Parasitology
Research Article

ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., 69 (4) 2017: 555-574

Helminth Parasites in the Alien *Lepomis gibbosus* (L.) (Centrarchidae) from the Lake Atanasovsko Wetlands, Bulgaria: Survey of Species and Structure of Helminth Communities

Borislav Stoyanov, Yasen Mutafchiev, Plamen Pankov & Boyko B. Georgiev*

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Street, 1113 Sofia, Bulgaria

Abstract: A helminthological survey of the alien Pumpkinseed Sunfish, *Lepomis gibbosus* (L.), from the Lake Atanasovsko Wetlands, Bulgarian Black Sea coast, is based on the examination of 107 host individuals studied in spring, summer and autumn of 2012 and 2013. Six helminth species are recorded: metacercariae of *Posthodiplostomum centrarchi* Hoffman, 1958 with prevalence (P) 8.41%, mean intensity (MI) 1.88 and mean abundance (MA) 0.16; monogeneans *Onchocleidus similis* Mueller, 1936 (P 31.78%, MI 4.41, MA 1.40) and *O. dispar* Mueller, 1936 (P 3.74%, MI 1.00, MA 0.04), both specific parasites of centrarchids; adult liver nematode *Schulmanela petruschewskii* (Shulman, 1948) (P 3.74%, MI 17.74, MA 0.66); larvae of nematodes *Spiroxys contortus* (Rudolphi, 1819) (P 10.28%, MI 2.90, MA 0.30) parasitic as adults in pond turtles and *Contracaecum* sp. (P 1.87%, MI 1.00, MA 0.02) parasitic as adults in fish-eating birds. Though *L. gibbosus* harbours three alien parasites (*P. centrarchi*, *O. similis* and *O. dispar*), there is no evidence for spillover infections in fishes in the Lake Atanasovsko Wetlands. However, its negative impact on native populations might be by parasite spillback (participation in life cycles of native parasites in fishes, reptiles and birds). Helminth component communities and infracommunities studied have lower species richness, diversity and abundance compared to those in the host's native range, which is consistent with the Enemy Release Hypothesis.

Key words: Trematoda, Monogenea, Nematoda, biological invasion, spillback infections

Introduction

The pumpkinseed sunfish, *Lepomis gibbosus* (L.), is a North American freshwater fish, which has been introduced into Europe as an ornamental fish in 1880s; currently, it is widespread in Europe, from Portugal to the River Dnieper (Ukraine), being an alien species in this continent (Kottelat & Freyhof 2007). In Bulgaria, *L. gibbosus* was initially recorded in 1921 in the Svishtov Marsh, Danubian Riverside (Drenski 1922). Currently, it is widespread throughout freshwater habitats in the country (Uzunova & Zlatanova 2007, Uzunova et al. 2008, 2010, 2012).

Introduced species may have significant and diverse negative impacts on the local biota, human health and economy (see Vitousek et al. 1996, 1997), including by introducing parasites and other disease agents (Hanel et al. 2011). On the other hand, introduced species, once established in the new environment, may be infected by local parasites, thus changing their transmission rate and infection burden in native hosts (Dunn 2009, Kelly et al. 2009, Dunn et al. 2012). In North America, the diversity of helminths in *L. gibbosus* includes 49 trematode, 28 monogenean, 12 cestode, 21 nematode and 6 acanthocephalan species (Hoffman 1999). By contrast, according to Buriola et al. (2007), the helminth diversity in *L. gibbosus* in Europe is

*Corresponding author: bbg@ecolab.bas.bg

poorer and comprises of 3 trematode, 7 monogenean, 4 cestode, 7 nematode and 6 acanthocephalan species. Data on several additional helminth species (not mentioned by Buriola et al. 2007) from *L. gibbosus* in Europe can be found in the works by Shulman (1948), Kulakovskaya & Koval' (1973), Pilecka-Rapacz & Sobecka (2008), Košuthová et al. (2009), Çolak (2013), Moshu (2014), Soylu (2014), Havlátová et al. (2015), Stoyanov et al. (2017) and Kvach et al. (2017). In Bulgaria, *L. gibbosus* has been reported as a host of two ancyrocephalid monogeneans, i.e. *Onchocleidus similis* Mueller, 1936 and *O. dispar* Mueller, 1936 (Margaritov 1968, Ondračková et al. 2011), the capillariid nematode *Schulmanela petruschewskii* (Shulman, 1948) and the pomphorhynchid acanthocephalan *Pomphorhynchus laevis* (Zoega in Müller, 1776) (see Kakacheva-Avramova 1977). Until recently, the helminthological data about *L. gibbosus* from this country have been reported only from the Danube River. Recently, metacercariae of the diplostomid trematode *Posthodiplostomum centrarchi* Hoffman, 1958 were reported in *L. gibbosus* from several localities in Europe, including the Lake Atanasovsko Wetlands, Bulgarian Black Sea coast, partly based on specimens obtained in the course of the present survey (Stoyanov et al. 2017).

To the best of our knowledge, there are no data on the structure of the helminth communities in *L. gibbosus* in its non-native geographical range in the Palaearctic. Therefore, we have used data from our survey to characterise helminth communities in this host species. Furthermore, a recent study of parasite communities in the same host from its native range in Canada (Chapman et al. 2015) provides the opportunity to compare helminth communities in native and non-native habitats of this fish species.

The aim of the present article is to report on the helminth diversity in the pumpkinseed sunfish, *L. gibbosus*, from the Lake Atanasovsko Wetlands and to characterise the structure of the helminth communities in this host species.

Materials and Methods

In May, July and September 2012–2013, we examined 107 individuals of pumpkinseed sunfish, *L. gibbosus*, collected from freshwater habitats in the northern part of the Lake Atanasovsko Wetlands (Table 1). The Lake Atanasovsko (42°34′55″N, 27°28′12″E) is a coastal wetland area consisting of a shallow hypersaline lagoon (mean depth 30 cm) used in salt production and divided by dikes into ponds with various salinity; it is surrounded by several channels, freshwater ponds and marshes (Vassilev et al. 2013). The fishes were caught by bait nets and traps from a freshwater pond and its connecting channel. They were kept alive in containers with aerated water from their habitat. In the laboratory, each fish was dissected under a stereomicroscope within next 24 hours. The isolated helminths were placed in saline for c. 10 min, then fixed in hot saline and transferred to 70% ethanol. Subsequently, trematodes were processed as described by Stoyanov et al. (2017). Monogeneans were mounted in glycerine-jelly (Gussev 1983) and measured according to the protocols proposed by Malmberg (1970), Beverley-Burton & Suriano (1980a) and Gussev (1985). Nematodes were mounted in temporary glycerine mounts. The metrical data are presented as the range, followed by the mean and the number of measurements taken (n) in parentheses. The standard deviation is given only when $n \geq 30$. The measurements are in micrometres.

The infection parameters and the parasite community terminology follow Bush et al. (1997). The prevalence criteria 10% and 50% were adopted for identifying rare, intermediate and common species as used by Chapman et al. (2015) for parasite communities of the same host in Canada. The significance of seasonal dynamics in mean intensity, mean abundance and species richness was assessed using Kruskal-Wallis H test. Several parameters of helminth communities were calculated (Magurran 1988). At the component community level, these were Shannon's Diversity Index (H') and Simpson's Dominance Index. At the infracommunity level, these were the number of helminth species per fish and the number of individuals per fish (mean ± standard error, range and median) as well as values of Shannon's Diversity Index (H') and Simpson's Dominance Index (Table 7).

Results

Systematic survey of helminth species

We recorded six helminth species in 54 out of 107 fish individuals studied. These were metacercariae of *Posthodiplostomum centrarchi* (Trematoda), the monogeneans *Onchocleidus similis* and *O. dispar* (Monogenea), and the nematodes *Schulmanela petruschewskii* (adults), *Spiroxys contortus* (third-stage larvae) and *Contracaecum* sp. (third-stage larvae). In order to support our species identifications and to complement data on the morphology and the variation of the helminth species recorded, we provide here brief descriptions and illustrations based on specimens from the Lake Atanasovsko Wetlands.

Table 1. Host sample size (n), prevalence (P%), intensity (I), mean intensity ± standard error (MI±SE), mean abundance ± standard error (MA±SE) and species richness (±SE) of seasonal samples of helminth parasites from *Lepomis gibbosus* (L.) from the Lake Atanasovsko Wetlands (Bulgaria). Statistically significant differences (Kruskal-Wallis H test) in bold; level of confidence p≤0.05.

Helminth species	Infection parameters	May 2012, n=12	July 2012, n=31	September 2012, n=16	May 2013, n=10	July 2013, n=7	September 2013, n=31	K-W (H)*	p-values	Total n=107
Posthodiplostomum centrarchi Hoffman, 1958	P%	–	19.35	–	–	14.29	6.45			8.41
	I (Range)	–	1–4	–	–	3	1–4			1–4
	MI±SE	–	1.50±0.50	–	–	3.00	2.50±1.50	0.000000	1.000	1.88±0.45
	MA±SE	–	0.29±0.14	–	–	0.42	0.16±0.13	8.512669	0.1302	0.16±0.06
Onchocleidus similis Mueller, 1936	P%	16.67	45.16	12.50	30.00	–	41.94			31.78
	I (Range)	1–12	1–14	3	1–15	–	1–14			1–15
	MI±SE	6.50±5.50	4.14±1.04	3.00	7.66±4.05	–	3.84±1.11	0.000000	1.000	4.41±0.73
	MA±SE	1.08±0.99	1.87±0.60	0.37±0.26	2.30±1.57	–	1.61±0.57	10.20161	0.0697	1.40±0.30
O. dispar Mueller, 1936	P%	8.33	6.45	6.25	–	–	–			3.74
	I (Range)	1	1	1	–	–	–			1
	MI±SE	1.00	1.00	1.00	–	–	–	0.000000	1.000	1.00
	MA±SE	0.08	0.06	0.06	–	–	–	3.450305	0.6309	0.04±0.02
Schulmanela petruschewskii (Shulman, 1948)	P%	–	3.23	–	30.00	–	–			3.74
	I (Range)	–	35	–	1–23	–	–			1–35
	MI±SE	–	35.00	–	12.00±6.35	–	–	0.000000	1.000	17.75±7.29
	MA±SE	–	1.12±1.12	–	3.60±2.46	–	–	**21.12771**	**0.0008**	0.66±0.40
Spiroxys contortus (Rudolphi, 1819)	P%	16.67	–	6.25	10.00	–	22.58			10.28
	I (Range)	1	–	1	3	–	1–8			1–8
	MI±SE	1.00	–	1.00	3.00	–	3.71±1.10	0.000000	1.000	2.90±0.77
	MA±SE	0.17±0.11	–	0.06	0.30	–	0.83±0.36	10.34451	0.0660	0.30±0.12
Contracaecum sp.	P%	16.67	–	–	–	–	–			1.87
	I (Range)	1	–	–	–	–	–			1
	MI±SE	1.00	–	–	–	–	–	0.000000	1.000	1.00
	MA±SE	0.17±0.11	–	–	–	–	–	**15.98413**	**0.0069**	0.02±0.01
Total	P%	33.33	61.29	18.75	60.00	14.29	67.74			50.47
	I (Range)	1–12	1–35	1–3	1–23	3	1–14			1–36
	MI±SE	4.50±2.07	5.47±1.70	2.67±0.67	10.33±3.37	3.00	3.85±0.75	2.587506	0.7633	5.11±0.80
	MA±SE	1.50±0.41	3.35±0.53	0.50±0.11	6.20±1.23	0.43±0.17	2.61±0.30	**14.87630**	**0.0109**	2.58±0.22

Class Trematoda
Family Diplostomidae Poirier, 1886
***Posthodiplostomum centrarchi* Hoffman, 1958, metacercariae**

Site of infection: body cavity, liver.

Description and remarks: Metacercariae collected in the course of the present study were described by Stoyanov et al. (2017). Kvach et al. (2017) considered this taxon as "*Posthodiplostomum* cf. *minimum centrarchi* Hoffman, 1958" but Blasco-Costa & Locke (2017) followed our concept (Stoyanov et al. 2017) and recognised it as a distinct species.

Class Monogenea
Family Ancyrocephalidae Bychowsky & Nagibina, 1968
***Onchocleidus similis* Mueller, 1936**

Site of infection: gills.

Description (based on 17 specimens; Fig. 1, 2A-C; for metrical data, see Table 2): Body fusiform, widest approximately at mid-body; anterior end with cephalic lobes bearing terminal parts of cephalic glands (Fig. 1). Eye spots two pairs, anterior (smaller) and posterior, yellowish-brown, consisting of large granules; situated dorsally, at level of pharynx or slightly anterior to it (Fig. 1). Haptor distinct, trapezoid, with rounded angles (Fig. 1). Hamuli two pairs (ventral and dorsal), very strong, similar in shape and size, without spurs (Fig. 2A); hamulus shaft elongate, slender, smooth; inner root conical, elongate; outer root vestigial; point gentle, with tapering and slightly curved tip. Ventral bar bow-shaped, with extended and rounded lateral ends (Fig. 2A). Dorsal bar bow-shaped, with more broadly open anterior arms than ventral bar, with extended lateral ends (Fig. 2A). Ventral and dorsal bars not articulating with one another. Marginal hooks 14 in number, differing in size and shape, grouped in seven pairs situated as follow: pair I largest, central, ventral, with tips directed anteriorly; pair II smallest, between hamuli, ventral, directed posteriorly; pairs III and IV dorsal and lateral; pairs V, VI, and VII ventral and lateral (Fig. 2B); sickle half-moon like, shafts robust, straight or slightly curved. Cirrus complex, shaft sclerotised, elongate-conical tube, straight, entirely encircled by single spiral filament, tip pointed or slightly curved; cirrus base inflated, not encircled by spiral filament (Fig. 2C). Accessory piece teepee-like sheath, transparent, almost entirely enveloping cirrus, not articulating with cirrus base; with proximal circular orifice with sclerotised edge, through which cirrus may protrude (Fig. 2C).

Remarks: The morphology of the studied specimens corresponds to the diagnosis of the genus *Onchocleidus* Mueller, 1936 as proposed by Wheeler & Beverley-Burton (1989): two pairs of hamuli (ventral and dorsal), with transverse bars not articulating with one another (Fig. 2A); marginal hooks 7 pairs, dissimilar in shape, size and position, pairs III and IV dorsal (remaining pairs ventral); cirrus shaft elongate-conical tube, straight, with inflated base, encircled by a spiral filament (Fig. 2C); accessory piece teepee-shaped sheath, transparent, with proximal circular orifice, through which cirrus protrudes (Fig. 2C). The present material is identified as *O. similis* based on the following characters: hamuli similar in shape and size, with vestigial outer roots, shaft elongate, slender and smooth, without spurs (Fig. 2A); cirrus shaft straight, encircled by

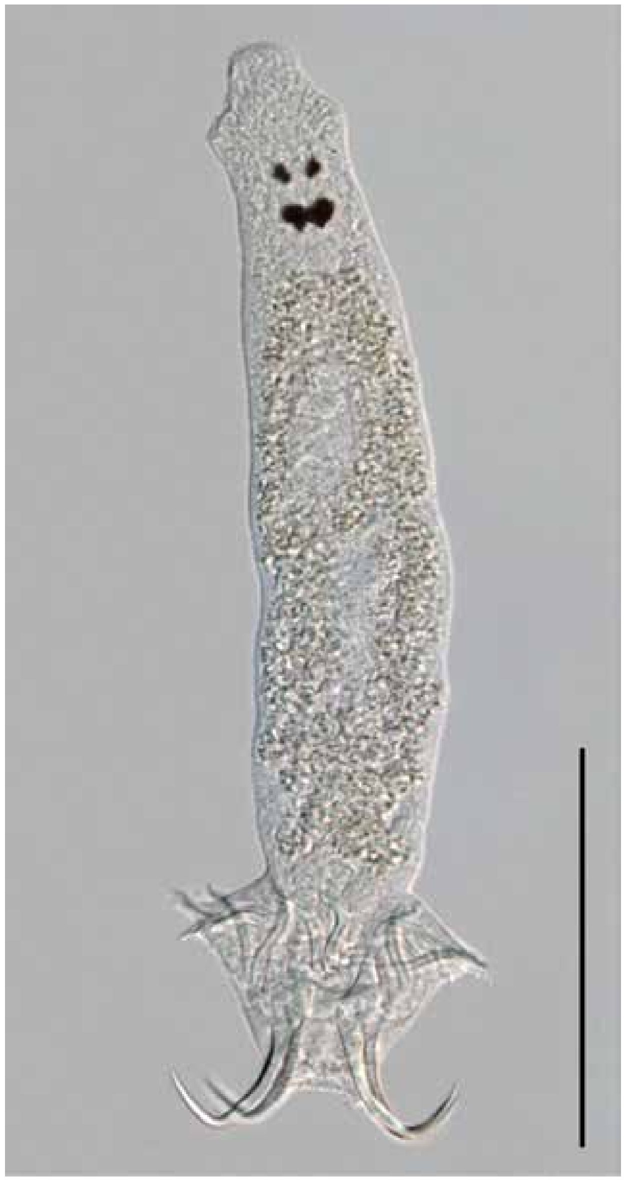

Fig 1. *Onchocleidus similis* Mueller, 1936 from *Lepomis gibbosus* (L.) from the Lake Atanasovsko Wetlands, Bulgaria. General view. Scale bar: 100 µm.

Table 2. Metrical data of *Onchocleidus similis* Mueller, 1936 from pumpkinseed sunfish *Lepomis gibbosus* (L.) in Europe and North America.

Locality	Ontario (Canada)	Komárno (Slovakia)	River Danube (Bulgaria)	River Durance (France)	Lake Atanasovsko (Bulgaria)
Sources	**Beverley-Burton & Suriano (1981)**	**Vojtek (1958)**	**Ondračková et al. (2011)**	**Havlátová et al. (2015)**	**Present study**
Characters	**Mean (Range; n)**	**Range**	**Mean (Range; n)**	**Range (n=10)**	**Mean ± SD (Range; n)**
BTL	280 (190–350; 20)	360–450	–	–	300.0 (218–455; 17)
BMW	78 (60–100; 20)	100	–	–	62.3 (35–87; 17)
HL	45 (31–50; 20)	–	–	–	64.4 (46–85; 17)
HW	60 (52–63; 20)	–	–	–	76.1 (53–104; 17)
PHL	–	–	–	–	16.4 (12–25; 10)
PHW	–	–	–	–	17.0 (13–28; 10)
DHTL	55 (48–61; 20)	36–42	46.8 (33.0–56.7; 10)	41.3–57.0	45.2 (40–54; 17)
DHBL	47 (41–52; 20)	29–34	39.8 (28.1–49.2; 10)	35.6–49.2	38.0 (31–46; 17)
DHORL	1 (1–2; 20)	–	1.7 (1.1–2.4; 10)	1.7–3.0	2.0 (1–3; 17)
DHIRL	12 (8–16; 20)	8–11	11.6 (7.6–14.1; 10)	8.8–13.6	10.1 (9–13; 17)
DHPL	19 (16–24; 20)	13–15	16.8 (12.6–19.7; 10)	14.7–19.7	17.2 (14–21; 17)
DBTL	26 (24–28; 20)	18–21	23.1 (17.8–27.9; 10)	18.9–26.0	21.1 (16–26; 17)
DBTW	–	–	6.5 (4.5–9.4; 10)	5.5–9.3	5.7 (4–7; 17)
DBMW	6 (5–8; 20)	3–4	3.2 (2.6–4.1; 10)	2.8–5.1	3.3 (2–5; 17)
VHTL	56 (50–61; 20)	33–41	45.3 (29.2–56.4; 10)	39.7–56.6	45.5 (38–55; 17)
VHBL	47 (42–52; 20)	29–33	37.9 (26.1–47.1; 10)	32.5–46.3	38.0 (32–47; 17)
VHORL	2 (1–4; 20)	–	2.1 (1.2–2.8; 10)	1.2–2.9	2.1 (2–3; 17)
VHIRL	13 (11–14; 20)	8–10	11.1 (6.4–13.8; 10)	9.9–14.2	10.0 (8–12; 17)
VHPL	18 (14–20; 20)	11–14	15.3 (11.2–17.6; 10)	14.4–17.5	17.8 (15–21; 17)
VBTL	28 (25–30; 20)	17–21	23.6 (18.7–28.2; 10)	18.8–26.9	20.3 (15–25; 16)
VBTW	–	–	6.9 (4.7–8.7; 10)	6.5–9.6	5.5 (4–8; 16)
VBMW	6 (5–7; 20)	3–4	3.6 (2.8–4.4; 10)	3.3–5.7	3.2 (2–4; 16)
MHTL I	29 (27–31; 20)	18–26*	29.3 (19.6–35.9; 10)	27.9–35.2	27.9±3.1 (20–35; 33)
II	11 (10–12; 20)	18–26*	11.5 (10.5–12.7; 10)	11.2–12.7	10.4±0.7 (10–12; 32)
III	28 (27–31; 20)	18–26*	27.5 (19.2–31.8; 10)*	25.9–31.3*	26.1±2.7 (18–31; 34)
IV	27 (25–28; 20)	18–26*	27.5 (19.2–31.8; 10)*	25.9–31.3*	24.5±3.2 (18–31; 34)
V	27 (25–28; 20)	18–26*	24.7 (17.1–30.6; 10)*	23.3–29.4*	23.9±3.1 (19–31; 34)
VI	26 (24–28; 20)	18–26*	24.7 (17.1–30.6; 10)*	23.3–29.4*	23.0±3.7 (17–33; 32)
VII	26 (24–27; 20)	18–26*	24.7 (17.1–30.6; 10)*	23.3–29.4*	24.5±4.2 (16–32; 33)
CSL	31 (25–34; 20)	26–33	29.4 (27.5–31.2; 10)	24.4–32.4	24.8 (17–31; 17)
APL	22 (20–24; 20)	16–20	–	–	17.0 (11–24; 5)

*Lengths of different pairs not separately given.

Abbreviations: APL, accessory piece length; BTL, body total length; BTW, body total width; CSL, cirrus length; DBMW, dorsal bar median width; DBTL, dorsal bar total length; DBTW, dorsal bar total width; DHBL, dorsal hamulus base length; DHIRL, dorsal hamulus inner root length; DHORL, dorsal hamulus outer root length; DHPL, dorsal hamulus point length; DHTL, dorsal hamulus total length; HL, haptor length; HW, haptor width; MHTL, marginal hook I to VII pairs total length; PHL, pharynx length; PHW, pharynx width; VBMW, ventral bar median width; VBTL, ventral bar total length; VBTW, ventral bar total width; VHBL, ventral hamulus base length; VHIRL, ventral hamulus inner root length; VHORL, ventral hamulus outer root length; VHPL, ventral hamulus point length; VHTL, ventral hamulus total length.

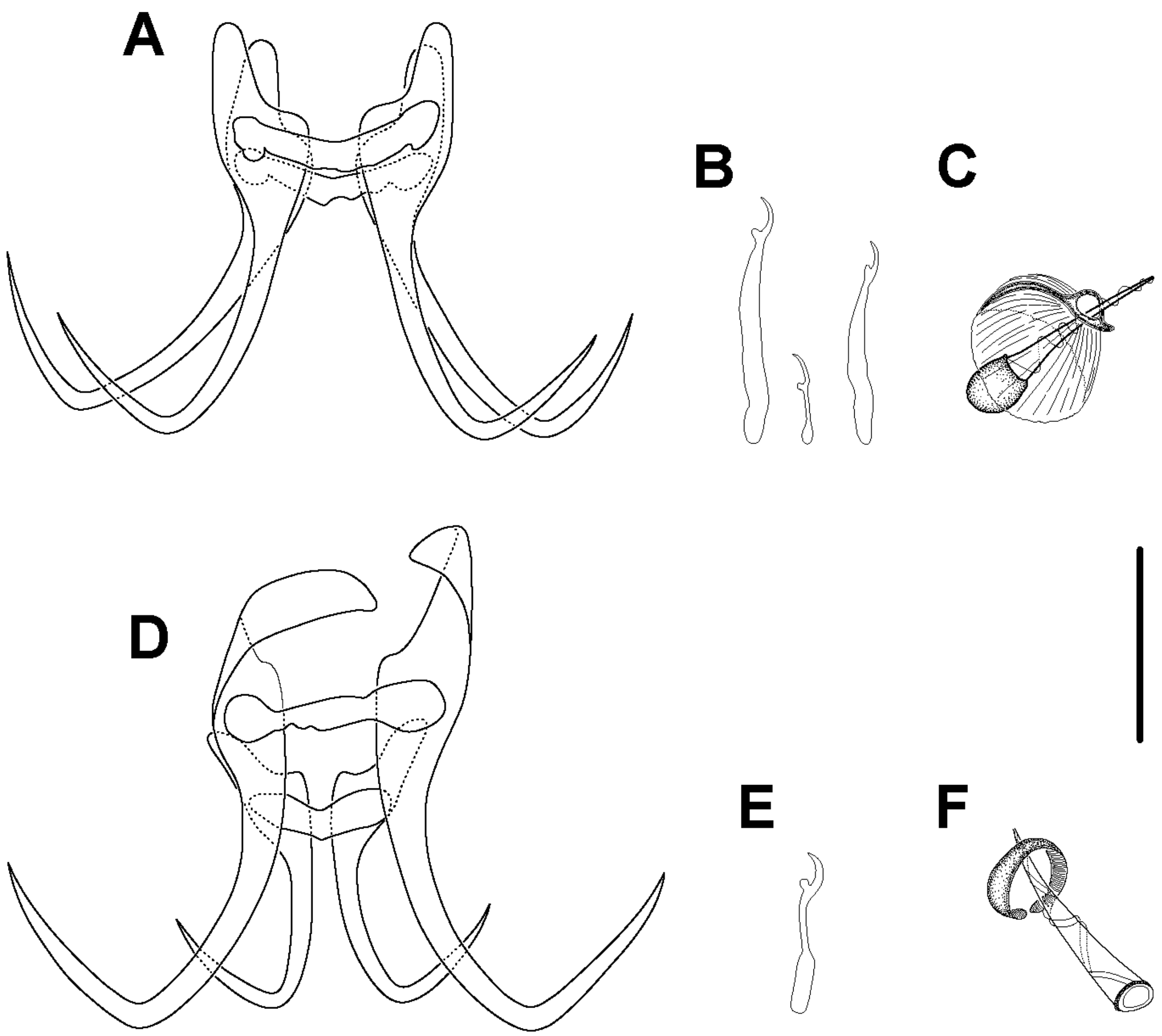

Fig. 2. A-C. *Onchocleidus similis* Mueller, 1936 from *Lepomis gibbosus* (L.) from the Lake Atanasovsko Wetlands, Bulgaria. A. Dorsal and ventral hamuli with transverse connective bars. B. Marginal hooks; left – pair I, central – pair II and right – pair VII. C. Cirrus with accessory piece. D-F. *Onchocleidus dispar* Mueller, 1936. D. Dorsal and ventral hamuli with transverse connective bars. E. Marginal hook. F. Cirrus with a part of accessory piece. Scale bar: 20 µm.

a single spiral filament; accessory piece represents an unsclerotised sheath with a proximal circular orifice with sclerotised edge (Fig. 2C). In addition, the overlap of metrical characters of studied specimens with those in the previous descriptions (Table 2) also supports their identification as *O. similis*.

In Europe, four species of the genus *Onchocleidus* have been recorded: *O. similis*, *O. dispar* Mueller, 1936 and *O. acer* Mueller, 1936, all from *L. gibbosus* (see Havlátová et al. 2015) and *O. principalis* Mizelle, 1936 from the largemouth bass *Micropterus salmoides* (Lacepède) (see Maitland & Price 1969, Galli et al. 2007). The present specimens can be differentiated from the other congeneric species in Europe as follows: similar in shape and size ventral and dorsal pairs of hamuli (Fig. 2A) *vs* ventral pair significantly smaller than dorsal pair and differing in shape in *O. dispar* (Fig. 2D); cirrus shaft encircled by a single spiral filament (Fig. 2C) *vs* cirrus shaft encircled by a double spiral filament in *O. dispar* (Fig. 2F); both pairs of hamuli without spurs (Fig. 2A) *vs* presence of flat finger-like spurs on inner curve of blade in *O. acer* (Figs. 9, 11 by Beverley-Burton & Suriano 1980b). *Onchocleidus principalis* and *O. similis* can be distinguished from one another by the structure of the cirrus complex: cirrus shaft helical, corkscrew-like, cirrus base encircled by indistinct spiral filament; accessory piece distally bifurcate, partially encircling cirrus shaft in *O. principalis* (see Wheeler & Beverly-Burton 1989) *vs* cirrus shaft elongate-conical tube, straight, entirely encircled by a spiral filament, tip pointed or slightly curved; cirrus base inflated, not encircled by spiral filament in *O. similis* (see Beverly-Burton & Suriano 1981; present study). In addition, *O. principalis* has not been recorded on *L. gibbosus* (see Hoffman 1999, Galli et al. 2007, Havlátová et al. 2015). Collins & Janovy (2003) have regarded it as oioxenous to the largemouth bass.

Onchocleidus dispar **Mueller, 1936**
Site of infection: gills.

Description (based on 4 specimens; Fig. 2D-F); for metrical data, see Table 3): Body fusiform, with maximum width approximately at mid-body; anterior extremity with cephalic lobes bearing terminal parts of cephalic glands. Eye spots two pairs, anterior and posterior, yellowish-brown, consisting of large granules; anterior eyespots smaller than posterior; situated dorsally, at level of pharynx or slightly anterior to it. Haptor distinct, with wider anterior part, gradually tapering posteriorly and passing into posterior part possessing almost parallel lateral margins and rounded posterior end. Hamuli in two pairs, ventral and dorsal, differing in shape and size, without spurs (Fig. 2D). Hamuli of ventral pair with conical inner root and vestigial outer root; hamulus shaft slender, smoot; point gentle, slightly curved, with tapering tip. Ventral bar bow-shaped, with extended and rounded lateral ends (Fig. 2D). Hamuli of dorsal pair distinctly larger than those of ventral pair, with elongate inner root and vestigial outer root; hamulus shaft slender, smoot; point slender, with slightly curved tip. Dorsal bar straight, with extended and slightly elongated lateral ends (Fig. 2D). Ventral and dorsal bars not articulating with one another. Marginal hooks tiny, 14 in number, different in size (Table 3), forming seven pairs; sickle half-moon like; shaft with robust distal part, passing into thinner and elongate proximal part (Fig. 2E). Cirrus complex, shaft sclerotised, elongate-conical tube, straight or curved, encircled by double spiral filament; cirrus passing through sclerotised distal ring (Fig. 2F).

Remarks: Morphologically, these specimens correspond to the diagnosis of *Onchocleidus* (see Wheeler & Beverley-Burton 1989). Their species identification is based on the hamuli dissimilar in shape and size (dorsal pair distinctly larger than ventral pair), without spurs (Fig. 2D); cirrus elongate-conical tube, straight or slightly curved, less than 40 μm long (15–25 μm long in our specimens), encircled by a double spiral filament (Fig. 2F). In addition, the metrical characters overlap with those from the previous descriptions of *O. dispar* (Table 3). The present material shows some metrical differences compared to previous descriptions: average body length, haptor width, ventral hamulus base length, dorsal bar total length, ventral bar total length and median width, and larger outer processes of both pairs of hamuli compared to those in the description by Beverley-Burton & Suriano (1980b) (Table 3). In addition, our specimens have smaller dorsal bar total length, ventral hamulus inner root length and point length, marginal hook total length (pair I) and cirrus length than those described by Ondračková et al. (2011) on the basis of specimens from the Danubian basin in Bulgaria (Table 3). Rubtsova (2015a) reported larger total length of body, dorsal hamulus inner root length, ventral bar total length and marginal hook total length (pairs I and II) in *O. dispar* from Ukraine than those in the present material (Table 3). The observed differences expand the knowledge of the intraspecific variation of *O. dispar*.

Class Adenophorea

Family Capillariidae Railliet, 1915

Schulmanela petruschewskii **(Shulman, 1948)**
Site of infection: liver.

Description (based on 2 male and 7 female mature individuals; Fig. 3A-F; for metrical data, see Table 4):

General: Body yellowish-brown, thread-like, strongly elongate, with maximum width approximately at mid-body, gradually narrowing to both ends; anterior end rounded, (Fig. 3G). Bacillary bands two, hardly distinct. Muscular oesophagus, narrow, short. Stichosome composed of irregular in shape stichocytes, arranged in three longitudinal rows; each stichocyte with single large nucleus. Nerve ring surrounding muscular oesophagus at posterior level of its anterior quarter.

Male: Stichocytes 133–137 (135, n = 2) in number. Length of muscular oesophagus and stichosome 27–33% (30%, n = 2) of total body length. Ratio muscular oesophagus length : stichosome length 1:6.0–6.1 (1:6.0, n = 2). Spicule sclerotised, covered with numerous transverse grooves except smooth ends; proximal spicule end slightly expanded; distal end narrow, slightly rounded (Fig. 3H). Spicular sheath partially covered with numerous tiny, flat spines (Fig. 3H). Tail short, with membranous bursa with broadly-rounded tip (Fig. 3I).

Female: Stichocytes 117–144 (129, n = 7) in number. Length of muscular oesophagus and stichosome 13–22% (16%, n = 7) of total body length. Ratio oesophagus muscular part length : stichosome length 1:5.3–8.8 (1:6.4, n = 7). Vulva distinct, slightly muscular (Fig. 3B). Rectum long, opens sub-terminally (Fig. 3D). Tail short, rounded (Fig. 3D). Eggs ellip-

Table 3. Metrical data of *Onchocleidus dispar* Mueller, 1936 from pumpkinseed sunfish *Lepomis gibbosus* (L.) in Europe and North America. For abbreviations, see Table 2.

Locality	Ontario (Canada)	Komárno (Slovakia)	River Danube (Bulgaria)	River Durance (France)	Kakhovske Reservoir (Ukraine)	Lake Atanasovsko (Bulgaria)
Sources	Beverley-Burton & Suriano (1980)	Vojtek (1958)	Ondračková et al. (2011)	Havlátová et al. (2015)	Rubtsova (2015a)	Present study
Characters	Mean (Range; n=20)	Range	Mean (Range; n=8)	Range (n=2)	Mean (Range; n=3)	Mean (Range; n)
BTL	410 (320–512)	350–560	–	–	500	320.0 (185–400; 4)
BMW	100 (90–150)	90–120	–	–	–	98.2 (55–125; 4)
HL	75 (50–100)	–	–	–	–	52.3 (46–57; 3)
HW	125 (100–150)	–	–	–	–	62.0 (53–67; 3)
PHL	–	–	–	–	–	25.3 (24–27; 3)
PHW	–	–	–	–	–	25.0 (21–29; 3)
DHTL	71 (60–85)	49–60	65.2 (61.3–68.1)	63.6–69.1	61.7 (60–65)	57.5 (52–64; 4)
DHBL	52 (45–65)	36–44	50.1 (47.8–51.9)	50.0-53.9	46.3 (42–52)	43.7 (40–50; 4)
DHORL	0	–	1.3 (1.0–1.7)	1.1–1.5	1.2 (1.1–1.3)	1.5 (1–2; 4)
DHIRL	27 (23–30)	19–23	26.5 (25.0–28.1)	22.7–25.2	27.7 (26–30)	19.0 (13–25; 4)
DHPL	28 (25–30)	21–25	25.1 (23.0–26.6)	23.2–25.6	23.7 (22–25)	24.5 (23–27; 4)
DBTL	30 (28–35)	23–26	27.8 (27.0–28.9)	30.1–30.7	29.7 (24–38)	23.5 (20–25; 4)
DBTW	–	–	6.8 (6.3–7.6)	7.8–8.1	4.0 (3–5)	5.5 (5–6; 4)
DBMW	4 (3–5)	4–6	4.4 (3.9–5.0)	4.0–4.6	–	3.2 (3–4; 4)
VHTL	40 (35–45)	29–33	36.1 (34.3–39.0)	35.2–38.7	37.0 (35–40)	31.5 (29–35; 4)
VHBL	33 (30–40)	23–26	28.1 (27.2–29.9)	28.7–32.0	27.3 (26–29)	25.5 (24–28; 4)
VHORL	0	–	1.3 (1.0–1.7)	1.2–1.5	1.4 (1.2–1.5)	1.5 (1–2; 4)
VHIRL	10 (7–12)	9–11	26.5 (25.0–28.1)	8.3–11.2	13.0 (10–15)	9.0 (8–10; 4)
VHPL	20 (15–25)	14–16	25.1 (23.0–26.6)	15.3–17.7	19.3 (16–25)	15.2 (15–16; 4)
VBTL	21(17–25)	13–16	17.2 (16.5–18.1)	15.7–18.0	22.0 (20–22)	14.5 (13–16; 4)
VBTW	–	–	5.1 (4.7–5.4)	5.7–7.2	3.0	4.7 (4–6; 4)
VBMW	6 (5–7)	3–4	3.1 (2.9–3.4)	4.0–4.0		2.5 (2–3; 4)
MHTL I	16 (15–20)*	15–19*	14.2 (12.5–16.8)	15.1–17.7	17.0 (16-19)	10.0 (10–10; 2)
II	16 (15–20)*	15–19*	14.1 (13.1–14.8)	13.9–14.2	17.0 (16-19)	13.5 (12–15; 4)
III	16 (15–20)*	15–19*	19.5 (17.6–20.7)*	17.2–18.4*	17.0 (16-19)	19.1 (15–26; 7)
IV	16 (15–20)*	15–19*	19.5 (17.6–20.7)*	17.2–18.4*	17.0 (16-19)	16.7 (15–18; 7)
V	16 (15–20)*	15–19*	18.1 (16.2–20.6)*	17.6–18.7*	17.0 (16-19)	16.1 (13–19; 6)
VI	16 (15–20)*	15–19*	18.1 (16.2–20.6)*	17.6–18.7*	17.0 (16-19)	15.6 (15–16; 5)
VII	16 (15–20)*	15–19*	18.1 (16.2–20.6)*	17.6–18.7*	17.0 (16-19)	14.5 (13–16; 4)
CSL	31 (26–35)	26–29	27.4 (25.8–29.8)	27.2–28.3	27.0 (25–30)	19.2 (15–25; 4)
APL	20 (17–25)	18–21	–	–	–	–

*Lengths of different pairs not separately given.

Table 4. Metrical data of *Schulmanela petruschewskii* (Shulman, 1948) from various freshwater fishes from Europe.

Host	***Cobitis taenia* L.; *Lepomis gibbosus***		***Lepomis gibbosus*; *Oncorhynchus mykiss* (Walbaum)**		**Various freshwater fishes**		***L. gibbosus***	
Locality	Lake Kagul (Ukraine)		River Po, Piedmont (Italy)		Europe		Lake Atanasovsko (Bulgaria)	
Source	Shulman (1948)		Ghittino (1961)		Moravec (2001a)		Present study	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
Character	Range	Range	Range*	Range*	Range	Range	Range (Mean; n)	Range (Mean ± SD; n)
BTL	4,720	6,600–12,000	5,000–6,000	12,000–15,000	5,750–8,660	10,140–14,760	5,631–7,045 (6,338; 2)	12,044–16,200 (13,917; 7)
BMW	33–81	30–70	76	90–150	68–95	122–149	50–68 (59; 2)	80–116 (96; 7)
BWV	–	–	–	–	–	–	–	53–86 (72; 7)
BBW	–	–	–	–	36	54–63	25 (25; 1)	30–38 (35; 3)
OTL	–	–	–	2,500–3,000	1,490–2,080	1,990–2,580	1,863–1,918 (1,891; 2)	1,823–2,708 (2,170; 7)
MOL	182	156	200–350	250–330	228–354	282–321	265–270 (268; 2)	270–326 (293; 7)
MOW	–	–	–	–	–	–	18–23 (21; 2)	22–26 (25; 7)
SL	–	–	2,380–2,400	2,110–2,520	–	–	1,598–1,648 (1,623; 2)	1,538–2,433 (1,877; 7)
SW	–	–	–	–	–	–	40–50 (45; 2)	58–68 (61; 7)
NR	120	80–90	–	–	69–99	84–108	75–75 (75; 2)	75–90 (82; 7)
VAE	–	1,200–3,300	–	2,500–3,000	–	–	–	2,030–2,828 (2,396; 7)
VOE	–	–	–	140–150	–	60–120	–	120–325 (229; 7)
SPL	–	–	200–280	–	210–279	–	158–195 (177; 2)	–
SPW	–	–	8–11	–	12–15	–	10–13 (12; 2)	–
RL	81	81	–	–	–	210–231	–	63–130 (104; 7)
TL	–	–	–	–	18–27	12–15	21–25 (23; 2)	5–13 (10; 6)
BL	–	–	–	–	9	–	8–8 (8; 2)	–
EGL	–	63–64	–	60–65	–	60–69	–	52–60 (58; 19)
EGW	–	28–30	–	28	–	30–36	–	22–26 (24; 19)
WT	–	–	–	–	–	2–3	–	1–2 (1.4; 19)
PPH	–	5	–	–	–	5–6	–	2–4 (3.2±0.4; 38)

*Ghittino (1961) reported additional metrical data for several characters. Males: body total length 8,000; body minimum width 17–20; body median width 60–73 and spicular sheath diameter 42. Females: body total length 20,000–22,000; body width at anterior extremity 20–23; body width at level posterior to vulva 70; eggs length without polar plugs 56–59.

Abbreviations: BBW, bacillary band width; BL, bursa length; BMW, body maximum width; BTL, body total length; BWV, body width at vulva; EGL, egg length; EGW, egg width; MOL, muscular oesophagus length; MOW, muscular oesophagus maximum width; NR, nerve ring, distance from anterior extremity; OTL, oesophagus total length; PPH, egg polar plugs height; RL, rectum length; SL, stichosome length; SPL, spicule length; SPW, spicule width; SW, stichosome maximum width; TL, tail length; VAE, vulva, distance from anterior extremity; VOE, vulva, distance from proximal end of oesophagus; WT, egg wall thickness.

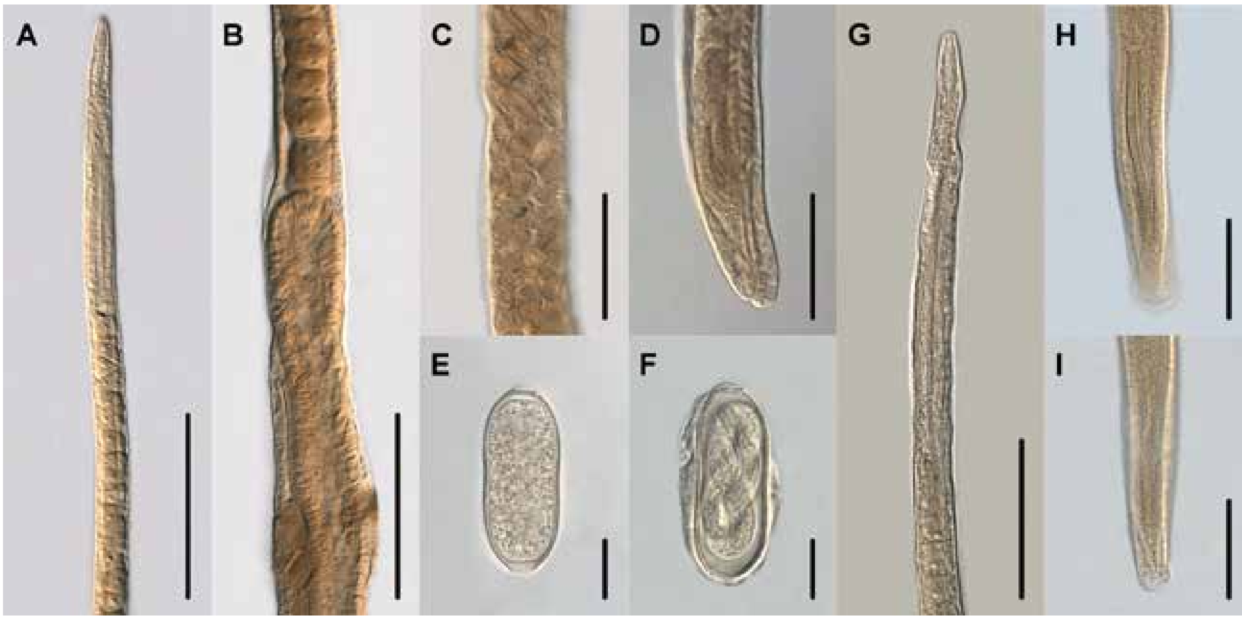

Fig. 3. *Schulmanela petruschewskii* (Shulman, 1948) from *Lepomis gibbosus* (L.) from the Lake Atanasovsko Wetlands, Bulgaria. A-D. Female. A. Anterior end of body with muscular oesophagus and anterior part of glandular oesophagus with stichocytes. B. Oesophago-intestinal junction, vulva and anterior portion of uterus with eggs. C. Uterus with eggs. D. Posterior end of body with rectum, anal opening and tail, lateral view. E. Unembryonated egg. F. Egg with a developing embryo. G-I. Male. G. Anterior end of body with muscular oesophagus and anterior part of stichosome with stichocytes. H. Posterior end of body with spicule and spicular sheath covered with spines. I. Tail with bursa, lateral view. Scale bars: A, B – 200 µm; C, D, G, H, I – 100 µm; E, F – 20 µm.

tical, yellowish-brown (Fig. 3C), with polar plugs, protruded or not; wall relatively thick, smooth; filled with homogeneous, slightly granular content (Fig. 3E) or developing embryo (Fig. 3F).

Remarks: The specimens studied correspond to the diagnosis of the monotypic genus *Schulmanela* Ivashkin, 1964 as proposed by Moravec (2001a). The main diagnostic features of this genus include the presence of three longitudinal rows of stichocytes; spicule with numerous transverse grooves, only spicule ends smooth, with proximal end slightly expanded and distal end rounded (Fig. 3H); spicular sheath partially covered with numerous tiny spines (Fig. 3H); tail of males with membranous bursa (Fig. 3I). The ranges of the majority of metrical characters of our specimens overlap those included in previous descriptions (Table 4). The specific site of infection (liver) and the freshwater fish host also support the placement of the present material in *Schulmanela*. The oesophagus total length as percentage of the total body length is 27–33% (30%; 2) in males and 13–22% (16%; 7) in females in the present specimens and this fits to 19–33% in males and 15–20% in females reported for *S. petruschewskii* (see Moravec 2001a). The present individuals possess smaller body maximum width, glandular oesophagus length and spicule length of males as well as egg width than those described by Ghittino (1961) (Table 4). In addition, our specimens possess larger ratio muscular oesophagus length : stichosome length 1:6.0–6.1 (1:6.0; 2) in males and 1:5.3–8.8 (1:6.4; 7) in females *vs* 1:2.1–3.7 in males and 1:4.7–5.0 in females in the description presented by Ghittino (1961). Our specimens possess smaller bacillary band width, spicule length and bursa length in males as well as body maximum width, rectum length, eggs width and eggs polar plugs length than those given by Moravec (2001a) (Table 4). It could be speculated that the differences observed may be a result of the different host species, i.e. *Oncorhynchus mykiss* (Walbaum, 1792) for the specimens described by Moravec (2001a) and (or) reflect intraspecific variations (Table 4).

Class Secernentea

Family Anisakidae Railliet & Henry, 1912

***Contracaecum* sp., third-stage larvae**

Site of infection: free in body cavity.

Description (based on two third-stage larvae; Fig. 4A-C; for metrical data, see Table 5): Body fusiform, yellowish-brown. Anterior end truncate, with weakly developed primordial lips and conical ventral larval tooth (Fig. 4A). Cuticle bearing external transverse striations, cuticle ticker and striations more distinct at body ends; cuticular spines absent. Excretory pore opening just posteriorly to cephalic tooth (Fig. 4A). Oesophagus muscular, elongate, with small glandular ventriculus at proximal end. Ventriculus irregular in shape, with posterior claviform ventricular appendix (Fig. 4B),(Table 5). Intestine light brownish, thick-walled, with broad lumen. Anterior intestinal caecum robust, elongate-conical, with rounded tip (Fig. 4B), extending to level posterior to nerve ring, twice shorter than oesophagus. Ratio intestinal caecum length : ventricular appendix length 1:1.75–2.21 (1:1.98). Ratio intestinal caecum length : oesophagus length 1:2.17–2.24 (1:2.20, n = 2). Rectum tubular, short. Genital primordium indistinct. Tail conical, with bluntly pointed tip (Fig. 4C).

Remarks: The morphology of the present specimens is in agreement with the generic diagnosis of *Contracaecum* Railliet & Henry, 1912 (see Moravec 1994): cuticle without spines; oesophagus with ventriculus on proximal end; posterior ventricular appendix and anterior intestinal caecum present (Fig. 4B); excretory pore opens on anterior end, posteriorly to cephalic tooth (Fig. 4A); tail conical (Fig. 4C). The present material is not identified at the species level. Currently, it is considered that the species identification of *Contracaecum* larvae from fishes based only on their morphology is not possible (Moravec 1994, Moravec et al. 2016). The present specimens differ by their metrical characters from the majority of larvae of *Contracaecum* spp. from Europe described in detail by Moravec (1994). In addition, our specimens have a smaller ratio intestinal caecum length : ventricular appendix length (1:1.75–2.21) compared to 1:3–4 in *C. microcephalum* (Rudolphi, 1809), a widespread parasite of various fish-eating birds (Pelecanidae, Ardeidae and Ciconiidae) in Europe (Moravec 1994). In contrast, the same ratio is 3:1 in *C. rudolphii* Hartwich, 1964, a parasite of Phalacrocoracidae and Anatidae (*Mergus* spp.), as described by Moravec (1994). The metrical data of the present specimens are more similar to those of third-stage larvae of *C. micropapillatum* (Stossich, 1890), a parasite in digestive system of Pelecanidae, Ardeidae, Phalacrocoracidae and Podicipedidae; however, they differ from them by the body maximum width and the distance of the nerve ring to the anterior body extremity, which are larger in the present material (Table 5).

Ten *Contracaecum* spp. have been recognized as parasites (larvae and adults) of numerous fish-eating birds and marine mammals (definitive hosts) and fishes (intermediate or paratenic hosts) (Moravec 1994). In the region of the Black Sea, eight species have been recorded (Gvozdev et al. 1983). Furthermore, two additional species of this genus have been described as parasites of Dalmatian pelicans (*Pelecanus crispus* Bruch, 1832) from adjacent regions in Northern Greece (Mattiucci et al. 2010). Therefore, we suppose that the third-stage larvae found by us belong to a species with unknown life cycle and further studies, combining morphological and molecular techniques, are needed for their identification.

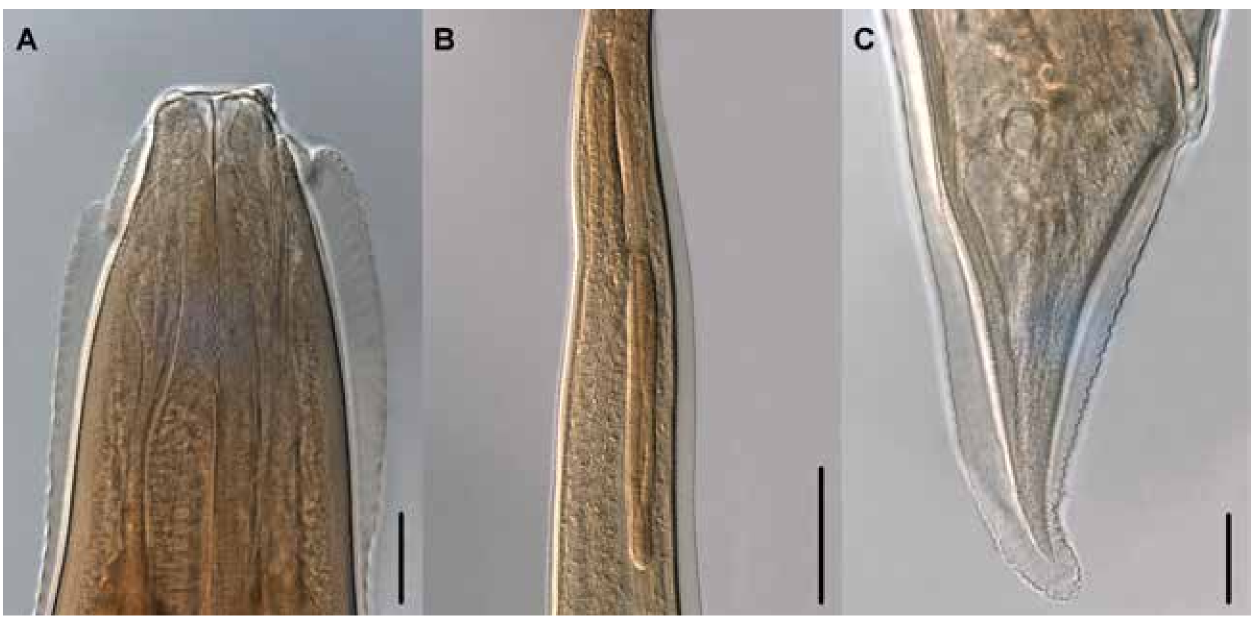

Fig. 4. *Contracaecum* sp., third-stage larvae from *Lepomis gibbosus* (L.) from the Lake Atanasovsko Wetlands, Bulgaria. A. Anterior end of body (lateral view), with cephalic tooth and excretory pore. B. Oesophago-intestinal junction, with anteriorly directed intestinal caecum and posteriorly directed ventricular appendix. C. Anal opening and tail, lateral view. Scale bars: A, C – 20 μm; B – 100 μm.

Table 5. Metrical data of *Contracaecum* spp. (third-stage larvae) described from freshwater fish species in Europe.

Species	*C. microcephalum* (Rudolphi, 1809)	*C. micropapillatum* (Stossich, 1890)	*C. osculatum* (Rudolphi, 1802)	*C. ovale* (Linstow, 1907)	*C. rudolphii* Hartwich, 1964	*Contracaecum* sp.
Host	Various fishes	Various fishes	Various fishes	Various fishes	Various fishes	*Lepomis gibbosus* (L.)
Locality	Europe	Europe	Europe	Europe	Europe	Lake Atanasovsko (Bulgaria)
Source	Moravec (1994)					Present study
Character	Range	Range[a]	Range	Range	Range	Range (Mean; n)
BTL	1,310–2,000 or 3,600–7,800*	430–6,000	3,810–22,000	3,800–4,100	780–910 or 15,000–24,000[b]	2,632–3,681 (3,157; 2)
BMW	56–70 or 150–240*	10–40	150–530	210–250	900–2,100	105–165 (135; 2)
BWA	–	–	–	–	–	43–63 (53; 2)
OL	210–230 or 400–480*	110–600	550–1,710	600	–	278–415 (347; 2)
OW	–	–	–	–	–	13–25 (19; 2)
VL	16–17	–	–	40–50	20–30	16–25 (21; 2)
VW	10–20	–	–	30–40	10–30	17–18 (18; 2)
VAL	190–240 or 420–500*	80–570	540–1,580	680–800	150–200 or 500–650[b]	225–410 (318; 2)
VAW	–	–	–	–	–	21–41 (31; 2)
NR	116–120	50–60	–	–	140–150	125–160 (143; 2)
ICL	6–43 or 210–270*	10–500	290–890	350–400	1,450–2,250	128–185 (157; 2)
ICW	–	–	–	–	–	26–44 (35; 2)
TL	50–90	50–150	90–210	–	–	95–105 (100; 2)

*Larger (fully-developed) larvae from naturally infected fishes; [a]From experimentally infected fishes; [b]More developed larvae

Abbreviations: BMW, body maximum width; BTL, body total length; BWA, body width at anus; ICL, intestinal caecum length; ICW, intestinal caecum maximum width; NR, nerve ring, distance from anterior extremity; OL, oesophagus length; OW, oesophagus width; TL, tail length; VAL, ventricular appendix length; VAW, ventricular appendix width; VL, ventriculus length; VW, ventriculus width.

The records of *Contracaecum* spp. (larvae) from *L. gibbosus* from North America and Europe are few. In North America, Dechtiar (1972) has reported light infection with *Contracaecum* sp. in this host from several wetlands in Ontario (Canada) and Aliff et al. (1977) have recorded *Contracaecum* sp. in the same host from Georgia (USA); unfortunately, descriptions of these larvae have not been published. In Europe, only three records of *Contracaecum* spp. (third-stage larvae) are known in *L. gibbosus* from Italy, Poland and United Kingdom (Buriola et al. 2007, Pilecka-Rapacz & Sobecka 2008, Hockley et al. 2011), with no morphological or genetic data on parasites found.

Family Gnathostomatidae Railliet, 1895

Spiroxys contortus (Rudolphi, 1819), third-stage larvae

Site of infection: encapsulated in the intestinal wall and stomach wall.

Description (based on ten third-stage larvae; Fig. 5A-D; for metrical data, see Table 6): Body elongate, slightly tapering to both ends, with maximal width approximately at level of oesophageal-intestinal junction; yellowish-brown. Anterior end with two triangular lateral pseudolabia, each with two distinct papillae (Fig. 5A-B). Cuticle smooth, bearing fine external transverse striations. Oesophagus straight, divided into anterior short and narrow muscular part with broad distal end and longer and wider posterior glandular portion, with grainy appearance. Nerve ring surrounding glandular oesophagus, slightly posterior to its anterior end. Excretory pore posterior to anterior end of glandular oesophagus. Deirids small, spine-like, lateral, at same level, inclined into posterior direction (Fig. 5C). Tail short, conical, with tapering tip (Fig. 5D).

Remarks: Morphologically, these specimens correspond to the genus *Spiroxys* Schneider, 1866 as diagnosed by Moravec (1994). The present material is identified as *Spiroxys contortus*, since this is the only species of the genus in Europe (Moravec 1994). Most of metrical characters of our specimens correspond to previous data about third-stage larvae of *S. contortus* (Table 6). Our specimens have slightly smaller body length and body maximum width as well as smaller distance of and excretory pore to the anterior extremity compared to those described by Moravec (1994) (Table 6). *S. contortus* is a widespread stomach parasite of the European pond turtle *Emys orbicularis* (L.) (frequent in the wetland studied) and Caspian turtle *Mauremys caspica* (Gmelin, 1774) (definitive hosts); its life-cycle includes copepods as intermediate hosts and fishes, larvae of aquatic insects, snails, amphibians and reptiles as paratenic host in Europe (Sharpilo 1976, Moravec 1994). From Bulgaria, larvae of *S. contortus* have been recorded from *Misgurnus fossilis* (L., 1758) (Cobitidae) from the River Danube (Kakacheva-Avramova 1983). From *L. gibbosus*, larvae of this parasite species have been recorded in Poland (Pilecka-Rapacz & Sobecka 2008).

Table 6. Metrical data of *Spiroxys contortus* (Rudolphi, 1819) (third-stage larvae) from various freshwater fishes in Europe.

Host	**Various freshwater fishes**	***Lepomis gibbosus* (L.)**
Locality	Europe	Lake Atanasovsko
Source	Moravec (1994)	Present study
Characters	Range	Range (Mean; n)
BTL	2,530–3,000	1,390–2,622 (1,898;10)
BMW	84–95	45–70 (57; 10)
BAW	–	28–45 (35; 9)
PL	15–18	10–18 (16; 10)
OTL	–	560–858 (653; 10)
MOL	88–120	84–103 (90; 10)
MOW	–	13–23 (18; 10)
GOL	549–775	475–754 (561; 100
GOW	–	28–50 (38; 10)
NR	–	117–165 (138; 6)
EP	200	128–215 (166; 9)
DD	237–299	162–305 (227; 10)
TL	60	38–83 (58; 9)

Abbreviations: BAW, body width at anus; BMW, body maximum width; BTL, body total length; DD, deirids, distance from anterior extremity; EP, excretory pore, distance from anterior extremity; GOL, glandular oesophagus length; GOW, glandular oesophagus maximum width; MOL, muscular oesophagus length; MOW, muscular oesophagus maximum width; NR, nerve ring, distance from anterior extremity; OTL, oesophagus total length; PL, pseudolabia length; TL, tail length.

Helminth communities

The component helminth community in *Lepomis gibbosus* in the Lake Atanasovsko Wetlands, based on six seasonal samples in 2012-2013, consisted of six species (Tables 1 and 7). These were metacercariae of one trematode species, two species of monogeneans and three species of nematodes (one represented by adult parasites and two occurring in fishes as larvae). Based on all the samples, none of the helminth species could be classified as common; two species, the host-specific monogenean *Onchocleidus similis* and the larval nematode *Spiroxys contortus* (known from a wide range of freshwater fish species) had preva-

Fig. 5. *Spiroxys contortus* (Rudolphi, 1819), third-stage larvae from *Lepomis gibbosus* (L.) from the Lake Atanasovsko Wetlands, Bulgaria. A. Anterior end of body, lateral view. B. Anterior end of body, dorso-ventral view. C. Deirids, dorso-ventral view. D. Tail, lateral view. Scale bars: 20 μm.

lence corresponding to the category of intermediate species; the remaining three species corresponded to the category of rare parasites (Tables 1 and 7). None of the seasonal samples revealed the presence of a common species, too; the number of the intermediate species varied in seasonal samples between one and three (i.e. above-mentioned *O. similis* and *S. contortus* as well as *Contracaecum* sp. recorded only in May 2012, see Table 7). While *O. similis* and *S. contortus* were intermediate in five and three seasonal samples, respectively, *Posthodiplostomum centrarchi* was intermediate in two samples, and *Schulmanela petruschewskii* and *Contracaecum* sp. were intermediate each in only one seasonal sample (Table 1). *Onchocleidus dispar* was present as a rare species in all the seasonal samples in 2012 but was not recovered in 2013 (Table 1).

None of the helminth species exhibited statistically significant differences relative to its mean intensity (Table 1). Concerning the mean abundance, only *S. petruschewskii* and *Contracaecum* sp. had significant seasonal differences, the former having a maximum in May 2013 and the latter appearing only in May 2012 (Table 1).

The Shannon's Diversity Index calculated on the basis of all samples was 1.20, varying in seasonal samples between 0 ("one-species community") in July 2013 and 0.98 in July 2012. The value of the Simpson's Dominance Index was 0.38 based on all the samples, ranging 0.43-1.00 in seasonal samples (Table 7).

At the infracommunity level, out of 107 examined pumpkinseeds, 50.47% (54 individuals) were infected with helminth parasites. The mean number of helminth species per examined fish individual (species richness of infracommunities) varied between 0.14 (July 2013) and 0.71 (September 2013) in seasonal samples (Table 7); the difference in the species richness was significant (Kruskal – Wallis H = 12.92193, p = 0.0241). Based on all the samples, the mean number of helminth species per fish individual was 0.60. In all the seasonal samples, the number of helminth species per examined fish varied between 0 and 2, except for July 2013 when the maximum number of helminth species was 1. The mean number of parasite individuals per examined fish (i.e. total mean abundance, see Table 1) was between 0.43 and 6.20 in seasonal samples and was also statistically significant (Table 1). Shannon's Diversity Index in seasonal samples varied between 0 and 0.41, and the values of the Simpson's Dominance Index were consistently high (Table 7).

Discussion

Our study has revealed six helminth species in the pumpkinseed sunfish (*Lepomis gibbosus*) from the Lake Atanasovsko Wetlands – one trematode, two monogenean and three nematode species. These represent a relatively small proportion of the totally 16 trematodes, 12 monogeneans, 8 cestodes, 18 nematodes and 8 acanthocephalans recorded in this host

Table 7. Comparison of parameters of the helminth communities in *Lepomis gibbosus* (L.) from Ontario (Canada) and the Lake Atanasovsko Wetlands, Bulgaria.

	Canada			Bulgaria						
Localities	**Gunn Creek**	**Gray's Creek**	**Hoople Creek**	**Lake Atanasovsko**						
Sampling period	June 2012	June 2012	June 2012	May 2012	July 2012	September 2012	May 2013	July 2013	September 2013	Total
Source	Chapman et al. (2015)			Present study						
Fish specimens studied	17	16	16	12	31	16	10	7	31	107
Helminth species number	14	15	16	4	4	3	3	1	3	6
Component communities										
Shannon's Diversity Index (H')	1.49	1.59	2.03	0.88	0.98	0.26	0.51	0.00	0.83	1.20
Simpson's Dominance Index	–	–	–	0.55	0.43	0.59	0.48	1.00	0.49	0.38
Dominant structure										
Common species number	3	3	1	0	0	0	0	0	0	0
Intermediate species number	9	11	15	3	2	1	3	1	2	2
Rare species number	2	1	0	1	2	2	0	0	1	4
Infracommunities										
Mean number of species per fish ± SE	5.06±0.5	5.69±0.5	4.69±0.4	0.58±0.26	0.74±0.12	0.25±0.14	0.70±0.21	0.14±0.14	0.71±0.10	0.60±0.06
Median	–	–	–	0	1	0	1	0	1	1
Range	–	–	–	0–2	0–2	0–2	0–2	0–1	0–2	0–2
Mean number of individuals per fish ± SE	37.0±3.68	28.63±3.54	14.69±1.91	1.50±1.07	3.35±1.23	0.50±0.30	6.20±2.68	0.43±0.43	2.61±0.67	2.58±0.51
Median	–	–	–	0	1	0	1	0	1	1
Range	–	–	–	0–13	0–36	0–4	0–23	0–3	0–14	0–36
Shannon's Diversity Index (H')										
Mean ± SE	–	–	–	0.41±0.17	0.11±0.06	0.19±0.19	0.08±0.08	0.00	0.03±0.03	0.10±0.03
Median	–	–	–	0.48	0	0	0	0	0	0
Simpson's Dominance Index										
Mean ± SE	–	–	–	0.71±0.13	0.92±0.04	0.88±0.13	0.95±0.05	1.00	0.98±0.02	0.93±0.02
Median	–	–	–	0.68	1	1	1	1	1	1

species in its non-native geographical range in Europe (Shulman 1948, Žitňan 1966a, 1966b, Molnár 1969, Kulakovskaya & Koval' 1973, Kakacheva-Avramova 1977, Lambert 1977, Kiškaroly 1978, Kritscher 1980, 1988, Nedeva 1988, Kiškaroly & Tafro 1989, Pazooki & Székely 1994, Piasecki & Flandysz 1994, Moravec 2001b, Cojocaru 2003, Buriola et al. 2007, Pilecka-Rapacz & Sobecka 2008, Košuthová et al. 2009, Hockley et al. 2011, Ondračková et al. 2011, Çolak 2013, Moshu 2014, Soylu 2014, Havlátová et al. 2015, Rubtsova 2015b). The possible explanation of the lower species diversity is the relative isolation of the wetlands studied (not associated with a large river basin) as well as the dynamic conditions associated with the frequent changes of the water level.

We have recorded metacercariae of one trematode species, *Posthodiplostomum centrarchi* that appear specific parasite to centrarchid fishes both in the native geographical range in North America (Hoffman 1999) and in the invasive range in Europe (Stoyanov et al. 2017, Kvach et al. 2017). Currently, this parasite has a wide geographical range in Europe: Portugal, Spain, Czech Republic, Slovakia and Bulgaria (Stoyanov et al. 2017, Kvach et al. 2017), with metacercariae recorded only in alien centrarchids *L. gibbosus* and *Micropterus salmoides*. This parasite species has not been recorded in native fish species in Europe. From the Lake Atanasovsko Wetlands, we have examined from various freshwater and brackish habitats other native and alien fishes: *Knipowitschia caucasica* (Berg) (Gobionellidae) – 186 individuals; *Pseudorasbora parva* Temminck & Schlegel (Cyprinidae) – 13, *Gambusia holbrooki* Girard (Poeciliidae) – 111, *Gasterosteus aculeatus* L. (Gasterosteidae) – 134, *Pungitius platygaster* (Kessler) (Gasterosteidae) – 1, and *Syngnathus abaster* Risso (Syngnathidae) – 13; we have not found metacercariae of *P. centrarchi* in them. Therefore, we can conclude that the presence of *P. centrarchi* does not influence other fish species in its non-native range in Europe. The life cycle of *P. centrarchi* in Europe has not been studied – the only definitive host documented is *Ardea cinerea* L. (Stoyanov et al. 2017) and there are no data on first intermediate hosts. Therefore, further studies are needed in order to understand the potential impact of *P. centrarchi* on avian and mollusc communities in invaded wetlands.

The monogeneans *Onchocleidus similis* and *O. dispar* have been described from centrarchid fishes in North America, the former from *Lepomis gibbosus* (type host) and *L. cyanellus* Rafinesque and the latter from *L. gibbosus* (type host) and nine further centrarchid species (Wheeler & Beverley-Burton 1989). The first records in Europe of both *O. similis* and *O. dispar* were from *L. gibbosus* in the Romanian part of the Danube (Roman 1953). Subsequently, they have been recorded from the same host species in Norway, United Kingdom, France, Italy, Czech Republic, Slovakia, Hungary, Bosnia and Herzegovina, Croatia, Serbia, Ukraine, Turkey (European part) and Bulgaria (Vojtek 1958, Molnár 1963, Lambert 1977, Kiškaroly 1977, Kiškaroly & Tafro 1988, Galli et al. 2003, Sterud & Jørgensen 2006, Ondračková et al. 2011, Hockley et al. 2011, Çolak 2013, Soylu 2014, Rubtsova 2015a). Out of Europe, Maneepitaksanti & Nagasawa (2013) reported *O. dispar* from *Lepomis macrochirus* Rafinesque from Japan and Přikrilová et al. (2015) found it from *Micropterus salmoides* in South Africa. In Bulgaria, both species have been reported on *L. gibbosus* from the Danube (Margaritov 1968, Ondračková et al. 2011). Grupcheva & Nedeva (1999) reported single individuals of *O. similis* on the gills of the Prussian carp *Carassius gibelio* (Bloch) (Cyprinidae) (from two out of 143 host individuals studied, each in a different season) from the Zhrebchevo Reservoir, Bulgaria. To our knowledge, this is the only record of *O. similis* from a non-centrarchid host species. Further studies are needed in order to assess the impact of *Onchocleidus* spp. associated with alien centrarchids on native fish populations in Europe.

In the wetlands studied, we have not found other non-native monogeneans associated with *L. gibbosus* in European waters. These are *Actinocleidus recurvatus* Mizelle & Donahue, 1944 known from many countries, including from the Danubian Basin (Ondračková et al. 2011) as well as *Actinocleidus oculatus* (Mueller, 1934), *Onchocleidus acer* Mueller, 1936, *Cleidodiscus robustus* Mueller, 1934 (Ancyrocephalidae) and *Gyrodactylus macrochiri* Hoffman & Putz, 1964 (Gyrodactylidae) (see Havlátová et al. 2015). Possibly, these species have not reached the territory of Bulgaria in their non-native geographical range.

Schulmanela petruschewskii is a widespread nematode occurring as adult in liver of various freshwater fish species throughout Europe. Its life cycle includes oligochaetes as intermediate hosts (Moravec 1994, 2001a). Moravec (1994) has summarised its host records from Europe from 28 fish species of the families Salmonidae, Cyprinidae, Cobitidae, Ictaluridae, Centrarchidae and Percidae. From Bulgaria, it has been reported from *Lepomis* *gibbosus* from the Danube as well as from cyprinids *Phoxinus phoxinus* (L.), *Barbus cyclolepis* Heckel, *Cyprinus carpio* L., *Gobio gobio* (L.) and *Rhodeus sericeus amarus* (Bloch) from various parts of the country (Kakacheva-Avramova 1983). Therefore, the alien *L. gibbosus* participates as an alternative definitive host in the life cycle of this native parasite occurring in a wide range of fish host species.

Our results demonstrate that *L. gibbosus* can participate as a paratenic host in the life cycle of *Spiroxys contortus*, a native nematode parasite of pond turtles. In addition, though the recorded *Contracaecum* sp. is not identified at the species level, it could be speculated that this is a native parasite of fish-eating birds, which are frequent in the habitats examined.

In summary, the studied population of *L. gibbosus* from the Lake Atanasovsko Wetlands harbours six helminth species, out of them three alien and three native. We have not found any indication for impact of alien parasites on the local fish populations. Therefore, for the moment, there is no evidence for negative effects of the introduced parasites on native populations in the Lake Atanasovsko Wetlands (i.e., no parasite spillover, see Dunn et al. 2012 for definition). However, we found that the alien *L. gibbosus* participates in the life cycles of native parasites of fishes, pond turtles and fish-eating birds, i.e. there is evidence for its parasite spillback effect (Kelly et al. 2009) on native populations.

The present description of helminth communities in *L. gibbosus* and the data on metazoan (parasitic worms and unionid glochidia) parasite communities in the same host species in Canada (Chapman et al. 2015) allow comparisons of structural parameters of helminth communities in this host species in its native and invasive geographical range (Table 7). The data from the native range in Ontario (Chapman et al. 2015) were based on a single sample for each of the three localities studied in June 2012. The unionid glochidia were recorded at two localities with low prevalence and abundance (Chapman et al. 2015), thus enabling similarities and differences from the present results to be interpreted mostly in view of the structure of the helminth communities. The comparison reveals that the species richness of helminth parasites in the native range (14-16 species) is considerably higher than that in the studied host population in the invasive range – 6 species (Table 7), despite that the data from the invasive range have been based on greater sampling efforts (107 host individuals compared to 16, 16 and 17 individuals examined from the localities in Ontario). There is no parasite species, which is common for the populations studied in Canada and Bulgaria. Data from the native range include five ancyrocephalid monogenean species specific to centrarchid fishes, 12 trematode (six occurring as adults and six as metacercariae) and larvae of two cestode species and of two acanthocephalan species (Chapman et al. 2015). The prevalence (93.8-100.0%) and mean intensity (up to 13.5-14.0) of gill monogeneans are much higher in the native habitats (compared with the total prevalence of 31.78% and the maximum mean intensity up to 7.66, see Table 1) in the present material. The diversity of the parasite component communities is higher in the native geographical range (Table 7). In Ontario, the helminth component communities have substantial participation of common and intermediate species while those in the Lake Atanasovsko Wetlands consist of intermediate and rare species only (Table 7).

At the infracommunity level, the mean number of parasite species per infected fish is 4.69-5.69 in native habitats and 0.14-0.74 in invasive habitats, and the mean size of the infracommunities was 14.69-37.0 and 0.43-6.20 in native and invasive habitats, respectively (Table 7).

On the basis of these comparisons, we can conclude that helminth communities in the invasive geographical range of *L. gibbosus* are less diverse and less abundant compared to those in the native range of the host in North America. These structural differences in helminth communities are consistent with the Enemy Release Hypothesis explaining the success of invaders by postulating that an alien species introduced to a new region may experience a reduced regulation by its natural enemies, including parasites (Torchin et al. 2003, Mitchell & Power 2003).

Acknowledgments: We are grateful to Ministry of Environment and Waters of the Republic of Bulgaria for licenses (NCZP-151/11.05.2012 and NCZP-168/29.04.2013) to carry out studies in Atanasovsko Lake Reserve. The field study was based at Atanasovsko Lake Field Station of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences; the support of the staff of this station is acknowledged. G. P. Vasileva provided useful comments in the course of the present study. K. Popov and P. Nikolov assisted in the field work. S. Georgieva (České Budějovice) kindly helped with obtaining some literature sources. Facilities developed in the frames of the projects WETLANET (FP7 CAPACITIES Grant 229802), CEBDER (National Science Fund, Ministry of Education, Youth and Science of the Republic of Bulgaria, Grant DO 02-15/2009) and Phylogenetic ultrastructure markers for parasitic worms. Funded by National Science Fund, Ministry of Education, Youth and Science of the Republic of Bulgaria (YS DO 02-271/18.12.2008) were used in the course of the present study.

References

Aliff J. V., Smith D. & Lucas H. 1977. Some metazoan parasites from fishes of middle Georgia. Transactions of the American Microscopical Society **96** (1): 145–148.

Beverley-Burton M. & Suriano D. M. 1980a. *Cleidodiscus robustus* Mueller, 1934 (Monogenea: Ancyrocephalinae) from *Lepomis gibbosus* L. (Pisces: Centrarchidae) in Ontario, Canada: anatomy and systematic position. Canadian Journal of Zoology **58**: 654–660.

Beverley-Burton M. & Suriano D. M. 1980b. *Haplocleidus dispar* (Mueller, 1936) and *Pterocleidus acer* (Mueller, 1936) (Monogenea: Ancyrocephalinae) from *Lepomis gibbosus* L. (Pisces: Centrarchidae) in Ontario, Canada: anatomy and systematic position. Canadian Journal of Zoology **58**: 661–669.

Beverley-Burton M. & Suriano D. M. 1981. *Onchocleidus ferox* (Mueller, 1934) and *O. similis* Mueller, 1936 (Monogenea: Ancyrocephalinae) from *Lepomis gibbosus* L. (Pisces: Centrarchidae) in Ontario, Canada: anatomy, systematic position, and possible evolution. Canadian Journal of Zoology **59**: 1161–1171.

Blasco-Costa I. & Locke S. A. 2017. Life history, systematics and evolution of the Diplostomoidea Poirier, 1886: progress, promises and challenges emerging from molecular studies. Advances in Parasitology **98**: 167–225.

Buriola E., Trotti G. C., Parenti U., Pizzinat A. & Pizzolato F. 2007. Metazoan parasites of pumpkinseed sunfish, *Lepomis gibbosus* (Linnaeus, 1758) in Torino province (Piedmont, Italy). Bollettino di Museo Regionale di Scienze Naturali (Torino) **24** (2): 281–289.

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.

Chapman J. M., Marcogliese D. J., Suski C. D. & Cooke S. J. 2015. Variation in parasite communities and health indices of juvenile *Lepomis gibbosus* across a gradient of watershed land-use and habitat quality. Ecological Indicators **57**: 564–572.

Cojocaru C. 2003. Cercetări privind ihtioparazitofauna Banatului. Annals of West University of Timisoara, Seria Biology **2003**: 1–30.

Çolak H. S. 2013. Metazoan parasites of fish species from Lake Sığırcı (Edirne, Turkey). Turkish Journal of Veterinary and Animal Sciences **37**: 200–205.

Collins M. R. & Janovy J. 2003. Host specificity among Ancyrocephalinae (Monogenoidea) of Nebraska sunfish. Journal of Parasitology **89** (1): 80–83.

Dechtiar A. O. 1972. Parasites of fish from Lake of the Woods, Ontario. Journal of the Fisheries Research Board of Canada **29**: 275–283.

Drenski P. 1922. [*Eupomotis gibbosus* Linné, a new species for the Bulgarian freshwater ichthyofauna.] Svedeniya po Zemledelieto **3** (10): 6–7. (In Bulgarian).

Dunn A. M. 2009. Parasites and biological invasions. Advances in Parasitology **68**: 161–184.

Dunn A. M., Torchin M. E., Hatcher M. J., Kotanen P. M., Blumenthal D. M., Byers J. E., Coon C. A. C., Frankel V. M., Holt R. D., Hufbauer R. A., Kanarek A. R., Schierenbeck K. A., Wolfe L. M. & Perkins S. E. 2012. Indirect effects of parasites in invasions. Functional Ecology **26**: 1262–1274.

Galli P., Stefani F., Benzoni F., Crosa G. & Zullini A. 2003. New records of alien monogeneans from *Lepomis gibbosus* and *Silurus glanis* in Italy. Parassitologia **45**: 147–149.

Galli P., Strona G., Benzoni F., Crosa G. & Stefani F. 2007. Monogenoids from freshwater fish in Italy, with comments on alien species. Comparative Parasitology **74** (2): 264–272.

Ghittino P. 1961. Su una capillariosi epatica in trote di allevamento e in altri teleostei delle acque libere del bacino del Po in Piemonte, con descrizione di una nuova specie (*Capillaria eupomotis*). Rivista di Parassitologia **22** (3): 193–204.

Grupcheva G. I. & Nedeva I. L. 1999. Parasite fauna of the crucian carp (*Carassius auratus gibelio* Bloch.) in the Zrebchevo reservoir (Bulgaria). Acta Zoologica Bulgarica **51** (1): 115–122.

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

Gussev A. V. 1985. Class Monogenea, subclass Polyonchoinea, order Dactylogyridea. 1 Family Dactylogyridae, 2 Family Ancyrocephalidae. In: Gussev A. V. (Ed.) [Key to parasites of freshwater fishes of the fauna of USSR. Volume 2, Part 1. Parasitic Metazoans.] Leningrad: Nauka, pp. 10–253. (In Russian).

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

Hanel L., Plesník J., Andreska J., Luks S., Novák J. & Plíštil J. 2011. Alien fishes in European waters. Bulletin Lampetra **7**: 148–185.

Havlátová L., Ondračková M. & Přikrylová I. 2015. Monogenean parasites of *Lepomis gibbosus* Linnaeus introduced into the River Durance, France. Helminthologia **52** (4): 323–330.

Hockley F.A., Williams C.F., Reading A.J., Taylor N.G.H. & Cable J. 2011. Parasite fauna of introduced pumpkinseed fish *Lepomis gibbosus*: first British record of *Onchocleidus dispar* (Monogenea). Diseases of Aquatic Organisms **97**: 65–73.

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

Kakacheva-Avramova D. 1977. Study on the helminths 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).

Kelly D. W., Paterson R. A., Townsend C. R., Poulin R. & Tompkins D. M. 2009. Parasite spillback: a neglected concept in invasion ecology? Ecology **90**: 2047–2056.

Kiškaroly M. 1977. Istraživanje parazitofaune riba slatkovodnih ribnjaka BiH, A. Monogeni trematodi 1. I. Ciprinidni ribnjaci. Veterinaria (Sarajevo) **26** (2-3): 195–208.

Kiškaroly M. 1978. Istraživanje parazitofaune riba slatkovodnih ribnjaka BiH, I. Ciprinidni ribnjaci C. Digeni trematodi 1. (sistematika i morfologija). Veterinaria (Sarajevo) **27** (1): 93–99.

Kiškaroly M. & Tafro A. 1988. Prilog poznavanju helmintofaune riba jednog dijela Dunava. Veterinaria (Sarajevo) **37** (2-3): 211–222.

Kiškaroly M. & Tafro A. 1989. Prilog poznavanju helmintofaune riba akumulacionog jezera kod Bačke Topole. Veterinaria (Sarajevo) **38** (1-2): 81–89.

Košuthová L., Koščo J., Letková V., Košuth P. & Manko P. 2009. New records of endoparasitic helminths in alien invasive fishes from the Carpathian region. Biologia, Section Zoology **64** (4): 776–780.

Kottelat M. & Freyhof J. 2007. Handbook of European freshwater fishes. Kottelat & Freyhof: Cornol & Berlin. 646 p.

Kritscher E. 1980. Die Fische des Neusiedlersees und ihre Parasiten III. Acanthocephala. Annalen des Naturhistorischen Museums in Wien **83**: 641–650.

Kritscher E. 1988. Die Fische des Neusiedlersees und ihre Parasiten VII. Trematoda: Monogena und Zusammenfassung. Annalen des Naturhistorischen Museums in Wien, Serie B **90**: 407–421.

Kulakovskaya O. P. & Koval' V. P. 1973. [Parasitic fauna in fishes in the Danube basin.] Kiev: Naukova Dumka. 212 p. (In Russian).

Kvach Y., Jurajda P., Bryjová A., Trichkova T., Ribeiro F., Přikrylová I., Ondračková M. 2017. European distribution for metacercariae of the North American digenean *Posthodiplostomum* cf. *minimum centrarchi* (Strigeiformes: Diplostomidae). Parasitology International **66** (5): 635–642.

Lambert A. 1977. Les Monogènes Monopisthocotylea parasites des poissons d'eau douce de la France méditerranéenne. Bulletin du Muséum National d'Histoire Naturelle (Paris), Zoologie **299**: 177–214.

Maitland P. S. & Price C. E. 1969. *Urocleidus principalis* (Mizelle, 1936), a North American monogenetic trematode new to the British Isles, probably introduced with the largemouth bass *Micropterus salmoides* (Lacépède, 1802). Journal of Fish Biology **1**: 17–18.

Maneepitaksanti W. & Nagasawa K. 2013. First record of *Onchocleidus dispar* (Monogenea: Ancyrocephalidae), a gill parasite of bluegill (*Lepomis macrochirus*), from Japan. Biogeography **15**: 67–71.

Magurran A. E. 1988. Ecological diversity and its measurement. New Jersey: Princeton University Press. 125 p.

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

Margaritov N. 1968. Über die Fischparasiten aus dem bulgarischen Donauabschnitt. In: Limnologische Berichte der X. Jubiläumstagung Donauforschung, Sofia, 10–20 Oktober 1966, Bulgarien, pp. 429–433.

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**: 207–224.

Mitchell C. E. & Power A. G. 2003. Release of invasive plants from fungal and viral pathogens. Nature 421: 625–627.

Molnár K. 1963. Mono- és Digenetikus mételyek halakból. Állattani Közlemények **50** (1-4): 103–107.

Molnár K. 1969. Beiträge zur Kenntnis der Fischparasitenfauna Ungarns IV. Trematoden. Parasitologia Hungarica **2**: 119–136.

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

Moravec F. 2001a. Trichinelloid nematodes parasitic in cold-blooded vertebrates. Praha: Academia. 430 p.

Moravec F. 2001b. Checklist of the metazoan parasites of fishes of the Czech Republic and the Slovak Republic (1873-2000). Praha: Academia. 168 p.

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**: 251–254.

Moshu A. 2014. [Helminths of fishes from waters of Prut-Dniester interfluve potentially dangerous to human health.] Chişinău: Eco-TIRAS, 88 p. (In Russian).

Nedeva I. 1988. [To the biology of *Bothriocephalus acheilognathi* Yamaguti, 1934 (fam. Bothriocephalidae).] Khelmintologiya **26**: 32–38. (In Bulgarian).

Ondračková M., Dávidová M., Přikrylová I. & Pečínková M. 2011. Monogenean parasites of introduced pumpkinseed *Lepomis gibbosus* (Centrarchidae) in the Danube River Basin. Journal of Helminthology **85**: 435–441.

Pazooki J. & Székely C. 1994. Survey of the paratenic hosts of *Anguillicola crassus* in Lake Velence, Hungary. Acta Veterinaria Hungarica **42** (1): 87–97.

Piasecki W. & Flandysz M. 1994. Preliminary survey on parasite fauna of pumpkinseed sunfish, *Lepomis gibbosus* (Linnaeus, 1758) (Pisces, Teleostei, Centrarchidae) from warm-water canal of the "Pomorzany" power plant in Szczecin, Poland. Acta Ichthyologica et Piscatoria **24** (1): 87–100.

Pilecka–Rapacz M. & Sobecka E. 2008. Parasitic nematodes of pumpkinseed sunfish (*Lepomis gibbosus* L., 1758) from warm-water canal of a power plant in Szczecin, Poland. Wiadomości Parazytologiczne **54** (3): 213–216.

Přikrilová I., Malherbe W., Davidova M. & Smit N. 2015. *Onchocleidus dispar* (Mueller, 1936) (Monogenea: Ancyrocephalinae) on the largemouth bass *Micropterus salmoides* (Lacepède, 1802) in South Africa: a transcontinental traveller. In: 9th International Symposium on Fish Parasites, Valencia, Spain, 31 August – 4 September 2015. pp. 183.

Roman E. 1953. [Parasite fauna of sunfish *Lepomis gibbosus* (L.), acclimatized in the Danube.] Doklady Akademii Nauk SSSR **89** (4): 765–768. (In Russian).

Rubtsova N. Y. 2015a. First record of *Onchocleidus dispar*, an alien monogenean from introduced pumpkinseed fish *Lepomis gibbosus* (Pisces, Centrarchidae) in Ukraine. Scientia Parasitologica **6** (3): 83–88.

Rubtsova N. Y. 2015b. Fauna of parasites of the upper part of the Kakhovske Reservoir on the 60-th year of its existence. Vestnik Zaporiz'kogo Nazional'nogo Universitetu, Biologichni Nauki **1**: 39–48.

Sharpilo V. P. 1976. [Parasitic worms of reptiles in the fauna of the USSR.] Kiev: Naukova Dumka. 288 p. (In Russian).

Shulman S.S. 1948. A new species of round worms parasitic in liver of fishes. Izvestiya Vsesoyuznogo Nauchno-Issledovatel'skogo Instituta Ozernogo i Rechnogo Ribnogo Khozyaystva **37**: 235–238. (In Russian).

Soylu E. 2014. Metazoan parasites of fish species from Lake Gala (Edirne, Turkey). Ege Journal of Fisheries and Aquatic Sciences **31** (4): 187–193.

Sterud E. & Jørgensen A. 2006. Pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758) (Centrarchidae) and associated parasites introduced to Norway. Aquatic Invasions **1** (4): 278–280.

Stoyanov B., Georgieva S., Pankov P., Kudlai O., Kostadinova

A. & Georgiev B. B. 2017. 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**: 1–20.

Torchin M. E., Lafferty K. D., Dobson A. P., McKenzie V. J. & Kuris A. M. 2003. Introduced species and their missing parasites. Nature **421**: 628–630.

Uzunova E. & Zlatanova S. 2007. A review of the fish introductions in Bulgarian freshwaters. Acta Ichthyologica et Piscatoria **37** (1): 55–61.

Uzunova E., Velkov B., Studenkov S., Georgieva M., Nikolova M., Pehlivanov L. & Parvanov D. 2008. Growth, age and size structure of the introduced pumpkinseed (*Lepomis gibbosus* L.) population from small ponds along the Vit River (Bulgaria). Bulgarian Journal of Agricultural Science **14** (2): 227–234.

Uzunova E., Georgieva M., Nikolova M., Studenkov S. & Traykov I. 2010. Pumpkinseed (*Lepomis gibbosus*) distribution and abundance in littoral zones of sand-pit lakes. Bulgarian Journal of Agricultural Science **16** (3): 275–283.

Uzunova E. P., Georgieva M., Pavlova M. N., Studenkov S. & Popov V. 2012. Variation of abundance and demographic structure of the introduced pumpkinseed, *Lepomis gibbosus* (Actinopterygii: Perciformes: Centrarchidae), in relation to littoral habitats and water body use. Acta Ichthyologica et Piscatoria **42** (2): 121–130.

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, Publication of Bulgarian Biodiversity Foundation.

Vitousek P. M., d'Antonio C. M., Loope L. L. & Westbrooks R. 1996. Biological invasions as global environmental change. American Scientist **84**: 468–478.

Vitousek P. M., d'Antonio C. M., Loope L. L., Rejmánek M. & Westbrooks R. 1997. Introduced species: a significant component of human-caused global change. New Zealand Journal of Ecology **21** (1): 1–16.

Vojtek J. 1958. *Urocleidus* Mueller, 1934, Nový rod žábrohlístů (Trematoda, Monogenea) PRO ČSR. Biologia (Bratislava) **13** (8): 612–615.

Wheeler T.A. & Beverley-Burton M. 1989. Systematics of *Onchocleidus* Mueller, 1936 (Monogenea: Ancyrocephalidae): generic revision. Canadian Journal of Zoology **67**: 136–157.

Žitňan R. 1966a. Trematodes of fishes from the River Hron. Helminthologia **7**: 201–220.

Žitňan R. 1966b. Cestoidea a Trematoidea rýb tečúcich vôd potiskej nížiny (helminty rýb potiskej nížiny (ČSSR). II). Biologia (Bratislava) **21** (10): 763–770.

Received: 15.10.2017
Accepted: 13.11.2017