

## New data on pipefishes' and seahorse's endohelminths off Crimean coasts of the Black Sea

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### Summary

A total of 357 Syngnathidae fishes (*Syngnathus abaster* Risso, 1827, *S. typhle* Linnaeus, 1758 and *Hippocampus hippocampus* (Linnaeus, 1758) caught in different habitats along Crimean Black Sea shelf were examined for presence of endohelminths and revealed to be hosts of 15 helminth species. The fishes are second intermediate hosts for five “birds” digenean species and nematodes (larvae of three species and immature adults of one more species) completing life-cycles in waterbirds and fish; for two acanthocephalans and three cestodes larvae ending development in fish. We suggest, basing on data on feeding of the Black Sea predatory fish and waterbirds, that Syngnathid fishes are paratenic hosts in parasitic systems of most cestodes, nematodes and acanthocephalans. All the trematodes found are generalists at metacercarial stage; specialists *Timoniella imbutiformis* and *Aphallus tubarium* use Syngnathidae as definitive hosts. Cestodes, nematodes as well as acanthocephalans found are generalists, too. Based on infection indices, *S. typhle* are main final host and *H. hippocampus* are main 2nd intermediate hosts for *T. imbutiformis*; Syngnathids are accidental hosts for other trematodes as well as for all the cestodes, nematodes and acanthocephalans. *Cryptocotyle concava* and *Pygidiopsis genata* are important as Syngnathid fishes' threats, especially in marine protected aquatoria, being potential agents of “black spot disease”.

**Keywords:** Syngnathidae; helminths; Black Sea; marine protected areas

### Introduction

Syngnathid fishes are a typical component of fish communities in the Black Sea shallow coastal waters, and are very important in terms of biodiversity. Seven species of pipefishes (*Nerophis ophidion* (Linnaeus, 1758), *Syngnathus abaster* Risso, 1827, *S. acus* Linnaeus, 1758, *S. schmidtii* Popov, 1927, *S. tenuirostris* Rathke, 1837, *S. typhle* Linnaeus, 1758, *S. variegatus* Pallas, 1814) and one, *Hippocampus hippocampus* (Linnaeus, 1758) (Vasil'eva, 2007), or two (as believe Bilecenoglu *et al.* (2014) meaning the presence also of *H. guttulatus* Cuvier, 1829) species of seahorses, have been known from there.

These small fishes are also known to contribute to the helminth life cycle that extending to water birds and some predatory Black Sea fish (Gaevskaya & Korniychuk, 2003). Only a few specimens of the Black Sea Syngnathidae fish were studied by helminthologists in the middle of XX century; so, information on the species composition of Syngnathidae helminths in the sea is extremely scarce even in relation to the Black Sea coast of the Crimea – the Black Sea region, the parasite fauna there is being studied most thoroughly (Gaevskaya & Korniychuk, 2003). Some modern data on Syngnathidae helminth fauna in the Black Sea are available (Korniychuk *et al.*, 2016); nevertheless, corrections are needed now in taxonomic status of some parasite species had previously

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been listed as well as appropriate quantitative data. Being small-size fishes, Syngnathidae are not active migrants (Svetovidov, 1964; Vasil'eva, 2007) and one could expect existence of different local population of Syngnathidae in the Black Sea (at least, there are evidence for special *S. typhle* population in the Sea of Marmara and the Black Sea (Wilson & Veraguth, 2010), with originality of their parasite fauna.

Syngnathid fishes are known as endangered part of some Black Sea coastal marine ecosystems: *S. tenuirostris*, *S. typhle*, *H. hippocampus* are included to the Red Book of Crimea (Ivanov, Fateryga, 2015) and Black Sea Red Data Book (Dumont, 1999), *S. variegatus* – to Red Book of Crimea (2015) and they can certainly be affected by physical degradation and destruction of their habitats, chemical pollutants, eutrophication, sedimentation of dispersed silts on seagrasses or other changes in water quality, intensive mining of sea sands, large-scale hydraulic construction, noise pollution, invasive species and even climate change (Boltachev & Karpova, 2012; Felipe *et al.*, 2016) as well as by parasites and diseases and helminthosis are one of most important of them. Nevertheless, being important part of coastal marine ecosystems, they are not commercially important food fishes and that's why Syngnathidae are still little studied fish hosts in the Black Sea. So, the purpose of the investigation was to study, based on original data,

the modern state of helminth fauna of the Black Sea Syngnathidae off Crimean coasts, with special attention to helminths as possible fish disease agents, and to specify the role these fishes play in helminths parasite systems using quantitative indices.

## Materials and Methods

### The Study Area

Syngnathidae fish hosts (black-striped pipefish *Syngnathus abaster* Risso, 1827, broadnosed pipefishes *S. typhle* Linnaeus, 1758 and short-snouted seahorses *Hippocampus hippocampus* (Linnaeus, 1758) were caught by hand nets (depths 0–2 m) in different *Zostera* spp. on silty sands byocenoses (Milchakova *et al.*, 2015) along Crimean Black Sea shelf (Fig. 1, Table 1) during spring – autumn seasons in 1995 – 2017. Sampling points were as follows: Sevastopol, river Chernaya estuary (44°36'29"N 33°35'54"E), Sevastopol, Streletskaya Bay (44°36'17"N 33°28'08"E), Sevastopol, Kazachya Bay (44°34'30"N 33°24'45"E), Kerch Strait, Naberezhnoe (45°08'20"N 36°25'00"E), Karkinit Gulf, Steregucshee (45°44'56"N 33°14'25"E), Karkinit Gulf, Sari-Bulat Bay (45°52'30"N 33°32'30"E). Previously, there were no data on the helminth fauna and infection rates of Syngnathidae in these areas of the Black Sea except for one preliminary fauna report (Kornychuk *et al.*, 2016).

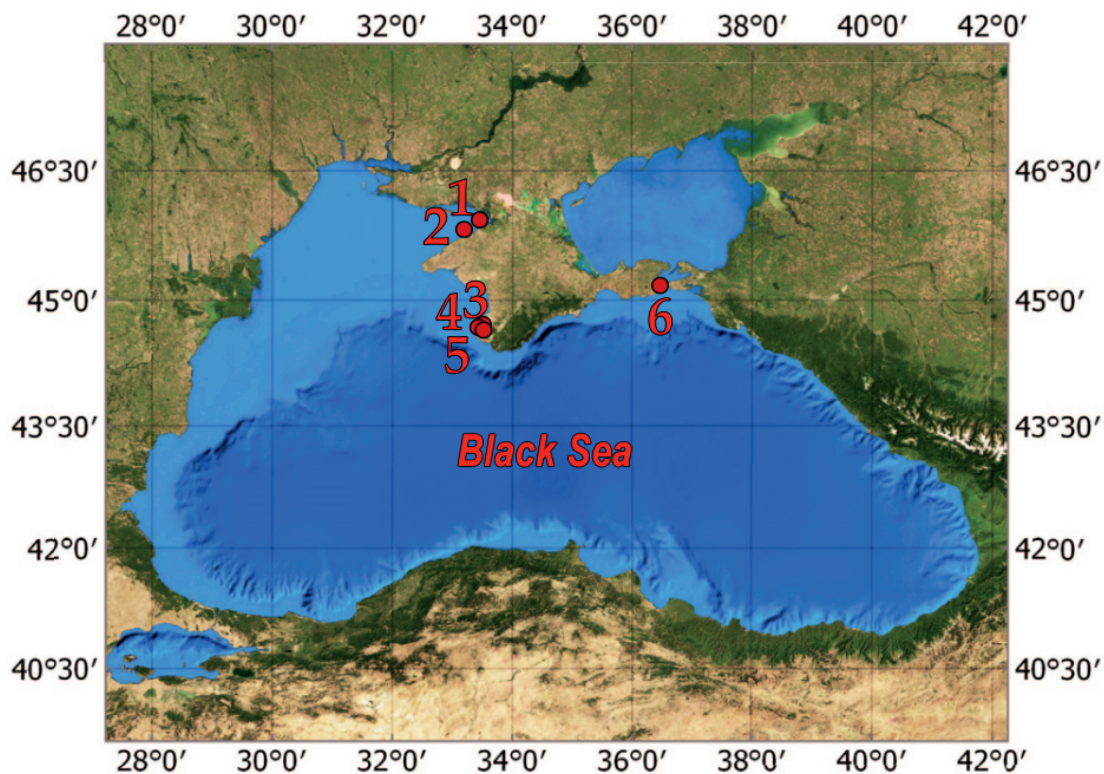


Fig. 1. Sampling sites: 1. Karkinit Gulf, The Crimean Nature Reserve, "Swan Islands" branch (Sari-Bulat Bay); 2. Karkinit Gulf, Steregucshee; 3-5. Sevastopol (3 – river Chernaya estuary, 4 – Streletskaya Bay, 5 – Kazachya Bay, aquatoria of State Zoological Reserve "Kazachya Bay"); 6. Kerch Strait, Naberezhnoe (MapQuest Open Aerial)

### Fish Material and Sample collection

We made parasitological dissections of 357 adult fishes: 152 specimens of *S. abaster*, 110 *S. typhle* and 95 *H. hippocampus*. Fish hosts were identified to species using conventional keys (Svetovidov, 1964; Vasil'eva, 2007).

All helminths were acquired from fresh fish. For light microscopy, digeneans and cestodes had been stored in 70 % ethanol were prepared as whole mounts as follows: stained with acetocarmine differentiated in tap water, destained in 70 % acid ethanol, dehydrated in a graded series (70 – 100°) of ethanol, cleared in clove oil and mounted in Canada balsam under a coverslip on glass slides (Bykhovskaya-Pavlovskaya, 1985). Nematodes and acanthocephalans were studied after clearing in a mixture of glycerin and lactic acid (Bykhovskaya-Pavlovskaya, 1985). Species identification was taken using an Olympus CX-41 microscope with digital camera CAM-SC50 and CellSens Standard v. 1.18 software. All total mounts are deposited in the Collection of Marine Parasites of the A.O. Kovalevsky Institute of Biology of the Southern Seas RAS, Sevastopol; data are available at <http://marineparasites.org> and were reinvestigated in the frames of this study.

All our samples were collected during warm hydrological season of the year (May – October, the marine water temperature above 16°C), this let us summarize data collected in different years but in one and the same region.

Some ecological indexes were calculated: prevalence (the percentage of infected fish hosts in a sample); intensity (the mean number of parasites per infected fish host) and abundance (the mean number of infected for all hosts (Bush *et al.*, 1997); appropriate data for the helminths listed in the present study are given in the Table 1.

### Ethical Approval and/or Informed Consent

Formal consent is not required for this study.

### Results

Fifteen helminth species were found from the studied pipefishes and seahorses inhabiting the biocenoses of the Black Sea shelf of the Crimea, they were six Trematoda, three Cestoda, four Nematoda and two Acanthocephala (Table 1). All the parasites found are heteroxenous (with multiple-host life-cycle) utilizing the fish hosts as definitive, intermediate or paratenic hosts.

Trematoda species recorded during the course of the study were two species of Cryptogonimidae Ward, 1917 family, namely *Timoniella imbutiformis* (Molin, 1859) Brooks, 1980 – *maritae* and metacercaria (in fish gills, muscles, serosa) and *Aphallus tubarium* (Rudolphi, 1819) Poche, 1926 *maritae*, three Heterophyidae Leiper, 1909 – *Galactosomum lacteum* (Jägerskiöld, 1896) Looss, 1899 metacercaria in fish gill muscles, *Pygidiopsis genata* Looss, 1907 metacercaria in fish hosts gut walls and *Cryptocotyle concava* (Creplin, 1825) Lühe, 1899 metacercaria in gills, serosa and

fish gut wall as well as Opecoelidae gen. sp. metacercaria in gills, serosa and gut walls of fish.

The most diverse digenean fauna among all studied regions was registered in Karkinit Bay (five species).

The fauna of Syngnathidae cestodes near Crimean Black Sea coasts revealed to be very poor, it is represented by larvae of three species from three orders: *Bothriocephalus gregarius* Renaud Gabrion & Pasteur, 1983 nomen nudum (Bothriocephalidea Kuchta, Scholz, Brabec & Bray, 2008), *Progrillotia dasyatidis* Beveridge, Neifar & Euzet, 2004 (Trypanorhyncha Diesing, 1863) and *Scolex pleuronectis* Müller, 1788 ("Tetraphyllidea" Van Beneden, 1850 relics) species complex (Table 1). Of the three fish species examined, the larvae of all these cestodes were found only in *S. abaster* in the estuary of the Chernaya River. Infectiveness of *S. abaster* with *B. gregarius* plerocercoids is rather high (Table 1) but this parasite species was found only in 4 % of the examined fish. *P. dasyatidis* plerocercoids was found once and only in one specimen of *S. abaster*, this implies an accidental parasitization of this host.

Four nematodes species ending their development in waterfowl, marine and brackish water fish were found in Syngnathidae studied, they belong to three families: Anisakidae Skrjabin & Karokhin, 1945, Raphidascarididae Hartwich, 1954, and Acuariidae Railliet, Henry & Sisoff, 1912 (Table 1). Indexes of invasion of needlefish by nematode larvae were extremely low (Table 1) that means minor significance of Syngnathidae in the parasite systems of these helminths in the Black Sea.

All larvae of nematodes of the genus *Contraecum* found in needlefish were at the third-stage larva of development; larvae of *C. rudolphii* (*spiculigerum*) Hartwich, 1964 were encapsulated in the intestinal walls and on the mesentery of host fish, and *C. multipapillatum* (Drasche, 1882) Lucker, 1941 larvae were localized in the bile ducts of fish.

Only one representative of the Nematoda Raphidascarididae family, *H. aduncum*, was found. In the studied Syngnathidae fish, most of the larvae were at third-stage of development and were located on the mesentery in the body cavity of the fish; once, an immature female was found in the intestinal lumen of *S. abaster* (Table 1).

Acanthocephalans in gut of Syngnathidae studied were represented by two species, *Acanthocephaloides propinquus* (Dujardin, 1845) Meyer, 1933 and *Telosentis exiguus* (von Linstow, 1901) (Table 1). All the found worms (*A. propinquus*, a male and *T. exiguus*, a male and a female) were immature.

As some of the fishes studied were caught in marine protected areas, we for the first time list the parasites found there as a part of their fauna check-lists:

#### The Crimean Nature Reserve, "Swan Islands" branch:

Trematoda: *Timoniella imbutiformis* met., *maritae*; *Aphallus tubarium* *maritae*; *Galactosomum lacteum* met.; *Cryptocotyle concava* met.; Opecoelidae gen. sp. met.

Nematoda: *Contraecum rudolphii* (*spiculigerum*) larvae; *Contra-*

Table 1. Modern data on species composition of Syngnathidae helminths off the Black Sea shelf of Crimea, with infection indices.

Fish species	FishNo	Location	Parasite species	II*	PI†	AI‡
<i>Syngnathus abaster</i>	91	Sevastopol: river Chernaya estuary	" <i>Scolex pleuronectis</i> " l. <sup>1</sup> <i>Bothriocephalus gregarius</i> l. <i>Progrillotia dasyatis</i> l. <i>Acanthocephaloides propinquus</i> * <i>Contracaecum rudolphii</i> l. <i>Hysterothylacium aduncum</i> l. III <i>H. aduncum</i> l. IV <i>Timoniella imbutiformis</i> met. <i>Cryptocotyle concava</i> met.	1-4/2.5 ± 1.5 2-18/8 ± 4 1 1 1 3 1 2 2	2.3 5 1 1 1 3 1 1.1 2.2	0.06 ± 0.05 0.3 ± 0.2 0.01 ± 0.01 0.01 ± 0.01 0.01 ± 0.01 0.03 ± 0.03 0.01 ± 0.01 0.02 ± 0.02 0.04 ± 0.03
	16	Sevastopol:	<i>Telosentis exiguus</i>	1	13	0.13 ± 0.09
	22	Streletskaia Bay	<i>T. imbutiformis</i> met.	1	4.6	0.05 ± 0.05
	20	Sevastopol: Kazachya Bay**	<i>Cryptocotyle concava</i> met. <i>T. imbutiformis</i> met. <i>Pygidopsis genata</i> met.	1 2	5 10	0.05 ± 0.05 0.2 ± 0.14
	18	Sevastopol: river Chernaya estuary	<i>H. aduncum</i> l. III <i>T. imbutiformis</i>	1-15/5.7 ± 5 2-24/13 ± 11	17 11.1	0.9 ± 0.8 1.89 ± 1.35
	12	Kerch Strait, Naberezhnoe	<i>C. rudolphii</i> l.	1	8.3	0.08 ± 0.08
	11	Sevastopol: Streletskaia Bay	<i>T. imbutiformis</i>	1-72/19.44 ± 8.1	81.8	15.91 ± 6.93
	1	Sevastopol: Streletskaia Bay	<i>Aphallus tubarium</i> <i>T. imbutiformis</i>	4 of 1 <sup>2</sup> 7 of 1	- -	- -
	15	Karkinit Gulf: Steregucshee	" <i>S. pleuronectis</i> " l. <i>Contracaecum multipapillatum</i> l. <i>C. rudolphii</i> l. <i>A. tubarium</i> <i>C. concava</i> met. <i>T. imbutiformis</i>	1 1-2/1.5 ± 0.5 1-11/3 ± 0.9 1-2/1.5 ± 0.5 4-18/9 ± 4.5 1-27/12.8 ± 2.7	7 13 67 9.1 13.6 68.2	0.07 ± 0.07 0.2 ± 0.1 2.1 ± 0.7 0.13 ± 0.1 1.2 ± 0.8 8.7 ± 2.3
	22	Karkinit Gulf: Steregucshee	<i>C. rudolphii</i> l. <i>C. multipapillatum</i> l. <i>H. aduncum</i> l. III <i>Cosmocephalus obvelatus</i> <i>T. imbutiformis</i> <i>T. imbutiformis</i> met. <i>A. tubarium</i>	1-23/5.4 ± 2.6 1-5/2.3 ± 1.3 1 2 2-27/19.2 ± 5.1 3-6/4.5 ± 1.5 1-2/1.5 ± 0.5	38 14.3 5 5 71.4 28.6 28.6	2.1 ± 1.1 0.3 ± 0.2 0.05 ± 0.05 0.1 ± 0.1 13.7 ± 5.0 1.3 ± 0.9 0.4 ± 0.3

29	Kerch Strait, Naberezhnoe	<i>H. aduncum</i> l. III <i>T. exiguus</i> <i>T. imbutiformis</i> met.	1-2/1.5 ± 0.5 2 1-8/3.3 ± 2.3	7 3.4 8.8	0.1 ± 0.08 0.1 ± 0.07 0.3 ± 0.2
37	Karkinit Gulf, Sari-Bulat Bay*	<i>C. rudolphii</i> l. <i>C. multipapillatum</i> l. <i>T. exiguus</i> <i>T. imbutiformis</i> met. <i>A. tubarium</i>	1-2/1.5 ± 0.5 1 1 2-78/31.9 ± 11.9 1/1	5 3 3 23.2 2.9	0.1 ± 0.06 0.03 ± 0.03 0.03 ± 0.03 7.7 ± 3.6 0.03 ± 0.03
34		<i>Galactosomum lacteum</i> met. <i>C. concava</i> mtc. Opocoeilidae gen. sp. met.	2-6/3.3 ± 1.3 1-9/4.3 ± 1.8 1-6/2.5 ± 1.2	0.9 11.8 11.8	0.3 ± 0.2 0.5 ± 0.3 0.3 ± 0.2

II\* – intensity of infection, worms per host, min–max / mean±SE; PI† – prevalence, %; AI‡ – abundance, worms per host, mean±SE; 1 – larvae; 2 – if less than 15 fishes were dissected, then the number of fish infected vice total number of fish studied is given; \* – aquatoria of Crimean Nature Reserve, "Swan Islands" branch; \*\* – aquatoria of State Zoological Reserve "Kazachya Bay".

*caecum multipapillatum* larvae; *Hysterothylacium aduncum* larvae III; *Cosmocephalus obvelatus* larvae III.  
Acanthocephala: *Telosentis exiguus*.

#### The State Zoological Reserve "Kazachya Bay":

Trematoda: *Timoniella imbutiformis* met.; *Pygidiopsis genata* met.

### Discussion

The pipefishes and a seahorse studied are Atlantic-Mediterranean species broadly distributed along Black Sea coasts and inhabiting seaweeds at depth up to about 20 m (Vasil'eva, 2007). In the Black sea they are known to feed with small-sized prey: fish fries and small-sized adult fishes and crustaceans (Svetovidov, 1964; Vasil'eva, 2007; Gürkan & Uncumusaoglu, 2012); on the other hand, they themselves are known in the Black Sea as food objects of rays *Raja clavata* Linnaeus, 1758 (Saglam & Bascinar, 2008), dolphins *Delphinus delphis ponticus* Barabasch-Nikiforov, 1935 (Svetovidov, 1964), turbot *Scophthalmus maeoticus* (Pallas, 1814) fry – under experimental conditions (Shishkina *et al.*, 2007), sea gulls *Larus argentatus* Pontoppidan, 1763 (Sten'ko, 1983) that determine complex ways of helminth circulations in Black Sea coastal zone due to presence of pipefishes and seahorses.

Trematoda are the most constant and most numerous (meaning species number) part of the Syngnathidae helminth community (40 % of the total number of registered helminth species). They are represented mainly by larval stages (Table 1) of which three species finish development in birds and two are in fish. All species of trematodes, the metacercariae of which are found in needlefish, are broadly specific at this developmental stage to the Black Sea bottom pelagic fishes (generalists).

*T. imbutiforme* was found from Syngnathidae hosts in all localities, contributed substantially to the observed trematode community's composition studied and is considered to be the most common ("core") digenean species for the Black Sea *S. typhle*: *maritae* infect more than 50 % of the broadnosed pipefishes studied; at metacercarial stage this species play the same role for *H. hippocampus* as they were found in more than 15 % of long-snouted seahorses.

Other trematodes found in the studied Syngnathidae were recorded sporadically and at low rates of invasion. Most all of them are generalists, marine and brackish species (excepting marine species, *A. tubarium*, which is specific to Syngnathidae only at marita stage).

Black Sea seahorses, black-striped and broadnosed pipefishes revealed to be intermediate hosts of trematodes *C. concava* and *P. genata* (Heterophyidae) known as broad specific ones to bottom-dwelling and pelagic Black Sea fishes (generalists) (Gaevskaia & Korniychuk, 2003). The life cycle of these trematodes is complex and the adult parasites are located in the piscivorous bird's intestine; of *P. genata* and *C. concava* are known from the area of Karkinit Bay in several bird species and are numerous:

for example, in *Larus argentatus* abundance of *P. genata* was 47 and *C. concava* 331 ind. per host (Sten'ko, 1983) indicating that these helminths are core species in the local parasite fauna. The cercaria of these trematodes penetrate the skin of a fish, encyst there becoming metacercaria and marking parasitized individuals with visible to the naked eye black dots (produced by host melanocytes migrating through the cysts' fibrous wall) and cause so called "black spot disease" (BSD) (Stunkard, 1930; Bush *et al.*, 2001). Heavy infections by metacercaria can reduce fish host physical condition and survival (Sindermann, 1990).

The cestodes *B. gregarius* is a nomen nudum (Kuchta & Scholz, 2017) and before the publication of its description from the type host from the native range, we use this name accompanying it with this mark. *B. gregarius* is a typical marine species but it can be transferred into estuarine biocenoses by their hosts; earlier, larvae of this species were registered from *S. abaster* in the Lake Donuzlav (Miroshnichenko, 2008), the salinity of which is equal to that of the Black Sea (18 – 19 ‰) (Ivanyutin, 2019). We found *B. gregarius* plerocercoids without blastocyst both as at the Black Sea typical salinity (at the mouth of the Chernaya River) as in fresh waters (upstream of the Chernaya River).

According to (Solonchenko, 1982), the only second intermediate hosts of *B. gregarius* in the Black Sea are Gobiidae. Probably, the black-striped pipefish is also the second intermediate host for this cestode species as the cestode larvae we found in pipefish were at the plerocercoid stage without blastocyst, had a well-formed scolex with slit-like bothria, and the infection of *S. abaster* was high (Table 1). In addition, planktonic crustaceans, in particular, *Acartia (Acartiura) clausi* Giesbrecht, 1889 (the first intermediate host of *B. gregarius*) are included in the spectrum of food items for both Gobiidae and Syngnathidae (Svetovidov, 1964; Solonchenko, 1982; Vasil'eva, 2007). And, finally, the final host for *B. gregarius*, the kalkan flounder *Scophthalmus maeoticus* (Pallas, 1814), feeds on both Gobiidae (Svetovidov, 1964; Vasil'eva, 2007) and Syngnathidae (Shishkina *et al.*, 2007). Consequently, the infection of the flounder can occur both through the gobies that dominate in its diet, and through the pipefishes. As *S. abaster* can feed on larvae and juvenile fish, including Gobiidae (Didenko *et al.*, 2018), infection of black-striped pipefish with *B. gregarius* plerocercoids can occur not only through crustaceans, but also through infected small gobies.

The *P. dasyatidis* larvae we found in the Black Sea *S. abaster* were at the plerocercoid stage without blastocyst. The life cycle of this cestode species is not known; we recorded earlier it's plerocercoids in 8 species of Black Sea teleost fishes from other families (second intermediate hosts), with much higher indexes of invasion (Polyakova *et al.*, 2017; Polyakova, 2020). The very low infection rates of black-striped pipefish with *P. dasyatidis* larvae implies that *S. abaster* is an occasional host for these cestodes. There are no data on the feeding of *Dasyatis pastinaca* (the final host of this cestode species) by pipefishes and seahorses; however, *Hippocampus* sp. and *Syngnathus* sp. are known from the stomachs

of other Black Sea rays, *Raja clavata* Linnaeus, 1758, (Saglam & Bascinar, 2008). We hope, further detailed studies of Cestodes in Syngnathidae fish will help to reveal the role of these fish in the life cycle of *B. gregarius* and *P. dasyatidis* in the Black Sea.

Larvae of *Contracecum* Railliet & Henry, 1912 nematodes were previously recorded in the Black Sea from *S. typhle* and *S. abaster* and were identified as *C. microcephalum* (Chernyshenko, 1955, Gaevskaya *et al.*, 1975, Mange, 1993). Previously (Korniychuk *et al.*, 2016) we also mentioned *C. microcephalum* (Rudolphi, 1809) l. and *C. rudolphii* Hartwich, 1964 l. from both *S. typhle* and *H. guttulatus* on the basis of conventional morphological features (Gaevskaya *et al.*, 1975; Moravec, 1994). Nevertheless, now we reinvestigated the collected material and believe the larvae of nematodes of the genus *Contracecum* from the pipefish and the seahorse we previously mentioned as *C. microcephalum* and *C. rudolphii* (Korniychuk *et al.*, 2016), morphologically corresponding to the larvae of two another species of the genus, namely, *C. rudolphii* (*spiculigerum*) and *C. multipapillatum*; their presence in the Black Sea is confirmed on the basis of analysis of nucleotide sequences of partial LSU rDNA and ITS rDNA (Pronkina & Spiridonov, 2018, 2020). So, it is impossible now to say exactly what species these larvae mentioned by (Chernyshenko, 1955, Gaevskaya *et al.*, 1975, Mange, 1993) belong to, but we have no reasons to deny it wasn't *C. microcephalum*.

The range of definitive hosts of *Contracecum* spp. nematodes is quite wide; adults and LIV parasitize fish-eating birds and marine mammals. Piscivorous birds are the most significant (main) final hosts in the Azov-Black Sea basin; *C. rudolphii* is more common in cormorants *Phalacrocorax* Brisson, 1760 and *C. multipapillatum* – in pelicans *Pelicanus* Linnaeus, 1758 (Smogorzhevskaya, 1976; Korniyushin *et al.*, 2004). The first intermediate hosts of *C. rudolphii* were identified only in experiments (crustaceans) (Mozgovoy *et al.*, 1968; Moravec, 2009); the natural first intermediate hosts for both *C. rudolphii* and *C. multipapillatum* have not been found yet. *Contracecum* spp. third-stage larvae are known from marine, brackish and freshwater fish. We assume that the main way of realizing the life cycle of these nematodes is trixenous and fish in the Black Sea (including Syngnathidae) are obligatory intermediate hosts and definitive hosts (fish-eating birds) become infected while eating them.

Syngnathidae infection with nematodes *H. aduncum* third-stage larva occurs when fish eat infected planktonic crustaceans (first intermediate hosts). In this case, the nematodes penetrate the intestinal walls of the fish and encyst on the serous membrane of their internal organs, i.e. Syngnathidae are the second intermediate hosts of these nematodes. The find of an immature *H. aduncum* female in the intestinal lumen of *S. abaster* speaks in favor of the infection of this pipefish via infected pelagic planktivorous fish. Nematodes *C. obvelatus* belongs to fam. Acuariidae which finish their development in birds and are known as epizootics agents of domestic and wild ducks (Smogorzhevskaya, 1976; Korniyushin *et al.*, 2004). The larvae of these nematodes can enter the final

host, birds, in two ways: directly through amphipods and mysids (first intermediate hosts) or through fish (paratenic hosts) previously infected by nematodes larvae via amphipods and mysids (Moravec, 1994). Probably, both ways are possible in the case of pipefish infection.

Acanthocephala *A. propinquus* and *T. exiguus* are generalists, but *A. propinquus* mature and abundant mainly in Gobiidae (Kvach, 2006) and *T. exiguus* – in pelagic fish, mainly in Atherinidae (Belofastova & Grintsov, 2005). Judging by the extremely low rates of infection and as the worms we found were not sexually mature, pipefishes and seahorses are occasional or abortive hosts for the both species of acanthocephalans in the studied areas of the Black Sea. Nevertheless, immature *A. propinquus* could move via food nets: it is known that the final host of *A. propinquus*, the grass goby *Zosterisessor ophiocephalus* (Pallas, 1814), becomes infected not only when eating isopods *Idotea balthica* (Pallas, 1772) (the first intermediate host), but also due to cannibalism and predation on other fish, including Syngnathidae (Smirnov, 1986).

As mainly larval helminthes persist in Syngnathidae fishes, we believe no reason to expect significant seasonal fluctuations in the composition of syngnathids helminths fauna.

As pipefishes and seahorses are fishes with low mobility and small home ranges, marine reserves are thought to be effective for conservation purposes. Conservation statuses for *H. hippocampus* is “no available information”, “vulnerable” for *S. abaster*, “least concern” for *S. typhle* (Yankova *et al.*, 2014). In the region studied, the pipefishes and the seahorse occur in at least two marine protected areas, namely Crimean Nature Reserve (“Swan Islands” branch in Karkinit Gulf) and State Zoological Reserve “Kazachya Bay” in aquatoria of Sevastopol city. Moreover, all the studied Syngnathidae species are abundant there and are the basis part of the faunistic diversity in the western part of Karkinit Gulf (Belogurova *et al.*, 2020).

List of major threats protected marine fishes are affected in the Black Sea (Yankova *et al.*, 2014) doesn't include fish diseases and helminthoses. Nevertheless, it was shown (Rosenqvist & Johansson 1995; Mazzi 2004) that *Cryptocotyle*-BSD affect *S. typhle* female fecundity (unparasitized females provide fertilizing more eggs) and sexual attractivity (males preferentially mate with fish females with lower *Cryptocotyle* cysts loads). Some researchers associate BSD also with growth retardation, body weight loss and higher mortality of young infected fish, which could regulate the host population density (Lemly & Esch, 1985). Anisakidae larvae (for example, *C. rudolphii*) are also known to cause lesions of fish host pancreas due to mechanical compression and displacement of liver in the case of high infection (destruction of the parenchyma, disorders of blood microcirculation, fatty degeneration of hepatocytes) and inflammation of the bile ducts (Hauck & May, 1977; Popuk, 2017). So, some helminths we found from Syngnathids in the frames of this study are potentially important as threats in marine protected aquatoria being potential agents of pipefishes and seahorses' diseases.

## Conflicts of Interest

Authors state no conflict of interest.

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