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Research Article

Distribution of parasitic larvae of non-native *Sinanodonta woodiana* on fish hosts across an invasion gradient

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Abstract

The Chinese pond mussel (*Sinanodonta woodiana*) is a highly invasive freshwater bivalve, recognised for its broad host range and ecological plasticity. Its obligate parasitic larvae (glochidia) must attach to fish hosts to complete development, potentially disrupting native host-parasite interactions. We investigated patterns of natural infection by glochidia of *S. woodiana* and native unionid species across six sites in Poland that differ in habitat type and invasion history. Using whole-body dissections of 601 wild fish belonging to eight species, we quantified glochidia prevalence, intensity of infection and attachment site preferences. Glochidia of *S. woodiana* were widespread and present on all fish species, with the greatest loads on *Carassius gibelio* and *Rhodeus amarus*. In contrast, native mussels (primarily *Unio* spp.) infected only a subset of hosts and at lower prevalence. Habitat type strongly influenced parasite loads, with higher infection levels observed in lentic compared to lotic habitats. Glochidia of *S. woodiana* attached both to gills and fins, whereas native unionids predominantly targeted the gills. This spatial disparity may reflect differences in infection strategies or host immune responses. Although previous studies have shown that prior infection by *S. woodiana* can reduce the success of subsequent native mussel infections, our study found simultaneous infections and no significant association between native and invasive glochidia under natural conditions. Our findings underscore the generalist nature and potential ecological dominance of *S. woodiana*, while highlighting risks posed to native mussel reproduction. Understanding these interactions is crucial for developing effective conservation strategies for native unionid populations in freshwater ecosystems under pressure from biological invasions.

Key words: Biological invasion, freshwater mussels, glochidia load, host infection, host-parasite interactions, sympatry gradient

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Introduction

Invasive species are significant drivers of ecological disruption, capable of altering trophic interactions, displacing native taxa and restructuring host-parasite dynamics (Shine 2010; Lagrue and Poulin 2015). In freshwater ecosystems, these effects can be particularly pronounced due to the high connectivity of habitats and the sensitivity of native symbioses (Bell et al. 2024). Introduced species

* Deceased on 26 August 2023.

may act as novel hosts or parasites, modulate immune responses or trigger spill-over and spill-back processes, reshaping parasite transmission pathways and community stability (Santicchia et al. 2020).

Unionid mussels seem particularly vulnerable to such disruption, given their obligate parasitic larval stage, which makes them highly dependent on specific fish hosts. Unionids reproduce by internal fertilisation: sperm released by males is taken in by females through the inhalant siphon and embryos develop within specialised gill chambers known as marsupia (Bauer and Wächtler 2001). The resulting larvae, known as glochidia, must attach to the gills, fins or skin of a suitable host fish to complete metamorphosis into juvenile mussels (Modesto et al. 2018); without successful attachment, glochidia perish. Host specificity varies widely amongst unionid species, from generalists capable of infesting many fish taxa to specialists reliant on a narrow range of hosts (Douda et al. 2012; Lopes-Lima et al. 2017). Due to this dependency, unionids are particularly sensitive to alterations in fish community composition caused by habitat degradation or biological invasions (Haag 2012; Lopes-Lima et al. 2017). While most unionid mussels suffer population declines, others are able to expand their range.

Amongst freshwater mussels, the Chinese pond mussel *Sinanodonta woodiana* (Lea, 1834) stands out as one of the most successful invasive bivalves in Europe. Native to East and Southeast Asia, particularly the River Yangtze and Amur Basins, the species was unintentionally introduced into Europe during the 1960s via the import of Asian cyprinid fish carrying glochidia (Konečný et al. 2018). The parasitic larvae attached to these fish allowed *S. woodiana* to colonise aquaculture ponds in Romania, rapidly spreading to other regions as stocked fish were translocated (Mehler et al. 2024). In Poland, the species was first recorded in the thermally altered Konin lake system in the 1980s, facilitated by elevated water temperatures that favoured larval development (Kraszewski and Zdanowski 2007). Since then, *S. woodiana* has expanded into both lentic and lotic environments across the country, forming a gradient of historical associations with host fish, ranging from long-established populations in artificial lakes to more recent occurrences in natural river systems (Urbańska and Andrzejewski 2019). This ongoing expansion illustrates the species' high ecological plasticity and ability to exploit anthropogenically disturbed and natural habitats.

The ecological success of *S. woodiana* may partly stem from its compatibility with a wide range of fish hosts from various taxonomic groups, including species with no shared co-evolutionary history (Douda et al. 2012; Donrovich et al. 2017). In Europe, successful metamorphosis of *S. woodiana* glochidia has been observed on a wide spectrum of fish species, including common cyprinids, as well as representatives of other families (Donrovich et al. 2017; Modesto et al. 2018). Amongst these, the European bitterling *Rhodeus amarus* (Bloch, 1782) is particularly notable due to its reproductive strategy, which involves parasitism of unionid mussels and suggests a co-evolutionary process with native European mussels (Smith et al. 2004; Reichard et al. 2012). The genus *Rhodeus* is of East Asian origin, with only a single species group occurring in the West Palaearctic and most diversity restricted to East Asia (Bryja et al. 2010; Chang et al. 2014). This phylogenetic history likely contributes to its compatibility as a host of *S. woodiana*. Interestingly, *R. amarus* is largely resistant to infection by glochidia of native unionid species (Reichard et al. 2006, 2010). In these interactions, glochidia of European unionid mussels typically attach to the gills of fish (from which they often appear to be shed). In contrast, *S. woodiana* glochidia tend to attach to fins and skin more frequently (Douda et al. 2012). Such differences in attachment sites may affect the immune response and host tolerance. Furthermore, experimental evidence shows that a prior infection with *S. woodiana* glochidia may reduce susceptibility to subsequent infection by native mussels, likely due to immune priming or localised tissue damage (Donrovich et al. 2017). This cross-immunisation effect may impair the reproductive success of native mussel species and represents a subtle, but significant threat to native mussel faunas associated with *S. woodiana* invasion.

Most information on host use by *S. woodiana* in Europe comes from experimental studies (Douda et al. 2012; Donrovich et al. 2017; Huber and Geist 2019). In this study, we examined patterns of natural infection by glochidia of the invasive *S. woodiana* and native unionid mussels in wild fish populations across six sites in Poland along a gradient of *S. woodiana* invasion history. By sampling natural fish communities across lentic and lotic habitats, we aimed to assess host use patterns and glochidia load under natural ecological conditions. Specifically, we evaluated which fish species carried glochidia of native or invasive mussels and how host identity, habitat type and potential co-infections influenced infection outcomes. We hypothesised that: (1) *S. woodiana* will infest a broader range of fish hosts than native unionid species; (2) infection intensity by *S. woodiana* will be higher in lake habitats than in rivers, reflecting differences in mussel preferences or host-parasite availability; and (3) the spatial distribution of glochidia on fish will differ between mussel taxa, with *S. woodiana* attaching more frequently to fins and native unionids primarily targeting the gills.

Material and methods

Sample collection

The study was conducted at six sites in central and southern Poland that differ in the duration of *Sinanodonta woodiana* presence. The sites were selected, based on previously reported occurrences of *Rhodeus amarus* and freshwater mussel communities with stable populations of *S. woodiana*, supported by a pilot survey conducted one year before sampling, confirming the presence of both species. The timeline for the first confirmed record of *S. woodiana* at each site was established, based on a *S. woodiana* distribution database by Mehler et al. (2024), supplemented by personal communication with A. M. Łabęcka and corresponds to the sympatry gradient described by Halabowski et al. (2025). The selected sites represent a gradient of three stages (groups) of *S. woodiana* occurrence: (1) recent invasion group – Pilica River (first record of *S. woodiana* in 2018; 51°50'01.4"N, 21°16'12.8"E) and Soła River (first record of *S. woodiana* in 2017; 50°00'43.9"N, 19°12'00.0"E); (2) intermediate invasion group – Krajskie Oxbow Lake (first record of *S. woodiana* in 2010; 50°00'46.7"N, 19°31'51.2"E) and Narew River (first record of *S. woodiana* in 2015; 53°02'49.4"N, 21°32'25.3"E); (3) old invasion group – Licheńskie Lake (first record of *S. woodiana* in the early 1980s, which is also the first known locality of this species in Poland; 52°18'46.8"N, 18°20'58.4"E) and Pątnowskie Lake (first record of *S. woodiana* in the early 1980s; 52°18'24.0"N, 18°16'00.6"E) (Fig. 1). All sites supported stable populations of *S. woodiana* and *R. amarus* alongside

Figure 1. Map of study area.

native unionids and fish communities. Sampling was conducted in June 2023, during the peak reproductive season of *S. woodiana*, following a spring 2023 field survey confirming mussel maturity. Notably, this period does not fully coincide with the reproductive peaks of all native unionid species, which may have different seasonal patterns of glochidia release (e.g. *Anodonta anatina* (Linnaeus, 1758) peaks in colder months; *Unio* spp. in late spring to early summer). Fish species (bitterling *Rhodeus amarus*, roach *Rutilus rutilus* (Linnaeus, 1758), rudd *Scardinius erythropthalmus* (Linnaeus, 1758), perch *Perca fluviatilis* Linnaeus, 1758, Prussian carp *Carassius gibelio* (Bloch, 1782), white bream *Blicca bjoerkna* (Linnaeus, 1758), chub *Squalius cephalus* (Linnaeus, 1758) and topmouth gudgeon *Pseudorasbora parva* (Temminck & Schlegel, 1846)) were collected by electrofishing (EFGI 650, BSE Bretschneider Spezialelektronik, Chemnitz, Germany) with adherence to standards and subject to the necessary approvals. Immediately after capture, individuals were anaesthetised using clove oil solution (~ 0.03%) and then euthanised with an overdose (Javahery et al. 2012). All fish were preserved in 4% buffered formalin and transported to the laboratory at the Department of Ecology and Vertebrate Zoology of the University of Lodz, Poland. For each fish species, we collected at least 20 individuals per site (individuals of the same fish species with comparable body sizes), although this was not always possible (Table 1).

Table 1. Infection characteristics of *Sinanodonta woodiana* glochidia on different host species overall and from six study sites separately. Mean host body size and its 95% confidence intervals (across all populations), prevalence and the number of sampled fish, mean abundance, intensity of infection and maximum intensity of infection (for overall sample only). Abbreviations: TL – total length, 95% CI – 95% confidence interval, prev (N) – prevalences (sample size), abund – abundance, int inf – intensity of infection (mean), Max Ii – maximum intensity of infection.

	Overall						Lake Krajskie			Lake Licheńskie		
Host species	TL	95% CI	prev (N)	abund	int inf	Max Ii	prev (N)	abund	int inf	prev (N)	abund	int inf
Rhodeus amarus	51.01	47.3–54.8	50 (141)	1.2	2.4	13	60 (25)	1.4	2.3	92 (25)	3.1	3.3
Rutilus rutilus	74.23	69.7–78.7	16 (100)	0.2	1.4	4	0 (3)	–	–	32 (19)	0.5	1.5
Perca fluviatilis	86.94	81.6–92.3	35 (69)	0.6	1.8	8	44 (9)	0.6	1.3	100 (1)	2	2
Scardinius erythropthalmus	79.02	73.4–84.7	44 (63)	1	2.3	8	50 (4)	1.8	3.5	65 (23)	1.4	2.1
Blicca bjoerkna	77.83	71.3–84.4	38 (47)	0.9	2.2	7	–	–	–	74 (19)	1.7	2.4
Squalius cephalus	75.36	66.9–83.8	11 (28)	0.1	1	1	–	–	–	–	–	–
Pseudorasbora parva	61.86	56.8–67.0	17 (77)	0.5	2.9	8	7 (15)	0.1	1	35 (26)	1.3	3.8
Carassius gibelio	60.05	54.9–65.2	54 (76)	2.9	5.4	52	62 (26)	2.3	3.8	70 (20)	7	10

	Narew River			Lake Pątnowskie			Pilica River			Soła River		
Host species	prev (N)	abund	int inf	prev (N)	abund	int inf	prev (N)	abund	int inf	prev (N)	abund	int inf
Rhodeus amarus	76 (17)	1.6	2.1	40 (25)	0.6	1.5	0 (29)	0	0	45 (20)	0.9	1.9
Rutilus rutilus	33 (21)	0.5	1.4	8 (25)	0.1	1.5	0 (25)	0	0	14 (7)	0.1	1
Perca fluviatilis	25 (20)	0.3	1	63 (8)	1.1	1.8	33 (6)	0.8	2.5	28 (25)	0.7	2.4
Scardinius erythropthalmus	9 (11)	0.1	1	40 (25)	0.9	2.3	–	–	–	–	–	–
Blicca bjoerkna	8 (13)	0.3	4	0 (1)	0	0	15 (13)	0.2	1	100 (1)	1	1
Squalius cephalus	–	–	–	–	–	–	20 (5)	0.2	1	9 (23)	0.1	1
Pseudorasbora parva	–	–	–	0 (1)	0	0	0 (10)	0	0	12 (25)	0.1	1
Carassius gibelio	–	–	–	36 (25)	0.6	1.8	–	–	–	40 (5)	1.2	3

Dissections and glochidia identification

In the laboratory, all fish were measured (total length) to the nearest 1 mm and weighed to the nearest 10 mg. Gills and fins were carefully removed and examined separately under a stereomicroscope for attached glochidia (Bresser Science ETD-201, Germany), as was the external surface of the fish body (skin). All glochidia were gently detached using fine needles and tweezers, counted and assigned to one of three attachment sites: gills, fins or skin. Glochidia were identified to species level based on morphological characters using an optical microscope (Bresser Researcher Trino, Germany), based on diagnostic characters (Jokela et al. 1991; Pekkarinen and Englund 1995a, 1995b; Pekkarinen 1996; Bauer and Wächtler 2001; Sayenko and Soroka 2013; Ćmiel et al. 2021). However, as species-level identification is challenging for European unionid species, we pooled *Unio* species (*Unio pictorum* (Linnaeus, 1758) and *U. tumidus* Philipsson in Retzius, 1788) to a single category. All glochidia identified as *Anodonta anatina* (Linnaeus, 1758) are referred to as *Anodonta* sp. as its morphology overlaps with glochidia of *A. cygnea* (Linnaeus, 1758).

Data analysis

Infection parameters were calculated as follows. Prevalence is the proportion of infected individuals (irrespective of infection intensity or attachment site). Mean abundance is the mean number of parasite individuals in the sample of hosts, including infected and non-infected host individuals. Intensity of infection is the number of parasite individuals in the sample of infected host individuals. These parameters are presented for each host species, collated for the entire dataset (pooled across sampling sites) and separately for individual sites.

To test the effects of host species on parasite load, we used a Generalised Linear Mixed Model (GLMM) in the *glmmTMB* package, with mean abundance as the response variable and host species identity as the main factor. Models included individual host body mass, which is predicted to correlate positively with mean parasite abundance. For models targeted to test the entire dataset, sampling sites were always included as random effects to account for the non-independence of data collected from the same host community. As mean abundance included many zeros (non-infected host individuals), we used a negative binomial error distribution. When testing native mussel glochidia load, models with negative binomial error were still overdispersed and we instead used a Tweedie distribution. Tukey's HSD tests were used for post hoc comparison amongst host species. The robustness of fitted models was checked using diagnostic tools in the *DHARMa* package.

Results

Sinanodonta woodiana glochidia

Glochidia of *Sinanodonta woodiana* were attached to all fish species collected, with overall (i.e. across six sampling sites) species-specific prevalence of 11–50% and intensity of infection between 1 and 52 glochidia per host fish. Overall, *Carassius gibelio* was the most infected host; its mean prevalence was 50% (36–70% across sites) and its mean intensity of infection was 5.4 glochidia per fish (1.8–10 across sites). *Rhodeus amarus* was also a common host for *S. woodiana* glochidia (prevalence 50%, intensity of infection 1–13 glochidia per fish). In contrast, *Squalius cephalus* was the least infected host species, with only 11% prevalence of *Sinanodonta woodiana* glochidia overall and never harbouring more than a single glochidium per fish (Fig. 2). Full details of overall and site-specific parasitological parameters (prevalence, mean abundance, intensity of infection, and sample size) are provided in Table 1.

The differences in *S. woodiana* glochidia infection amongst host species were statistically significant (relaxed negative binomial GLMM, species effect: $\chi^2 = 72.0$, d.f. = 7, n = 601, $P < 0.001$) and post-hoc contrasts (Tukey method, at $P = 0.05$) revealed four overlapping groups of species with varying intensity of glochidia parasitism (Table 2). Fish body mass was a significant predictor of infection (GLMM, body mass effect (log-transformed): estimate (95% confidence interval): 1.50 (1.29–1.74), $\chi^2 = 28.9$, d.f. = 1, $P < 0.001$). Species- and site-specific effects of body mass on *S. woodiana* glochidia infection are presented in Fig. 3.

Glochidia of European mussels

Glochidia of European unionid species were rarely attached to host fishes during the sampling season. Infection by *Unio* glochidia was relatively high, especially on *Perca fluviatilis* in the River Narew, with *Rutilus rutilus*, *Scardinius erythropthalmus* and *Pseudorasbora parva* also infected at non-negligible prevalence and intensity of infection (Suppl. material 1: fig. S1, table S1). The overall prevalence

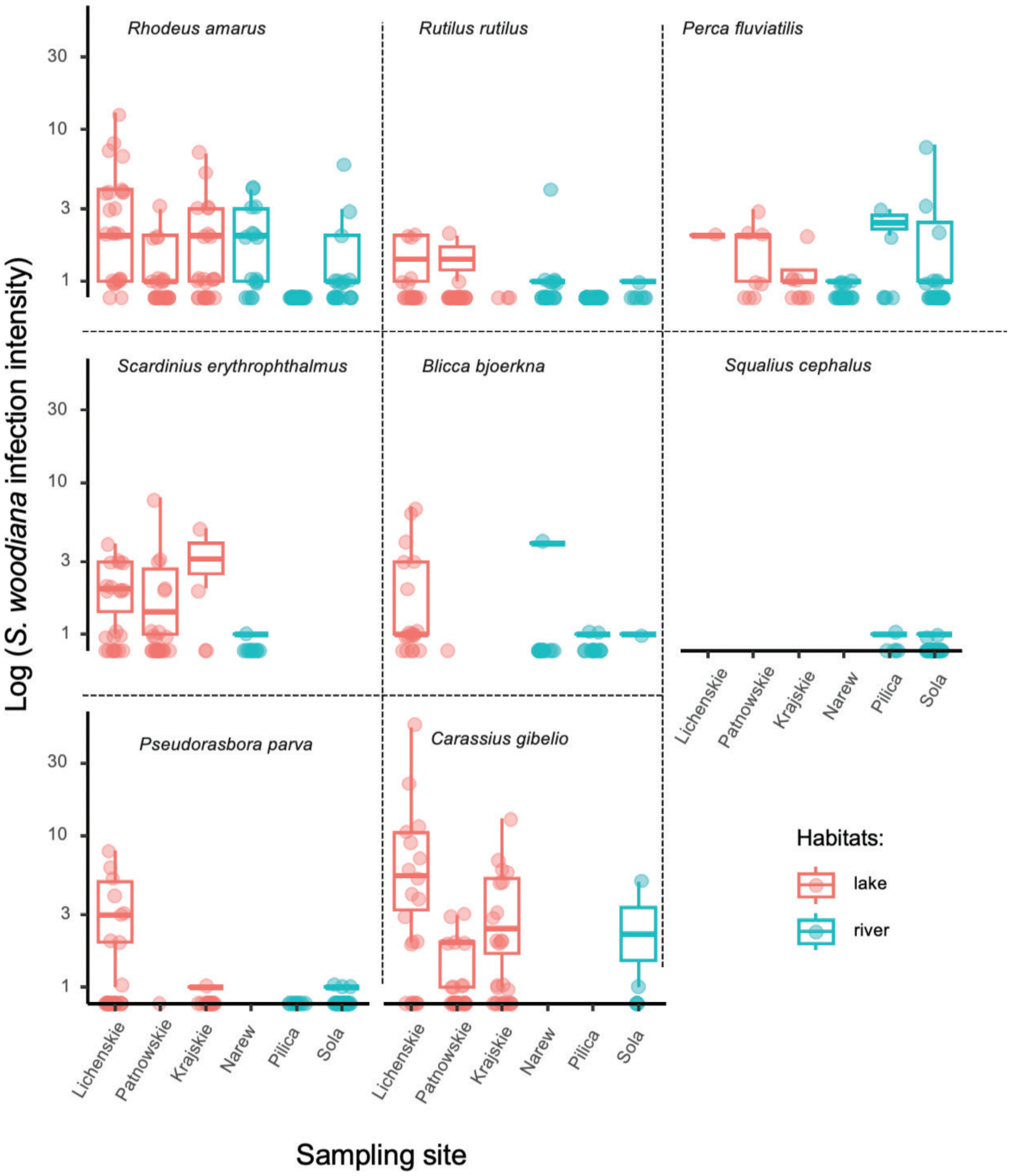

Figure 2. Abundance of *Sinanodonta woodiana* glochidia on eight host species across six sampling sites. Median, interquartile range and recorded data points are visualised. Lake and river habitats are separated by colour. Sites are ordered from those with most ancient presence of *S. woodiana* through intermediate presence to most recent presence.

of *Unio* spp. glochidia was 12.3% and intensity of infection varied between 1 and 67 glochidia per fish (Suppl. material 1: fig. S1, table S1). *Unio* glochidia load significantly varied across species (negative binomial GLMM for four host species with at least three infected individuals, $\chi^2 = 52.6$, d.f. = 3, $P < 0.001$), with Tukey post-hoc contrasts identifying that *Perca fluviatilis* had a higher glochidia load than *Pseudorasbora parva*, *R. rutilus* and *S. erythropthalmus* (Suppl. material 1: table S1). Other species had negligible prevalence of *Unio* glochidia (0–2 infected fish) (Suppl. material 1: fig. S1). Four fish individuals were infected by glochidia of *Anodonta* (most likely *A. anatina*) (overall prevalence 0.7%, intensity of infection 1–3 glochidia), namely two *S. erythropthalmus*, one *Rhodeus amarus* and one *P. parva*. These glochidia were recorded on fish sampled on 24 June 2023 in the Soła River and the Krajskie Oxbow Lake and on 22 June 2023 in the Licheńskie Lake.

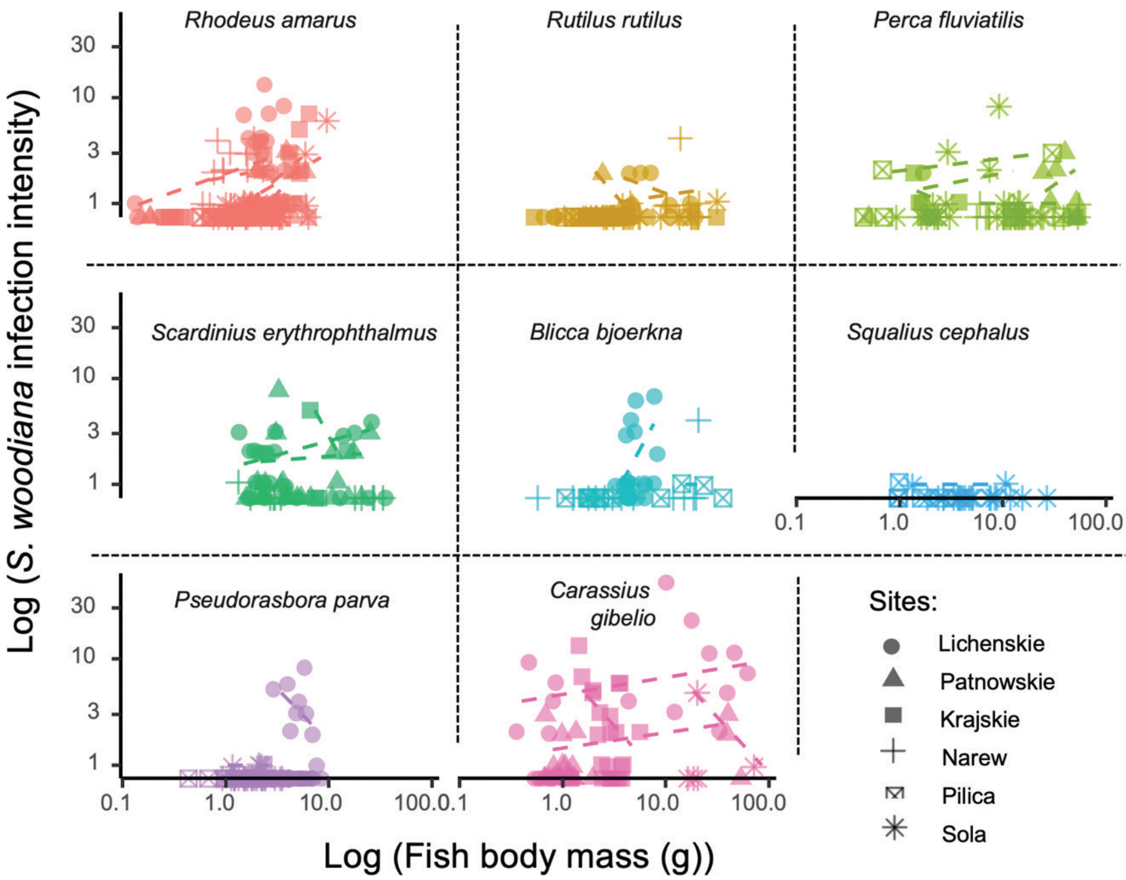

Figure 3. The relationship between fish body mass (log-transformed) and abundance of *Sinanodonta woodiana* glochidia (log-transformed) for each host species. Sites are denoted by different symbols. Site-specific trend-lines are visualised using "lm" method with the geom_smooth function in ggplot2 package.

Table 2. Results of the statistical test comparing *Sinanodonta woodiana* glochidia load across species, with pairwise contrasts denoted by letters. Host species are ranked according to their infection rates. Groups refer to groups of species with statistically (Tukey post-hoc test at P = 0.05) different glochidia load; two letters refer to inclusion to two groups.

Coefficient	Infection	Conf. Int (95%)	P-value	Group
Carassius gibelio	0.90	0.43–1.86	0.772	A
Rhodeus amarus	0.66	0.33–1.30	0.229	AB
Scardinius erythropthalmus	0.32	0.14–0.70	0.004	B
Perca fluviatilis	0.29	0.13–0.66	0.003	B
Blicca bjoerkna	0.22	0.10–0.52	0.001	BC
Pseudorasbora parva	0.15	0.07–0.33	<0.001	BC
Squalius cephalus	0.08	0.02–0.34	0.001	BC
Rhodeus rutilus	0.09	0.04–0.22	<0.001	C

We detected no statistical association between the presence or abundance of *Sinanodonta woodiana* glochidia and infection by native *Unio* glochidia. The GLMM (with Tweedie error distribution of glochidia abundance and with binomial error distribution for glochidia presence/absence data, with log-transformed body mass as a covariate and species and site as random effects) did not demonstrate any significant relationship between infection by the two mussel species (all P > 0.05). The tests were completed for overall glochidia load and fish gills (where most *Unio* glochidia were found).

Spatial aspect of glochidia attachment

The glochidia of all mussel species tended to infect host gills more often than host fins. In *S. woodiana* the ratios were 37 (gills):18 (fins) in *Blicca bjoerkna*, 54:9 in *Scardinius erythropthalmus*, 32:6 in *P. parva*, 26:24 in *Perca fluviatilis*, 16:7 in *Rutilus rutilus* and 2:1 in *Squalius cephalus*. However, in the most common hosts, the ratios were reversed: 81:87 in *Rhodeus amarus* and 67:155 in *C. gibelio*. Glochidia of native mussels were attached mainly on the gills, with the ratios of 866:14 (98.4% on gills) in *Unio* and 5:1 (83% on gills) in *Anodonta*.

Glochidia abundance on host gills and fins was positively correlated (Spearman test comparing infections within individual fish, *Sinanodonta woodiana*: rho = 0.26, $P < 0.001$, Fig. 4; *U. pictorum*: rho = 0.25, $P < 0.001$). For *S. woodiana*, where the ratio of glochidia on host gills and fins was comparable, host species with higher infection rates were more likely to have *S. woodiana* glochidia attached to their fins (*C. gibelio*, *R. amarus*, *P. fluviatilis*), while host species with lower infections tended to have more on their gills (Fig. 4).

We compared *S. woodiana* infection on gills and fins separately. While *S. woodiana* infections on gills varied across species (relaxed negative binomial GLMM, species effect: $\chi^2 = 27.2$, d.f. = 7, n = 601, $P < 0.001$), post-hoc contrasts revealed that *R. amarus* and *Scardinius erythropthalmus* had a higher *Sinanodonta woodiana* glochidia load on their gills than *Rutilus rutilus* (Suppl. material 1: fig. S2). No other species difference was statistically significant (all other $P > 0.13$). In contrast, host species differences in *S. woodiana* glochidia load on fins were substantial (relaxed negative binomial GLMM, species effect: $\chi^2 = 120.5$, d.f. = 7, n = 601, $P < 0.001$) and mainly arose from *C. gibelio* and *Rhodeus amarus* possessing a much higher fin glochidia load than all other host species (Tukey method $P < 0.05$) (Suppl. material 1: fig. S3).

Discussion

Our study revealed marked differences in host utilisation patterns between the invasive mussel *Sinanodonta woodiana* and native European unionid species. The invasive mussel displayed a significantly broader host range and higher infection loads at all sampled locations. These findings are consistent with previous studies demonstrating that *S. woodiana* can successfully parasitise a variety of fish species, including cyprinids and non-cyprinids, due to low host specificity (Douda et al. 2012; Donrovich et al. 2017). In contrast, native mussels, such as *Unio pictorum*, *U. tumidus* and *Anodonta anatina*, exhibited narrower host specificity and primarily targeted local cyprinid hosts (Bauer and Wächtler 2001; Lopes-Lima et al. 2017; Modesto et al. 2018). These differences may reflect distinct evolutionary trajectories, with *S. woodiana* evolving in ecologically and taxonomically more diverse fish communities in East Asia, favouring a generalist mode of parasitism (Konečný et al. 2018). We note that our sampling took place during the reproductive peak of *S. woodiana* (but not of all native unionid species), which likely contributed to its overall prevalence. Seasonal differences in reproduction amongst unionids are well-documented (Bauer and Wächtler 2001; Labecka and Domagała 2018), with European *Anodonta* spp. having its infection peaks during the coldest months and European *Unio* spp. in late spring and early summer. Therefore, while our results suggest a higher reproductive output in *S. woodiana*, interspecific seasonal variation of glochidia release of European unionids contributed to this outcome.

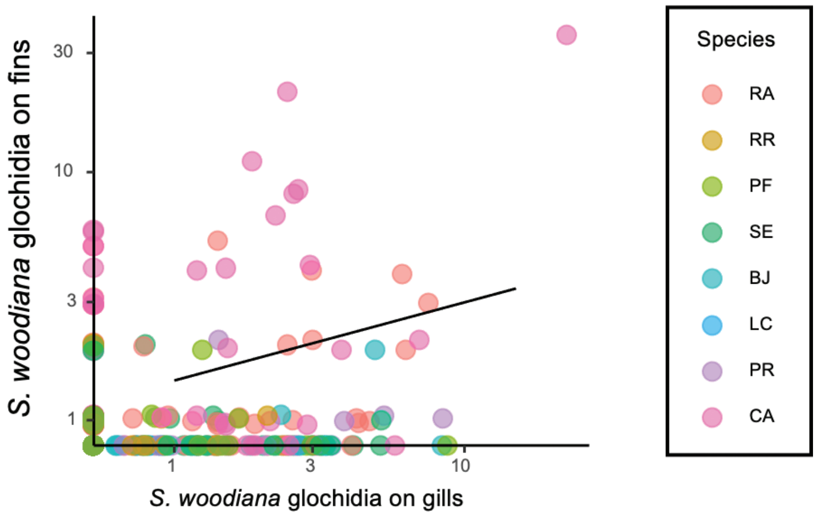

Figure 4. Relationship between *Sinanodonta woodiana* glochidia on the gills and fins of host fish. Each host species is depicted by different colour. Abbreviations: RA – *Rhodeus amarus*, RR – *Rutilus rutilus*, PF – *Perca fluviatilis*, SE – *Scardinius erythropthalmus*, BB – *Blicca bjoerkna*, SC – *Squalius cephalus*, PP – *Pseudorasbora parva*, CG – *Carassius gibelio.*

In our study, *S. woodiana* glochidia were found on all fish species sampled, yet infection intensity varied considerably amongst host taxa. Species of East Asian origin, such as *Carassius gibelio* and *Pseudorasbora parva*, hosted significantly more glochidia than other hosts. *Rhodeus amarus* also hosted significantly more glochidia than other fish species. This result suggests retained compatibility between *S. woodiana* and bitterling, whose phylogenetic origin is in East Asia (Chang et al. 2014). Douda et al. (2017) demonstrated experimentally that glochidia of *S. woodiana* exhibit greater success on cyprinids with a shared evolutionary origin, supporting the idea of legacy co-evolution re-established in the novel ranges of co-expanding lineages (Reichard et al. 2012). While *S. woodiana* is a generalist parasite, our results highlight asymmetries in host use between fishes of East Asian and European origin, likely linked to past co-evolutionary associations (Douda et al. 2012). The close evolutionary relationships between unionid mussels and their host fish often result in highly specialised parasitic strategies (Bauer and Wächtler 2001; Barnhart et al. 2008). These relationships involve adaptations in both larval morphology and behaviour that optimise attachment, survival and transformation on specific host taxa. For example, many native unionids exhibit morphological traits of glochidia, such as size, hooks and hinge structure, that match closely with the gill morphology of their preferred hosts (Bauer and Wächtler 2001). In contrast, invasive species like *S. woodiana* appear to circumvent such specialisation, successfully exploiting novel host species in new environments. Therefore, its ecological plasticity likely contributes to its successful establishment and spread in European waters (Watters 1997; Douda et al. 2012; Douda et al. 2024; Mehler et al. 2024). *Rhodeus amarus*, although native to Europe, not only shares a phylogenetic origin with East Asian bitterlings, but is also uniquely associated with mussels through its use of mussels for oviposition (Van Damme et al. 2007; Reichard et al. 2012). While *S. woodiana* effectively parasitises *R. amarus* (this study), the fish does not use *S. woodiana* for spawning (Reichard et al. 2007; Marčić et al. 2024; Halabowski et al. 2025). This asymmetry likely reflects shared evolutionary traits with historical hosts. In contrast, native unionids tend to achieve higher parasitic success on European fish species, such as *Rutilus rutilus* (Douda et al. 2017).

Although the infection intensity of *S. woodiana* was highest at sites with long-established populations, these patterns were primarily associated with lentic habitats. This result suggests that the apparent effect of time since introduction may be confounded by habitat characteristics. Lentic systems, such as lakes and reservoirs, likely promote higher encounter rates between hosts and parasites due to fish aggregation and more stable environmental conditions, facilitating transmission (Barnhart et al. 2008). Conversely, lotic systems may limit glochidia retention and reduce contact time with hosts, lowering transmission efficiency (Bauer and Wächtler 2001). Consequently, elevated infection levels in lentic sites may reflect ecological context rather than increased local adaptation resulting from prolonged sympatry. These trends are supported by data presented in Figs 2, 4, 5, showing greater glochidia loads on fins of *C. gibelio* and *Rhodeus amarus*, which also exhibited high susceptibility to parasitism. Given that the initial occurrence of *S. woodiana* was associated with lentic systems (especially reservoirs), we could not have accounted for this confounding effect in our study design. Environmental variation also plays a critical role in mediating infection outcomes. Variables such as temperature, turbidity and oxygen concentration modulate host-parasite contact rates, larval development and transformation success (Barnhart et al. 2008; Haag 2012). These factors likely differ significantly between lotic and lentic systems, further complicating the interpretation of contrasting infection rates between lotic and lentic habitats. Longitudinal and experimental studies are needed to disentangle these interacting drivers.

We found no evidence that different mussel species compete for attachment sites on the host fish body. Glochidia from different species were often present on the same host, but they did not show signs of spatial exclusion. This finding suggests that site saturation was unlikely at the time of sampling. However, competition may have been underestimated due to the low abundance of native glochidia. Interference through immune mechanisms might still occur; Donrovich et al. (2017) demonstrated that prior infection by *S. woodiana* can trigger an immune response in fish that reduces

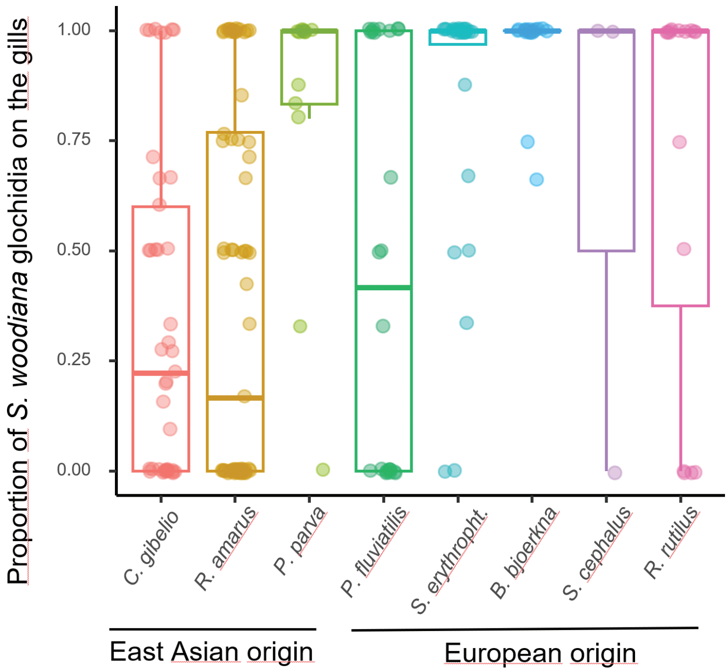

Figure 5. Proportion of *Sinanodonta woodiana* glochidia on the host fish gills (1: 100% of glochidia on host gills, 0: 100% of *S. woodiana* glochidia on host fins).

the success of subsequent infections by native mussel larvae. Amongst the fish species examined, *C. gibelio* and *R. amarus* showed the highest total glochidia loads, with a pronounced tendency for attachment on the fins. In contrast, fish species with lower glochidia numbers had them primarily on the gills. This pattern suggests that gill attachment may represent a widespread baseline strategy for *S. woodiana*, while fin colonisation is more typical for hosts that are either more compatible or more heavily infected. The even distribution of glochidia on gills across host species, combined with more selective fin attachment, supports this interpretation.

Finally, the implications of our findings extend to conservation management. Native unionids are globally threatened and often rely on specific host relationships that may be disrupted by invasive species (Lopes-Lima et al. 2017; Sousa et al. 2023). The introduction of *S. woodiana* poses both direct and indirect challenges, including altered host availability, immune interference and potential competitive suppression. We have established that natural co-infection between *S. woodiana* and native unionids is possible.

In conclusion, *S. woodiana* combines a suite of ecological and life-history traits, such as generalist host use (this study), broad habitat tolerance (Paunović et al. 2006; Douda et al. 2024), asynchronous reproduction (Douda et al. 2012; Labecka and Domagała 2018) and possible immune modulation (Donrovich et al. 2017), which collectively contribute to its invasive potential. Its ability to exploit co-evolved hosts and override host defences supports its establishment across various environments and regions. Future studies should assess its long-term population dynamics, larval viability and immunological interactions under natural conditions to better predict its ecological impacts on native biota and provide information for conservation efforts for native mussels.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

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Author contributions

D.H.: conceptualisation, methodology, investigation, visualisation, formal analysis, writing – original draft, writing – review and editing; K.P., G.Z., J.G and C.S.: investigation, writing – review and editing; M.P.: investigation, M.R.: conceptualisation, methodology, investigation, visualisation, formal analysis, writing – original draft, writing – review and editing, supervision.

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Data availability

All data generated or analysed during this study were uploaded to the Figshare repository (https://doi.org/10.6084/m9.figshare.29168393).

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Supplementary material 1

Additional information

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Data type: docx

Explanation note: **table S1**. Infection characteristics of *Unio* glochidia on different host species overall and from six study sites separately. Prevalence and the number of sampled fish (prev (N)), mean abundance (abund), intensity of infection (i) and maximum intensity of infection (max int, for overall sample only). **fig. S1**. Abundance of *Unio* glochidia on eight host species across six sampling sites. Median, interquartile range and recorded data points are visualised. Lake and river habitats are separated by colour. Sites are ordered from those with most ancient presence of *Sinanodonta woodiana* through intermediate presence to most recent presence. **fig. S2**. Abundance of *Sinanodonta woodiana* glochidia on the gills of eight host species across six sampling sites. Median, interquartile range and recorded data points are visualised. Lake and river habitats are separated by colour. Sites are ordered from those with most ancient presence of *S. woodiana* through intermediate presence to most recent presence. **fig. S3**. Abundance of *Sinanodonta woodiana* glochidia on the fins of eight host species across six sampling sites. Median, interquartile range and recorded data points are visualised. Lake and river habitats are separated by colour. Sites are ordered from those with most ancient presence of *S. woodiana* through intermediate presence to most recent presence.

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