PRIMARY RESEARCH PAPER

New phylogenetic insights on some species of Unionidae from Switzerland (Bivalvia, Palaeoheterodonta, Unionidae)

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Abstract Switzerland's drainage systems are divided into three major European river basins, i.e. the Po, the Rhône, and the Rhine basins. Until recently 32 species of freshwater mussel species (i.e. belonging to the genera *Anodonta* and *Unio*) were recognized for the country, albeit their identity and number remain uncertain especially, given the recent recognition of *Unio mancus* Lamarck, 1819 from the Rhône in France, and *Unio elongatulus* C. Pfeiffer, 1825 and *Anodonta exulcerata* Porro, 1838 from the Po basin in Italy. In this study, we molecularly assess Swiss populations of freshwater mussels to understand the identity and number of species as well as to characterize their distributions within this geologically differentiated Alpine country. We collected 125 specimens in 42 lakes and rivers representing the three major basins and performed a phylogenetic investigation of the collected specimens using two mitochondrial markers (COI & 16S) and one nuclear marker (28S). COI Haplotype networks are then presented for the identified species. Our new findings show that *Unio elongatulus* inhabits water bodies north of the main Alpine arc. No living populations of *Unio mancus* could be detected in Switzerland. *Anodonta exulcerata* is recorded from two localities north of Lake Maggiore and in the Swiss part of Lake Lugano. *Anodonta anatina* (Linnaeus, 1758) shows genetic differences between southern alpine and northern alpine populations. Our genetic data from Swiss populations of unionid species provides new records and knowledge concerning freshwater

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mussels from Central Europe and specifically from the Alpine region.

Introduction

The hydrogeological network of Switzerland is connected to three major European river basins, i.e. the Po, the Rhône, and the Rhine basin. The latter two rivers have their sources and upper potamic habitats in the Swiss Alps. Both rivers play an important role in the water contribution to large parts of Europe. This makes the area a crucial ecological hub for freshwater flora and fauna, including 32 species of freshwater bivalves. Members of the family Unionidae Rafinesque, 1820 represent the largest freshwater mussels in body size. Unfortunately, public awareness is still practically nonexistent for the water cleansing benefits provided by these remarkable biofilterers.

Swiss naturalists were long fascinated by the autochthonous bivalve fauna; Studer (1789) recorded five species of unionid mussels for Switzerland, and later in 1820, he already listed six species of *Unio*, and two of *Anodonta*. In the following years, Swiss waterbodies were studied by numerous malacologists: Bourguignat (1862) added four *Unio* and eight *Anodonta* species from the Lake Lucerne describing several new species; Brot (1867) discussed the Unionidae living in Lake Geneva, and Godet (1911) reported on the forms of *Unio crassus* Philipsson, 1788 from Lake Neuchâtel. The investigations of all these authors were hindered by an increasingly chaotic system of names assigned to the level of species, subspecies, variety, forms etc., purely based on morphology of shells combined with hypothetical biogeographic consideration. The first to apply a more modern species concept was Schnitter (1922), who revised the unionid species of Switzerland and realised the importance of phenotypic plasticity (Ortmann, 1920), which had been the cause of an overestimation of the number of taxa. Despite this knowledge of the shell plasticity, the classical discrimination of taxa based on conchological characters, was continued for the next 40 years. Haas (1969) hypothesised the existence of hybrids in *U. pictorum* (Linnaeus, 1758) and *U. mancus* after investigating animals with ambiguous

shell morphology. Later, enzyme variability analyses by Nagel and Badino (2001) lead to further clarification, rebutting Haas' hypothesis. More advanced genetic methods (COI analysis) generated increasingly more molecular data whilst ushering Unionidae taxonomy into twenty first Century assessment standards (Lopes-Lima et al., 2017b; Pfeiffer et al., 2019). Currently, six species of Unionidae are recognized for Switzerland (Lopes-Lima et al., 2017a). Since this last conservation assessment of European freshwater mussels, distinct *Unio* and *Anodonta* species have been recognized around Switzerland. Due to their ecological impact, it is important to monitor their presence in this small Alpine country.

This work's primary aim was prompted by Froufe et al. (2017) upon the recovery of a cryptic species of *Anodonta* in Lake Lugano, northern Italy. This species was formally resurrected under the name *Anodonta exulcerata* Porro, 1838 by Riccardi et al. (2020). Since a section of Lake Lugano overlaps into Switzerland, detecting this species there became important for assessing other Swiss freshwater ecosystems. Unfortunately, the shell morphology of *A. exulcerata* does not allow a distinction from the similar *A. anatina*; however, *A. exulcerata* can be clearly identified using the conventional mitochondrial markers COI and 16S in a molecular phylogenetic analysis.

The second aim of this study is to investigate the *Unio* populations living in the catchment areas that flow into the Mediterranean, and if they have been able to cross the European watershed between the Rhône and the Rhine system? *U. mancus* and *U. elongatulus* were identified as target species. The first species was reported for northern Italy and the Ticino for many decades, however, Prié & Puillandre (2014) confirmed that *U. elongatulus* and *U. mancus* represent two separate species which can be distinguished using COI in a molecular analysis. Froufe et al. (2017) recognized that the historical records for"*U. mancus* "in northern Italy belong to *U. elongatulus*, which is a widespread unionid taxon in the area north of the Apennines to coastal Croatia (Lopes-Lima et al., 2017a).

The third aim of our investigation is to discover if living populations of *U. mancus exist* in the northwestern and central part of Switzerland. The species was reported from the Doubs in the French part of the Jura. The last records of living populations in the Swiss part of the Doubs were reported in 2003

(Rüetschi et al., 2010). The species' known distribution covers Northern, Central and Southern France and Western and Southern Italy together with the Tyrrhenian Islands, Corsica, Sardinia and Sicily. However, a (re?)-dispersal along the Rhône system and potentially also the Doubs is theoretically given, particularly as the French freshwater canal system connects all larger rivers in eastern France. Specimens of *Unio tumidus* Philipsson, 1788 were included to represent the unionid fauna of the lakes in the Central Plateau of Switzerland.

To molecularly assess and delimit the selected Swiss species, we conducted phylogenetic analyses using two mitochondrial (COI, 16S) and one nuclear marker (28S) from 128 specimens culled from Swiss rivers and lakes. We enriched our dataset with sequences of the target species from areas outside Switzerland (Online Resource 4) to illustrate how Swiss species are nested in the larger context. Although a specimen of *U. crassus* was accidentally collected and added to the analysis to differentiate the trees. It was, however, not the target of this investigation.

Material and methods

Taxon sampling

Forty-two sites encompassing the major lakes and river systems of the Rhine, Rhône and Po basins were sampled during the years 2018–2020 (Fig. 1). One to eight individuals were collected at each site, resulting in a total of 125 specimens. To meet our research aim, only specimens from the target *Unio* species, i.e. *U. mancus* and *U. elongatulus* and *U. tumidus* were sampled. For *Anodonta* Lamarck, 1799, we included both species, *Anodonta cygnea* (Linnaeus, 1758) and *A. anatina* to detect possible *A. exulcerata*.

The mussels were preserved on site in 100% ethanol. Preliminary determinations were made at the locality by the diver teams; specimens were then labelled and catalogued. All specimens are stored in 80% ethanol and preserved in the wet collection of the Natural History Museum Bern, Switzerland. In addition to the Swiss specimens, tissue snippets of 10 unionid specimens from the wet collection of the Senckenberg Museum Frankfurt (SMF), Germany, were added to the study. These snippets were
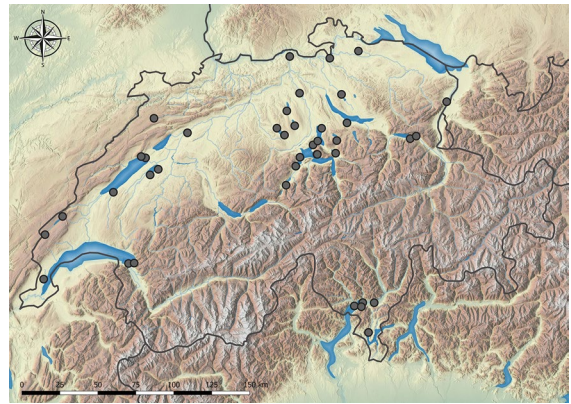

Fig. 1 Sampling sites in Switzerland (2018–2020)

supplied without taxonomic information, acting as a positive control for the extraction and PCR procedure performed in the laboratories at the NMBE (Online Resource 2).

A standardised field protocol was developed in cooperation with the SZKF (info fauna—Schweizerisches Zentrum für die Kartografie der Fauna (SZKF/CSCF)) by the research diver teams. Contained in the field log sheet is a complete set of physical, chemical and environmental data. These data include date, central coordinates, altitude, area searched, water temperature, general information on the dominant substrates and vegetation according to water depth, as well as information on the mussels observed, fish and other biota, with special attention to neozoa. This database will be available via the SZKF portal.

DNA extraction, markers description and amplification protocol

In a separate, sterile laboratory from that where the PCR is conducted, mantle snippets (somatic tissue) of approx. 5 mm^3 size were cut with sterile surgical blades from each specimen. Somatic tissue in Unionidae contains (F) maternal mitochondria, and by clipping tissue from the mantle (F), mitochondria can be harvested (Froufe et al., 2016). Additional snippets were cut and stored for later research. Pre-extraction digestion was performed using the protocol of the Qiagen Blood and Tissue Kit (Qiagen cat. nr. 69506). Subsequent extraction was done by using a QIAcube extraction robot (DNeasy Blood Tissue and Rodent tails, standard protocol). Three markers commonly

used in bivalves were selected: (1) the cytochrome c oxidase subunit I gene (COI; 710 bp amplicon length, *LCO22/HCO700* primer pair) (Froufe et al., 2014); (2) the 16S ribosomal RNA gene (16S; 480 bp amplicon length, *16Sar/16Sbr* primer pair) (Weigand et al., 2013) and (3) part of the 28S rRNA gene (28S; 810 bp amplicon length, *RD1.3f/RD4b* primer pair) (Whiting, 2002).

The PCR master mix consisted of 12.5 μl of GoTaq G2 HotStart Green Master Mix (Promega M7423), 6.5 μl nuclease free H_2O (Sigma-Aldrich, W4502). For each reaction, 1 μl of each primer and 2 μl template DNA were added.

The PCR protocol for each marker described are as follows: For the *LCO22/HCO700* (COI) primer pair the thermal regime begins with 3 min at 95 °C, followed by 35 cycles of 1 min at 95 °C, 1 min at 45 °C and 1 min at 72 °C and finishes at the end with 5 min at 72 °C. For the *16Sar/16Sbr* (16S) primer pair, the conditions were 5 min at 95 °C in the first step, followed by 34 cycles of 30 s at 95 °C, 25 s at 52 °C and 45 s at 72 °C, and finally, 5 min at 72 °C. For the *RD1.3f/RD4b* (28S) primer pair, the protocol begins with 3 min at 95 °C, followed by 45 cycles of 30 s at 95 °C, 45 s at 50 °C and 60 s at 72 °C with a final elongation step of 5 min at 72 °C. PCR products were displayed together with a negative control and a 1000 bp ladder (BenchTop 100 bp DNA Ladder, G8291) in an agarose 1% gel for assessment of quality and primer efficiency. LGC (LGC Genomics Berlin) performed PCR product purification and sequencing with the above primers. The generated sequence data are downloadable from GenBank (Online Resource 2).

Phylogenetic analyses and species delimitation

From the initial 135 specimens, 128 were successfully sequenced for the analyses. The AB1 sequences were processed in Geneious Ver.9.1.8 (Biomatters Ltd.). Every sequence was verified through a BLAST analysis (National Center for Biotechnology Information (NCBI)). Wherever possible, we focused on BLAST results, providing vouchers from the latest publications involving Unionidae e.g., *A. exulcerata* MF414281 (Froufe et al. 2017). For comparing the Swiss data in a broader geographical context, a selection of sequences from the latest publications (Froufe et al., 2017; Klishko et al., 2018; Marrone et al. 2019;

Lopes-Lima et al., 2021) were added to the phylogenetic analysis (Online Resource 2). The final sample size for a three-marker approach (COI, 16S, 28S) is 172 and the sample size for the ASAP analysis consisted of 246 COI sequences.

For the alignment, the MAFFT v.7.222 plugin of Geneious (Katoh & Standley, 2013) was implemented. The L- INS-i algorithm and the 1PAM/*k*=2 matrix chosen for the alignment construction. Every gene alignment was verified and, when needed, improved by excluding positions of unreliable parts of the alignment. The final alignment length after editing was as follows (original first MAFFT alignment length in brackets): COI 654 bp (702 bp), 16S 497 bp (539 bp) and 28S 644 bp (827 bp) summing up to a total of 1795 bp in the concatenated alignment block. The final alignment length for the COI phylogeny was 546 bp. Substitution saturation was assessed using Xia's test in DAMBE v.7.058 with default settings (Xia et al., 2003; Xia, 2017, 2018).

Species partitions analysis was performed on the Assemble Species by Automatic Partitioning (ASAP) web tool (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html) (Puillandre et al., 2021), using the Kimura (K80) (Kimura, 1980) substitution model with the settings for the transition/transversion rate ratio (ts/tv) = 2 and the standard advanced options for 5 best partition suggestions for 242 COI sequences, excluding *Potomida littoralis* (Cuvier, 1798), *Microcondylaea bonellii* (Férussac, 1827) and *Sinanodonta woodiana* (I. Lea, 1834) from the analysis.

The protein coding COI fragment was defined in two partitions: the first two codon positions as one partition and the third codon position as a second. Both 16S and 28S alignment data sets were not partitioned and were handled each as a single group for the analyses. The final data block contained 172 individuals. Partitionfinder Ver. 2.1 (Lanfear et al., 2012, 2017) was implemented using the greedy algorithm settings to look for the optimal evolutionary models for the partitions and analysis methods through the corrected Akaike Information Criterion (AICc).

Maximum Likelihood (ML) analysis was computed with RAxML (Stamatakis, 2014) and IQTree (Nguyen et al., 2015; Chernomor et al., 2016; Minh et al., 2020) setting GTR + I + G as substitution model. For RAxML, the settings were set for a rapid bootstrap analysis with 2000 replicates in a single run. For the IQTree analysis a standard bootstrap

analysis was performed with the settings of 100 bootstrap alignments and 1000 replicates for the SH-aLRT (approximate likelihood ratio test (aLRT) and Shimodaira–Hasegawa (SH)) (Guindon et al., 2010; Anisimova et al., 2011).

Bayesian Inference (BI) was performed using MrBayes v3.2.2×64 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003; Ronquist et al., 2012). The substitution model was chosen according to the Partitionfinder definition: the best model for COI and 16S was GTR+I+G and for 28S GTR+G. The settings for the Monte Carlo Markov Chain (MCMC) parameter were as follows: four chains and four separate runs for 20×10^6 generations, temperature set to 0.1, with a tree sampling frequency of 1000 and a burn in of 25%. For convergence diagnostics, Tracer (Rambaut et al., 2018) was implemented to analyse the effective sample size (ESS).

Tree display and editing was performed using FigTree v1.4.4 (https://github.com/rambaut/figtree/releases). MrBayes and RAxML were performed through UBELIX (http://www.id.unibe.ch/hpc), the HPC cluster at the University of Bern. The IQTree analysis was performed through the IQ-Tree web server http://iqtree.cibiv.univie.ac.at/. The haplotype networks were built with Popart v.1.7 (Leigh & Bryant, 2015) (http://popart.otago.ac.nz), using the TCS algorithm (Clement et al., 2002).

Results

Sequence analyses and species delimitation

Sequence numbers obtained per species are given in Online Resource 1. The substitution saturation assessment for the sequences using Xia's test showed no signs of saturation ($P = 0.00$). Tracer analysis of the BI run showed no anomaly in the ESS scores (> 200). ASAP suggested one result out of 5 possible partition solutions, it consists of 9 groups in 242 specimens (Online Resource 3).

Phylogeny and distribution patterns in Switzerland

The concatenated phylogenetic tree shown in Fig. 2 is a combination of the Bayesian Inference (BI) and the Maximum Likelihood analysis (RAxML & IQTree) for a dataset of 172 specimens. It shows high support

for the clades in the tree topology in that *Unio* and *Anodonta* are monophyletic. The *U. tumidus* clade splits early from the greater *Unio* cluster as shown in Fig. 2. The *U. crassus* group splits before the *U. pictorum/U. mancus/U. elongatulus* cluster (Fig. 3). The *Anodonta* clade splits in the three *Anodonta* groups (Fig. 2): *A. exulcerata* is sister group to *A. cygnea*, the *A. anatina* cluster is the counterpart to this group. The topology of the *A. anatina* cluster suggests four branches, with only one being supported by the node values: group A, consisting of Iranoturanic individuals (Fig. 4).

The haplotype network for *U. elongatulus* (Fig. 5) shows that the Swiss Rhine population shares the same haplotype as the populations from the Padano-Adriatic basins (Po, Reno) (Marrone et al., 2019). In Fig. 6, the haplotype network for *A. anatina* shows three distinguishable haplogroups displaying the topology shown in the phylogenetic tree. In Fig. 7, *Anodonta cygnea* displays one central haplogroup (central European), one Italian/Iberian and two distinct Turkish haplotypes. In Fig. 8, *A. exulcerata* shows 2 Croatian haplotypes, a central Italian/Swiss haplogroup and an Italian haplotype. The haplotypes with the corresponding sequences are given in Online Resource 1.

Discussion

BLAST and ASAP species delimitation

As stated by Froufe et al. (2017), an identification of species using morphological traits is rather difficult in the genus Unio. This pattern is in line with the display of high plasticity witnessed in shell morphology in members of Anodontini Rafinesque, 1820 (Zieritz & Aldridge, 2009; Zieritz et al., 2010; Riccardi et al., 2020). This situation is also known for other freshwater bivalves as well, e.g. Hyriidae Swainson, 1840 (Baker et al., 2003; Yusseppone et al., 2018). The BLAST results and ASAP partitioning facilitates the assignment of the individuals to their respective species identification.

Our investigation could not reveal any living population of *U. mancus* in Switzerland. However, *U. elongatulus* specimens from Switzerland could be confirmed by BLAST. Six specimens were first identified as *U. tumidus* in the field.

Fig. 2 Concatenated phylogenetic tree obtained by Bayesian Inference (BI) analysis and Maximum Likelihood (ML) analysis of three genes (COI, 16S and 28S). ML was computed with RAxML and IQTree performing a standard bootstrap analysis. Support values indicated at the nodes. Subpopulations of *A. anatina* are labelled as A, B, C and D
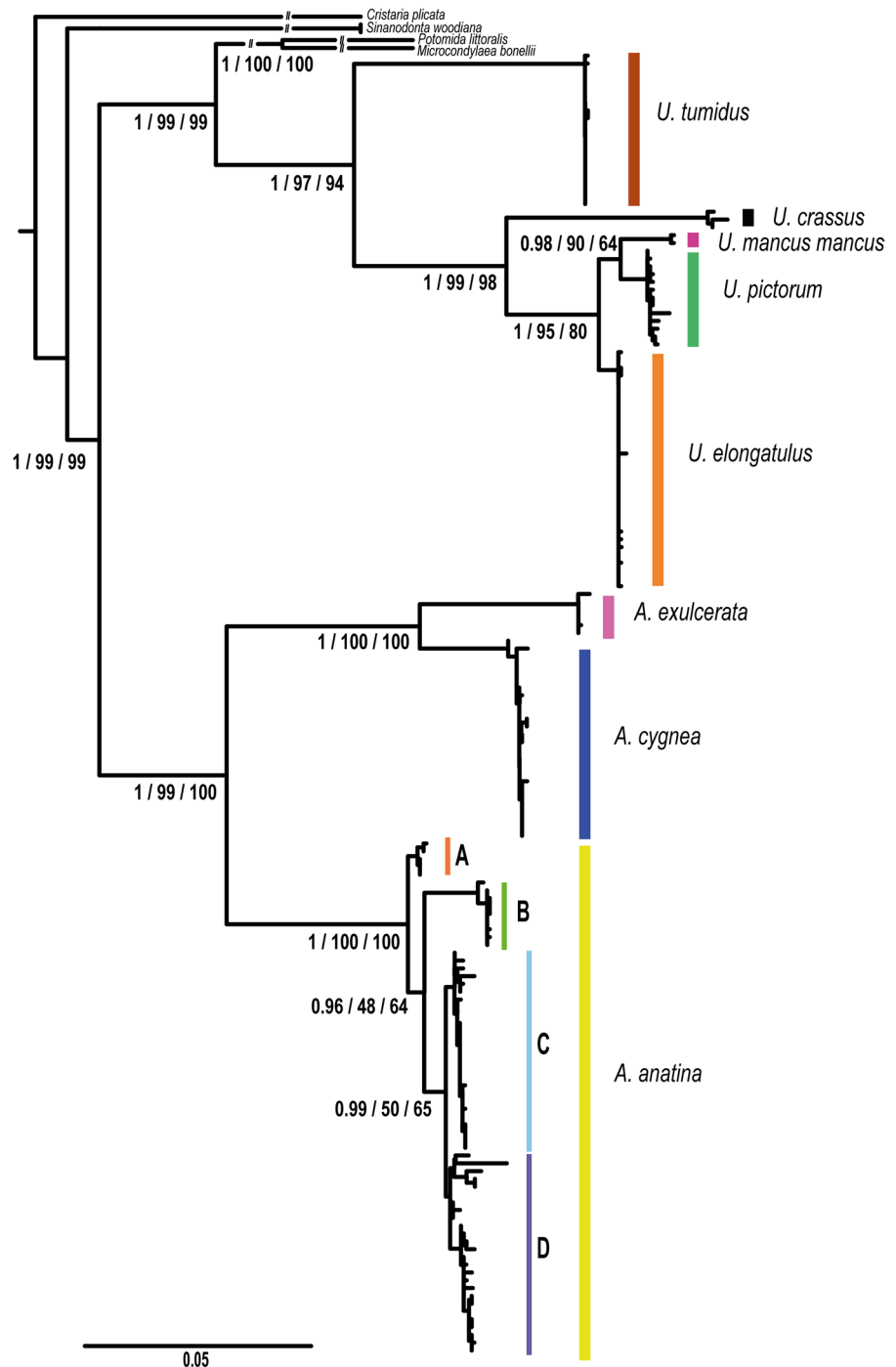
Anodonta exulcerata specimens however, do occur in the Swiss sampling shown in BLAST. The Swiss population belongs to the Italian haplogroup AE03 (Froufe et al., 2017) (Ticino⁄Lake Maggiore⁄Po) as shown in Fig. 8. Despite the variance displayed in the

haplotype network, the ASAP analysis classified all the *A. anatina* individuals to one group. The Ticino population (AA01 and AA02) is geographically and genetically distinct from all other Swiss *A. anatina* populations (Fig. 6). In contrast, the Rhine basin
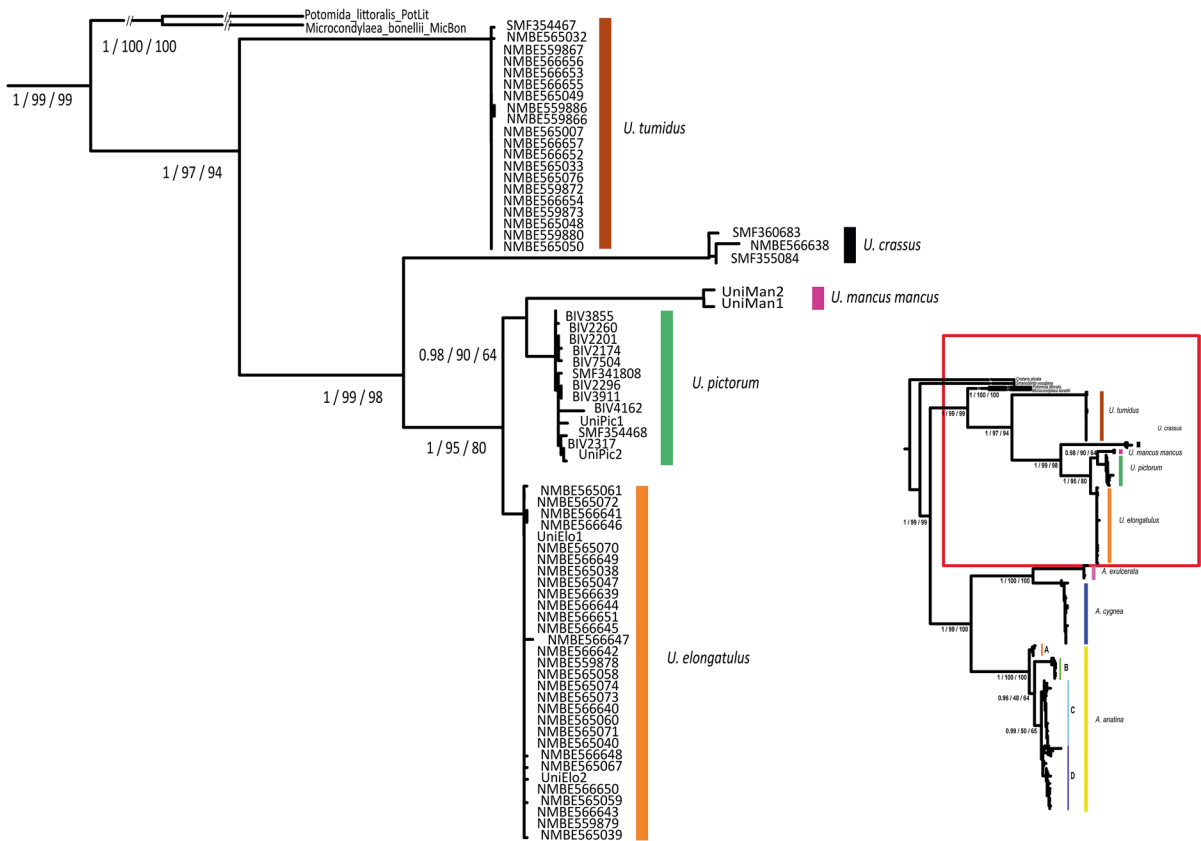
Fig. 3 Close up of the *Unio* clades in the concatenated phylogenetic tree

populations form a fragmented pattern throughout the Swiss water systems. The Rhine population shares on the one side, haplotypes with eastern Russian and Ukrainian populations (AA12 and AA144). The west European (AA03, AA04 and AA06) haplogroups include Portuguese, Spanish, French, Czech, Italian and West-Ukrainian specimens. This pattern could be explained by multiple refugia for *A. anatina* during the last glacial periods (Froufe et al., 2014).

The topology in the haplotype network of *A. cygnea* shows a central homogenous haplogroup (AC02). Although the additional sequences used for the haplotype network cover Croatia, Sweden, Poland and France in the haplogroup (AC02), no higher diversity is indicated as observed in *A. anatina*.

Molecular phylogeny

In the concatenated phylogeny (COI, 16S and 28S) in Fig. 2, the node splitting *Unio* and *Anodonta* is

supported with a posterior probability of BI = 1 and bootstrap values of BS = 99% and BS = 99%, with *P. littoralis and M. bonelli* changing position close to the *Unio* clade. The Position of *Gonideinae* Ortmann, 1916 is different in Froufe (2017: 3266, Fig. 6), which is explainable with the greater data set used in the analysis. For the species clades, the topology is coincident with that of Lopes-Lima et al. (2017b), Froufe et al. (2017), Marrone et al. (2019) and Riccardi et al. (2020).

Anodonta exulcerata

The haplotype network shows that AnoExu1 (Froufe et al., 2017) forms a haplogroup with the Swiss group, defining it as the Lake Maggiore haplotype. There were no geographical indications on AnuExu1 in Froufe et al. (2017). We identified one additional *A. exulcerata*, NMBE 565817 (Ceresio, Agno Camping) in the concatenated phylogeny. However, due
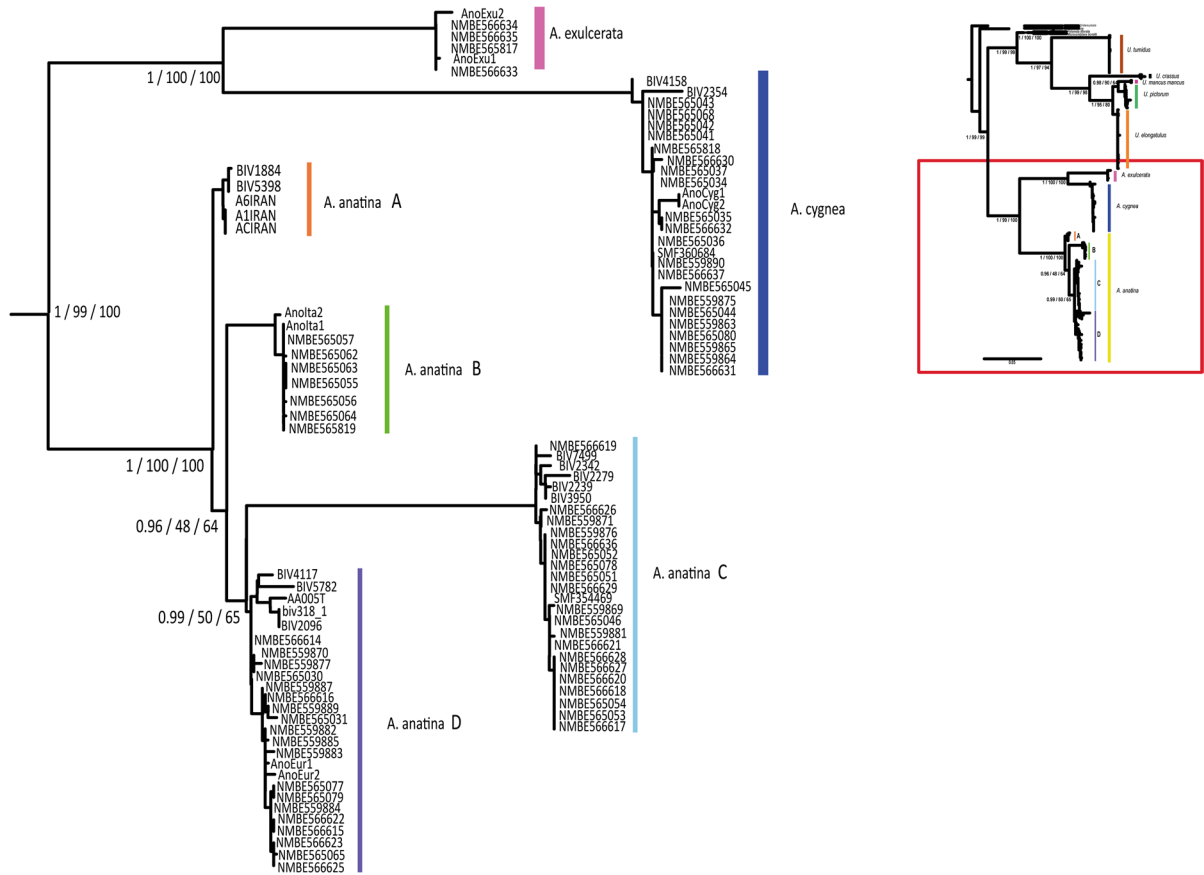
Fig. 4 Close up of the *Anodonta* clades in the concatenated phylogenetic tree

to the missing COI sequence, it was not possible to detect it in the preceding BLAST analysis. The three *A. exulcerata* individuals from Switzerland originate from a rather small pond, Laghetto Demanio in Gudo, which is situated north of Lake Maggiore, upstream of the Ticino River in the Magadino plain. The pond constitutes a remnant tributary before the river was canalized. Thus, the enclosed fauna represents a population isolated from the canalised mainstream. *Anodonta exulcerata* NMBE 565817 originates from Lake Lugano, which is connected via the River Tresa to Lake Maggiore (Fig. 9). Riccardi's (2020: 3, Table 1) sampling at Lake Lugano resulted in four *A. cygnea* specimens. These findings imply that *A. exulcerata* has a wider distribution in the southern Swiss and northern Italian lakes and rivers. Further investigation of the surrounding ponds and tributary remnants could well harbour additional populations warranting future consideration.
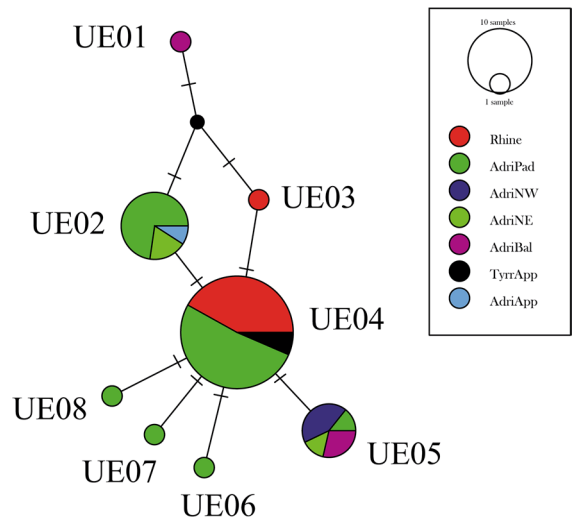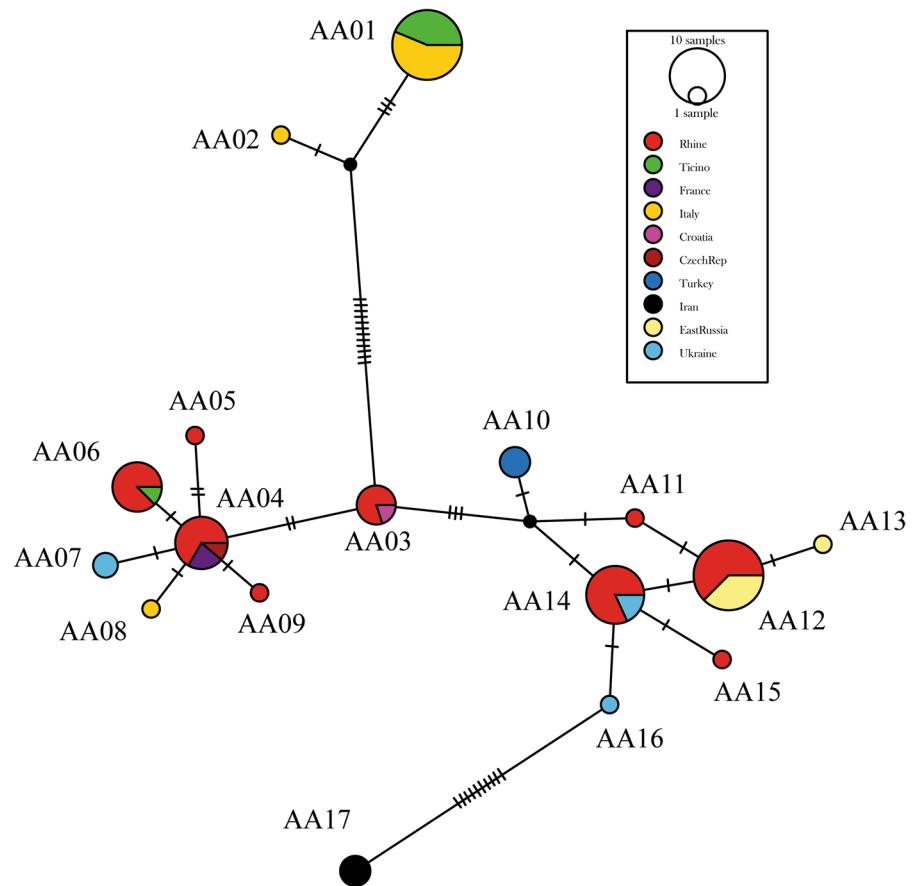

Fig. 5 Haplotype network (TCS) *Anodonta anatina*

**Fig. 6** Haplotype network (TCS) *Unio elongatulus*

Unio mancus

The species has been split into three subspecies: *U. mancus mancus*, *U. mancus requienii* Michaud, 1831 and *U. mancus turtonii* Payraudeau, 1826. Froufe et al. (2017) found that the latter two subspecies only live in affluents of western Italy and considered uniting them into a single subspecies (i.e., *U. mancus turtonii*). In this case, *Unio mancus mancus* is restricted to the western region of France, Pyrenees and eastern Spain. This hypothesis was recently refuted by Marrone et al. (2019) due to anthropogenic influence in the Tyrrhenian drainage basin such that vicariant speciation of *U. mancus requienii* could not be tested. Moreover, the COI haplotype network in Marrone et al. (2019: 347, Fig. 7) used a broader COI dataset, showing well-defined haplotypes for all three subspecies. In addition, Prié & Puillandre (2014) show well-supported subspecies clades in Fig. 2 (COI, 16S, 28S) and in the Online Resource 4 (16S). Despite the same genetic markers, the presented concatenated

phylogenetic tree did not show any *U. mancus* from Switzerland. When we revisited their sampling localities at the Swiss Doubs, only old shell fragments and no living *Unio* sp. individuals were found. However, the voucher collection in NMBE houses fresh shells collected in the mid 1980's from the Doubs near St. Ursanne, indicating that the species has recently been exterminated. Additional sampling should focus on Lake Geneva, which potentially harbours this species as well.

Unio elongatulus

The distribution of *U. elongatulus* spans from north of the Apennines to the coastal region of Croatia (Froufe et al., 2017). The new records of *U. elongatulus* north of the Alps extend the northern distribution range of this species (Fig. 10). Moreover, since the populations occur scattered all over central and north-eastern Switzerland, no biogeographical pattern can be detected. This probably results from stocking

Fig. 7 Haplotype network (TCS) *Anodonta cygnea*
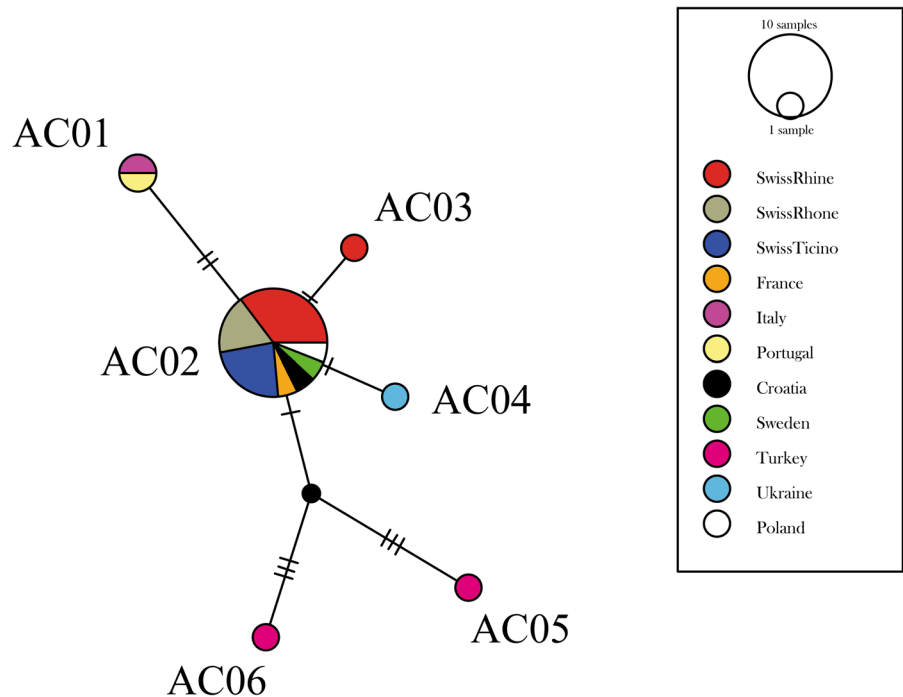
events of potential host fish species throughout Switzerland. Such human mediated transport can easily move glochidia via infected host fishes northwards over the central Alpine chain. Host fishes for *U. elongatulus* include salmonids, freshwater blennies and cyprinids (Prié et al., 2012). Anthropogenic interference involving similarly infected host fishes with *U. elongatulus* and *S. woodiana* has also been reported in Florence, Italy (Froufe et al. 2017).

Our *U. elongatulus* samples cluster together with the neotype MG967432 from Vipava (= Vipacco) River near Gabria Inferiore (Marrone et al., 2019), thus confirming the correct species identification. The *U. elongatulus* specimens do not show any subclade

Fig. 8 Haplotype network (TCS) *Anodonta exulcerata*

formations in the single gene and three-marker phylogenetic tree. The haplotype network shows that the Rhine population is part of a haplotype with the Padano—Adriatic basins' specimens (Marrone et al., 2019). Consequently, the entire *U. elongatulus* sampling from Switzerland originates from the southern alpine region. This opens new dispersal possibilities for this species towards Germany via the Rhine River such that the entire Swiss Rhine drainage system can be expected to be populated by *U. elongatulus* in near future.

The non-target taxa for this investigation showed interesting topologies in the phylogenetic analyses. Although not part of the main investigation, they are briefly pointed out here.

Anodonta cygnea

The low genetic variation *A. cygnea* observed in the single gene phylogeny and haplotype network does not change with additional molecular data. The clade is still compact and short-branched, which is a contrast to the higher variation shown in *A. anatina*. Possible explanations for this low variability could be that either the distribution of the individuals or certain populations appeared recently or there is
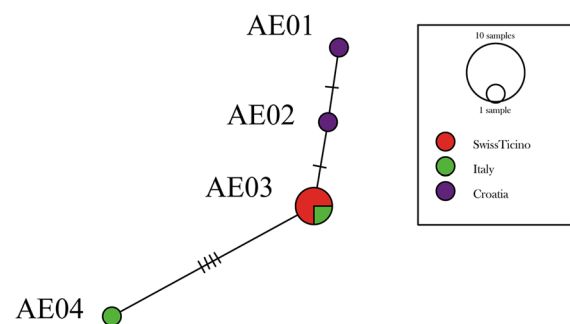
anthropogenic distribution involving infected host fish distribution. The haplotype network shows no variation. This may also be an artefact of the sequence choice and certainly requires a larger data set with a broader geographical scope.

Anodonta anatina

The *A. anatina* clade indicates a topology with a partitioning into three subclades, but without full support in the node values. The subpopulations in Fig. 4 are congruent with the haplogroups depicted in Fig. 6. The results of the phylogenetic analysis present a similar topology as shown by Froufe et al. (2014: 7, Fig. 2), where the main cluster is partitioned into a clade encompassing the Ebro and Adriatic basins, a pan-European clade and the Iberian clades. The partitioning into these clades was to be expected ie. recently, a Transbaikal distribution of *A. anatina* was reported showing the inclusion of Ukrainian and East-Russian individuals into the pan-European *A. anatina* haplogroups (Klishko et al., 2018). However, partition A, the Turanic partition, is distinctly separated from the main *A. anatina* cluster with high node support in the phylogenetic tree. In the haplotype network, the AA17 haplogroup separates from that of the Ukrainian (AA16) by 10 mutations.

Partition B individuals of *A. anatina* (Lake Maggiore) were labelled during the field work as *Anodonta* sp.. BLAST analysis identified them as closely related to the *A. anatina* specimens, which belong to the biogeographical clade of the Italian/Ebro group (Froufe et al., 2014). In the haplotype network, they form the haplogroup consisting of AA01 and AA02 and are separated by 12 mutations.

Partition C in the *A. anatina* clade consists of the haplotypes AA11 to AA16. The East-Russian individuals originate from the Lake Baikal area. Together with the oriental individuals (AA10), it may be considered the EU-Oriental-Russian group.

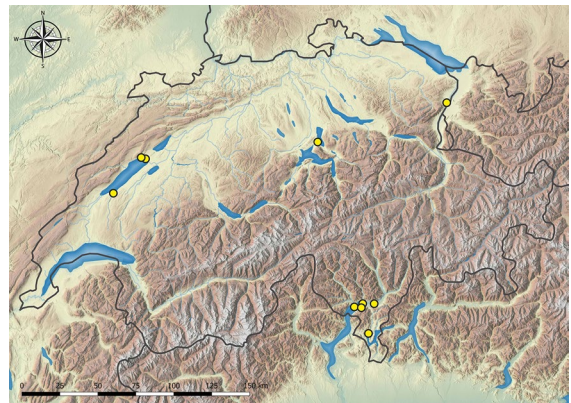**Fig. 9** Distribution of *Anodonta exulcerata* in Switzerland

Fig. 10 Distribution of *Unio elongatulus* in Switzerland

Table 1 List of primer pairs used in PCR and sequencing

Gene	Primer	Sequence (5–3)	Approx. amplicon lengths	References
COI	*LCO22*	GGTCAACAAAYCATAARGATATTGG	710	Froufe (2014)
	HCO700	CAGGGTGACCAAAAAAYCA		
16S	*LR-N-13398 (16Sar)*	CGCCTGTTTATCAAA AAC AT	480	Weigand et al. (2013)
	LR-J-12887 (16Sbr)	CCGGTCTGAACTCTGATCAT		
28S	*RD1.3f*	GGATTCCCTYAGTAAGKGCG	810	Whiting (2002)
	RD4b	CCTTGGTCCGTGTTTCAAGAC		

These specimens were part of the broader EU haplotype group in Klishko (2018: 7, Fig. 6).

Partition D is represented by the haplotypes AA03 to AA09. Individuals originate from West-Ukraine (AA07) over the Rhine basin into France (AA04), forming the Pan-European group.

By implementing a larger dataset in future investigations, the relationships between the different European groups in the *A. anatina* clade can likely be resolved.

Unio tumidus

Unio tumidus was chosen to support the structuring of the phylogenetic and ASAP analyses. In the phylogenetic tree it forms a well-defined compact clade, separating *U. tumidus* from *U. crassus* and *U. elongatulus* with high support (BI = 1; BS = 97%; BS = 94%). Future investigations covering a pan European context will do well to integrate Swiss *U. tumidus* genetic data derived from this work.

Conclusions

In our study, we present significant genetic data that enhances the known distribution of the family Unionidae in Central European freshwater systems. Of the 120 Swiss individuals, 46 specimens belong to *A. anatina*, 21 to *A. cygnea*, 4 to *A. exulcerata*, 1 to *U. crassus*, 19 to *U. tumidus* and 29 *U. elongatulus*. This information is listed together with the coordinates and GenBank accession numbers in the Online Resource 2.

We show that *Anodonta exulcerata* occurs in southern Switzerland along the River Ticino and is likely to inhabit other lakes and rivers in the area. To adequately assess the distribution of *A. exulcerata*, more lakes in the Tessin region need to be investigated. Although *Unio mancus* was not verified in Switzerland, further investigations are needed in the Swiss Rhône, the Lake Geneva and the Doubs system to detect cryptic populations. We recorded *U. elongatulus* from several sites north of the Alps, which proved to be closely related to those found in northern Italy. This is an alarming finding that has up to now not been reported. As this new invasion of *U. elongatulus* jeopardizes the ecology of the Rhine drainage system, the biome inhabited by indigenous species will drastically change, shifting the IUCN threatened status more towards extinction. In addition, our findings indicate that the *A. anatina* records in Switzerland show a north to south and eastern distribution pattern, and that *Anodonta cygnea* is the second most identified *Anodonta* species in the sampling.

The ecological importance of freshwater bivalves for river and lake systems is evident: being filter feeders, freshwater mussels greatly improve the water quality. A healthy mussel population in a river can retain up to 50% of the seston (Pusch et al., 2001). The size of the filtered particles differs for example between the species: *A. anatina* and *U. pictorum* retain particle sizes from 4 μm upwards and certain North American species filter particles smaller than 1 μm (Prié, 2017). Therefore, high biodiversity in freshwater mussel populations is a priority. The Red List for the molluscs of Switzerland (Rüetschi et al., 2010) listed a concerning number of large freshwater mussel species as threatened: *Anodonta anatina* VU; *Unio crassus*; *Unio mancus* EN; *Unio pictorum* EN; *Unio tumidus*. Specifically, *Unio crassus* in Switzerland has shown how a once widespread species rapidly suffered population decline since the 1950s. The species became almost extinct due to intensive farming and the introduced muskrat (*Ondatra zibethicus* (Linnaeus, 1766)) (Vicentini & Pfändler, 2001; Vicentini, 2004; Schwarzer & Neubert, 2014; Carlevaro & Stucki, 2020). Today, there are less than ten known live populations of *U. crassus* in Switzerland (Rüetschi et al., 2010). Current measures to protect the highly vulnerable populations of *U. crassus* showed promising results in the Canton of Zürich. One declining population could be stabilized with the method of ex situ infection of host fishes (Vicentini, 2006; Schwarzer, 2021). Despite all efforts, one of the three last known populations in the Canton of Zürich got lost (Isabelle Flöss, personal communication, July 21, 2021). Our scientific investigation widens the knowledge of large, Swiss indigenous freshwater bivalves. The genetic data is paramount for continuing the protection and monitoring of these important mussel species. In light of agricultural pollution, these natural water filters deliver an exceptional ecosystem service. Lastly, the data collected during this study will serve well to improve the classification of unionid taxa recorded in the IUCN Red List for Switzerland (Rüetschi et al., 2010).

Data availability Sequence data are downloadable from GenBank (https://www.ncbi.nlm.nih.gov/genbank/), species distribution data are downloadable from CSCF database (http://www.cscf.ch/cscf/de/home.html)

Code availability Not applicable.

Declarations

Conflict of interest No conflicts to report.

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