

INVADING NON-NATIVE POPULATIONS REPLACE NATIVE ONES OF THE ENDANGERED FRESHWATER SNAIL *THEODOXUS FLUVIATILIS* IN THE RIVER RHINE

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ABSTRACT

In the past, the freshwater snail *Theodoxus fluviatilis* was abundant in the river Rhine, but was considered to be extinct in the Upper and High Rhine in 2004. We found individuals of *T. fluviatilis* for the first time at sites in the southern part of the Upper Rhine and High Rhine in 2020, where this species went extinct 16 years ago. There are several distinct haplotypes of this species; some of them occurring in geographically separated regions of Europe. We used mitochondrial DNA (cytochrome c oxidase subunit I, COI) to trace the origin of the newly arrived *T. fluviatilis*. We examined 269 individuals collected at 29 sites and compared their sequence data with known haplotypes of *T. fluviatilis* and other species of *Theodoxus* deposited in GenBank. We also analysed a historical sample from 1995 (collected before the species went extinct), which revealed that the haplotype of the native *T. fluviatilis* in the High Rhine at that time was F28. In the recent samples, however, we found *T. fluviatilis* with the haplotypes F31 and “euxinus”, which are reported in the Ponto-Caspian region. Both haplotypes are cryptic invaders, colonizing the Rhine via the Rhine–Main–Danube canal and adversely affecting the remaining native populations of *T. fluviatilis* in Western Europe. Our findings are important for this species management by nature conservation authorities: lineages of endangered species should be preserved and supported, while the spreading of invasive, non-native lineages (or species) should be prevented.

Keywords: biological invasion; COI-sequencing; conservation; cryptic invader; non-native haplotype; Ponto-Caspian origin; tracing non-native species

Introduction

The world's freshwater ecosystems are significantly affected by biological invasions, with non-native taxa nowadays making up a large fraction of the species, individuals and biomass (Haas et al. 2002; Ojaveer et al. 2002; Strayer 2010). However, not all invasive taxa are easily identifiable. Cryptic invasions are defined as “the occurrence of a species or genotype that was not previously recognized as alien in origin or not distinguished from other aliens” (Novak 2011). There are two basic types of cryptic invasions. Firstly, *interspecific cryptic invasion* refers to the invasion of a non-native species that goes unnoticed due to misidentification as a native or another non-indigenous species, with which it is often closely related (Morais and Reichard 2018). Secondly, *intraspecific cryptic invasion* refers to the invasion of another lineage of a species into a region where a distinct native lineage of the same species already exists. Cryptic invasions form a minor part of current research on biological invasions, despite their potential to influence native species and affect native communities to a similar extent as standard biological invasions (Morais and Reichard 2018).

Molecular markers and mitochondrial DNA sequence data have frequently been used to trace the origin and spread of cryptic invaders in freshwater bodies (Morais and Reichard 2018). Examples of intraspecific invasions are reported in several lakes and rivers in Africa by non-native genotypes of the freshwater gastropod *Melanoides tuberculata* from Asia (Van Bocxlaer et al. 2015), in Western and Central Europe by non-native races of the freshwater snail *Gyraulus parvus* from North America (Lorenková et al. 2021) and the Atlantic by distinct haplotypes of the hydrozoan *Gonionemus vertens* from the Pacific (Govindarajan and Carman 2016).

Theodoxus fluviatilis (Linnaeus, 1758) is a neritid freshwater snail with a distribution extending from Western Russia to Iberia and from southern Scandinavia to the Balkan peninsula and north Africa (Morocco; Zettler et al. 2004). Individuals attach themselves to a hard substrate, such as rocks, cobbles or other pieces of stone, in calcium-rich waters (Fretter and Graham 1962). This species is dioecious with a life cycle lasting 2–3 years (Kirkegaard 2006). Up to 100 eggs are laid in yellowish spherical capsules, which are attached to any hard substrate (Fretter and Graham 1962). Bunje (2005)

reports the geographical distribution of genetic lineages of *T. fluviatilis* in Europe. Sequence analysis of mitochondrial cytochrome c oxidase subunit I (COI) reveal that this snail has geographically nested clades displaying a distribution consistent with fragmentation and restricted dispersal. Certain regions in Europe harbour distinct native haplotypes. For example, the haplotypes F01 and F03 occur in central and northern Germany, while the haplotype F28 is only recorded at two sites in western France (Bunje 2005).

In the river Rhine and its tributaries, *T. fluviatilis* was the only abundant species of neritid until 1920 (Zettler 2008). In the following decades, its abundance decreased dramatically, most probably due to severe water pollution, resulting in the near extinction in the 1970s (Meinert and Kinzelbach 1985). During the period 1970–1986, numerous wastewater treatment plants were constructed along the river, resulting in improvements in water quality (Baur and Schmidlin 2007). Populations of *T. fluviatilis* partly recovered in the Upper and High Rhine (Kinzelbach 1987). However, another dramatic decrease occurred around the year 2000. In an attempt to preserve the native *T. fluviatilis* in Switzerland, a founder sample was translocated from the High Rhine into Lake Zurich in 1997 (Müller and Steinmann 2008). It is claimed that *T. fluviatilis* was extinct in the High Rhine and the southern part of the Upper Rhine by 2004 (Rey et al. 2005). In Switzerland, *T. fluviatilis* is included on the Red List as critically endangered (Rüetschi et al. 2012), while it is highly threatened in Germany (Jungbluth and Knorre 2011). Nature conservation authorities are obliged to preserve and support populations of species on Red Lists. In contrast, in cases of invasive, non-native species or lineages, nature conservation authorities are obliged to prevent their further spread.

In 2006, *T. fluviatilis* was refound in the Upper Rhine near the mouth of the river Main in Germany (Westermann et al. 2007). It was assumed it recolonized the Rhine via the Rhine–Main–Danube Canal, which was opened in 1992 (Westermann et al. 2007). Indeed, isolated populations of *T. fluviatilis* were first recorded in Germany in the Danube near Regensburg in 2005, which rapidly spread in succeeding years (Hirschfelder et al. 2011). Genetic analysis revealed that the haplotype in these individuals is F31; a haplotype described from Ukraine near the Black Sea and, at that time, not yet recorded in rivers in Western Europe (Bunje 2005; Hirschfelder et al. 2011). Gergs et al. (2015) confirm the presence of haplotype F31 in all individuals examined in the river Rhine between Cologne and Karlsruhe in 2010–2013 and claim that the re-established *T. fluviatilis* populations that consist exclusively of the non-native haplotype F31 are intraspecific cryptic invaders in the river Rhine. In 2020, individuals of *Theodoxus* were recorded in both the southern part of the Upper Rhine and the High Rhine at sites where the native *T. fluviatilis* went extinct 16 years ago (present study).

We aimed to determine the source of the newly established *Theodoxus* populations in the southern part of the Upper Rhine and High Rhine and discuss the consequences for the conservation of this species. We analysed the mitochondrial DNA-sequence of the cytochrome c oxidase subunit I gene in each individual and compared the molecular sequences with those of individuals sampled at one site in the High Rhine before its assumed extinction and with other haplotypes of *T. fluviatilis* deposited in the NCBI/GenBank (www.ncbi.nlm.nih.gov) and the COI sequences of other species of *Theodoxus* (*T. danubialis* (C. Pfeiffer, 1828), *T. danasteri* (Lindholm, 1908) and *T. velox* V. Anistratenko, 1999) occurring in the Ponto-Caspian region (Sands et al. 2019, 2020). We also aimed to examine the wider geographical distribution of the native haplotype of *T. fluviatilis* formerly occurring in the High Rhine, which is unknown. In particular, we addressed the following questions: (1) Which haplotypes are present in the newly established populations of *T. fluviatilis* in the High Rhine and southern part of the Upper Rhine? (2) Which haplotype was present in the original, native population (now considered extinct) in the Upper Rhine and what is the distribution of this haplotype? (3) Was it possible to preserve *T. fluviatilis* with the native haplotype from the High Rhine by translocating it into Lake Zurich? and (4) Can individuals of *T. fluviatilis* with different haplotypes be visually distinguished by differences in shell patterning?

Material and Methods

Sampling and sites

Individuals of *T. fluviatilis* were obtained from 29 sites in Switzerland, France and Germany (Table 1, Fig. 1). The High Rhine emerges from Lake Constance, flows west, mainly on the border of Germany and Switzerland (Fig. 1A). In Basel, it flows to the north (now called Upper Rhine) and forms the southern part of the border between France and Germany. Then the Rhine merges with one of its main tributaries, the river Main. Cargo shipping on the Rhine is possible from Rotterdam (North Sea) to Rheinfelden, 20 km upstream of Basel (Baur and Schmidlin 2007). The Rhine–Main–Danube Canal connects the Rhine via the Danube with the Black Sea which, in turn, is connected by canals and rivers to the Caspian Sea. The Altrhein (site 22) is a remnant of the former Upper Rhine north of Basel running parallel to the Grand Canal d'Alsace. The Altrhein has a close to natural river bed and is fed with water from the Upper Rhine with a minimum discharge of 50 m³ per second (Wirth et al. 2010). However, when the flow of the Upper Rhine exceeds 1,400 m³ per second, surplus water is released through the Altrhein, thereby causing great fluctuations in discharge and water level.

Samples were hand collected in shallow water, except at sites 8 and 29, where SCUBA-divers collected

snails at a depth of 1–4 m. Individuals were preserved in 80%-ethanol before being photographed and utilized for DNA-extraction. We determined the geographical coordinates of the sites sampled using a GPS receiver and topographical maps (scale 1 : 25,000). For each individual sampled at sites 22 and 24, we recorded on which stone it was attached. In combination with the COI-sequences, this allowed an examination of the small-scale spatial distribution of different haplotypes (whether they occur separately on different stones or co-occur on stones).

Translocation of *T. fluviatilis* into Lake Zurich

In an attempt to preserve *T. fluviatilis* in Switzerland, 100–200 individuals were translocated from the High Rhine into Lake Zurich in October 1997 (Müller and Steinmann 2008). In the first years, the translocated native snail population grew exponentially partly with the aid of further translocations, both within the lake and the rivers Limmat and Sihl, with this species extending its distribution over almost the entire Lake Zurich (Müller 2016). After 2015, however, the populations declined and *T. fluviatilis* was rare in Lake Zurich in 2020 (P. Müller, unpubl. data). To examine whether the original haplotype of *T. fluviatilis* that is assumed to have become extinct in the High Rhine is still present in Lake Zurich, we determined the haplotypes of the individuals in the translocated population collected at various sites in 2013 and 2020 (Table 1).

DNA extraction, amplification and sequencing

We isolated total genomic DNA from each individual using a slightly modified CTAB-method (Doyle and Doyle 1987). DNA quantity and quality were measured using a NanoDrop (NanoDrop Technologies Inc., Wilmington, USA) and standardized for genetic analyses. A partial sequence of the mitochondrial COI gene (600 bp) was amplified by polymerase chain reaction (PCR) using the primers F4d (5'-TACTTTTRTATATATGTTTGGT-3') and R1d (5'-TGRTAWARAATDGGRTCWCCHCCVCC-3'; Bunje 2005). PCR reactions (40 µl) consisted of 10 µl of template DNA (20–25 ng/µl), 8 µl Master Mix (5x FIREPOL Master Mix, Solis BioDyne, Estonia), 1 µl of each primer (10 µM) and 20 µl sterile water. Amplification was done in an Eppendorf Mastercycler Pro (Vaudaux-Eppendorf AG, Schönenbuch, Switzerland) under the following conditions: initial 10 min. heat activation step at 95 °C, followed by 36 amplification cycles of denaturation at 95 °C for 50 seconds, annealing at 54 °C for 60 seconds and extension at 72 °C for 60 seconds, with a final extension step at 72 °C for 7 min. PCR products were cleaned-up using the NucleoSpin gDNA Clean-up kit (Macherey-Nagel, Oensingen, Switzerland) and sequenced by Macrogen Inc. (Amsterdam, The Netherlands) using a capillary ABI 3730 XL DNA Analyser (Applied Biosystems, Foster City, CA, USA).

The systematics and distribution of *Theodoxus* in the Ponto-Caspian region, the presumed source of the invaders, are not yet entirely resolved (Anistratenko et al. 2020). For example, recent molecular studies support the synonymy of *T. fluviatilis* and *Theodoxus euxinus* (Clessin, 1886) (Bunje and Lindberg 2007; Sands et al. 2019, 2020), while in the DNA-barcoding system *T. euxinus* is still considered as a distinct species (Barcode of Life Data System 2021). In our study, we considered the various haplotypes of *T. fluviatilis* and other *Theodoxus* species described in the literature (Bunje 2005; Bunje and Lindberg 2007; Gergs et al. 2015; Sands et al. 2019, 2020). Given the uncertain status of *T. euxinus*, we refer to its haplotype as *T. fluviatilis* haplotype “euxinus”.

We edited all sequences using CodonCode Aligner software (Codon Code Corporation, Centerville, MA, USA). Using the BLASTn tool, the molecular sequences of the individuals online were aligned to the sequences of the haplotypes of *T. fluviatilis* deposited in the NCBI/GenBank (www.ncbi.nlm.nih.gov) as well as to those of other *Theodoxus* species occurring in the Ponto-Caspian region (*T. danubialis*, *T. danasteri*, *T. velox*; Sands et al. 2019, 2020). Molecular sequences with at least 99.5% similarity to a described haplotype were considered as belonging to this haplotype. In total, we sequenced the COI from 269 individuals.

The haplotypes F28 (accession no AY765331) and TR01 (accession no KJ493817) of *T. fluviatilis* deposited in NCBI/GenBank differ in 3 base pairs (bp) from each other. Only a single COI sequence is stored from both haplotypes in GenBank. Thus, so far no information exists on the COI sequence variation in these haplotypes.

Periostracum patterning

The periostracum is the thin outer layer of the snail shell, composed of conchiolin, a type of protein. The periostracum colouration and its pattern are highly variable in *T. fluviatilis* (Zettler et al. 2004). The periostracum can be ornamented with a pattern of white drop-like spots on a dark-brown, black or redish background. In other individuals the periostracum is ornamented with zigzag stripes, while still others exhibit different combinations of white drop-like spots and zigzag stripes (Zettler et al. 2004). We analyzed the periostracum patterning in a “historical” sample of *T. fluviatilis* collected in the High Rhine near Augst (CH) in August 1997 before its presumed local extinction ($n = 50$) and in two recent samples obtained from the Altrhein (site 22, $n = 28$, collected on 15 September 2020; Table 1) and from the High Rhine (site 24, $n = 28$, collected on 14 September 2020). We photographed the shells of the snails using a digital microscope Keyence VHX-6000 (Keyence Corporation, Osaka, Japan). We then assigned each snail based on its periostracum patterning to one of five categories defined by Hirschfelder et al. (2011): D = ornamented with white drop-like spots (Fig. 3A); Z = pattern of dark zigzag

stripes (Fig. 3B, C); DZ = a combination of D and Z (Fig. 3D); HL = a combination of horizontal and longitudinal stripes; and M = monochrome, no particular ornamental pattern (this category did not occur in our samples). A few snails could not be assigned to one of these categories due to an eroded periostracum. Molecular sequencing of

the individuals made it possible to assign the periostracum patterning to the various haplotypes of *T. fluviatilis*.

Data analyses

Phylogenetic reconstruction of DNA-sequences of *T. fluviatilis* found in the river Rhine was done using the

Table 1 Overview of the sites of *Theodoxus fluviatilis* sampled, numbers of individuals examined and assigned to each haplotype.

Site sampled (waterbody, country)	Coordinates (N / E)	Altitude (m a. s. l.)	Date sampled	Number of individuals examined	Haplotype (No. of individuals assigned to haplotype) ⁵
1 Muttenz, Schweizerhalle (river Rhine, CH)	47.53265 / 7.67507	261	15.02.1995	5	F28 (5)
2 Zurich Riesbach, Höschgasse (lake Zurich, CH) ¹	47.35591 / 8.54916	405	17.05.2013	6	F28 (6)
3 Zurich Riesbach (lake Zurich, CH) ¹	47.35868 / 8.54712	405	04.09.2013	30	F28 (17), TR01 (12), F03 (1)
4 Zurich near Mythenquai (lake Zurich, CH) ²	47.35539 / 8.53617	405	17.05.2013	5	F28 (5)
5 Küsnacht near Goldbach (lake Zurich, CH) ³	47.32886 / 8.57284	405	16.05.2013	6	F28 (6)
6 Zurich, Höhe Schipfe (river Limmat, CH) ⁴	47.37257 / 8.54184	404	16.05.2013	6	F28 (6)
7 Zurich, near Gessnerbrücke (river Sihl, CH)	47.37661 / 8.53597	405	01.11.2020	25	F28 (9), TR01 (11), nd ⁶ (5)
8 Plobsheim, Ancienne gravière (quarry pond, F)	48.47831 / 7.74250	149	07.06.2015	20	F01 (13), F03 (6), F31 (1)
9 Montbozon, Haute-Saône (river Ognon, F)	47.46338 / 6.26415	241	31.05.2017	3	F28 (3)
10 Cenans, Haute-Saône (river Ognon, F)	47.42856 / 6.19654	232	30.05.2017	3	F28 (3)
11 Parcey, Doubs (river Loue, F)	47.01531 / 5.49436	196	22.06.2017	3	F28 (3)
12 Rahon, Doubs (river Orain, F)	46.98552 / 5.44935	194	22.06.2017	3	F28 (3)
13 Labeaume, Ardèche (river Beaume, F)	44.44952 / 4.30137	118	04.09.2017	3	F28 (3)
14 St. Martin-d'Ardèche (river Ardèche, F)	44.29965 / 4.56853	42	05.09.2017	3	F28 (3)
15 Méjannes-le-Clap, Gard (river Cèze, F)	44.26133 / 4.35057	95	14.09.2017	3	F28 (3)
16 Montclus, Gard (river Cèze, F)	44.26304 / 4.41988	84	05.09.2017	3	F28 (3)
17 St. André-de-Roquepertuis, Gard (river Cèze, F)	44.24816 / 4.44963	78	07.09.2017	3	F28 (3)
18 Goudargues, Gard (river Cèze, F)	44.20915 / 4.46955	69	13.09.2017	3	F28 (3)
19 Goudargues, Gard (river Source de Goudargues, F)	44.21574 / 4.46927	81	07.09.2017	3	F28 (3)
20 Sauve, Gard (river Vidourle, F)	43.94603 / 3.94701	94	06.09.2017	3	F28 (3)
21 Collias, Gard (river Gardon, F)	43.95289 / 4.48176	27	12.09.2017	3	F28 (3)
22 Istein, Altrhein (river Rhine, Ger)	47.66416 / 7.52127	230	15.09.2020	36	F31 (9), "euxinus" (27)
23 Basel, St. Alban-Rheinweg (river Rhine, CH)	47.55529 / 7.59780	248	17.09.2020	5	F31 (2), "euxinus" (3)
24 Basel, Rheinhalde (river Rhine, CH)	47.56059 / 7.62088	248	14.09.2020	32	F31 (24), "euxinus" (8)
25 Muttenz, Fuchsloch (river Rhine, CH)	47.53736 / 7.66400	261	17.09.2020	6	F31 (6)
26 Muttenz, Schweizerhalle (river Rhine, CH)	47.53370 / 7.67150	261	10.08.2020	12	F28 (1), F31 (11)
27 Pratteln, Rheinlehne (river Rhine, CH)	47.53176 / 7.68315	261	10.08.2020	10	F31 (10)
28 Pratteln, Saline (river Rhine, CH)	47.53173 / 7.68435	261	10.08.2020	7	F31 (7)
29 Stein am Rhein, Schiffflände (river Rhine, CH)	47.65982 / 8.85686	396	16.05.2020	19	F28 (18), F31 (1)

¹ 100–200 individuals collected in the river Rhine near Augst (CH; 47.53958 N, 7.71823 E) on 11.10.1997 were released in Lake Zurich (47.35591 N, 8.54924 E) on 12.10.1997

² collected in lake Zurich (CH; Zurich Riesbach, Höschgasse, 47.35591 N, 8.54916 E), released at this site on 28.05.2008

³ collected in lake Zurich (CH; Zurich Riesbach, Höschgasse, 47.35591 N, 8.54916 E), released at this site on 03.06.2008

⁴ collected in lake Zurich (CH; Zurich Riesbach, Höschgasse, 47.35591 N, 8.54916 E), released at this site on 26.06.2008

⁵ COI-sequences were assigned to haplotypes deposited in GenBank using the BLASTn tool; GenBank accession no. F28 AY765331; TR01 KJ493817; F01 AY765306; F03 AY765308; F31 AY765336; "euxinus" AY771300

⁶ could not be determined

package *phangorn* (Schliep 2011) in the statistical software R (version 3.6.3; R Core Team 2020). A maximum likelihood (ML) analysis was conducted that included all unique haplotypes with the substitution model TPM3u + I following Gergs et al. (2015). By using the *pml* function of the package *phangorn*, the likelihood of a phylogenetic tree based on given sequence alignments was computed and optimized with the function *optim.pml*. Support for reconstructed branches of the ML tree was estimated by bootstrapping with 1,000 replicates.

We used Contingency-tests to compare frequency distributions of periostracum patterns between native *T. fluviatilis* ("historical" sample) and invading *T. fluviatilis* (haplotypes F31 and "euxinus") and between the frequencies of the haplotype F31 and haplotype "euxinus". For these analyses we pooled individuals of the haplotype F31 collected at sites 22 and 24 as well as individuals of the haplotype "euxinus" from these two sites.

Results

Haplotypes recorded

We found that 97 individuals of *T. fluviatilis* belong to the haplotype F28. Analysis of the COI-sequence indicat-

ed that 78 individuals had a base-pair sequence identical to the sequence of F28 deposited in GenBank, while the sequence of 12 individuals differed in 1 bp, that of two individuals in 2 bp, that of three individuals in 3 bp and that of two individuals in 4 bp from the sequence in GenBank. Using the Alignment Megablast procedure of GenBank (www.ncbi.nlm.nih.gov), a query cover of 99% was found between the haplotypes F28 and TR01 with an identity of 99.8 ± 0.2 (\pm SE, range in bp difference: 0–5, median = 2). The query cover between TR01 and F28 was 99% (identity: 99.5 ± 0.3 , range in bp difference: 0–3, median = 1). Based on the small variation in the molecular sequence, the newly described haplotype TR01 (Gergs et al. 2015) can be considered as almost identical to the formerly described haplotype F28 (Bunje 2005) and thus as the original haplotype of *T. fluviatilis* in the High Rhine. In contrast, F01, F03 and F31 are distinct haplotypes of *T. fluviatilis*, differing by more than 5 bp from the haplotypes F28 and TR01. Furthermore, the haplotype "euxinus" collected in our study has a distinct, unique COI-sequence (accession no AY771300), which clearly differs from the other haplotypes of *T. fluviatilis* (Bunje 2005; Gergs et al. 2015; Sands et al. 2019, 2020). The BLAST tool also revealed that none of the COI-sequences of the 269 individuals examined could be assigned to the

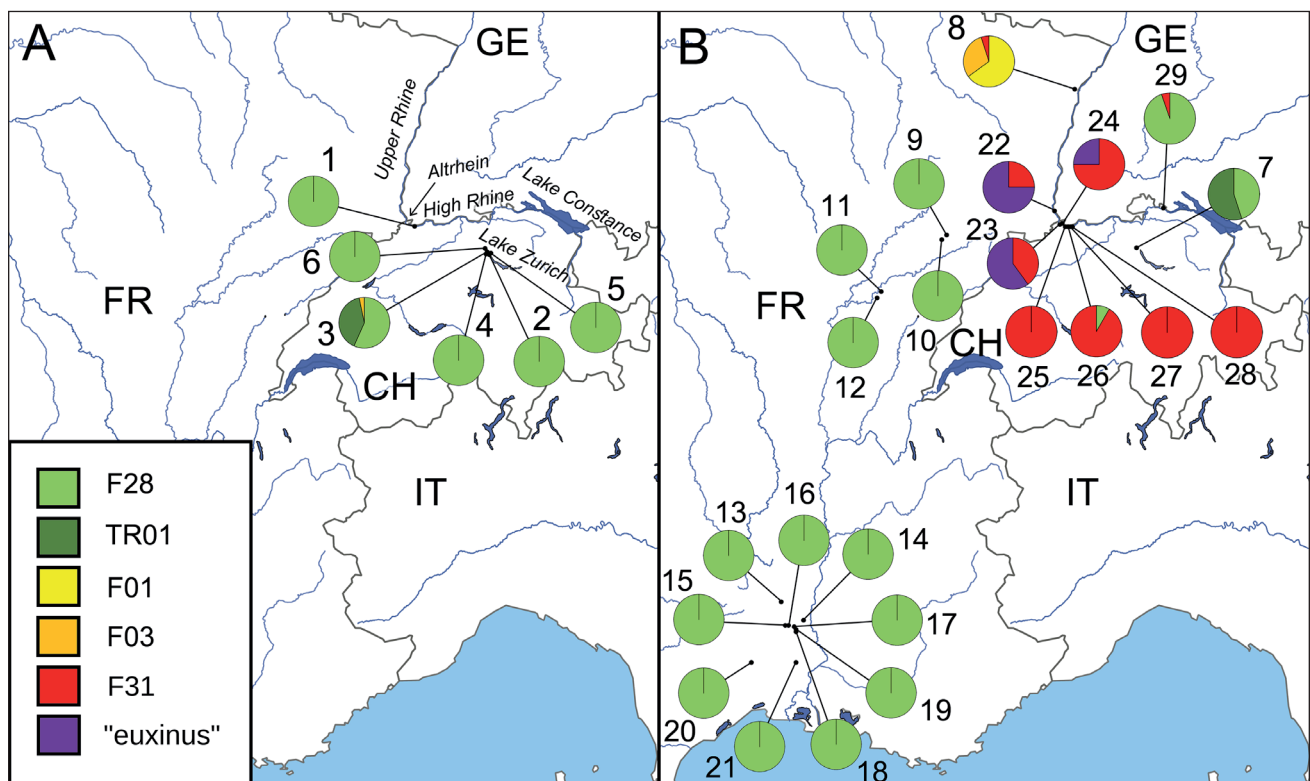


Fig. 1 Frequency distribution of *T. fluviatilis* haplotypes (in %) determined by COI-sequencing at each site sampled in this study. **A** Historical distribution of *T. fluviatilis* haplotypes at site 1 in 1995 (before the assumed extinction of the species in the High Rhine) and of populations translocated from this site into Lake Zurich in 1997 (analysed individuals were sampled in 2013). **B** Recent distribution of *T. fluviatilis* haplotypes. The originally native *T. fluviatilis* with haplotype F28 in the High Rhine was replaced by the invasive haplotypes F31 (sites 22–28) and "euxinus" (sites 22–24) in 2020. The native haplotype F28 can still be found in the High Rhine near Lake Constance (site 29), in several rivers in southwestern France (sites 9–21) and in the river Sihl near Lake Zurich (descendants of the translocated individuals). Site 8 (a quarry pond) harbours *T. fluviatilis* haplotypes found in central and northern Germany and the invasive haplotype F31. Sample sizes (number of individuals sequenced) and sampling dates are given in Table 1.

sequences of any other *Theodoxus* species deposited in GenBank.

Native haplotype in the High Rhine and its distribution

All individuals sampled in the High Rhine in 1995 before its presumed extinction 9 years later belonged to the native haplotype F28 (Table 1; site 1). Bunje (2005) found this haplotype at two sites in western France, but did not examine any specimens from the Upper Rhine or High Rhine. We extended our survey to rivers in southeastern France (e.g. to the river Ardèche and river Cèze; Table 1). All 39 individuals sequenced from sites 9–21 belonged to the haplotype F28, extending the so far known distribution of this haplotype considerably (Fig. 1). The majority of the descendants of individuals translocated from the High Rhine into Lake Zurich belonged to haplotype F28 (sites 2–6; Fig. 1). However, at site 3, the haplotypes F03 and TR01 were also recorded in Lake Zurich. The original haplotypes F28 and TR01 of *T. fluviatilis* from the High Rhine could still be detected in the river Sihl a few hundred metres from Lake Zurich (site 7) 23 years after their introduction into Lake Zurich.

Intraspecific cryptic invasion

COI-sequencing revealed that the newly established *T. fluviatilis* in the Upper and High Rhine belonged either to haplotype F31 or haplotype “euxinus”, indicating that these intraspecific invaders have now reached the upper sections of the Rhine (sites 22–29; Fig. 1). We detected the invasive haplotype F31 at low frequency also in a quarry 600 m from the Upper Rhine (site 8) and in the High Rhine where it emerges from Lake Constance (site 29; Fig. 1, Table 1). In contrast, individuals with haplotype “euxinus” were only recorded in sections of the Rhine downstreams of the dam of the hydroelectric power plant at Birsfelden near Basel (Fig. 1), indicating that individuals with this haplotype arrived later.

The ML analysis for phylogenetical reconstruction revealed that individuals of the invading haplotype F31 belong to the Danubian group with haplotypes F38 (originally found in Ukraine; Bunje 2005) and MT563453 (recorded as a newly arriving haplotype in the northern part of the Upper Rhine in 2018; Rothmeier et al. 2021) (Fig. 2). The invading haplotype “euxinus” is phylogenetically close to F30 (Fig. 2), a haplotype originally recorded in Ukraine (Bunje 2005). In contrast, F28, the native haplotype of *T. fluviatilis* in the High Rhine, is phylogenetically close to the haplotypes F22 and F23 (Fig. 2), both recorded in Northern Italy. The phylogenetical relationship of these three haplotypes can partly be explained by our new finding that the geographical range of haplotype F28 includes southeastern France (see above).

Periostracum patterning

Native *T. fluviatilis*, represented by a historical sample from 1997 at a site in the High Rhine, mainly had white drop-like spots on their shells (Figs 3A, 4), while shells of

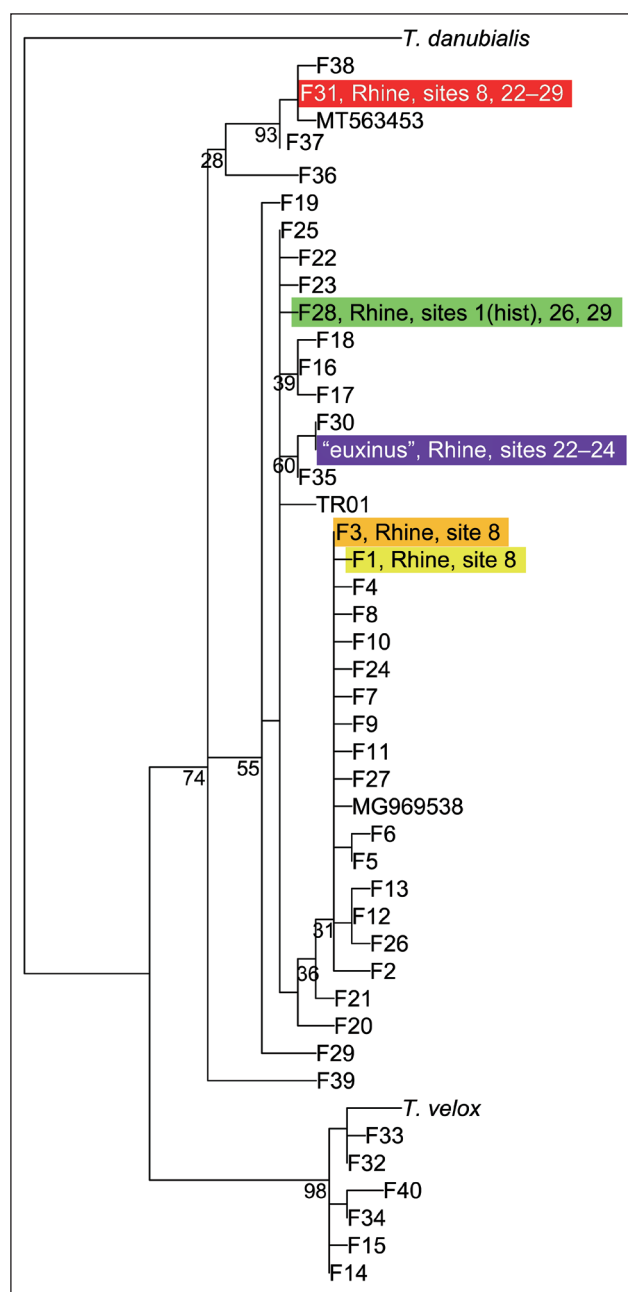


Fig. 2 Maximum likelihood tree of unique haplotypes of *T. fluviatilis* recorded in the river Rhine, rooted with the haplotype of *Theodoxus danubialis* as outgroup. Only bootstrap values ($n = 1,000$) of main branches are presented. See Table 1 for detailed information on the haplotypes found at the sites sampled. For further information on haplotypes and classification of groups see Bunje (2005), Gergs et al. (2015) and Rothmeier et al. (2021).

the invading *T. fluviatilis* (haplotypes F31 and “euxinus”) were characterized by dark zigzag stripes or a combination of zigzag stripes and drop-like spots (Figs 3B–D, 4). Individuals of both invading haplotypes differed significantly in periostracum patterning from native *T. fluviatilis* in the historical sample (Fig. 4A). However, invading individuals of the haplotypes F31 and “euxinus” did not differ in periostracum patterning (Fig. 4B).

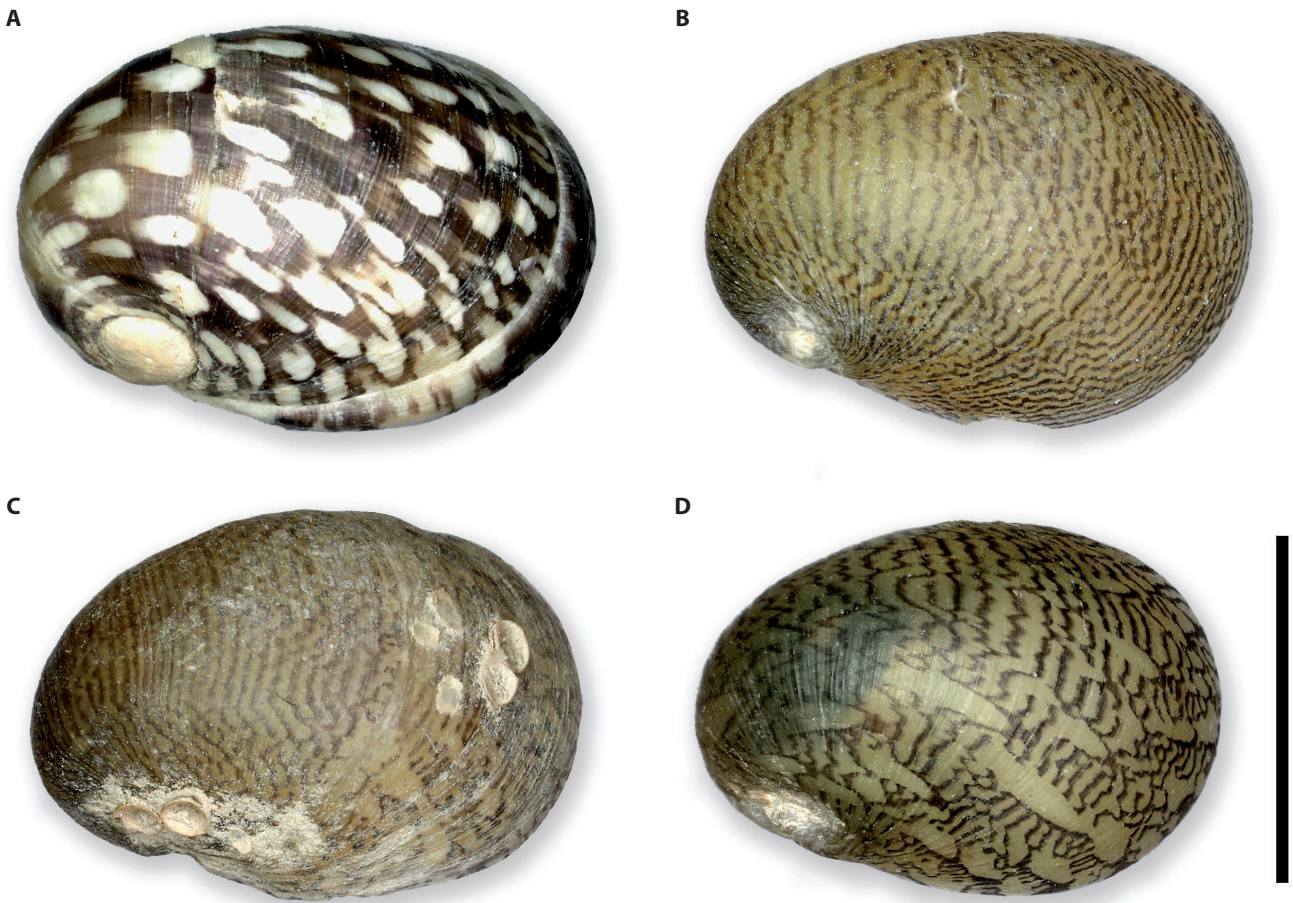


Fig. 3 Shells of *T. fluviatilis* from different sites. **A** Native *T. fluviatilis* of a historical sample from the High Rhine near Muttenz, CH (site 1; coll. P. Müller in August 1997). **B** Non-native haplotype “euxinus” from the Altrhein near Istein, GER (site 22; coll. B. Baur on 15.09.2020). **C** Non-native haplotype F31 from the High Rhine, Rheinhalde, Basel, CH (site 24; coll. B. Baur on 14.09.2020). **D** Non-native haplotype “euxinus” from the Altrhein near Istein, GER (site 22; coll. B. Baur on 15.09.2020). Scale bar indicates 5 mm.

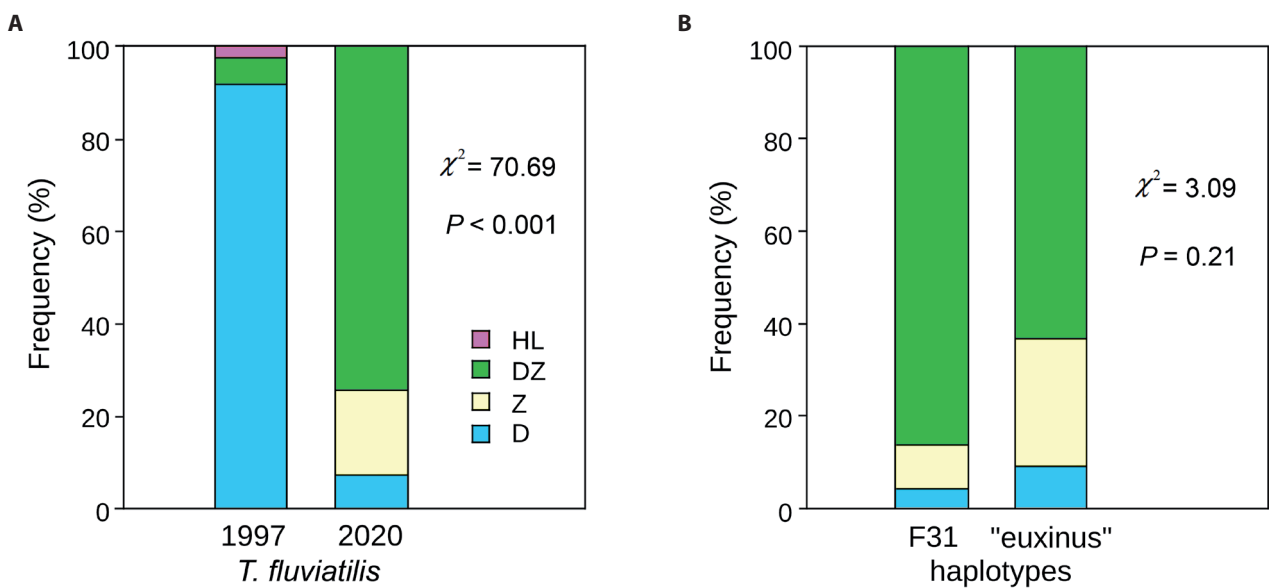


Fig. 4 Comparison of frequency distributions of periostracum patterning in *T. fluviatilis*. **A** Native *T. fluviatilis* from the High Rhine (collected in 1997 before the presumed local extinction of the species; $n = 50$) versus invading *T. fluviatilis* (haplotypes F31 and “euxinus” combined; $n = 44$) from the Upper Rhine and High Rhine in 2020. **B** Haplotype F31 (sites 22 and 24 combined; $n = 22$) versus haplotype “euxinus” (sites 22 and 24 combined; $n = 22$) from the Upper Rhine and High Rhine in 2020. D = white drop-like spots, Z = zigzag stripes, DZ = a combination of D and Z, and HL = a combination of horizontal and longitudinal stripes. The single individual with HL-type was not included in the statistical analysis.

Discussion

This study revealed that the newly established *T. fluviatilis* populations in the southern part of the Upper Rhine and High Rhine exhibit the non-native haplotypes F31 and “euxinus”. Both haplotypes can be considered as cryptic invaders (cf. Gergs et al. 2015) with a Ponto-Caspian origin. The detection of the haplotype “euxinus” at three sites is the first record of this haplotype in Western Europe (Germany and Switzerland). The correct recognition of cryptic invasions is crucial to understanding their effect on assemblages of native species and ecosystems. However, the intrinsic difficulty of identifying cryptic invaders is that they are, by definition, camouflaged and cannot be distinguished morphologically from native species already present or from known native species that may occur in other water bodies (Morais and Reichard 2018).

There is evidence from breeding experiments that periostracum patterning in *T. fluviatilis* can be influenced by environmental conditions such as water quality, type of food and mineral intake (Neumann 1959). However, the invading individuals (haplotypes F31 and “euxinus”) found in the Rhine show identical periostracum patterns to those reported in the Danube in Austria in 2001 (Schultz and Schultz 2001) and near Regensburg (Germany) in 2005 (Salewski and Hirschfelder 2006), indicating little influence of the environmental conditions of the two river systems. We showed that the periostracum patterns of the invading *T. fluviatilis* haplotypes F31 and “euxinus” did not differ from each other. Thus, visual differentiation between the two invaders is not possible. However, the periostracum patterns of both invading haplotypes differed from that of *T. fluviatilis* originally native to the High Rhine (F28), confirming the results of a similar comparison made by Hirschfelder et al. (2011) in the Danube near Regensburg (Germany). It should be pointed out that this finding relates to frequency distributions of periostracum patterning based on many shells. On the basis of its periostracum pattern an individual snail cannot be assigned to one of the two non-native haplotypes.

The diversity of haplotypes recorded in this study can hardly be compared with those reported in earlier studies (Bunje 2005, 2007; Bunje and Lindberg 2007; Gergs et al. 2015; Sands et al. 2019, 2020), because the latter focused on phylogeographical aspects and were frequently based on small numbers of individuals sampled per site; haplotype information is sometimes only available for one specimen per locality. Bunje (2005) demonstrated the presence of genetically distinct groups within *T. fluviatilis* that are separated by major geographical features (mountain chains, river systems) in Europe. However, the elimination of natural barriers between water sheds often triggers the invasion of non-native species, among them also cryptic invaders (Morais and Reichard 2018). The linear environment of rivers, streams and small

connected lakes allows non-native species to migrate along channelled fluvial paths, thereby increasing their distribution. The opening of the Rhine–Main–Danube Canal in 1992 increased the invasion rate of non-native vertebrate and invertebrate species into the Rhine drainage (Bij de Vaate et al. 2002; Baur and Schmidlin 2007; Leuven et al. 2009). It is assumed that transportation by cargo ships is responsible for the rapid spread of the invasive *T. fluviatilis* haplotype F31 from the Ponto-Caspian region to western Europe (Westermann et al. 2007; Gergs et al. 2015), as might be the case for the haplotype “euxinus”. Hull fouling on commercial ships and recreational boats and transport of gravel are considered as important vectors for the spread of non-native gastropods in aquatic habitats (Mineur et al. 2007). In the Danube, the cryptic invader *T. fluviatilis* (later determined as haplotype F31 from the Ponto-Caspian region) was first recorded in Austria in 2001 (Schultz and Schultz 2001), 2005 in Germany near Regensburg (Salewski and Hirschfelder 2006), 2006 in the northern part of the Upper Rhine near Mainz (Westermann et al. 2007), 2012 in Karlsruhe (Gergs et al. 2015) and reached the High Rhine in 2020 (present study). One may assume that the spread of this invasive snail is less rapid upstream of Basel, where cargo shipping stops, as has been shown for other non-native invertebrates (Schmidlin and Baur 2007; Schmidlin et al. 2012). However, our data demonstrate that *T. fluviatilis* (haplotype F31) has already colonized a locality close to Lake Constance (site 29). The spread of this non-native haplotype cannot exclusively be explained by transport by commercial ships. For example, we also recorded *T. fluviatilis* (haplotype F31) in a quarry pond 600 m apart from the Upper Rhine. Passive dispersal by waterbirds could also contribute to the spread of *T. fluviatilis* (Frisch et al. 2007).

The rediscovery of the native *T. fluviatilis* haplotype F28 at site 26 in the High Rhine was rather surprising. It was assumed that it went extinct in the High Rhine in 2004 (Rey et al. 2005; Westermann et al. 2007). Our results indicate that a few individuals survived in this section of the river or recolonized it later. Another surprise was the discovery of the native *T. fluviatilis* haplotype F28 at site 29, where the High Rhine emerges from Lake Constance. At this site, no neritid snail was found during 37 dives between 2010 and 2018, until the first record on 16 May 2020 (P. Steinmann, unpubl. data). Furthermore, no individuals of *T. fluviatilis* were observed during eight dives at nearby localities in Lake Constance and in the High Rhine in the previous decade (P. Steinmann, unpubl. data). Subsequent dives revealed that it had spread at site 29 over a distance of 320 m along the river bank of the High Rhine in summer 2020. We do not know how *T. fluviatilis* established this new population in the High Rhine. Most interestingly, however, one out of 29 individuals exhibited the non-native haplotype F31 from the Ponto-Caspian region. Thus, the frequency of the cryptic invader’s haplotype was still low in this population in

summer 2020. Future investigations will reveal whether the non-native haplotype F31 will become more abundant and displace the native haplotype F28 at this site.

In the northern part of the High Rhine the non-native haplotypes occupy broader ecological niches than the native haplotypes, persisting even in anthropogenically modified and disturbed habitats such as harbours (Rothmeier and Martens 2019). Non-native haplotypes of *T. fluviatilis* may also have a higher tolerance of the increase in water temperature in the river Rhine, as reported for other invading molluscs from warm regions (Müller and Baur 2011). Furthermore, *T. fluviatilis* individuals of haplotype F31 were less infested by the trematode *Plagioparus* cf. *skrjabini* (also an invader from the Caspian region) than individuals of the native haplotype (Rothmeier et al. 2021). The presence of both non-native parasites and non-native hosts (or hosts of non-native haplotypes) outside their natural range may have an effect on native biodiversity.

There is increasing evidence that introduced plant and animal taxa frequently hybridize with native taxa, leading to a growing concern that these hybridizations may compromise the genetic integrity of native taxa to the point of causing extinctions (Largiadier 2007). Hybridization events can only be demonstrated by analysing nuclear DNA. We sequenced mitochondrial DNA, which is maternally inherited. We are therefore not able to answer the question whether invading *T. fluviatilis* individuals of haplotypes F31 and “euxinus” hybridize with native individuals of haplotype F28.

Conclusions

In addition to water quality and quantity, invasive species are an important conservation challenge in freshwater habitats (Lysne et al. 2008). In the case of *T. fluviatilis* in the High Rhine, individuals with the native haplotype F28 are of conservation concern and recorded in the Red Lists of Germany and Switzerland (Jungbluth and Knorre 2011; Rüetschi et al. 2012). Nature conservation authorities are obliged to preserve and promote these populations. In contrast, individuals with the haplotypes F31 and “euxinus” are exotic invaders. In this case, nature conservation authorities are obliged to prevent the further spread of these lineages. Removing non-native species or strains from aquatic habitats is almost impossible. Re-introduction programmes with either the release of captive-propagated native snails and/or the translocation of eggs, juveniles and/or adults from viable populations aimed at restoring species or haplotypes in water bodies in their historical range, where they no longer exist, or to augment extant populations, are possible. Any re-introduction must be planned carefully because they are associated with risks such as reduction in genetic variability, introduction of disease infected individuals into the wild or the potential that habitat quality does not

match the actual needs of the introduced individuals (IUCN/SSC 2013). The endangered *Theodoxus prevostianus* (Pfeiffer, 1828) was successfully re-introduced into a spring in Hungary (Fehér et al. 2017). However, while the translocation of *T. fluviatilis* of the native haplotype F28 into Lake Zurich was very successful in the first few years, 20 years later, this translocation seems to have failed, for as yet unknown reasons. In the river Rhine, the two cryptic invaders replaced the native lineage of *T. fluviatilis* (original haplotype F28) at sites where it went extinct. Future research will show whether the invading individuals (haplotypes F31 and “euxinus”) outcompete the native lineage of *T. fluviatilis*, where it is still present.

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REFERENCES

- Anistratenko VV, Sitnikova TY, Kijashko PV, Vinarski MV, Anistratenko OY (2020) A review of species of the genus *Theodoxus* (Gastropoda: Neritidae) of the Ponto-Caspian region, with considerations on available type material. *Ruthenica* 30: 115–134.
- Barcode of Life Data System (2021) Advancing biodiversity science through DNA-based species identification. Version 4. <http://www.barcodinglife.com>. Accessed 14 December 2021.
- Baur B, Schmidlin S (2007) Effects of invasive non-native species on the native biodiversity in the river Rhine. In: Nentwig W (ed) *Biological Invasions*. Ecological Studies 193. Springer, Berlin, Germany, pp 257–273.
- Bij de Vaate A, Jazdzewski K, Ketelaars HAM, Gollasch S, Van der Velde G (2002) Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. *Can J Fish Aquat Sci* 59: 1159–1174.
- Bunje PME (2005) Pan-European phylogeography of the aquatic snail *Theodoxus fluviatilis* (Gastropoda: Neritidae). *Mol Ecol* 14: 4323–4340.
- Bunje PME (2007) Fluvial range expansion, allopatry, and parallel evolution in a Danubian snail lineage (Neritidae: *Theodoxus*). *Biol J Linn Soc* 60: 603–617.
- Bunje PME, Lindberg DR (2007) Lineage divergence of a freshwater snail clade associated with post-Tethys marine basin development. *Mol Phylogenet Evol* 42: 373–387.
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small amounts of leaf tissue. *Phytochem Bull* 19: 11–15.
- Fehér Z, Majoros G, Ötvös S, Bajomi B, Solymos P (2017) Successful reintroduction of the endangered black nerite, *Theodoxus prevostianus* (Pfeiffer, 1828) (Gastropoda: Neritidae) in Hungary. *J Mollusc Stud* 83: 240–242.
- Fretter V, Graham A (1962) *British Prosobranch Molluscs*. Ray Society, London, UK.
- Frisch D, Green AJ, Figuerola J (2007) High dispersal capacity of a broad spectrum of aquatic invertebrates via waterbirds. *Aquat Sci* 69: 568–574.

- Gergs R, Koester M, Grabow K, Schöll F, Thielsch A, Martens A (2015) *Theodoxus fluviatilis*' re-establishment in the River Rhine: a native relict or a cryptic invader? *Conserv Genet* 16: 247–252.
- Govindarajan AF, Carman MR (2016) Possible cryptic invasion of the Western Pacific toxic population of the hydromedusa *Gonionemus vertens* (Cnidaria: Hydrozoa) in the Northwestern Atlantic Ocean. *Biol Invasions* 18: 463–469.
- Haas G, Brunke M, Streit B (2002) Fast turnover in dominance of exotic species in the Rhine river determined biodiversity and ecosystem function: an affair between amphipods and mussels. In: Leppäkoski E, Gollasch S, Olenis S (eds) *Invasive aquatic species of Europe. Distribution, impacts and management*. Kluwer Academic Publisher, Dordrecht, The Netherlands, pp 426–432.
- Hirschfelder H-J, Salewski V, Nerb W, Korb J (2011) Schnelle Ausbreitung einer Schwarzmeeform der Gemeinen Kahnschnecke *Theodoxus fluviatilis* (Linnaeus, 1758) in der bayerischen Donau. *Mitt deutsch malakol Ges* 85: 1–10.
- IUCN/SSC (2013) Guidelines for reintroduction and other conservation translocations. Version 1.0. IUCN Species Survival Commission, Gland, Switzerland.
- Jungbluth JH, von Knorre D (2011) Rote Liste und Gesamtartenliste der Binnenmollusken (Schnecken und Muscheln; Gastropoda et Bivalvia) Deutschlands. In: Binot-Hafke M, Balzer S, Becker N, Gruttke H, Haupt H, Hofbauer N, Ludwig G, Matzke-Hajek G, Strauch M (eds) *Rote Liste gefährdeter Tiere, Pflanzen und Pilze Deutschlands, Band 3: Wirbellose Tiere (Teil 1)*. Naturschutz Biol Vielf 70: 647–708.
- Kinzelbach R (1987) Die Tierwelt im Rhein nach dem November 1986. *Natur Landsch* 62: 521–526.
- Kirkegaard J (2006) Life history, growth and production of *Theodoxus fluviatilis* in Lake Esrom, Denmark. *Limnologia* 36: 26–41.
- Largiader CR (2007) Hybridization and introgression between native and alien species. In: Nentwig W (ed) *Biological Invasions. Ecological Studies* 193. Springer, Berlin, Germany, pp 275–292.
- Leuven RSEW, Van der Velde G, Baijens I, Snijders J, Van der Zwart C, Lenders HJR, Bij de Vaate A (2009) The river Rhine: a global highway for dispersal of aquatic invasive species. *Biol Invasions* 11: 1989–2008.
- Lorenková E, Beran L, Nováková M, Horsáková V, Rowson B, Hlaváč JC, Nekola JC, Horsák M (2021) Invasion at the population level: a story of the freshwater snails *Gyraulus parvus* and *G. laevis*. *Hydrobiologia* 848: 4461–4671.
- Lysne, SJ, Perez KE, Brown KM, Minton RL, Sides JD (2008) A review of freshwater gastropod conservation: challenges and opportunities. *J North Amer Benthol Soc* 27: 463–470.
- Meinert W, Kinzelbach R (1985) Die limnischen Schnecken und die Muscheln von Rheinland-Pfalz (Mollusca: Gastropoda et Bivalvia). *Mainzer Naturwissensch Arch, Beih* 4: 1–58.
- Mineur F, Johnson MP, Maggs CA, Stegenga H (2007) Hull fouling on commercial ships as a vector of macroalgal introduction. *Mar Biol* 151: 1299–1307.
- Morais P, Reichard M (2018) Cryptic invasions: A review. *Sci Total Environm* 613/614: 1438–1448.
- Müller O, Baur B (2011) Survival of the invasive clam *Corbicula fluminea* (Müller) in response to winter water temperature. *Malacologia* 53: 367–371.
- Müller P (2016) Flussnixenschnecke. Bericht und Standorte Kanton Zürich 2016. Unpublished Report, Zurich, Switzerland.
- Müller P, Steinmann P (2008) Die Flussnixenschnecke *Theodoxus fluviatilis*, Kartierung des einzigen Schweizer Vorkommens in Zürich-Riesbach 2007–2008. Unpublished Report, Zurich, Switzerland.
- Neumann D (1959) Morphologische und experimentelle Untersuchungen über die Variabilität der Farbmuster auf der Schale von *Theodoxus fluviatilis*. *Z Morphol Ökol Tiere* 48: 349–411.
- Novak SJ (2011) Geographic origins and introduction dynamics. In: Simberloff D, Rejmánek M (eds) *Encyclopedia of Biological Invasions*. University of California Press, Berkeley and Los Angeles, USA, pp 273–280.
- Ojaveer H, Leppäkoski E, Olenin S, Ricciardi A (2002) Ecological impact of Ponto-Caspian invaders in the Baltic Sea, European inland waters and the Great Lakes: an inter-ecosystem comparison. In: Leppäkoski E, Gollasch S, Olenin S (eds) *Invasive aquatic species of Europe. Distribution, impacts and management*. Kluwer Academic Publisher, Dordrecht, The Netherlands, pp 412–425.
- R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Version 3.6.3, <https://www.R-project.org/>.
- Rey P, Ortlepp J, Kury D (2005) Wirbellose Neozoen im Hochrhein. Ausbreitung und ökologische Bedeutung. BAFU, Bern, Schriftenr Umwelt 380: 1–88.
- Rothmeier LM, Martens A (2019) Die Neubesiedlung des Oberrheins durch *Theodoxus fluviatilis*: die Ausbreitung im Rheinhafen Karlsruhe. *Lauterbornia* 86: 39–45.
- Rothmeier LM, Sahn R, Watermann B, Grabow K, Koester M, Cichy A, Martens A (2021) The Ponto-Caspian parasite *Plagiorporus cf. skrjabini* reaches the River rhine system in Central Europe: higher infestation in the native than in the introduced Danubian form of the gastropod *Theodoxus fluviatilis*. *Hydrobiologia* 848: 2569–2578.
- Rüetschi J, Stucki P, Müller P, Vicentini H, Claude F (2012) Rote Liste Weichtiere (Schnecken und Muscheln). Gefährdete Arten der Schweiz, Stand 2010. Bundesamt für Umwelt, Bern, und Schweizer Zentrum für die Kartografie der Fauna, Neuenburg. *Umwelt-Vollzug* 1216: 1–148.
- Salewski V, Hirschfelder H-J (2006) Erstnachweis der Gemeinen Kahnschnecke *Theodoxus fluviatilis* in der deutschen Donau. *Lauterbornia* 56: 85–90.
- Sands AF, Neubauer TA, Nasibi S, Harandi MF, Anistratenko VV, Wilke T, Albrecht C (2020) Old lake versus young taxa: a comparative phylogeographic perspective on the evolution of Caspian Sea gastropods (Neritidae: *Theodoxus*). *Roy Soc Open Science* 6: 190965.
- Sands AF, Sereda SV, Stelbrink B, Neubauer TA, Lazarev S, Wilke T, Albrecht C (2019) Contributions of biogeographical functions to species accumulation may change over time in refugial regions. *J Biogeogr* 46: 1274–1286.
- Schliep KP (2011) Phangorn: phylogenetic analysis in R. *Bioinformatics* 27: 592–593.
- Schmidlin S, Baur B (2007) Distribution and substrate preference of the invasive clam *Corbicula fluminea* in the river Rhine in the Region of Basel (Switzerland, Germany, France). *Aquat Sci* 69: 153–161.
- Schmidlin S, Schmera D, Ursenbacher S, Baur B (2012) Separate introductions but lack of genetic variability in the invasive clam *Corbicula* spp. in Swiss lakes. *Aquat Invasions* 7: 73–80.
- Schultz H, Schultz O (2001) Erstnachweis der Gemeinen Kahnschnecke, *Theodoxus fluviatilis* (Linnaeus, 1758) in Österreich (Gastropoda: Neritidae). *Annal Naturhist Mus Wien* 103B: 231–241.
- Strayer DL (2010) Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshw Biol* 55 (Suppl. 1): 152–174.
- Van Bocxlaer B, Clewing C, Mongindo Etimosundja J-P, Kankonda A, Ndeo OW, Albrecht C (2015) Recurrent camouflaged in-

- vasions and dispersal of an Asian freshwater gastropod in tropical Africa. *BMC Evol Biol* 15: 33.
- Westermann F, Schöll F, Stock A (2007) Wiederfund von *Theodoxus fluviatilis* im nördlichen Oberrhein. *Lauterbornia* 59: 67–72.
- Wirth A, Schmera D, Baur B (2010) Native and alien macroinvertebrate richness in a remnant of the former river Rhine: a source for recolonisation of restored habitats. *Hydrobiologia* 652: 89–100.
- Zettler ML (2008) Zur Taxonomie und Verbreitung der Gattung *Theodoxus* Montfort, 1810 in Deutschland. Darstellung historischer und rezenter Daten einschliesslich einer Bibliografie. *Mollusca* 26: 13–72.
- Zettler ML, Frankowski J, Bochert R, Röhner M (2004) Morphological and ecological features of *Theodoxus fluviatilis* (Linnaeus, 1758) from Baltic brackish water and German freshwater populations. *J Conchol* 38: 305–316.