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FISH CESTODES OF THE KARADAG NATURE RESERVE AND ADJACENT WATER AREAS OF THE BLACK SEA

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The first data on marine fish parasites in Karadag nature reserve water area were published at the beginning of the 20th century. By the beginning of the 21st century, information on the fauna of cestodes in this area of the Black Sea included data on 19 species recorded in 24 fish species. However, taxonomy of this class of helminths has changed significantly over the last decade, and regional fauna needs to be revised. The aim of this work is to revise the species composition of fish cestodes in the water area of the Karadag nature reserve and adjacent areas on the basis of new data obtained and in accordance with current systematics of Cestoda. The material for this study was the collections of cestodes gathered by the staff of IBSS RAS Environmental Parasitology Department in the area of the Karadag nature reserve in different years, as well as our own collections of 1754 specimens of rays and teleosts of 53 species (2005–2018). The area studied is Black Sea coastal area from Meganom Cape to Ordzhonikidze village (southeastern part of Crimea), including various marine biotopes of the Karadag nature reserve. Voucher preparations of all types of cestodes used in this study were deposited in a subcollection of marine parasites of the World Ocean hydrobionts collection of IBSS RAS. Totally 20 cestode species were found in 17 fish species. Nine species, namely *Progrillotia dasyatidis*, *Parachristianella trygonis*, *Dollfusiella aculeata*, *Rhinebothrium walga*, *Caulobothrium* sp., *Rhabdotobothrium* sp., *Acanthobothrium* sp. 5, 7, and Anthocephaliidae gen. sp. 2., were reported for the common stingray *Dasyatis pastinaca* in the area under study for the first time. Cestodes belonging to the new species *Acanthobothrium* sp. 1, 2, 4 were found in the thornback ray *Raja clavata*. Of 19 species previously known in Karadag area, only 8 adult mature cestodes were recorded: "*Bothriocephalus scorpii*", "*B. gregarius*", *Echinobothrium typus*, *Grillotia erinaceus*, *Prochristianella papillifer*, *Echeneibothrium variabile*, *Cairaeanthus ruhnkei*, and *C. healyae*. In addition, larvae of the complex species "*Scolex pleuronectis*" were found in teleost fish. Larvae of cestode *Progrillotia dasyatidis* were found for the first time in the water area studied in 8 teleost fish species; this data contribute to the information on the participants in the life cycle of this helminth. Four cestode species, namely *Hepatoxylon trichiuri* larvae, *Nybelinia lingualis* larvae, *Tetrarhynchobothrium tenuicolle*, and *Anthobothrium cornucopia*, which were previously reported from this area, were not found in the present survey. Moreover, recent analysis of the occurrence and synonymy of species of orders Trypanorhyncha and Onchoproteocephalidea revealed that the previous identification of the cestodes in elasmobranchs as *Grillotia* (*Christianella*) *minuta* and *Acanthobothrium coronatum*, as well as identification of the cestodes in teleosts as *Tentacularia* sp. larvae, is incorrect due to the absence of their specific definitive hosts in the Black Sea. On the other hand, among representatives of *Acanthobothrium* spp. registered in *D. pastinaca* and *R. clavata* in Karadag water area, we found 7 morphologically different new taxa identified to the species level. The cestodes found belong to 6 orders: Bothriocephalidea, Diphyllidea, Trypanorhyncha, "Tetraphyllidea" relics, Rhinebothriidea, and Onchoproteocephalidea. The most species diversity of cestodes in both species of rays is registered among representatives of the orders Trypanorhyncha and Onchoproteocephalidea (5 species each), the least – in the orders

Diphyllidea and "Tetraphyllidea" relics (1 species each). Thus, 12 species were added to the fauna of the cestodes parasitizing fish in Karadag area, and 8 of them are obviously representatives of new taxa.

Keywords: cestodes, fish, fauna, systematics, reserve, Crimea, Black Sea

On the territory of Crimea there is a large range of protected natural objects – from natural monuments of local importance to national and international reserves. Fauna of fish parasites in the water area of the Karadag natural reserve began to be studied in the early 20th century, and today it is the most thoroughly studied among fauna of protected water areas of Crimea [5].

The first data on the fauna of fish cestodes of Karadag water area were published in 1931 [4]. In this work, 3 cestode species were recorded, 2 species of which were larvae identified only to the genus level. After almost 30-year break, the study of fish cestodes in this area has been resumed [8, 9, 10, 11, 12, 13, 18]. In most works focused on fish cestodes of Karadag, only the fact of occurrence of a particular type of helminth in a particular host is indicated. Only [13] provides a brief description and schematic drawings of the found adult mature cestode species from the common stingray *Dasyatis pastinaca* (L., 1758) and the thornback ray *Raja clavata* L., 1758, as well as from teleosts: the Black Sea brill *Scophthalmus maeoticus* (Pallas, 1814) and the black scorpionfish *Scorpaena porcus* L., 1758.

At the beginning of the 21st century, the annotated list of fish parasites of Karadag included data on 19 cestode species registered in 24 fish species before 2002 [10]. This list of cestode species is currently inaccurate due to significant changes in their taxonomy [31, 35, 36, 37, 38]; it does not show their current species composition.

The aim of this work is to identify a modern species composition of fish cestodes in the water area of the Karadag nature reserve and adjacent water areas.

MATERIAL AND METHODS

The material for this study was the collections of cestodes obtained by researchers of IBSS RAS Environmental Parasitology Department in the Karadag nature reserve in 1994 and 2006, as well as our own samples collected in 2005–2018 from rays and teleosts (Table 1), caught in biotopes of the Karadag nature reserve (Biostation, Kuzmichev Rocks, and Malaya Putstsolanovaya and Serdolikovaya bays), as well as in water areas adjacent to the reserve (Meganom and Tolsty capes, Lis'ya Bay, and Koktebel and Ordzhonikidze villages).

Totally 1754 fish specimens of 53 species were examined by the method of incomplete parasitological dissection in the waters of Karadag and adjacent areas.

Fishes were identified by [3]. Cestodes were maintained in fresh water for 10–20 minutes to relax muscles and to evaginate tentacles and cirrus before fixing it in 70° ethanol and preparing total slides [2]. The cestodes were stained with acetocarmine and alum carmine by the standard method [19]; after dehydration with alcohol (70–100°) and enlightenment in clove oil, they were put into Canadian balsam. The infestation of fish with cestodes was assessed by the following parameters: prevalence (%) and intensity (worms per host) of infestation, as well as abundance index (worms per individual host) [1]. Calculations of the parameters were performed in Statistica 6 and PAST 3 programmes [30]. All types of cestodes, used in this study, were deposited in a subcollection of marine parasites of the World Ocean hydrobionts collection of IBSS RAS [27].

RESULTS

Of 53 fish species in the area studied, in 17 species 20 cestode species were found (Table 1). The greatest cestode species richness (12 species) was identified in *Dasyatis pastinaca*. Seven species were identified in *Raja clavata*; 1 species of adult mature cestode was identified in the teleost *Scophthalmus maeoticus*, and 1 – in the teleost *Scorpaena porcus*. The larvae of two cestode species (*Progrillotia dasyatidis* and the complex species "*Scolex pleuronectis*") were identified in 14 teleost species.

Table 1. Cestode infestation parameters in fish of the water area of the Karadag nature reserve and adjacent areas (1994–2018)

Cestode species	Species of the host (number of specimens)	II, min – max / mean ± SE	PI[1]	AI, mean ± SE
Parachristianella trygonis Dollfus, 1946	*Dasyatis pastinaca* (L., 1758) (11)	1 – 6 / 4	2 of 11	0.6
Progrillotia dasyatidis Beveridge, Neifar & Euzet, 2004		10 – 73 / 42	2 of 11	8
Dollfusiella aculeata Beveridge, Neifar & Euzet, 2004		1 – 14 / 7	3 of 11	2
Prochristianella papillifer (Poyarkoff, 1909) Dollfus, 1957 (syn. *P. trigonicola* Dollfus, 1946)		2 – 11 / 7	2 of 11	1.2
Acanthobothrium sp. 5		1 – 4 / 3	2 of 11	1
Acanthobothrium sp. 7		2 – 5 / 4	2 of 11	1
Caulobothrium sp.		1 – 1508 / 472	6 of 11	257
Cairaeanthus ruhnkei Kornyushin & Polyakova, 2012		1	1 of 11	0.1
C. healyae Kornyushin & Polyakova, 2012		1 – 3 / 2	3 of 11	0.6
Anthocephaliidae gen. sp. 2		5 – 6 / 5.5	2 of 11	1
Rhinebothrium walga (Shipley & Hornell, 1906)		1 – 12 / 5	3 of 11	1.3
Rhabdotobothrium sp.		1 – 35 / 13	4 of 11	5
Echinobothrium typus Van Beneden, 1849	*Raja clavata* L., 1758 (11)	1	1 of 11	0.1
Progrillotia sp.		3 – 103 / 28	5 of 11	13
Grillotia erinaceus (Van Beneden, 1858)		1 – 13 / 7	5 of 11	3.1
Echeneibothrium variabile Van Beneden, 1850		1 – 11 / 4	4 of 11	1.3
Acanthobothrium sp. 1		2 – 12 / 6.3	4 of 11	2.3
Acanthobothrium sp. 2		3 – 46 / 18	6 of 11	10
Acanthobothrium sp. 4		1 – 4 / 3	4 of 11	1
"*Bothriocephalus gregarius*" Renaud, Gabrion & Romestand, 1984	*Scophthalmus maeoticus* (Pallas, 1814) (3)	42 – 54	2 of 3	–
"*B. scorpii*" (Müller, 1779)	*Scorpaena porcus* L., 1758 (107)	1 – 3 / 2 ± 0.3	6	0.1 ± 0.04

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Cestode species	Species of the host (number of specimens)	II, min – max / mean ± SE	PI[1]	AI, mean ± SE
Progrillotia dasyatidis larvae	*Gobius niger* L., 1758 (6)	1 – 32	2 of 6	–
	Gobius bucchichi Steindachner, 1870 (5)	1 – 8	2 of 5	–
	Mullus barbatus L., 1758 (53)	1 – 86 / 23 ± 12	13	3.4 ± 2
	Trachurus mediterraneus (Steindachner, 1868) (129)	1	0,8	0.01 ± 0.01
	Gaidropsarus mediterraneus (L., 1758) (42)	1 – 4 / 2.5 ± 1.5	5	0.12 ± 0,1
	S. porcus	1 – 9 / 3.4 ± 0.6	16	0,5 ± 0,2
	Atherina boyeri Risso, 1810 (119)	1 – 4 / 2 ± 0.3	12	0.2 ± 0.06
	Salaria pavo (Risso, 1810) (59)	1	2	0.02 ± 0.02
"*Scolex pleuronectis*" Müller, 1788 larvae	*S. porcus*	1 – 8 / 5 ± 1.3	5	0,2 ± 0,1
	M. barbatus	1 – 4 / 2.5 ± 1.5	4	0,1 ± 0.08
	T. mediterraneus	2	0.8	0.02 ± 0.02
	Symphodus ocellatus Forsskål, 1775 (55)	1 – 38 / 20 ± 9	4	0.7 ± 0.7
	G. mediterraneus	1 – 7 / 4 ± 3	5	0.21 ± 0.18
	Atherina hepsetus L., 1758 (52)	1	2	0.02 ± 0.02
	Gobius niger	1	2 of 6	–
	Gobius bucchichi	3 – 38	3 of 5	–
	Neogobius melanostomus (Pallas, 1814) (14)	1	1 of 14	0.07
	Ponticola eurycephalus (Kessler, 1874) (44)	1	2.3	0.02 ± 0.02
	Aidablennius sphynx (Valenciennes, 1836) (301)	1 – 2 / 1.7 ± 0.3	1	0.02 ± 0.01
	Spicara smaris (L., 1758) (36)	14	3	0.4 ± 0.4

Note: II is intensity of infestation, worms per host; PI is prevalence of infestation, %; AI is abundance index, worms per individual host. [1] – if less than 15 fishes were dissected, then the number of fish infested from the total number of fish studied is given.

For the first time in the area under study, 9 cestode species were found in *D. pastinaca*: *Progrillotia dasyatidis*, *Parachristianella trygonis*, *Dollfusiella aculeata*, *Caulobothrium* sp., *Rhinebothrium walga*, *Rhabdotobothrium* sp., *Acanthobothrium* sp. 5, 7, and Anthocephaliidae gen. sp. 2 (Table 1). In this water area, cestodes *Acanthobothrium* sp. 1, 2, 4 were found in *R. clavata*. Two species of cestode larvae [*Hepatoxylon trichiuri* (Holten, 1802) and *Nybelinia lingualis* (Cuivier, 1817)], previously recorded, were not found in teleosts, whereas two species of adult mature cestodes [*Tetrarhynchobothrium tenuicolle* Diesing, 1854 and *Anthobothrium cornucopia* (Rud., 1819)] were not found in rays.

For the first time in Karadag water area, *Progrillotia dasyatidis* larvae were found in 8 teleost species. Previously, we found the larvae of this cestode in the same fish species in Sevastopol water area. The larvae were without a blastocyst; they localized in the gallbladder, sometimes in the intestinal lumina of fish. These teleosts are the second intermediate hosts for *P. dasyatidis*, which ends its development in the definitive host *D. pastinaca*. The larvae of cestode *P. dasyatidis* were found in demersal fish (*Gobius* spp., *Scorpaena porcus*, *Gaidropsarus mediterraneus*, and *Salaria pavo*), as well as in pelagic fish (*Mullus barbatus*, *Trachurus mediterraneus*, and *Atherina boyeri*). The highest abundance of *P. dasyatidis* larvae was identified in *M. barbatus*, *S. porcus*, and *A. boyeri* (Table 1). No data on the species composition of the first intermediate hosts of *P. dasyatidis* are available in literature. Free larvae, having no blastocyst, of this cestode from 7 species of demersal teleosts of three families (Soleidae Bonaparte, 1833, Scophthalmidae Chabanaud, 1933, and Batrachoididae Jordan, 1896), caught off the coast of Portugal, have recently been described [36]. Our data on the finding of *P. dasyatidis* larvae in Black Sea teleosts contribute to the information on the participants of this helminth's life cycle.

The analysis of cestode infestation of 2 ray species in the area under study revealed that the mass species found in most *D. pastinaca* studied was *Caulobothrium* sp., with the maximum abundance of 1508 spec. per ind. (Table 1). Cestodes, following in number and occurrence in the common stingray, were *Dollfusiella aculeata*, *Progrillotia dasyatidis*, and *Rhabdotobothrium* sp. The most abundant cestode in *Raja clavata* was *Progrillotia* sp., with the abundance reaching 103 specimens in one thornback ray. The second most abundant and most common species was *Acanthobothrium* sp. 2.

Adult mature forms of cestodes of the genus *Bothriocephalus* Rud., 1808, "*B. gregarius*" and "*B. scorpii*", were found in their definitive hosts: the Black Sea brill and the black scorpionfish. "*B. gregarius*" intensity of infestation (42–54 worms per host) of the Black Sea brill in Karadag water area is comparable to that of other areas along the coast of Crimea. The definitive host of "*B. scorpii*" in the Black Sea is *Scorpaena porcus*. According to [13], "*B. scorpii*" was found in Karadag water area in 28 % of black scorpionfish with an intensity of 1–2 worms per host. According to [9], up to 30 % of black scorpionfish were infested with this cestode with an intensity of 1–3 worms per host and an abundance of 0.5 worms per individual host. In [11, p. 10] there are no quantitative data on the infestation of the black scorpionfish with this cestode, and it is only stated that its occurrence in fish is high. During our study, "*B. scorpii*" was found on average in 6 % of black scorpionfish; compared with the 1960s [13] and 2000s [9], the occurrence decreased by almost 5 times. To date, this species is quite rare not only in the area under study, but along the whole coast of Crimea as well.

DISCUSSION

As a result of the audit of the cestode species composition in fish of Karadag water area and adjacent areas, the representatives of 6 orders were found: Bothriocephalidea, Diphyllidea, Trypanorhyncha, "Tetraphyllidea" relics, Rhinebothriidea, and Onchoproteocephalidea (Table 2).

Previously, in Karadag water area the infestation with only one cestode representative of the order Bothriocephalidea, "*Bothriocephalus scorpii*", was recorded in the black scorpionfish and the Black Sea brill [11, 13]. The only and incomplete description with schematic drawings of this species in the Black Sea is based on cestodes from the black scorpionfish and the Black Sea brill [13]. To date, this species is a complex one; it is registered in more than 50 genera of marine fish from families and orders that are not related phylogenetically.

Table 2. Fauna of fish cestodes of the Karadag nature reserve and adjacent water areas of the Black Sea (according to own and literary data)

Cestode taxa	Fish species
Bothriocephalidea Kuchta, Scholz, Brabec & Bray, 2008[1]	
Bothriocephalidae Blanchard, 1849	
"*Bothriocephalus scorpii*"	*Scorpaena porcus*
"*B. gregarius*"	*Scophthalmus maeoticus*
Diphyllidea Van Beneden in Carus, 1863	
Echinobothriidae Perrier, 1897	
Echinobothrium typus	*Raja clavata*
Trypanorhyncha Diesing, 1863	
Eutetrarhynchidae Guiart, 1927	
Parachristianella trygonis	*Dasyatis pastinaca*
Prochristianella papillifer	
Dollfusiella aculeata	
Dollfusiella aculeata larvae	*Chelidonichthys lucernus*, *Scomber scombrus*, *Mullus barbatus*, *Belone belone*, *Trachinus draco*, *Pegusa nasuta*
Tetrarhynchobothrium tenuicolle[2]	*Raja clavata*
Progllotiidae Palm, 2004	
Progrillotia dasyatidis	*Dasyatis pastinaca*
Progrillotia dasyatidis larvae	*Gobius niger*, *G. bucchichi*, *Mullus barbatus*, *Trachurus mediterraneus*, *Gaidropsarus mediterraneus*, *Scorpaena porcus*, *Atherina boyeri*, *Salaria pavo*
Lacistorhynchidae Guiart, 1927	
Grillotia erinaceus	*Raja clavata*
Tentaculariidae Poche, 1926	
Nybelina lingualis[2] larvae	*Sarda sarda*
Sphyriocephalidae Pintner, 1913	
Hepatoxylon trichiuri[2] larvae	*Trachinus draco*
"Tetraphyllidea" Van Beneden, 1850 relics: Family incertae sedis	
Anthobothrium cornucopia[2]	*Dasyatis pastinaca*
Caulobothrium sp.	
Scolex pleuronectis larvae	*Scorpaena porcus*, *Neogobius syrman*, *Gobius niger*, *G. bucchichi*, *Pomatoschistus minutus*, *Crenilabrus ocellatus*, *C. scina*, *C. tinca*, *Trachurus mediterraneus*, *Merlangius merlangus*, *Mullus barbatus*, *Platichthys flesus*, *Sciaena umbra*, *Uranoscopus scaber*, *Ophidium rochei*, *Pegusa nasuta*, *Spicara flexuosa*, *Syngnatus abaster*, *Chelon auratus*, *C. saliens*, *Mugil cephalus*, *Arnoglossiis kessleri*, *Gymnammodytes cicerellus*, *Atherina hepsetus*
Onchoproteocephalidea Caira, Jensen, Waeschenbach, Olson & Littlewood, 2014	
Onchobothriidae Braun, 1900	
Acanthobothrium sp. 1	*Raja clavata*
Acanthobothrium sp. 2	
Acanthobothrium sp. 4	
Acanthobothrium sp. 5	*Dasyatis pastinaca*
Acanthobothrium sp. 7	

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Cestode taxa	Fish species
Rhinebothriidea Healy, Caira, Jensen, Webster & Littlewood, 2009	
Rhinebothriidae Euzet, 1953	
Rhinebothrium walga	*Dasyatis pastinaca*
Rhabdotobothrium sp.	
Anthocephaliidae Ruhnke, Caira & Cox, 2015	
Cairaeanthus ruhnkei	*Dasyatis pastinaca*
C. healyae	
Anthocephaliidae gen. sp. 2	
Echeneibothriidae de Beauchamp, 1871	
Echeneibothrium variabile	*Raja clavata*

Note: [1] – classification of orders and families according to [25, 34, 37]; [2] – cestode species, not found by us in fish in Karadag water area.

A type species of the genus, *Bothriocephalus scorpii* sensu stricto, parasitizes only in the shorthorn sculpin *Myoxocephalus scorpius* (L., 1758) (Scorpaeniformes: Cottidae) inhabiting the northern parts of the Pacific and Atlantic oceans [34]. On the basis of the results of the electrophoresis of protein composition of cestodes named "*B. scorpii*" from the Black Sea brill in the Black Sea, these cestodes were redefined as "*B. gregarius*", but without describing morphological features [20]. According to [33, 34], "*B. gregarius*" is *nomen nudum*, as its description from a typical host from the native range has not yet been published. As a result of studying the morphological features of cestodes named "*B. scorpii*" (from the black scorpionfish) and "*B. gregarius*" (from the Black Sea brill) in the Black Sea (Crimea, Caucasus), as well as investigations of the ribosomal genes 18S and 28S of these cestodes [16], both morphological and genetic features were revealed which confirm independence of these species and their inconsistency with the type species *Bothriocephalus scorpii* sensu stricto. So far, we use the names of cestodes "*B. scorpii*" and "*B. gregarius*" in this study (Table 2).

Another species of this genus, *B. atherinae* Chernyschenko, 1949, was first observed in the big-scale sand smelt *Atherina boyeri* in Odessa area [21], and then – in Karadag water area [11]. The taxonomic status of this species is still not clear. It was transferred to the genus *Ptychobothrium* Lönnberg, 1889 as *P. atherinae* (Chernyschenko, 1949) [7]. However, some researchers consider the old name to be valid [33], and others consider this cestode as a species with an undefined generic status [34]. It should be noted that the registration of this species off the coast of Karadag is doubtful, since it prefers brackish-water biotopes. Thus, in the Black Sea *B. atherinae* was identified in the big-scale sand smelt only in three areas of the northwestern part of the sea: in Odessa water area (salinity of 3–14 ‰), as well as in Berezansky (4–12 ‰) and Tiligulsky (4–12 ‰) estuaries [21, 22]. Our data also confirm the association of this species to low salinity biotopes. Throughout our studies, only 2 specimens of *B. atherinae* were found and only in 2 of 280 big-scale sand smelts studied in the brackish-water biotope of Karkinitsky Bay. In polyhaline waters along the coast of Crimea [in Sevastopol water area (17–18 ‰, 545 specimens of the big-scale sand smelt were dissected), Karadag (17–18 ‰, 119 specimens), and the Kerch Strait (12–15 ‰, 65 specimens)], this cestode was not found. Therefore, the recording of *B. atherinae* in Karadag polyhaline area (16–18 ‰) is doubtful. Thus, only two of three cestode species of the order Bothriocephalidea in Karadag fish are evidently parasitic: "*B. scorpii*" and "*B. gregarius*" (Table 2).

Two specimens of *Echinobothrium typus*, the only representative of the order Diphyllidea in the Black Sea, were first found in *Raja clavata* in Karadag water area in the early 1960s [13]. This cestode species was registered again in this ray species in Karadag water area

in the late 1980s [12]. According to some authors, *E. typus* is common for this host (without indicating the quantitative parameters of infestation). However, *E. typus* was found by us only in Sevastopol water area (124 specimens of rays were examined, a prevalence of 15 %, with an abundance of 19 worms per individual host). In other areas, rays (166 specimens) were not infested with this cestode. For the first time, in 2018 one immature specimen of *E. typus* was found in *R. clavata* (Table 1).

According to [5, 9, 10, 11, 13], 13 cestode species of order Trypanorhyncha are found in fish in this water area. Of them, 8 are represented by adult mature forms (*Christianella minuta* (Van Beneden, 1849), *Tetrarhynchobothrium minutus* Van Beneden, 1850, *T. erinaceus* Van Beneden, 1861, *T. tenuicolle*, *Tetrarhynchus tenuicolle* Diesing, 1854, *Progrillotia louiseuzeti* Dollfus, 1969, *Grillotia erinaceus*, and *Prochristianella trigonicola*) parasitizing in two ray species and in the shark Picked dogfish *Squalus acanthias* L., 1758; 5 are larvae (*Hepatoxylon trichiuri*, *Nybelinia lingualis*, *Tetrarhynchobothrium* sp., *Tentacularia* sp., and *Eutetrarhynchus* sp.) recorded in teleosts.

Totally 5 cestode species of this order were identified by us in *Raja clavata* and *Dasyatis pastinaca*: *Dollfusiella aculeata*, *Grillotia erinaceus*, *Prochristianella papillifer*, *Parachistianella trygonis*, and *P. dasyatidis* (*P. louiseuzeti* sensu [6, 11]) (Tables 1, 2). The larvae of the cestode *P. dasyatidis* were identified in teleosts for the first time, while the larvae of *N. lingualis* and *H. trichiuri*, previously found in these fish, were not identified. According to [36], of the fish inhabiting the Black Sea, the second intermediate hosts of the latter two species are the Atlantic bonito *Sarda sarda* (Bloch, 1793) and the greater weever *Trachinus draco* L., 1758; the definitive hosts are *Raja clavata* and *Squalus acanthias*. We did not study the Atlantic bonito, the greater weever, and the shark Picked dogfish in Karadag water area, and the amount of *R. clavata* studied was small (11 specimens). This may explain the absence of these cestode species in our samples.

Previous analysis [15] of the occurrence and synonymy of Trypanorhyncha species showed that the identification of *Grillotia (Christianella) minuta* in elasmobranchs and *Tentacularia* sp. larvae in teleosts in the Black Sea is incorrect due to the absence of their specific definitive hosts in this water body. The names *Tetrarhynchobothrium erinaceus*, *T. minutus*, and *Tetrarhynchus tenuicolle* are not valid [36]. The representatives of the genus *Eutetrarhynchus* Pintner, 1913 were recorded in the Black Sea only in teleosts at the larval stage [5, 9, 10]. The adult mature specimens of these cestodes were first recorded in *D. pastinaca* in Sevastopol water area and identified as *Eutetrarhynchus spinifer* Dollfus, 1969 [6], but without description. The analysis of the morphology of Black Sea cestodes, previously identified as *E. spinifer*, showed inconsistency with the redescription of *Dollfusiella spinifer* (syn. *E. spinifer*), and these cestodes were redefined as *D. aculeata* [15].

Another trypanorinch species, *Tetrarhynchobothrium tenuicolle*, in *R. clavata* in the Black Sea (Romanian coast) was identified as *Rhynchobothrium tenuicolle* [23]. Once it was found by T. P. Pogorel'tseva in Karadag water area [13]. This species was not registered in the sea again. Thus, of 13 species of the order Trypanorhyncha, previously observed in fish in Karadag water area [10], in fact only 5 adult mature species parasitize in both ray species, and larvae of 2 cestode species were found in teleosts (Table 2).

Before our studies, 7 cestode species from 4 genera of the order "Tetraphyllidea" relics (*Echeneibothrium* Van Beneden, 1850, *Anthobothrium* Van Beneden, 1850, *Phyllobothrium* Van Beneden, 1849, and *Acanthobothrium* Van Beneden, 1849) were found in fish in Karadag water area [9, 11, 13, 18]. Three new orders were isolated from "Tetraphyllidea": Rhinebothriidea, Phyllobothriidea, and Onchoproteocephalidea [25]. Therefore, of the order "Tetraphyllidea" in Karadag water area only one representative

of *Anthobothrium*, *A. cornucopia*, parasitizes in *D. pastinaca* [13]. Previously, *A. auriculatum* (Rud., 1819) and *A. cornucopia* were identified in Black Sea rays. However, in our samples of tetraphyllideans from both ray species off the coast of Crimea these cestodes were not found.

Other representatives of the order "Tetraphyllidea" relics, cestodes of the genus *Caulobothrium* Baer, 1948, were first recorded by us in the Black Sea in *D. pastinaca* in the waters of Sevastopol (Kazach'ya Bay); they were found again in Karadag and Kerch Strait areas [14]. The specimens studied differ from 7 valid species of this genus [37], and in this work they are defined as *Caulobothrium* sp. To date, it is not clear to which family cestodes of this genus belong; so far, they have been identified as a separate group designated as Clade 4 [37, p. 378]. The authors claim that *Caulobothrium* spp. are found only in rays of the family Myliobatidae Bonaparte, 1838 [*Myliobatis* L., 1758], and the identification of cestodes of this genus in rays of the families Dasyatidae Jordan, 1888 [*Himantura* (Bleeker, 1852)] and Urolophidae Müller & Henle, 1841 [*Urolophus* Müller & Henle, 1837] in the Caribbean Sea, as well as in the Pacific and Atlantic oceans, requires additional confirmations. Thus, in the area studied the infestation of rays with only one species of the genus *Caulobothrium* was confirmed (Table 2).

According to [9, 10, 11, 13, 23] and analysis of our samples, cestodes of 3 families of the order Rhinebothriidea are found in Karadag rays: Echeneibothriidae (*Echeneibothrium*), Anthocephaliidae (*Cairaeanthus* Kornyushin & Polyakova, 2012 and Anthocephaliidae gen. sp. 2), and Rhinebothriidae (*Rhinebothrium* Linton, 1890 and *Rhabdotobothrium* Euzet, 1953) [14, 15].

The only representative of the family Echeneibothriidae recorded in *Raja clavata* in the Black Sea is *Echeneibothrium variabile*, and it is described only on the basis of immature specimens [13, 23]. According to [39], "*E. variabile*" is a complex species, and *E. variabile* sensu stricto is highly specific to *R. clavata*; the identification of this cestode species in different ray species and in other areas requires confirmation. In the cestode samples from *R. clavata* from Karadag and Sevastopol water areas, mature specimens, morphologically identical to this species from a typical host from the native range, were found [39]. It should be noted that *E. variabile* was found in Karadag water area in both ray species [9, 10, 11]. We have never registered parasitization of *E. variabile* in *D. pastinaca*. Given the specificity of *E. variabile* to *R. clavata*, we consider its identification in *D. pastinaca* in the Black Sea to be incorrect.

The cestode species composition of *D. pastinaca* in Karadag water area was replenished with new representatives: Anthocephaliidae gen. sp. 2, *Rhinebothrium walga*, and *Rhabdotobothrium* sp. [14, 15]. In cestode samples of the common stingray, caught along the coast of Crimea, cestodes of the genus *Cairaeanthus* (*Phyllobothrium* sensu [13, 23]) with two species, *C. ruhnkei* (syn. *P. lactuca* sensu [13, 23]) and *C. healyae* (syn. *P. gracilis* sensu [13, 23]), were identified by us [32]. In Karadag water area, *C. healyae* was first found in the late 1980s in 45 % of rays *D. pastinaca* and *R. clavata* with an intensity of 1–17 worms per host [12]. The annotated list indicates the identification of another species of this genus, *C. ruhnkei* [10]. According to [10, p. 476], 35 % of *Raja clavata* and *Scorpaena porcus* were infested with *C. ruhnkei* with an intensity of 1–5 worms per host; it was noted that for the first time both *Cairaeanthus* species were found in Karadag water area by T. P. Pogorel'tseva. However, according to [13, pp. 148–150], both cestode species were found only in *D. pastinaca* and in other water areas: *C. ruhnkei* was recorded in the Kerch Strait, and *C. healyae* – in Kerch and Novorossiysk water areas. Cestodes of the genus *Cairaeanthus* spp. are highly specific parasites of *D. pastinaca* [32]. Therefore, identifications of adult mature *Cairaeanthus* spp. not only in *R. clavata* [11], but also in the black scorpionfish [10], are obviously incorrect, since teleosts are the second, or paratenic, hosts for elasmobranch cestodes.

Both species of *Cairaeanthus* are found in the Black Sea, and only *C. ruhnkei* is registered in the Sea of Azov. According to [24, p. 17], this group of cestodes (*Cairaeanthus* spp.) parasitizes in cooler waters, since, while studying cestodes of rays of the genus *Dasyatis* Rafinesque, 1810 from tropical and subtropical areas, cestodes of the genus *Cairaeanthus* were not identified. Thus, 6 cestode species of the order Rhinebothriidea parasitize in rays of Karadag water area: *Echeneibothrium variabile*, *Cairaeanthus healyae*, *C. ruhnkei*, *Rhinebothrium walga*, *Rhabdotobothrium* sp., and Anthocephaliidae gen. sp. 2 (Table 2).

According to [10, 11, 13], only two cestode species of the genus *Acanthobothrium* parasitize in two ray species in Karadag water area: *A. coronatum* (Rud., 1819) and *A. dujardinii* Van Beneden, 1849. Prior to our studies, three species of this genus were recorded in the Black Sea: besides the species mentioned above, the only Black Sea endemic, *A. ponticum* Borcea, 1934, was identified [10, 11, 13, 23]. It is necessary to emphasize, that in our samples of cestodes of this genus from both ray species off the coast of Crimea and Caucasus, we did not find cestodes that would correspond, according to their morphological characteristics, to *A. coronatum*, *A. dujardinii*, and *A. ponticum* previously found. On the other hand, among representatives of *Acanthobothrium* spp. found in *D. pastinaca* and *R. clavata* in Karadag water area, we identified 7 morphologically different species-level taxa. *Acanthobothrium* sp. 1, 2, 4 were registered in *R. clavata*, and *Acanthobothrium* sp. 5, 7 were found in *D. pastinaca* (Tables 1, 2) [14, 17].

The analysis of the morphology, specificity, and synonymy of *A. coronatum*, *A. dujardinii*, and *A. ponticum* in Black Sea rays [15] revealed that the identification of the highly specific species *A. coronatum* [40] in these fish in this water area is incorrect due to the absence of the definitive hosts of this cestode species, sharks of the genus *Scyliorhinus* (Blainville, 1816). Descriptions of cestodes identified as *A. dujardinii* [13, 23] from rays of the Black Sea do not correspond to typical descriptions of this species from the English Channel [28, 29, 40]. The systematic position of *A. ponticum* is still not clear. Some researchers indicated it as a possible junior synonym for *A. crassicolle* Wedl, 1855 [29], others – as a species with an unclear systematic position [40], and others – as a valid species [26], without restudying the typical material, which location is unknown.

In our samples of *Acanthobothrium* spp. from *D. pastinaca* off the coast of Crimea (Karkinitsky Bay, Sevastopol, Karadag, and the Kerch Strait) and Caucasus, specimens found were initially identified as *A. crassicolle* [5]. However, having studied the additional material on the morphology of these cestodes and the sequence of their 18S and 28S ribosomal genes, we have revealed morphological and genetic features [17] which allowed us to identify these cestodes as a new species. So far, in this work, we use the name *Acanthobothrium* sp. 7. Thus, in the composition of the order Onchoproteocephalidea, 5 cestode species infest Karadag rays (Table 2).

Conclusion. As a result of the revision of the fauna of fish cestodes of the Karadag nature reserve and adjacent areas, it is recorded that the current species composition of these helminths includes 20 species from 19 genera and 6 orders. For the first time, 12 species were recorded in Karadag rays: *Progrillotia dasyatidis*, *Parachristianella trygonis*, *Dollfusiella aculeata*, *Rhinebothrium walga*, *Acanthobothrium* sp. 1, 2, 4, 5, 7, *Rhabdotobothrium* sp., *Caulobothrium* sp., and Anthocephaliidae gen. sp. 2. Larvae of *Progrillotia dasyatidis* were found in teleosts of Karadag for the first time. This new data supplement the knowledge about the features of the life cycles of cestodes of this genus. In fish of this water area, of the previously recorded 19 cestode species, finding of only 8 was confirmed: "*Bothriocephalus scorpii*", "*B. gregarius*", *Echinobothrium typus*, *Grillotia erinaceus*, *Prochristianella papillifer*, *Echeneibothrium variabile*,

Cairaeanthus ruhnkei, and *C. healyae*. The larvae of cestodes *Hepatoxylon trichiuri* and *Nybelinia lingualis* were not found in teleosts; *Tetrarhynchobothrium tenuicolle* and *Anthobothrium cornucopia* were not found in rays. As a result of the analysis of current taxonomy, synonymy, and specificity to the definitive hosts of cestodes of the orders Trypanoryncha and Onchoproteocephalidea, it was found out, that the initial identification and description of 3 species, *Tentacularia* sp. larvae, *Grillotia (Christianella) minuta*, and *Acanthobothrium coronatum*, from Black Sea fish were incorrect. The greatest cestode species richness was identified among the representatives of the orders Trypanorhyncha and Onchoproteocephalidea (5 species each), and the lowest cestode species richness was identified among the representatives of the orders Diphyllidea and "Tetraphyllidea" relics (1 species each). An increase in the species composition of elasmobranch cestodes of Karadag water area can occur due to the study of these helminths infesting *Squalus acanthias*, which was practically not studied in this area.

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ЦЕСТОДЫ РЫБ АКВАТОРИИ КАРАДАГСКОГО ПРИРОДНОГО ЗАПОВЕДНИКА И ПРИЛЕГАЮЩИХ РАЙОНОВ ЧЁРНОГО МОРЯ

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Данные о паразитах морских рыб акватории Карадага появились в первой половине XX века. К началу XXI столетия сведения о фауне цестод этого района Чёрного моря включали информацию о 19 видах, зарегистрированных у 24 видов рыб. Между тем за последнее десятилетие таксономия этого класса гельминтов претерпела существенные изменения, в свете чего региональные фауны нуждаются в ревизии. Цель работы — провести ревизию видового состава цестод рыб акватории Карадагского природного заповедника и прилегающих районов на основе новых сборов и в соответствии с современной систематикой класса Cestoda. Материалом для исследования послужили как коллекции цестод, собранные сотрудниками отдела экологической паразитологии ФИЦ ИнБЮМ в районе Карадагского заповедника в разные годы, так и собственные сборы

от 1754 экз. скатов и костистых рыб 53 видов (2005–2018). Район исследования — прибрежная акватория Чёрного моря от м. Меганом до пос. Орджоникидзе (Юго-Восточный Крым), в том числе различные морские биотопы Карадагского заповедника. Ваучерные препараты всех видов цестод, использованных в данном исследовании, депонированы в подколлекции морских паразитов коллекции гидробионтов Мирового океана ФИЦ ИнБЮМ. У 17 видов рыб обнаружено 20 видов цестод. Впервые в районе исследования у ската *Dasyatis pastinaca* зарегистрированы цестоды 9 видов: *Progrillotia dasyatidis*, *Parachristianella trygonis*, *Dollfusiella aculeata*, *Rhinebothrium walga*, *Caulobothrium* sp., *Rhabdotobothrium* sp., *Acanthobothrium* sp. 5, 7 и Anthocephaliidae gen. sp. 2. У другого вида ската, *Raja clavata*, найдены цестоды, относящиеся, очевидно, к новым видам *Acanthobothrium* sp. 1, 2, 4. Из 19 ранее известных в районе Карадага видов подтверждены находки только 8 половозрелых цестод: «*Bothriocephalus scorpii*», «*B. gregarius*», *Echinobothrium typus*, *Grillotia erinaceus*, *Prochristianella papillifer*, *Echeneibothrium variabile*, *Cairaeanthus ruhnkei* и *C. healyae*. Кроме того, у костистых рыб обнаружены личинки сборного вида «*Scolex pleuronectis*». Личинки цестоды *Progrillotia dasyatidis* найдены впервые в акватории заповедника у 8 видов костистых рыб, что расширяет сведения об участниках жизненного цикла этого гельминта. У рыб Карадага не обнаружены ранее регистрировавшиеся в районе 4 вида цестод: *Hepatoxylon trichiurid* larvae, *Nybelinia lingualis* larvae, *Tetrarhynchobothrium tenuicolle* и *Anthobothrium cornucopia*. Кроме того, недавно проведённый анализ встречаемости и синонимики видов отрядов Trypanorhyncha и Onchoproteocephalidea выявил, что ранее выполненное определение *Grillotia (Christianella) minuta* и *Acanthobothrium coronatum* у эласмобранхий и *Tentacularia* sp. larvae — у костистых рыб Чёрного моря ошибочно ввиду отсутствия в этом водоёме их специфичных окончательных хозяев. С другой стороны, среди найденных *Acanthobothrium* spp. у скатов *D. pastinaca* и *R. clavata* в районе Карадага мы идентифицировали 7 морфологически различных новых таксонов видового уровня. Обнаруженные цестоды относились к 6 отрядам: Bothriocephalidea, Diphyllidea, Trypanorhyncha, «Tetraphyllidea» relics, Rhinebothriidea и Onchoproteocephalidea. Наибольшее видовое богатство цестод у обоих видов скатов отмечено среди представителей отрядов Trypanorhyncha и Onchoproteocephalidea (по 5 видов), наименьшее — среди Diphyllidea и «Tetraphyllidea» relics (по 1 виду в каждом). Таким образом, фауна цестод, паразитирующих у рыб в районе Карадага, дополнена 12 видами, 8 из которых являются, очевидно, представителями новых таксонов.

Ключевые слова: цестоды, рыбы, фауна, систематика, заповедник, Крым, Чёрное море