2.3)	1.6-2.3 (1.7)	1.6-2.3 (1.8)
Central circle diameter	16.2-19.1 (18.1 ± 0.24)	16.8-19.5 (18.4 ± 0.19)	12-13 (12.5 ± 0.07)*	12-13.2 (12.8 ± 0.07)*

All measurements are in µm and based on 20 trichodinid specimens measured for each species. Mean values with superscript letter (*) represent statistically significant differences at P<0.05 when compared between March and May.

the months January, February and March when the stream mouth was in connection with the Black Sea, salinities of about 3, 4 and 3 ‰, respectively, were recorded and the dimensions of both trichodinid species were found to be slightly smaller in agreement with the findings of Lom (1970a) and Gaze (1995) who reported smaller specimen sizes in marine populations.

Host specificity in trichodinids seems to be highly variable, some species such as *T. domerguei*, *T. acuta* and *T. nigra* infest a large number of host species (Lom and Stein 1966, Lom 1970a, Calenius 1980) while *T. tenuidens* parasitises only one or two host species (Lom 1970b, Calenius 1980). It must be noted that several other fish species also inhabit the sampling site in this study, including *Aphanius chantrei* Gaillard, 1895, *Neogobius melanostomus* Pallas, 1811, *Mugil cephalus* L., 1758 and *Liza aurata* Risso, 1810, but *T. tenuidens* was not recorded on these fish species in contrast to *T. domerguei* which infected *N. melanostomus* as well. These findings on the host specificity of these trichodinid species also agree with the results of above mentioned authors.

The site of infection of trichodinids on host fish was categorised by Van As and Basson (1987) into four different groups based on site preference. Of the total number of trichodinid specimens, regardless of the actual species, counted throughout the study period, the infestation prevalence (%) and mean intensity levels were the highest on the gills as was shown in Table 1. *Trichodina tenuidens* was the dominant species and recorded mainly from the gills and rarely from the skin in accordance with Lom and Stein (1966), Calenius (1980) and Gaze (1995). On the other hand, most of the skin specimens were *T. domerguei* in this study. The above mentioned authors also reported this species to be skin specific and rarely found on the gills as well.

Seasonal and temperature dependant variations on the occurrence of trichodinids have been shown to occur (Özer and Erdem 1998, 1999). Spring was reported to be the mostly favoured season for trichodinid multiplication by the above mentioned authors and an increase was observed between the months February and May as in the present study. The obvious increase seen in both the infestation prevalence and mean intensity levels (Fig. 2) could be a result of the increase in temperature as the protozoan infestations in fish are strongly dependant on the ecological conditions such as temperature.

The number of studies on the existence of trichodinid parasites on both male and female fish is rare and almost no statistically significant difference in their existence is found (Özer 2000). However, the difference on the mean intensity levels of the trichodinids found on both sexes of fish in the present study is statistically significant. Pickering (1977), Pickering and Christie (1980) and Urawa (1992) attributed these differences to several factors such as rhythmical changes in epidermis thickness of male fish, a decrease in the number of AB-positive mucous cells and an increase in PAS-positive mucous cells.

The size of three-spined sticklebacks was a factor affecting the number of *T. domerguei* and *T. tenuidens* in this study and the differences in the parasite mean intensities among the different fish length classes were statistically significant. In general, the severity of most ecto- and endoparasitic infections increases with the age of the host fish, possibly as a result of the greater accumulation period and/or the larger space for feeding and breeding of the parasite. Özer and Erdem (1998) noted a tendency to increase in the mean intensity of *Trichodina* spp. in relation to the length of common carp. Our findings on the intensity levels of *T. domerguei* and *T. tenuidens* agree with those reported by the above mentioned authors.

Studies on the parasite fauna in farmed and wild fish in Turkey are quite rare. Özer and Erdem (1998, 1999) and Özer (2000) carried out extensive studies on the occurrence of trichodinids on common carp in the Sinop region of Turkey. This is the first study conducted on the trichodinids of three-spined sticklebacks found in a brackish environment in Turkey and *T. tenuidens* represents a new species record for Turkey.

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