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Helminth Parasites of the Three-spined Stickleback *Gasterosteus aculeatus* L., 1758 (Actinopterygii: Gasterosteidae) from a Black Sea Coastal Wetland, Bulgaria

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Abstract: Totally, 134 individuals of *Gasterosteus aculeatus* from the Lake Atanasovsko Wetland, Bulgaria, were examined for helminth parasites. Five helminth species were identified: the trematode *Posthodiplostomum brevicaudatum* (metacercariae), the monogenean *Gyrodactylus arcuatus*, the trypanorhynch cestode *Progrillotia dasyatidis* (plerocerci) and third-stage larvae of the ascaridoid nematodes *Contracaecum* sp. and *Hysterothylacium* sp. Of them, *G. arcuatus* was the most prevalent species, with prevalence (P%) 68.66, followed by *P. brevicaudatum* (P% 22.39). The remaining species were weakly represented: *Hysterothylacium* sp. (P% 4.48), *P. dasyatidis* (P% 3.73) and *Contracaecum* sp. (P% 0.75). The present study is the first record of *G. aculeatus* as a host of a species of *Contracaecum* from the Black Sea basin. *Gyrodactylus arcuatus* is a new record for the Bulgarian fauna.

Key words: *Gasterosteus aculeatus*, helminths, parasites, Black Sea

Introduction

The three-spined stickleback *Gasterosteus aculeatus* L. is a small-sized euryhaline fish occurring in the temperate and subarctic shallow coastal and inland waters throughout the Holarctic Region (Wootton 1976, Mattern 2007). Globally, it has been known as a host of more than 100 helminth species (Wootton 1976, Hoffman 1999, Barber 2007, 2013, Poulin et al. 2011, Moravec 2013). In Bulgaria, *G. aculeatus* has been recorded as a host of only three parasitic worm species: the trematode *Posthodiplostomum brevicaudatum* (von Nordmann, 1832) (metacercariae) (Stoyanov et al. 2017a), the monogenean *Gyrodactylus rarus* Wegener, 1910 (Margaritov 1959) and the trypanorhynch cestode *Progrillotia dasyatidis* Beveridge, Neifar & Euzet, 2004 (plerocerci) (Stoyanov & Georgiev 2022).

Lake Atanasovsko Wetland is an area of a primary conservation importance; it has been declared as a Ramsar site and a nature reserve (Vassilev et al. 2013). It is characterised by a low diversity of fish species (eighth species in total) but two of them, *Knipowitschia caucasica* (Berg) and *G. aculeatus*, are highly abundant (Stefanov 2006). The helminth fauna of *K. caucasica* was characterised in a previous work (Stoyanov et al. 2018). The aim of the present survey is to examine the helminth fauna in

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the three-spined stickleback from the Lake Atanasovsko Wetland.

Materials and Methods

Totally, 134 individuals of *G. aculeatus* (42 from brackish and 92 from freshwater habitats) were examined in May, July and September 2012–2013 from the northern part of the Lake Atanasovsko Wetland (42°34′42″N, 27°28′27″E). No fish were caught in September 2012. This wetland represents a complex of highly variable in their salinity water bodies, from freshwater and brackish (5–25 ‰) to hyperhaline (150–340 ‰) shallow coastal habitats; these include a lagoon partially used in salt production, a system of drainage and supply canals and marshes surrounding the lagoon (Ivanov et al. 1964, Vassilev et al. 2013).

The fish was caught by seines and traps and kept alive in containers with aerated water taken from the respective habitats. In the laboratory, each fish was dissected under a stereomicroscope within the next 24 hours. The recovered helminths were fixed in hot saline and transferred to 70 % ethanol. Subsequently, the monogeneans were mounted in glycerine-jelly microscopy slides (Gussev 1983). They were deposited in the Helminthological Collection of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Accession Nos. IBER-BAS M0162.1.1–4.7. The nematodes were studied as temporary glycerine mounts. The processing of trematodes and cestodes was previously described (Stoyanov et al. 2017a, Stoyanov & Georgiev 2022).

The measurements taken from monogeneans (Table 1) and the related terminology followed Malmberg (1970): BTL, body total length; BTW, body total width; CPL, cirrus pouch length; CPW, cirrus pouch width; DBMW, dorsal bar median width; DBTL, dorsal bar total length; HL, haptor length; HPL, hamulus point length; HRL, hamulus root length; HSL, hamulus shaft length; HTL, hamulus total length; HW, haptor width; MHFLL, marginal hook filament loop length; MHSDW, marginal hook sickle distal width; MHSHL, marginal hook shaft length; MHSL, marginal hook sickle length; MHSPW, marginal hook sickle proximal width; MHTL, marginal hook total length; PAPL, pharynx anterior part length; PAPW, pharynx anterior part width; PPPL, pharynx posterior part length; PPPW, pharynx posterior part width; PTL, pharynx total length; PTW, pharynx total width; VBBW, ventral bar basal width; VBMBL, ventral bar membrane length; VBMDP, ventral bar maximal distance between processes; VBMW, ventral bar median width; VBPL, ventral bar processes length; VBTL, ventral bar total length; VBTW, ventral bar total width.

The measurements of nematodes (Tables 2 and 3) and related terminology followed Moravec (1994, 2013): BMW, body maximum width; BTL, body total length; BWA, body width at anus; BWV, body width at ventriculus; EP, excretory pore, distance from anterior extremity; ICL, intestinal caecum length; ICW, intestinal caecum maximum width; IL, intestine length; IW, intestine maximum width; NR, nerve ring, distance from anterior extremity; OL, oesophagus length; OW, oesophagus maximum width; TL, tail length; VAL, ventricular appendix length; VAW, ventricular appendix maximum width; VL, ventriculus length; VW, ventriculus width.

The metrical data are presented as a range, followed by the mean and the number of measurements taken (n) in parentheses. The standard deviation is given only when n≥30. All measurements are in micrometres. The definitions of prevalence (P%), intensity (I, Range), mean intensity ± standard error (MI±SE) and mean abundance ± standard error (MA±SE), follow Bush et al. (1997).

Results

We found five helminth species from *G. aculeatus* from the Atanasovsko Lake wetland: one trematode, one monogenean, one cestode and two nematode species.

Trematoda

Family Diplostomidae Poirier, 1886
***Posthodiplostomum brevicaudatum* (von Nordmann, 1832), metacercariae**

Site of infection: Vitreous humour of the eye.

Infection characteristics: P% (22.39), I (1–10), MI (4.13±0.52), MA (0.93±0.19).

Description: see Stoyanov et al. (2017a).

Monogenea

Family Gyrodactylidae Cobbold, 1864
***Gyrodactylus arcuatus* Bychowsky, 1933**

Site of infection: skin, fins and gills.

Infection characteristics: P% (68.66), I (1–815), MI (49.62±12.54), MA (34.07±8.82).

Description (based on 35 specimens; for metrical data, see Table 1): Body fusiform, with greatest width approximately at level of uterus containing embryo (Fig. 1). Haptor transversely-oval, rarely circular, distinct. Hamuli strong, parallel, shaft slender; point slightly curved, sometimes straight, longitudinal groove not observed; root straight, stout (Fig. 2A, D). Ventral bar, median portion rectangular, with numerous longitudinal ridges; bases irregular in shape or approximately triangular; processes large, claviform, forwardly directed; membrane tongue-shaped, with numerous longitudinal ridges, outer edge well-defined, in few specimens entirely thickened; tip broadly rounded (Fig. 2A, D). Dorsal bar thin, with arch-shaped median portion, sometimes almost straight, with postero-medial notch (Fig. 2A, D). Marginal hook short, sickle point slender, downwardly inclined, not extended beyond sickle toe; sickle heel well-defined, prominent, rounded; sickle toe rhomboid, with relatively steep slope and pointed tip; filament loop gentle, attached to sickle heel, not exceeding half-length of hook shaft; hook shaft gentle, slender, distal end without narrowing, proximal end slightly expanded (Fig. 2B, C, E). Cirrus pouch globular, located posteriorly to pharynx; armed with 6–7 tiny spines arranged in single arched row and single, large, hook-like apical spine (Fig. 2F, G). Pharynx bipartite; anterior and posterior parts transversely-oval or globular, with small processes.

Cestoda

Family Progrillotiidae Palm, 2004
***Progrillotia dasyatidis* Beveridge, Neifar & Euzet, 2004, plecocerci**

Site of infection: Gallbladder.

Infection characteristics: P% (3.73), I (1–7), MI (2.40±1.17), MA (0.09 ± 0.06).

Description: see Stoyanov & Georgiev (2022).

Nematoda

Family Anisakidae Railliet & Henry, 1912
***Contracaecum* sp. 3, third-stage larva**

Site of infection: body cavity.

Infection characteristics: P% (0.75), I (1), MI (1.00), MA (0.01).

Description (based on one specimen; for metrical data, see Table 2): Body elongated, fusiform, with maximum width at level posterior to oesophageal-intestinal junction. Cuticle transversely striated, more visibly at anterior extremity, without spines (Fig. 3A). Anterior body end rounded, with weekly-developed lips and ventral conical larval toot (Fig. 3A). Excretory pore just posteriorly to larval toot (Fig. 3A). Cervical papillae minute, at level of nerve ring. Nerve ring oblique, approximately at middle of first half part of oesophagus. Oesophagus elongated, muscular, with oval ventriculus and posterior claviform appendix at proximal end (Fig. 3B). Intestine thick-walled, with relatively broad lumen, with anterior caecum (Fig. 3B). Intestinal caecum thick-walled, strongly elongated, with rounded tip (Fig. 3B), extended at level posterior to nerve ring. Rectum short, tubular, at proximal end surrounded by aggregation of glandular cells (Fig. 3C). Genital primordium, indistinct. Tail conical, with subterminal constriction, forming terminal pointed portion (Fig. 3C), representing 1.24 % of body length.

Fig. 1. *Gyrodactylus arcuatus* Bychowsky, 1933. General view. Scale-bar: 100 µm.

Family Raphidascarididae Hartwich, 1954

***Hysterothylacium* cf. *aduncum* (Rudolphi, 1802), third-stage larvae**

Site of infection: body cavity and liver.

Infection characteristics: P% (4.48), I (1–2), MI (1.33±0.21), MA (0.06±0.03).

Description (based on three well-preserved specimens; for metrical data, see Table 3): Body elongated, fusiform, maximum width at level posterior to oesophageal-intestinal junction. Cuticle

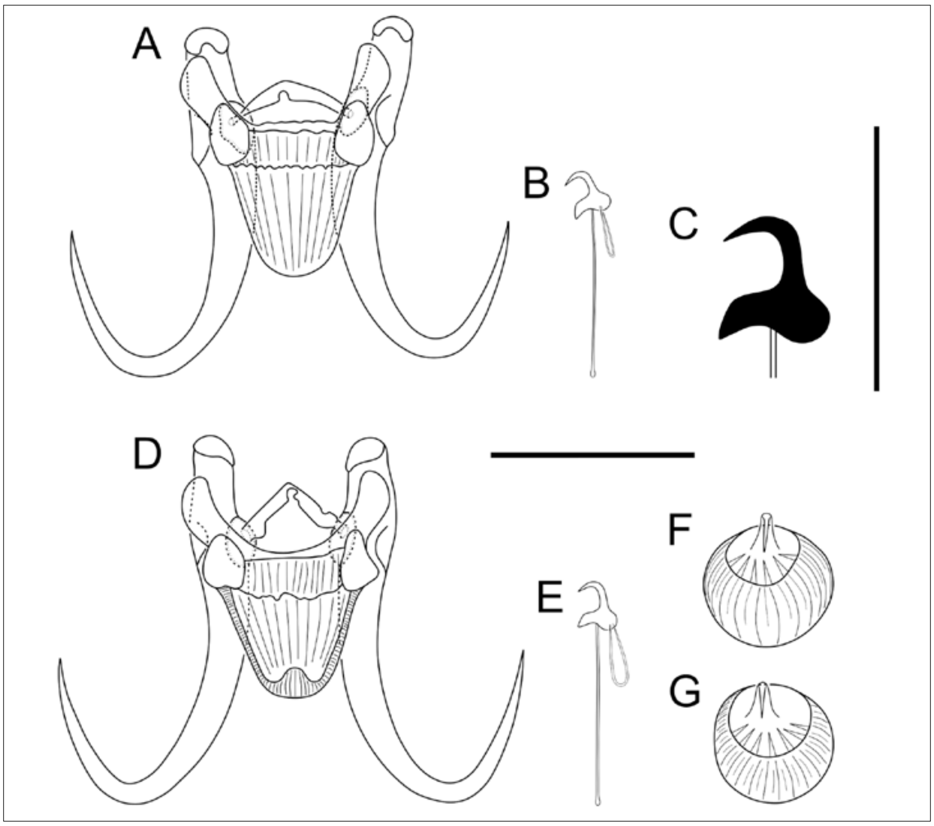

Fig. 2. *Gyrodactylus arcuatus* Bychowsky, 1933. **A–E** Haptoral sclerites: **A, D** Hamuli and connective bars, **B, E** Marginal hooks, **C** marginal hook sickle; **F, G** Variations in the structure of cirrus. Scale-bars: A, B, D–G 20 µm; C 10 µm.

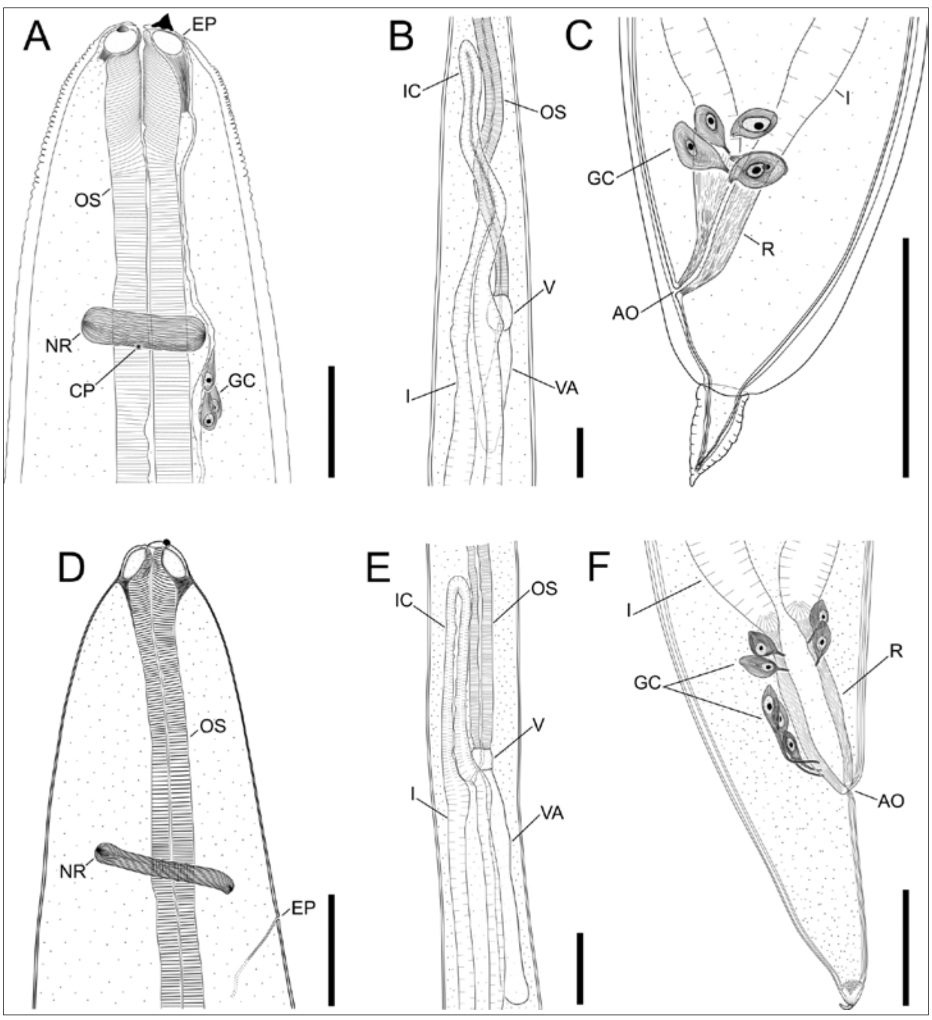

Fig. 3. Ascaridoid nematodes from *Gasterosteus aculeatus* from Atanasovsko Lake wetland. **A**–**C.** *Contracaecum* sp. 3, third-stage larva. **A.** Head end. **B.** Oesophago-intestinal junction. **C.** Tail. **D**–**F.** *Hysterothylacium* cf. *aduncum*, advanced third-stage larva. **D.** Head end; **E.** Oesophago-intestinal junction. **F.** Tail. **Scale-bars:** A, 100 µm; B–F, 200 µm. **Abbreviations:** AO, anal opening; CP, cervical papilla; EP, excretory pore; GC, glandular cells; GC, glandular cells; I, intestine; IC, intestinal caecum; NR, nerve ring; OS, oesophagus; R, rectum; V, ventriculus; VA, ventricular appendix.

Table 1. Metrical data of *Gyrodactylus* spp. from *Gasterosteus aculeatus* from the Holarctic Region. For abbreviations, see Materials and Methods.

Species	*G. alexanderi*	*G. avalonia*	*G. canadensis*	*G. branchicus*	*G. gasterostei*	*G. arcuatus*		
Locality	Stow Lake, California (USA)	Lake Ontario, Bay of Quinte (Canada)	Broad Lake, Bellevue (Canada)	North Sea, Gullmarsfjorden (Sweden)	Different localities (Germany)	Baltic Sea, Nämdö Island (Sweden)	River Rother and Harting Pond (UK)	Lake Atanasovsko Wetland (Bulgaria)
Sources	Mizelle & Kritsky (1967)	Hanek & Fernando (1971)	Hanek & Threlfall (1969)	Malmberg (1970)	Gläser (1974)	Malmberg (1957)	Harris (1985)	Present study
Characters	Range (Mean; n)	Range	Range	Range (Mean; n)	Range (n ~ 70)	Range (Mean; n)	Range (Mean ± SD; n)	Range (Mean ± SD; n)
BTL	598–841 (698; 19)	615–710	–	376–680 (468; 10)	550–950	280–490 (460; 10)	–	240–478 (320±51; 35)
BTW	97–204 (128; 19)	94–127	–	92–168 (119; 9)	130–210	80–120 (110; 10)	–	50–183 (89±26; 35)
HL	81–130 (101; 19)	69–78	–	64–120 (82; 10)	90–140	50–90 (80; 10)	–	27–57 (43±8; 34)
HW	98–151 (131; 19)	80–110	–	96–160 (123; 10)	110–180	70–120 (90; 10)	–	40–76 (60±9; 34)
PTL	–	–	–	41–64 (49; 10)	40–75	30–39 (34; 10)	–	25–40 (33; 20)
PTW	–	–	–	40–63 (47; 10)	35–70	37–47 (42; 10)	–	30–50 (35; 20)
PAPL	–	–	–	–	–	13–21 (18; 10)	–	14–28 (20; 20)
PAPW	–	–	–	40–63 (46; 10)	–	–	–	22–48 (31; 20)
PPPL	–	–	–	–	–	14–18 (16; 10)	–	10–22 (13; 20)
PPPW	–	–	–	37–60 (44; 10)	–	–	–	30–50 (35; 20)
CPL	–	–	–	10–15 (12; 6)	–	11–16 (14; 10)	–	7–15 (11; 23)
CPW	–	–	–	10–15 (13; 7)	15–20	12–16 (13; 10)	–	7–15 (12; 23)
HTL	72–82 (79; 19)	37–43	55–56	47–52 (49; 20)	53–69	40–46 (42; 10)	35–43 (39±2; 12)	33–43 (37±3; 69)
HSL	–	30–32	43–45	39–43 (41; 20)	40–52	–	28–34 (31±2; 12)	26–33 (29±2; 69)
HPL	–	17–18	13–14	14–15 (15; 20)	26–35	17–20 (19; 10)	17–24 (15.5±1.0; 12) Sic!	13–20 (16±2; 69)
HRL	12–19 (15; 19)	10–13	18–19	17–21 (18; 20)	15–23	8–12 (10; 10)	7–10 (8±1; 12)	5–13 (10±2; 69)
VBTL	37–44 (40; 19)	19–20	24–25	20–22 (21; 10)	20–30	22–27 (24; 10)	18–23 (21±1; 12)	15–23 (17±2; 35)
VBTW	–	–	–	20–23 (22; 9)	–	13–16 (14; 10)	17–20 (18±1; 12)	10–30 (22; 25)
VBMW	–	4–5	5–6	4–5 (5; 10)	5–7	–	–	3–5 (4±1; 35)
VBBW	–	–	–	7–10 (8; 20)	–	–	–	5–7 (6±1; 70)
VBPL	–	8–9	–	0.4–1.8 (0.8; 19)	2.0–3.5	–	6–8 (8±1; 12)	6–10 (7±1; 70)
VBMDP	–	–	–	18–21 (19; 9)	–	–	–	17–24 (21±2; 35)
VBMBL	–	12–13	–	12–15 (14; 9)	11–15	10–17 (13; 10)	7–10 (9±1; 12)	7–14 (11; 25)
DBTL	14–27 (18; 19)	7–18	21–22	21–25 (23; 9)	20–30	17–22 (19; 10)	10–14 (13±1; 12)	10–22 (16±3; 35)
DBMW	–	1–2	1.5	0.4–1.3 (1.1; 10)	1.3–2.5	1–2 (2; 10)	–	1–2 (2±0.4; 35)
MHTL	42–44 (43; 19)	21–23	40–41	28–32 (30; 10)	29–37	22–25 (22; 10)	19–25 (22±1; 12)	15–24 (21±1; 129)
MHSHL	–	–	–	22–25 (23; 10)	25–32	17–21 (19; 10)	14–18 (17±1; 12)	10–20 (16±1; 129)
MHSL	10–12 (11; 19)	4	7–8	7–8 (7; 10)	5.3–6.0	5–6 (5; 10)	4–6 (5±0.2; 12)	4–5 (4±0.4; 129)
MHSPW	–	–	–	4.8–5.3 (5; 10)	–	3–5 (4; 10)	–	3–4 (3±0.4; 129)
MHSDW	–	–	–	4–5 (5; 10)	–	–	–	3–3 (3±0.0; 129)
MHFLL	–	–	–	7–9 (8; 10)	9–12	7–7 (7; 10)	–	5–7 (6±1; 112)
Ratios:								
HL/BTL	–	–	–	1:4.7–7.0 (1:5.7; 10)	–	1:4.9–7.0 (1:6.4; 10)	–	1:5.0–11.3 (1:7.7±1.6; 34)
HRL/HSL	–	–	–	1:2.1–2.4 (1:2.3; 20)	–	–	–	1:2.4–5.6 (1:3.2±0.6; 69)

Table 2. Metrical data of *Contracaecum* spp. (third-stage larvae) harboured by various fish species from Europe. For abbreviations, see Materials and Methods. Legend: *When naturally infected fish – larvae larger; [a] From experimentally infected fish; [b] In a more advanced larva.

Species	***C. microcephalum***	***C. micropapillatum***	***C. osculatum***	***C. ovale***	***C. rudolphii***	***C. multipapillatum***	***Contracaecum* sp. 1**	***Contracaecum* sp. 2**	***Contracaecum* sp. 3**
Host	Various fishes					*C. auratus* (Risso)	*L. gibbosus* (L.)	*K. caucasica* (Berg)	*G. aculeatus* L.
Locality	Europe	Europe	Europe	Europe	Europe	Black Sea (Ukraine)	Lake Atanasovsko Wetland (Bulgaria)		
Source	Moravec (2013)					Pronkina & Spiridonov (2018)	Stoyanov et al. (2017)	Stoyanov et al. (2018)	Present study
Character	Range	Range [a]	Range	Range	Range	Range (Mean±SD; n)	Range (Mean; n)	Range (Mean; n)	n = 1
BTL	1,310–2,000[a] or 3,600–7,800*	430–6,000	3,810–22,000	3,800–4,100	435–450 or 3,970–4,870[b]	14,274–29,750 (23,266±816; 19)	2,632–3,681 (3,157; 2)	19,000–26,591 (22,796; 2)	13,141
BMW	56–70[a] or 150–240*	10–40	150–530	21–25	14–18 or 177–245[b]	575–1,125 (849±29; 19)	105–165 (135; 2)	870–1,050 (960; 2)	470
BWA	–	–	–	–	–	88–200 (148±9; 19)	43–63 (53; 2)	202–826 (514; 2)	156
OL	210–230[a] or 400–480*	110–600	550–1,710	600	476–525	–	278–415 (347; 2)	4,750	1,680
OW	–	–	–	–	–	–	13–25 (19; 2)	100	80
VL	16–17[a]	–	–	40–50	27–36	88–188 (123±6; 19)	16–25 (21; 2)	150	124
VW	10–20[a]	–	–	30–40	24–36	88–188 (142±6; 19)	17–18 (18; 2)	100	110
VAL	190–240[a] or 420–500*	80–570	540–1,580	680–800	449–552	500–760 (658±18; 19)	225–410 (318; 2)	700	530
VAW	–	–	–	–	–	100–160 (132±3; 19)	21–41 (31; 2)	150	111
NR	116–120[a]	50–60	–	–	171–219	179–375 (253±11; 19)	125–160 (143; 2)	270–380 (325; 2)	250
ICL	6–43[a] or 210–270*	10–500	290–890	350–400	294–318	1,527–3,500 (2,227±106; 19)	128–185 (157; 2)	2,780	1,130
ICW	–	–	–	–	–	125–550 (327±21; 19)	26–44 (35; 2)	332	150
TL	50–90[a]	50–150	90–210	–	90–120	125–280 (208±13; 20)	95–105 (100; 2)	121–292 (207; 2)	163
Ratios:									
VAL/OL	–	1 : 1.0–1.3	–	–	–	–	1 : 1.01–1.23 (1 : 1.12; 2)	1 : 6.78 (n = 1)	1 : 3.17
ICL/VAL	1 : 3–4[b]	–	–	–	–	3 : 1	1 : 1.75–2.21 (1 : 1.98; 2)	3.97 : 1 (n = 1)	2.13 : 1
ICL/OL	–	–	–	–	–	–	1 : 2.17–2.24 (1 : 2.20; 2)	1 : 1.71 (n = 1)	1 : 1.49

Table 3. Metrical data of *Hysterothylacium* spp. (third-stage larvae) harboured by various fishes from Europe. For abbreviations, see Materials and Methods.

Species	***H. aduncum***				***H. collare***	***H. fabri***	***H. cf. aduncum***
Host	Various teleost fishes	Various teleost fishes	Various teleost fishes	*Trachurus trachurus* (L.), *Micromesistius poutassou* (Risso), *Engraulis encrasicolus* (L.)	*Symphodus tinca* (L.), *S. roissali* (Risso)	Various teleost fishes	*Gasterosteus aculeatus* L.
Locality	Black Sea basin	Eastern Mediterranean Sea	Europe	Barcelona market (Spain)	Black Sea basin	Europe	Lake Atanasovsko Wetland (Bulgaria)
Source	Gaevskaya et al. (1975)	Petter & Millard (1988)	Moravec (1994, 2013)	Roca-Geronès et al. (2018b)	Gaevskaya et al. (1975)	Moravec (1994, 2013)	Present study
Character	Range	Range (Mean; n)	Range	Range (Mean; n)	Range	Range	Range (Mean; n)
BTL	7,000–10,350	3,000–12,100 (7,900; 14)	6,600–21,600	5,880–15,800 (9,390; 19)	4,000–4,060	1,840–2,160	13,425–15,996 (14,731; 3)
BMW	–	–	160–330	213–385 (276; 19)	168–280	80–90	290–340 (316; 3)
BWA	–	–	–	80–128 (102; 19)	49–89	–	111 (n=1)
OL	795–1,230	510–1,260 (895; 14)	1,000–2,000	880–1,800 (1,239; 17)	420–532	190–200	1,490–1,850 (1,612; 3)
OW	–	–	–	–	23–30	–	60–100 (75; 3)
VL	53–84	–	–	45–130 (90; 15)	59–76	15–18	63–93 (82; 3)
VW	50–74	–	–	–	53–56	18–27	55–90 (72; 3)
VAL	330–525	300–690 (478; 14)	400–680	340–690 (527; 14)	254–294	750–880	560–620 (594; 3)
VAW	22–47	–	–	–	–	–	51–62 (57; 3)
ICL	375–630	200–660 (457; 14)	330–920	290–930 (527; 16)	323–511	15–30	550–771 (689; 3)
ICW	40–56	–	–	–	33–89	–	91–105 (100; 3)
NR	–	–	280–450	255–445 (347; 16)	–	100–130	283–315 (301; 3)
EP	–	–	310–500	268–535 (397; 17)	–	120–140	350–390 (370; 3)
TL	96–171	80–170 (138; 14)	120–210	110–255 (168; 19)	92–132	80–110	188 (n=1)
Ratios:							
OL/BTL	–	1 : 5.70–10.20 (1 : 8.60±0.8; 14)	–	–	–	–	1 : 8.65–9.91 (1 : 9.18; 3)
VAL/OL	–	1 : 1.30–2.80 (1 : 2.00±0.2; 14)	–	–	–	–	1 : 2.64–6.05 (1 : 4.42; 3)
ICL/VAL	–	–	–	–	–	–	1.10–1.38 : 1 (1.22 : 1; 3)
ICL/OL	–	1 : 1.80–2.60 (1 : 2.10±0.2; 14)	–	–	–	–	1 : 1.94–2.71 (1 : 2.38; 3)

with delicate transverse striations, more visible at body extremities, without spines (Fig. 3D). Anterior body end with primordial lips and small ventral larval tooth (Fig. 3D). Excretory pore situated slightly posterior to nerve ring (Fig. 3D). Nerve ring oblique, approximately at middle of first half of oesophagus. Oesophagus strongly elongated, muscular, at proximal end with oval ventriculus and claviform, posterior ventricular appendix (Fig. 3E). Intestine thick-walled, with broad lumen and anterior caecum. Intestinal caecum finger-shaped (Fig. 3E), ended posterior to nerve ring. Rectum straight, short, partially surrounded by aggregations of glandular cells (Fig. 3F). Genital primordium indistinct. Tail conical, provided inside with caudal process of next stage of development, with mucron (Fig. 3F).

Discussion

Based on the examination of 134 individuals of *G. aculeatus*, we recorded five helminth species from the Lake Atanasovsko Wetland: one trematode, one monogenean, one cestode and two nematode species. Previously, we have provided morphological data and commented the identification and biology of the digenean *Posthodiplostomum brevicaudatum* (see Stoyanov et al. 2017a) and the cestode *Progrillotia dasyatidis* (see Stoyanov & Georgiev 2022). In this article, we describe and comment the monogeneans and nematodes from this host species.

Monogeneans

The monogeneans were identified as *G. arcuatus* based on the morphology of haptoral sclerites, which was consistent with the descriptions by Malmberg (1957, 1970) and Harris (1985). The identification was also supported by the overlapping ranges of most metrical characters with those in previous descriptions (Table 1). *Gasterosteus aculeatus* is the type-host of this parasite. The present specimens possessed some morphological differences compared to the previous descriptions. Five specimens had a distinct ventral bar membrane with thickened outer edge along its entire length (Fig. 2 D); this character had not been pointed out by previous studies of this species (Malmberg 1957, 1970, Harris 1983, 1985, Ergens 1985, Huyse et al. 2004, Paladini et al. 2009). Using SEM, Shinn et al. (1993) reported a partial thickening of the outer edge of the ventral bar membrane. In addition, the present worms had smaller average body total length and width, haptor length and width, pharynx total width, hamulus total length and ventral bar total length as well as bigger average ventral bar total width than the same characters as described by Malmberg (1957) (Table 1). Harris (1985) reported larger average ventral bar total length and smaller average ventral bar total width than the same measurements in our specimens (Table 1). Previous studies described considerable morphological variations of *G. arcuatus* (Malmberg 1964, 1970, Harris 1993), even in the frame of one geographical locality (Huyse et al. 2004). Moreover, the variations observed by us were minor and could be attributed to environmental factors (temperature and salinity), host size or host species affiliation. In gyrodactylids, the water temperature is a key factor influencing the size of attachment hard parts (Malmberg 1970, Ergens 1976, 1991, Ergens & Gelnar 1985, Mo 1991, 1993, Appleby 1996, Geets et al. 1999, Dmitrieva & Dimitrov 2002, Dávidová et al. 2005). In *G. arcuatus*, host size-dependent differences in the measurements of haptoral sclerites (Malmberg 1964) and body (Malmberg 1970) have been observed. In addition, the sexual reproduction of this species could lead to higher heterozygosity and, thus, to higher morphological variability (Harris 1993, Huyse et al. 2004). The differences recorded by us contribute to the knowledge of the intraspecific variation of the species.

Gyrodactylus arcuatus was originally described from fins and gills of the three-spined stickleback from the freshwater lakes Konch and Pert, Karelia, Russia (Bychowsky 1933). It is a euryhaline parasite with a disjunctive geographical distribution in the Holarctic (Malmberg 1970, Ergens 1985, Ziętara et al. 2012, Lumme et al. 2016). In the present study, it was found on the fins, skin and gills of the majority of the studied three-spined sticklebacks. We also recorded an accidental infection with *G. arcuatus* on the skin of the pumpkinseed sunfish *Lepomis gibbosus* (L.) (Centrarchidae) – two host specimens living in syntopy with *G. aculeatus*, each bearing a single monogenean. Such an accidental parasitism, however, should not be considered as an addition to the parasite fauna of the sunfish. The parasite studied is known of being able to attach to a wide range of fishes and even to tadpoles, which may serve to transport it to other populations of the primary host *G. aculeatus* (Volgar-Pastukhova 1959, Malmberg 1964, 1970, Prudhoe & Bray 1982, Bakke et al. 2002, Harris et al. 2004, Huyse et al. 2006, Ziętara et al. 2008). The temporal keeping of fish of both species in a common water tank is the most possible reason for the recorded accidental infection. Previously, a similar infestation was reported by Malmberg (1970). In the Palaearctic waters, the parasite has also been recorded on cyprinid, esocid, pleuronectid, ammodytid, zoarcid, gobiid, gobionellid, percid and salmonid species as well as on other gasterosteids of the genera *Pungitius* Coste and *Spinachia* Cuvier (Malmberg 1970, Ergens 1985, Huyse et al. 2006, Ziętara et al. 2008). In addition, Volgar-Pastukhova (1959) recorded an accidental parasitism of *G. arcuatus* on the skin of tadpoles of the European tree frog *Hyla arborea* (L.) from canals of the town of Vylkove, Danube Delta, Ukraine; currently, this host population is considered as belonging to *Hyla orientalis* Bedriaga (Dufresnes et al. 2016).

Currently, six gyrodactylids have been recognised as parasites of *G. aculeatus* worldwide: *G. alexanderi* Mizelle & Kritsky, 1967, *G. arcuatus*, *G. avalonia* Hanek & Threlfall, 1969, *G. branchicus* Malmberg, 1964, *G. canadensis* Hanek & Threlfall, 1969 and *G. gasterostei* Gläser, 1974 (Harris et al. 2004). All of them were originally described from *G. aculeatus* (Bychowsky 1933, Mizelle & Kritsky 1967, Hanek & Threlfall 1969, Malmberg 1964, Gläser 1974). Experimentally, *G. salaris* Malmberg, 1957, a common parasite of salmonid fishes, has also been recorded on *G. aculeatus* (Soleng & Bakke 1998). Of the typical stickleback monogeneans, *G. branchicus* and *G. gasterostei* have Palaearctic distribution (Malmberg 1970, Harris 1983, Ergens 1985, Ermolenko 1992), *G. canadensis* has Nearctic distribution (Cone & Wiles 1985, Hoffman 1999) whereas *G. alexanderi* has a disjunctive geographic distribution in the Holarctic Region (Harris 1983, 2008, Sokolov 2002, Hansen et al. 2012).

Recently, the Nearctic *G. avalonia* has been recorded on the sunfish *L. gibbosus* in the Black Sea basin – from a canal at the town of Vylkove, Danube Delta, Ukraine (Kvach et al. 2018). Previously, only once *L. gibbosus* has been recorded as an accidental host of *G. avalonia* from the Lake Ontario, Canada (Hanek & Fernando 1971). Due to the morphological similarity between *G. avalonia* and *G. arcuatus*, they have been suspected to be sibling species or even possible synonyms (Cone & Wiles 1985, Harris 1993, 2008, Paladini et al. 2011). We also confirm the similarity between *G. avalonia* and *G. arcuatus* (Table 1). Nevertheless, a minor morphological difference can be pointed out: dorsal bar with postero-medial notch in *G. arcuatus* (Fig. 13a, b and Fig. 8b of Malmberg 1957, 1970; Fig. 5 of Harris 1983; Fig. 419 of Ergens 1985; Fig. 2A, D; present study) *vs* dorsal bar without postero-medial notch in *G. avalonia* (Figs 1, 3–5 of Hanek & Threlfall 1969; Figs. 9–12 of Hanek & Fernando 1971; Fig. 3A of Kvach et al. 2018). This character is difficult for observation and its value for differentiation between the two species is not always easy for application.

The present material possesses substantial metrical differences (Table 1) distinguishing it from the remaining congeners parasitizing three-spined stickleback. However, the most striking characters distinguishing *G. arcuatus* from them are: hamulus root short and stout, ventral bar processes large and claviform in *G. arcuatus* (Fig. 2A, D) *vs* hamulus root elongated, ventral bar processes small and dome-shaped in *G. branchicus* (Fig. 454 of Ergens 1985), *G. gasterostei* (Fig. 3a, b of Gläser 1974, Fig. 499 of Ergens 1985), *G. alexanderi* (Fig. 1 of Mizelle & Kritsky 1967, Fig. 1 of Sokolov 2002) and *G. canadensis* (Fig. 6 of Cone & Wiles 1985). The shape of the marginal hook sickle is also a key character distinguished *G. arcuatus* from its congeners parasitising *G. aculeatus*.

Four gyrodactylids (*G. arcuatus*, *G. branchicus, G. gasterostei* and *G. rarus*) have been recorded on *G. aculeatus* from the Black Sea basin (Gaevskaya et al. 1975, Kakacheva-Avramova 1983, Ergens 1985, Miroshnichenko 1999, 2008a, b, Gaevskaya 2012, Özer & Öztürk 2017). Two of them, *G. gasterostei* and *G. rarus*, have been reported from Bulgaria. Margaritov (1959) recorded *G. rarus* on the fins of *G. aculeatus* in captivity. However, *G. rarus* is recognised as a specific gill parasite of the nine-spined stickleback *Pungitius pungitius* (L.) and its occurrence on other fish hosts is considered accidental (Malmberg 1964, 1970, Harris 1983, Harris et al. 2004, Raeymaekers et al. 2008). Unfortunately, Margaritov (1959) did not provide morphological data and the identification of his material is difficult to be evaluated; furthermore, his identification was based on Gussev (1955), which contained a misleading composite description of the species (Malmberg 1970, Harris 1983). The second species specific to *G. aculeatus* reported from Bulgaria (*G. gasterostei*) was, however, found on non-specific hosts: chub *Squalius cephalus* (L.) (Cyprinidae) and European perch *Perca fluviatilis* L. (Percidae) from Zrebchevo Reservoir, Bulgaria (Nedeva & Babacheva 1999). Therefore, the present study is the first record of *G. arcuatus* from Bulgaria.

Nematodes

The nematode larvae were identified as belonging to the genera *Contracaecum* Railliet & Henry, 1912 and *Hysterothylacium* Ward & Magath, 1917. In both genera, the species identification based on larval morphology is poorly worked out (Moravec 1994, 2013, Gaevskaya 2005, Moravec et al. 2016). The larval stages of the two genera can be distinguished based on the position of the excretory pore, opening at the anterior extremity of the body in *Con-*

tracaecum (Fig. 3A) and at the level of the nerve ring or posterior to it (Fig. 3D) in *Hysterothylacium* (Moravec 1994, 2013).

The genus *Contracaecum* consists of species with complex life-cycles, involving invertebrates and fishes as intermediate and (or) paratenic hosts and fish-eating birds and marine mammals as definitive hosts (Moravec 1994, 2013, Gaevskaya 2005). Currently, it comprises 64 species (NEMYS 2022). At least 13 species (larvae and adults) of *Contracaecum* have been known from Europe (Baruš et al. 1978, Moravec 1994, 2013, Gaevskaya 2005, Mattiucci et al. 2010, Pronkina & Spiridonov 2018). Larvae of this genus are commonly found in European freshwater fishes and the third-stage larval morphology of six species has been studied (Moravec 1994, 2013, Pronkina & Spiridonov 2018; see Table 2). Recently, we described larvae (not identified at the species level) from *Lepomis gibbosus* (reported as "*Contracaecum* sp. 1") and *Knipowitschia caucasica* (Berg) (Gobionellidae) ("*Contracaecum* sp. 2") from the area studied (Stoyanov et al. 2017b, 2018). In the present study, we identified a third congeneric species ("*Contracaecum* sp. 3") represented by a third-stage larva from the body cavity of *G. aculeatus* (sampled in May 2012).

The present larva possesses significant metrical differences compared to the previous descriptions of the European congeners (Table 2). In addition, it differs by the greater length ratio of intestinal caecum to ventricular appendix than those in *C. microcephalum* (Rudolphi, 1809) and *Contracaecum* sp. 1 but smaller than those ratios in *C. multipapillatum* (Drasche, 1882) and *Contracaecum* sp. 2 (Table 2). The present material has a larger length ratio of ventricular appendix to oesophagus than those in *C. micropapillatum* (Stossich, 1890) and *Contracaecum* sp. 1 but smaller than in *Contracaecum* sp. 2 (Table 2). It has a smaller length ratio of intestinal caecum to oesophagus than those in *Contracaecum* sp. 1 and *Contracaecum* sp. 2 (Table 2). In addition, the present larva has tail with subterminal constriction, forming terminal pointed portion (Fig. 3C) *vs* slender tail with rounded tip in *C. microcephalum*, *C. micropapillatum* and *C. rudolphii* Hartwich, 1964 (see Figs. 173 C, F and M in Moravec 2013) and slender tail with blunt tip in *C. osculatum* (Rudolphi, 1802) (see Fig. 173 J in Moravec 2013). Pronkina & Spiridonov (2018) reported a cupola-shaped tail in third-stage larvae of *C. multipapillatum*, which is not the case in our specimen. By the tail morphology, the studied larva differs also from the previously described congeners from the same wetland: the larvae from *L. gibbosus* have a conical tail with a bluntly-pointed tip (Fig. 4 in Stoyanov et al. 2017b) and those from *K. caucasica* have a conical tail with rounded tip (Fig. 4 in Stoyanov et al. 2018). The differences observed are substantial and therefore the *Contracaecum* larvae from this wetland belong to three distinct species.

In the Black Sea basin, eight *Contracaecum* spp. were reported from fish-eating birds (Gvozdev et al. 1983). Additionally, larvae of four further species were found in various fish hosts (Gaevskaya 2012, Pronkina & Spiridonov 2018). In an adjacent region, Mattiucci et al. (2010) described two new congeneric species in the Dalmatian pelican *Pelecanus crispus* Bruch from Northern Greece. Four species of *Contracaecum* (*C. circi* Oschmarin, 1953, *C. microcephalum*, *C. micropapillatum* and *C. rudolphii*) were recorded from various fish-eating birds from Bulgaria (Yanchev 1958, Jelyazkova-Paspaleva 1962, Kambourov & Vassilev 1972, Gvozdev et al. 1983, Petrova 1984). Of them, only larvae of *C. microcephalum* were found in freshwater fishes (Margaritov 1959, Kakacheva-Avramova 1983, Kirin 2001, Shukerova 2005, 2006, Shukerova et al. 2010, Shukerova & Kirin 2019). Margaritov (1960) reported unidentified *Contracaecum* larvae from several marine fishes off the Bulgarian Black Sea coast.

Globally, *G. aculeatus* is known as a host of several species of *Contracaecum* and numerous unidentified at the species level congeners (Moravec 1994, 2013, Hoffman 1999, Barber 2007, Poulin et al. 2011). In Europe, larvae of four species (*C. microcephalum*, *C. osculatum*, *C. ovale* (von Linstow, 1907) and *C. rudolphii*) as well as several unidentified congeners were recorded as its parasites (Moravec 1994, 2013, Poulin et al. 2011, Schade et al. 2016). *Contracaecum* spp. have not been reported from *G. aculeatus* by studies on it in the Black Sea basin (Gaevskaya et al. 1975, Kakacheva-Avramova 1983, Gaevskaya 2012, Özer & Öztürk 2017).

The genus *Hysterothylacium* comprises marine, estuarine and freshwater fish parasites (Gaevskaya 2005). Currently, it includes 97 valid species (NEMYS 2022). The life-cycles are not completely resolved (Gaevskaya 2005) but are considered similar in all species (Køie 1993, Klimpel et al. 2007). The best studied life-cycle is that of *H. aduncum*, comprising numerous invertebrates and fish species as intermediate hosts, diverse planktivorous fishes and some invertebrates as paratenic hosts, and many predatory teleosts and some elasmobranchs as definitive hosts (Køie 1993, Moravec 1994, 2013, Gaevskaya 2005, Gaevskaya et al. 2010). In rare cases, some herbivore fishes have also been found as definitive hosts (Gaevskaya 2005). Moravec (1994, 2013) considered clupeids as its proper definitive hosts. Seventeen congeneric species have been reported from various marine, migratory and freshwater fish species in Europe (Gaevskaya et al. 1975, Kakacheva-Avramova 1983, Petter & Millard 1987, Moravec 1994, 2013, Nadler et al. 2000, Gibson 2001, Mattiucci et al. 2005, 2014, Gaevskaya 2012). Adults and larvae of four species, i.e., *H. aduncum*, *H. bidentatum* (von Linstow, 1899), *H. collare* (Cobb, 1929) and *H. fabri* (Rudolphi, 1819) as well as an unidentified at the species level congeners, have been known from the Black Sea basin (Gaevskaya et al. 1975, Gaevskaya 2012). In Bulgaria, *H. aduncum*, *H. bidentatum* (Margaritov 1959, Kakacheva-Avramova 1977, Kakacheva-Avramova & Menkova 1978, Kirin et al. 2013) and *Hysterothylacium* sp. (Nachev & Sures 2009) were reported from the Danube and some inland waters. However, the morphology of third-stage larvae of only three species from the European waters, i.e., *H. aduncum*, *H. collare* and *H. fabri*, is known (Gaevskaya et al. 1975, Moravec 1994, 2013).

Morphologically, the present larvae resemble *H. aduncum* (third-stage larva). They possessed overlapping ranges of most of the characters as well as of the ratio of the length of intestinal caecum to the length of oesophagus compared to those in previous descriptions of *H. aduncum* (Table 3). Furthermore, the present material possesses conical tail with mucron provided inside with caudal process of the next developmental stage (Fig. 3F), resembling that of the advanced third-stage larva of *H. aduncum* (see fig. 172 C, D in Moravec 2013). By contrast, Gaevskaya et al. (1975) reported smaller body total length, oesophagus length, ventricular appendix length and width, intestinal caecum width and tail length in *H. aduncum* than those in the present material (Table 3). Petter & Millard (1988) reported for *H. aduncum* smaller body total length, oesophagus length, tail length and length ratio ventricular appendix to oesophagus than those in the present larvae (Table 3). Unlike the other European congeners, the present material possesses significant metrical differences in the most of the characters studied (Table 3). Thus, the sizes of intestinal caecum and ventricular appendix are almost equal in the present larvae *vs* markedly short intestinal caecum and much longer ventricular appendix in *H. fabri* (third-stage larvae) (Table 3). In addition, the present material possesses conical tail with mucron (Fig. 3F) *vs* tail with broadly rounded tip in *H. fabri* (see fig. 172 H in Moravec 2013).

Globally, *H. aduncum*, *H. auctum* (Rudolphi, 1802) and unidentified congeners have been recorded from *G. aculeatus* (Moravec 1994, 2013, Hoffman 1999, Barber 2007, Poulin et al. 2011, Schade et al. 2016). *Hysterothylacium aduncum* is a cosmopolitan euryxenous helminth species repeatedly recorded (larvae and adults) as a parasite of *G. aculeatus* from the Holarctic Region (Moravec 1994, 2013, Hoffman 1999, Palm et al. 1999, Barber 2007, Kirjušina & Vismanis 2007, Sokolov 2010, Poulin et al. 2011, Gaevskaya 2012). The second congener, *H. auctum,* is a poorly known species parasitizing mainly perciform fishes off the European and Japanese coasts (Moravec & Nagasawa 2000); previously, it was considered a junior synonym of *H. aduncum* (Gaevskaya 2005). Schade et al. (2016) recorded *H. auctum* in the intestine and body cavity of three-spined stickleback from the Sylt-Rømø Bight, North Sea, Germany.

The Lake Atanasovsko Wetland is a home of a great variety of potential intermediate, paratenic and definitive hosts of trophically-transmitted parasites such as the nematodes of the genera *Contracaecum* and *Hysterothylacium*. Totally, 157 aquatic and water-related terrestrial invertebrates (Hubenov et al. 2015) and eight permanently inhabiting the wetland fish species (Stefanov 2006) have been recorded. Furthermore, they are an important place for roosting, wintering and nesting of 317 bird species (Vassilev et al. 2013). Of them, 39 fish-eating bird species of the families Gaviidae, Podicipedidae, Phalacrocoracidae, Pelecanidae, Ardeidae, Ciconiidae, Threskiornithidae, Anatidae and Laridae have been recorded (Dimitrov et al. 2005), which is a prerequisite for the presence of *Contracaecum* spp. On the other hand, the poor fish fauna of this wetland might be a limit for the presence of typical fish parasites such as *Hysterothylacium* spp. represented here by only one species.

So far, larvae and adults of only four nematodes have been recorded as parasites of *G. aculeatus* in the Black Sea (Kornyychuk 2010, Gaevskaya 2012, Moshu 2014, Dmitrieva et al. 2015, Kornyychuk et al. 2016, Pronkina et al. 2017). These are *Eustrongylides tubifex* (Nitzsch in Rudolphi, 1819) (Dioctophymidae), *Raphidascaris acus* (Boch, 1779), *H. aduncum* (Raphidascarididae) and *Dichelyne minutus* (Rudolphi, 1819) (Cucullanidae). No previous data on nematodes from *G. aculeatus* have been reported from Bulgaria.

General comments

In the Black Sea basin, the helminth fauna of *G. aculeatus* comprises 21 species: 7 trematodes, 4 monogeneans, 3 cestodes, 4 nematodes and 3 acanthocephalans (Petrochenko 1956, Miroshnichenko 1999, 2008a, b, Lisitsina 2008, 2019, Gaevskaya 2012, Moshu 2014, Öztürk & Özer 2014, Dmitrieva et al. 2015, Kornyychuk et al. 2016, Özer & Öztürk 2017, Pronkina et al. 2017, Stoyanov et al. 2017a, Öztürk & Özer 2019, Stoyanov & Georgiev 2022). From parasitological point of view, *G. aculeatus* can be considered insufficiently studied in the Black Sea basin (Gaevskaya 2012), probably due to its non-commercial status. In the Lake Atanasovsko wetland, we recorded only five helminth species in this host. Among them, only *G. arcuatus* is a specific parasite to this fish. We found also larval forms of one cestode, one trematode and two nematode species, demonstrating that the three-spined stickleback plays a role in the transmission of parasites of birds and fishes as intermediate or paratenic host.

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References

Appleby C. 1996. Variability of the opisthaptoral hard parts of *Gyrodactylus callariatis* Malmberg, 1957 (Monogenea: Gyrodactylidae) from Atlantic cod *Gadus morhua* L. in the Oslo Fjord, Norway. Systematic Parasitology 33 (3): 199–207.

Bakke T. A., Harris P. D. & Cable J. 2002. Host specificity dynamics: observations on gyrodactylid monogeneans. International Journal for Parasitology 32 (3): 281–308.

Barber I. 2007. Host–parasite interactions of the three-spined stickleback. In: Östlund-Nilsson S., Mayer I. & Huntingford F. A. (Eds) Biology of the three-spined stickleback. Boca Raton: CRC Press. pp. 271–318.

Barber I. 2013. Sticklebacks as model hosts in ecological and evolutionary parasitology. Trends in Parasitology 29 (11): 556–566.

Baruš V., Sergeeva T. P., Sonin D. M. & Ryzhikov K. M. 1978. Helminths of Fish-Eating Birds of the Palaearctic Region I. Nematoda. Dordrecht: Springer. 318 p.

Bush A. O., Lafferty K. D., Lotz J. M. & Shostak A. W. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology 83 (4): 575–583.

Bychowsky B. 1933. [A new species of *Gyrodactylus* from lakes in Karelia.] Trudy Borodinskoy Biologicheskoy Stantsii Karelii 4: 51–55. (In Russian).

Cone D. K. & Wiles M. 1985. The systematics and zoogeography of *Gyrodactylus* species (Monogenea) parasitizing gasterosteid fishes in North America. Canadian Journal of Zoology 63 (4): 956–960.

Dávidová M., Jarkovský J., Matějusová I. & Gelnar M. 2005. Seasonal occurrence and metrical variability of *Gyrodactylus rhodei* Žitňan 1964 (Monogenea, Gyrodactylidae). Parasitology Research 95 (6): 398–405.

Dimitrov M., Michev T., Profirov L. & Nyagolov K. 2005. Waterbirds of Bourgas Wetlands. Results and evaluation of the monthly waterbird monitoring 1996-2002. Sofia & Moscow: Pensoft Publishers and Bulgarian Biodiversity Foundation. 160 p.

Dmitrieva E. & Dimitrov G. 2002. Variability in the taxonomic characters of Black Sea gyrodactylids (Monogenea). Systematic Parasitology 51 (3): 199–206.

Dmitrieva E. V., Yurakhno V. M., Kornyychuk Yu. M., Pronkina N. V., Polyakova T. A. & Popyuk M. P. 2015. Characterization of species diversity and structure of fish parasite communities from Karkinitsky Bay (Black Sea). Some problems on biodiversity conservation of aquatic biocenoses: proceedings of the International Conference, Rostov-on-Don, November 27, 2015. (In Russian).

Dufresnes C., Litvinchuk S. N., Leuenberger J., Ghali K., Zinenko O., Stöck M. & Perrin N. 2016. Evolutionary melting pots: a biodiversity hotspot shaped by ring diversifications around the Black Sea in the Eastern tree frog (*Hyla orientalis*). Molecular Ecology 25 (17): 4285–4300.

Ergens R. 1976. Variability of hard parts of opisthaptor of two species of *Gyrodactylus* Nordmann, 1832 (Monogenoidea) from *Phoxinus phoxinus* (L.). Folia Parasitologica 23 (2): 111–126.

Ergens R. 1991. Variability of the hard parts of poisthaptor of *Gyrodactylus leucisci* Žitňan 1964 (Monogenea: Gyrodactylidae). Folia Parasitologica 38 (1): 23–28.

Ergens R. R. 1985. Order Gyrodactylea Bychowsky, 1937. In: Bauer O. N. (Ed.) [Key to parasites of freshwater fish of the fauna of the USSR. Vol. 2. Parasitic metazoans.] Leningrad: Nauka. pp. 269–347. (In Russian).

Ergens R. & Gelnar M. 1985. Experimental verification of the effect of temperature on the size of hard parts of opisthaptor of *Gyrodactylus katharineri* Malmberg, 1964 (Monogenea). Folia Parasitologica 32 (4): 377–380.

Ermolenko A. V. 1992. Fish parasites of freshwater bodies in the continental part of the Sea of Japan basin. Vladivostok: DVO. RAS. 238 p. (In Russian).

Gaevskaya A. V. 2005. Anisakid nematodes and diseases caused by them in animals and man. Sevastopol: EKOSI-Gidrofizika. 223 p. (In Russian).

Gaevskaya A. V. 2012. Parasites and diseases of fishes in the Black Sea and the Sea of Azov: I. Marine, brackish and diadromous fishes. Sevastopol: EKOSI-Gidrofizika. 380 p. (In Russian).

Gaevskaya A. V., Gussev A. V., Deliamure S. L., Donez Z. S., Iskova N. I., Kornyushin V. V., Kovaleva A. A., Smogorzhevskaya L. A., Solonchenko A. I., Shtetin G. A., Shulman S. S., Margaritov N. M., Markevich A. P., Mordvinova T. N., Naydenova N. N., Nikolaeva V. M., Paruchin A. M. & Pogorelzeva T. P. 1975. [Handbook of parasites of vertebrate animals in the Black Sea and the Sea of Azov.] Kiev: Naukova Dumka. 552 p. (In Russian).

Gaevskaya A. V., Kornyichuk J. M., Machkevsky V. K., Pronkina N. V., Polyakova T. A., Mordvinova T. N. & Popyuk M. P. 2010. Characters of parasite system function of *Hysterothylacium aduncum* (Nematoda: Anisakidae) in the Black Sea. Marine Ecological Journal 9 (2): 37–50.

Geets A., Appleby C. & Ollevier F. 1999. Host-dependent and seasonal variation in opisthaptoral hard parts of *Gyrodactylus* cf. *arcuatus* from three *Pomatoschistus* spp. and *G. arcuatus* from *Gasterosteus aculeatus*: a multivariate approach. Parasitology 119 (1): 27–40.

Gibson D. I. 2001. Nematoda (parasitic). In: Costello M. J., Emblow C. & White R. (Eds) European register of marine species. A check-list of the marine species in Europe and a bibliography of guides to their identification. Collection Patrimoines Naturels, 50. Paris: Muséum National d'Histoire Naturelle, pp. 174–176.

Gläser H-J. 1974. Sechs neue Arten der *Gyrodactylus-wageneri*-Gruppe (Monogenea, Gyrodactylidae) nebst Bemerkungen zur Präparation, Determination, Terminologie und Wirtsspezifität. Zoologischer Anzeiger 192 (1/2): 56–76.

Gussev A. V. 1955. Monogenetic trematodes of fish of the Amur River system. Trudy Zoologicheskogo Instituta Academiya Nauk USSR 19: 171–398. (In Russian).

Gussev A. V. 1983. Methods for collecting and processing materials of monogeneans parasitizing fishes. Leningrad: Nauka. 48 p. (In Russian).

Gvozdev E. V., Vasilev I. & Vaidova S. M. 1983. Order Ascaridida. In: Kurashvili B. E. (Ed.) Nematodes and acanthocephalans of birds of Black Sea and Caspian Sea Regions. Tbilisi: Mecniereba, pp. 47–59. (In Russian).

Hanek G. & Fernando C. H. 1971. Monogenetic trematodes from the Bay of Quinte area, Ontario. II. Genus *Gyrodactylus* Nordmann, 1832. Canadian Journal of Zoology 49 (10): 1331–1341.

Hanek G. & Threlfall W. 1969. Monogenetic trematodes from Newfoundland, Canada. 1 New species of the genus *Gyrodactylus* Nordmann, 1832. Canadian Journal of Zoology 47 (5): 951–955.

Hansen H., Jøgensen A. & Mo T. A. 2012. Spin-off from routine parasite diagnostics of Atlantic salmon; first report of *Gyrodactylus alexanderi* in Norway. Bulletin of the European Association of Fish Pathologists 32 (1): 14–18.

Harris P. D. 1983. Studies on the biology of the Gyrodactyloidea (Monogenea). PhD thesis, London: Westfield College. xiv–318 p.

Harris P. D. 1985. Species of *Gyrodactylus* von Nordmann, 1832 (Monogenea: Gyrodactylidae) from freshwater fishes in southern England, with a description of *Gyrodactylus rogatensis* sp. nov. from the bullhead *Cottus gobio* L. Journal of Natural History 19 (4): 791–809.

Harris P. D. 1993. Interactions between reproduction and population biology in gyrodactylid monogeneans – a revue. Bulletin Français de la Pêche et de la Pisciculture 328: 47–65.

Harris P. D. 2008. The evolution of fish ectoparasite communities – the role of the ice ages. Wiadomości Parazytologiczne 54 (4): 287–296.

Harris P. D., Shinn A. P., Cable J. & Bakke T. A. 2004. Nominal species of the genus *Gyrodactylus* von Nordmann 1832 (Monogenea: Gyrodactylidae), with a list of principal host species. Systematic Parasitology 59 (1): 1–27.

Hoffman G. L. 1999. Parasites of North American freshwater fishes. Second Edition. Ithaca & London: Cornell University Press. 539 p.

Hubenov Z., Kenderov L. & Pandourski I. 2015. Invertebrate animals (Metazoa: Invertebrata) of the Atanasovsko Lake, Bulgaria. Historia Naturalis Bulgarica 22: 45–71.

Huyse T., Malmberg G. & Volckaert F. M. A. 2004. Four new species of *Gyrodactylus* von Nordmann, 1832 (Monogenea, Gyrodactylidae) on gobiid fishes: combined DNA and morphological analyses. Systematic Parasitology 59 (2): 103–120.

Huyse T., Pampoulie C., Audenaert V. & Volckaert F. M. A. 2006. First report of *Gyrodactylus* spp. (Platyhelminthes: Monogenea) in the Western Mediterranean Sea: molecular and morphological descriptions. Journal of Parasitology 92 (4): 682–690.

Ivanov K., Sotirov A., Rozhdestvenski A. & Vodenicharov D. 1964. [Lakes in Bulgaria.] in Trudove na Instituta po Hidrologiya i Meteorologiya 16. Sofia: DI. Nauka i Izkustvo. 242 p. (In Bulgarian).

Jelyazkova-Paspaleva A. 1962. [On the helminth fauna of wild birds from the Strandzha region]. Izvestiya na Tsentralnata Khelmintologichna Laboratoriya 7: 137–152. (In Bulgarian).

Kakacheva-Avramova D. 1977. Study on the helminth content of fishes from the Bulgarian part of the Danube. Khelmintologiya 3: 20–45. (In Bulgarian).

Kakacheva-Avramova D. 1983. Helminths of freshwater fishes in Bulgaria. Sofia: Publishing House of the Bulgarian Academy of Sciences. 262 p. (In Bulgarian).

Kakacheva-Avramova D. & Menkova I. 1978. [The rainbow trout – a host of *Contracaecum aduncum*.] Ribno Stopanstvo 5: 22–23.

Kambourov P. & Vassilev I. 1972. On the helminth fauna in certain wild aquatic birds (Anseres) in Bulgaria. Izvestiya na Tsentralnata Khelmintologichna Laboratoriya 15: 109–133.

Kirin D. 2001. Helminth parasites of *Cyprinus carpio* (L., 1758) (Osteichthyes, Cyprinidae) from Mesta River, Bulgaria. Comptes Rendus de l'Académie Bulgare des Sciences 54 (12): 89–92.

Kirin D., Hanzelová V., Shukerova S., Hristov S., Turceková L. & Spakulova M. 2013. Helminth communities of fishes from the River Danube and Lake Srebarna, Bulgaria. Scientific Papers. Series D. Animal Science 56: 333–340.

Kirjušina M. & Vismanis K. 2007. Checklist of the parasites of fishes of Latvia. Rome: FAO Fisheries Technical Paper 369/3. 106 p.

Klimpel S., Kleinertz S., Hanel R. & Rückert S. 2007. Genetic variability in *Hysterothylacium aduncum*, a raphidascarid nematode isolated from sprat (*Sprattus sprattus*) of different geographical areas of the northeastern Atlantic. Parasitology Research 101 (5): 1425–1430.

Kornyychuk Y. M. 2010. [New record of the nematode *Hysterothylacium aduncum* (Rud., 1802) from the Black Sea fishes.] Marine Ecological Journal 9 (3): 34.

Kornyychuk Y. M., Dmitrieva E. V., Yurakhno V. M., Polyakova T. A., Pronkina N. V., Popjuk M. P., Tarina N. A. & Rudenko M. R. 2016. [Parasite fauna of fishes in biocenoses of the reserve water off the Lebyazh'i islands.] Materials of the VIII International Scientific and Practical Conference, Simferopol, April 28–30, 2016. (In Russian).

Køie M. 1993. Aspects of the life cycle and morphology of *Hysterothylacium aduncum* (Rudolphi, 1802) (Nematoda, Ascaridoidea, Anisakidae). Canadian Journal of Zoology 71 (7): 1289–1296.

Kvach Y., Ondračková M., Kutsokon Y. & Dzyziuk N. 2018. New record of monogenean parasites on non-indigenous fishes in the Ukrainian Danube Delta. BioInvasions Records 7 (1): 65–72.

Lisitsina O. I. 2008. Acanthocephala. In Kornyushin V. V. (Ed.) Catalogue of helminths of vertebrates of Ukraine. Acanthocephala. Monogenea. Kiev: I.I. Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine, Ukrainian Scientific Society of Parasitologists. pp. 7–57. (In Russian).

Lisitsina O. I. 2019. Fauna of Ukraine, Vol. 31. Acanthocephala. Kyiv: Naukova Dumka. 224 p. (In Russian).

Lumme J., Mäkinen H., Ermolenko A. V., Gregg J. L. & Ziętara M. S. 2016. Displaced phylogeographic signals from *Gyrodactylus arcuatus*, a parasite of the three-spined stickleback *Gasterosteus aculeatus*, suggest freshwater glacial refugia in Europe. International Journal for Parasitology 46 (9): 545–554.

Malmberg G. 1957. Om förekomsten av *Gyrodactylus* på Svenska fiskar. Särtryck ur Skrifter Utgivna av Södra Sveriges Fiskeriförening Årsskrift 1956: 19–76.

Malmberg G. 1964. Taxonomical and ecological problems in *Gyrodactylus* (Trematoda, Monogenea). In: Ergens R. & Ryšavý B. (Eds) Parasitic worms and aquatic conditions. Prague: Publishing house of the Czechoslovak Academy of Sciences. pp. 203–230.

Malmberg G. 1970. The excretory systems and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea). Arkiv för Zoologi 2 (23): 1–235.

Margaritov N. 1959. [Parasites of some Bulgarian freshwater fishes] Varna, Publication of the Research Institute of Fisheries and Fishing Industry. 1–21. (In Bulgarian).

Margaritov N. 1960. [Parasites of some Bulgarian marine fishes.] Proceedings of the Research Institute of Fisheries and Fishing Industry, Varna 2: 195–213. (In Bulgarian).

Mattern M. Y. 2007. Phylogeny, systematics, and taxonomy of sticklebacks. In: Östlund-Nilsson S., Mayer I. & Huntingford F. A. (Eds) Biology of the three-spined stickleback. Boca Raton: CRC Press, pp. 1–40.

Mattiucci S., Farina V., Garcia A., Santos M. N., Marinello L. & Nascetti G. 2005. Metazoan parasitic infections of swordfish (*Xiphias gladius* L., 1758) from the Mediterranean Sea and Atlantic Gibraltar waters: implications for stock assessment. Collective Volume of Scientific Papers ICCAT 58 (4): 1470–1482.

Mattiucci S., Paoletti M., Solorzano A. C. & Nascetti G. 2010. *Contracaecum gibsoni* n. sp. and *C. overstreeti* n. sp. (Nematoda: Anisakidae) from the Dalmatian pelican *Pelecanus crispus* (L.) in Greek waters: genetic and morphological evidence. Systematic Parasitology 75 (3): 207–224.

Mattiucci S., Garcia A., Cipriani P., Santos M. N., Nascetti G. & Cimmaruta R. 2014. Metazoan parasite infection in the swordfish, *Xiphias gladius*, from the Mediterranean Sea and comparison with Atlantic populations: implications for its stock characterization. Parasite 21 (35): 1–13.

Miroshnichenko A. I. 1999. Parasites of Fishes in Crimea. In: Apostolov L. G., Dulitsky A. I., Bokov V. A., Ena A. V., Ena V. G., Korzhenevsky V. V., Kostin S. U., Sergeeva N. G. & Temirova S. I. (Eds) Points on the development of the Crimea: analytical, scientific and practical collected articles open to discussion. 11-th issue: Biological and landscape diversity in the Crimea: problems and perspectives. Simferopol: SONAT. pp. 123–124. (In Russian).

Miroshnichenko A. I. 2008a. Monogenea. In: Kornyushin V. V. (Ed.) Catalogue of helminths of vertebrates of Ukraine. Acanthocephala. Monogenea. Kiev: I.I. Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine, Ukrainian Scientific Society of Parasitologists. pp. 58–137. (In Russian).

Miroshnichenko A. I. 2008b. [Lists of Crimean fish parasites by hosts (with indication of water bodies and faunal complexes).] Scientific Notes of Taurida National V.I. Vernadsky University, series Geography 21 (3): 210–220. (In Russian).

Mizelle J. D. & Kritsky D. C. 1967. Studies on monogenetic trematodes XXXVI. Gyrodactylid parasites of importance to California fishes. California Fish and Game 53 (4): 264–272.

Mo T. A. 1991. Seasonal variations of opisthaptoral hard parts of *Gyrodactylus salaris* Malmberg, 1957 (Monogenea: Gyrodactylidae) on parr of Atlantic salmon *Salmo salar* L. in the River Batnfjordselva, Norway. Systematic Parasitology 19 (3): 231–240.

Mo T. A. 1993. Seasonal variations of the opisthaptoral hard parts of *Gyrodactylus derjavini* Mikailov, 1975 (Monogenea: Gyrodactylidae) on brown trout *Salmo trutta* L. parr and Atlantic salmon *S. salar* L. parr in the River Sandvikselva, Norway. Systematic Parasitology 26 (3): 225–231.

Moravec F. 1994. Parasitic nematodes of freshwater fishes of Europe. Dordrecht & Praha: Kluwer Academic Publishers & Academia. 473 p.

Moravec F. 2013. Parasitic nematodes of freshwater fishes of Europe. Praha: Academia. 601 p.

Moravec F. & Nagasawa K. 2000. Some anisakid nematodes from marine fishes of Japan and the North Pacific Ocean. Journal of Natural History 34 (8): 1555–1574.

Moravec F., van Rensburg C. J. & Van As L. L. 2016. Larvae of *Contracaecum* sp. (Nematoda: Anisakidae) in the threatened freshwater fish *Sandelia capensis* (Anabantidae) in South Africa. Diseases of Aquatic Organisms 120 (3): 251–254.

Moshu A. 2014. Helminths of fishes from waters of Prut-Dniester interriveran hydrographical area potentially dangerous to human health. Chişinău: Eco-TIRAS. 88 p.

Nachev M. & Sures B. 2009. The endohelminth fauna of barbel (*Barbus barbus*) correlates with water quality of the Danube River in Bulgaria. Parasitology 136 (5): 545–552.

Nadler S. A., Amelio S. D., Fagerholm H.-P., Berland B. & Paggi L. 2000. Phylogenetic relationships among species of *Contracaecum* Railliet & Henry, 1912 and *Phocascaris* Høst, 1932 (Nematoda: Ascaridoidea) based on nuclear rDNA sequence data. Parasitology 121 (4): 455–463.

Nedeva I. & Babacheva T. 1999. New Bulgarian fauna species belonging to Monogenea (van Beneden, 1858) Bychowsky, 1937. Experimental Pathology and Parasitology 3: 7–10.

NEMYS (Eds.) 2022. NEMYS: World Database of Nematodes. Ascaridoidea Baird, 1853. Accessed through: World Register of Marine Species at: https://www.marinespecies.org/aphia.php?p=taxdetails&id=22816 on 2022-07-09

NEMYS (Eds.) 2022. NEMYS: World Database of Nematodes. *Contracaecum* Railliet & Henry, 1912. Accessed through: World Register of Marine Species at: https://www.marinespecies.org/aphia.php?p=taxdetails&id=22849 on 2022-07-11

NEMYS (Eds.) 2022. NEMYS: World Database of Nematodes. *Hysterothylacium* Ward & Magath, 1917. Accessed through: World Register of Marine Species at: https://www.marinespecies.org/aphia.php?p=taxdetails&id=19962 on 2022-07-09

Özer A. & Öztürk T. 2017. Parasite diversity of the Black Sea fishes in Turkish coastal areas. In: Sezgin M., Bat L., Ürkmez D., Arici E. & Öztürk B. (Eds): Black Sea marine environment: The Turkish shelf. Istanbul: Turkish Marine Research Foundation (TÜDAV), Publication № 46. pp. 289–309.

Öztürk T. & Özer A. 2014. Monogenean fish parasites, their host preferences and seasonal distributions in the lower Kızılırmak Delta (Turkey). Turkish Journal of Fisheries and Aquatic Sciences 14 (2): 367–378.

Öztürk T. & Özer A. 2019. Digenean metacercariae parasitic in fishes in Sarıkum Lagoon Lake, Sinop, Turkish Black Sea coast: species diversity, seasonal occurrence and histopathological effects. Acta Zoologica Bulgarica 71 (3): 443–452.

Paladini G., Cable J., Fioravanti M. L., Faria P. J., Di Cave D. & Shinn A. P. 2009. *Gyrodactylus orecchiae* sp. n. (Monogenea: Gyrodactylidae) from farmed populations of gilthead seabream (*Sparus aurata*) in the Adriatic Sea. Folia Parasitologica 56 (1): 21–28.

Paladini G., Huyse T. & Shinn A. P. 2011. *Gyrodactylus salinae* n. sp. (Platyhelminthes: Monogenea) infecting the south European toothcarp *Aphanius fasciatus* (Valenciennes) (Teleostei, Cyprinodontidae) from a hypersaline environment in Italy. Parasites & Vectors 4 (1): 1–12.

Palm H. W., Klimpel S. & Bucher C. 1999. Checklist of metazoan fish parasites of German coastal waters. Berichte aus dem Institut für Meereskunde an der Christian-Albrechts-Universität Kiel 307: 1–148.

Petrochenko V. I. 1956. [Acanthocephalans of domestic and wild animals.] Vol. 1. Moscow: Izdatelstvo Academiya Nauk USSR 437 p. (In Russian).

Petrova K. 1984. Nematoda in wild birds from Bulgaria. In: Vassilev I. (Ed.): Fauna, taxonomy and ecology of helminths on birds. Sofia: Publishing House of the Bulgarian Academy of Sciences, pp. 172–184. (In Bulgarian).

Petter A. J. & Millard C. 1987. Ascarides de poissons de Méditerranée occidentale. Bulletin du Muséum National d'Histoire Naturelle, Paris, Section A: Zoologie, Biologie et Écologie Animales, 4e série 9 (4): 773–798.

Petter A. J. & Millard C. 1988. Larves d'Ascarides parasites de poissons en Méditerranée occidentale. Bulletin du Muséum National d'Histoire Naturelle, Paris, Section A: Zoologie, Biologie et Écologie Animales, 4e série 10 (2): 347–369.

Poulin R., Blanar C. A., Thieltges D. W. & Marcogliese D. J. 2011. The biogeography of parasitism in sticklebacks: distance, habitat differences and the similarity in parasite occurrence and abundance. Ecography 34 (4): 540–551.

Pronkina N. V. & Spiridonov S. E. 2018. Morphological and molecular characterisation of anisakid juveniles from the golden grey mullet of the Black Sea. Russian Journal of Nematology 26 (1): 87–92.

Pronkina N. V., Dmitrieva E. V., Polyakova T. A., Popyuk M. P. 2017. Life cycle of *Dichelyne minutus* (Rudolphi, 1819) (Nematoda: Cucullanidae) in estuarine biocenosis of the Black Sea. Biologiya Morya 43 (2): 117–124. (In Russian).

Prudhoe S. & Bray R. A. 1982. Platyhelminth parasites of the Amphibia. London & Oxford: British Museum (Natural History) and Oxford University Press. 217 p.

Raeymaekers J. A. M., Huyse T., Maelfait H., Hellemans B. & Volckaert F. A. M. 2008. Community structure, population structure and topographical specialisation of *Gyrodactylus* (Monogenea) ectoparasites living on sympatric stickleback species. Folia Parasitologica 55 (3): 187–196.

Roca-Geronès X., Montoliu I., Godínez-González C., Fisa R. & Shamsi S. 2018. Morphological and genetic characterization of *Hysterothylacium* Ward & Magath, 1917 (Nematoda: Raphidascarididae) larvae in horse mackerel, blue whiting and anchovy from Spanish Atlantic and Mediterranean waters. Journal of Fish Diseases 41 (10): 1463–1475.

Schade F. M., Raupach M. J. & Wegner K. M. 2016. Seasonal variation in parasite infection patterns of marine fish species from the Northern Wadden Sea in relation to interannual temperature fluctuations. Journal of Sea Research 113: 73–84.

Shukerova S. 2005. Helminth fauna of the Prussian carp, *Carassius gibelio* (Bloch, 1782), from the Srebarna Biosphere Reserve. Trakia Journal of Sciences 3 (6): 36–40.

Shukerova S. A. 2006. Helminth fauna of the common carp, *Cyprinus carpio* (Linnaeus, 1758), from the Srebarna Biosphere Reserve, Bulgaria. Scientific Articles. Ecology 2: 217–223.

Shukerova S. A. & Kirin D. A. 2019. Helminth communities of roach *Rutilus rutilus* (L., 1758) (Cypriniformes: Cyprinidae) from Srebarna Biosphere Reserve, Bulgaria. Acta Zoologica Bulgarica 71 (2): 285–292.

Shukerova S., Kirin D. & Hanzelová V. 2010. Endohelminth communities of the perch, *Perca fluviatilis* (Perciformes, Percidae) from Srebarna Biosphere Reserve, Bulgaria. Helminthologia 47 (2): 99–104.

Shinn A. P., Gibson D. I. & Sommerville C. 1993. An SEM study of the haptoral sclerites of the genus *Gyrodactylus* Nordmann, 1832 (Monogenea) following extraction by digestion and sonication techniques. Systematic Parasitology 25 (2): 135–144.

Sokolov S. G. 2002. The first record of *Gyrodactylus alexanderi* (Plathelminthes, Monogenea, Gyrodactylidae) in Eurasian fauna. Vestnik Zoologii 36 (5): 89–90.

Sokolov S. G. 2010. The parasites of sticklebacks (Gasterosteidae) from the Utkholok R. area (Northwestern Kamchatka). Bulletin of the North-East Scientific Center of FEB, RAS 3: 56–66.

Soleng A. & Bakke T. A. 1998. The susceptibility of three-spined stickleback (*Gasterosteus aculeatus*), nine-spined stickleback (*Pungitius pungitius*) and flounder (*Platichthys flesus*) to experimental infections with the monogenean *Gyrodactylus salaris*. Folia Parasitologica 45 (4): 270–274.

Stefanov T. 2006. Ichthyofauna of Bulgarian natural lakes. In: International Scientific Conference BALWOIS – 2006, Ohrid, Macedonia, 23–26 May 2006. http://balwois.com/balwois/administration/full_paper/ffp-628.pdf

Stoyanov B. & Georgiev B. B. 2022. Marine parasite in a freshwater wetland: new host and geographical records of *Progrillotia dasyatidis* (Cestoda: Trypanorhyncha) from *Gasterosteus aculeatus* (Actinopterygii: Gasterosteidae) in Bulgaria, with comments on its life-cycle. Journal of Helminthology 96: e70.

Stoyanov B., Georgieva S., Pankov P., Kudlai O., Kostadinova A. & Georgiev B. B. 2017a. Morphology and molecules reveal the alien *Posthodiplostomum centrarchi* Hoffman, 1958 as the third species of *Posthodiplostomum* Dubois, 1936 (Digenea: Diplostomidae) in Europe. Systematic Parasitology 94 (1): 1–20.

Stoyanov B., Mutafchiev Y., Pankov P. & Georgiev B. B. 2017b. Helminth parasites in the alien *Lepomis gibbosus* (L.) (Centrarchidae) from the Lake Atanasovsko Wetlands, Bulgaria: Survey of species and structure of helminth communities. Acta Zoologica Bulgarica 69 (4): 555–574.

Stoyanov B., Mutafchiev Y., Pankov P. & Georgiev B. B. 2018. Helminths and Helminth Communities of the Caucasian Dwarf Goby *Knipowitschia caucasica* (Berg) (Actinopterygii: Gobionellidae) from Lake Atanasovsko, Bulgaria. Acta Zoologica Bulgarica 70 (2): 225–240.

Vassilev V., Vassilev R., Yankov P., Kamburova N., Uzunov Y., Pehlivanov L., Georgiev B. B., Popgeorgiev G., Assyov B., Avramov S., Tzenova R. & Kornilev Y. 2013. National action plan for conservation of wetlands of high significance in Bulgaria, 2013–2022. Sofia: Bulgarian Biodiversity Foundation, 104 p.

Volgar-Pastukhova L. G. 1959. [Parasitic fauna of anurans in the Danube delta.] In: Polyanski Y. I. (Ed.) Ekologicheskaya parazitologiya. Leningrad: Izdatelstvo Leningradskogo Universiteta. pp. 58–95. (In Russian).

Wootton R. J. 1976. The biology of the sticklebacks. London: Academic Press. x–387 p.

Yanchev Y. 1958. [Studies on some helminths and helminthiases of white storks and pelicans.] Izvestiya na Zoologicheskiya Institut, Balgarska Akademiya na Naukite 7: 393–411. (In Bulgarian).

Ziętara M. S., Kuusela J., Veselov A. & Lumme J. 2008. Molecular faunistics of accidental infections of *Gyrodactylus* Nordmann, 1832 (Monogenea) parasitic on salmon *Salmo salar* L. and brown trout *Salmo trutta* L. in NW Russia. Systematic Parasitology 69 (2): 123–135.

Ziętara M. S., Lebedeva D., Muñoz G. & Lumme J. 2012. A monogenean fish parasite, *Gyrodactylus chileani* n. sp., belonging to a novel marine species lineage found in the South-Eastern Pacific and the Mediterranean and North Seas. Systematic Parasitology 83 (2): 159–167.

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