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Parasitic infections in the Mediterranean needlefish *Tylosurus acus imperialis* (Teleostei: Belonidae) off Tunisian coast

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ABSTRACT

Objective: To provide a current survey of parasitic infections in the Mediterranean needlefish *Tylosurus acus imperialis* (*T. acus imperialis*). The impact of host's size and sex on some characteristics of the infection was also examined.
Methods: Between 2004 and 2009, 126 specimens of *T. acus imperialis* were necropsied to study their parasites.
Results: Fifteen species of metazoan parasites were collected including 3 monogeneans (*Aspinatrium gallieni, Nudaciraxine imperium* and *Axinoides* sp.), 4 digeneans (*Lecithostaphylus tylosuri*, *Tetrochetus coryphaenae*, *Oesophagotrema mediterranea* and *Sclerodistomoides pacificus*), 1 cestode (*Ptychobothrium* sp.), 3 copepods (*Lernanthropus tylosuri*, *Caligodes laciniatus* and *Caligus* sp.), 1 isopod (*Irona nana*), 1 acanthocephalan (*Rhadinorhynchus* sp.), 1 nematode (anisakid larvae) and 1 annelid (piscicolid). Cestoda *Ptychobothrium* sp. was the most frequent species (72%). Prevalence and abundance of infection with *Ptychobothrium* sp. and *Rhadinorhynchus* sp. were positively correlated with the total length of the host. Host's sex did not seem to affect the infection parameters of parasites in *T. acus imperialis*.
Conclusions: This study provides first information on parasitic infections in the Mediterranean needlefish *T. acus imperialis* off Tunisia. Parasites can be used to understand phylogeny, biology and ecology of fish host and also as bioindicators of water quality.

1. Introduction

The Belonidae or needlefish is a relatively small family of beloniform fishes that comprise 34 recognized species in the oceans and freshwater of the world[1]. Among the three representatives belonids species off Tunisian coast, *Tylosurus acus imperialis* (Rafinesque, 1810) (*T. acus imperialis*) is exclusively the most frequent species during its spawning season, from May to July[2]. This epipelagic fish worldwide occurs in the Mediterranean Sea and it is also founded in the Eastern Atlantic, in the coast of Morocco, in the Cape Verde islands and in the Adriatic Sea[3,4]. It's the subspecies of *Tylosurus acus* (*T. acus*) mainly restricted to the Mediterranean Sea. Other subspecies of *T. acus* have been recognized according to their geographical distribution: *Tylosurus acus acus* (Lacépède, 1803) (*T. acus acus*) in the Western Atlantic; *Tylosurus acus rafale* (Collette & Parin, 1970) in the Gulf of Guinea and *Tylosurus acus melanotus* (Bleeker, 1850) throughout the Indo-Western Pacific, extending to the Eastern Pacific[1].

Despite its common occurrence, the parasite fauna of *T. acus imperialis* is poorly known. Raibaut *et al*. only reported copepods *Caligodes laciniatus* Krøyer, 1863 (*C. laciniatus*), *Lernanthropus tylosuri* Richiardi, 1880 (*L. tylosuri*) and *Parabomolochus bellones* Burmeister, 1835 from *T. acus imperialis* of the Mediterranean Sea[5]. Our studies have been focused so far on taxonomic features of specific taxa, such as mongenean *Nudaciraxine imperium* Châari, Derbel & Neifar, 2010 (*N. imperium*) and two digeneans species, *Oesophagotrema mediterranea* Châari, Derbel & Neifar, 2011 and *Lecithostaphylus tylosuri* Châari, Derbel & Neifar, 2013 (*L. tylosuri*) described from *T. acus imperialis* off Tunisia[6-8]. However, the quantitative aspect of parasites of *T. acus imperialis* has not previously been studied. In the Brazilian coast, Tavares *et al*. studied the metazoan parasites of the Western Atlantic needlefish *T. acus acus*[9].

The aim of this paper is to provide baseline information on the metazoan parasites of *T. acus imperialis* from Tunisian coast and analyze the parasitism host to evaluate the impact of host's size and

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sex on some characteristics of the infection, such as prevalence and mean abundance.

2. Materials and methods

Between 2004 and 2009, 126 specimens of *T. acus imperialis* measuring (78.41 ± 13.51) (29.7–110.5) cm of total length were collected from local fishermen using gill nets in the eastern coast of Tunisia (Figure 1). Samples were identified according to the method of Bauchot and Collette and Parin[3,10].

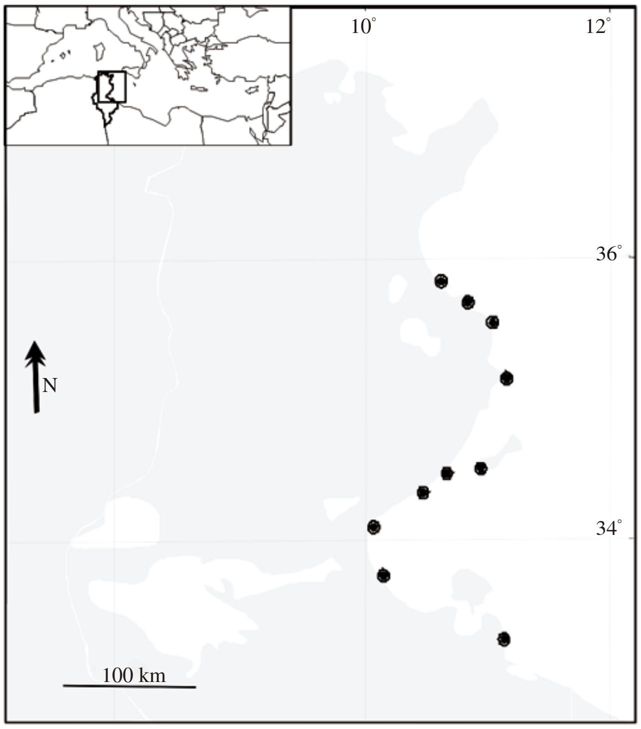

Figure 1. Map showing the study area and sampling sites of *T. acus imperialis* off Tunisian coast.

Fresh fish were subsequently examined for ectoparasites and endoparasites. Parasitological examination using a stereomicroscope under incident light included the skin, fins, gills, eyes, body cavity and visceral organs (stomach, intestine, liver, swim bladder, gallbladder and gonads). Platyhelminthes were fixed between slide and coverslip in 70% alcohol or in Bouin's fluid. Fixed specimens were stained with Semichon's acetic carmine, dehydrated using a graded ethanol series then cleared in clove oil and mounted in Canada balsam. Others parasites such as copepod, isopod, nematode and acanthocephalan were directly fixed in 70% alcohol for later examination. Parasites were identified to the lowest taxonomic level possible.

Infection parameters including prevalence, mean intensity and mean abundance were determined following Margolis *et al*. and Bush *et al*.[11,12]. Analysis was performed to evaluate the infections only for parasites with prevalence > 10%[13]. Spearman's rank correlation coefficient (r_s) was used to determine the possible correlation between the total length of the host and abundance of parasite. Pearson's correlation coefficient (r) was used as an indication of the relationships between the total length of the host and the prevalence of parasite with previous partition of fish samples into 10 cm length intervals.

The significance of host's sex differences on mean abundance and prevalence of parasites was assessed by ANOVA and *Chi*-square tests, respectively.

3. Results

A total of 1 976 individual parasites present with an average of (16 ± 12) parasites/fish were collected. Among the examined fishes, 94% was found to be parasitized by at least one of parasite species. Fifteen species of metazoan parasites were identified from *T. acus imperialis* off Tunisia (Table 1). Parameters of infection for each parasite taxa and location in the host were presented in Table 1. Digeneans parasites were the most important in species richness (four parasites species). *Ptychobothrium* sp. was the most dominant species, with 616 specimens (31.2% of parasites collected). Adult endoparasites represent 53.5% of all collected parasite, larval endoparasite 0.6% and ectoparasites 46.1%.

r_s and r indicated positive correlation with total host length in the mean abundance and the prevalence of *Ptychobothrium* sp. and *Rhadinorhynchus* sp. (Table 2).

Table 1

Infection parameters and infection site of the metazoan parasites of *T. acus imperialis* off the Tunisian coast.

Parasites		Site of infection	Prevalence (%)	Mean intensity	Mean abundance
Monogenea	*A. gallieni*	Gill cover, gills	55	3.07	1.70
	N. imperium	Gills	43	2.63	1.14
	Axinoides sp.	Gills	3	1.75	0.06
Digenea	*L. tylosuri*	Stomach	32	3.32	1.07
	Oesophagotrema mediterranea Châari, Derbel & Neifar, 2011	Oesophagus, vomer teeth	8	6.50	0.92
	Tetrochetus coryphaenae Yamaguti, 1934	Stomach	6	1.00	0.06
	Sclerodistomoides pacificus Kamegai, 1971	Gall bladder	1	2.00	0.02
Cestoda	*Ptychobothrium* sp.	Intestine	72	6.92	4.97
Copepoda	*L. tylosuri* (female)	G	52	2.72	1.43
	L. tylosuri (male)	G	25	2.03	0.50
	C. laciniatus	Eyes, gill cavity, nostrils	35	6.00	2.10
	Caligus sp.	Gill cavity	9	1.08	0.10
Isopoda	*I. nana*	Gill cavity	11	1.92	0.21
Acanthocephala	*Rhadinorhynchus* sp.	Intestine	34	5.52	1.87
Nematoda	Anisakid larvae	Intestine, liver	5	0.46	0.10
Annelida	Piscicolid	Gill cavity	5	1.33	0.06

A. gallieni: *Aspinatrium gallieni* Euzet & Ktari, 1971; *I. nana*: *Irona nana* Schioedte & Meinert, 1884.

Table 2

Correlations between the total length of *T. acus imperialis* and the mean abundance and the prevalence of its parasite species from the Tunisian coast.

Parasites	r_s	*P*	*r*	*P*
A. gallieni	0.141	0.119	0.184*	0.041
N. imperium	-0.106	0.242	-0.022	0.808
L. tylosuri	-0.183*	0.042	0.017	0.849
Ptychobothrium sp.	0.208*	0.020	0.199*	0.027
L. tylosuri (female)	0.039	0.670	0.152	0.093
L. tylosuri (male)	0.101	0.145	0.114	0.208
C. laciniatus	0.257*	< 0.001	0.152	0.094
I. nana	0.163	0.071	0.084	0.356
Rhadinorhynchus sp.	0.201*	0.026	0.184*	0.040

*: $P < 0.05$.

The host's sex did not influence the prevalence and mean abundance of infection with most parasites species in *T. acus imperialis* off Tunisian coast (Table 3).

Table 3

Effect of the sex on the prevalence and mean abundance of parasites in *T. acus imperialis* off Tunisia.

Parasites	Prevalence				Mean abundance			
	Female	Male	*Chi*-square	*P*	Female	Male	ANOVA	*P*
A. gallieni	57.41	55.22	0.04	0.84	1.57	1.85	0.19	0.65
N. imperium	35.19	52.24	3.32	0.06	0.63	1.64	6.18	0.01*
L. tylosuri	31.48	34.33	0.12	0.72	1.06	1.13	0.17	0.67
Ptychobothrium sp.	74.07	73.13	0.01	0.93	5.98	4.37	1.85	0.17
L. tylosuri (female)	53.70	53.73	0.00	0.99	1.56	1.39	0.24	0.62
L. tylosuri (male)	27.78	23.88	0.29	0.59	0.59	0.46	0.51	0.47
C. laciniatus	40.74	32.84	0.85	0.35	2.56	1.82	0.82	0.36
I. nana	12.96	10.45	0.27	0.60	0.20	0.24	0.05	0.81
Rhadinorhynchus sp.	42.59	28.36	2.85	0.09	2.56	1.40	2.22	0.13

*: $P \leqslant 0.05$.

4. Discussion

The present study increases our knowledge on the biodiversity of metazoan parasites of the Mediterranean needlefish *T. acus imperialis*. This is the first survey on the entire parasite fauna of *T. acus imperialis* off Tunisia. The parasite community of *T. acus imperialis* revealed the dominance of adult endoparasites (53.5% of all collected parasite) followed by ectoparasites (46.1% of all collected parasite). The high level of infection with endoparasites which are trophically transmitted could be related to the piscivorous behavior of the fish host. Needlefishes are carnivorous, feeding primarily on small fishes which they catch sideways in their beaks[1]. Piscivorous fishes occupy the top of the food web and may present a greater chance of infection[14,15].

Cestoda *Ptychobothrium* sp. was the most frequent species (72%) in the parasite fauna of *T. acus imperialis*. The high frequency of *Ptychobothrium* sp. may be an immune response of the fish exposed to an environmental stress. Fish possess defense mechanisms to counteract the impact of toxics. Immune defences can be modulated by the potential environmental pollutants but also by natural stimulants such as bacteria, viruses or parasites. The health status of organisms (parasitized/unparasitized) is important to understand the responses of immunological markers in fish[16]. It has also been demonstrated that infection with *Ptychobothrium* sp. in the carp *Cyprinus carpio* could develop an adaptive response to neutralise the oxidative stress normally induced by parasitism[17]. Moreover, parasitic infection like *Ptychobothrium* sp. can stimulate some components of the fish antioxidant system. The treatment of fish with copper was associated with a decrease in antioxidant enzymes activities especially in liver, more pronounced in healthy than in parasitized carp[18]. In addition, our result showed a positive significant correlation between infection parameters of the cestode *Ptychobothrium* sp. and the acanthocephalan *Rhadinorhynchus* sp. and the total length of the host. It appears that these intestinal parasites cannot release from the host and are accumulated as the fish grows. Infection with cestode *Ptychobothrium* sp. and acanthocephalan *Rhadinorhynchus* sp., which is more important in big fishes (specimens present in the coast off Tunisia were in size classes 70 and 80 cm of total length), could reflect the status of ecosystem in the coastal waters exposed to the pollution. The coexistence of various industrial and urban activities mainly in the Gulf of Gabes (Southern coast off Tunisia) may disrupt the stability of the ecosystem. Intestinal parasites particularly acanthocephalans and cestodes are demonstrated as bioindicators of heavy metal contamination in aquatic habitats. These parasites can accumulate heavy metals at concentrations that are orders of magnitude higher than those in the host tissues or the environment[19,20].

Ectoparasites monogenean *A. gallieni* and copepod *L. tylosuri* (female) could be also considered dominant species with prevalence > 50%[21]. The high level of infection with ectoparasites could be related to the gregarious behavior of the fish host that congregates seasonally on Tunisian coasts for spawning[2]. The temporal and spatial proximity of fish individuals within shoals potentially facilitates the transmission of parasites directly[22].

In the present study, we have identified fifteen parasites taxa in the Mediterranean needlefish *T. acus imperialis* off Tunisian coast. Tavares *et al*. reported fifteen metazoan parasites from the Western Atlantic needlefish *T. acus acus* off Brazilian coast including 3 monogeneans (*Chlamydaxine* sp., *Nudaciraxine* sp. and Mazocraeid), 4 digeneans [Didymozoid, *Parahemiurus merus* (Linton, 1910), *Rhipidocotyle* sp. and *Schikhoblotrema acuta* (Linton, 1910)], 1 cestode *Scolex pleuronectis* Müller, 1788, 2 nematods (*Hysterotylacium* sp. and *Pseudoterranova* sp.) and 5 copepods [*Caligus malabaricus* Pillai 1961, *Metacaligus* sp., *Caligus* sp., *L. tylosuri* Richiardi, 1880 and *C. laciniatus* (Krøyer, 1863)][9]. The parasite fauna composition of *T. acus imperialis* differs totally from that of *T. acus acus* excepting copepods *L. tylosuri*, *C. laciniatus* and *Caligus* sp. These copepods parasites species could be considered generalist that have broad specificity spectrum. Indeed, copepods *L. tylosuri*, *C. laciniatus* and *Caligus* sp. have been reported from several needlefishes around the world[23]. The qualitative change on the parasitism of *T. acus* subspecies can be due to the geographical distribution and phylogeny of hosts. Indeed, needlefishes *T. acus imperialis* and *T. acus acus* were recognized by Collette as different subspecies that live in completely different and separate ecosystems[1].

In our results, we observed that host's sex did not influence the prevalence and mean abundance of infection with parasites in *T. acus imperialis*. Males and females needlefish *T. acus imperialis* (sex ratio not different from 1) migrate to the coast to breed[2]. Therefore they are subject to the same environmental conditions and have probably the same diet. The absence of correlations in parasite prevalence and abundance with the sex of the host fish is a widely documented pattern and interpreted as a consequence of the absence of sexual differences in some biological aspects of the fish[24,25].

This study provides first information on parasitic infections in the

Mediterranean needlefish *T. acus imperialis*. Our findings can prove the use of parasites as a reliable tool to discriminate even subspecies of *T. acus*. Additionally, parasites can provide useful information which agrees with the biology and ecology of this host fish. Finally, intestinal parasites particularly cestode and acanthocephalan can be used as bioindicators to know the status of the coastal waters. Indeed, parasite communities can be affected by several environmental variables (physical or chemical conditions) and also by the ecological characteristics of the host[26]. Thus, the study of fish parasites communities were used as a tool to discriminate the host populations and identify contaminated habitats[27-30].

Conflict of interest statement

We declare that we have no conflict of interest.

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