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Seasonality and histopathology of *Trichodina puytoraci* Lom, 1962, a parasite of flathead mullet (*Mugil cephalus*) from Tunisia

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During a parasitological survey in Ghar El Melh Lagoon in Tunisia, 108 flathead mullet (Mugil cephalus L., 1758) were investigated for protozoan ectoparasites. Trichodina puytoraci Lom, 1962 infecting the gills of the examined fish were isolated and reported for the first time from this locality. The overall infestation prevalence and mean intensity level were 22.42 % and 70 ± 8.1 trichodinids per fish, respectively. The highest prevalence was observed in spring. The relationship between host's size and parasite prevalence was significant. The histopathological study revealed that M. cephalus heavily infested by T. puytoraci exhibited serious lesions such as hyperplasia of the epithelial cells, fusion and necrosis of secondary lamellae.

Key words: *Trichodina puytoraci*, *Mugil cephalus*, prevalence, mean intensity, histopathology, Tunisia

INTRODUCTION

Trichodinid Ciliophorans are one of the most common and widely dispersed groups of symbionts as parasites of aquatic invertebrate and vertebrate hosts (VAN AS & BASSON, 1989). The taxonomy of Trichodinids is based on the structure and the appearance of the adhesive disc and number and size of its constituents. All of these features can be revealed only by the silver impregnation technique of Klein (KLEIN, 1958). Consequently, the numerous species identified early without silver techniques are inadequately described (LOM & DYKOVA, 1992). Today, ten genera are described within the family Trichodinidae. The genus *Trichodina* Ehrenberg, 1838 is the largest of this family; more than 200 species have been described from fish (ASMAT *et al*., 2005). Trichodinids have a direct life cycle and they reproduce by binary fission. Most trichodinids are not pathogens, but under certain environmental conditions or when the fish are stressed by other factors, the parasite increases greatly its rate of infestation among fish and can become pathogenic and cause mass mortality of infested hosts. According to KHAN (2004), outbreaks and mass mortality of Atlantic cod (*Gadus morhua*) associated with *Trichodina murmanica* infection was reported in a coastal embayment of Newfoundland. The lesions most induced by this parasite are hyperplasia and necrosis of the epidermal cells (PADNOS & NIGRELLI, 1942; DAVIS, 1947; SARIG, 1971; HASSAN, 1999).

Trichodina puytoraci was originally described from mugilidae fish from the Romanian Black Sea coast (LOM, 1962). Later, this species has been reported from *Mugil cephalus* L.,

1758; *Liza aurata* Risso, 1810 and *Liza saliens* Risso, 1810 from different geographic localities (BYKOVSKAYA-PAVLOVSKAYA *et al*., 1964; KINNE, 1984; GRUPCHEVA *et al*., 1989; ÖZER & ÖZTÜRK, 2004; AL-BASSEL *et al*., 2007). However, the pathogenecity of this parasite has not been reported previously.

The present study reports for the first time the presence of *Trichodina puytoraci* on the gills of *Mugil cephalus* from Ghar El Melh Lagoon in northern Tunisia. The objectives were: description of *T. puytoraci* morphology; evaluation of the seasonal infestation rate in fish; assessment of the influence of the host's size on the parasitism; evaluation of histopathological changes caused by the ciliate.

MATERIAL AND METHODS

Fish were collected by nets from Ghar El Melh Lagoon situated in northeast Tunisia (37°10 N and 10°09 E) that communicates with the Mediterranean Sea by 3 open channels. This lagoon is characterized by a surface area of 3000 ha, a salinity ranging between 33.7-38.2 ‰ and 1 m depth.

A total of 108 *Mugil cephalus* were examined in the period from May 2007 to April 2008. Wet smears of gills were prepared and examined for the presence of Trichodinids. In positive samples, the smears were air dried and impregnated with a 2% aqueous solution of silver nitrate ($AgNO_3$) for 8 min and exposed to UV light for 20 min according to Klein's dry silver method (KLEIN, 1958) for observation of the adhesive disc. Examination of prepared slides and photographs was made by using a Nikon E600 microscope. Measurements of body, adhesive disc, central circle, denticulate ring, denticles, central part and border membrane were made according to the recommendations of LOM (1958), WELLBORN (1967) and VAN AS & BASSON (1989). All measurements are given in microns and based on 20 trichodinid specimens. For histological study, gills were fixed in 4% formaldehyde and processed using standard techniques with Hematoxylin-Eosin staining.

The prevalence and mean intensity levels of the trichodinids were determined according to BUSH *et al*. (1997). χ^2 test was conducted to evaluate the influence of the host's size on parasite prevalence.

RESULTS AND DISCUSSION

Morphology

Throughout the investigation period, *Trichodina puytoraci* was identified from the gills of *Mugil cephalus* for the first time in Tunisia. The specimens of *T. puytoraci* (Figs. 1,2) were characterized as of medium size, 47.2-54.4 (49.03 ± 2.02) µm in diameter. Adhesive disc concave, 41.6-52 (45.36 ± 3.6) µm in diameter. Central circle dotted with small particles, irregularly rounded. Denticulate ring, 28-32 (29.63 ± 1.72) µm in diameter, consists of 25-28 denticles with 7-8 radial pins per denticule. Denticles blade of 4.8-5.6 (4.9 ± 0.72) µm in length, broad and occupies most of the area between y-axes with distal surface parallel to border membrane when situated close it. Central part robust, 2.5-3 (2.6 ± 0.10) µm in width. The ray 6.4-7.2 (6.82± 1.28) µm is straight and slender of the same thickness throughout with rounded end. The denticule morphology and dimensions of *T. puytoraci* recorded in this study are similar to those reported by other authors (LOM, 1962; BYKOVSKAYA-PAVLOVSKAYA *et al.,* 1964; GRUPCHEVA *et al*., 1989; ÖZER & ÖZTURK, 2004; AL-BASSEL *et al*., 2007).

Fig. 1: Photomicrograph of silver nitrate impregnated adhesive disc of Trichodina puytoraci from the gill of Mugil cephalus. (scale bar = 12 µm)

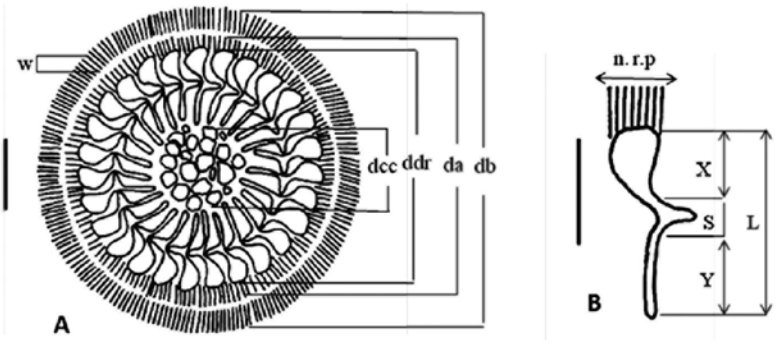

Fig. 2. A: Line drawing of T. puytoraci species; da: diameter of the adhesive disc; db: diameter of the body; dcc: central circle diameter; ddr: diameter of denticule ring; B: Schematic drawing of the denticule of T. puytoraci; nrp: number of radial pins; L: denticle length; S: central part length; X: blade length; Y: ray length. (Bar= 10 µm)

Parasite seasonal dynamics

The prevalence and mean intensity levels of *T. puytoraci* are variable with respect to the season. The prevalence increases from 8% in autumn, reaches a peak value of 36.66% in spring and then decreases to 3.33 % in summer (Fig. 3). The mean intensity increases from 30 ± 2.5 in autumn to 150 ± 22.5 in spring, then decreases to 10 ± 1.8 in summer (Fig. 3). Many authors have reported that trichodiniasis was prevalent throughout the year with a maximum rate of infestation during spring and winter (MCARDLE, 1984; ABU EL-WAFA, 1988; EL-KHATIB, 1989; HASSAN, 1999). Nevertheless, it seems that the presence of Trichodinids is closely linked to environmental conditions, particularly to temperature and salinity. In this study we noted that the highest prevalence and mean intensity level of *T. puytoraci* are recorded during spring, which correspond to the multiplication season for Trichodinids, while the lowest infestation rate occurred in summer. This is due to the fact that the high temperature of water in summer (29.33°C) prevents the proliferation of Trichodinids. It seems that the average temperature of water during spring (18.06 °C) favours the multiplication of *T. puytoraci*.

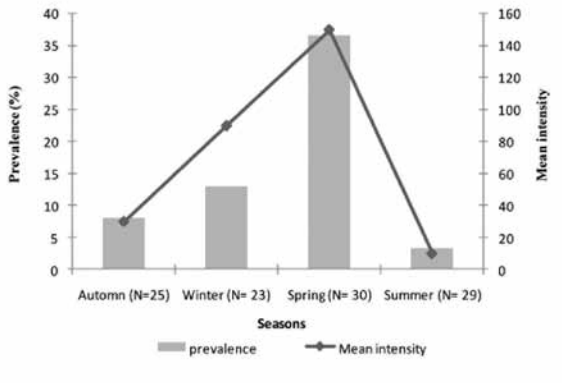

Fig. 3. Seasonal infestation prevalence (%) and mean intensity levels of Trichodina puytoraci

To study the influence of host's size on parasite prevalence, samples of *Mugil cephalus* measuring between 23 and 33 cm were grouped into 5 size classes of 2 cm amplitude and composed of N fish. The prevalence of *T. puytoraci* ranged from 6.89 % in the 29-31 cm class (N= 29) to 27.27 % in the 23-25 cm class (N= 11). The prevalence in the 25-27cm class (N = 31) and 27-29 cm class (N= 28) were 22.58% and 10.71%, respectively. Small fish between 23 and 25 cm were highly parasitized, while those belonging to the 31-33 cm class (N = 8) were not infested. We observed a gradual decrease in prevalence with the increase of fish size. This was further confirmed by a statistically significant difference ($\chi^2 = 9.66 > 9.38$) at the significance level of 95%, when comparing the parasitic frequencies to the size classes. It seems that young fish are more susceptible to the parasite than adults. This can be explained by the increase of the immune response with the age of the fish. In fact, fish can develop a specific immune response against pathogenic parasites

and over time this immune response becomes more intense and longer (DICKERSON & CLARK, 1996).

Trichodinid histopathology

Generally, the host response to ciliate infection was an inflammatory response. In the present study, the infestation with *T. puytoraci* caused serious pathological lesions in gills such as proliferation of the epithelium and fusion of secondary lamellae (Fig. 4A), hypertrophy and hyperplasia (Fig. 4B), degenerative and necrotic changes in the epithelium of secondary lamellae (Fig. 3C) and aneurysm in the apical region of the secondary lamellae (Fig. 4D). These pathogenic effects caused by *T. puytoraci* on *Mugil cephalus* are reported for the first time in this study. However, previous researchers have reported similar clinical symptoms (hypertrophy, hyperplasia and fusion of secondary lamellae etc.) in other fish caused by Trichodinids in cases of sever infestation (PADNOS & NIGRELLI, 1942; DAVIS, 1947; SARIG, 1971; HASSAN, 1999).

In conclusion, the *T. puytoraci* parasite of *M. cephalus* from Tunisian waters can become very pathogenic in particular environmental conditions, especially in spring when the temperature was favourable for Trichodinid proliferation. Young fish are affected to a greater degree by this species and are more susceptible than adults.

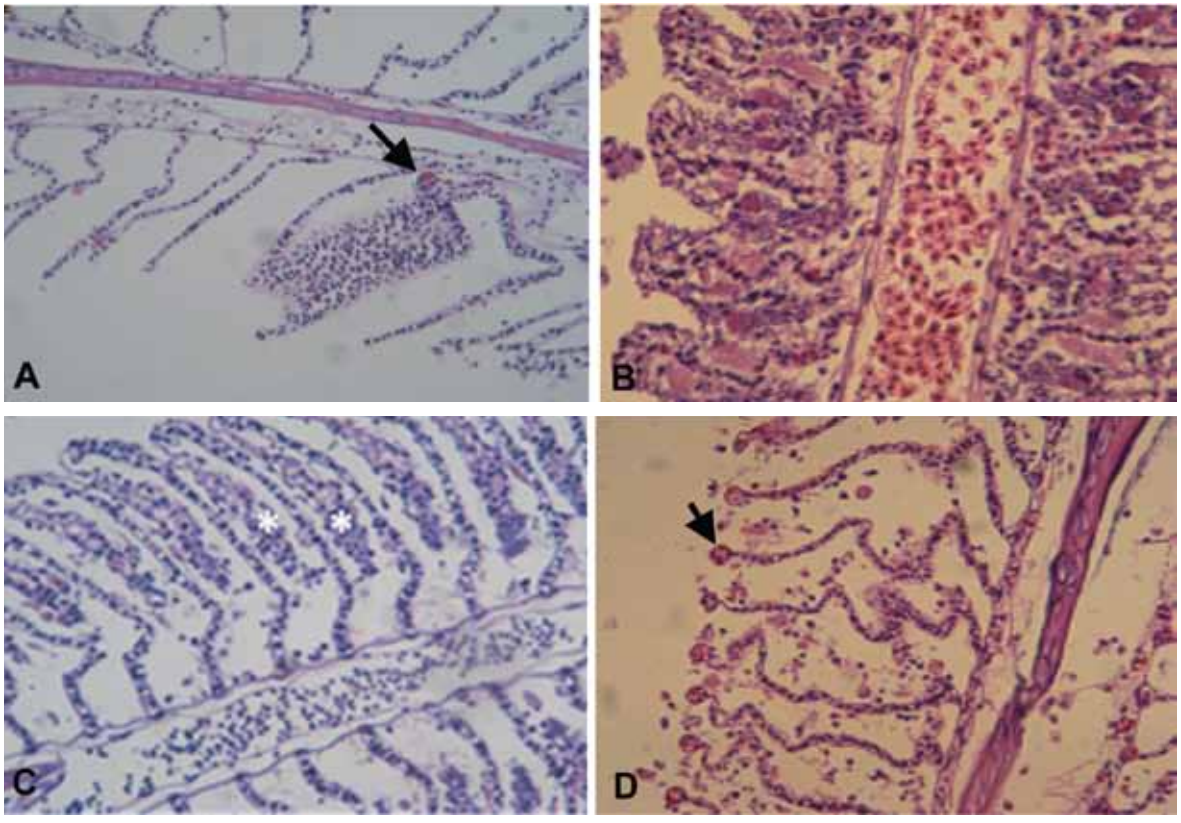

Fig. 4: Histological sections of infested gills showing the pathological lesions of T. puytoraci. A: Fusion of two secondary lamellae (T. puytoraci indicated by arrow); B: Pronounced hyperplasia in secondary lamellae; C: Degenerative and necrotic changes (*) in the epithelium; D: Aneurisms (arrow) in the apical region of secondary lamellae. (H&E 400x)

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Sezonska pojava i histopatologija parazita *Trichodina puytoraci* Lom, 1962 na ciplu glavašu (*Mugil cephalus*) iz Tunisa

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SAŽETAK

Tijekom parazitološkog pregleda 108 primjeraka cipla glavaša (*Mugil cephalus* L., 1758) u Ghar El Melh Laguna u Tunisu, istraživani su ektoparaziti iz grupe protozoa. Škrge pregledanih riba bile su zaražene s parazitom *Trichodina puytoraci* Lom, 1962 koji je izoliran i utvrđen po prvi put za ovaj lokalitet. Ustanovljena je ukupna prevalencija zaraze (22,42%) i srednja razina intenziteta (70 ± 8,1) trichodinida po primjerku. Najveća prevalencija uočena je u proljeće. Odnos između veličine domaćina i rasprostranjenosti nametnika je bio značajan. Histopatološka studija otkrila je da je cipal glavaš, *M. cephalus,* teško zaražen parazitom *T. puytoraci*, izložen ozbiljnim promjenama kao što su hiperplazija epitelnih stanica te spajanje i nekroza sekundarnih lamela.

Ključne riječi: *Trichodina puytoraci*, *Mugil cephalus*, pretežito, prosječni intenzitet, histopatologija, Tunis