

# PARASITIC FAUNA OF *SARDINELLA AURITA* VALENCIENNES, 1847 FROM ALGERIAN COAST

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**Abstract.** The parasitic fauna of *Sardinella aurita* Valenciennes, 1847 from the Gulf of Bejaia (eastern coast of Algeria) was studied. The parasites collected from 400 host fish specimens, comprised 10 taxa including 6 species of Digenea, 1 species of Copepoda, 1 species of Nematoda, 1 larva of Cestoda and an unidentified Microsporidian species. The Nematoda *Hysterothylacium* sp. and the Copepoda *Clavellisa emarginata* (Krøyer, 1873) are newly reported for *S. aurita*. The Digenean parasites were numerous, diverse and constituted the most dominant group (P = 33.63%). The checklist of all known parasite species collected from *S. aurita* in the Mediterranean Sea includes 13 species, among which eight are Digeneans.

## INTRODUCTION

*Sardinella aurita* Valenciennes, 1847, is a small widely distributed pelagic fish. It frequently occurs along the Algerian coastline as well as in Tunisia, Egypt, Greece and Sicily (Dieuzeide and Roland 1957; Kartas and Quignard 1976). The biology of *S. aurita* has been studied in the Mediterranean Sea by many researchers (Bounhioul 1921; Dieuzeide and Roland 1957; Chavance et al. 1985; Bouaziz et al. 2001; Hamida 2003; Bouaziz 2007). However, its parasitic fauna was investigated rarely (Derbel et al. 2012; Feki et al. 2015; Mansour et al. 2016), particularly along the Algerian coast. Taking into consideration the high economic importance of this fish, it is vital to know about its parasitic fauna. Pathogenic parasites induce diseases and may cause losses in the economic value of their hosts.

Microsporidia, for example, are among important protozoan parasites affecting fish (Dykova et al. 1980), in which they are transmitted both horizontally and vertically (MacNeil et al. 2003; Haine et al. 2004) and cause pathologies ranging from extensive muscle degeneration (Slothouber-Galbreath et al. 2004) to changes in the sex-ratio (Haine et al. 2007; Mautner et al. 2007). Microsporidia are common parasites of fish from different geographical areas (Caffara et al. 2010; Jithendran et al. 2011; Morsy et al. 2013; Abdel-Baki et al. 2015; Mansour et al. 2016; Frenette et al. 2016; Jones et al. 2017; Ovcharenko et al. 2017). The study of Microsporidia inducing xenomas in fish offers an insight into cell pathology. It is of interest since many of these species are important agents of fish diseases (Dykova 1995; Shaw et al. 1999). They have economic consequences and negative impacts when they cause

disease in commercially valuable fish (Yokoyama et al. 2002; Kent et al. 2014; Phelps et al. 2015; Mansour et al. 2016).

The aim of this study was to identify the parasitic fauna infecting *S. aurita* from the eastern coast of Algeria, and to establish a checklist of all known parasite species infecting *S. aurita* from the Mediterranean Sea.

## MATERIALS AND METHODS

During the period from October 2017 to October 2018, 400 specimens of *S. aurita* were collected from the Gulf of Bejaia (eastern coast of Algeria) and examined for parasites. The number of examined fish by age, size classes and months is indicated in Table 1.

The fish caught by local fishermen were transported to the laboratory immediately after landing. All parts of the body of each fish were carefully examined for attached parasites by both naked eye and with a binocular magnifying glass. Lesions and external changes were noted. For each fish specimen, the following parameters were recorded: total length (TL) in cm using an ichthyometer, total mass (Wt) and mass of gutted fish (We) in g using a precision balance (precision = 0.1g). Subsequently, the sex was determined. Once the parasites had been collected, they were cleaned and immediately preserved in pure ethanol (protozoan) and then transferred to 70% ethanol (for subsequent identification). Digenea and Cestoda (larvae) were stained, dehydrated in a graded series of ethanol dilutions (70°, 85°, 95° and 100°), cleared in clove oil and mounted in Canada balsam. Parasitic copepods were later cleared

Table 1. Number of examined fish specimens per months, size classes and age.

Months	October 2017	November 2017	December 2017	January 2018	February 2018	March 2018	April 2018	May 2018	June 2018	July 2018	August 2018	September 2018	October 2018
n	30	32	30	30	32	31	30	31	30	31	32	31	30
Size classes (cm)	[11–12]		[12–13]	[13–14]	[14–15]		[15–16]		[16–17]		[17–18]	[18–19]	
n	22		40	74	91		73		53		30	17	
Age (year)	[1–2]		[2–3]		[3–4]			[4–5]				[5–6]	
n	58		124		130			60				28	
Total													400

n: number of examined fishes.

Table 2. Parasitological indexes according to parasites species infecting *Sardinella aurita*.

Digenea	33.63	1.18	0.39		
<i>Aphanurus stossichii</i> (Monticelli, 1891)*	9.73	1.36	0.13	DT	E
<i>Parahemiurus merus</i> (Linton, 1910)*	5.31	1.25	0.06	DT	E
<i>Derogenes latus</i> Janiszewska, 1953*	0.44	1	0.004	DT	E
<i>Lecithochirium</i> sp.*	1.76	4	0.004	DT	E
<i>Hemiurus communis</i> Odhner, 1905*	0.44	1	0.004	DT	E
<i>Hemiurinae</i> sp.*	15.49	1.2	0.18	DT	E
Cestoda	0.88	0.5	0.004		
<i>Tetraphyllidea larvea</i> sp.*	0.88	0.5	0.004	DT	E
Copepoda	13.27	2.03	0.27		
<i>Clavellisa emarginata</i> (Krøyer, 1873)*	13.27	2.03	0.27	Gi	E
Nematoda	0.88	1.5	0.013		
<i>Hysterothylacium</i> sp.*	0.88	1.5	0.013	PC	E
Protozoan	23.45	1	0.226		
Unidentified Microsporidian*	23.45	1	0.226	PC/DT	/

P%: Prevalence (%); Im: Mean intensity; A: Mean abundance; M: Microhabitat; H/S: Host specificity; DT: Digestive tract; PC: Peritoneal cavity; Gi: Gills; E: Euryxenic; \*: Newly collected parasites on *S. aurita* from the east coast of Algeria.

in 85% lactic acid for 1 to 2 h before dissection. The parasites were identified using identification keys based on their morpho-anatomic characteristics.

To evaluate parasitism, we calculated three parasitological indices (Bush et al. 1997):

1. Prevalence (P%) = the number of hosts infested (n) / the number of fish specimens examined (H) × 100.
2. Mean intensity (Im) = the number of parasite specimens (n) / the number of infested hosts.
3. Age, estimated using otholithometry.

To examine protozoan parasites, the xenoma was crushed, and three types of smears were prepared: (fresh, fixed and stained). Fresh spores were observed using an optical microscope, and the diameter of the spores was measured using an ocular micrometer. The smears were left to dry in ambient temperature. After drying, the smears were stained with Giemsa and Haematoxylin-Eosin (H&E).

## RESULTS

### *Diversity of the collected parasites*

The ten parasite taxa observed for the first time on *Sardinella aurita* Valenciennes, 1847 from the eastern coast of Algeria are as follows: Digenea, *Aphanurus stossichii* (Monticelli 1891), *Parahemiurus merus* (Linton 1910), *Derogenes latus* Janiszewska, 1953, *Lecithochirium* sp., *Hemiurinae* sp. and *Hemiurus communis* Odhner 1905, Copepoda, *Clavellisa emarginata* (Krøyer 1873), Nematoda, *Hysterothylacium* sp., Cestoda, *Tetraphyllidea larvae* and an unidentified Microsporidian (Table 2).

The fish specimens infected with Microsporidia did not show any external alteration in their appearance in contrast to infections with other parasite taxa; no external morphological signs of disease were recorded. Macroscopically, the infection manifested itself in numerous visible xenomas in the general cavity of the host fish *S. aurita* (Figure 1A, white arrows). These xenomas are easy to extract from the organs to which

they are attached. The xenomas observed in infected fish varied in number (1 to 6) and in diameter (6 mm to 15 mm). Also, they differed in appearance, consistency and color. The vast majority of xenomas were found in the peritoneal cavity and were either i) rigid- and irregular-shaped with a hard chalky exterior containing a solid, crumbly, cream-coloured, dark yellowish substance free of any liquid; or of regular spherical shape with a highly flexible wall containing a white liquid. The microscopic examination of fresh wet smears, Giemsa stained smears, and smears stained with Hematoxylin-Eosin (H&E), prepared from the contents of the crushed xenomas, revealed a large number of mature ovoid- to

ellipse-shaped spores (Figure 1B, C). Each spore contained a large central vacuole occupying more than half of the spore volume (Figure 1C). The nematodes *Hysterothylacium* sp. and *Clavellisa emarginata* (Krøyer 1873) are newly reported from *S. aurita*. Our results (Table 2) show that *S. aurita* hosts an abundance of diverse parasite species, especially metazoan parasites. Generally, the parasites collected from *S. aurita* show euryxenic specificity to their host.

### Infection rates

The Digenea is the most diversified group (6 species) of metazoan parasites and the most represented in the metazoan parasite fauna of *S. aurita* ( $P = 33.6\%$  and  $Im = 1.18$  parasites per infected host) (Table 2).

The highest infection rates were recorded for *Hemiurinea* sp. ( $P = 15.5\%$ ), *Clavellisa emarginata* ( $P = 13.3\%$ ), *Aphanurus stossichii* ( $P = 9.7\%$ ) and *Parahemiurus merus* ( $P = 5.31\%$ ). According to our results, *Lecithochirium* sp. and *Clavellisa emarginata* show the highest mean intensity (respectively 4 and 2.03 parasites per infected host). The remaining parasite species have relatively low prevalence ranging from 0.4% to 1.8% (Table 2). Parasite attachment sites are variable, with the digestive tract (Digenea, Cestoda, Nematoda), the peritoneal cavity (Microsporidian) followed by the gills, which is a favourable environment for Copepoda, being the most common. The prevalence of infection with protozoan parasites is relatively the highest ( $P = 23.5\%$ ) (Table 2).

The identified parasites infect *S. aurita* throughout the year, the highest prevalence (more than 60%) being observed in summer (June and July particularly) and spring (March) (Figure 2C). All size classes of *S. aurita* are parasitized (Figure 2B), and especially size classes 11–12 cm and 18–19 cm. The most infected specimens are those older than 5 years ( $P = 75.0\%$ ;  $Im = 1\text{--}2$  parasite per infected host) (Figure 2A). Younger specimens (1–2 years) are less infected ( $P = 4.5\%$ ;  $Im = 1$  parasites per infected host) (Figure 2A). Our results show that male specimens were more infected ( $P = 62.4\%$ ) than the female ones ( $P = 54.9\%$ ).

The overall prevalence of Microsporidia was relatively significant ( $P = 23.5\%$ ). Our observations reveal that the specimens with the total length (TL) of 11–12 cm were the most infected with Microsporidian parasites.

### DISCUSSION

In this study, the parasitic fauna of *Sardinella aurita* was newly recorded off the Algerian coast. The following ten parasite taxa were collected: *Aphanurus stossichii*, *Parahemiurus merus*, *Derogenes latus*, *Lecithochirium* sp., *Hemiurinae* sp., *Hemiurus communis*, *Clavellisa*

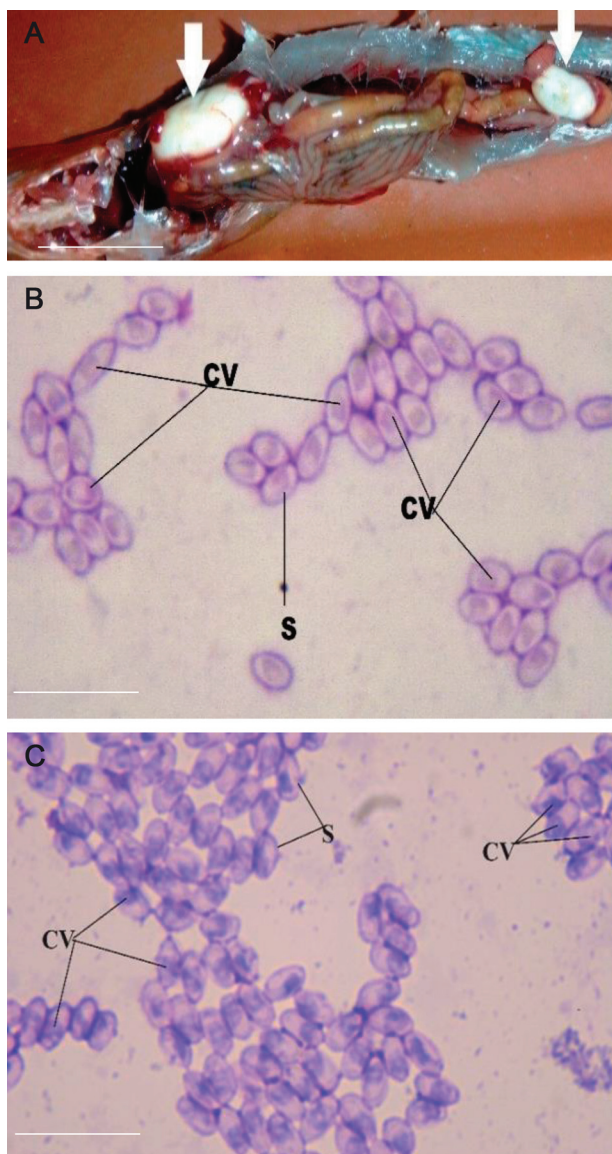


Figure 1. A, Xenomas attached to the general cavity of *Sardinella aurita* (white arrows); Scale bar: 2cm. An unidentified microsporidian observed in fresh wet smears and fixed smears stained with Haematoxylin-Eosin and with Giemsa; B, a large number of spores in a thin smear (Haematoxylin-Eosin staining  $\times 100$ ). Scale bar:  $0.2\ \mu\text{m}$ ; C, a large number of spores in a thin smear (Giemsa stain  $\times 100$ ). Scale bar:  $0.2\ \mu\text{m}$ . S: spores, CV: central vacuole.



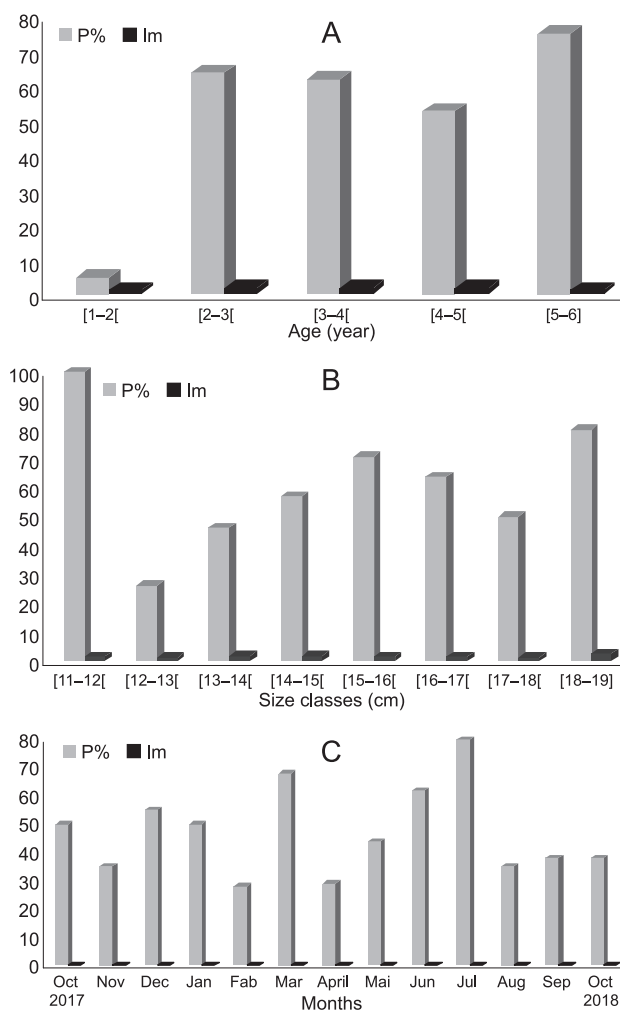


Figure 2. Parasitological indexes according to age (A), size class (B) and month (C).

*emarginata*, *Hysterothylacium* sp., *Tetraphylidea* larvae and an unidentified Microsporidian (molecular study will be required for species identification). Thirteen nominal parasite species of this host species are well known from their previous records from different localities in the Atlantic–Mediterranean region (Bartoli et al. 2000; Derbel et al. 2012; Feki et al. 2015 and the present study). The majority of the parasite species we identified had previously been reported from *S. aurita* off the coast of Tunisia (Derbel et al. 2012; Feki et al. 2015), and from other fish species caught in different localities (the Atlantic–Mediterranean region and the Black Sea) (Bartoli et al. 2000, 2005; Mackenzie et al. 2008; Merzoug et al. 2012; Ichalal et al. 2015; Öztürk and Özer 2016; Ider et al. 2018). The parasitic fauna of *S. aurita* is diverse and the Digeneans are well represented. Our results corroborate those reported by Derbel et al. (2012) and Feki et al. (2015).

The unidentified Microsporidian reported in this paper is its first record off the Algerian coastline. Its taxonomic position should be subject to further investigation using phylogenetic data. A microsporidian (*Glugea sardinensis*) was reported by Mansour et al. (2016) from the

Tunisian coast. Among the species identified from *S. aurita*, *Hemiurinae* sp.; *Aphanurus stossichii*, *Tetraphylidea* larvae were previously recorded by Ider et al. (2018) in *Boops boops* caught off the Algerian coast, *Hemiurus communis* and *Aphanurus stossichii* were reported by Ichalal et al. (2015) from *Trachurus trachurus* caught along the Algerian coastline. The parasites *Parahemiurus merus* and *Aphanurus stossichii* were identified by Merzoug et al. (2012) from *Sardina pilchardus* collected from the west coast of Algeria. The parasite *Derogenes latus* was recorded by Bartoli and Gibson (1991) in *Mullus surmuletus* captured in the western Mediterranean Sea. *Clavellisa emarginata*, recorded here, is newly reported along the northeastern coastline of Algeria. We collected one species of nematode *Hysterothylacium* sp. from *S. aurita*, which is a previously unknown host of this parasite. It is very important to update the inventory of the parasitic fauna infesting *S. aurita* because parasites have often been used as biological markers for discrimination among different stocks of *S. aurita* (Feki et al. 2015) in fish population studies (Mackenzie 2002; Mackenzie and Abaunza 2005) and for investigations of fish migratory movements (Oliva 2001). Our parasite indices show that rates of *S. aurita* infestation with all identified parasite species are relatively low, with the highest abundance of *Hemiurinae* sp. ( $P = 15.5\%$ ) and *Clavellisa emarginata* ( $P = 13.3\%$ ) being recorded. Our results do not corroborate those of Derbel et al. (2012), who reported a predominance of *Aphanurus stossichii* and *Parahemiurus merus* in the same host species. The infection rate was observed to vary according to months, which means that the seasonal timing of sampling is important.

The highest prevalence of parasites was recorded from March to July inclusive (2018). This result can be explained by an increase in water temperature, which may favour the population growth of parasites as has been observed for Digenean parasites. The same results were reported by De Kinkelin (1971). Ramdane et al. (2013) found that warm seasons clearly enhance parasitism in the specimens of *Boops boops* and *Mullus barbatus barbatus* caught in the coastal waters of Algeria. Ichalal et al. (2015) and Ider et al. (2018) recorded the highest infestation rates of *Trachurus trachurus* and *Boops boops* in spring. Microhabitat varies according to parasite species, while the digestive tract and gills were reported by Ichalal et al. (2015) and Ider et al. (2018) to be sites with the highest infestation rates. Increasing prevalence from April to July can be explained by a high abundance of prey species, i.e. intermediate hosts (zooplankton especially copepods) on which *S. aurita* feeds (Fedekar 2012). The phase of constant infestation rate observed from August to October might be related to low prey abundance. As reported by Bahar and Tuncay (2015), the emptiest stomachs of *S. aurita* from the Mediterranean Sea (in the Turkish Aegean) were encountered during autumn. Ichalal et

al. (2015), Ider et al. (2018) and Saadi et al. (2020) have shown that the trophic behavior of fishes can increase the likelihood of infection with parasites, especially with those infesting crustacean and molluscan hosts.

Planktivorous fishes exhibiting pelagic behavior become infected with Digeneans and parasitic nematode larvae through ingestion of their intermediate hosts such as euphausiids, and copepods (Ichalal et al. 2015; Smith 1983). In addition, intensive infestation of *S. aurita* in April–July precedes its first reproductive period, which occurs from July to October (Palomera and Sabatès 1990). Many authors (Abattouy et al. 2011; Eissa et al. 2018) have reported that the highest prevalence of infection with Helminths was recorded in summer. As for ectoparasites, warm water in spring and summer enhances proliferation of infective forms with consequent rises in infection rates.

The highest infection rates were observed in older and larger individuals, with Digeneans being the dominant parasite group. This result can be explained by *S. aurita* feeding habits. Juveniles of this species feed on

phytoplankton comprising a large quantity of diatoms (Fedekar 2012). However, adults feed on many species (groups) of zooplankton such as Copepods, Mollusks, Siphonophores, Teleostean eggs and larvae, Polychaeta, Chaetognatha and Tunicate (Lomiri et al. 2008; Fedekar 2012; Bahar and Tuncay 2015). Most of these species constitute potential prey (intermediate hosts) for parasites, especially for those with a complex life cycle like Digeneans. Pérez-del Olmo et al. (2007) linked the predominance of Digenea to the proliferation of intermediate hosts and the increase in copepod biomass. Small (1–2-year-old) fish have a low prevalence of infection. The rapid increase in infection observed in 2–3-year-old and older specimens may be explained by changes in *S. aurita* feeding behavior, i.e. shift towards larger prey (Crustaceans, Mollusks, etc.), which facilitates infection with Digeneans, and Nematodes. Three-year-old fish move to nurseries, facilitating their infection (endemi-topes) with mesoparasites and ectoparasites through feeding and contact (lateral transfer) respectively.

Abattouy et al. (2011) and Ichalal et al. (2015) reported

Table 3. Checklist of parasites collected from the Mediterranean on *Sardinella aurita*.

Parasites species	Studied area	References
<b>Digenea</b>		
<i>Lecithochirium</i> sp.	Tunisia	Derbel et al. 2012; Feki et al. 2015
	Algeria	Present study
<i>Parahemiurus merus</i> (Linton, 1910)	Tunisia	Derbel et al. 2012; Feki et al. 2015
	Algeria	Present study
<i>Aphanurus stossichii</i> (Monticelli, 1891)	Tunisia, Greece, Spain	Derbel et al. 2012; Feki et al. 2015; Sofronios and Papoutsoglou 1975; Lozano et al. 2001
	Algeria	Present study
<i>Derogenes latus</i> Janiszewska, 1953	Algeria	Present study
<i>Monorchis monorchis</i> (Stossich, 1890)	Greece	Sofronios and Papoutsoglou 1975
<i>Bacciger bacciger</i> (Rudolphi, 1819)	Greece	Sofronios and Papoutsoglou 1975
<i>Hemiurus appendiculatus</i> (Rudolphi, 1802)	Greece	Sofronios and Papoutsoglou 1975
<i>Hemiurus communis</i> Odhner, 1905	Algeria	Present study
<i>Lecithaster confusus</i> Odhner, 1905	Greece	Sofronios and Papoutsoglou 1975
<i>Hemiurinea</i> sp.	Algeria	Present study
<b>Monogenea</b>		
<i>Mazocraes</i> sp.	Tunisia	Feki et al. 2015
<b>Cestoda</b>		
Tetraphyllidean larva	Tunisia	Feki et al. 2015
	Algeria	Present study
<i>Calliobothrium</i> sp.	Greece	Sofronios and Papoutsoglou 1975
<b>Copepoda</b>		
<i>Mitrapus oblongus</i> (Pillai, 1964)	Egypt, Turkey	El-Rashidy and Boxshall 2009; Romero and Öktener 2010
<i>Clavellisa ilishae</i> Pillai, 1962	Egypt	El-Rashidy and Boxshall 2009
<i>Clavellisa emarginata</i> (Krøyer, 1873)	Algeria	Present study
<b>Nematoda</b>		
<i>Anisakis pegreffii</i>	Italy	Piras et al. 2014
<i>Hysterothylacium</i> sp.	Algeria	Present study
<b>Protozoa</b>		
<i>Glugea sardinellensis</i> (Thélohan, 1892)	Tunisia	Mansour et al. 2016
<i>Eimerias ardinae</i> (Thélohan, 1890)	Yugoslavia	Daoudi et al. 1989
Unidentified Microsporidian	Algeria	Present study

size-dependent infection rates in teleost hosts from the Mediterranean coasts of Morocco and Algeria. They postulated that intermediate prey, i.e. intermediate hosts of these parasites, constitute a significant part in the diet of large fish, which increases infection rates.

In summary, the rates of infection we recorded in *S. aurita* could be related to numerous factors: temperature, prey availability, host behavior, food availability, feeding behavior and reproductive physiology.

In this study, male specimens were found to be more infected ( $P = 62.4\%$ ) than females ( $P = 54.9\%$ ). Our results show that the number of parasites is related to sex, which is in agreement with the findings reported by Tantanasi et al. (2012) and Ichalal et al. (2015). This result corroborates those reported by Mansour et al. (2016), who recorded high infection ( $P = 70.0\%$ ) with *Glugea sardinellensis* (11–13 cm) in small fish specimens. Host fish pick up Microsporidian parasites through the ingestion of free spores from the water column (Weissenberg 1968). Intestinal infection with microsporidia in *S. aurita* off the coast of Tunisia was first reported by Mansour et al. (2016). A molecular study should be conducted in order to identify this Microsporidian to the species level.

The checklist confirms that *S. aurita* is infected with numerous and various parasite species (Table 3). Until now, *S. aurita* from the Mediterranean Sea has been known to get infected with 21 parasite taxa (protozoan and metazoan parasites). Digenea were the most represented parasite group (8 species). The groups of parasites collected by the authors were represented by only 2–3 taxa (Table 3).

*S. aurita* from the Algerian coast is infected with 10 parasite taxa (9 metazoan and 1 protozoan). *S. aurita* from the coastal waters of Algeria was not found to be infected with monogeneans, but it was found to be infected with 6 Digenean species. As for the other parasitic taxa that we have identified, each of them was represented by only 1 parasite species (Table 3).

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