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The freshwater mussels Unionidae (Mollusca: Bivalvia: Unionida) of the Lakes of Monticolo/Montiggl and Caldaro/Kaltern (Province of Bolzano/Bozen, Italy)

Abstract

The freshwater bivalves Unionidae are in dramatic decline globally. Due to wide shell variability, only the genus can be determined by traditional morphological methods, with genetic analyses being required for species identification.
From November 2018 to October 2020 four freshwater bodies in the Province of Bolzano/Bozen (Italy) (Small Lake and Large Lake of Monticolo/Montiggl, Lake Caldaro/Kaltern, and its main emissary Fossa Grande di Caldaro/Großer Kalterer Graben) have been explored to collect unionid bivalves by hand and scuba diving. Unionid presence, bathymetric distribution, biometric characteristics, and attachment of invasive allochthonous zebra mussel (*Dreissena polymorpha*) were detected.
Molecular barcoding analyses (COI) on foot snips from 25 specimens (19 *Anodonta* and 6 *Unio*) confirmed the presence of four native species: *Anodonta anatina*, *A. cygnea*, *A. exulcerata,* and *Unio elongatulus*. Most *Anodonta* specimens from both Small (78 %) and Large Lake of Monticolo (60 %) were parasitized by *Dreissena polymorpha* in numbers ranging from 1 to 81 per individual. The biomass of *D. polymorpha* amounted up to 41 % of *Anodonta*'s weight. *D. polymorpha* was not found in Lake Caldaro/Kaltern and its emissary Fossa Grande di Caldaro/Großer Kalterer Graben.

Keywords: molecular analyses, *Anodonta anatina*, *Anodonta cygnea*, *Anodonta exulcerata*, *Unio elongatulus*, South Tyrol

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Introduction

The freshwater bivalves of the Order Unionida Gray 1854 are in dramatic decline globally due to habitat degradation, water pollution, climate change, over-exploitation, and the introduction of non-native species (Lopes-Lima et al. 2017b, Ferreira-Rodríguez et al. 2019). The life cycle of freshwater mussels is characteristic and includes a parasitic stage (glochidium) using fishes as hosts (Castagnolo et al. 1980, Modesto et al. 2018). The order Unionida is represented in Europe by two families: the Margaritiferidae and the Unionidae, with 20 currently recognized species (Lopes-Lima et al. 2017a, Riccardi et al. 2022a). Seven species of the family Unionidae occur in the Italian peninsula, six of which are native: three *Anodonta* species (*A. anatina* (Linnaeus, 1758), *A. cygnea* (Linnaeus, 1758), *A. exulcerata* Porro, 1838), two *Unio* species (*U. elongatulus* Pfeiffer, 1825 and *U. mancus* Lamarck, 1819), *Microcondylaea bonellii* (Férussac, 1827) and the non-native *Sinanodonta woodiana* (Lea, 1834) (Froufe et al. 2017). In recent years, a neotype of *U. elongatulus* was designated and a redescription of *A. exulcerata* provided by using a combination of molecular, morphological, and anatomical data (Marrone et al. 2019, Riccardi et al. 2020). *A. exulcerata* is the most widespread species of *Anodonta* in Italy, occurring in Adriatic river drainages delimited by the Italian Alps in the North, the Apennine Mountains in the West and the Dinaric Alps in the East (Froufe et al. 2017). *A. cygnea* is present with a few populations in northern and central Italy. Two genetically distinct clades of *A. anatina* occur on the Italian peninsula: one restricted to the Ebro and Adriatic basins, the West Mediterranean Lineage, and the European

clade distributed across Europe and parts of Asia except for the Iberian Peninsula (Froufe et al. 2017). *Unio elongatulus* is present in the peri-Adriatic drainages of the Italian and Balkan peninsulas south to Albania. Conversely, *Unio mancus* occurs in the Apennine-Tyrrhenian drainages of the Italian peninsula, in Sardinia, Sicily, eastern Iberia, France and Corsica (Froufe et al. 2017, Marrone et al. 2019). *Microcondylaea bonellii* is an endangered species with only a handful of extant isolated populations in northern Italy (Po River basin and Brenta and Isonzo basins) and in Slovenia, Croatia, and Albania (Froufe et al. 2017, Riccardi et al. 2022b). The former occurrence of *M. bonellii* in the Province of Bolzano/Bozen in the outflow channel of Lake Caldaro has been reported in the literature (Nisters & Hellrigl 1996). The allochthonous invasive species *Sinanodonta woodiana*, native to East and South-East Asia, was reported for the first time in Italy in 1997 in Emilia-Romagna (Manganelli et al. 1998, Fabbri & Landi 1999). Since then, it has rapidly spread to many other regions of Italy (Cianfanelli et al. 2007, De Vico et al. 2007, Colomba et al. 2013, Renda & Niero 2014, Ercolini 2015, Cilenti et al. 2019) including Veneto (Niero 2003) and Lombardy in Lake Garda (Cappelletti et al. 2009) and Lake Maggiore (Kamburska et al. 2013).

All species of Unionidae are protected in the Province of Bolzano/Bozen by the Provincial Law n. 6, 12 May 2010. The Red List of Threatened Animal Species in South Tyrol (Nisters 1994) reports the presence in the Province of Bolzano/Bozen of *Anodonta anatina* and *Unio mancus*. Nisters & Hellrigl (1996) list three unionids in the checklist of animal species of South Tyrol: *Anodonta anatina, A. cygnea* and *Unio mancus*. More recently, *Anodonta anatina* and *Unio pictorum* were detected during monitoring of several water bodies in South Tyrol in 2014 and 2015 (Füreder et al. 2016). Recent genetic evidence (Araujo et al. 2017, Froufe et al. 2017, Marrone et al. 2019) shows that the *Unio elongatulus* populations present in South Tyrol were erroneously assigned to *U. mancus* or *U. pictorum*.

The aims of this study are to: 1. compile a species checklist of Unionidae in South Tyrol based on molecular analyses - DNA barcoding, 2. to collect new data on the occurrence and bathymetric distribution of unionids in four water bodies in South Tyrol, 3. to assess the occurrence and relative abundance of the allochthonous invasive zebra mussel *Dreissena polymorpha* (Pallas, 1771) on the shells of native unionids, and 4. to verify whether the allochthonous invasive *Sinanodonta woodiana* occurs in the four studied sites.

Study area

Unionids were searched in four water bodies of the Adige River basin in the Province of Bolzano/Bozen, Italy: 1. Small Lake of Monticolo/Montiggl (Lago Piccolo di Monticolo/Kleiner Montiggler See), 2. Large Lake of Monticolo/Montiggl (Lago Grande di Monticolo/Großer Montiggler See) (Fig. 1), 3. Lake Caldaro/Kaltern (Lago di Caldaro/Kalterer See) (Fig. 2) and 4. the drainage ditch Fossa Grande di Caldaro/Großer Kalterer Graben (Fig. 3).

The Small Lake of Monticolo (46°25'41" N 11°17'38" E; 514 m a.s.l; surface area 5.2 ha; volume 517,000 m^3 ; max.depth 14.8 m; mean depth 9.9 m) is a meso-eutrophic lake that receives water from internal springs, precipitation and runoff (Thaler & Tait 1981a).

The Large Lake of Monticolo (46°25'16" N 11°17'41"E; 492 m a.s.l.; surface area 17.8 ha; volume 1,490,000 m^3; max. water depth 12.5 m; mean depth 8.4 m) is a meso-eutrophic lake connected by its outflow (Angelbach, 5 km long) with Lake Caldaro (Thaler & Tait 1981b).

Lake Caldaro (46°23'11" N 11°15'20" E; 216 m a.s.l., surface area 131 ha; volume 6,000,000 m^3; max. water depth 5.6 m; average depth 3.5 m) is a mesotrophic lake (Provincia Autonoma di Bolzano – Alto Adige 2021a).

The Fossa Grande of Caldaro (46°21'51"N 11°15'26"E) is a drainage ditch flowing into the Adige River whose bottom is dredged every two or three years (Provincia Autonoma di Bolzano – Alto Adige 2021b).

Fig. 1: Small Lake (right) and Large Lake (left) of Monticolo.

Fig. 2: Lake Caldaro

Fig. 3: Fossa Grande di Caldaro/Großer Kalterer Graben

Materials and methods

Sampling

This study was carried out between November 2018 and October 2020. In the drainage ditch of Caldaro the mussels were collected by hand during low water periods in November 2018 and March 2019. In the lakes, the visual census and qualitative sampling were performed by scuba diving from April to October 2019 and 2020 (Tab. 1). Depth (± 0.1 m) of the position of each mussel was measured by a scuba dive computer Uwatec Aladin Prime.

Tab. 1: Field sampling and visual census dates.

1. Lago Piccolo di Monticolo	2. Lago Grande di Monticolo	3. Lago di Caldaro	4. Fossa Grande di Caldaro
21.04.2019	21.04.2019	10.05.2019	27.11.2018
03.09.2019	08.06.2019	02.06.2019	12.03.2019
13.06.2020	13.09.2019	05.06.2019	
14.10.2020	19.09.2019	21.05.2020	
	26.09.2019	28.05.2020	
	03.11.2019	02.06.2020	
	15.08.2020		
	10.10.2020		

Each collected unionid was identified morphologically to genus level, photographed, and measured with a steel Vernier caliper in mm (± 1 mm). The mussels were weighed before and after detaching and counting the zebra mussels attached to their shell.
In 2019, 8 individuals of *Anodonta* (2 from the Small Lake of Monticolo and 6 from the Large Lake of Monticolo) were marked with a waterproof pen on both valves and then returned to their site (Fig. 4). In 2020, the marked specimens were searched by scuba diving.
Eight specimens were transported to the laboratory for photo and video recording, then fixed in ethanol 70% and deposited in the zoological collection of the Museum of Nature South Tyrol, Bolzano/Bozen, Italy (*Unio elongatulus* catalogue numbers NMS Bozen Mol 7 – 8 and *Anodonta exulcerata* catalogue numbers NMS Bozen Mol 9 – 14).

Fig. 4: *Anodonta exulcerata*, identified with molecular analysis (GenBank accession number OP741121), marked with a progressive alphanumeric code and released back in Large Lake of Monticolo (Underwater photo by Massimo Morpurgo; 26.09.2019).

Molecular analyses

A small biopsy was taken from the foot of 25 specimens (19 *Anodonta* and 6 *Unio*) from the four sites in the field following Naimo et al. (1998) and the tissue was then transferred to 99% ethanol for subsequent molecular analyses. The F-type COI gene was amplified for all specimens using LCO_22me and HCO_700dy primers (Walker et al. 2006), with annealing temperatures ranging from 50°C to 55°C and PCR conditions as described in Froufe et al. (2014). Amplified DNA templates were purified and sequenced by a commercial company, Macrogen, using the same primers. Chromatograms were checked by eye using ChromasPro 2.1.8 (technelysium.com.au) and the alignment was performed using Bioedit 7.2.5 (Hall 1999). COI sequences were then run on BOLD (Ratnasingham & Hebert 2007) for barcode identification.

Results

Live individuals of both genera (81 *Anodonta* and 16 *Unio*) were recorded in total from the four sites. The molecular barcoding analyses (COI) of 19 *Anodonta* and 6 *Unio* identified the occurrence of four native species of unionids: *Anodonta anatina* (Linnaeus, 1758) West Mediterranean Lineage (Fig. 5), *A. cygnea* (Linnaeus, 1758), *A. exulcerata* Porro, 1838 and *Unio elongatulus* Pfeiffer, 1825 (Tab. 2). The resulting COI sequences have been deposited in GenBank under the accession numbers from OP741112 to OP741136 (Tab. 3). The endangered native *Microcondylaea bonellii* and the allochthonous invasive *Sinanodonta woodiana* were not found.

Tab. 2: Distribution of Unionidae and *Dreissena polymorpha* in the four investigated water bodies. The identification of the unionids is based on molecular barcoding analyses (COI).

	1. Lago Piccolo di Monticolo	**2. Lago Grande di Monticolo**	**3. Lago di Caldaro**	**4. Fossa Grande di Caldaro**
Anodonta anatina West Mediterranean Lineage	2 specimens		5 specimens	
Anodonta cygnea		3 specimens		
Anodonta exulcerata		2 specimens	1 specimen	6 specimens
Unio elongatulus	1 specimen	1 specimen	1 specimen	3 specimens
Dreissena polymorpha	abundant	abundant		

Fig. 5: *Anodonta anatina* (West Mediterranean Lineage), identified with molecular analysis (GenBank accession number OP741136), from the Small Lake of Monticolo with extended foot (Photo by Massimo Morpurgo; 13.06.2020).

Tab. 3: GenBank accession number, species, water body, latitude, longitude and sampling date of the 25 specimens collected for DNA analysis.

GenBank accession number	Species	Water body	Latitude	Longitude	Sampling date
OP741112	*Anodonta anatina* West Mediterranean Lineage	Lago Piccolo di Monticolo	46°25'48.2"N	11°17'40.1"E	03.09.2019
OP741113	*Anodonta anatina* West Mediterranean Lineage	Lago di Caldaro	46°23'05.7"N	11°15'36.9"E	10.05.2019
OP741114	*Anodonta anatina* West Mediterranean Lineage	Lago di Caldaro	46°23'05.7"N	11°15'36.9"E	02.06.2019
OP741115	*Anodonta anatina* West Mediterranean Lineage	Lago di Caldaro	46°23'05.7"N	11°15'36.9"E	02.06.2019
OP741116	*Anodonta exulcerata*	Lago di Caldaro	46°23'05.7"N	11°15'36.9"E	02.06.2019
OP741117	*Anodonta anatina* West Mediterranean Lineage	Lago di Caldaro	46°22'45.4"N	11°15'30.0"E	05.06.2019
OP741118	*Anodonta anatina* West Mediterranean Lineage	Lago di Caldaro	46°23'05.7"N	11°15'36.9"E	05.06.2019
OP741119	*Unio elongatulus*	Lago di Caldaro	46°23'05.7"N	11°15'36.9"E	10.05.2019
OP741120	*Anodonta cygnea*	Lago Grande di Monticolo	46°25'22.7"N	11°17'16.7"E	21.04.2019
OP741121	*Anodonta exulcerata*	Lago Grande di Monticolo	46°25'19.8"N	11°17'15.3"E	13.09.2019
OP741122	*Anodonta cygnea*	Lago Grande di Monticolo	46°25'17.3"N	11°17'22.1"E	19.09.2019
OP741123	*Anodonta cygnea*	Lago Grande di Monticolo	46°25'14.7"N	11°17'19.9"E	19.09.2019
OP741124	*Anodonta exulcerata*	Lago Grande di Monticolo	46°25'14.7"N	11°17'19.9"E	19.09.2019
OP741125	*Unio elongatulus*	Lago Grande di Monticolo	46°25'19.8"N	11°17'15.3"E	26.09.2019
OP741126	*Anodonta exulcerata*	Fossa Grande di Caldaro	46°19'47.2"N	11°14'52.0"E	27.11.2018
OP741127	*Anodonta exulcerata*	Fossa Grande di Caldaro	46°19'47.2"N	11°14'52.0"E	27.11.2018
OP741128	*Anodonta exulcerata*	Fossa Grande di Caldaro	46°19'47.2"N	11°14'52.0"E	27.11.2018
OP741129	*Anodonta exulcerata*	Fossa Grande di Caldaro	46°19'47.2"N	11°14'52.0"E	27.11.2018
OP741130	*Anodonta exulcerata*	Fossa Grande di Caldaro	46°19'47.2"N	11°14'52.0"E	27.11.2018
OP741131	*Anodonta exulcerata*	Fossa Grande di Caldaro	46°19'47.2"N	11°14'52.0"E	27.11.2018
OP741132	*Unio elongatulus*	Fossa Grande di Caldaro	46°19'47.2"N	11°14'52.0"E	27.11.2018
OP741133	*Unio elongatulus*	Fossa Grande di Caldaro	46°19'47.2"N	11°14'52.0"E	27.11.2018
OP741134	*Unio elongatulus*	Fossa Grande di Caldaro	46°20'48.0"N	11°15'38.6"E	12.03.2019
OP741135	*Unio elongatulus*	Lago Piccolo di Monticolo	46°25'47.7"N	11°17'39.8"E	13.06.2020
OP741136	*Anodonta anatina* West Mediterranean Lineage	Lago Piccolo di Monticolo	46°25'47.7"N	11°17'39.8"E	13.06.2020

The zebra mussel *Dreissena polymorpha* occurs in the two Lakes of Monticolo, while it was not found in Lake Caldaro and the drainage ditch Fossa Grande di Caldaro. Most *Anodonta* specimens from both Small (78 %) and Large Lake of Monticolo (60 %) were parasitized by *D. polymorpha* (Tab. 4) in numbers ranging from 1 to 81 per individual with an average of 19 (n = 16). The biomass of *D. polymorpha* amounted up to 41 % of *Anodonta*'s weight (Fig. 6).

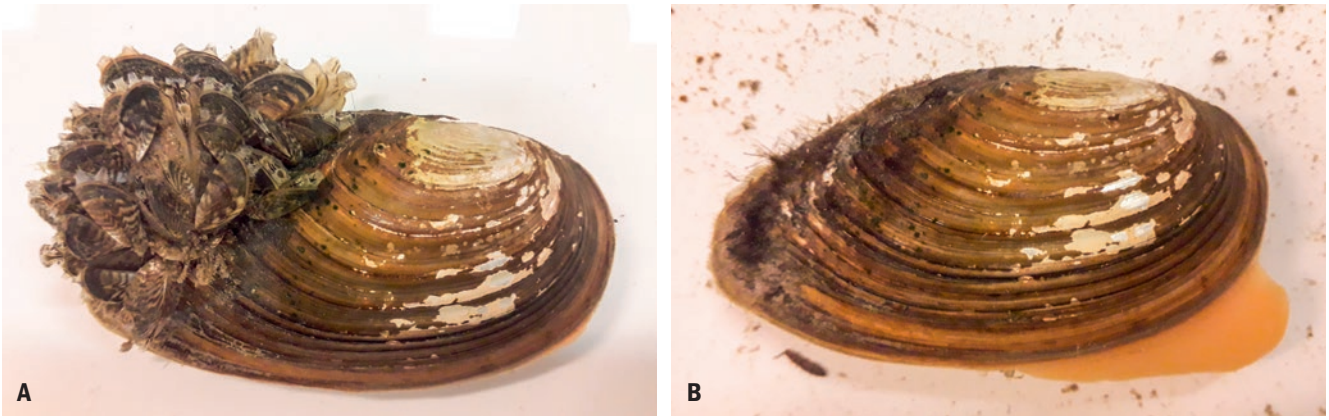

Fig. 6: a) *Anodonta cygnea*, identified with molecular analysis (GenBank accession number OP741120), from the Large Lake of Monticolo infested by 43 individuals of *Dreissena polymorpha*. b) Same specimen with extended foot and without zebra mussels (Photo by Massimo Morpurgo; 23.04.2019).

Tab. 4: *Anodonta* spp. and *Unio elongatulus* fouled by *Dreissena polymorpha*.

	1. Lago Piccolo di Monticolo	**2. Lago Grande di Monticolo**	**3. Lago di Caldaro**	**4. Fossa Grande di Caldaro**
Number of live individuals of *Anodonta* spp. examined	9	15	26	31
Percentage of *Anodonta* spp. fouled by *D. polymorpha*	78%	60%	0%	0%
Number of live individuals of *Unio elongatulus* examined	2	2	4	8
Percentage of *Unio elongatulus* fouled by *D. polymorpha*	0%	50%	0%	0%

Live *Anodonta* spp. were detected in Large Lake of Monticolo between 2.2 and 6.4 meters of depth (n = 15) (Fig. 7) and in Small Lake of Monticolo between 2.5 and 4.9 meters of depth (n = 9), while in Lake Caldaro from 1.2 to 2.7 meters of depth (n = 20) (Fig. 8).
Glochidia larvae were found in the gills of 2 dead specimens of *Anodonta exulcerata* collected on the banks of Fossa Grande di Caldaro on November 27, 2018. One *A. exulcerata* specimen collected on March 12, 2019, from the same site released mature glochidia in the lab aquarium.

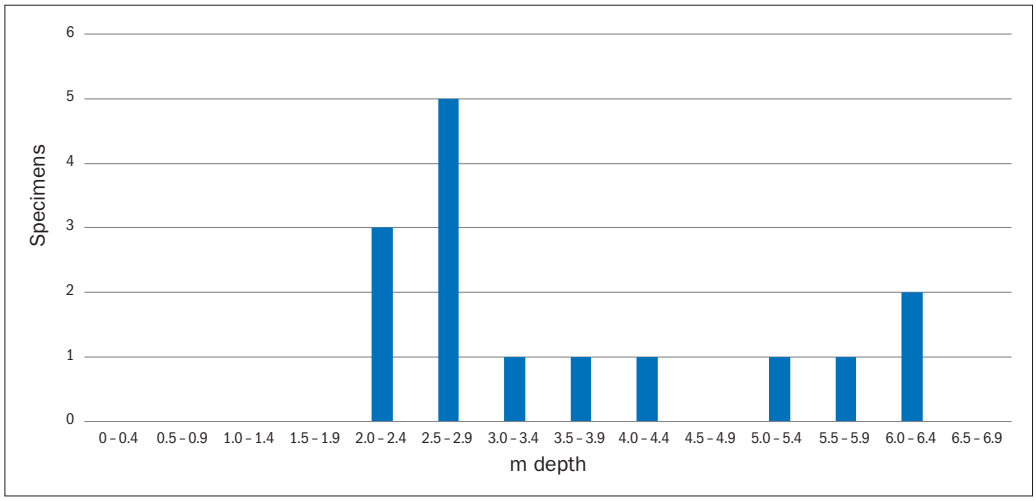

Fig. 7 Bathymetric distribution of *Anodonta* spp. (n = 15) in the Large Lake of Monticolo.

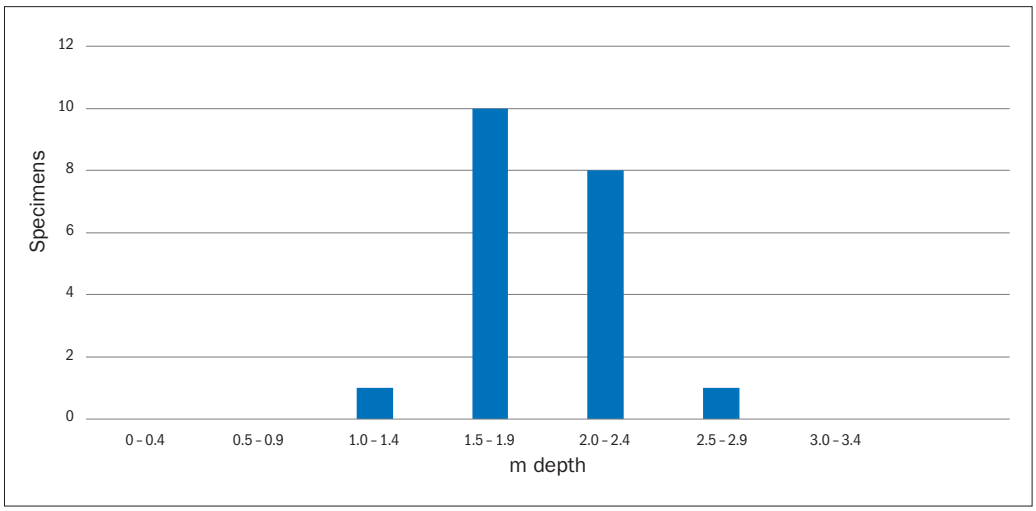

Fig. 8 Bathymetric distribution of *Anodonta* spp. (n = 20) in Lake Caldaro.

Five out of the 8 individuals of *Anodonta* spp. marked in 2019 were re-captured, apparently healthy, in 2020 in the two Lakes of Monticolo, almost in the same place where they had been released.

Discussion

Molecular identification allowed to update the species list of Unionidae (Mollusca: Bivalvia: Unionida) occurring in South Tyrol and increase the number of species reported in previous literature (Nisters 1994, Nisters & Hellrigl 1996, Füreder et al. 2016). The updated list thus includes:
Anodonta anatina (Linnaeus, 1758) West Mediterranean Lineage
Anodonta cygnea (Linnaeus, 1758)
Anodonta exulcerata Porro, 1838
Unio elongatulus Pfeiffer, 1825
All the 3 species of *Anodonta* native to the Italian peninsula (Froufe et al. 2017) were recorded, including *Anodonta exulcerata*, which was only recently re-validated (Froufe et al. 2017, Riccardi et al. 2020). The genetic analyses revealed that the only *Unio* species present in South Tyrol is *U. elongatulus* (Fig. 9 and Fig. 10), previously misidentified as *U. mancus* (Nisters 1994, Nisters & Hellrigl 1996) or *U. pictorum* (Füreder et al. 2016). *U. pictorum* is not present on the Italian peninsula but is widespread in Europe north of the Alps (Araujo et al. 2017, Froufe et al. 2017). In Italy, *U. mancus* is restricted to the river basins west of the Apennines, as well as to Sardinia and Sicily (Froufe et al. 2017, Marrone et al. 2019). On the one hand, correct taxonomic identification is essential for conservation policy (e.g. Prié et. al. 2012); on the other hand, the wide morphological plasticity of the Unionids may lead to misidentifications that can only be avoided by the application of molecular techniques. Unresolved taxonomy still hinders the proper legal protection of *Unio* species, like *Unio elongatulus* and *U. mancus*. The first is listed in the EU Habitats Directive (Annex V) and protected throughout the Europe Union by the Bern Convention (App. III), while the second is not. Moreover, there is still no IUCN conservation assessment of either *U. elongatulus* or *U. mancus* (Riccardi et al. 2022a).
U. elongatulus was found in all four sites of this study, although in low numbers. Recently, freshly dead shells of *Unio* were recorded in Fossa Piccola di Caldaro (Renate Alber, pers. com. 2022 and Franziska Zemmer, pers. com. 2022). Füreder et al. (2016) found living specimens of *Unio* only in Lake Caldaro and the in the large ditch of Caldaro out of 24 surveyed water bodies in South Tyrol potentially suitable for unionids. The distribution range of *Unio* in South Tyrol seems to be limited to the area of Lake Caldaro and related two ditches, and of the two Lakes of Monticolo (Fig. 11).

Fig. 9: Shells of *Unio elongatulus* collected in Fossa Grande di Caldaro (Photo by Massimo Morpurgo; 27.02.2019).

Fig. 10: *Unio elongatulus* from Fossa Grande di Caldaro in aquarium (Photo by Massimo Morpurgo; 14.03.2019).

Fig. 11: *Unio elongatulus* at 1.8 m depth in Lake Caldaro: a) completely buried in the sediment, b) the same specimen extracted from the sediment (Underwater photos by Massimo Morpurgo; 02.06.2019).

Fig. 12: *Anodonta* sp. partially buried in the sediment in Lake Caldaro (Underwater photo by Massimo Morpurgo; 02.06.2019).

The sympatric presence of *Anodonta exulcerata* and *A. cygnea* in Large Lake of Monticolo and of *A. exulcerata* and *A. anatina* in Lake Caldaro was observed (Fig. 12). Since not all the specimens from each lake were barcoded, we cannot exclude that all three species are present in the surveyed sites. The finding of *A. cygnea* in Large Lake of Monticolo brings the number of genetically confirmed Italian populations of the species to 4 (Froufe et al. 2017).

Collecting mussels can be challenging as they are often rare, spatially scattered, and difficult to detect. Sampling is often hampered by restrictive environmental conditions, such as too-high water levels, too-strong water currents, and too-high water turbidity. In lakes, unionids are generally studied in shallow littoral areas (<3 m depth; e.g. Huebner et al. 1990; Amyot & Downing 1991; Ravera et al. 2007; Crail et al. 2011), even though their distribution can extend much deeper (e.g. Cyr 2008). The sampling by diving carried out for this study allowed us to collect live specimens of the species present, while in a previous study (FÜREDER et al. 2016) almost only dead shells were retrieved by snorkeling in the Lakes of Monticolo.

From 20 surveys, only a small number (n =97) of live unionids was found in the three lakes and in the ditch (Tab. 4). Although quantitative estimates are not available, this suggests that populations are at low densities, particularly in the Lakes of Monticolo, compared to Lake Caldaro. Although admittedly speculative, we could hypothesize a negative impact from *Dreissena polymorpha*, which has been present in the first two lakes since 2001 (Morpurgo & Thaler 2002) but has no yet been detected in the third lake. Since mussels' density was never assessed, population trends are unknown. *D. polymorpha* is included in the IUCN list of the 100 world's worst invasive alien species (Lowe et al. 2000) and in the list of the 100 worst invasive species of Europe (DAISIE 2009). Firstly reported in Italy in Lake Garda in 1970 (Giusti & Oppi 1972, Franchini 1976), it can spread easily by boats (Quaglia et al. 2008), and has reached high densities in the Lakes of Monticolo on various solid substrates and unionids shells (Fig. 13).

Most *Anodonta* specimens from both Small (78%) and Large Lake of Monticolo (60%) were fouled by *Dreissena polymorpha*. *D. polymorpha* fouling on the posterior end of unionids hinders their locomotion, alters their balance, and can deform the unionid valves, causing valve occlusion and consequent suffocation (Mackie 1991). Moreover, zebra mussels can compete with unionids for available food. These mechanisms can affect mussels physiology and contribute to increased mortality (Sousa et al. 2011) (Fig. 14). None of the few *U. elongatulus* specimens collected in the Lakes of Monticolo was heavily fouled by zebra mussels, but it would be risky to put forward any hypothe-

Fig. 13: Invasive zebra mussels *Dreissena polymorpha* in Large Lake of Monticolo (Underwater photo by Massimo Morpurgo; 16.09.2016).

sis based on such a limited number of samples. According to Sousa et al. (2011), *Unio* species bury themselves deeper in the sediment than *Anodonta* spp., thus exposing less shell surface that can become fouled.

The fact that *Dreissena polymorpha* has never been found in Lake Caldaro (Bodon et al. 2005, Cianfanelli et al. 2010, Füreder et al. 2016, this study), although it receives an inflow from the Large Lake of Monticolo, where the species arrived more than twenty years ago, is still unexplained and deserves further studies.

The re-capture in Lakes of Monticolo of 5 marked *Anodonta* spp. specimens one year after their release show that the animals survived the biopsy performed for genetic analysis. Interestingly, they were not or just poorly fouled by *Dreissena polymorpha*.

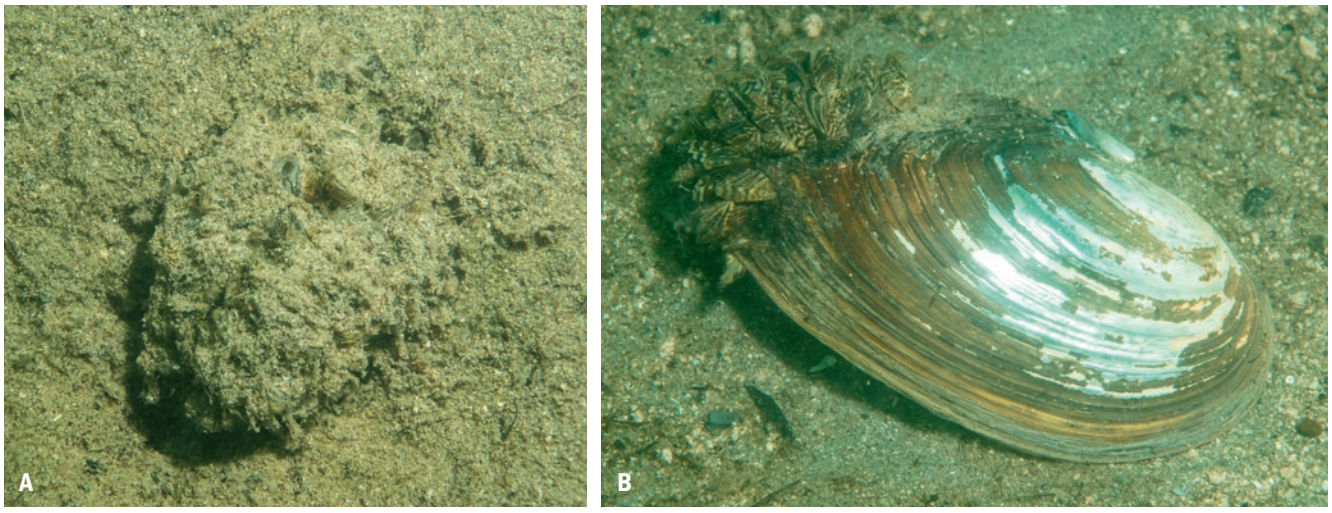

Fig. 14: *Anodonta* sp. highly infested by *Dreissena polymorpha* at 4.3 m depth in Large Lake of Monticolo: a) partially buried in the sediment, only the zebra mussels are visible; b) the same specimen extracted from the sediment (Underwater photos by Massimo Morpurgo: 09.06.2019).

Fig. 15: *Rhodeus amarus*: female (bottom) with a long ovipositor and male (top), specimens collected in South Tyrol (Photo in aquarium by Massimo Morpurgo; 24.11.2019).

The code written by a waterproof marker remained legible over time qualifying this economic and non-invasive marking method as a valid tool for the follow-up of freshwater mussel populations. The mark-release-recapture method, widely used to track animals in the wild, can be used to evaluate the demographic changes of the populations identified by this study.

The presence of *Microcondylaea bonellii* in the Fossa Grande di Caldaro reported by Nisters & Hellrigl (1996) was not confirmed by either this study or the previous one by Füreder et al. (2016). The species, in dramatic decline all over its distribution range (Riccardi et al. 2022b), is probably extinct in South Tyrol.

This study did not detect the presence of the allochthonous invasive *Sinanodonta woodiana*, which is spreading rapidly throughout Italy (e.g. Niero 2003, Cianfanelli et al. 2007, Cappelletti et al. 2009, Kamburska et al. 2013, Colomba et al. 2013, Ercolini 2015, Cilenti 2019) and is known to negatively impact native unionid populations (e.g. Cappelletti et al. 2009, Donrovich et al. 2017). For example, Fabbri & Landi (1999) and Niero (2003) reported that *S. woodiana* replaced *A. anatina* in the channels of Veneto and Emilia Romagna.

The European bitterling *Rhodeus amarus* (Bloch, 1782) poses a further threat to the native *Anodonta exulcerata* and *Unio elongatulus* in South Tyrol. Native to central Europe (Kottelat & Freyhof 2007), *R. amarus* is a small fish that has spread in South Tyrol by accidental (e.g. with restocking fish) and/or deliberate (anglers and aquarium hobbyists) introduction (Morpurgo 2005, Meraner 2015). Bitterling females deposit 80–100 eggs through a long ovipositor in the gills of unionids (Fig. 15). The males fertilize these eggs releasing sperm into the inhalant aperture of the freshwater mussels. The fertilized eggs develop inside the freshwater mussels (Kottelat & Freyhof 2007). The presence of bitterling eggs and embryos in the gills has physiological consequences for unionids because it causes a reduction in water circulation and thus competition for oxygen and impairment of filter-feeding (Prié 2017, Sousa et al. 2020). Soler et al. (2019) hypothesized that *R. amarus* can spawn in any European unionid mussel. In European areas where bitterling historically occurred, unionid species have developed adaptations against bitterling, such as expelling bitterling eggs and embryos. These adapta-

Fig. 16: *Anodonta exulcerata*, identified with molecular analysis (GenBank accession number OP741127), collected in Fossa Grande di Caldaro (Photo by Massimo Morpurgo; 27.02.2019).

tions are possibly missing in species from areas where the bitterling did not originally occur, as in the case of *A. exulcerata* and *U. elongatulus*. For these species, the additional physiological stress caused by the infestation of bitterling embryos may be problematic (Sousa et al. 2020), and should be investigated.
In addition to invasive species, the removal of aquatic vegetation and the excavation of sediments in the Caldaro ditches is a major threat to South Tyrolean freshwater mussels. Plenty of dead shells along the banks testify to mussel mortality during this periodic maintenance of the riverbed. This stress-induced mortality occurs when *A. exulcerata* (Fig. 16) is brooding the larvae (glochidia) in the gills, as indicated by the dead specimens retrieved from the banks of Fossa Grande di Caldaro in November 2018. Therefore, the reproductive contribution to the next generation is precluded (Fig. 17). To reduce the impact on the population the mussels should be returned to the water immediately after the excavation work. This simple and quick intervention should be mandatory for all the species of the family Unionidae that are protected in the Province of Bolzano by the Provincial Law n. 6, 12 May 2010 and for *Unio elongatulus* that is listed in the EU Habitats Directive (Annex V) and Bern Convention (App. III).

Fig. 17: a) Fossa Grande di Caldaro after excavation of bottom material on November 27, 2018; b) *Unio elongatulus* dead on the bank (Photos by Massimo Morpurgo).

Riassunto

I Molluschi Bivalvi d'acqua dolce della famiglia Unionidae sono drammaticamente in declino a livello globale. A causa della grande variabilità della forma del loro guscio, le caratteristiche morfometriche consentono di determinare il genere, ma solo l'analisi genetica garantisce l'identificazione esatta e certa delle specie.
Da novembre 2018 a ottobre 2020 quattro ambienti acquatici in Provincia di Bolzano: Lago Piccolo e Lago Grande di Monticolo, Lago di Caldaro e il suo principale emissario la Fossa Grande di Caldaro sono stati indagati alla ricerca di molluschi bivalvi della famiglia Unionidae. Tramite raccolte manuali e immersioni subacquee sono stati raccolti dati sulla distribuzione batimetrica e caratteristiche biometriche dei bivalvi nativi e sulla presenza di specie alloctone invasive, come la cozza zebrata (*Dreissena polymorpha*).
Le analisi molecolari (basate sul marcatore mitocondriale COI) effettuate su frammenti di tessuto del piede di 25 individui (19 *Anodonta* e 6 *Unio*) hanno permesso l'identificazione di quattro specie autoctone: *Anodonta anatina*, *A. cygnea*, *A. exulcerata* e *Unio elongatulus*.
Nel Lago Piccolo di Monticolo il 78 % e nel Lago Grande di Monticolo il 60 % dei molluschi *Anodonta* osservati presentava *Dreissena polymorpha* attaccate alla conchiglia in numero variabile da 1 a 81. Il peso complessivo delle *Dreissena* attaccate sulla conchiglia arrivava fino a oltre il 41 % del peso di *Anodonta*. *D. polymorpha* non è stata trovata nel Lago di Caldaro e nella Fossa Grande di Caldaro.

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