Knowl. Manag. Aquat. Ecosyst. 2026, 427, 17
© J.A. Dołęga et al., Published by EDP Sciences, 2026
https://doi.org/10.1051/kmae/2026010

www.kmae-journal.org

Knowledge & Management of Aquatic Ecosystems

Journal fully supported by Office français de la biodiversité

RESEARCH PAPER OPEN ACCESS

Baltic sculpin *Cottus microstomus* as a host for larvae of two species belonging to *Unio crassus* complex: *Unio crassus* Philipsson, 1788 and *Unio nanus* Lamarck, 1819

Jacek A. Dołęga[1,*], Adam M. Ćmiel[1], Dariusz Halabowski[2], Katarzyna Zając[1], Monika Mioduchowska[3] and Tadeusz Zając[1]

[1] Institute of Nature Conservation of Polish Academy of Sciences, al. A. Mickiewicza 33, 31-120 Kraków, Poland
[2] University of Lodz, Faculty of Biology and Environmental Protection, Department of Ecology and Vertebrate Zoology, Banacha 12/16, 90-237 Łódź, Poland
[3] University of Gdańsk, Faculty of Biology, Department of Evolutionary Genetics and Biosystematics, Wita Stwosza 59, 80-308 Gdańsk, Poland

Received: 30 October 2025 / Accepted: 2 May 2026

Abstract – Freshwater mussels of the family Unionidae require an obligatory parasitic larval stage on a specific fish host species to complete their ontogeny. This relationship can be highly specific to co-evolving species and geographically variable, although information regarding host suitability remains scarce. Recent advances in molecular and phylogenetic studies of fish have uncovered cryptic host species that have yet to be tested for their role in mussel development. The genus *Cottus*, particularly European bullhead *Cottus gobio*, is known to be a primary host for the threatened thick-shelled river mussel *Unio crassus* complex, a group of cryptic species of large freshwater mussels endemic to Europe. However, the recent revision of the geographical distribution of another *Cottus* species, Baltic sculpin *Cottus microstomus*, raises questions about its suitability as a host for species in *U. crassus* complex. We report that the transformation of mussel larvae from mixed population of *U. crassus* sensu stricto and *U. nanus* into juvenile mussels is successful when *C. microstomus* serves as the host, with an effectiveness comparable to that of the well-established host Eurasian minnow *Phoxinus phoxinus*. The sympatric distribution of *C. microstomus* and *U. crassus* complex is confined to several river drainages in Central and Eastern Europe, areas that are affected by anthropogenic factors, which may pose challenges to their conservation.

Keywords: parasite-host interaction / conservation / artificial infestation / glochidia / fish host

1 Introduction

Large freshwater mussels (Unionidae, hereafter referred to as naiads) require a mandatory parasitic larval phase to complete their ontogeny. Females produce tens of thousands, or even hundreds of thousands, of tiny parasitic larvae (larval length is approximately 0.05–0.45 mm), known as glochidia (Bauer, 2001). Once fully developed, glochidia are expelled by the female through the exhalant siphon into the water, where they passively float and attract a fish host to which they attach, typically to the gills or fins (Aldridge *et al.*, 2023) where they remain encysted for several weeks in the case of *Unio crassus* complex (Lamand *et al.*, 2016; Schneider *et al.*, 2017a) or even up to 11 months in *Margaritifera margaritifera* (Linnaeus, 1758) (Bauer and Vogel, 1987). During this period, the larvae metamorphose and detach from the host as juvenile mussels. This phase is crucial for naiad reproduction, and a key concern is that not all fish species are capable of successfully hosting larvae of every naiad species. Due to co-evolution, each naiad species has a specific set of host species (Bauer and Wachtler, 2001). Moreover, local co-evolution and co-diversification can further specialise this relationship (Bauer and Wachtler, 2001; Douda *et al.*, 2017; Neemuchwala *et al.*, 2023).

From the perspective of both biodiversity and nature conservation, coevolution is particularly important when threatened naiad species depend on sensitive fish hosts, which could pose a risk of cascading extinctions (Modesto *et al.*, 2018). This is the case for species belonging to *U. crassus* complex, including *Unio crassus* Philipsson, 1788 and *Unio nanus* Lamarck, 1819 – a globally endangered species (Lopes-Lima *et al.*, 2024, Lopes-Lima and Prié, 2024) endemic to Europe, and protected by both EU (Council of the European Communities, 1992) and national regulations

*Corresponding author: dolega@iop.krakow.pl

(Journal of Laws of the Republic of Poland, 2016) under the common name of *U. crassus*. One of the best-known hosts for larvae of this species complex is the European bullhead *Cottus gobio* Linnaeus, 1758 (Teleostei: Scorpaeniformes) (Douda *et al.*, 2012; Lamand *et al.*, 2016). This species is actually one of several within the *C. gobio* group (Freyhof *et al.*, 2005), a complex often referred to as *C. gobio* sensu stricto, whose members are locally considered vulnerable (VU) (Witkowski, 2009) and are similarly, legally protected under national (Journal of Laws of the Republic of Poland, 2016) and European law (Council of the European Communities, 1992). To date, this sculpins has been tested several times for its host role in artificial infestation experiments using *U. crassus* complex glochidia under controlled conditions, *e.g.*, by Douda *et al.* (2012), Taeubert *et al.* (2012a, 2012b) and Schneider *et al.* (2017a). The geographical origin of the fish used in these experiments suggests that, in fact, only *C. gobio* sensu stricto has been tested as a host to date. However, both morphological and molecular analyses have revealed that in the Oder, Vistula (Baltic Sea catchment), and Dniester (Black Sea catchment) river drainages, the sculpin species that co-occurs with the *U. crassus* complex is not the European bullhead *C. gobio*, but the Baltic sculpin *Cottus microstomus* Heckel, 1837 (Freyhof *et al.*, 2005; Sideleva *et al.*, 2018). A more recent study by Sideleva *et al.* (2022) shows that *C. microstomus* also occurs in river systems of the eastern Baltic Sea catchment, such as the Neman and Venta rivers (including the Krasnaya River).

Given the cases of strict co-evolution between naiads and their host species, it is crucial to identify the host species of *U. crassus* complex across its entire geographical range, particularly in relation to its threatened status. A key question concerns the role of species of the genus *Cottus*, which may serve as one of the most suitable hosts. In this study, we present the results of an experiment designed to assess whether *C. microstomus* is also a primary host for *U. crassus* complex larvae, as this fish species is found in the larger, eastern portion of *U. crassus* complex range.

2 Material and methods

2.1 Animal collection and acclimation

To test the effectiveness of *C. microstomus* as a host for mussel larvae, we conducted an artificial infestation using *U. crassus* complex glochidia under controlled conditions. As a reference species, we used the Eurasian minnow *Phoxinus phoxinus* (Linnaeus, 1758), which is widely used as a host in artificial infestation experiments and is known for its ability to transform *U. crassus* complex larvae (*e.g.*, Taeubert *et al.*, 2012a, 2012b; Douda *et al.*, 2012, 2014; Schneider *et al.*, 2017a; Geist *et al.*, 2023). Fish and mussel sampling took place in two rivers within the Nida River system, a tributary of the Vistula River in the Baltic Sea catchment. Five gravid females belonging to *U. crassus* complex and 11 specimens of *P. phoxinus* were collected from the Warkocz River near Niestachów, while 11 specimens of *C. microstomus* were collected from the Mierzawa River near Sędowice (both sites are located in the Świętokrzyskie Voivodeship, southern Poland). At the time of the experiment, only the Warkocz River supported a significant population of mussels belonging to *U. crassus* complex, allowing for their sampling without harm to the local population. The choice of site for obtaining *C. microstomus* specimens was deliberate: not only it is located in terra typica of the species, but also, during the recent rediscovery and redescription of Heckel's holotype from year 1837, non-type specimens from this site were confirmed as *C. microstomus* (Sideleva *et al.*, 2018). Specimens of the *U. crassus* complex had never been observed in the Mierzawa River, despite intensive surveys conducted since 1997 (unpublished data), thus ensuring that the collected *C. microstomus* specimens were naive to glochidia. A map showing the locations of animal collection sites and the cited geographic ranges of the discussed species is provided in Figure 1.

Fish were collected using a backpack electrofishing device (IUP-12A, prod. Radet, Poland) and transported to the mussel breeding facility in Krzyżanowice Średnie (Świętokrzyskie Voivodeship, southern Poland) in plastic bags filled with fresh river water and oxygen. At the site of sympatric occurrence of *P. phoxinus* and *U. crassus*, fish were inspected for visible glochidia attached to their fins or gills by gentle opening of the operculum. Only specimens appearing free of larvae were selected for the experiment. Even though *C. microstomus* is the only *Cottus* species inhabiting the Vistula river drainage, some anatomic traits were checked during fish collection: e. g. smooth dorsal profile (no hump behind the head), upper jaw reaching the anterior margin of an eye, lobular genital papilla occurrence in males and distinct narrowness of caudal peduncle (Sideleva *et al.*, 2018). Total length of all fish was measured to the nearest 1 mm. Fish body weight data were not collected, however, considering the different allometry of *C. microstomus* and *P. phoxinus*, body mass of each specimen was calculated using the length–weight relationship equation with coefficients specific to the species or family, as determined by Froese *et al.* (2014). At the breeding facility, fish were placed individually in 13-litre tanks within a recirculating aquaculture system for acclimation. Each tank, equipped with a surface overflow, was constantly supplied with aerated and purified water with controlled parameters. Encystation and transformation of larvae occurred within these tanks. The entire facility was air-conditioned, maintaining a constant temperature. Following previous findings that glochidia viability and metamorphosis of larvae depend on water temperature (*e.g.*, Zimmerman and Neves, 2002; Taeubert *et al.*, 2014; Lamand *et al.*, 2016; Benedict *et al.*, 2021), and that mussel reproduction is adapted to local thermal conditions (Schneider *et al.*, 2017b), we maintained a temperature of 16°C. This approximated natural environmental conditions while allowing efficient larval transformation and minimizing thermal stress to animals. Fish acclimation lasted for eight days.

Adult mussels (age class 5+) were located while wading in the Warkocz River, hand-collected, sexed, and inspected for fully developed, "snapping" glochidia using marsupial puncture, as described by Zając and Zając (2021). Five gravid females of *U. crassus* were transported in a box with fresh, aerated water and ice to the breeding facility, where they were placed in a 96-litre aerated tank. The tank was checked daily to monitor whether glochidia were released into the water, and whether the gentle opening of the valves in each specimen revealed any changes in the marsupial fill.

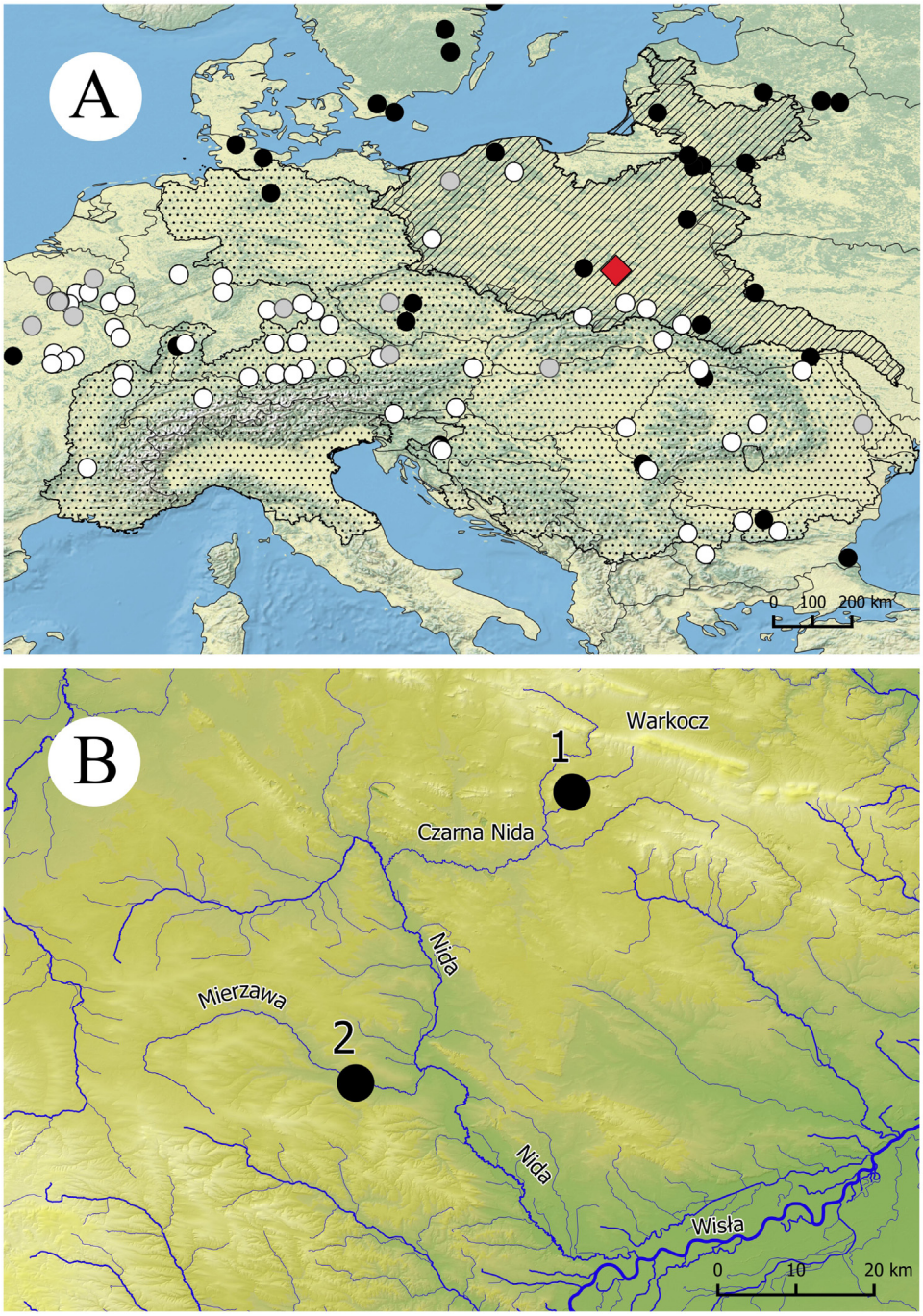

Fig. 1. A map of geographical ranges of discussed species and the study location; A) range of occurrence of *C. microstomus*: a polygon with hatched fill -spatial data was provided by IUCN (MfN Berlin, 2021b) and updated by authors to include drainages of Neman and Venta, as no available spatial data for Krasnaya river drainage was found; and *C. gobio*: a polygon with dotted fill -spatial data provided by IUCN: (MfN Berlin, 2021a); points indicate exact sites of collection of specimens used in the study by Lopes-Lima *et al.* (2024) to assess *U. crassus* complex genetic divergence (limited to the map range): black points - *U. crassus*, white points – *U. nanus*, grey points – sites of sympatric occurrence of both species; the red diamond indicates the area of this study; B) the sites of collection of animals in this study: 1- *P. Phoxinus* specimens used during artificial infestation, *U. crassus* complex specimens used in artificial infestation, *U. crassus* complex individuals used for genetic reassessment of the population inhabiting the river Warkocz; 2- *C. microstomus* specimens used during artificial infestation.

2.2 Infestation of fish and juvenile mussel collection

Once visible conglutinates of glochidia were observed in the tank and it was ensured that the females had emptied their marsupia, the mussels were removed from the tank. The contents were gently mixed, and all fish were simultaneously placed into the glochidia suspension to ensure a uniform infestation rate across both fish species. The fish were exposed

to glochidia for 20 min. During this period, the suspension tank was continuously aerated and hand-stirred to prevent glochidia sedimentation and to stimulate fish movement. Following the exposure, each fish was carefully returned to its individual 13-litre tank within the recirculating aquaculture system. At this stage, each tank was equipped with a net (mesh size 2 mm), which was fixed horizontally approximately 3 cm above the tank bottom, to prevent the fish from foraging on juvenile mussels detaching from their gills and sinking to the bottom. Juvenile mussels were collected every 3 to 4 days. On each occasion, the individual 13-litre tank was removed from the recirculating aquaculture system, and the fish were gently transferred using a fine mesh net to a temporary holding tank. The contents of the 13-litre tank were then flushed several times through a planktonic net (mesh size 0.01 mm), which led to a small tank. The contents of this tank were subsequently passed through a sieve (mesh size 0.15 mm), and the material retained in the sieve was transferred onto a petri dish. Transformed and viable juveniles were counted using a binocular magnifier (Delta Optical, SZ-450B). After the procedure, the tank was refilled with water, and all components were restored to their original configuration to allow the procedure to be repeated.

2.3 Statistical analyses

Statistical analyses were conducted using IBM SPSS Statistics. In order to investigate differences between glochidia transformation patterns in consecutive controls between both species of fish, a generalized mixed model with repeated measures (Log-Poisson) was constructed; dependent variable: number of transformed and vital juvenile mussels at given time post infestation of fish, grouping variable: host species, continuous predictor: fish weight, random effect: fish specimen ID. The random effect was used to account for potential differences between fish specimens, which may result from earlier acquisition of immunity to glochidia, or individual condition, infestation intensity or any other non-controlled factor resulting from testing a random group of wild fish. Differences between specific levels of variables were tested using pairwise contrasts post-hoc tests and are presented in the Supplementary Materials. Since juvenile mussels were collected every 3 or 4 days, in the descriptive statistics we refer to the "potential excystation period" and "potential parasitism period", which reflects respectively: the maximum possible time between first and last excystment of juvenile mussel from a host, and maximum possible time from infestation to excystment of the last juvenile, both determined for each host fish specimen. The mean parasitism duration was calculated based on the time each individual glochidium spent attached to the host, from initial attachment until collection of transformed juvenile mussel - these data were then pooled for each host species.

2.4 Molecular taxonomic verification of the *U. crassus* complex species from the Warkocz River

While much of the existing literature and legal frameworks refer to "*U. crassus*" as a single species, recent findings suggest that it should be regarded as a species complex. In their recent study, Lopes-Lima *et al.* (2024) identified 12 molecularly distinct species within what was previously recognised as "*U. crassus*", two of which are likely present in our study area: *U. crassus* sensu stricto and *U. nanus*. To reassess the taxonomic identity of *Unio* specimens from the Warkocz River under the revised taxonomic framework of the *U. crassus* complex, we reanalyzed mitochondrial cytochrome c oxidase subunit I (COI) barcode sequences previously obtained by our team (Kilikowska *et al.*, 2020). At the time of the original study, all specimens were assigned to *U. crassus* according to the taxonomy then in use.

Following the integrative taxonomic revision by Lopes-Lima *et al.* (2024), which recognized 12 geographically structured species within the *U. crassus* complex, we conducted a comparative molecular analysis to determine the species that inhabited the Warkocz River. Two previously published COI sequences represented two haplotypes from the Warkocz population (GenBank accession numbers KJ525912 and KJ525917; Kilikowska *et al.*, 2020) were analyzed together with one representative COI sequence for each of the 12 species described by Lopes-Lima *et al.* (2021, 2024). All sequences have been downloaded from GenBank (accession numbers listed in supplementary materials, Tab. S3).

The COI sequences were aligned using the MUSCLE algorithm implemented in MEGA X v10.0.3 (Kumar *et al.*, 2018) with default settings. All barcode sequences were trimmed to equal length, resulting in a final alignment of 614 bp. The alignment contained no gaps or ambiguous sites. To confirm the absence of pseudogenes, sequences were translated into amino acids using the invertebrate mitochondrial genetic code, and no stop codons were detected.

Uncorrected pairwise genetic distances (p-distances) were calculated in MEGA X. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated (complete deletion option). The resulting distance matrix was used to assess genetic divergence between haplotypes of *U. crassus* species complex from Warkocz River and reference sequences representing the newly delimited species within the *U. crassus* complex. All pairwise p-distance values are provided in Tab. S3 in supplementary materials.

3 Results

3.1 Artificial infestation

The Baltic sculpin *C. microstomus* demonstrated the ability to successfully transform glochidia released by females originating from a mixed population of *U. crassus* sensu stricto and *U. nanus* into viable juvenile mussels. Observations on equal groups of fish kept under identical conditions revealed a higher efficiency of larval transformation in *P. phoxinus* compared to *C. microstomus*. The number of successfully transformed juveniles per specimen in *C. microstomus* ranged from 44 to 134, mean = 82.2, SD = 27.27), while in *P. phoxinus* it ranged from 26 to 211 (mean = 114.1, SD = 63.67). In total, *C. microstomus* produced 904 juvenile mussels, while *P. phoxinus* produced 1255 juvenile mussels. The results of constructed GLZ (log-poisson) model showed that the difference in number of juveniles collected during the whole excystation period between species was not statistically

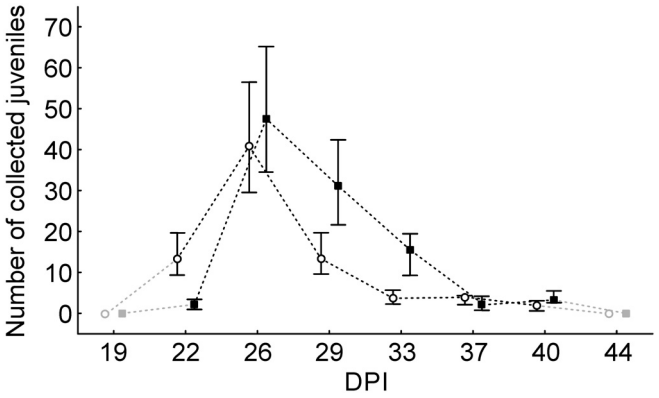

Fig. 2. The differences in number of juvenile mussels in consecutive counts between studied species: *C. microstomus* (empty circles) and *P. phoxinus* (solid squares); points: predicted average, whiskers: 95% confidence interval, DPI (Days post-infestation): days from exposition of fish to *U. crassus* glochidia; gray markers indicate last and first control in which no juveniles were detected (estimated start and finish point).

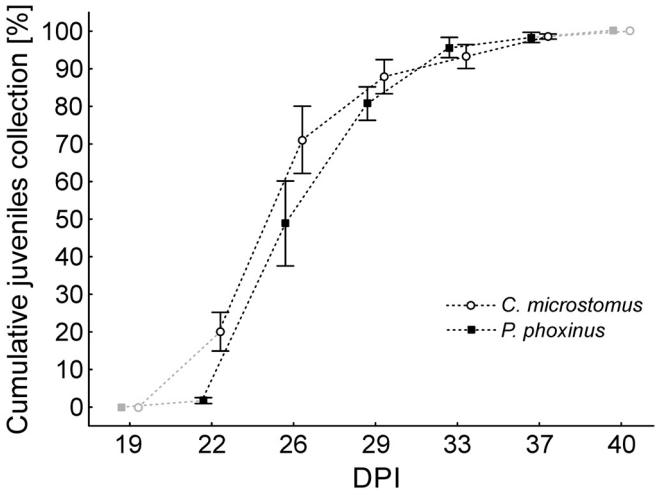

Fig. 3. Cumulative percentage of juvenile mussels collected from fish in consecutive steps: juveniles collected from *C. microstomus* (empty circles) and *P. phoxinus* (solid squares); points: predicted average, whiskers: 95% confidence interval, DPI (Days post-infestation): days from exposition of fish to *U. crassus* glochidia; grey markers indicate last and first control in which no juveniles were detected (estimated start and finish point).

significant ($F(1, 119) = 0.46$, $p = 0.501$) and also, that it was not significantly influenced by the fish weight ($F(1, 119) = 0.99$, $p = 0.323$). The number of juvenile mussels (pooled across both host species) differed significantly between most of the collections dates ($F = 246.2$, $df1 = 5$, $df2 = 119$, $p < 0.001$) indicating, that juvenile mussels release showed dynamic changes over the collection dates; there was no statistically significant difference in the number of juveniles collected on the 22nd and 33rd and on the 37th and 40th days post-infestation (see Tab. S1 in the Supplementary Materials).

The model also indicated a statistically significant interaction between host species and collection date ($F = 37.9$, $df1 = 5$, $df2 = 119$, $p < 0.001$), showing that the number of successfully metamorphosed juvenile mussels varied between species in a collection-specific manner (Fig. 2).

A random effect was also found to be statistically significant, although it explained less than 23% of the variation in the data ($\eta^2 = 0.223$, SE = 0.088, $p = 0.012$). To further illustrate the data, a figure displaying the mean percentage of juveniles collected at each time point has been included, which visually depicts the differences in excystation patterns for both host species in a cumulative diagram (Fig. 3).

Both host fish species exhibited similar maximum time frames for larval transformation. The first transformed, viable juveniles of *U. crassus* were collected from both species on the 22nd day post-infestation, and the last juvenile mussels detached from the fish by the 40th day after infestation, with a pronounced peak in juvenile collection on the 26th day (Fig. 2). No juveniles were collected during the control checks conducted on the 19th and 44th days post-infestation. The potential excystation period lasted from 18 to 21 days (mean = 20.45, SD = 1.21) in *C. microstomus* and from 11 to 21 days (mean = 18.36, SD = 3.88) in *P. phoxinus*. The potential parasitism period lasted from 37 to 40 days (mean = 39.45, SD = 1.21) in *C. microstomus* and from 33 to 40 days (mean = 37.91, SD = 2.77) in *P. phoxinus*. Mean duration of successful parasitism was 26.93 days (SD = 3.9 days) in *C. microstomus* and 28.39 days (SD = 3.5 days) in *P. phoxinus*.

The size of 11 specimens of *P. phoxinus* ranged between 48 mm and 90 mm (median: 72, SD = 11.51), while the size of 11 specimens of *C. microstomus* ranged between 63 mm and 127 mm (median: 82, SD = 17.56). The calculated weigth of 11 specimens of *P. phoxinus* ranged between 0.95 g and 6.86 g (median: 3.41, SD = 1.68), while for 11 specimens of *C. microstomus* it ranged between 1.94 g and 17.66 g (median: 4.45, SD = 4.36).

3.2 Mitochondrial divergence in the Warkocz River population of the *U. crassus* species complex

The uncorrected p-distances within the *U. crassus* species complex revealed substantial genetic differentiation between the two haplotypes obtained from the Warkocz River population (KJ525912 and KJ525917, Kilikowska *et al.*, 2020), which differed by 2.8%. The first haplotype (KJ525912) showed the lowest divergence from *U. nanus* ($p = 0.010$), followed by *U. vicarius* (0.023) and *U. bruguierianus*, *U. carneus*, and *U. crassus* sensu stricto (each 0.028). In contrast, the second haplotype (KJ525917) was nearly identical to *U. crassus* sensu stricto ($p = 0.003$) and showed low divergence from *U. gontierii* (0.010), while distances to other taxa were markedly higher (≥0.023). Both Warkocz haplotypes exhibited substantially greater divergence from more distantly related taxa such as *U. sesirmensis* (0.057 - 0.062) and *U. tumidiformis* (0.078 - 0.083). Overall, the observed pattern indicates pronounced mitochondrial heterogeneity within the Warkocz River population, with one haplotype closely affiliated with *U. crassus* sensu stricto and the other showing the strongest affinity to *U. nanus*, indicating the presence of two coexisting species from the *U. crassus* species complex in the Warkocz River: *U. crassus* sensu stricto and *U. nanus*.

4 Discussion

Unionid mussels (Unionida) represent one of the classic examples of tight coevolution, in which one group of organisms has effectively made its reproductive success dependent on another group (host fish). Consequently, the prospects for their conservation are closely linked to the availability of suitable host fish. In some studies on host suitability, binary tests (presence/absence) are performed to determine whether a given fish species is capable of transforming glochidia or not (reviewed in Lopes-Lima *et al.*, 2017). However, as recently demonstrated by Dołęga *et al.* (2025), the influence of fish species that reject larvae may also be indirect and depend on the probability of larval interception by dead-end hosts, as well as on the abundance of both fish and mussel species. In turn, Douda *et al.* (2014) identified differences in the ability of *U. crassus* to infest particular host fish species between nearby and recently isolated mussel populations, indicating a significant effect of even slight genetic differences among populations.

In both groups of animals, substantial progress has recently been made in phylogenetic research. Assuming after Douda *et al.* (2014) that phylogenetic differences, often not detectable without detailed genetic analyses, play an important role in shaping interactions between mussels and fish, studies incorporating recent taxonomic advances gain particular importance. Such an approach may explain many of the observed differences in infestation success and transformation efficiency among mussel and fish populations.

In the case of mussels, species identification can be challenging due to the high phenotypic plasticity of shell morphology (*e.g.*, Zając *et al.*, 2018, Egg *et al.*, 2025) and the lack of clear diagnostic morphological traits distinguishing recently described species (Lopes-Lima *et al.*, 2024). Furthermore, the possibility of hybridization demonstrated in our study further complicates species identification based solely on morphology. In fish, ongoing advances in phylogenetic analyses are leading to taxonomic revisions, revealing previously unrecognized diversity that may translate into differences in host suitability and transformation efficiency. There is therefore a need to effectively reinitiate research programmes aimed at assessing the susceptibility of different fish lineages to glochidial infestation and transformation, incorporating recent advances in genetics.

In the present study, we tested a mussel population whose precise species identity was uncertain, as the taxonomic revision by Lopes-Lima *et al.* (2024) had not yet been available at the time of sampling. Retrospectively, we can assign these individuals to two lineages (*U. crassus* and *U. nanus*) dominating in this part of Europe, which, according to our data, can hybridize. At the same time, following the recognition of a new potential fish host species, *C. microstomus*, we demonstrated successful transformation of larvae attributed to *U. crassus* in the traditional sense. This finding is of considerable importance, as all three taxa involved (*U. crassus*, *U. nanus*, and *C. microstomus*) are widespread in this part of Europe, and conservation strategies for the *U. crassus* complex must necessarily account for their interactions. Although our experiment does not allow us to determine precisely which mussel species is responsible for successful transformation, we can state with confidence that conservation of *C. microstomus* may contribute to the persistence of the co-occurring members of the *U. crassus* complex: *U. crassus* and *U. nanus*.

The results of the statistical model indicate, that overall transformation of *U. crassus/nanus* larvae on *C. microstomus* did not differ significantly from that observed on the well-established host species *P. phoxinus*. However, the rapid development of larvae on *C. microstomus*, with a statistically significant advancement in juvenile excystation (juvenile collection on 22nd day post infestation, Fig. 2), suggests that the ontogenetic transition is not hindered in this host, even in comparison to *P. phoxinus*. Although the overall time frames of juvenile mussel excystation and parasitism duration are similar in both hosts, a more detailed view on excystment timing—at the level of host individuals and even individual glochidia—reveals differences in the temporal patterns of transformation between the two host species. This is consistent with observations reported by other authors (Tab. S4, Supplementary Materials), where both transformation success and temporal parameters are usually lower for *Cottus* sp. than for *P. phoxinus*. The generally shorter duration of parasitism is suggested to play a minimal role for *U. crassus* complex larvae, as according to Taeubert *et al.* (2012b), glochidia of *U. crassus* doesn't grow significantly during encystation, confining benefits of parasitic stage of mussels life to dispersal rather than individual condition.

Given the diversity of methodologies and study objectives in the literature, direct comparisons remain difficult, as already noted by Douda (2013). In study by Douda *et al.* (2012) *C. gobio* and *P. phoxinus* were identified as primary hosts, each exhibiting high transformation rates (>50% of attached larvae). *C. gobio* hosted on average 365 glochidia per fish, with 57.3% successfully transforming c. 209 juveniles per fish, whereas in our study the mean number of juveniles detached from *C. microstomus* was 82.2 (SD = 27.27), however, initial infestation levels were not quantified in our study. In contrast, Taeubert *et al.* (2012a) reported that *C. gobio* lost approximately 90% of glochidia, classifying it as a relatively unsuitable host. They also noted variation in host suitability among populations of *C. gobio*, possibly reflecting obscured lineage-specific responses within the *C. gobio–U. crassus* interaction.

According to *in situ* observations by Schneider *et al.* (2018), during the reproductive period of *U. crassus*, *C. gobio* carried fewer glochidia per individual than *P. phoxinus*, despite both species being simultaneously active and exposed. This may suggest that *P. phoxinus* is a more effective host *in situ* possibly due to microhabitat preferences and behavioural traits that increase susceptibility to infestation (Ćmiel *et al.*, 2018). A more detailed comparison of literature data with the results of the present study is provided in Table S4 of the supplementary materials.

In the Warkocz River population, Mioduchowska *et al.* (2016) identified individuals exhibiting discordant mitochondrial lineages: F*cox1* haplotypes corresponded to *U. crassus*, whereas M*cox1* haplotypes were assigned to *U. nanus*. These lineages correspond to the northern and southern clades described in Poland and were later recognized as distinct species within the *U. crassus* complex by Lopes-Lima *et al.* (2024). This pattern provides clear evidence of interspecific

gene flow and hybridization within the complex. Such hybrids may result from secondary contact following historical divergence, but they may also indicate that speciation has proceeded under conditions of incomplete reproductive isolation, allowing ongoing genetic exchange. The coexistence of mitochondrial genomes from two nominal species within single individuals raises important questions regarding species boundaries and the applicability of traditional species concepts in freshwater mussels.

In this study, we demonstrated that *C. microstomus* is an effective host species for one or both species of the *U. crassus* complex occurring in Poland (*U. crassus and/or U. nanus*). Despite taxonomic differences and geographical separation from other *Cottus* species used in previous studies, *C. microstomus* supports successful infestation and transformation of glochidia of one or both of these two representatives of the *U. crassus* complex. Both *C. microstomus* and the *U. crassus* complex have wide distributions across diverse river habitats, yet both are highly susceptible to environmental changes, making them prone to co-extinction - a process documented for associated fish–mussel systems (Modesto *et al.*, 2018). Our findings demonstrate that uncovering cryptic host–parasite relationships is not only of academic interest but constitutes a prerequisite for effective conservation planning.

Acknowledgments

The authors would like to thank Mr. Michał Bień, the Director of Polish Angling Association in Kielce, for help in obtaining specimens of *C. microstomus* from the river Mierzawa. We are also very grateful to dr Michał Nowak, for turning our focus on taxonomy of European *Cottus* species. We would also like to sincerely thank the anonymous reviewers for their valuable comments, which contributed to the improvement of the manuscript. The study was conducted partially within the framework of LIFE+ project "Life4Delta" (LIFE17-NAT_PL_000018) and in part by statutory funds of the Institute of Nature Conservation, Polish Academy of Sciences. All procedures leading to creation of present manuscript were carried out in accordance with applicable ethics and current legal requirements in force in Republic of Poland.

Supplementary material

Tab. S1. p-values of pairwise contrasts post-hoc test for performed repeated Measures generalized Linear Model (log, Poisson) of the total number of juveniles collected in each control (aggregated for both species); DPI – days post infestation of fish.

Tab. S2. Results of pairwise contrasts post-hoc test for performed repeated Measures generalized Linear Model (log, Poisson) of the differences in number of juveniles collected in given control between both host species (*P. phoxinus* and *C. microstomus*); DPI – days post infestation of fish.

Tab. S3. Estimates of evolutionary divergence between COI sequences of *U. crassus* complex; references: A - Kilikowska et al. 2020, B - Lopes-Lima et al. 2021, C - Lopes-Lima et al. 2024; the referenced citations are included in the main text of the manuscript, in the "References" section.

Tab. S4. Reference to cited literature on host suitability for larvae of *U. crassus* complex (comparisons between *C. gobio* and *P. phoxinus*); A – results obtained from artificial infestation, N – results obtained from natural infestation; DPI – days post infestation; *- only data from a trial performed concurrently with *C. gobio* were considered in the table; where possible, data from the present study were presented in the same format as in the cited studies for comparison; the referenced citations are included in the main text of the manuscript, in the "Literature" section.

The Supplementary Material is available at https://www.kmae-journal.org/10.1051/kmae/2026010/olm.

References

Aldridge DC, Brian JI, Ćmiel A, *et al.* 2023. Fishing for Hosts: Larval spurting by the endangered thick-shelled river russel, *Unio crassus*. *Ecology* e4026. https://doi.org/10.1002/ecy.4026.

Bauer G, Wächtler K. 2001. Ecology and evolution of the naiads. In: Bauer G, Wächtler K. eds. Ecology and Evolution of the Freshwater Mussels Unionoida. *Ecol. Stud.*, vol 145. Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-642-56869-5_22.

Bauer G. 2001. Framework and driving forces for the evolution of naiad life histories. In: Bauer G, Wächtler K, eds. Ecology and Evolution of the Freshwater Mussels Unionoida. *Ecol. Stud.*, vol 145. Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-642-56869-5_13.

Bauer G, Vogel C. 1987. The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.). I. Host response to glochidiosis. *Arch Hydrobiol* 76: 393–402.

Benedict A, Geist J. 2021. Effects of water temperature on glochidium viability of *Unio crassus* and *Sinanodonta woodiana*: implications for conservation, management and captive breeding. *J Mollus Stud* 87. https://doi.org/10.1093/mollus/eyab011.

Council of the European Communities. 1992. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. *Official Journal of the European Union* L206: 7–50.

Ćmiel A, Zając K, Lipińska A, Zając T. 2018. Glochidial infestation of fish by the endangered thick- shelled river mussel *Unio crassus*. *Aquat Conserv: Mar Freshw Ecosyst* 28: 535–544.

Dołęga J, Zając TA, Ćmiel A. 2025. Evidence for dilution effect by *Gobio gobio*, a dead-end host in the *Unio crassus*–Cyprinidae coevolutionary system. *Sci Rep* 16: 2832.

dos Santos RCL, Sousa R, Lopes-Lima M, Teixeira A. 2024. The role of adaptive resistance in a widespread freshwater mussel species. *Aquat Conserv: Mar Freshw Ecosyst* 34: e4117.

Douda K, Horkỹ P, Bílỹ M. 2012. Host limitation of the thick-shelled river mussel: identifying the threats to declining affiliate species. *Anim Conserv* 15: 536–544.

Douda K, Liu H-Z, Yu D, *et al*. 2017. The role of local adaptation in shaping fish–mussel coevolution. *Freshw Biol* 62: 1858–1868.

Douda K, Sell J, Kubíková-Peláková L, Horký P, Kaczmarczyk A, Mioduchowska M. 2014. Host compatibility as a critical factor in management unit recognition: population-level differences in mussel–fish relationships. *J Appl Ecol* 51: 1085–1095.

Douda K. 2013. Quantifying the host relationships of endangered freshwater mussels – *Unio crassus* demonstrates a need for unifying methodologies. *Biol Conserv.* https://doi.org/10.1016/j.biocon.2012.12.030.

Egg S, Kuehn R, Geist J. 2025. Environmental niche differentiation and past colonization dynamics of two European freshwater mussels (*Unio crassus* and *Unio nanus*). *Diversity* 17: 779.

Freyhof J, Kottelat M, Nolte A. 2005. Taxonomic diversity of European *Cottus* with description of eight new species (Teleostei: Cottidae). *Ichthyol Explor Freshw* 16: 107–172.

Froese R, Thorson JT, Reyes RB, Jr. 2014. A Bayesian approach for estimating length-weight relationships in fishes. *J Appl Ichthyol* 30: 78–85.

Geist J, Thielen F, Lavictoire L, *et al*. 2023. Captive breeding of European freshwater mussels as a conservation tool: a review. *Aquat Conserv: Mar Freshw Ecosyst* 33: 1321–1359.

Jansen W, Bauer G, Zahner-Meike E. 2001. Glochidial mortality in freshwater mussels. In: Bauer G, Wachtler K. eds. Ecology and Evolution of the Freshwater Mussels Unionoida, Heidelberg: Springer-Verlag Berlin Heidelberg, pp. 185–211.

Journal of Laws of the Republic of Poland. 2016. Regulation of the Minister of the Environment of 16 December 2016 on the protection of animal species. *J Laws* 2016: item 2183.

Kilikowska A, Mioduchowska M, Wysocka A, *et al.* 2020. The patterns and puzzles of genetic diversity of endangered freshwater mussel *Unio crassus* Philipsson, 1788 populations from Vistula and Neman drainages (Eastern Central Europe). *Life* 10: 119.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 35: 1547–1549.

Lamand F, Roche K, Beisel JN. 2016. Glochidial infestation by the endangered mollusc *Unio crassus* in rivers of north-eastern France: *Phoxinus phoxinus* and *Cottus gobio* as primary fish hosts. *Aquat Conserv: Mar Freshw Ecosyst* 26: 445–455.

Lopes-Lima M, Geist J, Egg S, *et al.* 2024. Integrative phylogenetic, phylogeographic and morphological characterisation of the *Unio crassus* species complex reveals cryptic diversity with important conservation implications. *Mol Phylogenet Evol*. 195: 108046.

Lopes-Lima M, Gürlek ME, Kebapçı Ü, *et al.* 2021. Diversity, biogeography, evolutionary relationships, and conservation of Eastern Mediterranean freshwater mussels (Bivalvia: Unionidae). *Mol Phylogenet Evol* 163: 107261.

Lopes-Lima M, Prié V, Österling M, Zając TA. 2024. *Unio crassus*. *The IUCN Red List of Threatened Species* 2024: e. T210291828A215467836. https://www.iucnredlist.org/species/210291828/215467836 Accessed on 26 February 2026.

Lopes-Lima M, Prié V. 2024. *Unio nanus*. *The IUCN Red List of Threatened Species* 2024: e.T215447310A215447388. https://www.iucnredlist.org/species/215447310/215447388 Accessed on 26 February 2026.

Lopes-Lima M, Sousa R, Geist J, *et al.* 2017. Conservation status of freshwater mussels in Europe: state of the art and future challenges. *Biol Rev* 92: 572–607.

MfN Berlin. 2021. *Cottus gobio. The IUCN Red List of Threatened Species*. Version 2025-2. https://www.iucnredlist.org. Downloaded on 10 March 2026.

MfN Berlin. 2021. *Cottus microstomus. The IUCN Red List of Threatened Species*. Version 2025-2. https://www.iucnredlist.org. Downloaded on 10 March 2026.

Mioduchowska M, Kaczmarczyk A, Zając K, Zając T, Sell J. 2016. Gender-associated mitochondrial DNA heteroplasmy in somatic tissues of the endangered freshwater mussel *Unio crassus* (Bivalvia: Unionidae): implications for sex identification and phylogeographical studies. *J Exp Zool Part A Ecol Genet Physiol* 325: 610–625.

Modesto V, Ilarri MI, Souza AT, *et al*. 2018. Fish and mussels: importance of fish for freshwater mussel conservation. *Fish Fish* 19: 244–259.

Neemuchwala S, Johnson N, Pfeiffer J, *et al.* 2023. Coevolution with host fishes shapes parasitic life histories in a group of freshwater mussels (Unionidae: Quadrulini). *Bull Soc Syst Biol* 2: 1–25.

Schneider LD, Nilsson PA, Österling EM. 2017a. Evaluating temperature- and host-dependent reproduction in the parasitic freshwater mussel *Unio crassus*. *Hydrobiologia* 810: 283–293.

Schneider LD, Nilsson PA, Höjesjö J, Österling EM. 2017b. Local adaptation studies and conservation: parasite–host interactions between the endangered freshwater mussel *Unio crassus* and its host fish. *Aquat Conserv: Mar Freshw Ecosyst* 27: 1261–1269.

Schneider LD, Nilsson PA, Osterling EM. 2018. Evaluating temperature- and host-dependent reproductionin the parasitic freshwater mussel *Unio crassus*. *Hydrobiologia* 810: 283–293.

Schneider LD, Nilsson PA, Höjesjö J, *et al*. 2018. Effects of mussel and host fish density on reproduction potential of a threatened unionoid mussel: prioritization of conservation locations in management trade-offs. *Biodivers Conserv* 28: 259–273.

Sideleva V, Kesminas V, Zhidkov Z. 2022. A new species of the genus *Cottus* (Scorpaeniformes, Cottidae) from the Baltic Sea Basin and its phylogenetic placement. *Eur J Taxon* 834: 38–57.

Sideleva V, Naseka AM, Nowak M, Palandačić A. 2018. The finding of holotype and redescription of *Cottus microstomus* Heckel 1837 (Cottidae). *Ichthyol Res* 66: 249–257.

Taeubert JE, El-Nobi G, Geist J. 2014. Effects of water temperature on the larval parasitic stage of the thick-shelled river mussel (*Unio crassus*). *Aquat Conserv: Mar Freshw Ecosyst* 24. https://doi.org/10.1002/aqc.2385.

Taeubert JE, Gum B, Geist J. 2012a. Host-specificity of the endangered thick-shelled river mussel (*Unio crassus*, Philipsson 1788) and implications for conservation. *Aquat Conserv: Mar Freshw Ecosyst* 22: 36–46.

Taeubert JE, Martinez AMP, Gum B, Geist J. 2012b. The relationship between endangered thick-shelled river mussel (*Unio crassus*) and its host fishes. *Biol Conserv* 155: 94–103.

Witkowski A, Kotusz J, Przybylski M. 2009. The degree of threat to the freshwater ichthyofauna of Poland: Red list of fishes and lampreys—Situation in 2009. *Chrońmy Przyr Ojcz* 65: 33–52.

Zając K, Zając T, Ćmiel A. 2018. What can we infer from the shell dimensions of the thick-shelled river mussel *Unio crassus*. *Hydrobiologia* 810: 415–431.

Zając K, Zając T. 2021. Seasonal patterns in the developmental rate of glochidia in the endangered thick-shelled river mussel, *Unio crassus* Philipsson, 1788. *Hydrobiologia* 848: 3077–3091.

Zimmerman LL, Neves RJ. 2002. Effects of temperature on duration of viability for glochidia of freshwater mussels (Bivalvia: Unionidae). *Am Malacol Bull* 17: 31–35.

Cite this article as: Dołęga JA, Ćmiel AM, Halabowski D, Zając K, Mioduchowska M, Zając T. 2026. Baltic sculpin *Cottus microstomus* as a host for larvae of two species belonging to *Unio crassus* complex: *Unio crassus* Philipsson, 1788 and *Unio nanus* Lamarck, 1819. *Knowl. Manag. Aquat. Ecosyst.*, 427, 17. https://doi.org/10.1051/kmae/2026010