Trichodinid Parasites (Protozoa: Ciliophora: Peritrichida) of Invasive Gobiid Fish Inhabiting The Lower Kızılırmak Delta in Samsun, Turkey

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Abstract.- The trichodinid fauna of three gobiid species, the monkey goby Neogobius fluviatilis (Pallas, 1814), the tubenose goby Proterorhinus marmoratus (Pallas, 1814) and the marbled goby Pomatoschistus marmoratus (Risso, 1810) collected from the Lower Kızılırmak Delta in Samsun, Turkey were investigated during December 2010-November 2011. Three trichodinid species including Trichodina domerguei Wallengren, 1897, Trichodina heterodentata Duncan, 1977 and Paratrichodina corlissi Lom et Haldar, 1977 were identified on 222 gobiid fish specimens. The existence of trichodinid parasites in relation to the body parts of three gobiid species as well as their seasonal occurrences were determined and discussed. This paper is the first report on the trichodinid fauna on the monkey goby, the tubenose goby and the marbled goby in Turkey. Trichodina heterodentata Duncan, 1977 and Paratrichodina corlissi Lom et Haldar, 1977 are new records for Turkish fish parasite fauna, while T. heterodentata is a new parasite record for Neogobius fluviatilis, Proterorhinus marmoratus and Pomatoschistus marmoratus.

Key words: Trichodina heterodentata, T. domerguei, Paratrichodina corlissi, gobiid fishes.

INTRODUCTION

Gobiidae is distributed worldwide in both marine and freshwater habitats and the majority of species are invasive (Miller, 2004). Neogobius, Proterorhinus and Pomatoschistus are the most common genera of the this family. Neogobius fluviatilis and Proterorhinus marmoratus are invasive species based on the Ponto-Caspian basin. Their native habitats include the coastal zones of the Black and Caspian Seas, the Seas of Azov and Marmara (Miller, 2004; Prasek and Jurajda, 2005; Neilson and Stepien, 2011). Pomatoschistus marmoratus is a species of Mediterranean basin and widespread in the eastern Atlantic, Mediterranean, Black Sea, Azov Sea, Suez Canal (Miller, 2004; Keskin, 2010).

Many studies have been conducted on helminth parasite fauna of these gobiid fishes. (Kvach, 2002a, 2002b, 2004a, 2004b, 2005, 2007; Ondrackova et al., 2005; Eros et al., 2005; Krasnovyd et al., 2012). Till date, the number of published studies on the trichodinid parasites of gobiid fishes are very limited (Lom and Haldar, 1977; Grupcheva and Lom, 1980; Van As and Basson, 1989; Asmat and Sultana, 2005; Mitra and Haldar, 2005; Mitra and Bondyopadhyay, 2006). To date in Turkey, there is only one published study on the trichodinids of Neogobius melanostomus, which is not a target gobiid species investigated in the present study (Özer, 2003a). There is no published study so far on the trichodinid parasites of Neogobius fluviatilis, Proterorhinus marmoratus and Pomatoschistus marmoratus from Turkish coast of the Black Sea. Thus, more studies are needed to determine the trichodinid fauna of various gobiid species.

The aims of the present study were: to investigate and describe trichodinid parasites on Neogobius fluviatilis, Proterorhinus marmoratus and Pomatoschistus marmoratus from Turkish coast of the Black Sea to obtain information on the occurrence of trichodinid parasites in relation to the body parts of these gobiid species and seasons, to make a contributions to the trichodinid fauna of fish hosts, to establish a background for further studies on trichodinids, and to extend our knowledge on the distribution and morphological variability of these trichodinid species in a precise part of the Black sea.

MATERIALS AND METHODS

The gobiid fish specimens were collected
Specimens of the gobiid fishes were collected by fishing nets and electroshock device. Sampling was carried out on a monthly basis between the period December 2010-November 2011. For parasitological examination, fish were transported alive in local water directly to the Sinop Fisheries Faculty Laboratory. A total of 222 gobiid fish specimens were investigated. The materials of the present study were three gobiid species: *Neogobius fluviatilis* (161), *Proterorhinus marmoratus* (45) and *Pomatoschistus marmoratus* (16). Skin, fins and gills were examined under a light microscope, and scrapings of whole mucus from these parts of fish were taken on several slides. The total number of trichodinids was determined by screening and counting the entire mucus material on each slide. Air dried smears were stained in accordance with the Klein’s dry silver impregnation technique in order to study details of the adhesive disc (Klein, 1958). All morphological measurement were carried out by oil-immersion light microscopy (Nikon SE). All measurements are micrometres and follow the uniform specific characteristics proposed by Lom and Dykova (1992). In each case, maximum and minimum values were given, the arithmetic mean and standard error were followed in parentheses. In the case of radial pins, the mode was given instead of the arithmetic mean. The span of the denticle was measured from the tip of the blade to the tip of the ray. In the description of denticle elements, the format recommended by Van As and Basson (1989) was followed.

The infestation prevalence (P, %) and mean intensity (MI) levels of the trichodinids were determined according to Bush *et al.* (1997). The standard error (SE) of the mean intensity was calculated. The prevalence and mean intensity values of three trichodinids were given for pooled data rather than by each trichodinid species.

Normal distribution of the data was tested by using Kolmogorov-Smirnov test. Kruskal-Wallis test (Nonparametric ANOVA) was performed to
find out the significant differences in the mean intensity values of trichodinids for infestation sites this study was conducted. The difference between parasite loading seasonal were tested by the Mann-Whitney U-test. The analyses were carried out using the computer programmes GraphPad Instat 3.0 and SPSS 9.0.

RESULTS

The current study is the first to report trichodinid fauna from three gobiid fish species captured from their natural environment on the Black Sea coast of Turkey. During the present study, three trichodinid species including Trichodina domerguei Wallengren, 1897, Trichodina heterodentata Duncan, 1977 and Paratrichodina corlissi Lom et Haldar, 1977 were identified. Neogobius fluviatilis and Pomatoschistus marmoratus were found to be infested with above mentioned three trichodinid species, Pr. marmoratus was infested with only T. heterodentata. The site of infestation of the three trichodinids on these gobiid fishes were different. T. heterodentata was commonly found on the skin and fins of Proterorhinus marmoratus it was the reversed. T. domerguei was commonly found on the skin and fins of N. fluviatilis and Po. marmoratus rarely on the gills. Paratrichodina corlissi, however, was found only on the skin of the monkey goby and the marbled goby (Table I). In addition, proportions of 100:40:1 for T. heterodentata, T. domerguei, Paratrichodina corlissi were observed on the stained slides (Fig. 2, 3, Table II).

Trichodina domerguei Wallengren, 1897 (Figs. 2A, 3A; Table II)
A large trichodinid with disc-shaped body. The centre of the adhesive disc of the specimens impregnated with silver nitrate is clear with numerous dark granules. The sickle-shaped blade of denticle is broad filling large area between y axes. The distal margin of blade almost touches the border membrane. The apex of blade is round almost touching y+1 axis. Tangent point round. Posterior blade margin fairly curved. Blade apophysis present, but not clearly visible. Blade connection thin. Central part well developed, but thin and long tapering to rounded point fitting tightly into preceding denticle. Ray connection short and thin. Base of ray thin, with ray bulbous towards broads and rounded point. Rays short and curved in posterior direction with tips extending beyond y axes. Section of denticle above x axis to denticle below similar, ratio one. The morphometrical data are presented in Table II.

<table>
<thead>
<tr>
<th>Infestation Site</th>
<th>Neogobius fluviatilis (mainly T. heterodentata)</th>
<th>Pomatoschistus marmoratus (mainly T. heterodentata)</th>
<th>Proterorhinus marmoratus (only T. heterodentata)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>1.61%</td>
<td>1.05%</td>
<td>76%</td>
</tr>
<tr>
<td>Fins</td>
<td>1.90%</td>
<td>1.54%</td>
<td>20%</td>
</tr>
<tr>
<td>Gills</td>
<td>96.49%</td>
<td>97.4%</td>
<td>4%</td>
</tr>
</tbody>
</table>

Trichodina heterodentata Duncan, 1977 (Figs 2B, 3B; Table II)
A medium-sized trichodinid with disc-shaped body. The centre of the adhesive discs of the specimens impregnated with silver nitrate is dark-stained. T. heterodentata is a distinctive species characterised by the robust, strongly sickle-shaped denticle blades with pointed ends, the evenly tapering rays with pointed tips, the absence of any central inclusions and the prominence of the radial pins. The distal surface of blade is rounded and slants away from the border membrane. The denticle having strongly falcate blade with fine tangent and anterior blade apophysis, the anterior margin of blade sharply curves down. The apex of blade is round, touching y+1 axis. Blade apophysis visible, triangular with bluntly rounded point and thick. Blade connection is also thick. The central part is elongate and wide, tip rounded. Projection of central part not visible pointed and extending to slightly more than halfway towards the y-1 axis. Ray long, robust, situated between the axes y and y-1. The morphometrical data are presented in Table II.

![Diagrammatic structure of the denticles of trichodinids](image)


Paratrichodina corlissi *Lom et Haldar, 1977* (Figs. 2C, 3C; Table II)

A small trichodinid. The adhesive disc club-shaped. The border membrane is finely striated. The distal surface of blade smooth, straight, nearly in parallel with border membrane, and higher than the bluntly tangent point; the anterior and posterior margins of blade not very smooth and not parallel with each other; anterior margin of blade not extending beyond y+1 axis; posterior margin of blade L shaped with deep point; apophysis of blade present and posterior projection absent; central part not developed and cone-shaped with sharp point fitting loosely into preceding denticle and not extending half way to y-1 axis; shapes of the central part above and below the X-axis similar; ray connection very inconspicuous and barely distinguishable from ray; ray relatively thin, needle-shaped, obliquely attached and slanted a little posteriorly with a sharp point of ray. The morphometrical data are presented in Table I.

Seasonal prevalence and mean intensities of trichodinids infesting three gobiid species are reported in Table III. The maximum infestation prevalence and mean intensity values were recorded in *Po. marmoratus* (81.25%, 1003.84 ± 972.26). The minimum infestation prevalence (%) and mean intensity values was recorded from *N. fluvialis* (22.98%), while the minimum mean intensity value was in *Pr. marmoratus* (7.83 ± 1.75) (Table III).

Seasonal prevalence and mean intensities values of trichodinids recorded from both *N. fluvialis* and *Po. marmoratus* were similar, and the maximum values in both fish species were determined in spring. It was obvious that mean intensities values of trichodinids recorded from *Pr. marmoratus* was seasonally different from other two gobiid species (Table III).

**DISCUSSION**

Trichodinids are geographically a widely dispersed group of ectoparasites in freshwater, marine and euryhaline environments. So far, seven trichodinid species from five gobiid species have been reported worldwide (Table 4). There is only one study on the trichodinids of our target gobiids, namely *Neogobius fluvialis*, that was carried out in the Black Sea (Grupcheva and Lom, 1980).
Table II.- Morphometrical data, host and infestation site of of Trichodina domerguei, T. heterodentata and Paratrichodina corlissi (n: number of measured specimens) (range with arithmetic mean and standard error in parentheses) (all measurements in μm)

<table>
<thead>
<tr>
<th>Host</th>
<th>Trichodina domerguei (n: 20)</th>
<th>Trichodina heterodentata (n: 21)</th>
<th>Paratrichodina corlissi (n: 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of</td>
<td>Nf, Po</td>
<td>Nf, Pr, Po</td>
<td>Nf, Po</td>
</tr>
<tr>
<td>body</td>
<td>75-89 (80.0 ± 1.65)</td>
<td>45-64 (51.17 ± 3.09)</td>
<td>30-32 (30.75±0.48)</td>
</tr>
<tr>
<td>adhesive dics</td>
<td>55-71 (64.0 ± 2.12)</td>
<td>37-55 (43.42 ± 2.60)</td>
<td>26-28 (27.0±0.41)</td>
</tr>
<tr>
<td>denticular ring</td>
<td>38-46 (41.88 ± 1.10)</td>
<td>24-39 (27.16 ± 1.70)</td>
<td>16-18 (17.0±0.58)</td>
</tr>
<tr>
<td>Number of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>denticles</td>
<td>27-30</td>
<td>20-26</td>
<td>22-23</td>
</tr>
<tr>
<td>radial pins per denticle</td>
<td>9-10</td>
<td>7-8</td>
<td>5-6</td>
</tr>
<tr>
<td>Length of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>blade</td>
<td>8-10 (8.88 ± 0.38)</td>
<td>4-6 (4.75 ± 0.22)</td>
<td>3-3.5 (3.25±0.14)</td>
</tr>
<tr>
<td>ray</td>
<td>4-5 (4.78 ± 0.15)</td>
<td>5-8 (5.58 ± 0.34)</td>
<td>2-3 (2.38±0.24)</td>
</tr>
<tr>
<td>Span of denticle</td>
<td>16-20 (17.88 ± 0.49)</td>
<td>11-17 (12.88 ± 0.65)</td>
<td>7-8 (7.50±0.20)</td>
</tr>
<tr>
<td>Width of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>central part</td>
<td>2.5-3.5 (3.12 ±0.15)</td>
<td>1-3 (2.17 ± 0.21)</td>
<td>2-2.2 (2.05±0.05)</td>
</tr>
<tr>
<td>border membrane</td>
<td>4-5 (4.75 ± 0.15)</td>
<td>4-5 (3.33± 0.25)</td>
<td>1.7-2 (1.93±0.08)</td>
</tr>
</tbody>
</table>

Nf: Neogobius fluviatilis, Pr: Proterorhinus marmoratus, Po: Pomatoschistus marmoratus

Table III.- Seasonal infestation prevalence (%) and mean intensity levels of three trichodinid species (T. heterodentata, T. domerguei, and Paratrichodina corlissi) on three gobiid fish species (P (%): Prevalance, MI: Mean Intensity, SE:Standart Error, n: Examined fish number)

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neogobius fluviatilis</td>
<td>P (%): 17.64</td>
<td>16.12</td>
<td>37.50</td>
<td>22.98</td>
<td>188.10 ± 175.18</td>
</tr>
<tr>
<td></td>
<td>MI±SE: 745.33 ± 718.54 (51)</td>
<td>9.40 ± 6.35 (62)</td>
<td>8.77 ± 4.20 (48)</td>
<td>(0) (161)</td>
<td>81.25</td>
</tr>
<tr>
<td>Pomatoschistus marmoratus</td>
<td>P (%): 100</td>
<td>4.00 ± 0.00 (11)</td>
<td>8.73 ± 2.02 (24)</td>
<td>2.00 ± 0.00 (19)</td>
<td>7.83 ± 1.75 (45)</td>
</tr>
<tr>
<td>Proterorhinus marmoratus</td>
<td>P (%): 4.00 ± 0.00 (0)</td>
<td>62.5 (2)</td>
<td>5.26 (5)</td>
<td>40 (16)</td>
<td></td>
</tr>
</tbody>
</table>

Trichodina domerguei, T. heterodentata and Paratrichodina corlissi have so far been reported from several gobiid species excluding gobiids investigated in the present study. Thus, in the present study T. heterodentata on N. fluviatilis, Pr. marmoratus, Po. marmoratus and Paratrichodina corlissi on N. fluviatilis, t Po. marmoratus are the first reports in the Black Sea.

Trichodina domerguei is one of the most widely distributed trichodinid and it has been reported from variety of fish hosts living in freshwater, brackish and marine habitats (Lom and Stein, 1966; Lom, 1970; Xu et al., 1999; Özer, 2003a, b). T. domerguei was commonly found on the skin and fins of the monkey goby and the marbled goby, rarely on the gills. The present data is similar with those reported by Özer (2003a, b), Isaksen (2003), Rolbiecki (2006), Öztürk and Özer.
(2007) in that this species was found mainly on skin. Our findings on the morphological and morphometrical data of *T. domerguei* are also in agreement with the statements of the authors mentioned above.

*Trichodina heterodentata* is a cosmopolitan species and, since it was first described by Duncan (1977), more than 35 species of fishes in 14 families have now been recognized as hosts for this parasite (Martins et al., 2010). *T. heterodentata*, having a wide geographical distribution in 12 countries in different zoogeographical region (Dias et al., 2009) and host range, occurs on the gills and skin of marine and brackish fish. In addition, it has been reported from tadpole in freshwater (Martins et al., 2010). This study is the first report of *T. heterodentata* in Turkish waters of the Black Sea. The presence of *T. heterodentata* at the Palearctic Region following Egypt and Israel has been confirmed once again by the present study. *N. fluviatilis*, *Po. marmoratus* and *Pr. marmoratus* have been also added in host list of *T. heterodentata* indicating its low host specificity. In this study, *T. heterodentata* was the dominant species and commonly found on the skin and fins of the tubenose goby, rarely on the gills, for the monkey goby and the marbled goby it was the reversed. Dove and O’doghue (2005), Faurie (2006), Martins et al. (2010), Miranda et al. (2012) stated that this species was found mainly on skin. Our data are similar for the tubenose goby but different for the monkey goby and the marbled goby with the statements of the authors mentioned above. Findings reported by Al-Rasheid et al. (2000), Asmat (2004), Bondad-Reantaso and Arthur (1989) who found this species only on the gills are totally different from our data.

*Paratrichodina corlissi* was first identified on gills of *Gobio gobio* in ex-Czechoslovakia. Lom and Haldar (1977) was described on gills of *Gobio kessleri* in Bulgaria, later Tang et al. (2012) reported on the gill of *Letalurus punetaus* in China. This species was detected for the first time on skin of *Neogobius fluviatilis* and *Po. marmoratus* in the present study.

The seasonal prevalence and mean intensity levels of identified three trichodinid species had different values in each gobiid species in the present study (Table 3). Trichodinids often show seasonal changes in prevalence and intensity of infestation and occurrence of trichodinids is generally related to water temperature. Some authors reported peak levels of trichodinid infestation in spring and early summer (Özer and Erdem, 1999; Özer, 2003a, b). The highest mean intensity levels in the present study were determined in spring for *Po. marmoratus* (1154.80 ± 1151.40) and *N. fluviatilis* (745.33 ± 718.54). This result is also supported by the data presented by the authors mentioned above, even though the trichodinid species they studied were different. Seasonally, the mean intensity level from *Pr. marmoratus* was lower than the other two gobiid species (Table III). When the number of trichodinid species infesting gobids was considered, this could be the result of *Pr. marmoratus* having only one trichodinid species while the other gobids had three trichodinid species. Moreover, throughout the investigation period, *T. heterodentata, T. domerguei* and *P. corlissi* were present simultaneously in *N. fluviatilis* and *Po. marmoratus*. In the present study, *T. heterodentata* was possibly the main factor causing higher prevalence and mean intensity levels.

<table>
<thead>
<tr>
<th>Trichodinid species</th>
<th>Gobiid fishes</th>
<th>Reference</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Paratrichodina corlissi</em></td>
<td><em>Gobio kessleri</em></td>
<td>Lom and Haldar (1977)</td>
<td>Bulgaria</td>
</tr>
<tr>
<td><em>Trichodina jiroveci</em></td>
<td><em>Neogobius fluviatilis</em></td>
<td>Groupcheva and Lom (1980)</td>
<td>Bulgaria</td>
</tr>
<tr>
<td><em>Trichodina heterodentata</em></td>
<td><em>Glossogobius giurusi</em></td>
<td>Van As and Basson (1989)</td>
<td>South Africa</td>
</tr>
<tr>
<td><em>Trichodina glossogobiusi</em></td>
<td><em>Rhinogobius brunneus</em></td>
<td>Basson and Van As (1994)</td>
<td>South Africa</td>
</tr>
<tr>
<td><em>Trichodina haldari</em></td>
<td><em>Glossogobius giurusi</em></td>
<td>Asmat and Sultana (2005)</td>
<td>Bangladesh</td>
</tr>
<tr>
<td><em>Trichodina giurusi</em></td>
<td><em>Glossogobius giurusi</em></td>
<td>Mitra and Bandyopadyay (2006)</td>
<td>India</td>
</tr>
<tr>
<td><em>Trichodina domerguei</em></td>
<td><em>Neogobius melanostomus</em></td>
<td>Özer (2003a)</td>
<td>Turkey</td>
</tr>
</tbody>
</table>

Table IV. *Trichodinid species have been reported from gobiid species to date.*
in spring as being dominant species among the three. The situation was different for *Pr. marmoratus* regarding to mean intensity level. It was low due to host preference of *T. heterodentata* in favour of *N. fluviatilis* and *Po. marmoratus*.

In conclusion, this paper is the first research study conducted on the existing trichodinid fauna of *N. fluviatilis*, *Pr. marmoratus* and *Po. marmoratus*. This paper makes some valuable contribution to our knowledge about known trichodinid parasites; *Trichodina heterodentata* and *Paratrichodina corlissi* are new records for Turkish parasite fauna, While *T. heterodentata* and *Paratrichodina corlissi* are new parasite records for both *N. fluviatilis* and *P. marmoratus*. To date, the majority of research studies conducted on trichodinid species are related to host records and identification of their parasites rather than the determination of parasite infestation levels. Therefore, this study provides some new, valuable and ecologically significant results.

**ACKNOWLEDGEMENTS**

This study was supported financially by The Scientific and Technological Research Council of Turkey (TÜBİTAK) with the project number of 1100424. I thank to Prof. Dr. Ahmet ÖZER, Sinop University, Fisheries and Aquaculture Faculty, Turkey, for his comments on the text.

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(Received 25 April 2013, revised 6 August 2013)