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 gobioïd 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 Gobioïd. 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 canestrini*, 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 Lauen-Guay, 2005).

So far, a recent review has reported 326 fish species suitable for unionids (Modesto et al., 2018). Although gobioïd fishes are adapted to a benthic life style, world-wide only eight of these species (2.5%) were listed as hosts in that review. Gobioïd males are generally territorial, construct and guard...
mussels but also populations of many freshwater species, hosts of the parasitic stage of the mussels, are declining (Modesto 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 goboid species of Ponto-Caspian origin which are exotic in Central and Western Europe (Ondráčková et al., 2005; 2009; Antal et al., 2015; Śląpansky 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 (Śląpansky et al., 2016; Tremblay et al., 2016).

In this study we report (1) the first infestation of Gobioidei with glochidia of freshwater mussels in the wild in Europe outside the Ponto-Caspian region, (2) two Gobionellidae (‘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 goboid fishes as hosts of the parasitic stage of freshwater mussels world-wide.

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).

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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.

Fig. 1. A: 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.
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 Fischer 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 I 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.

<table>
<thead>
<tr>
<th>Table 1. <em>Knipowitschia panizzae</em> and <em>Pomatoschistus canestrinii</em> from Lake Trasimeno; number (N) of specimens with glochidia of <em>Anodonta anatina</em> 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).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
</tr>
<tr>
<td><em>K. panizzae</em></td>
</tr>
<tr>
<td><em>P. canestrinii</em></td>
</tr>
<tr>
<td><em>K. panizzae</em> males</td>
</tr>
<tr>
<td><em>K. panizzae</em> females</td>
</tr>
<tr>
<td><em>P. canestrinii</em> males</td>
</tr>
<tr>
<td><em>P. canestrinii</em> females</td>
</tr>
</tbody>
</table>

2.2 Material

2.2.1 Fishes

Sand gobies of the genera *Knipowitschia* and *Pomatoschistus* have a benthic life-style, are opportunistic feeders predating 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 in females. Fishes were sampled with the permission of Italian state laws on fisheries.

The systematics of the Gobioidei (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 gobid 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 umbal sculpture. The umbal 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.
P. fluviatilis and 37.5% of A. boyeri. From Fig. 7, 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/C0 examined 173 specimens of which 53 were infested (30.6%); T. tinca/C0 examined 140, infested 39 (27.9%); P. fluviatilis/C0 examined 615, 167 infested (27.2%) and A. boyeri/C0 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-infected 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 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...
female *Knipowitschia 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 (*K. panizzae* *n* = 70) vs. 18.6% (*P. canestrinii* *n* = 16) on the trunk and tail. The differences between the percentage frequencies were highly significant (chi-square test: *χ²* = 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 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: *χ²* = 68.87; *p* = 0.001).

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**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 percentage frequencies were highly significant (chi-square test: *χ²* = 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).
to date 33 gobioid species from four families
20 Gobiidae, 7 Gobionellidae (= Oxudercidae) and 2

Table 5. Infestation (mean number) of Knipovitschia 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.

<table>
<thead>
<tr>
<th>Date</th>
<th>Temperature</th>
<th>Infestation</th>
<th>K. panizzae</th>
<th>P. canestrinii</th>
</tr>
</thead>
<tbody>
<tr>
<td>03/03/2022</td>
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<td>2</td>
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<tr>
<td>03/21/2022</td>
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<td>1.6</td>
</tr>
<tr>
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<td>14.7 °C</td>
<td>yes</td>
<td>2.5</td>
<td>1.8</td>
</tr>
<tr>
<td>04/22/2022</td>
<td>16.3 °C</td>
<td>yes</td>
<td>*</td>
<td>1.5</td>
</tr>
<tr>
<td>05/04/2022</td>
<td>24.4 °C</td>
<td>no</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

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 (March 24–May) from 7 °C to 24.4 °C and during the period with attached glochidia (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 (~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 (Günter 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 Percottus glenni 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 (Reshetnikov, 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 gobiids 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; Patzer, 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 gobiod fish infested with glochidia of Unionidae (Bivalvia: Unionida). Gobioidi 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.

<table>
<thead>
<tr>
<th>Species (( n = 33 ))</th>
<th>Countries (( n = 17 ))</th>
<th>Publications (( n = 42 ))</th>
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<td>Australia §</td>
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<td>Ondračková et al. (2021)</td>
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<td></td>
<td>Ukraine</td>
<td>Kvach et al. (2009)</td>
</tr>
<tr>
<td>Goby</td>
<td>USA</td>
<td>Graf (1997)</td>
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<td></td>
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<td>Russia</td>
<td>Mineeva and Semenov (2021a)</td>
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<td></td>
<td>USA §</td>
<td>Muzzal et al. (1995)</td>
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<td>Padogobius bonelli *</td>
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<td>Nagel and Castagnolo (1991)</td>
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<td>Ponticola gorlap ***</td>
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<td>Mineeva and Semenov (2021b)</td>
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<td>Itoh (2019)</td>
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</tr>
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<td>Itoh et al. (2016b)</td>
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<tr>
<td>Gymnogobius urotaenia</td>
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<td>Itoh (2019)</td>
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<td>Hungary</td>
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<td>Italy</td>
<td>this study</td>
</tr>
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<td>Pomatoschistus canestrini</td>
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<td>Gobiomorphus cotidianus</td>
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<td>Hanrahan (2019)</td>
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<td>Gobiomorphus huttoni</td>
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<td>Hanrahan (2019)</td>
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<td>Oxyeleotris marmorata</td>
<td>Thailand §</td>
<td>Panha (1992)</td>
</tr>
<tr>
<td><strong>Odontobutidae (( n = 2 ))</strong></td>
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</table>
series of fish species infected by anodontin glochidia. Those authors were the first to study this issue in Lake Trasimeno (central Italy), but assumed that only one anodontin 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 anodontin mussels. *Sinanodonta woodiana*, the fourth anodontin 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 anodontin mussels are unable to swim actively and sink to the ground after they are released (Lefèvre 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 (Banarascu, 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 (Mierzewska 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., Tuytin 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 gobiid species but a high value in a sympatric goby (e.g., Mierzewska 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 gobiid 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

### Table 6. (continued).

<table>
<thead>
<tr>
<th>Species (n = 33)</th>
<th>Countries (n = 17)</th>
<th>Publications (n = 42)</th>
</tr>
</thead>
<tbody>
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<td><em>Odontobutis obscura</em></td>
<td>Japan §</td>
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</tr>
<tr>
<td><em>Percottus glenii</em></td>
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<td></td>
<td>Latvia</td>
<td>Kirjušina et al. (2014)</td>
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<td></td>
<td>Ukraine</td>
<td>Kvach et al. (2022)</td>
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</table>

*as Padogobius martensis; ** as Proterorhinus marmoratus; or *** as Neogobius iljini; § laboratory experimental infection.
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. canestrini 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. canestrini 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 gobid 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 (Taeubert 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 gobids 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 gobiod 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. canestrini serve as primary hosts or as a sink for glochidia of the freshwater mussel A. anatina in Lake Trasimeno. This may become increasing important because the Ebro P. canestrini populations (Schwalb et al., 2011; Douda et al., 2012; Froufe et al., 2014). Especially anodontin 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 gobiod species (Ondračková et al., 2005; 2009; Antal et al., 2015; Šlapansky et al., 2016; Tremblay et al., 2016). With their benthic life-style gobios occur close to the mussels and also come directly in contact with glochidia on the bottom of aquatic habitats. These introduced gobios 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 gobid species, K. panizzae and P. canestrini, translocated in Lake Trasimeno (Italy), are hosts for the parasitic stage of anodontin 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 anodontins (A. cygnæa 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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Proterorhýnis mármoreus (Ichthyolo) 69: 174


