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Finding of Parasites *Kudoa nova* and *Kudoa niluferi* (Myxosporea: Kudoidae) in the Muscles of Alien Gobies *Tridentiger trigonocephalus* and *Gobius cruentatus* (Actinopterygii: Gobiidae) in the Black Sea

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Abstract—The data on parasitizing of two species of myxosporeans of the genus *Kudoa* in the muscles of two species of alien gobies in the Black Sea off the coast of Sevastopol are presented. One isolate was found in the chameleon goby *Tridentiger trigonocephalus*, and the other one was found in the red-mouthed goby *Gobius cruentatus*. It is supposed that the source of infection for the alien fish were native gobies, in which the above species of parasites were previously known. A decrease in the size of spores and polar capsules as well as a slight deformation of the spore shape of *K. niluferi* were observed, which may be associated with parasitizing in an unusual host and in another part of the range. Molecular genetic identification of myxosporeans from two species of gobies confirms that the studied isolates belong to the genus *Kudoa*. Two 18S rDNA sequences of these isolates turned out to be very similar, but not identical to each other (differences of 4.87%), and also similar to two known species of goby myxosporeans from the Black Sea, *K. nova* and *K. niluferi*. The results obtained are the first evidence of parasitizing of both species of gobies by Myxosporea representatives in the area of invasion and also compensate for the absence of information on genomic variability of parasites of chameleon and red-mouthed gobies in the native range.

Keywords: *Kudoa nova*, *Kudoa niluferi*, *Tridentiger trigonocephalus*, *Gobius cruentatus*, parasites, fish, 18S rDNA, Black Sea

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INTRODUCTION

The active expansion of the chameleon goby *Tridentiger trigonocephalus* Gill, 1859 beyond its natural range is assumed to have started in the mid-1980s as a result of unintentional introduction with the transport of oysters (Courtenay et al., 1986) and via ballast waters (Eschmeyer et al., 1983), since this species often inhabits mussel and oyster farms. It is also known that breeding fish in aquariums was another vector of dispersal, since these gobies are unpretentious to the conditions of detention and have a bright natural color. Thus, one of the employees of the Sevastopol Marine Aquarium Museum admitted, that at the beginning of the 1980s, he released several dozen individuals of this fish species, brought by him from the Posyeta Bay (Sea of Japan), into the Sevastopol Bay near the building of the Institute of Biology of the Southern Seas, Russian Academy of Sciences (Boltachev and Karpova, 2017). This species was first recorded in the Black Sea fish fauna in the estuarine zone of the Chernaya River (Sevastopol) in 2006. The native range of the striped tripletooth goby is confined to the coastal and estuarine zones of the Sea of Japan, Yellow Sea, and South China Sea (Pacific Ocean basin, its western part). It is found in waters near South and East China and off the coast of Japan and the Philippines, as well as in Russia in the Khromi Bay in the Amur Liman, in Novgorod Bay, in the Posyeta Bay, and in the mouths and lower reaches of rivers flowing into the Peter the Great Gulf and Amur Bay (Parin et al., 2014). In view of the mass dispersal of representatives of the genus *Tridentiger* all over the world, we conducted an identification of the species caught in the Sevastopol region. The analysis of the

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mtDNA 16S rRNA gene confirmed that it precisely belongs to this species, *Tridentiger trigonocephalus* (Slynko et al., 2020). According to the data of ichthyologists, in the Black Sea, the chameleon goby reaches a maximum length of 9.8 cm with a weight of 7.2 g. This is a small fish that has no nutritional value and does not form large aggregations (Boltachev and Karpova, 2017).

The vector of invasion of the red-mouthed goby *Gobius cruentatus* Gmelin, 1789 into the Black Sea is most likely an accidental introduction via the ballast waters of ships or on the valves of transported mollusks that are objects of marine aquaculture. This fish species was first recorded in the Martynov Bay of Sevastopol in 2002 and later was found in different areas of the Sevastopol coastal waters. The species was also found in the Black Sea waters of Turkey. A steady trend of increasing abundance and distribution of the red-mouthed goby off the coast of southwestern Crimea to the Laspi Bay, as well as along the coast of the North Caucasus, was observed (Boltachev and Karpova, 2017). The native range of this purely marine species of gobies is the eastern Atlantic (from southwestern Ireland in the north to Senegal in the south) and the Mediterranean Sea (Parin et al., 2014). Owing to its rather large size (it reaches a length of 18 cm), the red-mouthed goby has nutritional value and is an object for sport fishermen; however, it does not form commercial aggregations and according to observations of underwater hunters is found only as solitary specimens in the Black Sea.

Analysis of published and Internet sources showed a complete lack of data on the parasitism of myxosporeans and parasites of other groups in the chameleon goby *T. trigonocephalus* in the native range. The parasitofauna of the red-mouthed goby is better known. There are several works on helminths *G. cruentatus* in natural habitats. The recent works, for example, indicate the presence of the trematode *Brachyphallus musculus* and nematode *Hysterothylacium aduncum* in a red-mouthed goby from the Adriatic Sea (Split, Croatia) (Walker et al., 1974) and the detection of metacercariae of the trematode *Galactosomum lacteum* on the optic nerve, brain, muscles, and connective tissues of the pharynx and esophagus of *G. cruentatus* in the southwestern part of the Mediterranean Sea (Culurgioni et al., 2006) and in the Gulf of Cagliari (southern Sardinia, Italy) (Culurgioni et al., 2007). Only six species of helminths have been found in the area of invasion, in the Black Sea (four species of trematodes, metacercaria *Metadena* sp. and *G. lacteum*, adult forms of *Magnibursatus skrjabini* and *Phyllodistomum* sp., as well as one species of nematodes, *H. aduncum*, and one species of cestodes, *Scolex pleuronectis*), in a red-mouthed goby off the coast of Sinop (Turkey) (Güven and Öztürk, 2017). In this regard, information about the parasitofauna of the above-mentioned gobies in the areas of invasion and the first finding of myxosporeans in these fish species, the source of which was probably native gobies, is of great interest.

MATERIALS AND METHODS

The parasitological part of the work is based on our own materials on the myxosporeans of two species of gobies that have penetrated into the Black Sea and inhabit the Sevastopol region. In 2010–2013, we studied eight specimens of the chameleon goby *Tridentiger trigonocephalus* caught with a drag net and a fishing rod in the mouth of the Chernaya River. In 2016–2019, eight specimens of the red-mouthed goby *Gobius cruentatus* taken with nets and an underwater gun were studied in the bay near the radiobiological building (RBB) of the Institute of Biology of the Southern Seas, Russian Academy of Sciences, and in Karantinnay Bay, as well as in the area of Cape Fiolent (Avtobat). The material was obtained by the method of incomplete parasitological dissections (Bykhovskaya-Pavlovskaya, 1985). The pieces of muscles compressed by the compression method were examined under a binocular microscope at ×15–25 magnification for the detection of pseudocysts of myxosporeans. Owing to the absence of the latter, blind smears obtained from muscle tissue were examined under an Olympus CX41 microscope equipped with a CX50 camera with Infinity Analyze software and phase contrast, at ×1000 magnification. Further, the smears were processed according to the generally accepted method using permanent preparations (Donets and Shulman, 1973). Measurements of parasites were based on 20 spores of *Kudoa nova* Naidenova, 1975 and 8 spores of *K. niluferi* Özer, Okkay, Gürkanlı, Çiftçi & Yurakhno, 2018 when examining gelatin-glycerin preparations. All measurements were made according to the common method (Lom and Dykova, 1992). The abundance of parasites was estimated using the standard parameters: prevalence and intensity of infection (Bush et al., 1997).

The study of the polymorphism of the 18S rRNA gene was based on the study of spores from the muscles of infected specimens of the red-mouthed goby *G. cruentatus* and the chameleon goby *Tridentiger trigonocephalus*. The collected material was fixed in 96% ethanol. The total DNA was extracted using the innuPREP DNA Mini Kit (Analytik Jena, Germany). As amplification mixtures, ready-to-use lyophilized reaction mixtures without primers (master mixes) were used, intended for DNA amplification in the volume of 20 μL (LLC Genlab Scientific-Production Company, Moscow). Amplification of 18S rRNA fragment with a length of 1000–1200 bp was conducted using the combination of the following primers: Myxgen4F (5'-GTGCCTTGAATAAATCAGAG-3') (Diamant et al., 2004) and 18R (5'-CTACGGAAACCTTGTTACG-3') (Whipps et al., 2003) or Kud6F (5'-TCACTATCGGAATGAACG-3') and 18R (5'-CTACGGAAACCTTGTTACG-3') (Whipps et al., 2003). A phylogenetic tree was constructed using the maximum

Table 1. Size of spores (μm) of *Kudoa nova* from different host gobies in the Black Sea

Size of spores and polar capsules of the parasite	*Tridentiger trigonocephalus* (present study)	11 species of the family Gobiidae in the Black Sea and Sea Azov (Naidenova et al., 1975)	*Neogobius melanostomus* in the Black Sea (Yurakhno, 1994)
Spore length	3.8	4.0–5.0	3.1
Spore thickness	5.39 ± 0.21 (5.10–5.85)	–	4.0–6.3
Spore width	6.62 ± 0.25 (6.32–7.30)	4.0–5.0	5.0–6.3
Length of polar capsules	2.02 ± 0.04 (1.94–2.15)	1.5–1.7	2.0–2.9
Width of polar capsules	1.12 ± 0.03 (1.05–1.19)	1.2	1.5–2.0

likelihood (ML) method and the GTR + G + I model with the calculation of bootstrap support for branch nodes (1000 replications) MEGA 6.0 software package (Tamura et al., 2013). For comparison, 18S rDNA sequences were used for 13 known species of myxosporeans of the genus *Kudoa*.

RESULTS AND DISCUSSION

In July 2012, *Kudoa nova* Naidenova, 1975 was found in the mouth of the Chernaya River in the chameleon goby *Tridentiger trigonocephalus*, whose native range is the Pacific seas, the Sea of Japan, Yellow Sea, and South China Sea. The average prevalence of this parasite was 13%. It was detected in 1 out of 8 fish specimens studied in 2010–2013 with the intensity of invasion of thousands of spores in a smear. The total length (TL) of the studied chameleon gobies was 5.0–8.0 cm; the length of the infected female had the maximum size for the sample.

K. nova was previously known in 26 species of hosts, in 15 native fish species (family Gobiidae) in the Azov-Black Sea basin and in 10 fish species of other families (Sparidae, Scombridae, Carangidae, Pomatomidae) in the Mediterranean Sea and the Atlantic Ocean, and in the native range of the chameleon goby, *K. nova* was detected in the snowy sculpin *Myoxocephalus brandtii* (Steindachner, 1867) (family Cottidae) from the Sea of Japan (Naidenova et al., 1975; Aseeva, 2005; Yurakhno and Gorchanok, 2011; etc.).

We suggest that the source of infection of alien *T. trigonocephalus* with *Kudoa nova* was native gobies, among which the latter has been living for several decades and which are also infected with this parasite. According to our data, the Black Sea species of gobies caught at the same time and in the same area, in the mouth of the Chernaya River, were rather highly infected with *Kudoa nova*. This parasite was detected along with the chameleon goby in the muscles of 1 out of 3 studied specimens of the black goby *Gobius niger*, 5–17% of the grass goby *Zosterisessor ophiocephalus*, 17–27% of the mushroom goby *Ponticola eurycephalus*, 25–73% of the round goby *Neogobius melanostomus*, and 92% of the tubenose goby *Proterorhinus marmoratus* (Yurakhno, 2014, 2015). It was earlier established that the chameleon goby was a new host for *K. nova* (Yurakhno, 2014). *Kudoa nova* did not form pseudocysts in the muscles of *T. trigonocephalus*, but was found in the form of "diffuse infiltration," which is quite common for the pattern of infection of Black Sea gobies with this parasite species. The size of the parasite spores varied within the limits known for specimens from the Azov-Black Sea species of gobies, with a slightly smaller width of the polar capsules; the shape of the spores corresponded to the original description (Naidenova et al., 1975) (Table 1).

In July 2018, *Kudoa niluferi* Özer, Okkay, Gürkanlı, Çiftçi & Yurakhno, 2018, the native range of which is the eastern Atlantic and the Mediterranean seas, was found in the red-mouthed goby *Gobius cruentatus* Gmelin, 1789 in the bay near the RBB (radiobiological building of the Institute of Biology of the Southern Seas, Russian Academy of Sciences). The average prevalence of this parasite was 13%. *K. niluferi* was detected in 1 out of 8 fish specimens caught in 2016–2019 with the intensity of invasion of a few spores in a smear. The total length of the studied red-mouthed gobies was 13.0–16.7 cm; the length of the infected male was 15.5 cm.

Previously, this Black Sea parasite was found in the round goby *Neogobius melanostomus* off the coast of Turkey (Özer et al., 2018). *K. niluferi* for the first time was recorded in the fauna of the Crimea and Russia as a whole. The red-mouthed goby was a new host for this parasite.

Kudoa niluferi did not form pseudocysts in the muscles of *G. cruentatus*, but was found in the form of "diffuse infiltration," which also took place when describing this myxosporean species from the Black Sea goby off the coast of Sinop (Turkey). The spores in

Table 2. Size of spores (μm) of *Kudoa niluferi* from different species of host gobies in the Black Sea

Size of spores and polar capsules of the parasite	*Gobius cruentatus* (present study)	*Neogobius melanostomus* (Özer et al., 2018)
Spore length	3.89	5.9 ± 0.1 (5.7–6.1)
Spore thickness	4.39 ± 0.29 (3.92–4.79)	7.5 ± 0.3 (7.0–8.1)
Spore width	5.98 ± 0.45 (5.48–6.85)	9.2 ± 0.2 (8.8–9.5)
Length of polar capsules:		
larger	2.03 ± 0.08 (1.89–2.14)	2.7 ± 0.1 (2.6–2.9)
medium	1.72 ± 0.11 (1.53–1.88)	2.6 ± 0.1 (2.4–2.8)
smaller	1.38 ± 0.18 (1.04–1.58)	2.3 ± 0.1 (2.2–2.4)
Width of polar capsules:		
larger	1.26 ± 0.05 (1.2–1.31)	2.3 ± 0.1 (2.1–2.6)
medium	1.17 ± 0.02 (1.15–1.19)	2.2 ± 0.1 (2.0–2.3)
smaller	0.94 ± 0.04 (0.9–0.98)	2.1 ± 0.1 (1.9–2.2)

our study had a slightly modified shape with slightly "crumpled" edges of the valves and much smaller sizes (Table 2), which may indicate parasitism in an unusual host, the parasite-host relationship with which has emerged relatively recently.

The results of the molecular genetic identification of myxosporeans from samples of two species of gobies confirm that the studied isolates belong to the genus *Kudoa*. Relatively short nucleotide sequences (~900 bp) of two samples of KR (NCBI MK541039) and 3M (NCBI MK541040) were quite similar, but not identical to each other (differences of 4.87%), and also similar to two known myxosporean species of gobies from the Black Sea, *K. nova* and *K. niluferi* (Fig. 1). Apparently, for a more accurate species diagnosis of the studied samples, it is necessary in the future not only to increase the size of the studied sample of myxosporeans but also to continue molecular typing of more extended rDNA regions.

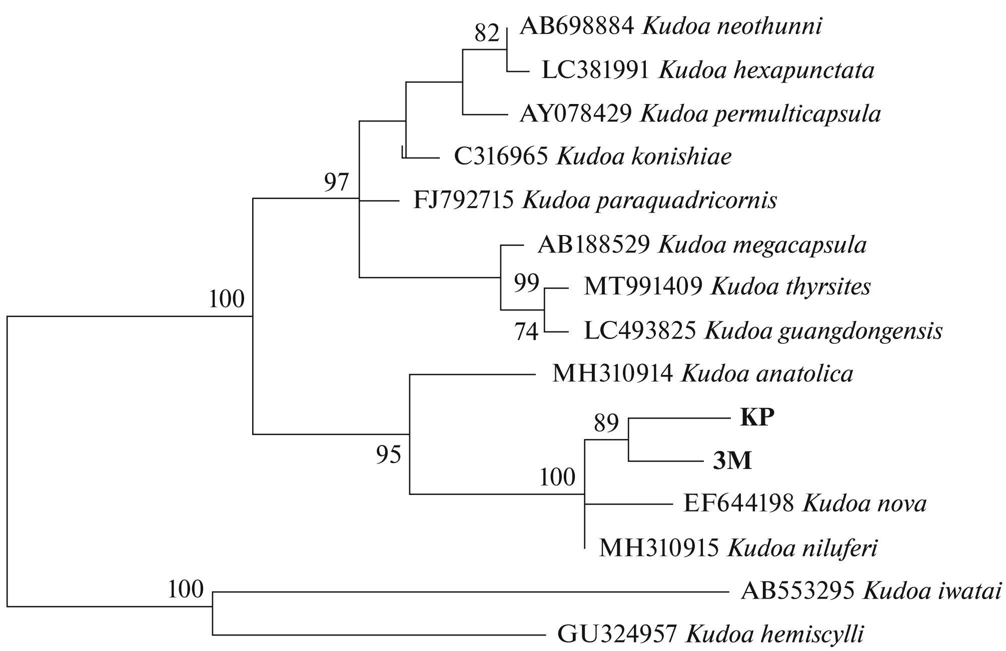

Fig. 1. The phylogenetic tree (maximum likelihood, GTR + G + I model, AIC = 3945.459) constructed on the basis of polymorphism of the 18S rRNA gene (863 bp) in 13 known species and two studied isolates (KP from the red-mouthed goby, 3M from the chameleon goby) of myxosporeans. Bootstrap support indices exceeding 70% are indicated in the branch nodes. The unit of measurement of branch lengths is indicated at the bottom of the figure, 0.5% of nucleotide substitutions.

CONCLUSIONS

It is shown that a Pacific alien species, the chameleon goby *Tridentiger trigonocephalus*, may be the host of tissue mixosporeans of the genus *Kudoa*, namely, *K. nova*, in the Black Sea, and an Atlantic-Mediterranean alien species, the red-mouthed goby *Gobius cruentatus*, may be the host of *K. niluferi*. *Kudoa niluferi* was for the first time detected in the fauna of the Crimea and Russia, and the red-mouthed goby was a new host for this parasite. Preliminary molecular genetic comparisons of 18S rDNA polymorphism confirmed that the myxosporeans of two goby species belong to the genus *Kudoa*. The applied morphological and morphometric methods demonstrated a decrease in the size of spores and polar capsules, as well as a slight deformation of the shape of spores for the putative species *Kudoa niluferi*, which may be due to parasitism in an unusual host and in another region. It is suggested that native gobies in which the above species of parasites were recorded earlier were the source of infection for the alien fish. Absence of data on the occurrence of parasites in *T. trigonocephalus* in the native range and the first finding of myxosporeans in the areas of invasion of both species of gobies are noted.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care of animals were followed.

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