

Site-specific attachment of *Anodonta anatina* (Bivalvia: Unionidae) glochidia on two new fish hosts translocated in Lake Trasimeno (Italy)

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Abstract – To complete their life cycle, freshwater mussels of the order Unionida depend on fishes as hosts for their obligatory parasitic larval phase, the glochidium. Here we report the first documentation of gobioid fishes as hosts of glochidia of unionid mussels in the wild in Italy and in southern Europe (outside of the Danube drainage area). We also examined the pattern of the attached glochidia. A recent review reported 326 fish species as suitable hosts for unionids, but only eight (2.5%) of these were Gobioidae. Host identification and the documentation of their benefits or threats for the interaction of the mussels with their hosts is important for conservation of freshwater mussels. But the co-existence of mussels with non-native fish species is only poorly understood, including the compatibility of these two groups. *Knipowitschia panizzae* and *Pomatoschistus canestrinii*, two translocated sand gobies, were sampled in 2022 at the east shore of Lake Trasimeno (Italy). Two species of anodontine mussels were present at this locality, the native *Anodonta anatina* and the translocated *Sinanodonta woodiana*. Genetic data revealed that both sand gobies hosted glochidia only of *A. anatina* but not of *S. woodiana*, possibly because of seasonal bias. About 50% of the specimens of both fish species were infested. The paired fins were the most prominent targets. The examined individuals of both sand gobies carried only few glochidia (max. 5). Nevertheless, the two species showed a divergent pattern of glochidia attachment.

Keywords: *Anodonta* / Glochidia / Parasite / *Knipowitschia* / *Pomatoschistus* / Italy / Tiber River Basin / Italy

1 Introduction

The unusual life cycle of freshwater mussels (Bivalvia, Unionida) is characterized by a close link to freshwater fishes via a temporary but obligatory parasitic stage. Specialized larvae, the glochidia, are able to attach on the external surface on various body parts and fins, where they encyst and metamorphose to free-living juvenile mussels (Kat, 1984; Douda *et al.*, 2012; Modesto *et al.*, 2018). Many, but not all, freshwater mussels are host generalists in so far as their glochidia can attach to various fish species (Haag and Warren, 1997; 2003; Douda *et al.*, 2012). Nevertheless, not all of these fish species are suitable as hosts. Some species are merely marginally suitable because only few glochidia can transform into juvenile mussels (Haag and Warren, 1997; summarized in Modesto *et al.*, 2018) or complete development into juveniles is unsuccessful, for example due to

an immune response of the fish host (Reuling, 1919; Jansen *et al.*, 2001; Ieshko *et al.*, 2016).

Altered fish host behavior due to glochidia infestation such as reduced migration behavior was described by Horky *et al.* (2014). Conversely, host behavior may affect the probability of the glochidia to attach. Glochidia of many mussel species sink to the ground after being released. Benthic and epibenthic fishes at or close to the bottom of a water body are more likely to become parasitized by these glochidia than pelagic fishes (McMahon and Bogan, 2001). The few studies on the pattern of glochidia attachment to the host showed that they do not attach randomly, but that certain body regions are more affected than others (Giusti *et al.*, 1975; Dartnall and Walkey, 1979; Dudgeon and Morton, 1984; Martel and Lauzon-Guay, 2005).

So far, a recent review has reported 326 fish host species for unionids (Modesto *et al.*, 2018). Although gobioid fishes are adapted to a benthic life style, world-wide only eight of these species (2.5%) were listed as hosts in that review. Gobioid males are generally territorial, construct and guard

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nests. During these activities and when eggs and larvae are fanned with fresh water, glochidia find ideal conditions for contact. Such a ‘glochidia friendly’ behavior was already mentioned by Waters (1994) for Centrarchidae (sunfishes), which also construct and guard nests.

Lake Trasimeno, the fourth largest freshwater lake in Italy (Giardino *et al.*, 2010; Lorenzoni *et al.*, 2010), was variously altered by anthropogenic activities (Giusti *et al.*, 1975; Mearelli *et al.*, 1990; Ludovisi and Gaino, 2010; Carosi *et al.*, 2019). This included numerous fish introductions (Lorenzoni *et al.*, 2006, 2010). Seventy-four percent of the 19 species documented for the lake are introduced, a mere five are native (Lorenzoni *et al.*, 2015; Carosi *et al.*, 2019). Two gobies of the ‘sand goby’ group were likely accidentally introduced with fish fry, namely *Knipowitschia panizzae* (Berg 1916) and *Pomatoschistus canestrinii* (Ninni 1883), both endemic to the Adriatic catchment. Today, these two sand gobies are acclimated and abundant in the lake (Ahnelt *et al.*, 2018).

Currently, four mussel species of the tribe Anodontini are documented for the lake. Three of them are native, the Duck mussel *Anodonta anatina* (Linnaeus 1758), the Swan mussel *A. cygnea* (Linnaeus 1758) and the Fretted mussel *A. exulcerata* Porro 1838, and one is introduced, the Chinese pond mussel *Sinanodonta woodiana* (Lea 1834) (Froufe *et al.*, 2017). Back in the 1970s, the development of a freshwater mussel and of the glochidia in Lake Trasimeno was already documented (Giusti *et al.*, 1975). Those authors, however, assumed that the lake was inhabited by only a single *Anodonta* species, *A. cygnea*. The authors based their study on extensive samples from three localities of the lake, and their analysis of the glochidia was likely a mix of more than one species, possibly of all three anodontin mussels (*S. woodiana* was first detected in Italy in 1996 (Maganelli *et al.*, 1998)). World-wide not only populations of freshwater mussels but also populations of many freshwater fishes, hosts of the parasitic stage of the mussels, are declining (Modesto *et al.*, 2018). The loss of host fish availability can have severe effects on freshwater mussel dispersion and thus on the status of their populations (Schwalb *et al.*, 2011; Douda *et al.*, 2012; Froufe *et al.*, 2014) in the worst case resulting in extinction cascades (Modesto *et al.*, 2018). Recently, it has been shown that glochidia of freshwater mussels also attach to gobioid species of Ponto-Caspian origin which are exotic in Central and Western Europe (Ondračková *et al.*, 2005; 2009; Antal *et al.*, 2015; Šlapansky *et al.*, 2016). Such introduced species may have severe effects on freshwater mussel populations in two ways. They could be beneficial as new hosts but may also act as a sink if the attached glochidia cannot develop to young mussels (Šlapansky *et al.*, 2016; Tremblay *et al.*, 2016).

In this study we report (1) the first infestation of Gobioidae with glochidia of freshwater mussels in the wild in Europe outside the Ponto-Caspian region, (2) two Gobiionellidae (‘gobies’ in the following) endemic to the Adriatic basin as new hosts for glochidia, (3) the infestation of these two gobies by only a single anodontin species and (4) the topography of glochidia attachment on these two hosts. We also present an extensive literature survey on gobioid fishes as hosts of the parasitic stage of freshwater mussels world-wide.

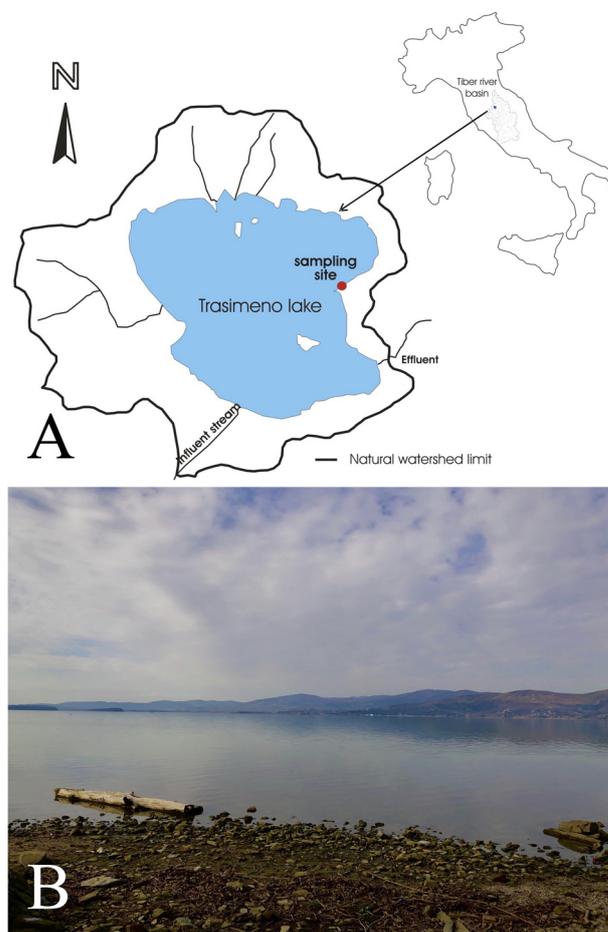


Fig. 1. A: map of Italy showing location of Lake Trasimeno and location of sampling site (red dot). B: sampling site at Monte del Lago. Photo A. Carosi.

2 Materials and methods

2.1 Study area

Lake Trasimeno (43°06' N, 12°07' E) measures 124 km², making it the fourth largest lake in Italy. It is shallow (average depth: 4.7 m; max. depth: 6.3 m), has no thermal stratification, the water transparency is low and the water temperature is about the same as the air temperature (Giardino *et al.*, 2010; Lorenzoni *et al.*, 2010; Ludovisi and Gaino, 2010; Carosi *et al.*, 2019). Nineteen fish species occur in the lake, most of them accidentally introduced for fishing purposes. The fish fauna is dominated by cyprinid fishes such as goldfish (*Carassius* sp.), rudd (*Scardinius hesperidicus* Bonaparte, 1845) and the tench (*Tinca tinca* (Linnaeus, 1758)) (Lorenzoni *et al.*, 2010).

From 3 March 2022 to 24 May 2022, five samplings were conducted on the east shore of the lake, at ‘Monte del Lago’ (43° 8.797' N; 12° 9.887' E) (Fig. 1A). The site is located in a shallow pebbly bottom area (Fig. 1B), in a littoral stretch free from the presence of *Phragmites australis* (Cav.) Trin. ex Steud. 1840, which, however, is abundant in the surrounding areas.

Table 1. *Knipowitschia panizzae* and *Pomatoschistus canestrinii* from Lake Trasimeno; number (N) of specimens with glochidia of *Anodonta anatina* attached to different parts of the body in total and by sex. A = anal fin, C = caudal fin, D1, D2 = first and second dorsal fin, P = pectoral fins and V = pelvic fins (fused to pelvic disc).

Species	N	P	V	A	D1	D2	C	Head	Trunk	Tail
<i>K. panizzae</i>	62	20	20	3	2	1	7	9	0	0
<i>P. canestrinii</i>	86	24	19	5	1	1	20	13	1	2
<i>K. panizzae</i> males	32	13	12	1	1	1	2	2	0	0
<i>K. panizzae</i> females	30	7	8	2	1	0	5	7	0	0
<i>P. canestrinii</i> males	34	10	7	1	0	0	10	4	1	1
<i>P. canestrinii</i> females	52	14	12	4	1	1	10	9	0	1

2.2 Material

2.2.1 Fishes

Sand gobies of the genera *Knipowitschia* and *Pomatoschistus* have a benthic life-style, are opportunistic feeders preying mostly on macroinvertebrates, and prefer soft bottoms (Miller, 2004). We investigated a total of 148 sympatrically occurring gobies: 62 specimens of the Adriatic dwarf goby *Knipowitschia panizzae* and 86 specimens of the Canestrini's goby *Pomatoschistus canestrinii*. The sex was determined based on the shape of the urogenital papilla, longer than wide and tapering in males, shorter and about as long as wide in females. Fishes were sampled with the permission of the state and regional institutions and were in accordance with Italian state laws on fisheries.

The systematics of the Gobioidae (as suborder of Gobiiformes) is still in progress. Therefore, we keep the Gobionellidae sensu Thacker (2009) on family level.

2.2.2 Mussels

Four species of Unionidae are documented for Lake Trasimeno, three native (*Anodonta anatina*, *A. cygnea*, *A. exulcerata*) and one non-native (*Sinanodonta woodiana*) (Froufe *et al.*, 2017; Riccardi *et al.*, 2020). Only two of these four unionid mussel species were present at the sampling locality of the gobiid fishes, the Duck mussel *Anodonta anatina* and the Chinese pond mussel *Sinanodonta woodiana*.

We investigated shells of a total of 12 mussels, eight *Anodonta anatina* and four *Sinanodonta woodiana*. These two species were distinguished based on their different umbonal sculpture. The umbonal ridges of *A. anatina* characteristically crossed the growth ridges, whereas those of *S. woodiana* were more elevated and further apart than those of *A. anatina*. Additionally, *A. anatina* has more elongated, rhombic shells, with the dorsal margin slightly bent and the lower edge characteristically thickened. In contrast, the shells of *S. woodiana* were more roundish, nearly circular.

Genetic data were retrieved from nine glochidia, five from *K. panizzae* and four from *P. canestrinii*.

2.3 Methods

The fishes were sampled along the shore of the lake by electrofishing, using a direct electric current with a power of 4,500 W. Specimens were euthanized by an overdose of phenoxyethanol and preserved in 99.8% ethanol.

The gobies were examined for the presence of parasites using a stereo microscope. The attachment sites of glochidia were individually documented for each specimen. The recorded attachment sites were grouped into fins, head, trunk and tail (from anus to base of caudal fin) for a total of nine attachment sites (Tab. 1). We did not differentiate between left and right side but summarized these data. To test if glochidia were attached on gills, nine of the largest infested specimens and six of the largest specimens without external glochidia infestation were examined.

Glochidia for genetic analysis were dissected from their cysts with aid of insect needles: four such glochidia from *P. canestrinii* and five from *K. panizzae*. DNA was extracted using the whole specimen with the Qiagen's DNA MicroKit following the associated protocol and elution in 25 µl elution buffer. The DNA concentration was measured with the Invitrogen Qubit Fluorometer from Thermo Fisher Scientific. The Qubit™ dsDNA HS Assay Kit with the associated standard protocol was used. To amplify the DNA barcoding fragment of the mitochondrial cytochrome oxidase 1 gene, the primer pair LCO1490_Mol1/HCO2198_Mol1 (Duda *et al.*, 2017) was used. PCRs were performed on a Master Gradient thermocycler (Eppendorf) in 25 µl elution buffer with 5 µl template DNA, using the QIAGEN Multiplex PCR Kit with 0.5 µM of each primer. Each PCR comprised 40 reaction cycles with an annealing temperature of 50°C. Control reactions were carried out for both DNA extractions and PCR amplifications. PCR products were purified using the QIAquick PCR Purification kit (Qiagen) and analyzed by direct sequencing (both directions) by Microsynth Austria GmbH using the PCR primer pairs.

Four voucher specimens of both goby species infested with parasitic glochidia are deposited in the Collection Mollusca of the Natural History Museum of Vienna with the register numbers NHMW-MO-113654 (*Knipowitschia panizzae*) and NHMW-MO-113655 (*Pomatoschistus canestrinii*). Further specimens (infested or not infested) are deposited in the Ichthyological Collection of the Natural History Museum of Vienna with the register numbers NMW 100508–100509 (*K. panizzae*) and NMW 100510–100511 (*P. canestrinii*). Shells of *Anodonta anatina* (NMHW-MO-113652) and *Sinanodonta woodiana* (NMW-MO-113653) are deposited in the Collection Mollusca of the Natural History Museum.

Giusti *et al.* (1975) gave inconsistent numbers regarding the infestation of fishes with glochidia in Lake Trasimeno. On page 105 (penultimate sentence), they mention that glochidia were found in 53% of *L. gibbosus*, 16.6% of *T. tinca*, 54.5% of

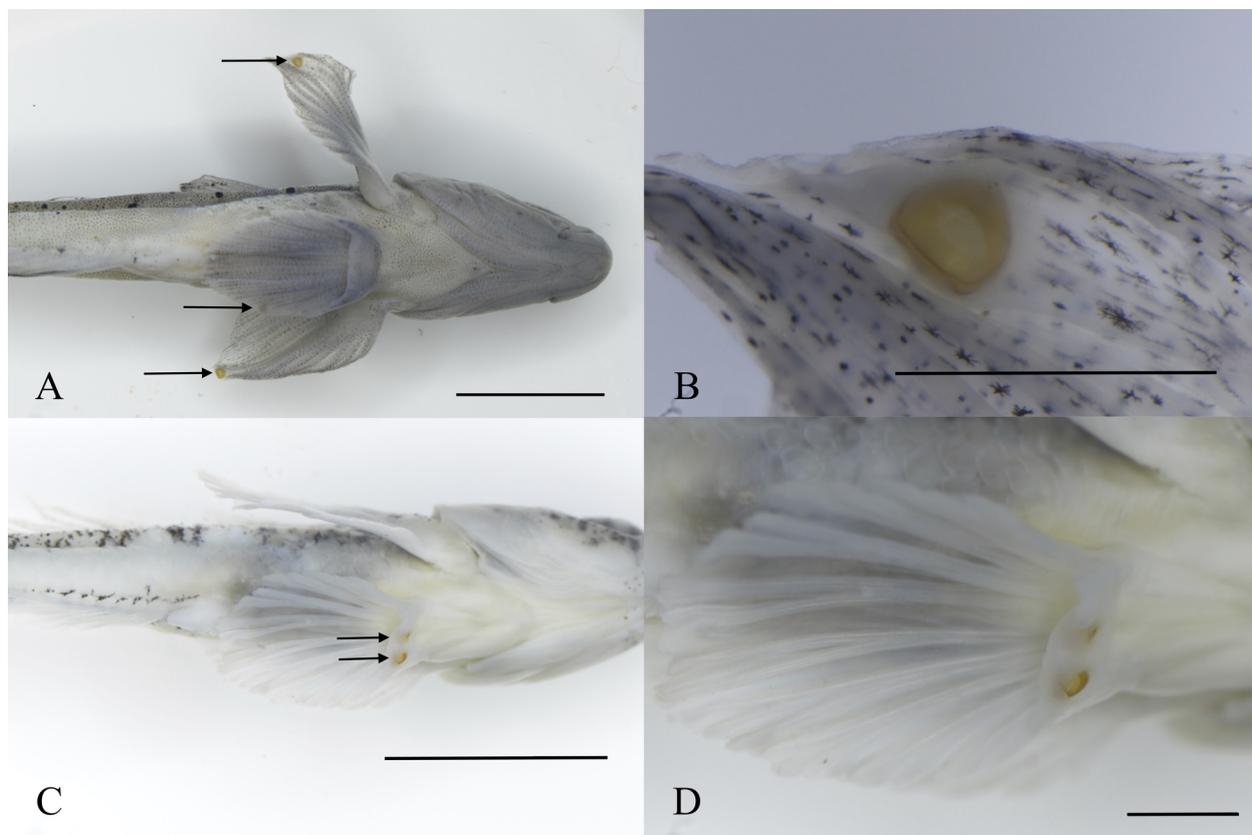


Fig. 2. Glochidia of *Anodonta anatina* (black arrows) attached to hosts *Pomatoschistus canestrinii* (NHMW-MO-113655) (A, B) and *Knipowitschia panizzae* (NHMW-MO-113654) (C, D). Parasites attached to A: the pectoral and the pelvic fins (united to pelvic disc); B: same specimen, encysted glochidium, right pectoral fin; C: base of pectoral disc; D: same specimen, pectoral disc enlarged. Scale of A, C = 5 mm; scale of B, D = 1 mm. Photos C. Hörweg, H. Ahnelt.

P. fluviatilis and 37.5% of *A. boyeri*. From Fig. 7, page 106, about the same percentage is shown for *P. fluviatilis*, but much lower values are given for *L. gibbosus* (about 35%) and *T. tinca* (about 6%). Nevertheless, the exact number of individuals of fish species examined and how many of them were infested is listed on page 109, Fig. 9: *L. gibbosus* – examined 173 specimens of which 53 were infested 53, which equals 30.6%; *T. tinca* – examined 140, infested 39 (27.9%); *P. fluviatilis* – examined 615, 167 infested (27.2%) and *A. boyeri* – examined 99, infested 31 (31.3%). We therefore follow these data in Fig. 9 of Giusti *et al.* (1975) based on the exact number of examined vs. infested specimens.

2.3.1 Statistical analysis

A Fisher's Exact test was used to assess differences in the proportion of infested specimens between *P. canestrinii* and *K. panizzae*, and between sexes. The comparison of the mean number of attached glochidia between the two fish species, and between sexes, was performed using a *t*-test. A chi-square test was used to assess differences between the two species and between sexes in the proportion of attached glochidia for target body regions.

The between-sex comparison of the average number of glochidia detected in the various body parts was performed by

MANOVA. All analyses were done using Dell STATISTICA 13 software.

3 Results

3.1 Infestation and topography of attachment

Glochidia-infested gobies were recorded during the sampling events in March and April. Specimens collected in May were parasite free.

We found that 51.4% ($n = 76$) of all sampled gobies ($n = 148$), collected on a single locality in Lake Trasimeno, had glochidia attached (Fig. 2). The maximum number per specimen was five for both *P. canestrinii* and for *K. panizzae*. Nevertheless, the vast majority of specimens were infested by only 1–2 glochidia, 77.1% ($n = 37$) in *P. canestrinii* and 64.3% ($n = 18$) in *K. panizzae*; 3–5 glochidia were found in 22.9% ($n = 11$) of *P. canestrinii* and in 35.7% ($n = 10$) of *K. panizzae* (Tab. 2).

More *P. canestrinii* were affected (55.8%) ($n = 48$) than *K. panizzae* (45.2%) ($n = 28$) (Tab. 3), but this difference was not significant based on the Fisher's Exact test ($p = 0.085$). Nevertheless, in the mean (mean intensity of infestation), the glochidia burden was larger in *K. panizzae* (2.2 vs. 1.8); again, this difference was not significant (*t*-test: $t = 2.97$; $p = 0.089$). The largest mean number of attached glochidia occurred in

Table 2. *Knipowitschia panizzae* and *Pomatoschistus canestrinii* from Lake Trasimeno; sampling dates from March – May 2022 with number of specimens in total and, in parentheses, females/males, of not infested and infested specimens.

Date	<i>Pomatoschistus</i> not infested	<i>Pomatoschistus</i> infested	<i>Knipowitschia</i> not infested	<i>Knipowitschia</i> infested
03/03/2022	11 (8/3)	12 (11/1)	10 (4/6)	5 (2/3)
03/21/2022	8 (4/4)	11 (6/5)	9 (0/9)	7 (0/7)
03/28/2022	7 (2/5)	6 (1/5)	15 (7/8)	15 (8/7)
04/22/2022	11 (6/5)	19 (9/10)	0	1 (1/0)
05/24/2022	0	0	0	0
Total	37 (20/17)	48 (28/20)	34 (11/23)	28 (11/17)

Table 3. Fish species of Lake Trasimeno infested with glochidia of *Anodonta anatina* * (this study) and *Anodonta* sp.**. Ab=*Atherina boyeri*, Kp=*Knipowitschia panizzae*, Lg=*Lepomis gibbosus*, Pc=*Pomatoschistus canestrinii*, Pf=*Perca fluviatilis*, Tt=*Tinca tinca*.

Total	Kp *	Pc *	Pf **	Lg**	Tt**	Ab**
	N = 62	N = 86	N = 615	N = 173	N = 140	N = 99
Infested	N = 28 45.2%	N = 48 55.8%	N = 167 27.2%	N = 53 30.6%	N = 39 27.9%	N = 31 31.3%
Glochidia/Ind	N = 2.2	N = 1.7	N = 5.2	N = 2.8	N = 1.6	N = 1.7

** from Giusti *et al.* (1975, Fig. 9).

Table 4. Percentage of glochidia attachment on the four most infested structures of *Knipowitschia panizzae* and *Pomatoschistus canestrinii* separated by species and, within species, by sex. C=caudal fin, P=pectoral fin and V=pelvic fin (sucking disc).

Species	P	V	C	Head
<i>K. panizzae</i>	32.3	32.3	11.3	14.5
<i>P. canestrinii</i>	27.9	22.1	23.3	15.1
<i>K. panizzae</i> males	40.6	37.5	6.3	6.3
<i>K. panizzae</i> females	23.3	26.7	16.7	23.3
<i>P. canestrinii</i> males	29.4	20.6	29.4	11.8
<i>P. canestrinii</i> females	26.9	23.1	19.2	17.3

female *K. panizzae* (3.9); males carried only 1.9. In *P. canestrinii* the respective values were 1.9 and 1.6, respectively (Tab. 4). The differences between the sexes are statistically significant for *K. panizzae* ($t=4.78$; $p=0.038$), but not for *P. canestrinii* ($t=1.09$; $p=0.301$). In both species, males were infested to a larger percentage than females (*P. canestrinii* 61.8% vs. 53.8%; *K. panizzae* 53% vs. 36.7%). This difference was more expressed in *K. panizzae*. However, in neither *P. canestrinii* ($p=0.507$) nor in *K. panizzae* ($p=0.499$) were the differences between the sexes statistically significant based on Fisher's Exact test. No glochidia were found on the gills ($n=15$ for both species).

3.2 Pattern of attached glochidia

All nine investigated regions of both species combined were targets of glochidia, although none were found on the first dorsal fin, the trunk and the tail in *K. panizzae* (Tab. 1).

In the mean number of glochidia per specimen was 1.8 on *P. canestrinii*. Except for the two dorsal fins of the males, glochidia occurred on all nine investigated structures, but in

different proportions (Tab. 1). Most affected were fins: pectoral fins 27.9% ($n=24$), pelvic fins 22.1% ($n=19$), caudal fin 23.3% ($n=20$), anal fin 5.8% ($n=5$), the head 15.1% ($n=13$), the two dorsal fins 1.1% ($n=1$) each, the trunk 1.1% ($n=1$) and the tail 2.3% ($n=2$). The differences between the frequencies were highly significant (chi-square test: $\chi^2=68.87$; $p=0.001$).

The mean number of glochidia per specimen was 2.2 on *K. panizzae*. They occurred on seven of nine structures, and in different proportions. In this species the trunk and tail were not affected (Tab. 1). Most affected were fins: pectoral fins 32.3% ($n=20$), pelvic fins 32.3% ($n=20$), caudal fin 11.3% ($n=7$), anal fin 4.8% ($n=3$), first dorsal fin 3.2% ($n=2$), second dorsal fin 1.6% ($n=1$) and head 14.5% ($n=9$). The differences between the percentage frequencies were highly significant ($\chi^2=45.99$; $p=0.001$).

By far the most glochidia were attached to the fins of both species; in *P. canestrinii* 81.4% ($n=70$) vs. 18.6% ($n=16$) on the entire body (head, trunk, tail). In *K. panizzae* the corresponding values were 85.5% ($n=53$) on the fins vs. 14.5% ($n=9$) on the entire body (Tab. 4).

Table 5. Infestation (mean number) of *Knipowitschia panizzae* and *Pomatoschistus canestrinii* with glochidia of *Anodonta anatina* and water temperature of Lake Trasimeno during sampling period. *April 22: only one, not infested, specimen of *K. panizzae* was collected.

Date	Temperature	Infestation	<i>K. panizzae</i>	<i>P. canestrinii</i>
03/03/2022	7.0 °C	yes	2	2.2
03/21/2022	10.8 °C	yes	1.9	1.6
03/28/2022	14.7 °C	yes	2.5	1.8
04/22/2022	16.3 °C	yes	*	1.5
05/24/2022	24.4 °C	no	–	–

We found only encysted glochidia on the fishes (Fig. 2). We could not determine the metamorphosis rate and whether the glochidia metamorphosed into juveniles or remained encysted.

3.3 Temperature-dependent infestation

Water temperature increased during the sampling period (3 March–24 May) from 7 °C to 24.4 °C and during the period with attached glochidia (3 March–22 April) from 7 °C to 16.3 °C (Tab. 5). No temperature-dependent infestation gradient was recorded for either fish species.

3.4 Sexual dimorphism and glochidia infestation

Of the nine investigated morphological structures, some were differently infested in males and females of both fish species. This involved six structures in *K. panizzae* but only three of the nine structures in *P. canestrinii* (Tab. 4). The paired fins of male *K. panizzae* were more infested than those of the females (pectoral fin 40.6% vs. 23.3%, pelvic fins 37.5% vs. 27.7%); in contrast, we detected more glochidia on the head (23.3% vs. 6.3%), on the caudal fin and, to a lesser extent, on the anal fin (16.7% vs. 6.3%, 6.7% vs. 3.1%) in females than in males. In *P. canestrinii* the differences between sexes were distinct only on the caudal fin (males 29.4% vs. females 19.2%), on the head (females 17.3% vs. males 1.8%) and on the anal fin (females 7.7% vs. males 3.1%).

Despite a seeming trend to sexual bias of certain body regions in both species, statistical analysis did not support sexual dimorphism. The average number of glochidia on the various body parts of the two sexes were not statistically significant (MANOVA), neither for *K. panizzae* ($F=1.045$, $p=0.411$) nor for *P. canestrinii* ($F=0.756$, $p=0.659$).

3.5 Literature survey

The literature survey yielded 42 publications from 1991 and 2021 which reported 33 gobioid species as hosts of glochidia of unionid mussels world-wide. Documentations came from four continents and 17 countries: Australia, Japan, New Zealand, Thailand, USA and 12 in Europe (Tab. 6).

Somewhat more than the half of these records (17 of 33 = 51.5%) are based on laboratory experimental infestations; only 48.5% are based on records from the wild. Including *K. panizzae* and *P. canestrinii* of the present study, to date 33 gobioid species from four families – 4 Eleotridae, 20 Gobiidae, 7 Gobionellidae (= Oxudercidae) and 2

Odontobutidae – are known as (potential) hosts for freshwater mussels (Tab. 6).

4 Discussion

We found a high prevalence (~50%) of the obligatory, time-limited parasitic stage of the freshwater mussel *A. anatina* on two gobies, the Adriatic dwarf goby *K. panizzae* and the Canestrini's goby *P. canestrinii*. These small, short-lived fishes (\pm annual) (Miller, 2004) were accidentally introduced in Lake Trasimeno, where they established large populations (Borroni, 1976; Freyhof, 1998). No records of infestation of these gobies with glochidia in their natural distribution area are documented. Such a report involving an infestation by *Anodonta* sp. is available only for a congener of the Adriatic dwarf goby, the Caucasian dwarf goby *K. caucasica* (Berg 1916), introduced in Hungary (Antal *et al.*, 2015).

Gobioid fishes are relatively rarely mentioned as hosts for glochidia of unionid mussels. Modesto *et al.* (2018) reported 326 fish host species, but only eight (2.5%) of them were gobioids. Our survey increased this number to 33 species (Tab. 6). Although the typically benthivorous gobioids now make up 9.4% of the known 351 fish host species, these fishes are relatively insignificant as potential hosts for freshwater mussels in species number. Note also that 17 of these 33 species, infestations are known only from laboratories (Tab. 6). This also includes *Padogobius bonelli* (Bonaparte 1846) (as *P. martensii* (Günther 1861)) from the Po River drainage area (northern Italy) (Nagel and Castagnolo, 1991). To our knowledge, no further record (either from the laboratory or the wild) of other gobioid host species is known from the Italian peninsula. The other gobioids reported from Europe as hosts for glochidia are all of Ponto-Caspian origin except for the Far East Asian *Perccottus glenii* Dybowski 1877. That species was introduced to the European part of Russia in the early 20th century and is currently spreading through eastern and central Europe (Reshentikov, 2013; Grabowska *et al.*, 2020). This makes the two sand gobies *K. panizzae* and *P. canestrinii* the first gobioid fishes with natural glochidia infestation in the wild in Italy and Europe outside of the Ponto-Caspian region and the Danube River watershed, or which are not of Ponto-Caspian origin.

Anadontin mussels are generalists in that they use taxonomically widely different fish species as hosts, mostly cyprinids, but also salmonids, esocids, gasterosteids or centrarchids (e.g., Dartnall and Walkey, 1979; Patzner, 2004; Blažek and Gelnar, 2006; Douda *et al.*, 2012; Lopes-Lima *et al.*, 2017). Giusti *et al.* (1975) also found a

Table 6. Literature survey of gobioid fishes infested with glochidia of Unionidae (Bivalvia: Unionida). Gobioides infested with glochidia: $n = 33$ (Graf (1997), who just mentioned “goby”, is not included in this number). The eight species listed by Modesto *et al.* (2018) are shaded in grey. Note, that for each species/country just one publication, as example, is listed.

Species ($n = 33$)	Countries ($n = 17$)	Publications ($n = 42$)
Gobiidae ($n = 20$)		
<i>Acanthogobius flavimanus</i>	Japan §	Itoh (2019)
<i>Afurcagobius suppositus</i>	Australia §	Klunzinger <i>et al.</i> (2012)
<i>Babka gymnotrachelus</i>	Poland	Ondračková <i>et al.</i> (2021)
Goby	Ukraine	Kvach <i>et al.</i> (2009)
<i>Neogobius fluviatilis</i>	USA	Graf (1997)
	Bulgaria	Kvach <i>et al.</i> (2018)
	Germany	Ondračková <i>et al.</i> (2021)
	Poland	Ondračková <i>et al.</i> (2021)
	Slovakia	Ondračková <i>et al.</i> (2021)
	Russia	Mineeva and Mineev (2020)
<i>Neogobius melanostomus</i>	Austria	Francová <i>et al.</i> (2011)
	Bulgaria	Francová <i>et al.</i> (2011)
	Czech Republic	Francová <i>et al.</i> (2011)
	Germany	Emde <i>et al.</i> (2014)
	Hungary	Molnár (2006)
	Poland	Rolbiecki (2006)
	Russia	Mineeva and Semenov (2021a)
	Slovakia	Ondračková <i>et al.</i> (2005)
	USA §	Muzzal <i>et al.</i> (1995)
<i>Padogobius bonelli</i> *	Italy §	Nagel and Castagnolo (1991)
<i>Ponticola gorlap</i> ***	Russia	Mineeva and Semenov (2021b)
<i>Ponticola kessleri</i>	Slovakia	Ondračková <i>et al.</i> (2005)
	Bulgaria	Ondračková <i>et al.</i> (2006)
	Germany	Ondračková <i>et al.</i> (2015)
	Ukraine	Kvach and Ondračková (2020)
<i>Proterorhinus semilunaris</i> **	Czech Republic	Koubkova and Barus (2000)
	Germany	Ondračková <i>et al.</i> (2015) Mierzejewska <i>et al.</i> (2014)
	Poland	Kvach and Ondračková (2020)
	Ukraine	
<i>Proterorhinus semipellucidus</i>	Russia	Kvach and Ondračková (2020)
<i>Pseudogobius olorum</i>	Australia §	Klunzinger <i>et al.</i> (2012)
<i>Rhinogobius</i> sp.	Japan §	Itoh (2019)
<i>Rhinogobius biwaensis</i>	Japan	Shimizu and Nagasawa (2018)
<i>Rhinogobius brunneus</i>	Japan	Shimizu and Nagasawa (2018)
<i>Rhinogobius flumineus</i>	Japan §	Shimizu and Nagasawa (2018)
<i>Rhinogobius fluviatilis</i>	Japan	Shimizu and Nagasawa (2018)
<i>Rhinogobius giurinus</i>	Japan §	Itoh <i>et al.</i> (2016b)
<i>Rhinogobius kurodai</i>	Japan	Shimizu and Nagasawa (2018)
<i>Rhinogobius nagoyae</i>	Japan §	Itoh <i>et al.</i> (2016b)
<i>Rhinogobius similis</i>	Japan §	Itoh (2019)
Gobionellidae (=Oxudercidae) ($n = 7$)		
<i>Gymnogobius</i> sp.	Japan	Itoh <i>et al.</i> (2016a)
<i>Gymnogobius castaneus</i>	Japan §	Itoh <i>et al.</i> (2016b)
<i>Gymnogobius urotaenia</i>	Japan §	Itoh (2019)
<i>Knipowitschia caucasica</i>	Hungary	Antal <i>et al.</i> (2015)
<i>Knipowitschia panizzae</i>	Italy	this study
<i>Pomatoschistus canestrinii</i>	Italy	this study
<i>Tridentiger brevispinis</i>	Japan §	Itoh <i>et al.</i> (2016b)
Eleotridae ($n = 4$)		
<i>Gobiomorphus basalis</i>	New Zealand §	Hanrahan (2019)
<i>Gobiomorphus cotidianus</i>	New Zealand §	Hanrahan (2019)
<i>Gobiomorphus huttoni</i>	New Zealand §	Hanrahan (2019)
<i>Oxyeleotris marmorata</i>	Thailand §	Panha (1992)
Odontobutidae ($n = 2$)		

Table 6. (continued).

Species (<i>n</i> = 33)	Countries (<i>n</i> = 17)	Publications (<i>n</i> = 42)
<i>Odontobutis obscura</i>	Japan §	Itoh <i>et al.</i> (2016b)
<i>Perccottus glenii</i>	Germany	Kvach <i>et al.</i> (2017)
	Hungary	Antal <i>et al.</i> (2015)
	Latvia	Kirjušina <i>et al.</i> (2014)
	Ukraine	Kvach <i>et al.</i> (2022)

*as *Padogobius martensis*; ** or as *Proterorhinus marmoratus*; or *** as *Neogobius iljini*; § laboratory experimental infection.

series of fish species infected by anadontin glochidia. Those authors were the first to study this issue in Lake Trasimeno (central Italy), but assumed that only one anadontin mussel, *A. cygnea*, occurred in the lake. Based on our results, their analysis probably involved a mix of more than one, possibly of all three native anadontin mussels. *Sinanodonta woodiana*, the fourth anadontin mussel, was first documented for Italy in 1996 (Maganelli *et al.*, 1998).

Giusti *et al.* (1975) reported relatively low infestation rates in Lake Trasimeno. About one quarter of *Perca fluviatilis* Linnaeus 1758 and *T. tinca* specimens, and only one third of *E. cisalpinus* Bianco and Delmastro 2011 (as *E. lucius* Linnaeus 1758) and of *Lepomis gibbosus* (Linnaeus 1758) specimens, carried glochidia (Table 3). We found glochidia on about half of the *K. panizzae* and *P. canestrinii* specimens, an infestation rate twice as high as for non-gobioid fishes in the lake. A possible explanation for this difference is the bottom-dwelling life style of both gobies. The glochidia of anadontin mussels are unable to swim actively and sink to the ground after they are released (Lefevre and Curtis, 1910; Paling, 1968; Dudgeon and Morton, 1984). Lake Trasimeno, however, is shallow (mean depth 4.7 m), and most fishes probably more or less often come close to the bottom or in direct contact with it. The tench *T. tinca*, for example, lives close to the bottom where it searches for food, even by digging in the sediment (Banareescu, 1999). Nonetheless, only about one quarter of the tench specimens were infested (Giusti *et al.*, 1975).

Another potential explanation for the high infestation rate of the two gobies compared to the other fishes in the lake is a different reaction of their immune systems. Naïve specimens are most at risk if infestation (Rogers-Lowery and Dimock, 2006; Modesto *et al.*, 2018). After a first infestation the immune system of perennial species learns to avoid or at least reduce infestation with glochidia. Accordingly, the young-of-the-year specimens of a host species generally carry a relatively higher glochidia burden than older conspecifics (Dodd *et al.*, 2005; Barnhart *et al.*, 2008; Donrovich *et al.*, 2017). Both sand goby species are annual and therefore all specimens are naïve in this respect.

Beyond the degree of infestation, we also investigated the pattern of glochidia attachment on the fish body. This pattern on *K. panizzae* and *P. canestrinii* was generally similar to those of the fishes investigated by Giusti *et al.* (1975), with most glochidia attached to the fins and the head. Nearly no glochidia were attached on the trunk or tail. This also agrees with other studies, which found preferable attachment on the fins and head (e.g., McMahon and Bogan, 2001; Šlapansky *et al.*, 2016; Mineeva and Semenov, 2021a, b). Giusti *et al.* (1975)

concluded that the negligible attachment to the trunk and tail was linked to the scale cover of these body parts.

A deviation from the observations of Giusti *et al.* (1975) was the infestation of the caudal fin of Canestrini's goby. Those authors documented a relatively low rate on this fin for the lake fishes. The rate was also similarly low on the caudal fin of *K. panizzae*, but it was twice as high on the caudal fin of *P. canestrinii* and similar to the infestation rate of the paired fins of this species. Both species occur sympatrically at the sampling station. The infestation rate of the caudal fin is about 4.5 times larger in *P. canestrinii* than *K. panizzae*. Sampling was conducted at the beginning of the spawning season, when males probably already started to occupy territories. Possibly differences in microhabitat use of the males of the two species are responsible for the distinct difference in glochidia attachment on the caudal fins of both species.

Šlapansky *et al.* (2016) also reported a higher infestation of the caudal fin in juvenile *Neogobius melanostomus* (Pallas 1814) and *Proterorhinus semilunaris* (Heckel 1837) but unfortunately most other studies fail to provide a detailed pattern of glochidia attachment (Mierzejewska *et al.*, 2014; Tremblay *et al.*, 2016; Mineeva and Mineev, 2020). Comparing the various studies of gobioid infestation leaves an ambivalent picture. Some studies report minor infestation rates, demonstrating that glochidia are rare gobioid parasites (e.g., Tyutin *et al.*, 2013; Kvach *et al.*, 2017, 2018; Zhokhov *et al.*, 2017; Mineeva and Mineev, 2020; Mineeva and Semenov, 2021a). Others, however, recorded low rates in some gobioid species but a high value in a sympatric goby (e.g., Mierzejewska *et al.*, 2014; Itoh *et al.*, 2016). Again, others found high glochidia burdens (e.g., Antal *et al.*, 2015; Šlapansky *et al.*, 2016). If repeated infection decreases the risk of further infestation by boosting the immune response, this could explain the divergent infestation rates of perennial gobioid fishes. For example, a high glochidia load was reported by Antal *et al.* (2015) and Šlapansky *et al.* (2016); the former authors investigated an annual species, the latter young-of-the-year of perennial species: both were naïve fishes and therefore probably more prone to infestation (Rogers-Lowery and Dimock, 2006; Modesto *et al.*, 2018).

Statistical analysis did not support sexual dimorphism in the susceptibility of *K. panizzae* and *P. canestrinii*. Nevertheless, a trend for sexual bias of certain body regions is apparent in both species. Overall, sexual dimorphism in glochidia infestation is apparently rare or not investigated. *Anodonta anatina* is a generalist accepting a wide array of fish hosts and does not prefer a particular sex or body region (Nagel, 1985; Barnhart *et al.*, 2008; Modesto *et al.*, 2018). We therefore assume that the sexual bias of attached glochidia in the

investigated gobies relate to the behavior of these fishes during breeding season, with males being more sedentary (guarding nests and fanning) and females more mobile in search of suitable mates.

The prevalence (number of fish with glochidia/total number of fish sampled) but not the mean abundance (glochidia per individual) in *A. anatina* in Lake Trasimeno was higher in *K. panizzae* and *P. canestrinii* than for other investigated fish species here (> 45% vs. < 32%). The mean abundance is relatively low in all examined species including the two gobies and was 1.6–2.2 for all investigated species except for *P. fluviatilis* (Giusti *et al.*, 1975; this study). Surprisingly, *P. fluviatilis*, an open-water species, carried most glochidia (5.2) rather than the inherently bottom-dwelling gobies (Tab. 3).

Because fishes we sampled in the wild were preserved immediately, we do not know if the larval glochidia successfully metamorphosed into juvenile mussels. It is therefore unclear if *K. panizzae* and *P. canestrinii* serve as primary hosts (high proportion of glochidia metamorphoses into juveniles) or as marginal hosts (low metamorphosis rates) and how they affect the recruitment of *A. anatina*. As primary hosts they could enhance the recruitment of this native mussel. In contrast, however, they could also limit the recruitment as a sink for glochidia, *i.e.*, if most glochidia that encyst fail to metamorphose to juveniles. The latter scenario has been suggested for a gobiid introduced into the Great Lakes (USA) (Barnhart and Baird, 2000; Tremblay *et al.*, 2016) and is supported by a laboratory infestation in which infested gobies lost more than 98% of glochidia within two weeks (Taubert *et al.*, 2012). Finally, Moore and Clearwater (2021) found that non-native fish species were only marginal hosts and a sink for native freshwater mussels, which could also be the case for juvenile gobiids with a high prevalence of glochidia (Šlapansky *et al.*, 2016).

The main advantage of a parasitic stage is thought to be the spreading of the mussel by host fishes (Kat, 1984; Haag and Warren, 1997). Most benthic gobioid species are not good swimmers and do not traverse large distances. This makes any substantial transport by the two gobies low. Nevertheless, for relative sedentary organisms such as mussels, even small-scale movements of fish hosts could aid dispersal. Further investigation is needed to test whether *K. panizzae* and *P. canestrinii* serve as primary hosts or as a sink for glochidia of the freshwater mussel *A. anatina* in Lake Trasimeno. This may become increasingly important because the Ebro+Italian clade of *A. anatina* (Froufe *et al.*, 2014, 2017) is locally restricted and apparently declining (Lopes-Lima *et al.*, 2017).

Freshwater mussels are of great ecological importance. They filter and process suspended particles thus clearing the water and also mix sediments (Modesto *et al.*, 2018). Many populations are diminishing because of water pollution, habitat destruction and the decline of host species (Douda *et al.*, 2012; Zieritz and Aldridge, 2011; Lopes-Lima *et al.*, 2017). World-wide not only populations of freshwater mussels but also populations of many freshwater fishes, hosts of the parasitic stage of the mussels, are declining (Modesto *et al.*, 2018). The loss of host fish availability can have severe effects on freshwater mussel dispersion and thus on the status of their

populations (Schwalb *et al.*, 2011; Douda *et al.*, 2012; Froufe *et al.*, 2014). Especially anadontin mussels which are generalists for fish hosts could benefit from exotic, introduced fish species where natural fish populations are diminishing. Recently, it has been demonstrated that glochidia of freshwater mussels also attach to introduced gobioid species (Ondračková *et al.*, 2005; 2009; Antal *et al.*, 2015; Šlapansky *et al.*, 2016; Tremblay *et al.*, 2016). With their benthic life-style gobioids occur close to the mussels and also come directly in contact with glochidia on the bottom of aquatic habitats. These introduced gobioids may have severe effects on freshwater mussel populations in two ways. They can be beneficial as new hosts but may also act as a sink if the attached glochidia cannot develop to young mussels (Šlapansky *et al.*, 2016; Tremblay *et al.*, 2016). The understanding of the fish-mussel relationship, including non-native fish species which get increasingly important in many aquatic habitats, is essential for conservation management of freshwater mussels (Lopes-Lima *et al.*, 2017; Modesto *et al.*, 2018).

5 Conclusions

Two gobiid species, *K. panizzae* and *P. canestrinii*, translocated in Lake Trasimeno (Italy), are hosts for the parasitic stage of anadontin freshwater mussels. Although two mussels, the native *A. anatina* and the east Asian *S. woodiana*, are sympatric at the sampling locality, both fish species were infested only with glochidia of *A. anatina*. Host identification and the documentation of their benefits or threats for the interaction of the mussels with their hosts is an important baseline for conservation of freshwater mussels. But the co-existence of mussels with non-native fish species is only poorly understood, including the compatibility of these two groups. Extended work is required to identify (i) if the glochidia of two more native anadontins (*A. cygnaea* and *A. exulcerata*) also attach on these fishes, (ii) if the absence of glochidia of the introduced *S. woodiana* is a seasonal bias and (iii) in terms of conservation management, if the glochidia develop to juvenile mussels or if the two sand gobies are a possible sink for the freshwater mussels of Lake Trasimeno.

Author's contribution

HA conceived the study and wrote the manuscript. AC and ML collected the fishes, conducted statistical analysis and contributed to the writing of the manuscript. LK genetically identified the mussel parasites and contributed to the writing of the manuscript. TL contributed to the data sampling. MD identified the adult mussel shells and contributed to the manuscript. All authors read and improved the final version of the manuscript.

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References

- Ahnelt H, Konecny R, Gabriel A, Bauer A, Pompei L, Lorenzoni M, Sattmann H. 2018. First report of the parasitic copepod *Lernaea cyprinacea* (Copepoda: Lerneidae) on gobioid fishes (Teleostei: Gobionellidae) in southern Europe. *Knowl Mang Aquat Ecosyst* 419: 34.
- Antal L, Székely C, Molnár K. 2015. Parasitic infections of two invasive fish species, the Caucasian dwarf goby and the Amur sleeper in Hungary. *Acta Vet Hung* 63: 472–484.
- Banarescu P. 1999. The Freshwater Fishes of Europe. Vol. 5/I. Cyprinidae 2. Part I: *Rhodeus* to *Capoeta*. Wiebelsheim: Aula Verlag, 426 p.
- Barnhart MC, Baird MS. 2000. Fish hosts and culture of mussel species of special concern: Annual report for 1999. Rep Missouri Dep Conserv, Columbia, Missouri, 21p.
- Barnhart MC, Haag WR, Roston WN. 2008. Adaptations to host infection and larval parasitism in Unionoida. *J N Am Benthol Soc* 27: 370–394.
- Blažek R, Gelnar M. 2006. Temporal and spatial distribution of glochidial larval stages of European unionid mussels (Mollusca: Unionidae) on host fishes. *Folia Parasit* 53: 98–106.
- Borroni I. 1976. Involontaria introduzione nel Lago Trasimeno (Umbria) di un gobide di acqua salmastra (*Knipowitschia panizzae*) e seguito di pratiche ittigeniche. *Mem Ital Idrobiol* 33: 297–304.
- Carosi A, Ghetti L, Padula R, Lorenzoni M. 2019. Potential effects of global climate change on fisheries in the Trasimeno Lake (Italy), with special reference to the goldfish *Carassius auratus* invasion and the endemic southern pike *Esox cisalpinus* decline. *Fish Manag Ecol* 26: 500–511.
- Dartnall HJG, Walkley M. 1979. The distribution of glochidia of the Swan mussel, *Anodonta cygnea* (Mollusca) on the Three-spined stickleback *Gasterosteus aculeatus* (Pisces). *J Zool, Lond* 189: 31–37.
- Dodd BJ, Barnhart MC, Rogers-Lowery CL, Fobian TB, Dimock RV. 2005. Cross-resistance of largemouth bass to glochidia of unionid mussels. *J Parasitol* 91: 1064–1072.
- Donrovich SW, Douda K, Plechingerová V, Rylková K, Horky P, Slavík O, Liu H-Z, Reichard M, Lopes-Lima N, Sousa R. 2017. Invasive Chinese Pond mussel *Sinanodonta woodiana* threatens native mussel reproduction by inducing cross-resistance of host fish. *Aquatic Conserv: Mar Freshw Ecosyst* 27: 1325–1333.
- Douda K, Horky P, Bílý M. 2012. Host limitation of the thick-shelled river mussel: identifying the threats to declining affiliate species. *Animal Conserv* 15: 536–544.
- Duda M, Schindelar J, Macek O, Eschner A, Kruckenhauser L. 2017. First record of *Trochilus clandestinus* (Hartmann, 1821) in Austria (Gastropoda: Eupulmonata: Hygromiidae). *Malacol Bohemoslov* 16: 37–43.
- Dudgeon D, Morton B. 1984. Site selection and attachment duration of *Anodonta woodiana* (Bivalvia: Unionacea) glochidia on fish hosts. *J Zool, Lond* 204: 355–362.
- Emde S, Kochmann J, Kuhn T, Plath M, Klimpel S. 2014. Getting what is served? Feeding ecology influencing parasite-host interactions in invasive Round goby *Neogobius melanostomus*. *PLoS ONE* 9: e109971.
- Francová K, Ondračková M, Polačik M, Jurajda P. 2011. Parasite fauna of native and non-native populations of *Neogobius melanostomus* (Pallas, 1814) (Gobiidae) in the longitudinal profile of the Danube River. *J Appl Ichthyol* 27: 879–886.
- Freyhof J. 1998. First record of *Pomatoschistus canestrinii* (Ninni, 1883) in Lake Trasimeno. *Riv Idrobiol* 37: 107–108.
- Froufe E, Lopes-Lima M, Riccardi N, Zaccara S., Vanetti I, Lajtner J, Teixeira A, Varandas S, Prié V, Zieritz A, Sousa R, Bogan AE. 2017. Lifting the curtain on the freshwater mussel diversity of the Italian Peninsula and Croatian Adriatic coast. *Biodivers Conserv* 26: 3255–3274.
- Froufe E, Sobral C, Teixeira A, Souse R, Varandas S, Aldridge DC, Lopes-Lima M. 2014. Genetic diversity of the pan-European freshwater mussel *Anodonta anatina* (Bivalvia: Unionoida) based on COI: new phylogenetic insights and implications for conservation. *Aquatic Conserv: Mar Freshw Ecosyst* 24: 561574.
- Giardino C, Bresciani M, Villa P, Martinelli A. 2010. Application of remote sensing in water resource management: the case study of Lake Trasimeno, Italy. *Water Res Manag* 24: 3885–3899.
- Giusti F, Castagnolo L, Moretti Farina L, Renzoni A. 1975. The reproductive cycle and the glochidium of *Anodonta cygnea* L. from Lago Trasimeno (Central Italy). *Monit Zool Ital* 9: 99–118.
- Grabowska J, Kvach Y, Rewicz T, Pupins M, Kutsokon I, Dykyy I, Antal L, Zieba G, Rakauskas V, Trichkova T, Čeirāns, Grabowski M. 2020. First insight into the molecular population. Structure and origins of the invasive Chinese sleeper, *Perccottus glenii*, in Europe. *NeoBiota* 57: 87–107.
- GrafDL. 1997. Sympatric speciation of freshwater mussels (Bivalvia: Unionoidea): a model. *Amer Malacol Bull* 14: 35–40.
- Haag WR, Warren ML. 1997. Host fishes and reproductive biology of 6 freshwater mussel species from the Mobile Basin, USA. *J N Am Benthol Soc* 16: 576–585.
- Haag WR, Warren ML. 2003. Host fishes and infection strategies of freshwater mussels in large Mobile Basin streams, USA. *J N Am Benthol Soc* 22: 78–91.
- Hanrahan NJ. 2019. Field and laboratory investigations of Echyridella menziesii (Unionida: Hyriidae) interactions with host fish. Masterthesis, University of Waikato, New Zealand.
- Horky P, Douda K, Maciak M, Závorka L, Slavík O. 2014. Parasite-induced alterations of host behaviour in a riverine fish: the effects of glochidia on host dispersal. *Freshw Biol* 59: 1452–1461.
- Ieshko EP, Geist J, Murzina SA, Veselov AE, Lebedeva DI, Ziuganov VV. 2016. The characteristics of the infection of juvenile Atlantic salmon with glochidia of the freshwater pearl mussel in rivers of Northwest Russia. *Knowl Manag Aquat Ecosyst* 417: 6.
- Itoh T. 2019. Host fish species for glochidia of the freshwater unionid mussel *Sinanodonta* sp. from Lake Biwako in autumn. *Venus* 77: 27–35.
- Itoh T, Saitoh Y, Satoh Y, Ito K. 2016a. A new record of host fish *Gymnogobius* sp. for glochidia of *Pronodularia japonensis*, and the availability of host species for the mussel at three paddy field ditches in the Kanto area of central Japan. *Jap J Limnol* 77: 281–291.
- Itoh T, Uesugi S, Kakino W. 2016b. Host fish species for glochidia of the freshwater unionid mussel *Cristaria plicata* in tanks. *Venus* 74: 79–88.
- Jansen W, Bauer G, Zahner-Meike E. 2001. Glochidial mortality in freshwater mussels. In Bauer G, Wächtler K, eds. Ecology and Evolution of the Freshwater Mussels Unionoida. Ecological Studies (Analysis and Synthesis), 145. Springer, Berlin, Heidelberg, 185–211.
- Kat PW. 1984. Parasitism and the Unionacea (Bivalvia). *Biol Rev* 59: 189–207.
- Kirjušina M, Lazdāne M, Zolos M. 2014. Parasitofauna of *Perccottus glenii* Dybowski, 1877 (Osteichthyes, Odontobutidae) in water bodies of the southern part of Latgale (Latvia). *Acta Biol Univ Daugavp* 14: 137–143.
- Klunzinger MW, Beatty SJ, Morgan DL, Thomson GJ, Lymbery AJ. 2012. Glochidia ecology in wild fish populations and laboratory

- determination of competent host fishes for an endemic freshwater mussel of south-western Australia. *Australia J Zool* 60: 26–36.
- Koubkova B, Barus V. 2000. Metazoan parasites of the recently established tubenose goby (*Proterorhinus marmoratus*: Gobiidae) population from the South Moravian reservoir, Czech Republic. *Helminthologia* 37: 89–95.
- Kvach, Y, Kutsokon Y, Janáč M, Dykyy I, Dzyziuk N, Dudliv I, Nazaruk K. 2022. Parasites of the invasive sleeper *Perccottus glenii* (Actinopterygii: Odontobutidae) in the region of the first introduction of the Carpathian population. *Ocean Hydrobiol Studies* 51: 1–9
- Kvach Y, Mierzejewska K, Dziekońska-Rynko J. 2009. The parasites of two gobiids (*Apollonia fluviatilis* and *Babka gymnotrachelus*) in native range of southern Ukraine. XIV Conference of the Ukraine Scientific Society of Parasitologists, Uzghorod, 21–24 September 2009: 146.
- Kvach Y, Ondračková M. 2020. Checklist of parasites of Ponto-Caspian gobies (Actinopterygii: Gobiidae) in their native and non-native ranges. *J Appl Ichthyol* 36: 472–500.
- Kvach Y, Ondračková M, Janáč M, Jurajda P. 2017. The parasite community of round goby *Neogobius melanostomus* (Pallas, 1814) (Actinopterygii: Gobiidae) newly introduced into the upper Elbe. *Knowl Manag Aquat Ecosyst* 418: 19.
- Kvach Y, Ondračková M, Trichkova T, Crobiniak O, Zamorov V, Jurajda P. 2018. Parasitization of monkey goby, *Neogobius fluviatilis* (Pallas, 1814) (Actinopterygii: Gobiidae), at localities with different salinity levels. *Oceanol Hydrobiol St* 47: 376–383.
- Lefevre G, Curtis WC. 1910. Studies on the reproduction and artificial propagation of fresh-water mussels. *Bull Bur Fish* 30: 105–201.
- Lopes-Lima M, Sousa R, Geist J, Aldrige DC, Araujo R, Bergengren J, Bernalaya Y, Bódis E, Burlakova L, Van Damme D, Douda K, *et al.* 2017. Conservation status of freshwater mussels in Europe: state of the art and future challenges. *Biol Rev* 92: 572–607.
- Lorenzoni M, Mearelli M, Ghetti L. 2006. Native and exotic fish species in the Tiber River watershed (Umbria – Italy) and their relationship to the longitudinal gradient. *Bull Fr Pêche Piscic* 382: 19–44.
- Lorenzoni M, Ghetti L, Pedicillo G, Carosi A. 2010. Analysis of the biological features of the goldfish *Carassius auratus auratus* (Linnaeus, 1758) in Lake Trasimeno (Umbria, Italy) with a view to drawing up plans for population control. *Folia Zool* 58: 56–70.
- Lorenzoni M, Giannetto D, Carosi A, Dolciami R, Ghetti L, Pompei L. 2015. Age, growth and body condition of big-scale sand smelt *Atherina boyeri* Risso, 1810 inhabiting a freshwater environment: Lake Trasimeno (Italy). *Knowl Manag Aquat Ecosyst* 416, 09.
- Ludovisi A, Gaino E. 2010. Meteorological and water quality changes in Lake Trasimeno (Umbria, Italy) during the last fifty years. *J Limnol* 69: 174–188.
- Maganelli G, Bodon M, Favilli L, Castagnolo L, Giusti F. 1998. Checklist delle species della fauna d'Italia, molluschi terrestri e d'acqua dolce. Errata ed addenda, 1. *Boll Malacol*, Roma 33: 151–156.
- Martel AL, Lauzon-Guay J-S. 2005. Distribution and density of glochidia of the freshwater mussel *Anodonta kennebecensis* on fish hosts in lakes of the temperate rain forest of Vancouver Island. *Can J Zool* 83: 419–431.
- McMahon RF, Bogan AE. 2001. Mollusca: Bivalvia. In Thorp JH, Covich AP eds. *Ecology and classification of North American freshwater invertebrates*. 2nd ed. Academic Press.
- Mearelli M, Lorenzoni M, Mantilacci L. 1990. Il Lago Trasimeno. *Riv Idrabiol* 29: 353–389.
- Mierzejewska K, Kvach Y, Stánczak K, Grabowska J, Wozniak M, Dziekońska-Rynko J, Ovcharenko M. 2014. Parasites of non-native gobies in the Włocławek Reservoir on the lower Vistula River, first comprehensive study in Poland. *Knowl Manag Aquat Ecosyst* 414: 01.
- Miller PJ. 2004. *The Freshwater Fishes of Europe*. Vol. 8/II. Gobiidae 2. Wiebelsheim: Aula Verlag, 477 p.
- Mineeva OV, Mineev AK. 2020. The first data on parasites of the Monkey goby *Neogobius fluviatilis* (Perciformes, Gobiidae) in the Saratov Reservoir. *Russian J Biol Inv* 11: 341–347.
- Mineeva OV, Semenov DY. 2021a. The parasite fauna of the round goby *Neogobius melanostomus* (Perciformes, Gobiidae) in the Kuybyshev reservoir (Middle Volga). *Russian J Biol Inv* 12: 83–93.
- Mineeva OV, Semenov DY. 2021b. First data on parasites of *Neogobius iljini* (Perciformes, Gobiidae) of the middle Volga. *Russian J Biol Inv* 12: 362–372.
- Modesto V, Ilarri M, Souza AT, Lopes-Lima M, Douda K, Clavero M, Sousa R. 2018. Fish and mussels: importance of fish for freshwater mussel conservation. *Fish Fisheries* 19: 244–259.
- Molnár K. 2006. Some remarks on parasitic infections of the invasive *Neogobius* spp. (Pisces) in the Hungarian reaches of the Danube River, with a description of *Goussia szekelyi* sp. n. (Apicomplexa: Eimeriidae). *J Appl Ichthyol* 22: 395–400
- Moore TP, Clearwater SJ. 2021. Non-native fish as glochidial sinks: elucidating disruption pathways for *Echydridella menziesii* recruitment. *Hydrobiologia* 848: 3191–3207.
- Muzzal PM, Pebles CR, Thomas MV. 1995. Parasites of the Round goby, *Neogobius melanostomus*, and Tubenose goby, *Proterorhinus marmoratus* (Perciformes: Gobiidae), from the St. Clair River and Lake St. Clair, Michigan. *J Helminthol Soc Wash* 62: 226–228.
- Nagel K-O. 1985. Glochidien und Fortpflanzungsbiologie von Najaden des Rheins (Bivalvia – Unionidae – Anadontinae). *Mainz Naturw Arch* 5: 163–175.
- Nagel K-O, Castagnolo L. 1991. Fish hosts for the glochidium of *Unio mancus*. *Riv Idrabiol* 30: 2–3.
- Ondračková M, Dávidová M, Blažek R, Gelnar M, Jurajda P. 2009. The interaction between an introduced fish host and local parasite fauna: *Neogobius kessleri* in the middle Danube River. *Parasitol Res* 105: 201–208.
- Ondračková M, Dávidová M, Pečniková M, Blažek R, Gelnar M, Valová Z, Černý J, Jurajda P. 2005. Metazoan parasites of *Neogobius* fishes in the Slovak section of the River Danube. *J Appl Ichthyol* 21: 345–349.
- Ondračková M, Janáč M, Borchering J, Grabowska J, Bartáková V, Jurajda P. 2021. Non-native gobies share predominantly immature parasites with local fish hosts. *J Vert Biol* 70: 21050.
- Ondračková M, Trichkova T, Jurajda P. 2006. Present and historical occurrence of metazoan parasites in *Neogobius kessleri* (Pisces: Gobiidae) in the Bulgarian section of the Danube River. *Acta Zool Bulgar* 58: 401–408.
- Ondračková M, Valová Z, Hudocvá I, Micháľková V, Šimková A, Borchering J, Jurajda P. 2015. Temporal effects on host-parasite associations in four naturalized goby species living in sympatry. *Hydrobiologia* 746: 233–243.
- Paling JE. 1968. A method of estimating the relative volumes of water flowing over the different gills of a freshwater fish. *J Exp Biol* 48: 533–544.
- Panha S. 1992. Infection experiment of the glochidium of a freshwater pearl mussel, *Hyriopsis (Limnoscapha) myersinana* (Lea, 1856). *Venus* 51: 303–314.
- Patzner RA. 2004. Bivalves and their host fishes. *Österr Fischerei* 57: 278–281 [in German with English summary].
- Reuling FH. 1919. Acquired immunity to an animal parasite. *J Infect Dis* 24: 337–346.
- Reshentikov AN. 2013. Spatio-temporal dynamics of the expansion of rotan *Perccottus glenii* from West-Ukrainian centre of

- distribution and consequences for European ecosystems. *Aquatic Inv* 8: 193–206.
- Riccardi N, Froufe E, Bogan AE, Zieritz A, Teixeira A, Vanetti I, Varandas S, Zaccara S, Nagel K-O., Lopes-Lima M. 2020. Phylogeny of European Anodontini (Bivalvia: Unionidae) with a redescription of *Anodonta exulcerata*. *Zool J Linn Soc* 189: 745–761.
- Rogers-Lowery CL, Dimock RV. 2006. Encapsulation of attached ectoparasitic glochidia larvae of freshwater mussels by epithelial tissue on fins of naïve and resistant host fish. *Biol Bull* 210: 51–63.
- Rolbiecki L. 2006. Parasites of the round goby, *Neogobius melanostomus* (Pallas, 1811), an invasive species in the Polish fauna of the Vistula Lagoon ecosystem. *Oceanologia* 48: 545–561.
- Schwalb AN, Cottenie K, Poos MS, Ackerman JD. 2011. Dispersal limitation of unionid mussels and implications for their conservation. *Freshw Biol* 56: 1509–1518.
- Shimizu T, Nagasawa K. 2018. Four species of acanthocephalans parasitic in freshwater gobies *Rhinogobius* ssp. in western and central Japan, with a list of the known parasites of *Rhinogobius* ssp. of Japan (1935–2018). *Bull Hirosh Univ Mus* 10: 37–52.
- Šlapansky L, Jurajda P, Janáč M. 2016. Early life stages of exotic gobiids as new hosts for unionid glochidia. *Freshw Biol* 61: 979–990.
- Taeubert J-E., Gum B, Geist J. 2012. Host-specificity of the endangered thick-shelled river mussel (*Unio crassus*, Philipsson 1788) and implications for conservation. *Aqu Cons Mar Freshw Ecosyst* 22: 36–46.
- Thacker CE. 2009. Phylogeny of Gobioidae and placement within Acanthomorpha, with a new classification and investigation of diversification and character evolution. *Copeia* 2009: 93–104.
- Tremblay MEM, Morris TJ, Ackerman JD. 2016. Loss of reproductive output caused by an invasive species. *R. Soc. Open Sci* 3: 150481.
- Tyutin AV, Verbitsky VB, Verbitskaya TI, Medyantseva EN. 2013. Parasites of aquatic animals in the Upper Volga Basin. *Russian J Biol Inv* 4: 54–59. [Originally published in *Russ Z Biol Inv* 2012 (4): 96–105; in Russian].
- Watters GT. 1994. An annotated bibliography of the reproduction and propagation of the Unionoidea (primarily of North America). Misc Contrib Ser Ohio Biol Surv Ohio State Univ.
- Zieritz A, Aldridge DC. 2011. Sexual, habitat-constrained and parasite-induced dimorphism in the shell of a freshwater mussel (*Anodonta anatina*, Unionidae). *J Morphol* 272: 1365–1375.
- Zhokhov AE, Pugacheva MN, Molodozhnikova NM. 2017. Parasites of the invasive goby *Proterorhinus semilunaris* (Pisces: Gobiidae) in Rybinsk Reservoir and checklist of their parasites of gobiids (Genus *Proterorhinus*) in Eurasia. *Russian J Biol Inv* 8: 18–33. Originally published in *Russ Z Biol Inv* 2016, 4: 24–40 [in Russian].

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