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Parasitofauna isolated from fish off the east Algerian coast

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Abstract

Between January 2011 and January 2013, parasitofauna of 1643 specimens, obtained both from wild sources (n=1545) and aquaculture (n=98) were studied. From 14 fish species, 17 parasitic species were isolated, of which three species represent a new geographical record. Despite their low infestation rate, some isolated parasite species induce serious necrosis, alterations and atrophies to the muscle, mouth cavity and tongue of their hosts by *Pyroderma cylindricum* (P=9.37%; I=1); *Lerneolophus sultanus* (P=9.09%; I=1), and *Ceratothoa oestroides* (P=0.62%; I=1), respectively. Similar pathological effects were observed in gill filaments infected by *Naeobranchia sygniformis* (P=27.27%; I=1). Among the most abundant isolated parasites was the nematode, *Anguillicola crassus* (P=42.53%; I=6.11) infecting the European eel, *Anguilla anguilla*, which caused an evident inflammatory reaction of the swim-bladder. The present study improves our knowledge on Algerian fish parasitofauna.

Introduction

Parasites are an essential component of every ecosystem (Zander et al., 2002), greatly contributing to overall aquatic biodiversity.

Although parasites of Algerian marine fishes have been studied extensively (Ramdane and

Trilles, 2008, 2010, 2012; Ramdane et al., 2007, 2009, 2013; Ider et al., 2014; Ichalal et al., 2015). Little attention has been focused on farmed and endangered species such as the European eel, *Anguilla anguilla* (L., 1758) (*Anguilla*; Anguillidae). The exception is the study conducted in the complex of wetlands (El-Kala) (Loucif et al.,

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2009) who recorded high prevalence of *Myxidium giardi* (Cépède, 1906) (*Myxidium*; Myxidiidae), and in the Estuary Mefrag (Annaba) (Boudjadi et al., 2009) about the infestation by *Anguillicola crassus* (Kuwahara, Niimi and Hagaki, 1974) (*Anguillicola*; Dracunculidae).

The aim of this study was to list identified parasitic taxa from a large number of Algerian fish, both from wild sources and aquaculture environments, and try to assess their potential pathogenic effect on the host.

Material and Methods

The study was conducted at three different localities along the east coast of Algeria (Figure 1); Azeffoune fish farm (1, n= 98 specimens), Gulf of Bejaia (2, n=1396 specimens) and Soummam

River (outfall) (3, n=149 specimens) from a total of 1643 fish specimens sampled (from 2011 to 2013). Farmed fishes were caught using landing net, while wild fish of the Gulf of Bejaia were randomly sampled from commercial fishing vessels in the fishing port of Bejaia. Wild fish from Soummam River were caught using a monofilament fishing line. Fish were placed in a transparent, labelled plastic bag (date, study area, etc.) and transported immediately (in less than 4 h post-fishing) to the laboratory in a cooler. Fish species were identified and measured to the nearest 1 mm using an ichthyometer, and total weight (Wt) was determined to the nearest 0.1 g using an electronic balance. The sex was determined by macroscopic observations of gonads. The body, fins, buccal cavity, branchial cavities, gills and swim-bladders were exam-

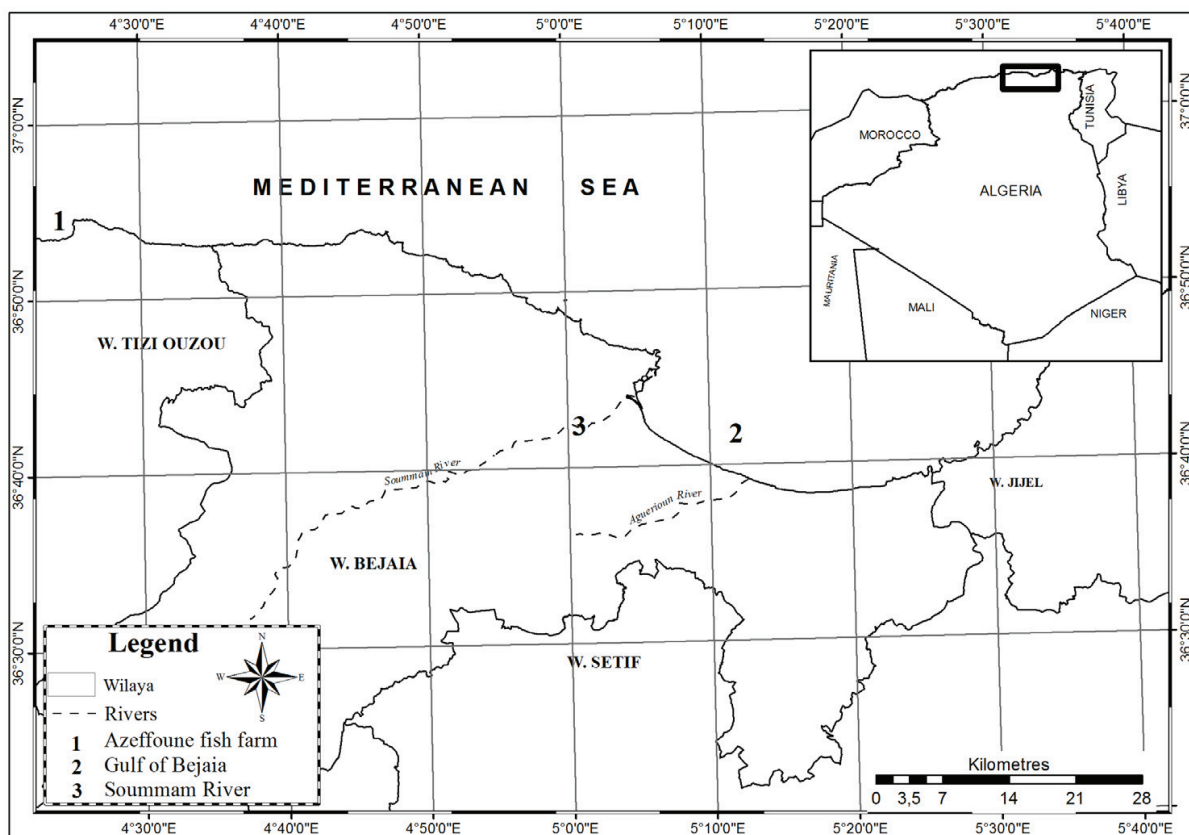


Figure 1. Study area and sampling stations, 1: Azeffoune fish farm; 2: Gulf of Bejaia; 3: Soummam River.

ined for the presence of parasites using a dissecting stereomicroscope. Collected parasites were fixed in 70% ethanol for later identification. Parasitological parameters (Prevalence (P %) = the number of infected hosts/ the number of examined hosts × 100; mean Intensity (I) = the total number of collected parasites / the number of infected hosts; and mean Abundance (Am) = the total number of collected parasites / the number of examined hosts) were calculated according to Bush et al. (1997), the results of which are summarised in Table 1.

To evaluate the pathological effect of parasites on the host, especially in the case of *A. crassus* and *Peroderma cylindricum* (Heller, 1865) (*Peroderma*; Pennellidae), a histological study was performed. Infected tissues (swim-bladders and muscles) were fixed in 10% buffered paraformaldehyde solution, dehydrated in increasing ethanol concentrations (70 to 95%), cleared in xylene and paraffin-embedded. Histological sections of 3µm thickness (thin sections providing clean and good observations) were performed using Leica RM2125 RTS rotary microtome. Sections were mounted on glass microscope slides, stained with Mayer's hematoxylin-eosin, mounted with Canada balsam and examined by Leica DM1000 LED microscope.

Results and Discussion

Diversity and infection rates of isolated parasites

Fourteen fish species have been sampled and identified. Parasite species associated with these fishes have been collected and identified, encompassing 16 metazoans and 1 protozoan taxa. Among the collected parasite species, three are new geographical records; *Clavellisa emarginata* (Kroyer, 1837) (*Clavellisa*; Lerneopodidae);

Caligus ligusticus (Brian, 1906) (*Caligus*; Caligidae); *Gotocotyla acanthus* (Parona et Perugia, 1896) (*Gotocotyla*; Gotocotylidae). The European eel, *Anguilla anguilla* from Soummam River is the new host record for The protozoan *Ichthyophthirius multifiliis* (Fouquet, 1876) (*Ichthyophthirius*; Hymenostomatida). One nematode *Anguillicola crassus* and four monogenean species were collected for the first time in the Gulf of Bejaia, although have been already reported from various Mediterranean localities: *Microcotyle mugilis* (Vogt, 1878) (*Microcotyle*; Microcotylidae) reported from Morocco (El Hafidi et al., 1998), *Pagellicotyle mormyri* (Lorenz, 1878) (*Pagellicotyle*; Microcotylidae) in Gulf of Lyon, France (Marc, 1963) and Tunisia (Naifar, 1995), *Atraster heterodus* (Lebedev et Parukhin, 1969) (*Atraster*; Microcotylidae) in Africa (Lebedev and Parukhin, 1969) and France (Euzet and Maillard, 1973), *Polylabris tubicirrus* (Paperna et Kohn, 1964) (*Polylabris*; Microcotylidae) in Israel (Paperna and Kohn, 1964), France (Euzet and Maillard, 1973), Spain (Lopez-Roman, 1973) and Montenegro coast of Adriatic (Radujkovic and Euzet, 1989), *G. acanthus* in Tunisia (Naifar, 1995) and *A. crassus* in Egypt (Koops and Hartman, 1989), Morocco (El Hillali et al., 1996) and Tunisia (Maamouri et al., 1999). Parasites isolated from Gulf of Bejaia parasitized the same hosts as reported in different localities of the Mediterranean Sea.

New host records were identified for *Ceratothoa oestroides* (Risso, 1826) (*Ceratothoa*; Cymothoidae) in the European pilchard *Sardina pilchardus* (Walbaum, 1792) (*Sardina*; Clupeidae) and *Nerocila maculata* (Milne Edwards, 1840) (*Nerocila*; Cymothoidae) in the tub gurnard *Trigla lucerna* (L., 1758) (*Trigla*; Triglididae). The wildlife fishes were more infected compared to those from

Table 1. Parasitological parameters of the collected parasite taxas.

Host	Sites	N	Lm (Min - Max)	Parasites groups	Parasites species	Fs	P%	Am	I
<i>Sparus aurata</i> (L., 1758)	1	83	17.31 (4.20 - 31.50)	/	/	/	0	0	0
<i>Sparus aurata</i> (L., 1758)	2	11	27.75 (20.90 - 33.50)	Crustacea	<i>Lernaeolophus sultanus</i> (Heller, 1865)	Pr	9.09	0.09	1
					<i>Naobranchia cygniformis</i> (Hesse, 1863)	Br	27.27	0.27	1
					<i>Gnathia</i> sp.	Br, Bc	45.45	17.81	39.2
<i>Dicentrarchus labrax</i> (L., 1758)	1	15	19.65 (7 - 32.10)	/	/	/	0	0	0
<i>Dicentrarchus labrax</i> (L., 1758)	2	11	32.26 (30.30 - 34)	Crustacea	<i>Lernanthropus kroyeri</i> (van Beneden, 1851)	Br	33.33	0.33	1
					<i>Caligus minimus</i> (Otto, 1821)	Br	33.33	0.33	1
<i>Sarpa salpa</i> (L., 1758)	2	30	26.3 (18 - 32)	Crustacea	<i>Caligus ligus- ticus</i> (Brian, 1906) **	Bc	3.33	0.033	1
<i>Sardina pilchar- dus</i> (Walbaum, 1792)	2	160	13.26 (10 - 20)	Crustacea	<i>Peroderma cylin- dricum</i> (Heller, 1865)	Mus	9.37	0.09	1
					<i>Ceratothoa oes- troides</i> (Risso, 1826) *	Bc	0.62	0.006	1
<i>Trigla lucerna</i> (L., 1758)	2	10	19.75 (16 - 25)	Crustacea	<i>Nerocila mac- ulata</i> (Milne Ed- wards, 1840)*	Cf	10	0.1	1
<i>Lithognathus mormyrus</i> (L., 1758)	2	330	16.61 (14 - 22)	Monogenea	<i>Pagellicotyle mormyri</i> (Lorenz, 1878)	Br	3.33	0.03	1
<i>Boops boops</i> (L., 1758)	2	664	18.95 (10 - 27.50)	Monogenea	<i>Atraster hetero- dus</i> (Lebedev et Parukhin, 1969)	Br	6.02	0.14	2.37

Table 1 Continued. Parasitological parameters of the collected parasite taxas.

Host	Sites	N	Lm (Min - Max)	Parasites groups	Parasites species	Fs	P%	Am	I
<i>Diplodus vulgaris</i> (Geoffroy Saint-Hilaire, 1817)	2	64	18 (14 - 29)	Monogenea	<i>Atraster heterodus</i> (Lebedev et Parukhin, 1969)	Br	15.62	0.16	1
					<i>Polylabris tubicirrus</i> (Paperna et Kohn, 1964)	Br	4.69	0.08	1.67
<i>Diplodus cervinus</i> (Lowe, 1838)	2	75	26.67 (10 - 55)	Monogenea	<i>Atraster heterodus</i> (Lebedev et Parukhin, 1969)	Br	10.67	0.11	1
					<i>Polylabris tubicirrus</i> (Paperna et Kohn, 1964)	Br	4	0.07	1.67
<i>Diplodus annularis</i> (L., 1758)	2	37	12.44 (10 - 17)	Monogenea	<i>Gotocotyla acanthus</i> (Parona et Perugia, 1896) **	Br	16.22	0.02	1.33
<i>Lichia glauca</i> (L., 1758)	2	3	28 (23 - 36)	Monogenea	<i>Gotocotyla acanthus</i> (Parona et Perugia, 1896) **	Br	33.33	1.33	4
<i>Mugil cephalus</i> (L., 1758)	3	50	27.49 (15 - 40)	Monogenea	<i>Microcotyle mugilis</i> (Vogt, 1878)	Br	10	0.12	1.2
<i>Anguilla anguilla</i> (L., 1758)	3	87	361.99 (197 - 822)	Nematoda	<i>Anguillicola crassus</i> (Kuwahara, Niimi and Hagaki, 1974)	Sb	42.53	2.6	6.11
				Protozoa	<i>Ichtyophthirius multifiliis</i> (Fouquet, 1876)	Af	2.3	0.02	1
<i>Alosa fallax algeriensis</i> (Regan, 1906)	3	12	17.04 (14 - 21)	Crustacea	<i>Clavellisa emarginata</i> (Kroyer, 1837) **	Br	25	0.25	1

*: New parasite for the host; **: New parasite for Algerian fauna; N: Number of examined fishes; Lm: mean length, Min-Max: Minimum and maximum; P (%): Prevalence; Am: mean Abundance; I: mean Intensity; Fs: Fixing site; Bc: Buccal cavity; Pr: Palate roof; Br: Branchial arches; Af: Anal fin; Cf: Caudal fin; Sb: Swim-bladder ; Mus: Muscle; 1: Azzefoune fish farm; 2: Gulf of Bejaia; 3: Soummam River.

aquaculture. The maximum prevalence and the mean intensity reached 45.45% and 39.2 parasites per infected host, respectively. For some species, infection rates were generally low and not exceeding 33.33% (*Lernanthropus kroyeri*, *Caligus minimus* and *G. acanthus*), with the exception of the nematode *A. crassus* in the swim-bladder of *A. anguilla* (P=42.53%; I=6.11 parasites per infected host) and the Crustacean, Praniza of *Gnathia* sp. infecting the gills and oral cavity of the wild gilthead sea bream, *Sparus aurata* (L., 1758) (*Sparus*; Sparidae) (P=45.45%; I=39.2 parasites per infected host). Crustaceans had higher rates of infection compared to monogeneans. Aquaculture-farmed fish from the floating cages of Azeffoune, *S. aurata* and the European sea bass, *Dicentrarchus labrax* (L., 1758) (*Dicentrarchus*; Moronidae) did not show parasitic infection in comparison with their congeners from the wild environment. This might be related to one or a combination of a few factors: the reduced contact with infected wild fish (no transfer of infective forms), indeed, the cages were well spaced and placed generally in suitable depths (40 m), with generally moderate currents; and/or the excellent quality of water and respected fish density in these cages did not promote the development of parasites. However, a good water quality of a recently installed fish farm (Azeffoune farm) could be easily altered over the coming years by fish excreta, the residue of fish feed, algae proliferation, reduced oxygen, etc., becoming an important issue without regular monitoring. As infected wild fish are attracted by food availability at aquaculture sites, enhanced interaction in that case might contribute to the development of infection in farmed fish.

In addition, aquaculture larvae and fingerlings

often had undergone antiparasitic treatments before being introduced into the cages, which would not prevent them from subsequent infection. In contrast, wild *S. aurata* and *D. labrax* were infected by several parasite species such as *Lernaeolophus sultanus* (Heller, 1865) (*Lernaeolophus*; Pennellidae), *Naobranchia cygniformis* (Hesse, 1863) (*Naobranchia*; Lernaeopodidae), *Gnathia* sp., *Lernanthropus kroyeri* (van Beneden, 1851) (*Lernanthropus*; Lernanthropidae), *Caligus minimus* (Otto, 1821) (*Caligus*; Caligidae).

Pathologic effects of selected parasites

The microhabitats of the identified parasite taxa were parasite-specific; the most infected organs were the gills and the oral cavity (Table 1), however parasitic infection of internal organs has not been assessed in this study. Parasites also showed a wide level of host-specificity, being oioxenic, stenoxenic or euryxenic (Table 2). Taxa with a strict specificity to their microhabitat showed elevated pathogenic effects, observed both at macroscopic and microscopic level. Likewise, adults of *Ceratothoa oestroides* showed visible effects in the host mouth in the form of alterations of the tongue of their host (Table 2, Figure 2A). The same effect was previously described by Ramdane et al. (2009). Our results corroborate those reported by that group for *Lernaeolophus sultanus* and *Naobranchia cygniformis* that caused extensive lesions in the form of perforations in the palate roof and gill arches (Table 2, Figure 2 B to C).

The female copepod *Peroderma cylindricum* caused lesions in epidermis, muscle and kidney inflicted by mechanical penetration of this parasite into the infected European pilchard specimens (Table 2; Figure 2D; Figure 3 A to B). Zamouri-Langar (1995) noticed that *P. cylindricum* weakened the

Table 2. Specificity and pathological effects of the collected parasite taxa.

Collected parasites species	Specificity/host	Specificity/ Fixing site	Impact of parasites on their hosts	
<i>Lernaeolophus sultanus</i>	Euryxenic	Strict	Perforation of palate roof	
<i>Naobranchia cygniformis</i>	Stenoxenic		Alteration of gill filaments	
<i>Gnathia</i> sp.	Euryxenic	Large	Alteration of gill filaments, dermis, buccal cavity	
<i>Lernanthropus kroyeri</i>	Oioxenic	Strict	Alteration of gill filaments	
<i>Clavellisa emarginata</i>	Stenoxenic		Unobserved	
<i>Caligus minimus</i>	Oioxenic		Alteration of the buccal cavity	
<i>Caligus ligusticus</i>			Unobserved	
<i>Peroderma cylindricum</i>			Muscle and kidney deterioration	
<i>Ceratothoa oestroides</i>	Stenoxenic		Alteration of the tongue	
<i>Nerocila maculata</i>			Alteration of the dermis of the fin	
<i>Anguillicola crassus</i>	Polyxenic		Degradation of swim-bladder and inflammatory reaction	
<i>Microcotyle mugilis</i>	Oioxenic		Unobserved	
<i>Pagellicotyle mormyri</i>				
<i>Atraster heterodus</i>				
<i>Polylabris tubicirrus</i>	Stenoxenic			
<i>Gotocotyla acanthus</i>				
<i>Ichtyophthirius multifiliis</i>	Euryxenic	Large		Hemorrhage

parasitised European pilchard, making them lose their swimming-balance.

The adults of the hematophagous nematode *Anguillicola crassus* parasitised the lumens of the swim-bladder (Table 2; Figure 4A) and caused the thickening of its different layers, with signs of acute inflammation and fibrosis (Table 2; Figure 2E; Figure 4 A to B). Larvae, L3 and L4 however, were located in the wall, resulting in the formation of nodules (Figure 4 D to E). Similar results were reported by Molnar et al. (1993) and Lefebvre et al. (2002).

The studied parasitofauna, from body surface

and digestive tract, reveals potential pathogenic parasite taxa. More investigations must be done on the protozoan parasites, especially Myxozoa, Ciliates, etc., which often exhibit more pathogenic effects to their hosts.

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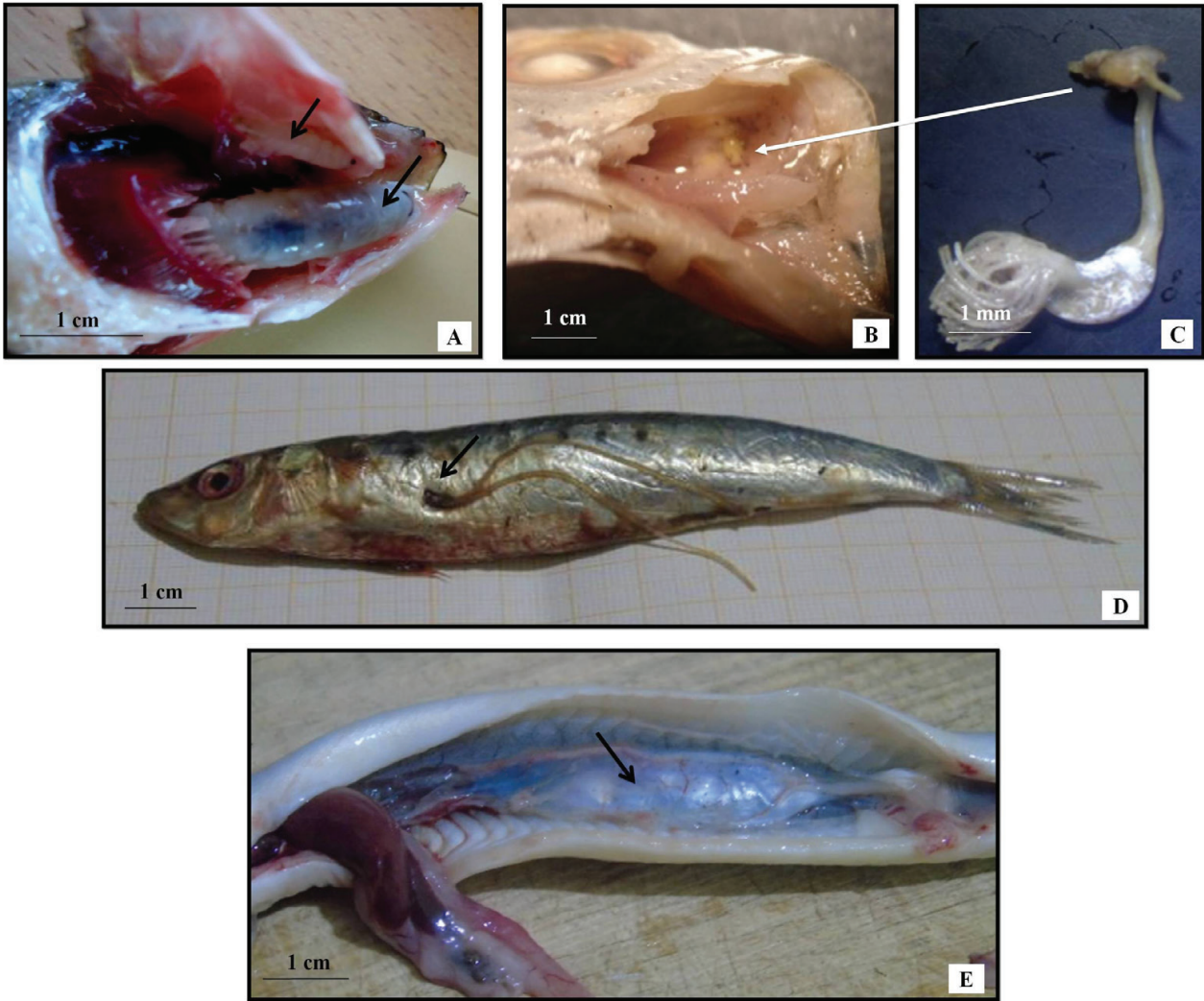


Figure 2. **A:** *Ceratothoa oestroides* parasitising the dorso-lateral (male) and ventral part of the buccal cavity (female) in the bogue *Boops boops*; **B to C:** Dorsal part of buccal cavity of *B. boops* perforated by the female copepod, *Lernaolophus soltani* by its anchor-shaped fixing appendix; **D:** *Pyroderma cylindricum* penetrating into European pilchard *Sardina pilchardus* body; **E:** Enlarged swim-bladder of European eel *Anguilla anguilla* by parasitising *Anguillicola crassus*.

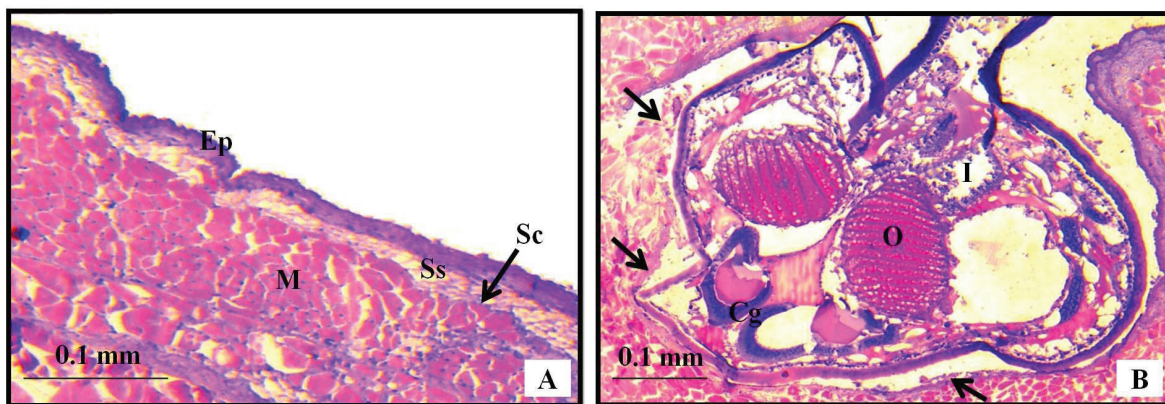


Figure 3. Histological sections in lateral musculature of European pilchard *Sardina pilchardus*; **A:** Skin of unparasitised European pilchard showing normal skin layers, (**Ep**) epidermis, (**Ss**) stratum spongiosum, (**Sc**) stratum compactum, (**M**) skeletal muscle; **B:** Penetration of *Peroderma cylindricum* in the muscle of its host, showing disruption of the dermis (black arrows) and the body section of the parasite copepod: (**I**) Intestine, (**O**) Ovary, (**Cg**) Cemental gland.

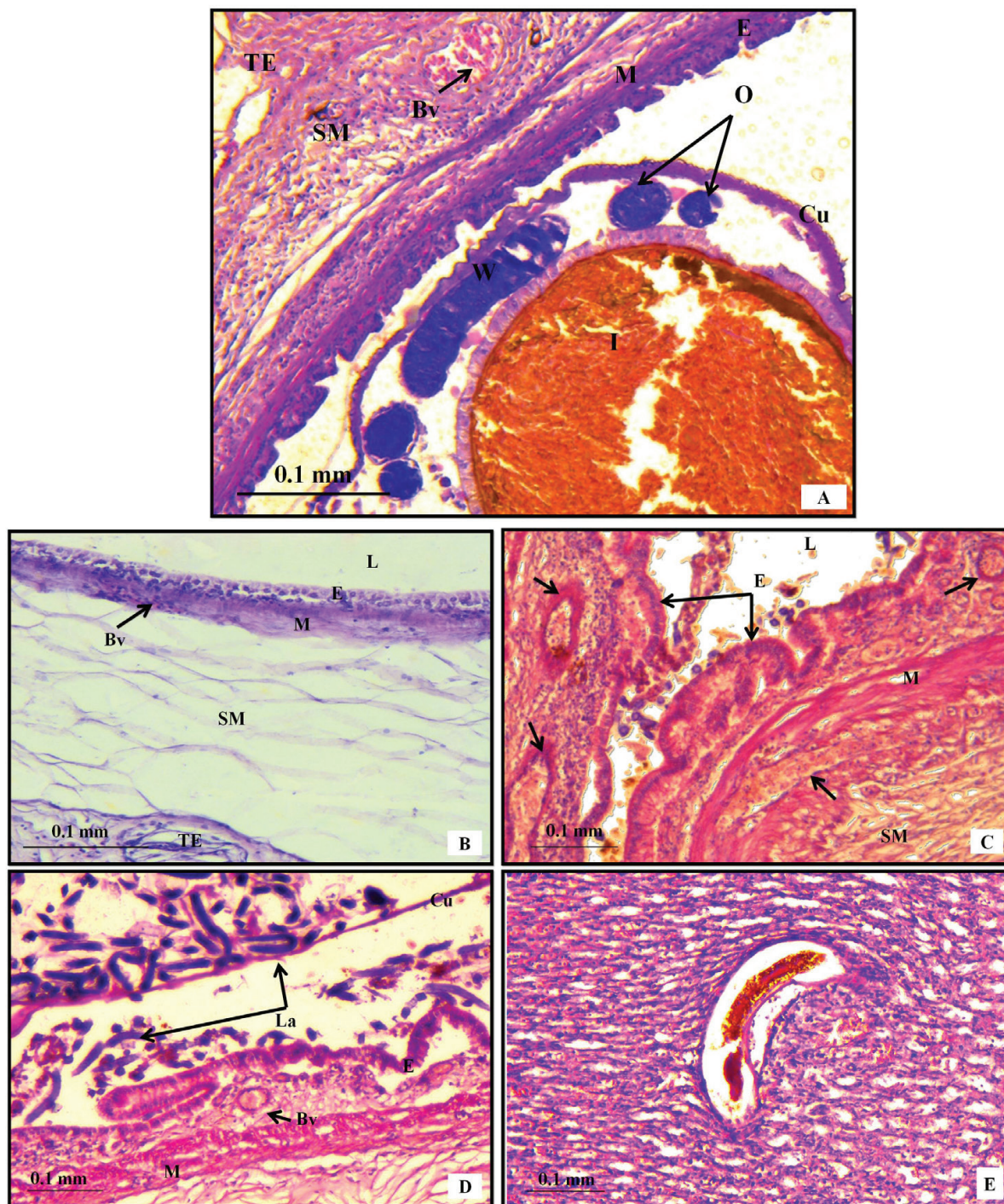


Figure 4. Histological sections of swim-bladder in the European eel *Anguilla anguilla*, **A:** *Anguillicola crassus* parasitising the swim-bladder lumen, (**Cu**) cuticle, (**W**) womb, (**O**) ovaries, (**I**) intestine; **B:** swim-bladder of an unparasitised eel with four healthy layers: (**TE**) tunica externa, (**SM**) submucosa, (**M**) muscularis mucosae, (**E**) epithelium, (**Bv**) blood vessel, (**L**) lumen; **C:** swim-bladder in parasitised eel showing thickening of layers (**SM**, **M**, **E**) and dilation of capillaries (black arrows); **D:** Release of *Anguillicola crassus* larvae (**La**) from female body, **E:** Larva of *Anguillicola crassus* entrapped in the *rete mirabile* of the swim-bladder.

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