

PARASITIC INFECTIONS OF TWO INVASIVE FISH SPECIES, THE CAUCASIAN DWARF GOBY AND THE AMUR SLEEPER, IN HUNGARY

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In recent years and decades, two new fish species, the Caucasian dwarf goby (*Knipowitschia caucasica*) and the Amur sleeper (*Perccottus glenii*) have become members of the Hungarian fish fauna. In a 14-month study on the parasite fauna of these species, the authors detected 11 parasite species in the Caucasian dwarf goby and 17 species in the Amur sleeper. All parasites found in dwarf goby belong to species commonly occurring also in native Hungarian fishes, but three species (*Goussia obstinata*, *Gyrodactylus perccottii* and *Nippotaenia mogurndae*) collected from the Amur sleeper are introduced species new for the Hungarian fauna.

Key words: Parasite, native, non-indigenous, invasive fish species, Ponto-Caspian, *Perccottus glenii*, *Knipowitschia caucasica*

In recent years and decades the Hungarian fish fauna has been expanded by several new invasive fish species. Of them, the appearance of a series of Ponto-Caspian gobies and the Amur sleeper *Perccottus glenii* in the territory of the Danube and Tisza Rivers (Bíró, 1972; Erős and Guti, 1997; Harka, 1998; Guti, 2000; Guti et al., 2003; Harka et al., 2005; Harka and Bíró, 2007; Takács et al., 2011) deserves special mention. The latter species was documented in the Balaton catchment area, where it had presumably arrived via fish transport from the Tisza catchment area (Erős et al., 2008; Reshetnikov, 2010). More recently, a new sand goby species, the Caucasian dwarf goby (*Knipowitschia caucasica*) has been found in the Tisza River basin (Halasi-Kovács et al., 2011; Harka et al., 2013, 2015a, 2015b).

The climate change will modify the ecological impacts of invasive species by enhancing their competitive effects on native species, and then the introduction of such species into ecosystems may contribute to the loss of biodiversity (Thomas et al., 2004; Rahel and Olden, 2008; Bellard et al., 2012). These invasive species also introduced a part of their parasite fauna to the new biotope, and

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since parasites can play an important role in determining the final outcome of competition between invasive and native species, monitoring the parasite fauna of invasive fish species is a very important task (Torchin et al., 2003; Prenter et al., 2004; Sokolov and Zhukov, 2014).

Data on the parasite fauna of these gobies in Central Europe have been presented by Molnár (2000, 2006), Francová et al. (2011) and Ondračková et al. (2012). Endoparasitic helminths of the Amur sleeper in the Carpathian basin were studied by Košuthová et al. (2004, 2008, 2009). Further data on the parasite infections of this fish species have been presented in the works of Mierzejewska et al. (2010, 2012) from Poland and of Kvach et al. (2013, 2014) and Zaichenko (2015) from the Ukraine. The enrichment of the parasite fauna of the Amur sleeper during its spread to the European part of Russia was thoroughly studied by Sokolov and Frolov (2012) and Sokolov (2013) in the original biotope of the fish in the Far East, and in the European part of Russia (Sokolov et al., 2012a, 2013). From the European stock of the Amur sleeper Sokolov and Moshu (2014) described a new species, *Goussia obstinata*, from the intestine. Little is known about the parasitic infection of small-sized sand gobies. Data have been reported only on their *Gyrodactylus* fauna. Of the latter, Osmanov (1971) described *G. bubyri* from the Caucasian dwarf goby, and Vanhove et al. (2014) described further *Gyrodactylus* spp. from *Knipowitschia* and *Economidichthys* spp.

This paper reports the results of a survey conducted for recording parasitic infections of two invasive fish species that have conquered water bodies in Hungary in recent years, the Caucasian dwarf goby and the Amur sleeper.

Materials and methods

Caucasian dwarf gobies were collected with hand nets from the Lake Tisza close to the city of Tiszafüred, while Amur sleepers were collected by electrofishing (Hans Grassl IG200/2B, Hans Grassl GmbH, Germany) from April 2014 to June 2015 (except in winter) from oxbows of the Tisza River and the channels close to it (Table 1). Altogether 66 dwarf goby and 169 Amur sleeper specimens were dissected from the main study area in the Tisza basin, and further six Amur sleeper specimens were derived from the tributary of the Zala River close to Lake Balaton (Table 1). Fish were collected in different periods of the year, starting in April and ending in November. As far as possible, different age groups were selected. From the dwarf goby, fingerlings measuring 1.5–1.7 cm in total length and one-year-old mature specimens achieving 3.5 to 3.7 cm in length were studied. The size of the Amur sleepers varied between 3 and 16 cm. The fish were placed into plastic bags filled with oxygen and sent to the laboratory for detailed parasitological dissection. The dissections were performed within three days of collection. Fish were anaesthetised with a drop of clove oil into their wa-

ter and their heads were cut afterwards. Dissections were made under a Zeiss stereomicroscope. All organs were checked for infection with parasites. Photos on parasites were taken using Nomarski differential interference contrast with an Olympus BH2 microscope and photographed with an Olympus DP 20 digital camera.

Table 1

List of sampling locations of Caucasian dwarf goby (*Knipowitschia caucasica*) and Amur sleeper (*Percottus glenii*)

Water body	Location	Code	Co-ordinates
<i>Percottus glenii</i>			
Belfő-csatorna	Tiszabercel	b	48°09'48.4"N 21°40'04.1"E
Cserőközi-Holt-Tisza	Tiszaderzs	c	47°31'18.5"N 20°39'50.0"E
Keleti-főcsatorna	Balmazújváros	k	47°37'43.9"N 21°22'18.3"E
Lónyai-főcsatorna	Gávavencsellő	l	48°08'38.4"N 21°37'47.0"E
Rakamazi-Nagy-morotva	Tiszanagyfalu	r	48°05'42.7"N 21°27'45.8"E
Tiszacsegei-Nagy-morotva	Tiszacsege	t	47°40'07.6"N 20°56'08.1"E
Zala River	Fenékpuszta	z	46°42'04.2"N 17°15'26.0"E
<i>Knipowitschia caucasica</i>			
Lake Tisza	Tiszafüred	lt	47°37'34.1"N 20°44'10.0"E

Results

A total of 11 parasite species were found in the Caucasian dwarf goby (Table 2). The most common infection was caused by an *Apiosoma* sp., a commensal organism, which, however, may become pathogenic after colonising the body surface of fish. Hundreds of these sessile ciliates covered mostly the fins of fish (Fig. 1A), but they were frequently found also in the gills. *Apiosoma* infection was frequently accompanied by the presence of the flagellate *Cryptobia branchialis* and some common ciliates such as *Trichodina mutabilis*, *Hemio-phrys branchiarum*, and *Chilodonella cyprini*. In a single case a trophont of *Ichthyophthirius multifiliis* was also recorded. Of the metazoan parasites, metacystodes of a *Proteocephalus* species were the most common in the intestine of this fish species. Scolices of metacystodes in the dwarf goby had four well-observable suckers and an inconspicuous fifth central sucker. Although these scolices had relatively long unsegmented strobilae, we were unable to identify them with an already described species (Fig. 1B). In the abdominal cavity, digenean metacercariae surrounded by a very thick wall and having a characteristic lemon shape (Fig. 1C) were frequently recorded. By their shape and thick wall resistant to compression these metacercariae were identified as *Apatemon gracilis*. Although less frequently, non-encysted specimens were also found (Fig. 1D), but their inner structure could not be studied in detail. Of the other

helminths commonly infecting Hungarian fishes, *Nicolla skrjabini* and *Camallanus truncatus* in the gut and metacercariae of an *Echinochasmus* sp. in the gill filaments were accidentally found. In fishes examined in the spring and summer period, glochidia larvae of an *Anodonta* species were frequently recorded. All parasites found can be regarded as members of the Hungarian parasite fauna, and most of them had been found earlier also in the River Tisza (Ergens et al., 1975).

Table 2

List of parasites found in 66 Caucasian dwarf goby (*Knipowitschia caucasica*) specimens caught in the Lake Tisza, Hungary

Name of parasite	Site of infection in fish	Number of infected fish
<i>Cryptobia branchialis</i> Nie (in Chen, 1956)	gill, fin	19
<i>Apiosoma</i> sp.	fin	54
<i>Trichodina mutabilis</i> Kazubski & Migala, 1968	gill, fin	13
<i>Hemiophrys branchiarum</i> (Weinrich, 1924)	gill, fin	5
<i>Chilodonella cyprini</i> Moroff, 1902	gill	3
<i>Ichthyophthirius multifiliis</i> Fouquet, 1876	gill	1
<i>Proteocephalus</i> sp. (l)	intestine	23
<i>Nicolla skrjabini</i> (Iwanitzky, 1928)	intestine	2
<i>Echinochasmus</i> sp. (l)	gill	1
<i>Apatemon gracilis</i> (Rudolphi, 1819)	abdominal cavity	11
<i>Camallanus truncatus</i> (Rudolphi, 1814)	gill, fin	14

l = larval stage

A total of 17 parasite species were found in the Amur sleeper (Table 3). Of them, three species (*Goussia obstinata*, *Gyrodactylus perccotti* and *Nippotaenia mogurndae*) belong to the original parasite fauna of this fish known also in its Far Eastern biotope (Fig. 2). Of the species common in other European fishes, the most frequently found species was *Trichodina mutabilis*, causing infection both in the fins and in the gills (Fig. 2A). Less frequently, in younger fish specimens, *Trichodinella epizootica* caused mixed infection with *T. mutabilis*. Of the other protozoans, compared to the members of the local fish fauna, relatively few species were recorded. Of them, the flagellate *Spironucleus elegans* was found to cause intensive infection in the intestine of some fish specimens. On the other hand, *Goussia obstinata*, a recently described species was frequently isolated from the gut of both young and old Amur sleeper specimens (Fig. 2B). *Gyrodactylus perccotti* was mostly found in the fins, but some of its specimens could be collected from the gills as well (Fig. 2C). *Nippotaenia mogurndae* infected only some of the stocks, but in these cases infection involving up to ten specimens could be found, located in different parts of the intestine. The species could be easily recognised by its large single oral sucker (Fig. 2D). Of the other cestodes, scolices of *Proteocephalus percae* were found in the rectum of one fish specimen. *Nicolla skrjabini*, a common trematode species with a wide host range was

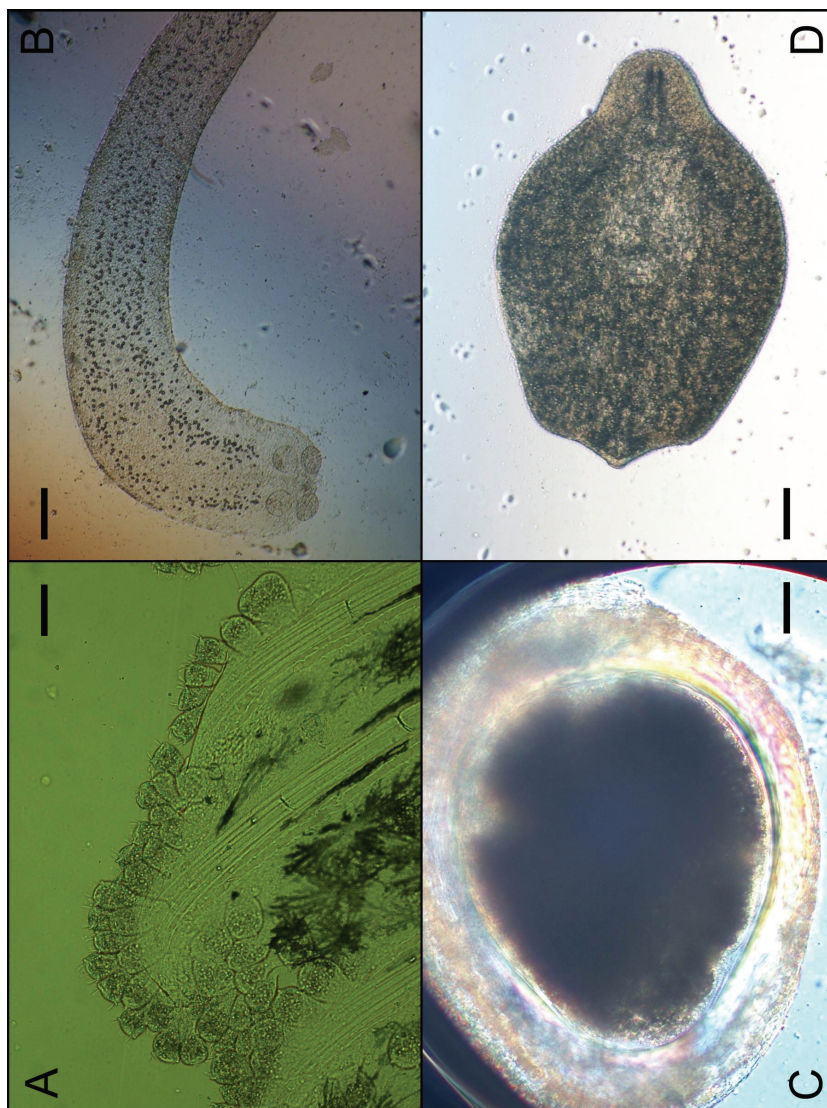


Fig. 1. Parasites from Caucasian dwarf gobies: A – Hundreds of specimens of an *Apatemon* sp. invading the fins of a dwarf goby. Bar = 500 μ m; B – Head end with five suckers of a *Proteocephalus* sp. from the gut of a dwarf goby. Bar = 2 mm; C – Thick-walled metacercaria of *Apatemon gracilis* from the abdominal cavity of a dwarf goby. Bar = 100 μ m; D – Early metacercaria of *Apatemon gracilis* from the abdominal cavity before encystation. Bar = 100 μ m

only recorded in two cases in Amur sleepers. Of the larval trematodes, the metacercariae of *Opisthioglyphe ranae* (Fig. 2E) infecting gill filaments were the most common. Other trematode larvae, among them the relatively large metacercariae of *Clinostomum complanatum* (Fig. 2F), occurred only incidentally. Like in the dwarf goby, glochidia of an *Anodonta* species were frequently recorded and caused well-observable infection in the fins and gills of the Amur sleeper as well (Fig. 2G).

Although heavy infections with the above parasites were often found, no fatal cases manifesting themselves in disease signs were recorded.

Table 3

List of parasites found in 169 Amur sleeper (*Perccottus glenii*) specimens from the region of the Tisza and Zala Rivers, Hungary

Name of parasite	Site of infection in fish	Number of infected fish	Location of infection [†]
<i>Spironucleus elegans</i> Lavier, 1936	intestine	8	b, c, l, r, h
<i>Apiosoma</i> sp.	fin	1	r
<i>Trichodina mutabilis</i> Kazubsky & Migala, 1968	gill, fin	70	b, c, k, r, t, z
<i>Trichodinella epizootica</i> Raabe, 1960	fin	4	l, r
* <i>Goussia obstinata</i> Sokolov & Moshu, 2014	intestine	31	b, l, r, z
* <i>Gyrodactylus perccotti</i> Ergens & Yuhhimenko, 1973	fin	32	b, c, k, l, r, t, z
* <i>Nippotaenia mogurndae</i> Yamaguti & Miyata, 1940	intestine	18	b, r
<i>Proteocephalus percae</i> (Müller, 1780) (l)	intestine	1	r, z
<i>Nicolla skrjabini</i> (Iwanitzky, 1928)	intestine	2	r
<i>Echinochasmus</i> sp. (l)	gill	9	l, r, z
<i>Petasiger</i> sp. (l)	gill	1	r
<i>Diplostomum spathaceum</i> (Rudolphi, 1819) (l)	eye lens	2	b
<i>Opishyoglyphe ranae</i> (Fröhlich, 1791) (l)	gill	14	b, c, l, r
<i>Clinostomum complanatum</i> (Rudolphi, 1819) (l)	gill	1	r
<i>Spiroxis contortus</i> (Rudolphi, 1819) (l)	intestine	4	k, z
<i>Ergasilus sieboldi</i> Nordmann, 1832	gill	1	r
<i>Anodonta</i> sp. (glochidium)	gill, fin	20	l, r, t

[†]See Table 1 for the explanation of location codes; *, introduced species; l = larval stage

Discussion

Having evaluated the parasitic infections of the two goby species in Hungary, we may conclude that they have introduced relatively few new parasites to Hungary, compared to the original Far Eastern parasite fauna of the Amur sleeper (Sokolov and Frolov, 2012; Sokolov, 2013) or to data from the European part of Russia, Moldova, the Ukraine, and Poland (Sokolov and Moshu, 2013; Kvach et al., 2014; Sokolov et al., 2014). The relatively low number of parasitic species in the

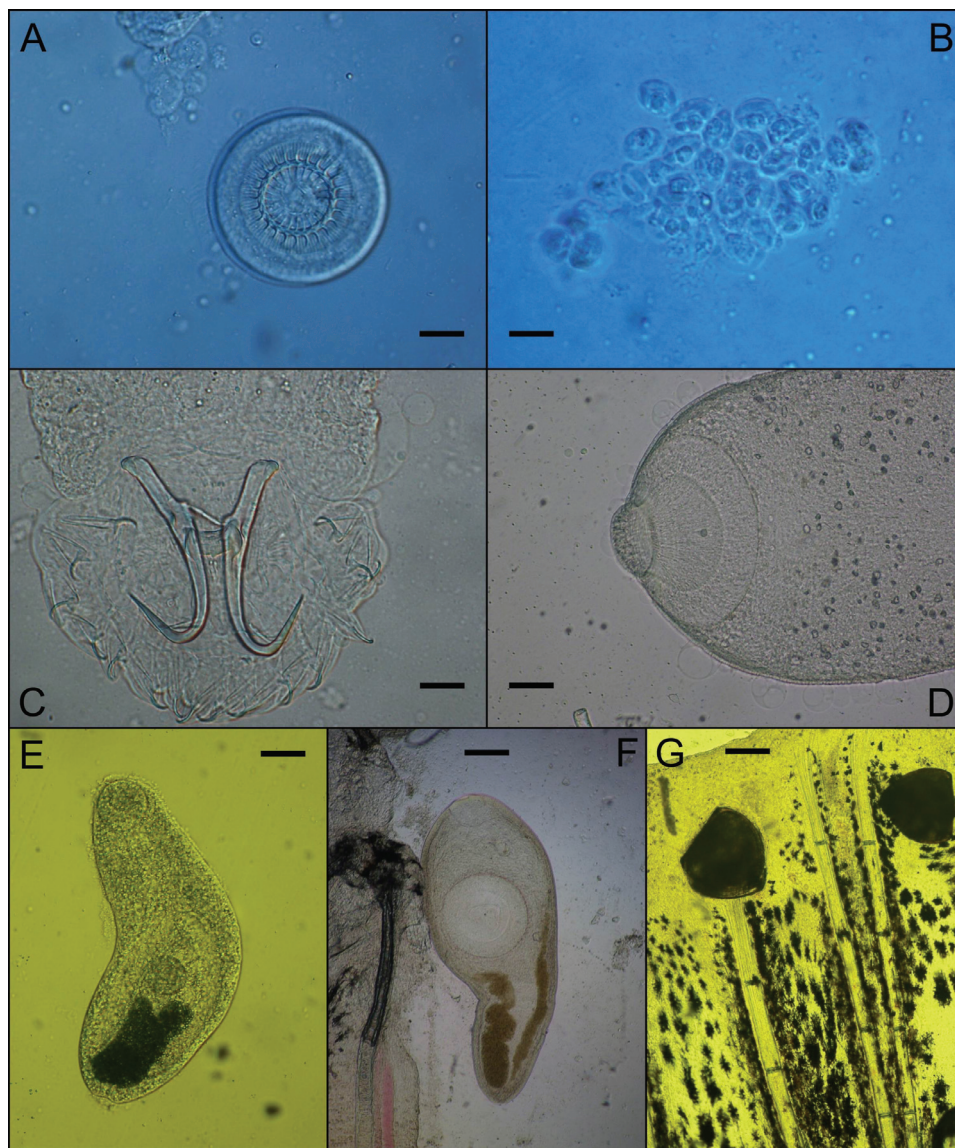


Fig. 2. Parasites from Amur sleeper: A – *Trichodina mutabilis* from the fins of a Amur sleeper. Bar = 30 μ m; B – Sporocysts of *Goussia obstinata* from the gut of an Amur sleeper. Bar = 20 μ m; C – *Gyrodactylus perccotti* from the fins of an Amur sleeper. Bar = 20 μ m; D – Head end of *Nipoptaenia mogurndae* with its single sucker. Bar = 400 μ m; E – Excysted metacercaria of *Opisthioglyphe ranae* from the gills of an Amur sleeper. Bar = 1 mm; F – Excysted metacercaria of *Clinostomum complanatum* from the gill arch of an Amur sleeper. Bar = 2 mm; G – Glochidia of an *Anodonta* species attached to the fins of an Amur sleeper. Bar = 0.4 mm

short-lived Caucasian dwarf goby is less surprising as this fish is a typical brackish-water species less common in freshwaters and its less studied parasite fauna seems to be of marine origin (Miller, 2004). Parasites found by us in this work are common parasites infecting Middle European freshwater fishes. Of the parasites found in these small fishes, an *Apiosoma* sp. deserves a mention, which infected the fins and gills of practically all of the fish examined, but other common flagellate and ciliate protozoans known from native fishes were also found (Molnár, 1979). Of the metazoan parasites, the common occurrence of a closely not identified metacercaria in the abdominal cavity presented a new record, because no similar thick-walled metacercariae had been recorded from Hungarian fishes up to this time. Despite the new record of this species, this parasite, which infects aquatic birds in its adult stage, cannot be regarded as an introduced parasite species. Larval stages of *Proteocephalus* spp. were found in the gut of both the dwarf goby and the Amur sleeper. They were, however, different in some respects. *Proteocephalus* larvae in the gut of the dwarf goby were in the metacercaria stage, having a scolex and an unsegmented strobila and located in different parts of the gut lumen, while larvae in the gut of the Amur sleeper had only scolices and were located in the rectum. Scolices of the species infecting the dwarf goby had a conspicuous small fifth (central) sucker, while the central sucker of scolices found in the rectum of the Amur sleeper was well observable. Although we suppose that the species bearing scolices with the primordia of proglottids corresponds to *P. torulosus*, until obtaining conclusive molecular evidence we designate it as *Proteocephalus* sp. On the other hand, scolices found accumulated in the rectum of Amur sleeper showed great similarity to scolices commonly occurring in the rectum of the carrier host ruffe (Molnár, 1966), and they were identified as larval stages of *P. percae*.

Contrary to that of the dwarf goby, the parasite fauna of the Hungarian Amur sleeper population contains, besides the common Hungarian fish parasites, three introduced species (*Nippotaenia mogurndae*, *Gyrodactylus perccotti* and *Goussia obstinata*) known from the original Far Eastern biotope of this fish. Sokolov and Frolov (2012) as well as Sokolov (2013), who studied the parasite fauna of the Amur sleeper in its original biotope in the Far East by taking into consideration also data previously reported by other researchers, found 83 parasite species in the Amur sleeper. In another paper providing a checklist of the parasite fauna of the non-native Amur sleeper population based on the data of European and Russian authors, Sokolov et al. (2014) counted 67 identified parasite species and 30 species identified only to the genus or family level. Although the specific parasite fauna of the Amur sleeper in its native population is rich in specific parasites, aside from worldwide-distributed fish parasites, only three common species (*Nippotaenia mogurndae*, *Gyrodactylus perccotti* and *Goussia obstinata*) and a less common one (*Henneguya alexeevi* Shulman, 1962) have arrived with it in Europe and become parasites of the non-native populations

(Kořuthová et al., 2004, 2008; Hanzelová et al., 2007; Ondračková et al., 2012; Sokolov et al., 2012a,b, 2013). Of the above specific parasite species of the Amur sleeper, *Gyrodactylus perccotti*, infecting 18% of the fins in the studied Hungarian fish material, proved to be the most common in our studies. This monogenean was first isolated in the Carpathian basin by Ondračková et al. (2012). The monogenean *Gyrodactylus* spp., which has a direct development cycle and can infect fish for a long time, can easily be transmitted by the host. However, another monogenean, *Ancyrocephalus curtus*, which is common in China and the Far Eastern territories of Russia, has not been introduced to Europe.

Nippotaenia mogurndae was found to infect about 10% of the Amur sleepers examined in Hungary. It was common in some collecting places, achieving 60% infection rate, while in other collecting places it was not recorded at all. This parasite has been thoroughly studied in Slovakia (Kořuthová et al., 2004, 2008, 2009; Hanzelová et al., 2007), Poland (Mierzejewska et al., 2010, 2012), Russia (Reshetnikov et al., 2011a,b) and the Ukraine (Kvach et al., 2013). The species develops via cyclops intermediate hosts, which makes possible its invasion also into Europe. It has been suggested that the infection of old specimens not feeding on plankton takes place by cannibalism (Reshetnikov et al., 2011a). Reshetnikov et al. (2011b) observed that the worms infect the host's gut only for a limited time. This means that the about 10% prevalence found in Hungarian fish specimens represents a relatively frequent infection.

The coccidian species *Goussia obstinata* with its 17% prevalence shows that it has found facilities for propagation in Hungary and, just like in Russia and Moldova (Sokolov and Moshu, 2013, 2014), causes frequent infection in Hungary as well. The species is long known from the original biotope but it was erroneously identified with *G. carpelli* and has only been recently described as *G. obstinata* (Sokolov and Moshu, 2014). Fish coccidia have a direct developmental cycle but tubificid carrier hosts promote their intensive invasion. They belong to species most commonly transmitted by invading species to the conquered new biotope, and have been described from several gobies in their new locations (Molnár, 2000, 2006). In this study they were found in both the Tisza and the Zala region.

With its 40% prevalence of infection, *Trichodina mutabilis* is the most common of the parasites known from Hungary, but the glochidial larvae of an *Anodonta* species also showed distinct signs on the fins and gills of Amur sleepers. The occurrence of encysted metacercariae of *Opisthioglyphe ranae* deserves mention because in Hungary these worms had never been diagnosed in fish before.

When considering the cause of colonisation of the hosts, a basic question was whether the dwarf goby and the Amur sleeper appeared in Europe as a result of the climate change or via introduction. Data suggest that the Amur sleeper had been introduced from the original biotope with unconsidered fish transfers

(Reshetnikov, 2004), and it is one of the most invasive fish species in Eurasia in the past few decades (Copp et al., 2005; Reshetnikov and Ficetola, 2011; Reshetnikov, 2013). The short-lived Caucasian dwarf goby probably originates from the Black Sea, so it represents a unique type of spread of a goby species. It may have come to the Tisza River basin from Romania. The downstream spread of the species is extremely rapid, and its presence has been demonstrated also in Serbia (Harka et al., 2013, 2015a,b). From a parasitological point of view the important questions to be answered were how many parasites unknown in Hungarian fish basins had been carried to the new biotope and how these species could propagate there. It seems that the Amur sleeper transferred to Hungary only three specific parasites (*Goussia obstinata*, *Gyrodactylus perccotti* and *Nippotaenia mogurndae*) which do not endanger native fishes and do not cause intensive infection in the introduced hosts. Both fish species can be infected with the common wide host range parasites of Hungarian origin, of which *Proteocephalus* spp. deserve mention. While the Amur sleeper plays the same role for scolices of *Proteocephalus percae* as the common carrier host ruffe, the bleak parasite *Proteocephalus* sp. (presumably *P. torulosus*) can infect, and develop in, the dwarf goby but never achieves full maturity.

The low parasite infection level of these two invasive fish species may have contributed to their successful invasion into Hungary.

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