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Parasitofauna of some mugilid and soleid fish species from Tunisian lagoons

Chiraz YEMMEN*, Mohamed Hédi KTARI and Sihem BAHRI

Department of Biology, Faculty of Sciences, University of Tunis El-Manar, 2092, Tunisia

**** Corresponding author, E-mail: chiraz_yemmen@yahoo.fr***

*A parasitological survey of important commercial fish from northern Tunisian lagoons was conducted from May 2009 to April 2010. A total of 771 fish belonging to six species: Flathead mullet (*Mugil cephalus *Linnaeus, 1758); Golden mullet (*Liza aurata *Risso, 1810); Leaping mullet* (Liza saliens *Risso, 1810) (Mugilidae); Common sole (*Solea solea *Quesnel, 1806); Egyptian sole (*Solea aegyptiaca *Chabanaud, 1927) and Senegalese sole (*Solea senegalensis *Kaup, 1958) (Soleidae) were examined. The total of 11 parasite species representing 7 genera (from four different phyla) was recorded:* Myxobolus Bütschli*, 1882;* Sphearospora Thélohan*, 1892;* Zschokkella Auerbach*, 1910;* Ceratomyxa Théloan*, 1892 (Myxozoa);* Trichodina Ehrenberg*, 1838 (Ciliophora);* Amyloodinium *Brown & Hovasse, 1946 (Sarcomastigophora) and* Solostamenides *Unnithan, 1971 (Platyhelminthes). Morphology, site of infection and prevalence of the parasites found during the survey are described.*

Key words: Parasitofauna, important commercial fish, Tunisian lagoons

INTRODUCTION

In Tunisia, mugilid and soleid fish species are among the most important resources of the Tunisian lagoons. These fish are of great economic value and they are highly valued for their flesh. Some species of mullet from Tunisian waters, especially *Mugil cephalus* and *Liza ramada*, have been found to be infected with several myxosporean species, all of which belonging to the genus *Myxobolus* Bütschli, 1882: *M. mülleri* Bütschli, 1882; *M. ramadae* Bahri, 1997; *M. exiguus* Thélohan 1895 infecting *L. ramada* and *M. bizerti* Bahri & Marques, 1996*; M. ichkeulensis* Bahri & Marques, 1996; *M. episquamalis* Egusa, Maeno & Sorimachi, 1990; *M. spinacurvatura* Maeno, Sorimachi, Ogawa & Egusa, 1990 parasite of *M. cephalus* (BAHRI *et al*., 1995; BAHRI, 1997; BAHRI & MARQUES, 1996). Nevertheless, no parasitological studies have been carried out on soleid fish from Tunisian waters until now.

In the present study a preliminary parasitological survey on some of the most economically important fish species, captured from four brackish-water lagoons situated in northern Tunisia over the last 2 years, was undertaken in order to define the distribution and the importance of parasites in Tunisian marine fish.

MATERIAL AND METHODS

During monthly samplings, from May 2009 to April 2010, a total of 771 fish belonging to 6 species: Flathead mullet (*Mugil cephalus)*;

Golden mullet (*Liza aurata*); Leaping mullet (*Liza saliens*) (Mugilidae); Common sole (*Solea solea*); Egyptian sole (*Solea aegyptiaca*) and Senegalese sole (*Solea senegalensis*) (Soleidae) were collected from four lagoons situated in northern Tunisia: Ghar El Melh lagoon (37°10′ N, 10°09′ E), Bizerte lagoon (37°8′ N, 9°48′ E), Ichkeul Lake (37°10' N, 9°33' E), and Tunis Lake (36°49' N, 10°15' E) (Fig. 1). Sampled fish were transported alive to the laboratory where they were measured, weighed and dissected. The surface of the body and all organs (intestine, gallbladder, kidney, liver and gills) were examined macroscopically and microscopically for visible parasite infection. Fresh smears of gills and skin were prepared and examined for the presence of Protozoans. In order to study details of the Trichodinid's adhesive disc, a silver impregnation method was performed (KLEIN, 1958). Monogeneans isolated from the gills and body surface were fixed in 70% ethanol. Fresh spores of myxosporean isolated from tissue cysts and from gallbladders were identified, described and measured according to the guidelines of LOM & ARTHUR (1989). The intensity of infection and prevalence of parasites were calculated according to BUSH *et al*. (1997).

RESULTS AND DISSCUSION

Myxozoa

Myxobolus bizerti Bahri & Marques, 1996, (Table 1, Fig. 2A, Fig. 3A) was found at the base of the primary gill lamellae of *Mugil cephalus* from Ghar El Melh and Bizerte lagoons. The parasite was detected in autumn when the water temperature varied from 18-21 °C. *M. bizerti* formed elongated whitish cysts 0.3-2.3 mm in length and 0.4-0.8 mm in width. The spores were mostly spherical and measured 12-14 µm in diameter. The polar capsules exceeded the mid-length of the spores, measuring 6-7 µm in length and 5.5-6 µm in width, and showing 6 to 7 filament coils. This species was previously described in *Mugil cephalus* from Ichkeul Lake by BAHRI & MARQUES (1996).

Myxobolus spinacurvatura Maeno, Sorimachi, Ogawa and Egusa, 1990 (Table 1, Fig. 2B, Fig. 3B). This species formed oval cysts, 0.5 to 3.8 mm in length and 0.2 to 3.3 mm in width, in the mesenteric vessels of *Mugil cephalus* from Ghar El Melh lagoon. The spores are ellipsoidal in front view, measuring 11-12.5 µm in length and 9.5-11.5 µm in width. Polar capsules were oval shaped and measured 4-5.5 µm in length and 2-3 µm in width. Each polar filament was coiled in 4 or 5 turns. All characteristics of this species are similar to those reported by other authors (BAHRI & MARQUES, 1996; MAENO *et al*., 1990). The heaviest infestation of *M. spinacurvatura* was detected in winter when the water temperature reached 11.2 °C.

Myxobolus sp. 1. (Table 1, Fig. 2C, Fig. 3C) This parasite formed various whitish cysts in the liver of *M. cephalus* from Ghar El Melh lagoon, measuring 0.5-2 mm in diameter. Spores were mostly rounded in frontal view and measured 12-14 µm in length and 10-12 µm in width. The polar capsules were pyriform and exceeded the mid-length of the spores. They measured 5-7 µm in length and 3-3.5 µm in width with 5 to 6 filament coils. According to the literature, two species belonging to the *Myxobolus* genus have been described from the liver of *M. cephalus*, *Myxobolus spinacurvatura* from Japanese coastal waters and *Myxobolus raibauti* Fall, Kpatcha, Diebakate, Faye & Toguebaye, 1997 from the coast of Senegal (MAENO *et al*., 1990; FALL *et al*., 1997). However, these species differ from our findings by the size and shape of their spores. During the sampling period, the highest level of *Myxobolus* sp. 1 infestation was noted in the coldest months when the water temperature varied from 10.2-12.2 °C.

Myxobolus sp. 2. (Table 1, Fig. 2D, Fig. 3D) The specimens of *Mugil cephalus* from Ghar El Melh lagoon, infested by *Myxobolus* sp. 2, presented whitish cysts on their hearts, measuring approximately 0.5-1 mm in length. The spores were spherical in frontal view, measuring 10-12 µm in length and 10-11 µm in width. The polar capsules were pyriform and reached half

Table 1. List of parasite species found on fish samples from Northern lagoons of Tunisia

Locality	Fish species	Parasites		Site of infection	Prevalence
		Species	**Family**		
Ghar El Melh lagoon	*Mugil cephalus* (n= 107)	*Myxobolus bizerti*	Myxobolidae	Gills	2.8%
		Myxobolus spinacurvatura	Myxobolidae	Mesenteric vessels	13.08%
		Myxobolus sp. 1	Myxobolidae	Liver	15.88%
		Myxobolus sp. 2	Myxobolidae	Heart	0.93%
		Sphaerospora sp.	Sphaerosporidae	Gallbladder	1.87%
		Trichodina lepsii	Trichodinidae	Gills	12.15%
		Solostamenides-mugilis	Microcotylidae	Gills	6.54%
	Liza aurata (n= 50)	*Trichodina lepsii*	Trichodinidae	Gills	6%
	Solea aegyptiaca (n= 121)	*Zschokkella* sp.1	Myxidiidae	Gallbladder	5.78%
		Ceratomyxa sp. 1	Ceratomyxidae	Gallbladder	9.09%
		Trichodina gobii		Gills	35.53%
		Trichodinidae		Gills	9.91%
		Amyloodinium ocellatum	Oodiniaceae		
	Solea solea (n=89)	*Zschokkella* sp.	Myxidiidae	Gallbladder	9%
		Ceratomyxa sp. 1	Ceratomyxidae	Gallbladder	16.85%
		Trichodina gobii	Trichodinidae	Gills	22.47%
		Amyloodinium ocellatum	Oodiniaceae	Gills	10.2 %
	Solea impar (n= 10)	*Ceratomyxa* sp. 2	Ceratomyxidae	Gallbladder	20 %
Bizerte lagoon	*Mugil cephalus* (n= 57)	*Myxobolus bizerti*	Myxobolidae	Gills	5.26 %
		Solostamenides mugilis	Microcotylidae	Gills	7 %
	Solea senegalensis (n= 150)	*Zschokkella* sp.	Myxidiidae	Gallbladder	4.66%
		Trichodina gobii	Trichodinidae	Gills	18.66%
		Amyloodinium ocellatum	Oodiniaceae	Gills	10.52%
	Solea solea (n=40)	*Zschokkella* sp.	Myxidiidae	Gallbladder	9%
		Trichodina gobii	Trichodinidae	Gills	5 %
		Amyloodinium ocellatum	Oodiniaceae	Gills	12.5%
Ichkeul Lake	*Liza saliens* (n=80)	*Trichodina lepsii*	Trichodinidae	Gills	50%
				Gills	48%
Tunis Lake	*Solea solea* (n=84)	*Trichodina gobii*	Trichodinidae	Gills	63.84%

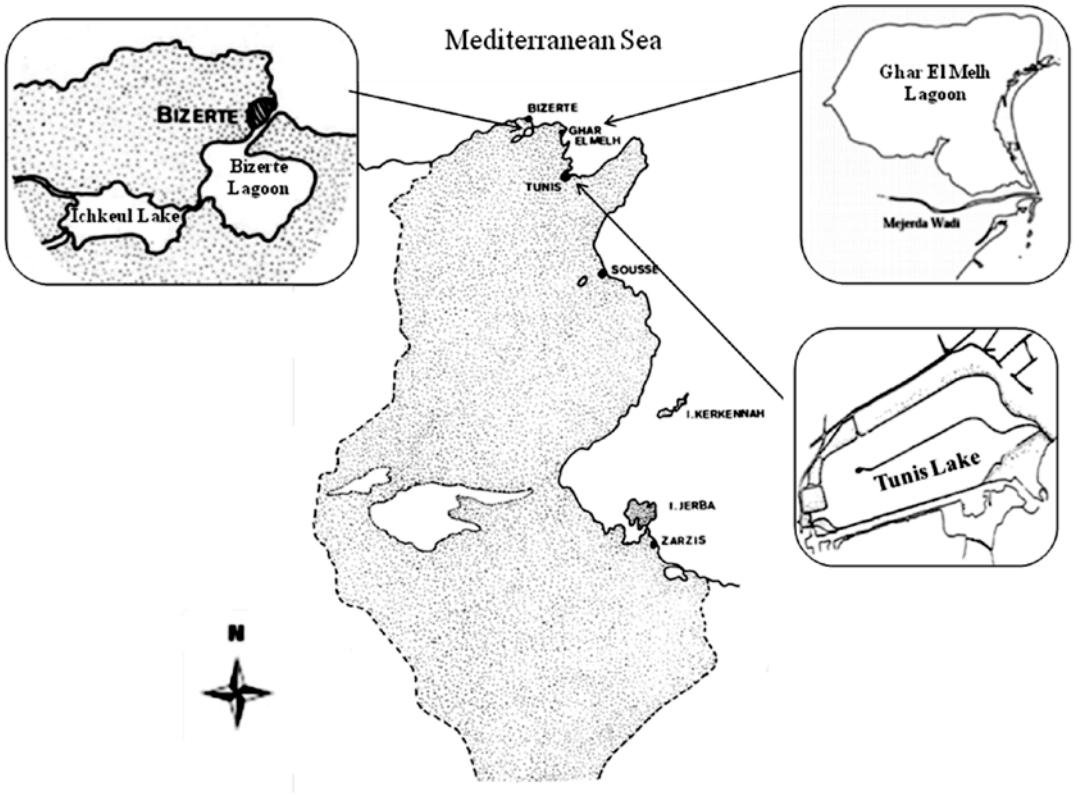

Fig. 1. Maps of study area showing the locations of the 4 sampling sites

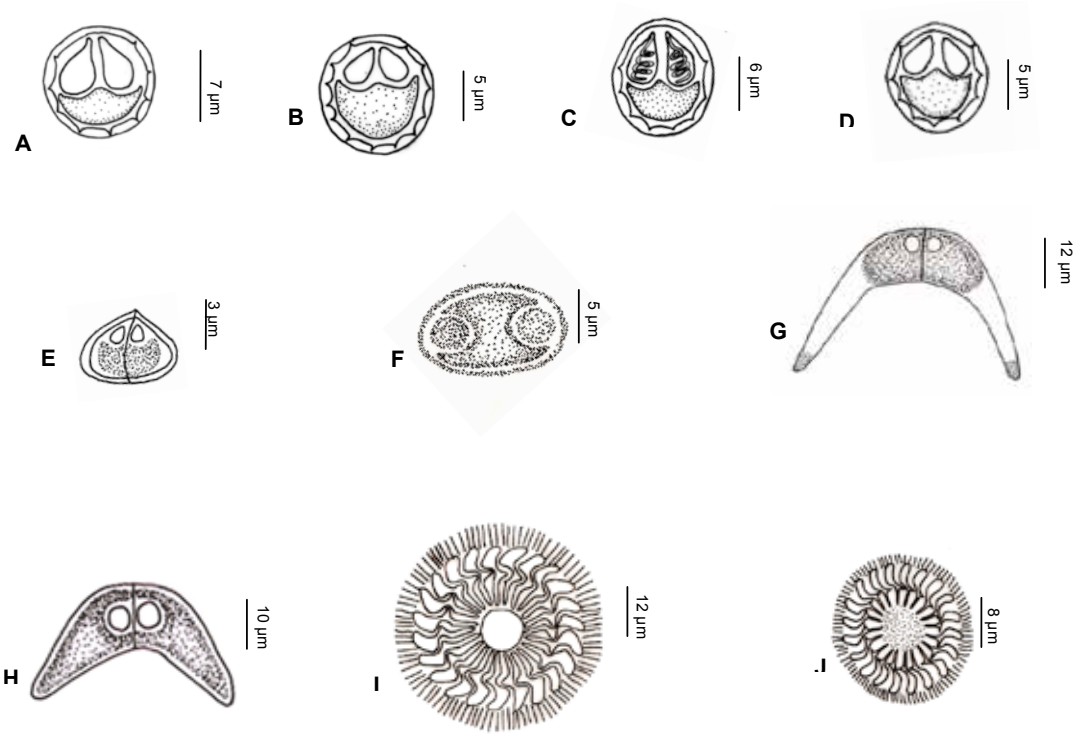

Fig. 2. A: Myxobolus bizerti; B: Myxobolus spinacurvatura; C: Myxobolus sp. 1; D: Myxobolus sp. 2; E: Sphaerospora sp.; F : Zschokkella sp., G: Ceratomyxa sp.1; H : Ceratomyxa sp. 2; I: Trichodina gobii; J: Trichodina lepsii

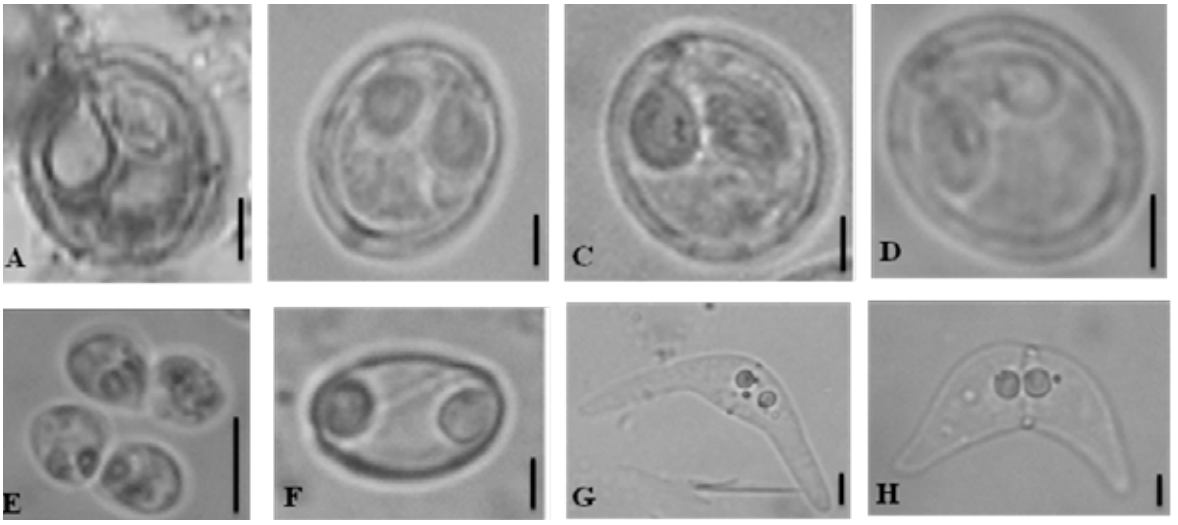

Fig. 3. Mature spores of eight Myxosporeans species isolated from Mugilid and soleid fish from Tunisia. A: Myxobolus bizerti; B: Myxobolus spinacurvatura; C: Myxobolus sp. 1; D: Myxobolus sp. 2; E: Sphaerospora sp.; F:Zschokkella sp. ;G: Ceratomyxa sp. 1; H: Ceratomyxa sp. 2. (Bar = 5 µm)

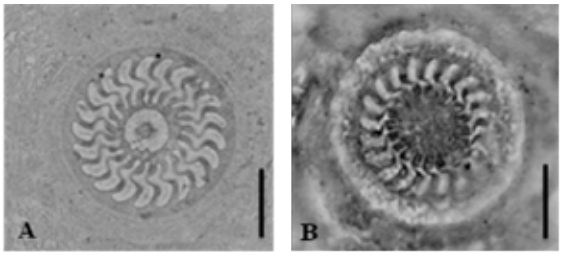

Fig. 4. Photomicrograph of silver nitrate impregnated adhesive disc of Trichodinids. A: Trichodina gobii from the gills of soleid fish; B: Trichodina lepsii from the gills of Mugilid fish. (Bar = 20 µm)

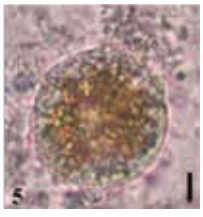

Fig. 5. Trophont of Amyloodinium ocellatum from the gills of soleid fish. (Bar = 20 µm)

of the spore length. They measured 4-5.5 µm in length and 2-2.5 µm in width with 4 to 5 filament coils. This is the first report of *Myxobolus* species on the heart of Mugilidae fish. Nevertheless, several species of *Myxobolus* have already been described on the hearts of other hosts: *M. baueri* Chernova, 1970 in *Tinca tinca* from Russian coasts (CHERNOVA, 1970); *M. bulbocordis* Masoumian, Baska & Malnar, 1996 in *Barbus sharpeyi* from Iranian coasts (MASOUMIAN *et al*., 1996) and *Myxosoma cartilaginis* Hoffman, Putz & Dunbar, 1965 infecting *Leponis macrochirus* from the coasts of the USA (HOFFMAN *et al*., 1965). The spores of these species did not have morphological similarities with our findings. During this survey, the present species was noted only during winter when the water temperature reached its minimum value of 10.2 °C.

Sphearospora sp. (Table 1, Fig. 2E, Fig. 3E) Free spores of *Sphaerospora* sp. were detected from the gallbladder of *M. cephalus* from Ghar El Melh lagoon. Mature spores were almost triangular in frontal view and measured 4.8-5.6 µm in length and 4-6 µm in width. The suture line was visible, straight and thin. Polar capsules were equal in size, pyriform and measured 1.6-1.8 µm in length, 0.8-0.9 µm in width and each of which contained a polar filament with 3 to 4 coils. Among the 78 *Sphaerospora* species (LOM & DYKOVÁ, 2006), only two species present morphological similarity with the present species. *Sphearospora mugili* Yurakhno & Maltsev, 2002 a parasite of the gallbladder of *Mugil cephalus* from the Black Sea, the Sea of Azov and the Atlantic Ocean (YURAKHNO & MALTSEV, 2002) and *Sphaerospora dicentrarchi* Sitjá-Bobadilla & Alvarez-Pellitero, 1992 the histozoic parasite of the connective tissue of

Dicentrarchus labrax from the Mediterranean Sea (SITJÁ-BOBADILLA & ALVAREZ-PELLITERO, 1992). However, our species differs from *S. mugili* by its larger spores and smaller polar capsules. On the other hand, our finding presents a similar shape with *S. dicentrarchi*, but differs from it by its smaller polar capsules and sites of infestation in the host.

Zschokkella sp. (Table 1, Fig. 2F, Fig. 3F) was found in the gallbladder of Soleidae fish from Ghar El Melh and Bizerte lagoons. Spores appeared only in summer. Mature spores of *Zschokkella* sp. were ellipsoidal in front view with rounded ends. The suture line was clearly visible and slightly curved. On each spore valve, 8 to 9 distinct striations parallel to the suture line were visible. The spores measured 13.6-14.4 µm in length and 10.4-11.2 µm in width. Polar capsules were spherical and equal in size, 3.2-4 µm in diameter, located in the spore ends and opening slightly subterminally at the two opposite ends of the spore. Each polar capsule had five filament coils. According to our knowledge, this is the first report of *Zschokkella* species from Soleidae fish. Among the 68 species of *Zschokkella* described in the literature, only *Zschokkella mugilis* (SITJÀ-BOBADILLA & ALVAREZ-PELLITERO, 1993b) in Mugilids from the Mediterranean sea and *Zschokkella acheilognathi* Kudo, 1916 parasite of the gallbladder of *Acheilognathus lanceolatum* from Japan (SITJÁ-BOBADILLA *et al*., 1993b; KUDO, 1919) presented morphological similarities with our species, although differed from it by spore dimensions.

Ceratomyxa sp. 1. (Table 1, Fig. 2G, Fig. 3G). Specimens of *Ceratomyxa* sp. were found in the gallbladder of two hosts, *Solea solea* and *Solea aegyptiaca* from Ghar El Melh lagoon. Infection with *Ceratomyxa* sp. 1 was detected only in summer when the water temperature reached its maximum value of 29 °C. Mature spores were elongated and crescent-shaped with rounded ends and measured 8-11 µm in length and 48-54 µm in width. The two polar capsules were spherical and almost equal in size, from 3.2 to 4 µm in diameter, opposed near the suture line, and each contained a polar filament with 5 to 6 coils. Trophozoites were polysporic and were freely floating in bile or logging on the epithelium of the gallbladder. According to our knowledge, this is the first report of *Ceratomyxa* species on soleid fish. Among the 200 species of C*eratomyxa* reported worldwide (GUNTER *et al*., 2009), only *Ceratomyxa protopsettae* Fujita, 1923 presents a similarity in the general morphology of the spore with our finding. Indeed this species was described from the gallbladder of 14 species of flatfish in Japan (FUJITA, 1923) and characterized by a strongly arcuate spore with unequal valves measuring 10 to 12 µm in length, 50 to 65 µm in width and spherical polar capsules of 4 to 6 µm in diameter. However, our species differs from *C. protopsettae* by its smaller size, equal valves and was less arcuated. Nevertheless, a study of the *Ceratomyxa* of the present study was undertaken to confirm its taxonomic identity.

Ceratomyxa sp. 2. (Table 1, Fig. 2H, Fig. 3H). Spore of *Ceratomyxa* sp. 2 was found in the gallbladder of *Solea impar* from Ghar El Melh lagoon during summer. Mature spores were more or less arcuate with anterior margin convex and posterior margin concave, valves equal in size with rounded ends. Spores measured 8-10 µm in length and 32-34 µm in width. The two polar capsules were spherical and equal in size, of 3.5-4 µm in diameter, and showing 4 to 5 filaments coils. Trophozoites were disporic and freely floating in bile. The present species shows similarity in shape with *Ceratomyxa uncinata* Meglitsch, 1960 parasitizing lemon sole (*Pleotretis flavilatus)* from New Zealand (MEGLITSCH, 1960). Therefore, *C. uncinata* differs from the present species by having longer spores (10.9-14.1 µm), sharply bent and unequal valves.

Ciliophora

Trichodina gobii Raabe, 1959, (Table 1, Fig. 2I, Fig. 4A) was found for the first time in Soleidae fish as well as in Tunisian waters. This ciliate was characterized as medium in size, 36.8-40.8 µm in diameter; the adhesive disc,

concave, 27.2-32.8 µm in diameter, consisted of 20 to 22 denticles. The similarity in the denticle shape and dimensions of the present species is shared with *T. gobii* originally described from *Gobius minutes* from the Black Sea. The heaviest infestation with *T. gobii* was noted in winter when the water temperature reached its minimum of 10.2 °C.

Trichodina lepsii Lom, 1962 (Table 1, Fig. 2J, Fig. 4B). Specimens of *Trichodina lepssi* were identified from the gills of *Mugil cephalus* and *Liza aurata* from Ghar El Melh lagoon as well as from the gills of *Liza saliens* from Ichkeul Lake. This species was characterised by its smaller size, 32-37.6 µm in diameter; the adhesive disc, concave, 20.8-28 µm in diameter, consisted of 20 to 22 denticles. Populations of *Trichodina lepsii* harvested during this study showed significant resemblance in the size and shape of the denticle with those reported in the literature (LOM, 1962; BYKOVSKAYA-PAVLOVSKAYA *et al*., 1964; KINNE, 1984; GRUPCHEVA *et al*., 1989: AL-BASSEL *et al*., 2007). Along with *Trichodina lepsii* most of the *Mugil cephalus* were also parasitized with *Trichodina puytoraci.* The heaviest infestations were in spring when the water temperatures reached 18°C.

Sarcomastigophora

Amyloodinium ocellatum Brown*,* 1931, (Table 1, Fig. 5) was identified on the gills of Soleidae fish from Ghar El Mel and Bizerte lagoons. Trophontes of *A. ocellatum* measured 80-90 µm in diameter. The heaviest infestations were in summer when the water temperature reached 29 °C. Under mariculture conditions, this parasite has caused fatal epizooties and mass mortality of fish throughout the world (LOM & DYKOVÁ, 1992; CRUZ-LACIERDA *et al.*, 2004; ROBERTS-THOMSON *et al.*, 2006).

Solostamenides mugilis Vogt, 1878, (Table 1) was found attached on the gill filaments of *Mugil cephalus* from Ghar El Melh and Bizerte lagoons. Specimens of *Solostamenides mugilis* were characterized by a fusiform body 8-10 mm in length and 1-1.5 mm in width. Haptor relatively short clearly marked off from body proper. Buccal organs small, oesophagus long, wide, with conspicuous diverticula intestine bifurcates at level of genital atrium. Testes (60-66) in number in post ovarian intercaecal field, not extending posteriorly to haptor. Sperm duct conspicuous, coiling strongly in midline. This species is a common parasite of Mugilidae fish from the Mediterranean (EUZET & COMBES, 1969; EUZET *et al*., 1993).

In the present study we have reported seven parasite species which have been previously described by other authors worldwide: *Myxobolus bizerti*, *Myxobolus spinacurvatura*, *Trichodina gobii*, *Trichodina puytoraci*, *Trichodina lepsii*, *Amyloodinium ocellatum* and *Solostamenides mugilis*. Among these parasites, the following species were reported for the first time from Tunisian waters: *Trichodina gobii* and *Trichodina lepsii*. Furthermore, we have identified six more species of myxosporean, *Myxobolus* sp. 1, *Myxobolus* sp. 2, *Sphaerospora* sp., *Zschokkella* sp., *Ceratomyxa* sp. 1 and *Ceratomyxa* sp. 2. The taxonomic identity of these parasites based on molecular analysis will be subsequently presented.

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Parazitofauna nekih vrsta cipala i listova u tuniskim lagunama

Chiraz YEMMEN*, Mohamed Hédi KTARI i Sihem BAHRI

Odjel za biologiju, Prirodoslovno-matematički fakultet, Sveučilište u Tunisu, El-Manar, 2092, Tunis

**Kontakt adresa, e-mail: chiraz_yemmen@yahoo.fr*

SAŽETAK

Obavljen je parazitološki pregled važnih komercijalnih riba iz laguna sjevernog Tunisa. Istraživanje je trajalo od svibnja 2009. do travnja 2010. godine. Ulovljeno je ukupno 77 jedinki riba koje spadaju u šest istraživanih vrsta: cipal bataš (*Mugil cephalus* Linnaeus, 1758); cipal zlatar (*Liza aurata* Risso, 1810); cipal dugaš (*Liza saliens* Risso, 1810) (Mugilidae); list (*Solea solea* Quesnel, 1806); egipatski list (*Solea aegyptiaca* Chabanaud, 1927) i senegalski list (*Solea senegalensis* Kaup, 1958) (Soleidae).

Zabilježeno je ukupno 11 vrsta parazita iz 7 rodova (iz četiri različita koljena): *Myxobolus* Bütschli, 1882; *Sphearospora* Thélohan, 1892; *Zschokkella* Auerbach, 1910; *Ceratomyxa* Théloan, 1892 (Myxozoa); *Trichodina* Ehrenberg, 1838 (Ciliophora); *Amyloodinium* Brown & Hovasse, 1946 (Sarcomastigophora) i *Solostamenides* Unnithan, 1971 (Platyhelminthes). Tijekom znanstvenog istraživanja proučavani su: morfologija, mjesto infekcije i učestalost parazita.

Ključne riječi: Parazitofauna, važne komercijalne vrste riba, tuniske lagune