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Helminth Parasites of Black-striped Pipefish *Syngnathus abaster* Risso, 1827 (Actinopterygii: Syngnathidae) from a Black Sea Coastal Wetland, Bulgaria

Borislav Stoyanov, Yasen Mutafchiev & Boyko B. Georgiev*

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria
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Abstract: In a helminthological survey, the black-striped pipefish *Syngnathus abaster* (13 individuals) from the Lake Atanasovsko Wetlands, Bulgaria, three helminth species were recorded. These were the cryptogonimid trematode *Timoniella imbutiformis* (subadult and mature individuals), the opisthorchid trematode *Cryptocotyle concava* (metacercaria) and the raphidascaridid nematode *Hysterothylacium* cf. *aduncum* (third-stage larvae). Of them, *T. imbutiformis* was the most prevalent parasite species (15.38%). The other two helminth species were with lower prevalence, 7.69% each. Previously, these helminth species have not been reported as parasites of *S. abaster* in Bulgaria. This is the first record of *Timoniella imbutiformis* for the fauna of Bulgaria.

Key words: Cryptogonimidae, Lake Atanasovsko, morphology, new records, Opisthorchiidae, Raphidascarididae

Introduction

The black-striped pipefish *Syngnathus abaster* Risso, 1827 is a euryhaline demersal species, with no commercial value, inhabiting the shallow coastal waters, lower reaches of the rivers and coastal wetlands of the Iberian Peninsula, the Mediterranean, Black and Caspian Sea basins (Reshetnikov et al. 2003, Kottelat & Freyhof 2007). Currently, it invades the middle and upper stretches of the rivers in the Black Sea and Caspian Sea basins (Kottelat & Freyhof 2007). Monteiro & Vieira (2017) reported a possible occurrence of the fish off the Atlantic coast of France and the Baltic coast of Germany. The species is not enough studied from parasitological point of view, probably due to its non-commercial value (Moshu & Trombitskiy 2012, Kornyychuk et al. 2022, Yuryshynets et al. 2023). In Bulgaria, its helminth fauna is poorly studied and comprises only nine taxa recorded in the Danube River (Ondračková et al. 2012).

The Lake Atanasovsko Wetlands are an area of a great conservation importance as a Ramsar site, managed nature reserve and site of the Natura 2000 network in accordance with the Birds and Habitats Directives (code BG0000270, see Vassilev et al. 2013). These wetlands represent a complex of highly variable in their salinity and hydrological regime shallow coastal habitats: a hypersaline lagoon partially used in salt production, a system of freshwater and brackish water canals and ponds and freshwater marshes (Ivanov et al. 1964, Vassilev et al. 2013).

*Corresponding author: boyko_georgiev@yahoo.com

Its fish fauna is rather poor, consisting of eight species permanently inhabiting the wetlands; the dominant species are the euryhaline Caucasian dwarf goby *Knipowitschia caucasica* (Berg, 1916) and the three-spined stickleback *Gasterosteus aculeatus* L., 1758 (Stefanov 2006). The helminth fauna of these two species has previously been described (Stoyanov et al. 2018, 2023). In addition, the helminth fauna of the invasive pumpkinseed sunfish *Lepomis gibbosus* (L., 1758), inhabiting freshwater habitats adjacent to the lagoon, has also been studied (Stoyanov et al. 2017b).

In this article, we present the results of the helminth fauna study of the black-striped pipefish *S. abaster* occurring in the Lake Atanasovsko Wetlands.

Materials and Methods

Survey of the helminth parasites of the fishes in the Lake Atanasovsko Wetlands (42°34′42″N, 27°28′27″E) was carries out in May, July and September of 2012 and 2013. We sampled totally 565 individuals of seven fish species. Only 13 individuals of the black-striped pipefish *S. abaster* were captured and examined in September 2012 and July 2013. The fish was caught in the brackish habitats by drop nets for pier fishing and telescopic landing nets. Fish specimens were kept alive in containers filled with aerated water taken from the respective habitats. In the laboratory, each fish was dissected under a stereomicroscope within 24 hours. The recovered helminths were placed in saline for approximately 10 minutes, then fixed in hot saline and transferred to 70% ethanol. Subsequently, the trematodes were stained in iron acetocarmine, dehydrated in ascending ethanol series, cleared in dimethyl phthalate and mounted in Canada balsam. The nematodes were studied as temporary mounts in glycerine.

Measurements taken from the trematodes were: ATL, anterior testis length; ATW, anterior testis width; BMW, body maximum width; BTL, body total length; CSL, circumoral spines length; CSW, circumoral spines width; EGL, egg length; EGW, egg width; FBL, forebody length; HBL, hind body length; OL, oesophagus length; OSDVS, distance of oral sucker to ventral sucker; OSL, oral sucker length; OSW, oral sucker width; OVL, ovary length; OVW, ovary width; OW, oesophagus width; PHL, pharynx length; PHW, pharynx width; PL, prepharynx length; PTL, posterior testis length; PTW, posterior testis width; PTDPB, distance of posterior testis to posterior extremity of body; PW, prepharynx width; SRL, seminal receptacle length; SRW, seminal receptacle width; VSDAB, distance of ventral sucker to anterior extremity of body; VSL, ventral sucker length; VSW, ventral sucker width.

Measurements taken from the nematode were: BMW, body maximum width; BTL, body total length; BWA, body width at anus; EP, excretory pore, distance from anterior extremity; ICL, intestinal caecum length; ICW, intestinal caecum maximum width; NR, nerve ring, distance from anterior extremity; OL, oesophagus length; OMW, oesophagus maximum width; TL, tail length; VAL, ventricular appendix length; VAW, ventricular appendix maximum width; VL, ventriculus length; VW, ventriculus width.

Metrical data are presented as the range, followed by the mean and the number of measurements taken (n). The standard deviation (SD) is given only when n≥30. All measurements are given in micrometres.

The infection characteristics prevalence (P%), intensity (I, Range), mean intensity ± standard error (MI±SE) and mean abundance ± standard error (MA±SE) as well as related terminology are as defined by Bush et al. (1997).

Results

In the present survey, we identified three helminth species (two trematode and one nematode species) as parasites of *S. abaster* from Lake Atanasovsko. They have been recovered from three (out of 13) fish individuals caught in a brackish habitat.

Trematoda

Cryptogonimidae Ward, 1917
***Timoniella imbutiformis* (Molin, 1859) Brooks, 1980 (subadult and mature individuals)**

Site of infection: intestine.

Infection characteristics: P% (15.38), I (18–24), MI (21.00±3.00), MA (3.23±1.18).

Description (based on 12 mature specimens; for the metrical data, see Table 1): Body elongated, with maximum width at level of ventral sucker; shape of anterior extremity depending on degree of protrusion of oral sucker (Fig. 1A, B); posterior extremity rounded (Fig. 1A). Tegument spinous; spines tiny, uniform (Fig. 1A, B). Eyespots two, dark-brown, forming compact granulated masses, sometimes strongly diffused (Fig. 1A, B, E); located at level of pharynx. Oral sucker funnel-shaped, thick-walled, terminal, armed with 17–18 large circumoral spines arranged in single crown (Fig. 1A, B). Ventral sucker circular to slightly transversely-

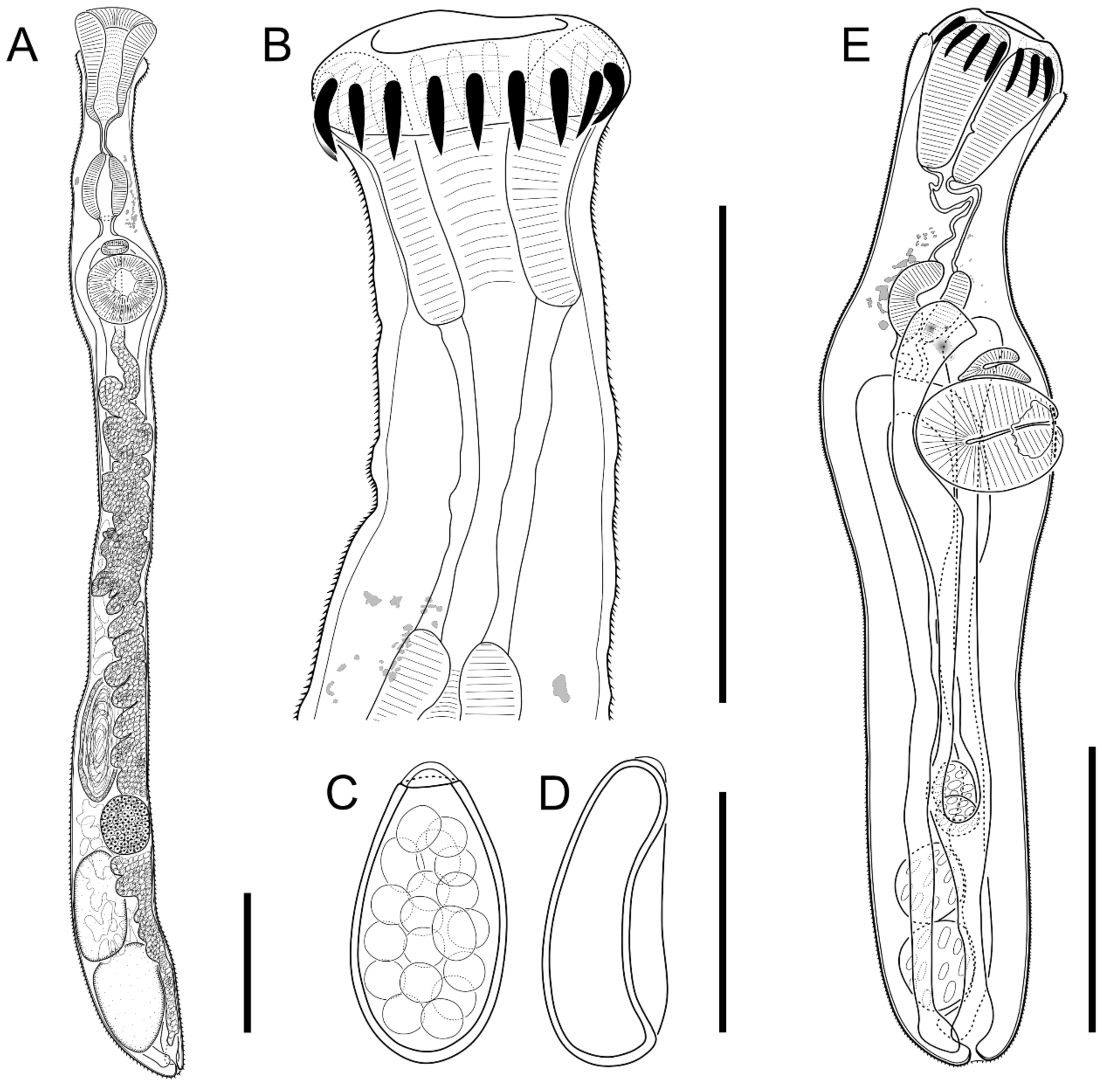

Fig. 1. *Timoniella imbutiformis* (Molin, 1859) Brooks, 1980, mature individual. A. General view. B. Head end with circumoral spines. Eggs: C. Operculated egg; D. Strongly deformed egg. Immature individual. E. General view. Scale-bars: A–B, 250 µm; C–D, 20 µm; E, 200 µm.

oval, muscular, in anterior quarter of body; no ventrogenital sac (Fig. 1A). Prepharynx straight, longer than oesophagus. Pharynx elongate-oval, muscular (Fig. 1A). Oesophagus short. Intestinal bifurcation located anterior to ventral sucker (Fig. 1A). Caeca, extending close to posterior extremity of body (Fig. 1A). Testes two, oval, in tandem, contiguous, close to posterior extremity (Fig. 1A). Cirrus and cirrus sac absent. Seminal vesicle tubular, sinusoidal. Gonotyl present, sucker-like, with transverse slit-like genital pore, immediately to anterior rim of ventral sucker, median (Fig 1A). Ovary oval, compact, in hindbody, pretesticular, sinistral, near to anterior testis (Fig. 1A). Vitellarium follicular, forming two dorsolateral fields in hindbody, extending approximately from mid-body to level of posterior testis slightly overlapping it. Seminal receptacle elongate-oval, thick-walled, in hindbody, immediately pre-ovarian, dextral (Fig. 1A). Uterus tubular, strongly coiled, in hindbody, between ventral sucker and posterior testis, sometimes extending in post-testicular region, close to posterior extremity of body; packed with numerous eggs (Fig. 1A). Excretory vesicle Y-shaped, arms bifurcated approximately at level to or slightly posterior to ovary, extending anteriorly to level of pharynx (Fig. 1E); opens via terminal pore. Eggs elongate-oval, yellowish-brown, not embryonated, with operculum; pole opposite to operculum rounded (Fig. 1C); some eggs deformed (Fig. 1D).

Remarks: Due to the strongly developed uterus, which partially masks some of the inter-

Table 1. Metrical data on *Timoniella imbutiformis* (Molin, 1859) Brooks, 1980 in fishes from the European waters. For abbreviations, see Materials and Methods.

Host	***Dicentrarchus labrax* (L.)**			***Syngnathus typhle* L.**		***S. typhle***				***S. abaster* Risso**		
Locality	**Mediterranean Sea, Costal lakes and Gulf of Lion (France)**			**Baltic Sea (Germany)**		**Baltic Sea, Salzhaff Bay (Germany)**		**Black Sea, Budaki Lagoon, Sevastopol Bay, Gulf of Odessa (Ukraine)**		**Lake Atanasovsko Wetlands (Bulgaria)**		
Source	**Maillard (1973)**			**Reimer et al. (1996)**		**Kvach et al. (2018)**				**Present study**		
Characters	**Range**	**Mean**	**n**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean ± SD**	**n**
BTL	2,074–3,944	2,978	10	980–1,987	–	744–1,629	1,199	1,003–1,996	1,442	1,495–2,184	1,878	12
BMW	240–442	333	10	154–267	–	113–242	175	129–290	194	183–300	220	11
FBL	–	–	–	–	–	259–432	363	180–535	428	575–780	636	12
HBL	–	–	–	–	–	438–1,196	836	634–1,535	1,014	862–1,431	1,242	12
OSL	200–304	225	10	111–194	–	79–130	101	101–165	132	105–253	182	12
OSW	166–277	229	10	79–199	–	65–120	93	96–170	136	135–275	177	12
OSDVS	–	–	–	–	–	–	–	–	–	235–418	293	12
CSL	50–74	63	10	13–42	–	20–29	24	26–50	41	28–45	36	22
CSW	–	–	–	–	–	5–9	8	8–10	10	5–8	5	22
VSL	–	–	–	99–142		69–113	96	89–179	128	113–160	134	12
VSW	216–304	263	10	40–142	–	74–125	100	92–154	125	120–160	141	11
VSDAB	–	–	–	–	–	–	–	–	–	413–553	474	12
PL	144–225	209	10	40–167	–	58–170	129	131–223	193	60–140	108	5
PW	–	–	–	–	–	–	–	–	–	15–23	18	5
PHL	96–151	121	10	70–90	–	34–64	52	48–79	67	92–138	105	12
PHW	–	–	–	50–67	–	41–74	58	55–88	74	68–105	85	12
OL	–	–	–	25–50	–	30–73	47	46–85	60	48		1
OW	–	–	–	–	–	–	–	–	–	20		1

Table 1. Continuation.

Host	*Dicentrarchus labrax* (L.)			*Syngnathus typhle* L.		*S. typhle*				*S. abaster* Risso		
Locality	Mediterranean Sea, Costal lakes and Gulf of Lion (France)			Baltic Sea (Germany)		Baltic Sea, Salzhaff Bay (Germany)		Black Sea, Budaki Lagoon, Sevastopol Bay, Gulf of Odessa (Ukraine)		Lake Atanasovsko Wetlands (Bulgaria)		
Source	Maillard (1973)			Reimer et al. (1996)		Kvach et al. (2018)				Present study		
Characters	Range	Mean	n	Range	Mean	Range	Mean	Range	Mean	Range	Mean ± SD	n
OVL	–	–	–	82–109	–	23–129	80	59–136	106	103–145	112	10
OVW	–	–	–	65–109	–	21–147	81	19–113	64	88–135	107	9
SRL	–	–	–	52–102	–	28–70	42	28–84	55	130–205	171	4
SRW	–	–	–	50–72	–	26–61	38	28–65	45	55–100	75	4
ATL	297–411	330	10	137–265	–	40–159	107	70–200	121	163–263	216	7
ATW	158–227	193	10	72–97	–	39–172	109	54–181	112	108–155	133	7
PTL	297–436	363	10	137–265	–	47–205	127	75–217	124	118–238	200	11
PTW	168–217	194	10	72–97	–	41–181	110	58–200	111	115–183	143	11
PTDPB	–	–	–	–	–	51–69	61	48–94	67	35–68	44	11
EGL	24–27	26	10	26–33	28	20–31	25	23–28	25	20–25	24 ±1.5	60
EGW	9–12	11	10	11–15	13	12–15	13	13–14	13	10–13	11±1.5	60
Ratios:												
BTL/BMW	6–11:1	9:1	10	5–10:1	7:1	–	–	–	–	7–10:1	9:1	11
OSW/VSW	1–1:1	1:1	10	1:1	–	–	–	–	–	1–2:1	1:1	11
OSW/PHW	–	–	–	–	–	–	–	–	–	2–3:1	2:1	12
BTL/ BMW%	–	–	–	–	–	5–12%	7%	6–10%	8%	10–14%	12%	11
FBL/BTL%	–	–	–	–	–	26–41%	31%	11–41%	31%	29–42%	34%	12

nal structures such as caeca and excretory vesicle (Fig. 1A), they have been observed in detail only in the subadult individuals (Fig. 1E). In addition, the circumoral spines have been observed in full number only in a few mature specimens; in most of the specimens, they were partially or completely lost.

Opisthorchiidae Looss, 1899
***Cryptocotyle concava* (Creplin, 1825) Lühe, 1899, metacercaria**

Site of infection: intestine.

Infection characteristics: P% (7.69%), I (1), MI (1), MA (0.08)

Description (based on one excysted specimen): Body oval, 343 × 193, slightly narrowing to posterior extremity (Fig. 2). Forebody and hindbody 60% and 40% of total length, respectively. Tegument spinous; spines tiny, uniform, lacking on posterior body extremity (Fig. 2). Eyespots two, dark-brown, one of them compact granulated masse, another strongly diffused; dorsolateral, approximately at level of intestinal bifurcation. Oral sucker primordial, circular, 45 × 45, subterminal (Fig. 2). Prepharynx absent. Pharynx elongate-oval, 35 × 25, muscular (Fig. 2). Oesophagus short, 25 × 10. Intestinal bifurcation in first third of body, at distance of 103 from anterior extremity of body. Caeca extending close to posterior extremity of body, curved behind testes (Fig. 2). Ventrogenital sac primordial, oval, 38 × 38, post-equatorial, median, with rudimental ventral sucker on anterior edge (Fig. 2). Genital primordia adjacent to excretory vesicle, in hind body. Ovary transversely-elongated, rather V-shaped, 89 × 15, pretesticular, at level between ventrogenital sac and excretory vesicle (Fig. 2). Testes two, transversely-oval, slightly lobed, 55 × 30 and 45 × 33, opposite, at level just posterior to excretory vesicle arms (Fig. 2). Excretory vesicle Y-shaped, robust, opens terminally via pore (Fig. 2).

Nematoda

Raphidascarididae Hartwich, 1954
***Hysterothylacium* cf. *aduncum*, third-stage larvae**

Site of infection: body cavity.

Infection characteristics: P% (7.69), I (5), MI (5), MA (0.38).

Identification based on five specimens.

Remarks: The same parasite species has previously been reported in the three-spined stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) from the same area (Stoyanov et al. 2023). Therefore, the present description is based only on the metrical data (Table 2). For morphology, see Fig. 3A–C.

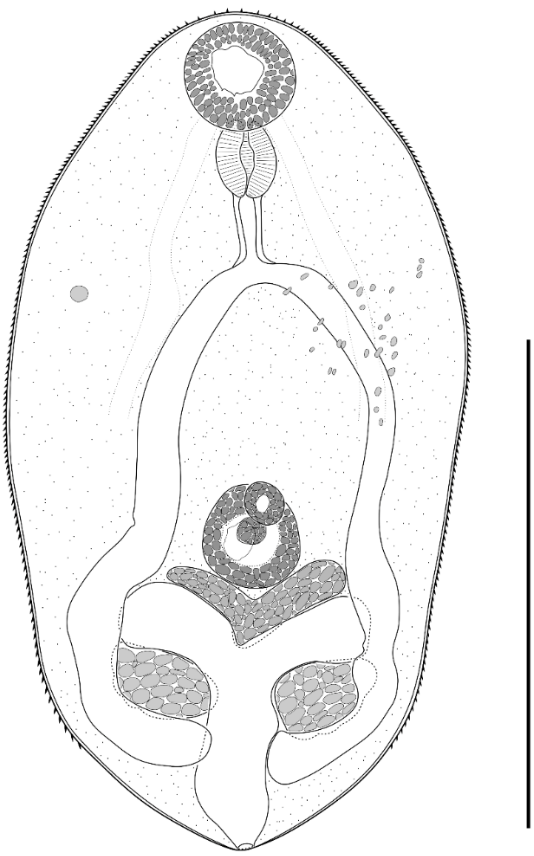

Fig. 2. *Cryptocotyle concava* (Creplin, 1825) Lühe, 1899, metacercaria. General view. Scale-bar: 200 µm.

Discussion

In the present study, we found three helminth species as parasites of *Syngnathus abaster*, i.e., the cryptogonimid trematode *Timoniella imbutiformis* (subadult and mature individuals), the opisthorchid trematode *Cryptocotyle concava* (metacercaria) and the raphidascaridid nematode *Hysterothylacium* cf. *aduncum* (third-stage larvae). Of them, *T. imbutiformis* was the most prevalent parasite species (15.38%), with relatively high intensity (18–24), mean intensity (21.00±3.00) and mean abundance (3.23±1.18). The other two helminth species have been found once and with low infection rates.

The morphology of the cryptogonimid trematode studied corresponds to the generic diagnosis of *Timoniella* Rebecq, 1960 (Miller & Cribb 2008). They are identified at species level based on the presence of 17–18 circumoral spines and caeca ending close to the posterior extremity of body (Fig. 1A, B). These characters are consistent with those in previous species descriptions (Maillard 1973, Brooks 1980, Kvach et al. 2018). Moreover, the present material has overlapping ranges with those in previews de-

Table 2. Metrical data on *Hysterothylacium* spp. (third-stage larvae), parasitising various fishes in Europe. Abbreviations (see Materials and Methods).

Species	***H. aduncum* (Rudolphi, 1802)**					***H. collare* (Cobb, 1929)**	***H. fabri* (Rudolphi, 1819)**	***H.* cf. *aduncum***					
Host	**Various teleosts**	**Various teleosts**			**Various teleosts**	***Symphodus tinca* (L.), *S. roissali* (Risso)**	**Various teleosts**	***Gasterosteus aculeatus* L.**			***Syngnathus abaster* Risso**		
Locality	**Black Sea basin**	**Eastern Mediterranean Sea**			**Europe**	**Black Sea, Crimea (Ukraine)**	**Europe**	**Lake Atanasovsko Wetlands (Bulgaria)**			**Lake Atanasovsko Wetlands (Bulgaria)**		
Source	**Gaevskaya et al. (1975)**	**Petter & Milard (1988)**			**Moravec (1994, 2013)**	**Gaevskaya et al. (1975)**	**Moravec (1994, 2013)**	**Stoyanov et al. (2023)**			**Present study**		
Character	**Range**	**Range**	**Mean ± SD**	**n**	**Range**	**Range**	**Range**	**Range**	**Mean**	**n**	**Range**	**Mean**	**n**
BTL	7,000–10,350	3,000–12,100	7,900	14	6,600–21,600	4,000–4,060	1,840–2,160	13,425–15,996	14,731	3	10,640–14,800	12,850	4
BMW	–	–	–	–	160–330	168–280	80–90	290–340	316	3	230–270	253	4
BWA	–	–	–	–	–	49–89	–	111	–	1	98–120	109	5
OL	795–1,230	510–1,260	895	14	1,000–2,000	420–532	190–200	1,490–1,850	1,612	3	1,150–1,555	1,404	5
OMW	–	–	–	–	–	23–30	–	60–100	75	3	50–100	71	5
VL	53–84	–	–	–	–	59–76	15–18	63–93	82	3	45–75	62	5
VW	50–74	–	–	–	–	53–56	18–27	55–90	72	3	50–83	72	5
VAL	330–525	300–690	478	14	400–680	254–294	750–880	560–620	594	3	243–535	368	5
VAW	22–47	–	–	–	–	–	–	51–62	57	3	40–70	58	5
ICL	375–630	200–660	457	14	330–920	323–511	15–30	550–771	689	3	308–515	434	5
ICW	40–56	–	–	–	–	33–89	–	91–105	100	3	28–95	69	5
NR	–	–	–	–	280–450	–	100–130	283–315	301	3	188–340	271	5
EP	–	–	–	–	310–500	–	120–140	350–390	370	3	230–445	358	5
TL	96–171	80–170	138	14	120–210	92–132	80–110	188	–	1	105–203	165	5
Ratios:													
OL/BTL	–	1 : 5.70–10.20	1 : 8.60±0.8	14	–	–	–	1 : 8.65–9.91	1 : 9.18	3	1 : 9.25–9.61	1 : 9.39	4
VAL/OL	–	1 : 1.30–2.80	1 : 2.00±0.2	14	–	–	–	1 : 2.64–6.05	1 : 4.42	3	1 : 2.52–6.40	1 : 4.21	5
ICL/VAL	–				–	–	–	1.10–1.38 : 1	1.22 : 1	3	0.96–1.90 : 1	1.25 : 1	5
ICL/OL	–	1 : 1.80–2.60	1 : 2.10±0.2	14	–	–	–	1 : 1.94–2.71	1 : 2.38	3	1 : 2.62–5.05	1 : 3.39	5

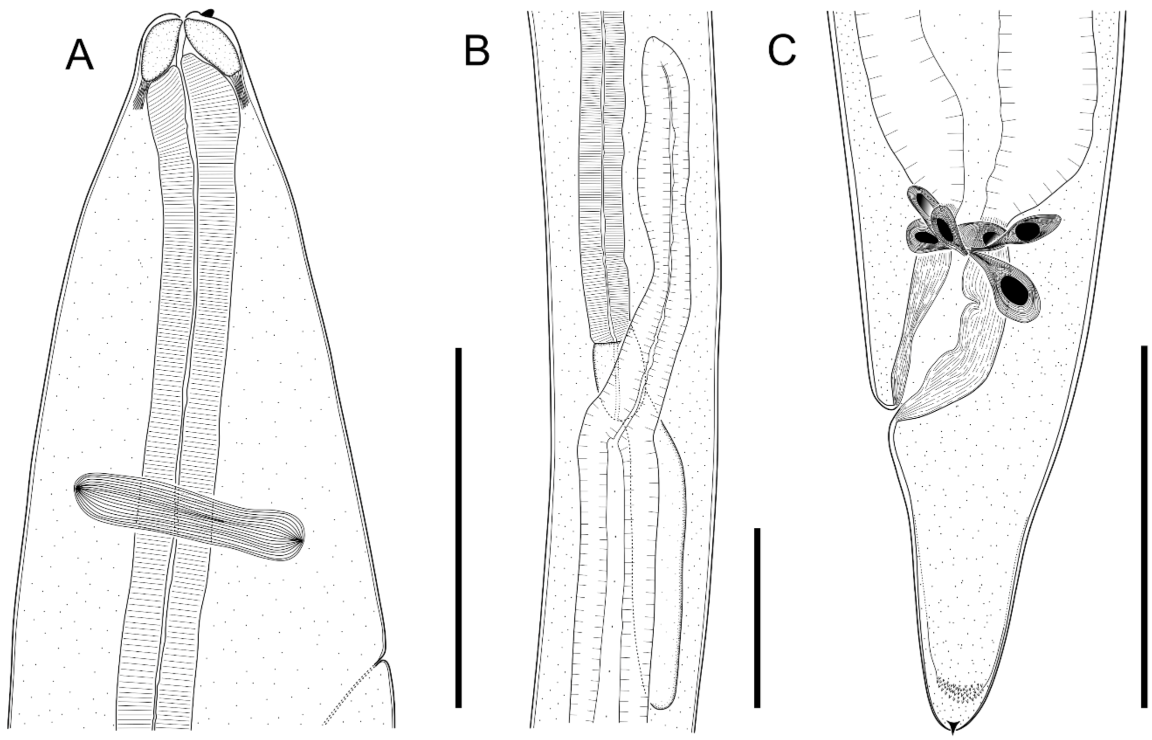

Fig. 3. *Hysterothylacium* cf. *aduncum*, advanced third-stage larva. A. Head end; B. Oesophago-intestinal junction. C. Tail. Scale-bars: 200 µm.

scriptions of *T. imbutiformis* in the most of the characters (Table 1). The metrical differences are minor and can be recognised as intraspecific variability.

The genus *Timoniella* is represented by six species (WoRMS 2023) parasitic in fishes and reptiles (Miller & Cribb 2008). Two of them, i.e., *T. praeterita* (Looss, 1901) Maillard, 1974 (type species) and *T. imbutiformis* (Molin, 1859) Brooks, 1980 have been reported as fish parasites from the European waters (Brooks 1980, McDowall & James 1988, El-Darsh & Whitfield 1999, Palm et al. 1999, Gibson 2001, Sterud 2002, Culurgioni et al. 2010, 2014, 2015, Gaevskaya 2012, Radujković & Šundić 2014, Kvach et al. 2018). Until recently, *T. balthica* (Reimer, Hildebrand, Scharberth & Walter, 1996) Miller & Cribb, 2008 was the third congener known from Europe (Reimer et al. 1996); however, a recent taxonomic study has proved it as a junior synonym of *T. imbutiformis* (see Kvach et al. 2018). The two species (*T. praeterita* and *T. imbutiformis*) have identical life-cycles, comprising gastropods of the genus *Hydrobia* Hartmann as a first intermediate host and teleost fishes as second intermediate and definitive hosts (Maillard 1973, 1974). They can be distinguished from one another by the average number of circumoral spines (22 *vs* 18) and the presence of cyclocoel opening via subterminal uroproct *vs* caeca ending close to posterior extremity of body in *T. praeterita* and *T. imbutiformis*, respectively (Maillard 1974). The morphological characteristics of the present material (Fig. 1A, B, E) clearly show its correspondence to *T. imbutiformis*.

Syngnathus abaster is a definitive host to the parasite studied. The pipefish has repeatedly been reported as its second intermediate or definitive host (Gaevskaya et al. 1975, Gaevskaya 2012, Moshu & Trombitskiy 2012, Kornyychuk et al. 2016, 2022, Kvach et al. 2018, Yuryshynets et al. 2023). *T. imbutiformis* is known as a euryxenous parasite of various gobiid, gobionellid, moronid, pleuronectid, atherinid, mugilid, syngnathid and gasterosteid species from the Northeastern Atlantic as well as from the Baltic, Mediterranean and Black Seas (Nicoll 1914, Maillard 1973, Reimer et al. 1996, El-Darsh & Whitfield 1999, Palm et al. 1999, Domnitch & Sarabeev 2000, Bartoli & Gibson 2007, Kvach & Oğuz 2009, Kvach 2010, Kornyychuk et al. 2016, 2022). It was the only species of this genus reported from the Black Sea basin (Gaevskaya et al. 1975, Domnitch & Sarabeev 2000, Kvach & Oğuz 2009, Kvach 2010, Gaevskaya 2012, Moshu & Trombitskiy 2012, Ondračková et al. 2012, Kornyychuk et al. 2016, 2022, Kvach et al. 2018, Yurakhno 2020). No data on the presence of *Timoniella* spp. have been known from Bulgarian waters (Kakacheva-Avramova 1983, Ondračková et al. 2012).

The morphology of the opisthorchid metacercaria found coincides with the generic diagnosis of *Cryptocotyle* Lühe, 1899 (Gaevskaya et al. 1975, Pearson 2008). It is identified at species level as *Cryptocotyle concava* based mainly on the opposite position of the testicular primordia. The testicle position in *Cryptocotyle* spp. is considered a species-specific characteristic (Gaevskaya et al. 1975, McDonald 1981, Martynenko 2016a). The parasite has been found in the intestine of a fish specimen studied. Due to the scarcity of the present material, however, any metrical comparisons would be unreliable.

The genus *Cryptocotyle* comprises 14 valid species (WoRMS 2023). Species of this genus have complex life-cycles involving hydrobiid and littorinid gastropods as first intermediate hosts, fishes as second intermediate hosts and fish-eating birds, rarely carnivore mammals and even humans, as definitive host (Wootton 1957, Sudarikov et al. 2006, Honcharov et al. 2022). Tatonova & Besprozvannykh (2019) recorded a bythiniid snail as a first intermediate host of *C. lata* Tatonova & Besprozvannykh, 2019 from the Russian Far East. *Cryptocotyle* spp. are known as stenoxenous parasites in relation to their first intermediate host and euryxenous to the second intermediate and definitive hosts. In heavy infections, they cause the so-called "black spot disease" resulting into exhaustion and dead of infected fish (Gaevskaya et al. 1975, Honcharov et al. 2022). In the Black Sea basin, four species of this genus have been recorded: *C. concava* (type-species), *C. cryptocotyloides* (Issaitschikov, 1923) Witenberg, 1929, *C. jejuna* (Nicoll, 1907) Ransom, 1920 and *C. lingua* (Creplin, 1825) Fischoeder, 1903 (Gaevskaya 2012). Three of them (*C. concava*, *C. jejuna* and *C. lingua*) are known from Bulgaria (Kakacheva-Avramova 1983, Yanchev et al. 1984, Chipev & Kostadinova 1995, Kostadinova 1995, 1997). *Cryptocotyle concava* has opposite testes (see Fig. 2) *vs* oblique testes in *C. jejuna* and *C. lingua* (Gaevskaya et al. 1975, McDonald 1981, Martynenko 2016a). Moreover, *C. concava* possesses slightly lobed testes (Fig. 2) *vs* deeply incised testes in *C. cryptocotyloides* (Gaevskaya et al. 1975, McDonald 1981). In addition, *C. concava* has oval body (Fig. 2) *vs* elongated in *C. jejuna* and *C. lingua* (Gaevskaya et al. 1975, McDonald 1981, Martynenko 2016a). Therefore, though based on a single specimen, this identification of this metacercaria seems justified.

Cryptocotyle concava has a Holarctic distribution (Martynenko 2016b), parasitising a variety of gobiid, gobionellid, pleuronectid, atherinid, osmerid, mullid, silurid, syngnathid and gasterosteid fish species (Wootton 1957, Naydenova 1974, Gaevskaya et al. 1975, Zander et al. 1984, Donoghue 1988, Alvik et al. 1995, Sudarikov et al. 2006, Krasnovyd et al. 2012, Kornyychuk et al. 2022, Duflot et al. 2023, Yuryshynets et al. 2023). *S. abaster* has previously been reported as its host from Ukraine, Moldova and Spain (Moshu & Trombitskiy 2012, Moshu 2014, Kornyychuk et al. 2022, Almeida et al. 2023, Yuryshynets et al. 2023). As adult, *C. concava* parasitizes the alimentary tract of various piscivorous birds of the families Gaviidae, Podicipedidae, Phalacrocoracidae, Ardeidae, Anatidae and Laridae (Gaevskaya et al. 1975). In Bulgaria, *C. concava* has been reported from gobiid fish species (*Gobius* sp.) from Lake Varna (Margaritov 1960, Kakacheva-Avramova 1983) as well as from fish-eating birds of the families Gaviidae, Ardeidae and Laridae (Yanchev et al. 1984, Chipev & Kostadinova 1995, Kostadinova 1995, 1997).

In the present survey, the raphidascaridid nematode larvae have been found free in the body cavity. The species identification in *Hysterothylacium* Ward & Magath, 1917 based on the larval morphology is considered uncertain (Moravec 1994, 2013, Gaevskaya 2005). Recently, we reported identical larvae in the three-spined stickleback *G. aculeatus* from the same area (Stoyanov et al. 2023).

The genus *Hysterothylacium* comprises 98 valid taxa (Nemys eds. 2023) parasitic in a variety of invertebrates and fishes serving as intermediate or paratenic hosts as well as predatory teleosts and some elasmobranchs as definitive hosts (Køie 1993, Moravec 1994, 2013, Gaevskaya 2005, Gaevskaya et al. 2010). Seventeen species of this genus are known from Europe (Gaevskaya et al. 1975, Kakacheva-Avramova 1983, Petter & Millard 1987, Moravec 1994, 2013, Gibson 2001, Gaevskaya 2012, Mattiucci et al. 2014). The morphology of the third-stage larva of only three species is known: *H. aduncum* (Rudolphi, 1802) Deardorff & Overstreet, 1981, *H. collare* (Cobb, 1929) Mosgovoy, 1951 and *H. fabri* (Rudolphi, 1819) Deardorff & Overstreet, 1980 (Gaevskaya et al. 1975, Moravec 1994, 2013). Morphologically, our larvae resembled those of *H. aduncum* (third-stage larva). They possessed a conical tail with a mucron at the tip, provided inside with the caudal process of the next stage of development (Fig. 3C), corresponding to that of the advanced third-stage larva of *H. aduncum* (see Fig. 172 C, D of Moravec 2013). Furthermore, our larvae have overlapping ranges in the most of the characters studied with those in previews descriptions of *H. aduncum* (Table 2), being most similar to *H. cf. aduncum* from *G. aculeatus* from the same habitat

(Table 2). Few minor differences can be pointed out: larger body maximum width (maybe due to preparation on slide), ventricular appendix length, intestinal caecum length as well as larger average body total length, intestinal caecum width and smaller length ratio of intestinal caecum to oesophagus (Table 2). However, our larvae have considerable metrical differences in the most of the characters compared to the other two European congeneric species with known larval morphology (Table 2). Furthermore, the present material has cuticle with very fine transverse striations and tail with mucron (Fig. 3A, B, C) *vs* deeply striated cuticle and tail without mucron in *H. collare* (larvae) (Gaevskaya et al. 1975). In comparison, our larvae have intestinal caecum and ventricular appendix of almost similar size *vs* markedly short intestinal caecum and much longer ventricular appendix in *H. fabri* (third-stage larvae) (Table 2). Furthermore, they possess conical tail with mucron (Fig. 3C) *vs* tail with broadly rounded tip without mucron in *H. fabri* (Moravec 2013). The reliable species identification of the species of this genus occurring in the studied wetlands needs future studies, especially based on molecular comparison of larval and adult nematodes.

Third-stage larvae and an immature female of *H. aduncum* were recorded in the black-striped pipefish from the Black Sea (Kornyychuk et al. 2022). This nematode species is a cosmopolitan euryxenous parasite of a great variety of marine, estuarine and freshwater fish species (Margaritov & Dimov 1969, Gaevskaya et al. 1975, 2010, Moravec 1994, 2013, Gaevskaya 2005). In the Black Sea basin, ca. 50 fish species have been recorded as its second intermediate or definitive hosts (Gaevskaya et al. 2010). Dimitrov (1989) reported 28 teleosts and elasmobranchs as second intermediate and definitive hosts of this parasite species off the Bulgarian Black Sea coast. So far, it has been reported as a parasite of *S. abaster* only from Ukraine (Kornyychuk et al. 2022). Tepe & Oğuz (2013) reported *H. aduncum* as a parasite of the greater pipefish *Syngnathus acus* L. from the southeastern Black Sea (Artvin Province, Turkey). This nematode has not been found in *S. abaster* in Bulgaria so far.

The Lake Atanasovsko Wetlands are home of a great variety of aquatic and water-related terrestrial invertebrates (Hubenov et al. 2015), which favour the life-cycles of trophically transmitted fish parasites (see also Stoyanov et al. 2015, 2018). Moreover, these wetlands harbour a species-rich avifauna comprising 39 piscivorous bird species (Dimitrov et al. 2005) providing a solid basis for the development of avian parasites (Stoyanov et al. 2017a, b, 2018, 2023). On the other hand, however, the species-poor fish fauna of these wetlands is a prerequisite to a limitation on the species richness and diversity of the local parasite communities (Stoyanov et al. 2017b, 2018, 2023, present study). The helminth species richness recorded in *S. abaster* in Lake Atanasovsko represents a negligible proportion of the known diversity recorded in its whole geographical range consisting of 85 helminth species – 46 trematode, 3 monogenean, 15 cestode, 15 nematode and 6 acanthocephalan taxa (Kurochkin 1964, Prévot 1973, Gaevskaya & Kornyychuk 2003, Semenova et al. 2007, Miroshnichenko 2008, Yevtushenko 2008, Amin et al. 2011, Gaevskaya 2012, Moshu & Trombitskiy 2012, Ondračková et al. 2012, Moshu 2014, Kornyychuk et al. 2022, Almeida et al. 2023, Yuryshynets et al. 2023). The possible explanations of the small diversity of helminth parasites in *S. abaster* in Lake Atanasovsko may include both small sample size and peculiar conditions in the wetlands differing from those in marine and riverine habitats.

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