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PRIMARY RESEARCH PAPER

Invasion at the population level: a story of the freshwater snails *Gyraulus parvus* and *G. laevis*

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Abstract Biological invasions are common among freshwater molluscs, with the North American planorbid gastropod *Gyraulus parvus* being reported from Europe (Germany) by the 1970s. It has since spread across Central and Western Europe, mostly living in artificial and highly modified habitats. However, considerable conchological and anatomical similarity exists between it and the native European *G. laevis*. Using four other European and one North American *Gyraulus* species as outgroups, separate phylogenetic analyses using mitochondrial and nuclear DNA sequences show that *G. parvus* and *G. laevis* are in fact part of the same species-level clade, with the former having nomenclatural priority. However, the structure within the mitochondrial tree suggests a North American origin of the invasive populations. It also makes it possible to track down the distribution of both races. Although native and non-native races in Europe tend to possess some differences in conchology and ecology, the degree of overlap makes it impossible to accurately distinguish between them without the DNA barcode data. Our results change the outlook on the conservation of the rare native race. While interspecific competition among snail species is rare, invasion on an intraspecific level may represent a serious threat for native populations.

Keywords Freshwater snail · *Gyraulus* · Molecular data · Invasive race · Planorbidae · Phylogeny · Non-native · Genotype

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Introduction

Biological invasions are among the most severe current threats to biodiversity and ecosystem health (Williamson, 1999; Mack et al., 2000). Six mollusc species (three gastropods and three bivalves) appear on a list of 100 of the world's worst invasive species (Invasive Species Specialist Group ISSG 2015). Exotic aquatic mollusc populations are often unintentionally introduced through global trade (Leuven et al., 2009; Van Leeuwen et al., 2013). These may subsequently be further spread via natural means including waterfowl (Kappes & Haase, 2012; Van Leeuwen et al., 2012).

So far, 13 freshwater mollusc species have been introduced to outdoor environments in Central Europe, including five pulmonate gastropods all of which are of North American origin (Horsák et al., 2013). *Gyraulus parvus* (Say, 1817) is one of these. It was first recorded in Europe from near Speyer, south-west Germany in 1973 (Meier-Brook, 1983; Glöer & Meier-Brook, 1998). It has since become widely distributed throughout Central Europe (Patzner, 1997; Beran & Horsák, 2002; Fehér et al., 2004; Gloër, 2019), Western Europe (Boschi & Heim, 2011; Jansen, 2015) and Mediterranean islands (Hughes, 1995; Falkner et al., 2002). Although known from Iceland (Meier-Brook, 2002), it has never been reported from Britain or Ireland (Rowson et al., 2021) and Northern Europe. In North America, *G. parvus* occupies all types of natural and anthropogenic aquatic habitats (Clarke, 1981; Burch, 1982) while in the invaded regions, most records are limited to man-made ponds and reservoirs. The first European record, for instance, originated from an artificial pond near a highway (Meier-Brook, 1983). In the Czech Republic, it is mostly found in artificial and modified standing water bodies such as fishponds, reservoirs, and sand-pits (Lorencová et al., 2015).

Gyraulus parvus is conchologically similar to the native European *G. laevis* (Alder, 1838), differing only by having its penultimate whorl distinctly elevated and its body whorl prominently deflected (Meier-Brook, 1983). However, these characters do not allow for clear demarcation, with there being a notable overlap in shell morphology, produced also by high phenotypic variation within populations (Meier-Brook, 1983; personal observation). Therefore, only the widening of the distal half of the vas deferens has been thought to provide a reliable diagnosis: both halves are equally wide in *G. laevis,* while *G. parvus* has the proximal half narrower (Meier-Brook, 1983; Beran & Horsák, 2002; Horsák et al., 2013).

In reality, though, the preference of *G. parvus* to artificial and modified habitats has been a principal trait used to distinguish it from *G. laevis*, which mainly occupies mesotrophic pools and wetlands. *Gyraulus laevis* has been reported to have recently become very rare across almost the whole of Europe (Glöer, 2019 and more references therein) except for the United Kingdom where it still occurs in large stable populations (Kerney, 1999; Rowson et al., 2021). However, because high levels of morphological plasticity are well known from within the genus (Jung & Burch, 1991; Clewing et al., 2015), it seemed useful to subject the *G. parvus* and *G. laevis* taxonomic concepts to empirical confrontation using mitochondrial and nuclear DNA sequence data. We hypothesize that these two entities could potentially be members of the same species-level clade, meaning the invasion occurs at the population level. To have this insight might also help to assess the current conservation status of rapidly vanishing *G. laevis* populations.

Methods

Samples, DNA extraction, PCR amplification and sequence analysis

A priori identifications of *G. parvus* and *G. laevis* samples were conducted based on the critical diagnostic shell characters of Burch (1982) and Meier-Brook (1983); see Fig. 1 for representative shells of each species. We used specimens collected between 1998 and 2020 across Central Europe, in conjunction with two North American *G. parvus*, one sourced from near the type locality (Online Resource 1). Two samples of *G. laevis* collected in the Czech Republic before the 1960s were accessed from the National Museum in Prague. Five additional *Gyraulus* species: *G. albus* (O. F. Müller, 1774), *G. circumstriatus* (Tryon, 1866), *G. crista* (Linnaeus, 1758), *G. riparius* (Westerlund, 1865) and *G. rossmaessleri* (Schmidt, 1852), and *Planorbis planorbis* (Linnaeus, 1758) were used for outgroup comparisons. These samples were collected mostly in Europe (Czech Republic, Slovakia, Austria, Croatia, Serbia), with two North

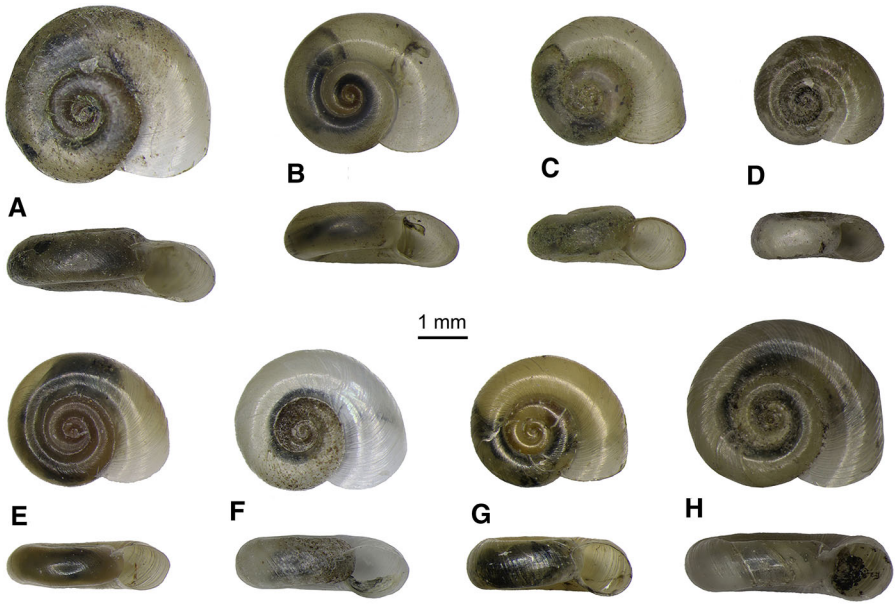

Fig. 1 Variation in shell morphology among individuals from *Gyraulus parvus* species complex used in phylogenetic analyses; representing three morphotypes being traditionally considered nominal species: **A**–**D**, *G. parvus*; **E**–**F**, *G. laevis*; **G**–**H**, *G. circumstriatus*. *G. parvus*: **A** G238, Austria (47.8519 N, 15.0422E); **B** G309, Czech Republic (50.0704 N, 14.4213E); **C** G320, Czech Republic (50.6398 N, 13.7522E); **D** G394, USA (41.4773 N, -74.9118E); *G. laevis*: **E** G292, Croatia (44.1515 N, 15.8865E); **F** G237, Czech Republic (48.7799 N, 16.7556E); *G. circumstriatus*: **G** G332, Canada (50.9213 N, -117.5771E); **H** G393, USA (48.7766 N, -114.1308E). Note that although planorbids are sinistral in coiling, they carry their shells in a way that makes them appear to be dextral, as photographed

American populations sampled to represent *G. circumstriatus* (Online Resource 1).

Specimens were either fixed in 96% ethanol or allowed to desiccate at ambient temperature and humidity. DNA was extracted from 61 specimens using the E.Z.N.A. Mollusc DNA Kit (Omega BioTek) following the manufacturer's instructions and stored at −20 °C. Due to the small, tightly coiled shells of *Gyraulus* species, shell destruction was necessary for sufficient DNA yield. Before DNA isolation, the gastropods were microscopically photographed for documentation. Shell photographs of selected specimens are in Fig. 1; photographs of all specimens are available upon request.

We amplified two mitochondrial regions [cytochrome c oxidase subunit I (COI) and cytochrome b (CytB)], and two nuclear genes [internal transcribed spacers1 and 2 of the rRNA gene cassette (ITS1, ITS2)] using the primers and protocols listed in Table 1. Amplifications were checked by gel electrophoresis and PCR products were purified using ExoSAP (Affymetrix) and sequenced at the OMICS Core facility, BIOCEV (CZ) using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) or SEQme s.r.o. (CZ) using Gerbera Sequencing Kit v3.1 (SEQme).

Forward and reverse strands were assembled and proofread using Geneious Prime 2020.2.4 (Biomatter Ltd.), aligned with Mafft v.7 online version (Kuraku et al., 2013; Katoh et al., 2019) and manually corrected. The final alignment was trimmed to remove primer sequences. All nucleotide sequences have been deposited in the GenBank database, with the accession numbers provided in Online Resource 1.

Data analysis

DNA sequence data were analyzed separately for concatenated mtDNA (COI + CytB) and nDNA (ITS1 + ITS2) fragments. To identify robust and strongly supported topological features we used four different methods of phylogeny reconstruction. Neighbor-joining (NJ) trees were generated in MEGA v.6.0 (Tamura et al., 2013), using maximum composite distance including transitions and transversions with pairwise gap deletion. Maximum parsimony

Table 1 Forward (F) and reverse (R) primer sequences used for genetic analysis, annealing temperatures for PCR, and authors of primer design

Region	Name	Sequence	Anneal	Source
COI (F)	L1490-Alb (F)	5′-ACTCAACGAATCATAAAGATATTGG-3′	46 °C	Gittenberger et al. (2004a, b)
COI (R)	BCO (R)	5′-GTATCGGCTGTAAAATAAGC-3′	46 °C	Haase et al. (2003)
CytB (F)	CytB_F_Gyr	5′-GTKTTACCATGAGGRCAAATATC-3′	45 °C	Author design, V. Horsáková
CytB (R)	CytB_R_Gyr	5′-GCAAAYAAAAAATATCATTCTGG-3′	45 °C	Author design, V. Horsáková
ITS1 (F)	ITS1_F	5′-TAACAAGGTTTCCGTATGTGAA-3′	52 °C	Armbruster and Bernhard (2000)
ITS1 (R)	ITS1_R	5′-TCACATTAATTCTCGCAGCTAG-3′	52 °C	Nekola et al. (2018)
ITS2 (F)	ITS2_F	5′-CTAGCTGCGAGAATTAATGTGA-3′	52 °C	Wade and Mordan (2000)
ITS2 (R)	ITS2_R	5′-GGTTTCACGTACTCTTGAAC-3′	52 °C	Nekola et al. (2018)

(MP) trees were inferred in the TNT software (Goloboff et al., 2008) using traditional search with 1000 replicates of Wagner trees, one random seed and TBR branch swapping algorithm, treating gaps as missing data. For Maximum likelihood (ML) and Bayesian inference (BI), the data were partitioned by genes, with two separate partitions (1st + 2nd, and 3rd positions) being created for the protein-coding mitochondrial fragments. ML analysis was performed in RAxML v 8.2 (Stamatakis, 2014), using 500 search replicates and the GTR + G models, applied separately for each gene partition. Node support for NJ, MP, and ML was assessed by 1000 non-parametric bootstrap replicates (Felsenstein, 1985). For BI, the best-fitting nucleotide substitution models were selected using jModelTest v. 2.1.10 (Darriba et al., 2012) based on the Akaike Information Criterion separately for each gene partition. BI was performed in MrBayes v3.2.6 (Huelsenbeck & Ronquist, 2001), running four simultaneous MCMC chains (one cold and three heated) for 10,000,000 generations, sampling every 1000 generations, with 25% of trees being discarded as burn-in. Four independent searches of tree space were run, and parameter estimates were checked for convergence in Tracer 1.6 (Drummond & Rambaut, 2007). The tree search was considered stable with the effective sample sizes of all parameters > 200 and the standard deviation of split frequencies lower than 0.01. Values of node support in NJ, MP, and ML, and posterior probabilities of BI, are shown only for the nodes that were highly supported by at least one of the methods (> 70% bootstrap support, or > 95% posterior probability). The QUIDDICH R package (Kühn & Haase, 2020) was used to search for diagnostic bases in the nDNA tree. This analysis was done post hoc, to test whether the structure revealed in the mtDNA data is reflected also in the nDNA data, potentially based on a very small number of characters. Final trees were visualised in the Interactive Tree Of Life (iTOL v.5.7) online tree annotator (Letunic & Bork, 2019). The same procedure and methods were used to generate a COI-only tree incorporating 28 additional *G. parvus*, *G. laevis*, and *G. circumstriatus* sequences archived in GenBank or representing unpublished data from the Czech Republic and the UK (including Wales, Scotland and Northern Ireland) (Online Resource 2). The relationships between individual COI genotypes of *G. parvus*/*G. laevis*/*G. circumstriatus* were visualized using a minimum spanning haplotype network constructed in PopART v. 1.7 (Leigh & Bryant, 2015).

Results

DNA sequences were obtained from 53 specimens for concatenated COI + CytB and 49 for ITS1 + ITS2 (see Online Resource 1 for details). The amplicons consisted of 591–615 bp for COI, 360 bp for CytB, 665–816 bp for ITS1, and 846–952 bp for ITS2. The total amplicon length for the concatenated mtDNA (COI + CytB) and nDNA fragments (ITS1 + ITS2) varied between 951–975 bp and 1511–1740 bp, respectively. The numbers of variable sites per amplicon were as follows: 167 for COI, 122 for CytB, 311 for ITS1, and 182 for ITS2.

Planorbis planorbis could not be used in the nDNA alignment because it was not possible to obtain an

unambiguous alignment with the ingroup. We observed a certain amount of length variability among *Gyraulus* sequences. While *G. parvus*/*G. laevis*/*G. circumstriatus* sequences all possessed 615 bp COI amplicons, *G. albus*, *G. crista*, *G. riparius* and *G. rossmaessleri* sequences had 15 bp gaps at the upstream end of COI, with *G. riparius* having an additional 9 bp gap ca 300 bp downstream.

The four different reconstruction methods (NJ, MP, ML, and BI) yielded very similar tree topologies; inconsistencies were limited to deeper nodes with low support. The support values/posterior probabilities were high and consistent for all major clades, with no ambiguously assigned specimens. Therefore, only one representative tree (BI) was used to visualize the results, separately for mtDNA and nDNA fragments (Fig. 2). All four methods yielded highly supported monophyletic clades for each of the species *Gyraulus riparius*, *G. crista*, *G. rossmaessleri*, and *G. albus*. All four also yielded a clade consisting of all European and North American individuals of *G. parvus*, *G. laevis*, and *G. circumstriatus* (support of 99–100% in all reconstruction methods for both mtDNA and nDNA; Fig. 2). The latter clade showed virtually no genetic variation in the nDNA tree (mean sequence divergence within the clade 0.2%), while it was more variable in the mtDNA tree (mean sequence divergence 2.6%). In the mtDNA tree, three groups could be distinguished within this clade: one containing European populations identified as either *G. laevis* or (less frequently) *G. parvus*; a second containing samples from both Europe and North America, identified almost exclusively by shell morphology as *G. parvus*; and a third containing only material identified as North American *G. circumstriatus* (Fig. 2). When the specimens were assigned into these three groups based on mtDNA and checked post facto for diagnostic nDNA bases, none were found to exist (for alignment input into the analysis see Online Resource 3). Two museum samples of *G. laevis* collected before the 1960s in the Czech Republic possessed mtDNA and nDNA sequences identical to populations identified as *G. laevis*. Mean sequence divergences between all clades are shown in Table 2. The observed topology remained unchanged following the addition of 28 additional GenBank or unpublished COI sequences (17 from North American specimens labelled *G. parvus* or *G. circumstriatus*, seven from the Czech Republic labelled *G. parvus*, and four from the UK labelled *G. parvus*/*laevis*) to an alignment consisting of only COI sequences. All these additional sequences were assigned to either the group conchologically assigned to *G. parvus* (including all four from the UK) or to *G. circumstriatus* (Online Resource 2).

The haplotype network of all individuals of the focal *G. parvus* group showed three major COI haplotype clusters (Fig. 3). The most numerous and diverse cluster contained individuals collected from both Europe and North America, mostly showing a *G. parvus* shell phenotype (Online Resource 2). The other two clusters contained less diverse haplotypes of European populations showing a *G. laevis* phenotype, and relatively diverse haplotypes of North American samples, including those showing a *G. circumstriatus* phenotype (Fig. 1).

Discussion

Are *G. parvus* and *G. laevis* part of the same species-level clade?

Yes, we can conclude they are, with *Gyraulus parvus* having nomenclatural priority. While DNA sequence analyses clearly confirm the species-level status for *G. albus*, *G. crista*, *G. riparius, G. rossmaessleri* and *G. parvus*, they do not validate the species-level status of *G. laevis* (Fig. 2). There are no diagnostic base pairs in the nDNA sequences that separate *G. laevis* from the remainder of *G. parvus*. Although reasonably well-supported races exist in the mtDNA tree, their base pair variability is three times smaller than that seen between the other analyzed *Gyraulus* species. Some morphological trends are present (Fig. 1) which are consistent with the a priori differences identified between *G. parvus* and *G. laevis* (Meier-Brook, 1983). The level of overlap is so great, however, that it is often not possible to accurately identify mtDNA races using morphological features alone. Nevertheless, habitat data may help. There does appear to be a rather strong propensity for the North American race to inhabit disturbed, eutrophic wetlands and the European race to inhabit undisturbed mesotrophic native wetlands. Such habitat preferences could influence the ecophenotypic response of the species, inducing the phenotypic response of different shell forms as already observed for other *Gyraulus* species (Clewing et al., 2015).

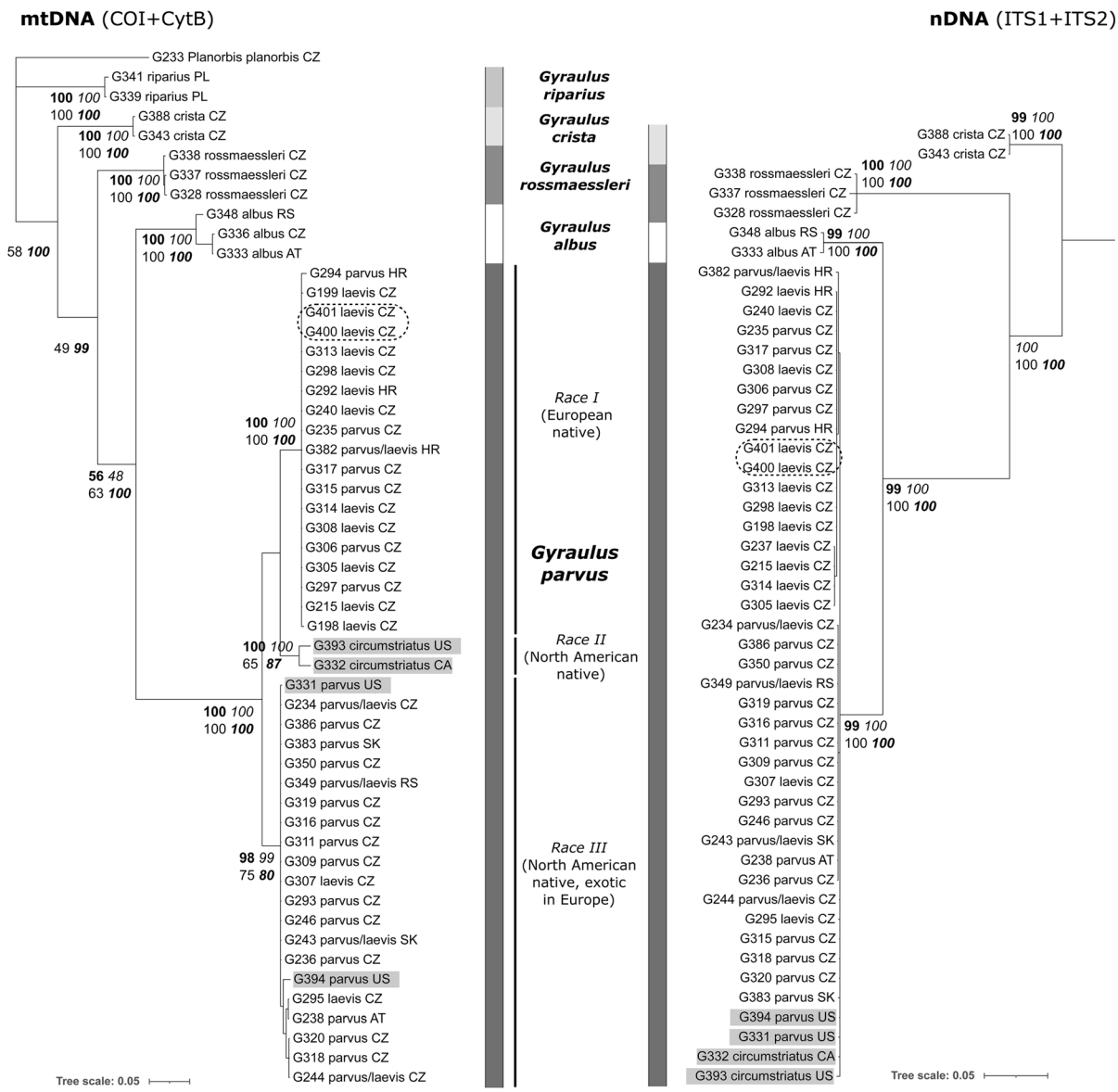

Fig. 2 Phylogenetic tree of *Gyraulus* spp. based on the Bayesian inference of mitochondrial DNA (COI + CytB, left) and nuclear DNA (ITS1 + ITS2, right). Support values are shown next to the corresponding nodes as follows: **Neighbor joining**, upper left, bold; *Maximum parsimony*, upper right, italic; Maximum likelihood, lower left, normal font; and posterior probabilities for ***Bayesian inference***, lower right, bold italic. Values are shown only for the nodes that were highly supported by at least one of the methods (> 70% bootstrap support in NJ, MP, and ML, or > 95% posterior probability in BI). Specimens from North America are highlighted in grey colour. Two samples collected before 1960s, i.e. prior to the first record of North American *G. parvus* in Europe, are marked by dashed line. Species labels refer to morphology-based a priori species identifications. For samples location see Online Resource 1. Samples labelled as *parvus/laevis* could not be reliably identified due to unclear features

Our data also suggest that North American *G. circumstriatus* might simply represent another race within *G. parvus*. However, with respect to this species, we are unwilling to conduct a taxonomic act at this time due to the small number of observed individuals with coincident DNA sequence and shell data ($n = 2$), combined with the lack of DNA sequence from populations near the type locality in Connecticut.

Table 2 Mean sequence divergences between the *Gyraulus* clades in the ITS1 + ITS2 construct (below diagonal, italic) and the COI + CytB construct (above diagonal)

	G. crista	*G. rossmaessleri*	*G. albus*	*G. riparius*	*G. parvus*	Race I	Race II	Race III
G. crista		121.3 (12.6)	138.8 (14.5)	122.0 (12.8)	142.0 (14.8)	140.1 (14.6)	143.0 (14.9)	150.5 (15.7)
G. rossm	*210.2 (14.6)*		129.7 (13.5)	144.2 (15.2)	148.6 (15.5)	149.8 (15.6)	146.8 (15.3)	155.7 (16.2)
G. albus	*229.5 (16.2)*	*276.2 (19.2)*		147.7 (15.5)	141.3 (14.7)	142.5 (14.8)	139.7 (14.5)	145.8 (15.2)
G. riparius	*n.a.*	*n.a.*	*n.a.*		152.0 (16.0)	152.8 (16.1)	150.5 (15.8)	159.5 (16.8)
G. parvus	*226.2 (15.7)*	*270.6 (18.5)*	*106.8 (7.4)*	*n.a.*				
						Race I	42.5 (4.4)	49.8 (5.1)
						Race II		54.1 (5.5)

Numbers of base pair differences per sequence are shown, and uncorrected p-distances in percentages are given in parentheses. Sequence data not available for the particular construct are labelled n.a

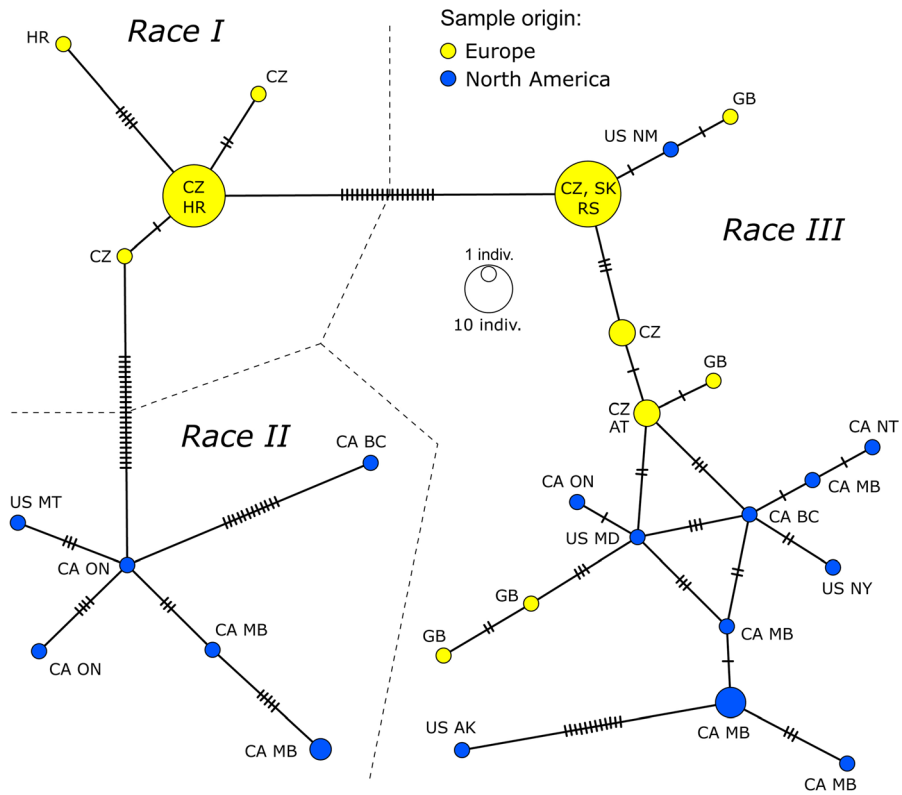

Fig. 3 Minimum spanning haplotype network of specimens G. parvus group analysed for Online Resource 2 based on the COI fragment. The clusters are color-coded to display the geographic origin of individuals. The origin of samples is shown by official country codes

While GenBank COI sequences (Online Resource 2) support the existence of another race within *G. parvus*, additional work across the reported range of *G. circumstriatus* will be required to test this hypothesis.

Hazard of an invasion at the population level

The pattern identified here with both native and exotic races of a species that can exist within a region is a

reminder that invasions can occur across many genetic scales. The well-known example of a race-level invasion, resembling our data, is the multiple introductions of the Eurasian race of the wetland grass *Phragmites australis* to North America. The existing rare native American genotype has stayed restricted to high-quality undisturbed sites, while the extremely aggressive non-native Eurasian race has overwhelmed many disturbed wetland sites (Saltonstall, 2002; Meyerson & Cronin, 2013). Similar examples of non-native genotypes have been reported across taxonomic spectra, including freshwater molluscs (Genner et al., 2004). In most of these cases, non-native races outcompete the native race and/or threaten the original genotypes by hybridization (e.g. Genner et al., 2004; Meyerson & Cronin, 2013; Raw et al., 2013).

In general, competition on an interspecific level is assumed to be rare in aquatic gastropods (Dillon, 2000; Raw et al., 2013; but see Richards et al., 2001) because non-native snails in the invaded areas utilize mainly free niches of anthropogenically modified and disturbed habitats (Cope & Winterbourn, 2004). Invasion on an intraspecific level might have more direct consequences on the native species in question than the invasion of an unrelated species. In our data, the existence of highly diverse *G. parvus* haplotypes (Fig. 3) suggests multiple introductions from North American populations to Europe. Multiple introductions of an invasive species were observed to increase invader success through increased propagule pressure, genetic diversity (Schierenbeck & Ellstrand, 2009), and potential adaptation to local conditions (Roman, 2006). The introduction of the non-native North American race also opens an important issue of parasitic infection. Aquatic gastropods are common intermediate hosts of many trematodes, including avian schistosomes, causing cercarial dermatitis (swimmer's itch) reported also for *G. parvus* in North America (Laman et al., 1984). Schistosome cercariae were observed to attack non-native *G. parvus* populations also in the invaded areas of Europe (Obwaller et al., 2001; Duras, 2014), with even a risk of introducing non-native schistosomes (Duras, 2014). As schistosome cercariae may also attack humans, the use of molluscicides to control the snail intermediate host in swimming areas is being commonly applied (Leighton et al., 2000; Duras, 2014). These interventions, however, may also damage populations of native mollusc species as an indirect consequence of the abundant presence of the non-native *G. parvus* race.

Possible course of the invasion inferred from genetic data

The data presented may explain the puzzling existence of large stable populations of *G. laevis* reported from the UK (Kerney, 1999). Available COI sequence data from the UK all lies within the mtDNA clade that includes all North American and exotic European material—including those collected from near the *G. parvus* type locality along the Delaware River near Philadelphia. Because the *G. laevis* type locality was a highly modified wetland associated with abandoned quarries near Newcastle (Alder, 1838), it is possible that "*G. laevis*" is based on an early introduction of the North American race into the UK. This is in line with early twentieth-century shells in museum collections, including from Brora, canals at Burnley, and "near London" being initially identified as *G. parvus* (Rowson et al., 2021). Two possible non-mutually exclusive mechanisms for this can be advanced: First, the North American race was carried to the UK over the last half-millennium in freshwater ballast within the holds of transcontinental ships (Ville, 1988). From this, it was able to rapidly expand into a wide range of natural and artificial habitats (Kerney, 1999) via migratory waterfowl or human-assisted vectors (Van Leeuwen et al., 2013), representing a cryptic invasion. Second, it is possible that the UK was naturally colonized from North America via migrating waterfowl (Van Leeuwen et al., 2012) in the early to mid-Holocene. The existence of subfossil British shells of the *G. laevis* phenotype (Rowson et al., 2021) favours the former hypothesis—that of introduction in recent centuries. However, the detection of multiple haplotypes in the UK, from only four analysed individuals, may suggest repeated introductions. To address these hypotheses will require substantial genetic screening of modern and old museum of *G. parvus*/*laevis* material from across Britain and Ireland.

Both *G. parvus* races appear to have originally possessed different ranges, with the North American race being known on the continent from at least as far back as the mid-Pliocene (Taylor, 1966). *Gyraulus parvus* is also a common late glacial fossil in the Great Lakes region from marl lake sediments of recently

deglaciated terrains (Yu, 2000). The European race has been reported as a common Pleistocene and Early Holocene fossil across Europe (Ložek, 1964; Meier-Brook, 1984; Kerney, 1999; Gittenberger et al., 2004a, b; Hošek et al., 2017). Because we were unable to secure tissue samples from "*G. laevis*" populations that range across central Asia east to Kamchatka (Zhadin, 1952; Meier-Brook, 1983), we cannot assess the complete range of the European race at this time. It seems at least possible that it represents an originally Siberian element that expanded west into Europe during the full glacial, given that identically appearing fossil shells are known from European glacial loess sediments (Ložek, 1964). However, the general range of fossil and extant sites reminds us of a number of land snails that were more common in Europe during the last glacial period which today reside in central Asian mountain ranges (e.g. *Pupilla alpicola*, *P. loessica*, *Vallonia tenuilabris*, *Vertigo parcedentata*, *V. pseudosubstriata*; Horsák et al., 2015). We would thus not be surprised to discover that this race actually extends east into western and central Beringia, with native European populations representing glacial relicts at the far western range limit of the race. Conservation of the native *G. laevis* might then need to grapple with twin threats: long-term warming coupled with a cryptic, intraspecific invasion.

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Author contributions EL, VH, JCN and MH contributed to the conception and design of the study. Material preparation and data collection were performed by all authors. VH, MN and EL ran the analyses. The first draft of the manuscript was written by EL and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability Data are uploaded and available in the GenBank database.

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Informed consent All authors consent to the publication being submitted for peer review.

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