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Morphological, ecological and histopathological studies of *Trichodina gobii* Raabe, 1959 (Ciliophora: Peritrichida) infecting the gills of *Solea aegyptiaca*

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Summary

Specimens of *Solea aegyptiaca* caught from Ghar El Melh lagoon in Tunisia were investigated for trichodinid ectoparasites. One species of the genus *Trichodina* was recorded on the gills of *S. aegyptiaca*. This species is identified as *Trichodina gobii* Raabe, 1959 which is reported for the first time on the soleidae fishes from Tunisian waters. The prevalence and the mean intensity level of these ciliophorans varied throughout the year with maximum rate of infection during the winter. Taxonomic descriptions of this species based on silver nitrate method and scanning electron microscopic observations are provided in this paper. Histopathological examinations of gills infested with *T. gobii*, revealed several lesions such as hyperplasia and lamellar epithelium lifting of the secondary lamellae.

Key words: ecology, histopathology, SEM study, *Solea aegyptiaca, Trichodina gobii*

Introduction

Trichodinids are one of the largest and most widely dispersed groups of ectoparasites in freshwater, marine and euryhaline environments. Today more than 260 species representing ten genera were described within the family of Trichodinidae from the skin, gills, urinary bladder of fishes, amphibians and from the integument and the genital tract of invertebrates (Lom and Dykova, 1992). Some species inhabiting inhabit also the digestive tract of marine fishes (Da Cunha and Pinto, 1928; Basson et al., 1990). The genus *Trichodina* is the largest of this family; about 200 species of *Trichodina* have been described from fishes by silver impregnation technique (Asmat et al., 2005). In the case of massive infestation, some species of Trichodinids become highly pathogenic and may cause severe damage

and even the death of their host (MacArdle, 1984; Hassan, 1999). In Tunisia, there are a few studies mentioned the presence of Trichodinids on marine fishes, all of them without presenting any taxonomic study. The aim of the present work is to identify the *Trichodina* species that occur on the gills of *S. aegyptiaca* - one of the important economical fish in Tunisian waters, to follow the variations of the prevalence and the mean intensity of this parasite during the investigation period, and to evaluate its pathogenicity.

Material and methods

From September 2009 to August 2010, a total number of 130 specimens of *S. aegyptiaca* measuring 16.2 to 28.4 cm were caught by gill net from Ghar El Melh lagoon which is situated in Northeast of Tunisia (37°10′ N and 10°09′ E). During the investigation period, host fishes were transported alive in local water directly to the laboratory. Wet smears of gills were prepared and examined in order to detect the presence of Trichodinids. Smears from infested infected fishes were air-dried and impregnated for 10 min in 2 % aqueous $AgNo_3$ solution (Klein's silver impregnation technique) (Klein, 1958), washed in distilled water, and exposed to ultraviolet light for 20 to 25 min, in order to study details of the adhesive disc and the aboral ciliary spiral. Preparations and photographs were made under a Nikon E 600 microscope. All measurements are in micrometers and based on 30 trichodinid specimens and follow the uniform specific characteristics given by Lom (1958). The description of denticule elements follows the format recommended by Van As and Basson (1989). The intensity of infestation and the seasonal prevalence of parasites were calculated according to Bush et al. (1997).

For histological study, gills were fixed in 4% formaldehyde and processed using standard technique with haemotoxylin-eosin staining and examined microscopically.

For scanning electron microscopic study, gills were fixed for 1 hour in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate at pH 7.2, then dehydrated in a graded acetone series and dried using CO_2 in an Emitech K850 critical point dryer. After mounting, gills were coated with gold/palladium in a Quorum Technologies SC7640 sputter coater and examined with a Hitachi S-3400N scanning electron microscope at an acceleration voltage of 10 kV.

Results

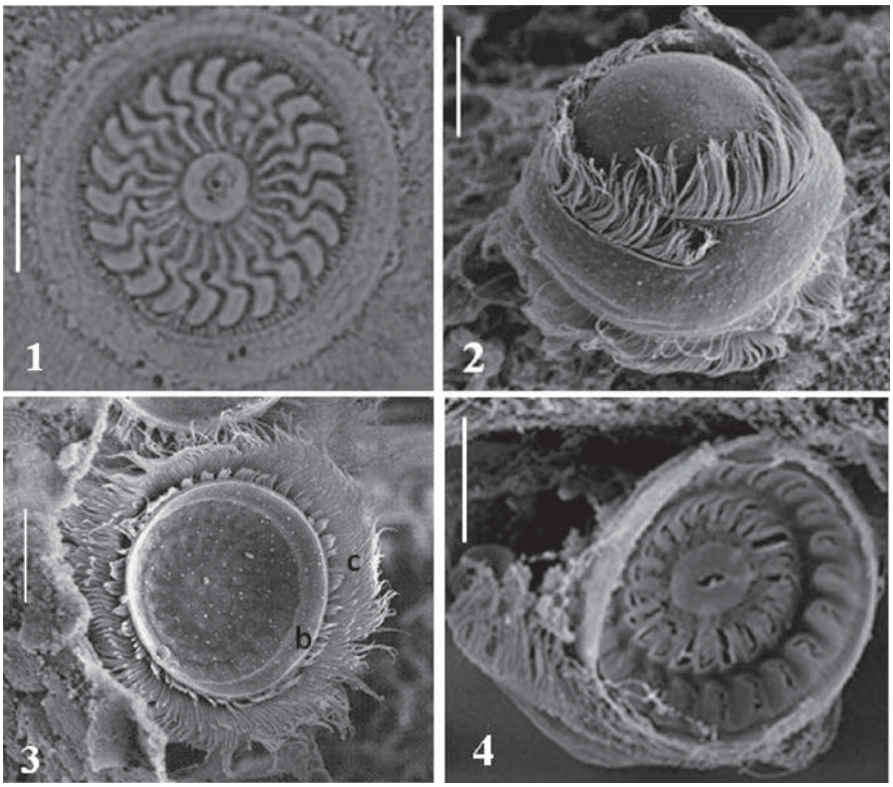
Morphological data of *Trichodina gobii* Raabe, 1959

All collected specimens from the gills of *Solea aegyptiaca* are assigned to *T. gobii*. Parasite presents a medium size with 36.8-40.8 (38.5 ± 1.4) µm in diameter. Adhesive disc concave, 27.2-32.8 (29.8 ± 1.7) µm in diameter, with a well defined central circle 7.2-9.6 (8.3 ± 0.9) µm and surrounded by a border membrane of 3.2-4.5 (3.6 ± 0.5) µm in width (Fig. 1, Table 1). Diameter of denticulate ring 20.2-29.6 (24.6 ± 2) µm, consists of 20-23 denticles, with length of 6.2-7 (6.4 ± 0.6) µm. Blade 4-5.6 (4.6 ± 0.4) µm less curved and more or less rectangular, slightly narrowing to rounded distal ends and filling large portion of sectors between y and y-1 axes; tangent point almost rounded, situated slightly lower than distal margin. Posterior margin curve forms small narrow arch with y axes; deepest point on same level as apex. Section connection blade is relatively thick. Blade apophysis and posterior projection are absent. Sections of central part above and below x axis are similar in shape. Central part is thick with rounded point extending more than half way to y axis. Thorns 2.4-4 (3.24 ± 0.2) are slender and smooth with rounded end. Apophysis of thorn is not visible. Section connection of thorn is thinner than those connecting blade. Number of radial pins per denticles is 7-8.

SEM observations of the ciliate show a spherical shape in oral view. The surface topography was smooth (Fig. 2). There are two ciliary bands well developed, one adoral and the other around the aboral adhesive disk (Fig. 3). The adoral ciliary band is composed of two rows of closely-set cilia. The buccal region is therefore flanked by two bands of cilia (Fig. 2). The adhesive disk is surrounded by a well developed border membrane, and contains fine vertical stria over its entire surface (Figs 3, 4). These strias on the internal surface of the border membrane are the radial pins.

Prevalence and Mean intensity

The prevalence and the mean intensity of *T. gobii* fluctuate during the sampling period. Indeed, the prevalence ranged from 12.03 % to 84.4 %. The prevalence reached its maximum in winter, when water temperature was low (12 °C), then decreased along with increased temperature and reached its minimum in summer when the temperature was at maximum (29.33 °C) (Fig. 5).



Figs 1-4. Photomicrographs of *Trichodina gobii* from the gills of *Solea aegyptiaca*. 1 – Silver impregnated adhesive disc of *T. gobii*; 2-4 – SEM micrographs of *T. gobii*: 2 – oral view showing adoral spiral entering infundibulum; 3 – aboral view showing border membrane (b) and ciliary band (c); 4 – aboral view showing denticulate ring. Scale bars = 12μm.

The mean intensity level of *T. gobii* has increased from 29.27 ± 3.08 in autumn and reached a peak value of 117.66 ± 29.5 in winter, when the water temperature was low, then mean intensity decreased gradually with the increasing of temperature and reached its minimum in summer (11.63 ± 1.04) (Fig. 6).

Histopathology

Histopathological examination of infested fishes infested by with the present species, revealed significant gill changes. In most cases fusion of secondary lamellae and hyperplasia were observed (Fig. 7). We noted also, an intense lamellar epithelium lifting (epithelium detachment) in secondary lamellae (Fig. 8). In addition to these lesions, an excessive accumulation of mucus on gills was also seen on gills of in infested fishes.

Discussion

The present species of *Trichodina* collected from the gills of *S. aegyptiaca*, was compared with previously described Trichodinid species with similar morphological characters. *T. jadranica* Raabe, 1958 is one of the most frequently reported trichodinids from various marine fishes, especially flatfishes.

Based on the silver impregnated specimens, the species of *Trichodina* collected from *S. aegyptiaca* are very similar to *T. jadranica* by the well defined central circle, a low number of denticles and a relatively smaller body. However, our species is different by less curved and more or less rectangular blades slightly narrowing to rounded distal ends. The denticle blades of *T. jadranica* are distinctly curved, almost sickle-shaped with a sharp tangent point distally. The present species shows similarity in the denticles shape which is almost rectangle-like blades with *T. gobii* Raabe, 1959 originally described from *Gobius minutes* from the Baltic Sea. The similarity in the denticle shape and dimensions of the present species is also shared with the others populations of *T. gobii* already described from various hosts (Table 1). The SEM observations of *T. gobii* are reported for the first time in this study. The ciliate was spherical in shape and the surface topography was smooth, typically for the most Trichodinids (Qi et al., 1995).

It seems that *T. gobii* has low host specificity

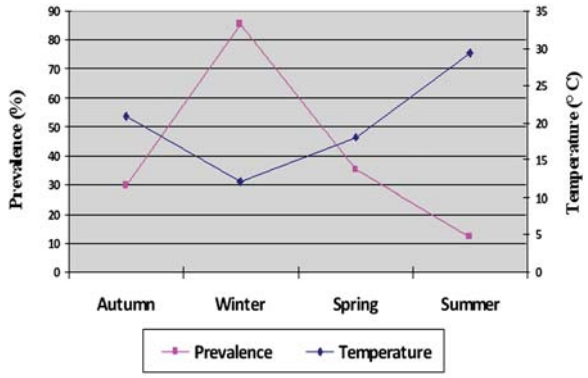


Fig. 5. Seasonal variations of temperature (°C) and prevalence of infection of *T. gobii*.

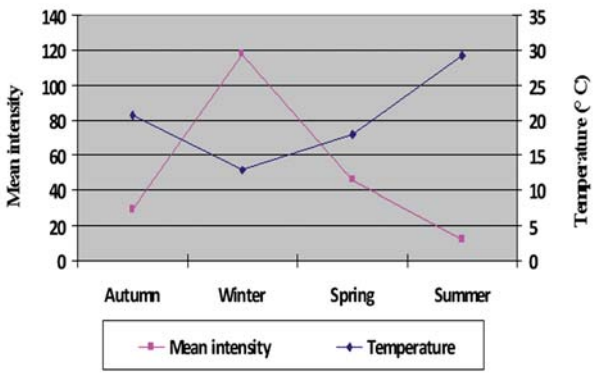


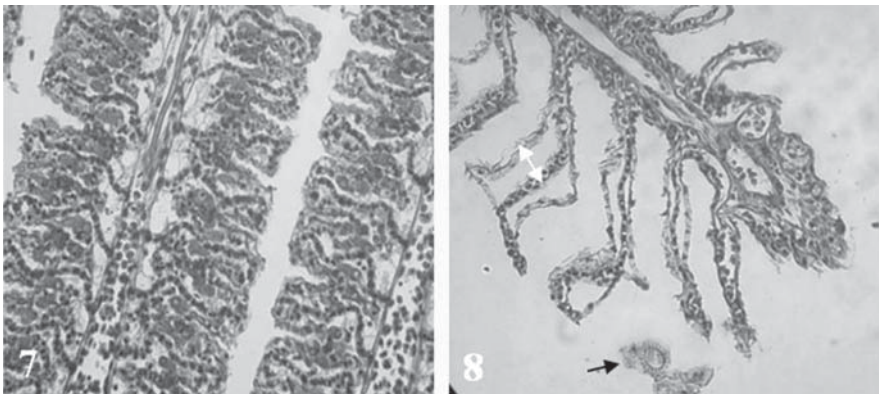
Fig. 6. Seasonal variations of temperature (°C) and mean intensity level of *T. gobii*.

and a widely geographical distribution, it can occur in both marine and freshwater environments. This study reports for the first time the presence of *T. gobii* on soleidae fishes from Tunisian coast.

T. gobii was present throughout the year with higher rate of infestation during the coldest season. It seems that the high temperature of water, prevent the proliferation of the ciliate. The present finding agree with those of McArdle (1984), Abu El-Wafa (1988), El-Khatib (1989) and Hassan (1999), who mentioned that trichodinids were prevalent all over the year with maximum rate of infestation during

Table 1. Morphometric data (in micrometers) of *Trichodina jadranica* and *Trichodina gobii* from different hosts.

	Raabe (1958)	Raabe (1959)	Lom (1970)	Grupcheva et al. (1989)	Su and White (1995)	Madsen et al. (2000)	This paper
Species	*T. jadranica*	*T. gobii*	*T. gobii*	*T. gobii*	*T. gobii*	*T. gobii*	*T. gobii*
Host	*Mullus barbatus*	*Gobius minutus*	*Callionymus lyra*	*Crenilabrus ocellatus, C. griseus, Neogobius malanostomus*	*Nesogobius* sp.	*Anguilla anguilla*	*Solea aegyptiaca*
Site	Gills	Gills	Gills	Gills	Gills	Gills	Gills
Locality	Adriatic Sea	Baltic Sea	Brittany coast of France	Black Sea, Blalchik	Tasmania, Australia	Eel farm, Denmark	Lagoon of Ghar El Melh, Tunisia
Body diameter	34-43	25-35	28-41 (36)	35-50	40-55(45.1)	–	36.8-40.8 (38.5 ± 1.4)
Adhesive disc	28-38	25-35 (30)	22-31 (27)	20-27	24-37(32.3)	31-41(37±4.1)	27.2-32.8 (29.8 ± 1.7)
Border membrane width	–	–-	3	2,9-3,6	3.1	–	3.2-4.5 (3.6 ± 0.5)
Denticle ring diameter	16-22	13-18 (15,5)	14-18 (16)	12,5-16	13-21(16 .6)	19-29(23±2.8)	20.2-29.6 (24.6 ± 2)
Denticle number	22-25	19-23 (20-21)	18-23 (22)	19-21	20-24(23)	24-27(26±0.9)	20-23
Radial pins per denticle	8	6	6-7	6	6-9(7)	6-8	7-8
Denticle Span	–	–	–	7.3-7.8	–	–	–
Denticle length	–	–	4,5-5	2.9-4.3	4-5(4.5)	–	6.2-7 (6.4 ± 0.6)
Blade length	3-4	3.5-4.5 (4)	2.2 – 3	2.9-3.4	4-5(4.7)	–	4-5.6 (4.6 ± 0.4)
Central part width	–	–	1.5	1.5	1-1.5 (1.1)	–	1-1.5
Ray length	2.5-3	3-4 (3.5)	2-2.8	2.9	3-4 (3.7)	–	2.4-4 (3.24 ± 0.2)
Central circle diameter	–	–	–	–	7-8 (7.5)	–	7.2-9.6 (8.3 ± 0.9)



Figs 7, 8. Histopathology of infested fishes. 7 – Histological section of infested gills showing pronounced hyperplasia in secondary lamellae; 8 – intense lamellar epithelium lifting in secondary lamellae (white arrow) (*T. gobii* indicated by black arrow).

winter. Fluctuations of prevalence and mean intensity of *T. gobii* observed in this study are connected in relation with the ecological conditions such as temperature, oxygen and salinity. This result has been mentioned by Maslin-Leny (1988), Hassan (1999), Ogut and Palm (2005) and Kristmundsson et al. (2006).

In the present study it is shown, that the infestation with *T. gobii* caused serious pathological lesions in gills, such as hyperplasia of the epithelial cells, clubbing and fusion of the gill filaments, which have been previously reported by many authors (Padnos and Nigrelli, 1942; Davis, 1947; Sarig, 1971; Hassan, 1999). In addition to these lesions, massive production of mucus was observed on infested fishes as a defence mechanism produced by the host to eliminate the parasite.

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