

Rapid Communication**First record of quagga mussel, *Dreissena bugensis* Andrusov, 1897, in Italy: morphological and genetic evidence in Lake Garda**Nico Salmaso^{1,*}, Francesca Ciutti², Cristina Cappelletti², Massimo Pindo¹ and Adriano Boscaini¹¹Research and Innovation Centre, Fondazione Edmund Mach, via E. Mach 1, 38098, San Michele all'Adige, Italy²Technology Transfer Centre, Fondazione Edmund Mach, via E. Mach 1, 38098, San Michele all'Adige, Italy

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OPEN ACCESS**Abstract**

Here, we report the first record of quagga mussel, *Dreissena bugensis* Andrusov, 1897, in Italy. This mollusc is native of the Dnieper River drainage of Ukraine and is one of the most aggressive invaders in freshwater ecosystems. The spread of quagga mussels throughout Europe and Northern America begun during the 1940s and 1989, respectively. Individuals of quagga mussel were identified in February and March 2022 in two stations located in the shallow (Bardolino) and deeper basins (Castelletto di Brenzone) of Lake Garda. Samples, collected with an Eckman grab, allowed identifying individuals of quagga mussel attached on macrophytes, stones and other mollusc shells. The identification of the individuals was carried out both through the analysis of morphological characters and genetic and phylogenetic analyses using the mitochondrial COI gene. The discovery of quagga mussel is the last of a long series of reports of non-indigenous species introduced into Lake Garda. Lacking direct connection by rivers or canals with other waterbodies colonized by *D. bugensis* at the northern side of the Alps, a more probable cause of introduction of this new species into Lake Garda can be due to unintentional overland transport through recreational boats and fishing gear. With this new discovery, Lake Garda has confirmed its pivotal role as a southern Alpine corridor for the introduction of non-indigenous species previously established at the northern border of the Alps. Considering its high colonization rate and compared with other invasion patterns observed in Europe and North America, it can be assumed that *D. bugensis* will soon establish itself with dominant populations throughout Lake Garda. At the same time, it can also be assumed that it will soon spread to other Italian water bodies.

Key words: invasive molluscs; mitochondrial COI; genetic analysis; phylogenetic analysis; alpine lakes; southern alpine corridor; invasion

Introduction

Along with habitat alteration, pollution, population growth and overexploitation, non-indigenous species (NIS) are included among the top five causes of biodiversity loss in Europe and worldwide (Tyus 2011). Their introduction and establishment cause the accelerated impairment of aquatic and riparian habitats and the ecosystem services they sustain (MedECC 2020). When they become invasive, NIS are a major threat to native plants and animals, causing important economic damages (Kettunen

et al. 2009; Walsh et al. 2016; Haubrock et al. 2021). Invasive alien species (IAS) are thought to be involved in over 70% of the 21st century's extinctions of native freshwater species, whereas 42% of current endangered species are impacted significantly by IAS (Rankin 2004). These threats have been the object of regulations enacted by the EU parliament (European Commission 2019).

Inland waters are particularly affected by the continuous introduction of NIS and establishment of IAS (Havel et al. 2015; Thomaz et al. 2015). Most of the routes of specific introduction and spread were proved or thought to be mediated by anthropogenic interventions, caused by the intentional or unintentional transport of species into new regions or by habitat alterations. On the other hand, acting at the global level, climate change may favor large shifts of species over wide latitudinal ranges (Sukenik et al. 2012; Hansen et al. 2017; Chaudhary et al. 2021).

One of the most exceptional cases of rapid colonization of freshwater ecosystems was that of *Dreissena bugensis* Andrusov, 1897 (“quagga mussel”, also known as *Dreissena rostriformis* Deshayes 1838 and *Dreissena rostriformis bugensis* Andrusov, 1897). This species is a bivalve mollusc belonging to the Dreissenidae family (Stepien et al. 2013; Wesselingh et al. 2019) and native of the southern Dnieper River drainage. Typical features of ecological relevance of this family are the presence of a byssus that allows adults and juveniles to attach to substrates or hosts (acting as dispersal vectors), and the production of numerous free-swimming larvae (*veligers*) that favour rapid dispersal.

The spread of quagga mussels throughout Europe begun during the 1940s from the North Black Sea region through rivers in Ukraine (Bij de Vaate et al. 2013). In Eastern Europe this species was first recorded in the upper Volga River in 1997 and in 2000 was identified in several other downstream watersheds (Orlova 2013). In Northern America the first quagga mussel was collected from Lake Erie in 1989; since then, this species soon spread from the Eastern to the Western coast (Lindsay et al. 2018).

The expansion of *D. bugensis* in Western Europe begun in 2004, when individuals of this species were discovered in the Romanian section of the Danube River. In 2006 this bivalve was detected in the Rhine–Meuse estuary (The Netherlands) (Molloy et al. 2007) and then, in 2007, in the Main River, a tributary of the Rhine River (Germany) (Van der Velde and Platvoet 2007). Successively, between 2009 and 2010, the quagga mussel was discovered for the first time also in the Albert Canal and the Meuse River, in Belgium (Marescaux et al. 2012) and, in 2011, in the southern section of the Moselle River, in France (Bij de Vaate and Beisel 2011). Since then, the presence of quagga mussel in Western Europe was confirmed in other regions, including the valley of the River Thames, in UK (Ainscough et al. 2015) and Ireland (Baars et al. 2022). In the Alpine Lake district, the colonization by quagga mussel was confirmed only in the northern perialpine

region, with individuals of the species identified, in the period spanning from 2014 to 2021, in the lakes Geneva, Hongrin, Neuchâtel, Biel, Murten, Constance, and the River Rhine (Haltiner et al. 2022, and references therein), as well as since 2019 in Lake Bourget (<https://www.cisalb.fr/>).

Bij de Vaate et al. (2013) hypothesized that western Europe was colonized by quagga mussels through two separate events: one from Eastern Europe through upstream dispersal in the Danube River (the “southern corridor”) and the other via jump dispersal as a primary (from the Ponto-Caspian region) or a secondary introduction (from secondary colonized areas). Using different approaches, besides dispersal through the southern corridor, Bij de Vaate (2010) and Marescaux et al. (2016) hypothesized a second invasion to Western Europe from North America via transatlantic shipping. In this context, it is interesting to note that, contrary to the hypothetical existence of other future invasion corridors (such as a “western corridor” around the western border of the Alps), no hypotheses were formulated on the potential colonization by *D. bugensis* of the regions south of the Alps.

In this work we report the first occurrence of *D. bugensis* in Italy. This NIS was identified in late winter 2022 along the eastern shores of Lake Garda. The identification was based on the morphological characterization of specimens, supported by genetic and phylogenetic analyses. The causes of its introduction and further potential spread will be briefly discussed.

Materials and methods

Study area

Lake Garda is the largest Italian lake and one of the largest water bodies in the Alpine region. Surface, volume, and maximum depth are 368 km², 49 × 10⁹ m³ and 350 m, respectively. The lake is located at a mean altitude of 65 m a.s.l.. Lake Garda is formed by two basins (Figure 1). In the north-western basin the shores are steep, and the bottom extends over 20 km to depths between 300 and 350 m. The shallower south-eastern basin has a maximum depth of around 80 m, therefore representing only a small portion of the lake’s overall volume (less than 7%).

Since the 1990s, Lake Garda underwent important changes in the trophic status and thermal structure (Salmaso et al. 2018, 2020). Maximum concentrations of total phosphorus (TP) in the water column (0–350 m) were recorded around 2000 (20–25 µg P L⁻¹). Soon after, TP showed a rapid decrease, reaching concentrations of around 15 µg P L⁻¹ in the last five years. At present, the lake is oligo-mesotrophic. At the same time, Lake Garda showed a significant warming trend. The linear increase in water temperatures in the 0–50 m layer between 1991 and 2018 was around 0.030 °C yr⁻¹ (Salmaso et al. 2020).

Owing to its intrinsic naturalistic value, the beauty of the landscape and the oligo-mesotrophic nature of the waters, Lake Garda showed an impressive

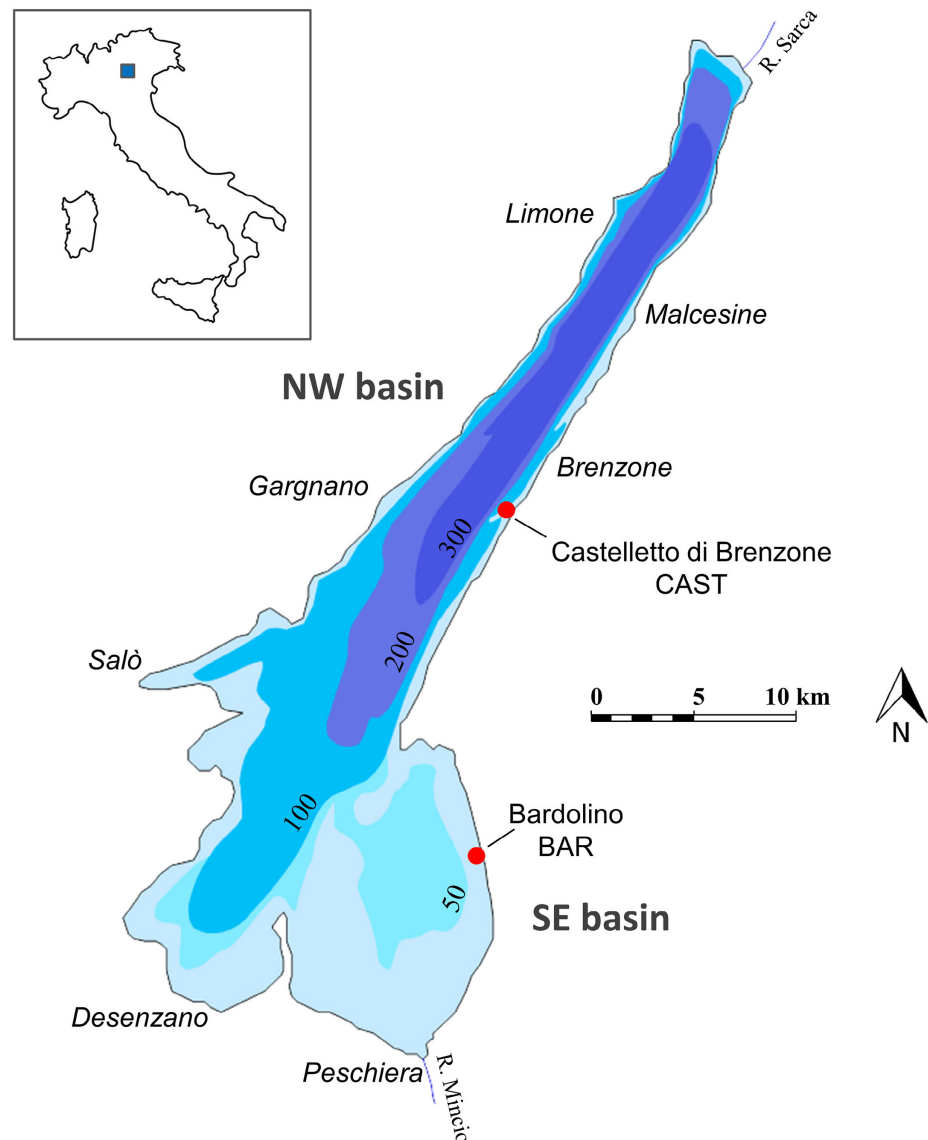


Figure 1. Map of Lake Garda. The areas where *Dreissena bugensis* was found in February and March 2022 are marked with a red circle (Table 1).

development of tourism since the 1960s and 1970s. The areas along the shores underwent impressive changes, due to the adaptation of facilities dedicated to the reception of tourists. In the last years, Lake Garda had 24 million tourist visits per year (Finotto 2017). Of these, 85% of visitors travelled from Germany and other central and northern European countries. Main tourist activities on the lake included swimming, sailing, surfing, and recreational boating (Betta and Maccagnan 2010).

Sampling and morphological identification

Sixty-six specimens of quagga mussel were collected in the SE and NE shores of Lake Garda, in the Province of Verona, with the logistic support of the Environmental Agency of the Veneto Region (Figure 1). Molluscs were collected using an Eckman grab in front of the villages of Bardolino at 10 m depth and Castelletto di Brenzone at 15 meters depth. The distance

Table 1. Location of the sampling sites, substrata where the organisms were collected, and maximum length of the organisms. The last two columns report the sample codes and accession numbers of the five COI sequences deposited in GenBank.

Sampling station	Basin	Sampling dates (2022)	Latitude	Longitude	Substratum	Individuals (n)	Maximum length (mm)	DNA Sample code	Accession number
Bardolino	SE	17 February 15 March	45.5316031	10.7227848	macrophyte	60	12.9	BAR1	OP143817
								BAR2	OP143818
Castelletto	NW	8 February	45.6804898	10.7415734	stones and mollusc shells	6	16.6	CASTDB	OP143819
								CAST1	OP143820
								CAST2	OP143821

along the coast between the sampled stations is around 20 km. The molluscs were then brought to the laboratory for taxonomic identification. Shells length (the longest antero-posterior distance) was measured using a calibre to the nearest 0.1 millimetre. For subsequent genetic analyses, some individuals were frozen at -20°C (next section).

DNA extraction, genetic and phylogenetic analyses

Genetic analyses were performed on two and three specimens collected along the shores at Bardolino and Castelletto, respectively (Figure 1; Table 1). Total genomic DNA was extracted from the whole animals using the DNeasy Blood and Tissue kit (Qiagen, Inc.) according to manufacturer's Protocol. DNA concentrations, measured with a NanoDrop ND-8000 (ThermoFisher Scientific Inc., MA, USA), were in the range $74\text{--}204\text{ ng }\mu\text{L}^{-1}$. Genetic determinations were carried out by the amplification of a fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene, using the universal primers by Folmer et al. (1994), namely LCO1490 (5' GGTCAAC AAATCATAAAGATATTGG 3') and HC02198 (5' TAAACTTCAGGGT GACCAAAAATCA 3'). Amplification was performed using 1X PCR Buffer (Thermo Fisher Scientific), 0.2 mM of dNTPs mix (ThermoFisher Scientific), 2.5 mM MgCl_2 , 0.8 μM of the two primers, 1.25 U of AmpliTaq Gold[®]DNA Polymerase (ThermoFisher Scientific), 2 μL of DNA templates diluted to the range $8\text{--}10\text{ ng }\mu\text{L}^{-1}$, and the necessary volume of ddH₂O to reach a final volume of 25 μL . The cycling protocol included an initial denaturation step at 95°C for 10 min; 35 cycles of DNA denaturation at 95°C for 45 seconds, primer annealing at 48°C for 40 seconds, and strand elongation at 72°C for 60 seconds; and a final elongation step at 72°C for 7 min. PCR products were checked and separated by 1.5% agarose gel electrophoresis stained with Midori Green Advance (Nippon Genetics). DNA sizes were preliminarily evaluated with a commercial DNA ladder 100–5000 bp GeneRuler Express DNA Ladder (ThermoFisher Scientific). Before COI sequencing, PCR products were cleaned with Exonuclease plus Shrimp Alkaline Phosphatase Illustra ExoProStar 1-Step (GE Healthcare, Little Chalfont, UK) according to the manufacturer's instructions. The same primers as in the PCR were used with the BigDye Terminator Cycle Sequencing technology (Applied Biosystems), according to the manufacturers' protocols. After purification in automation using the CleanDTR bead Kit (CleanNA), products were run on an Automated Capillary Electrophoresis Sequencer 3730XL DNA Analyzer latest version (Applied Biosystems).

Preliminary checking of chromatograms and trimming of low-quality 5' and 3' ends were performed with Chromatogram Explorer Lite v. 5.0.2 (Heracle Biosoft). Forward and reverse sequences were then assembled using UGENE v. 42 (Okonechnikov et al. 2012). Sequences were deposited to GenBank with accession numbers OP143817–OP143821; in GenBank, *D. bugensis* is considered a homotypic synonym of *D. rostriformis bugensis*. The identity of the new COI sequences from Lake Garda was preliminarily verified using megablast. All the five specimens shared the same sequence, therefore only two were included in the successive phylogenetic analysis. To evaluate the phylogenetic position of the new molluscs isolated from Lake Garda, a selection of homologous sequences was imported from GenBank. These included representatives of *Dreissena rostriformis/bugensis*, *D. polymorpha* Pallas 1771 (including *D. p. anatolica*), *D. caputlacus* Schütt 1993, *D. carinata* Dunker 1853 (*D. presbensis*) and *D. blanci* Westerlund 1890. *Congeria kusceri* Bole, 1962 was included as outgroup. The phylogenetic analysis was computed in Linux Ubuntu 22.04 using MEGA X 11.011 (Tamura et al. 2021). Sequences were aligned with MUSCLE (Edgar 2004) and, after trimming to the shortest sequence, a subsequent alignment of 564 bp was obtained. The phylogenetic tree (maximum likelihood, 500 bootstraps) was computed with the HKY+G best-fitting evolutionary model.

Results

Morphological characteristics

Benthic samples collected with an Eckman grab in the two sites allowed identifying the individuals of quagga mussels in different substrata, including macrophytes, stones and mollusc shells (Table 1). Just from a first visual inspections, the outline shapes and colour patterns of the new molluscs collected in Lake Garda looked different from the common shells of *D. polymorpha* (Figure 2). The morphology of the new shells was congruent with the illustrations and descriptions reported for *D. bugensis/D. rostriformis bugensis* in Rosenberg and Ludyanskiy (1994), Claxton et al. (1997), Ram et al. (2012), and Teubner et al. (2016). Specifically, the new organisms were characterized by pale colour near the hinge, a rounded transition of the ventral and dorsal shell surfaces, and convex ventral shell surface. These characters contrast with those of *D. polymorpha*, which has striped colour patterns, sharp-angled transition of the ventral and dorsal shell surfaces, and flat or concave ventral shells. In the two sampling stations, the maximum length of the individuals of quagga mussel was 12.9 (Bardolino) and 16.6 mm (Castelletto di Brenzone) (Table 1).

Genetic and phylogenetic analysis of individuals collected in Lake Garda

After trimming low-quality ends of raw sequences and obtaining the contigs, the length of the five final sequences was between 592 and 620 base pairs (bp).

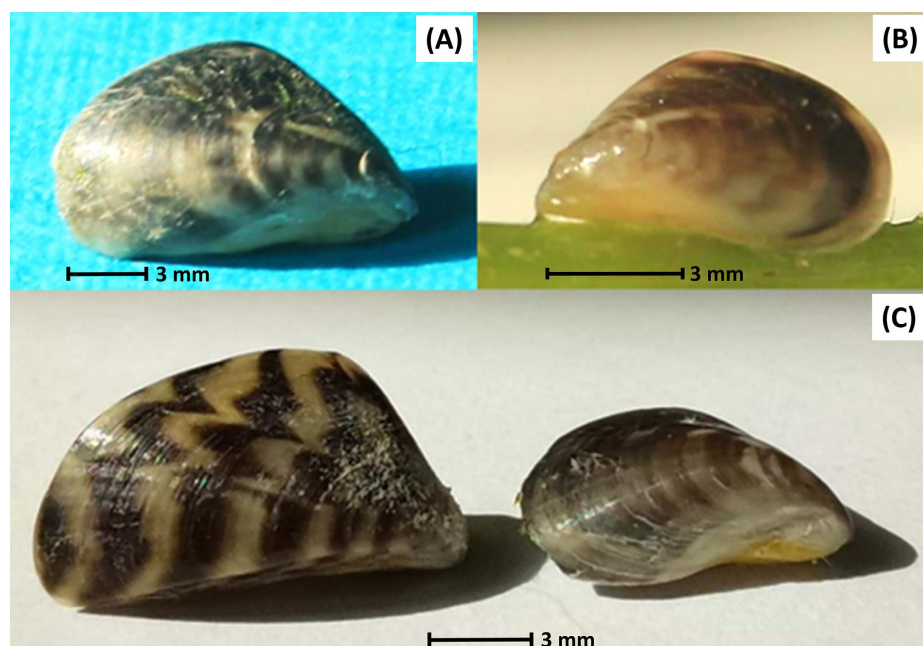


Figure 2. Dreissenids collected in Lake Garda; (A, B) *Dreissena bugensis*; (C) Left, *Dreissena polymorpha* and, right, *Dreissena bugensis*.

Table 2. Megablast analysis of the COI sequence “CAST2” (620 bp) obtained from Lake Garda (Table 1). The table reports only the results with 100% percent identity and query cover \geq 95%. “GenBank species” indicates the original name reported in GenBank: Dbugen, *D. bugensis*; Drostr, *D. rostriformis*; Drostr_bugen, *D. r. bugensis*. The last column reports the locality where the specimens were collected or the haplotype.

Accession number	Percent identity	Query cover	GenBank species	Locality / Haplotype
MF469065			Drostr_bugen	River Saone (tributary R. Rhône), France (Prié and Fruget 2017)
MF469064	100%	100%	Drostr_bugen	
MF469063			Drostr_bugen	
KJ881409	100%	100%	Drostr	Haplotype Q1 (Marescaux et al. 2016)
NC_057514	100%	100%	Drostr	Danube River in Vienna, Austria (Calcino et al. 2020)
JX945980	100%	100%	Drostr_bugen	River Meuse, France (Marescaux et al. 2012)
JN133735	100%	100%	Dbugen	Chicago, USA
EF080861	100%	100%	Drostr_bugen	Hollands Diep, in the Rhine delta, The Netherlands (Molloy et al. 2007)
AF495877	100%	100%	Dbugen	Warm water reservoir 10 km north of Kiev, Ukraine (Van der Velde et al. 2010)
EU484436	100%	99%	Dbugen	Lake Superior, USA (Grigorovich et al. 2008)
DBU47651	100%	98%	Dbugen	Lake Ontario, USA (Baldwin et al. 1996)
DQ840132	100%	95%	Dbugen	“pop_variant=”Black Sea” (Gelembiuk et al. 2006)

In the common region, all sequences were identical, sharing a unique haplotype. After megablast analysis, all the sequences showed a percent identity of 100% with several representatives of *D. bugensis*, *D. r. bugensis* and *D. rostriformis*. The similarity with other *Dreissena* species was always $<$ 91%. The results of the megablast query using the sequence CAST2 (620 bp) indicated a perfect match with several haplotypes characterized in Eastern Europe (Ukraine and Black Sea region), Western Europe (Austria, France, The Netherlands) and North America (lakes Ontario and Superior) (Table 2).

In the phylogenetic analysis, the different species of *Dreissena* were clustered into circumscribed clades (Figure 3). The taxonomic attributions of the species at the tips of the tree were those reported in GenBank. All the specimens classified as *D. bugensis*, *D. r. bugensis* and *D. rostriformis* were

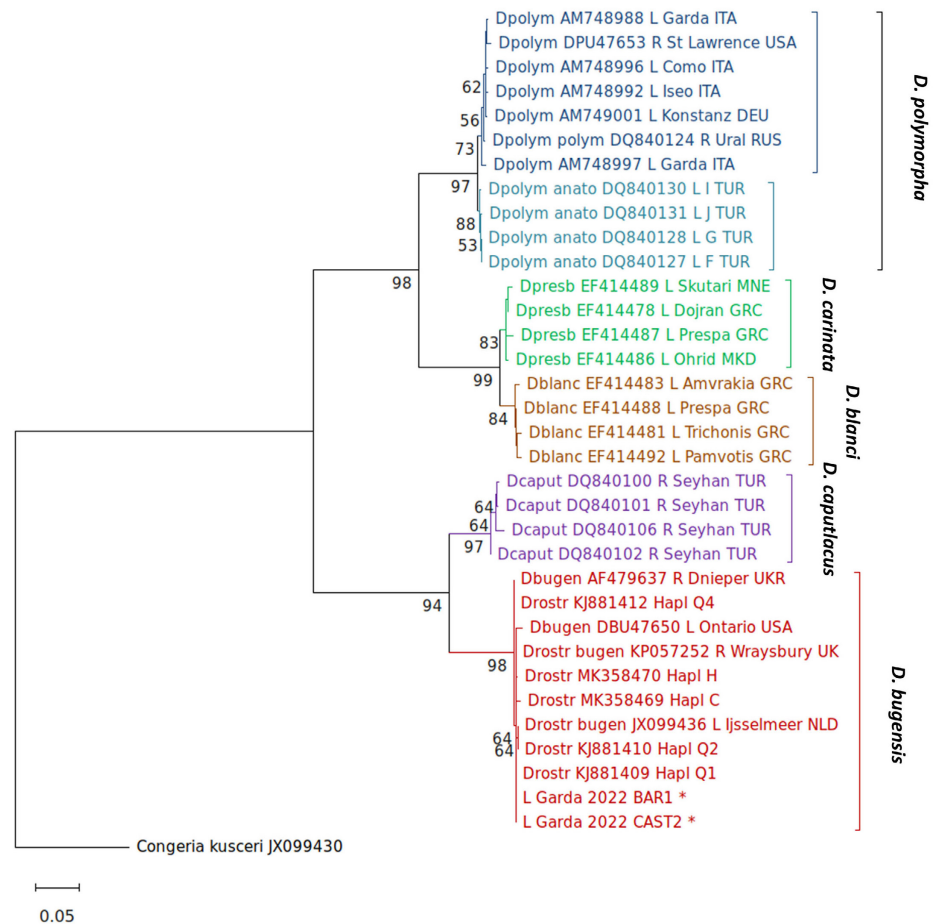


Figure 3. Maximum likelihood (ML) rooted topology of distinct species of *Dreissena* based on the alignment of the COI gene (564 bp). Values at nodes are bootstrap percentages from ML tree (500 replications; bootstrap values < 50 not shown). The species names on the tips were those recorded from GenBank. The individuals of quagga mussel collected in Lake Garda in 2022 are marked with an asterisk (Table 1). The sequences are identified by a code reporting the species code, the accession number, the sampling location (L, lake; R, river) or the haplotype, and, when relevant, the country. Species codes: Dpolym, *D. polymorpha*; Dpolym_anato, *D. p. anatolica*; Dpresb, *D. carinata* (*D. presbensis*); Dblanc, *D. blanci*; Dcaput, *D. caputlacus*; Dbugen, *D. bugensis*; Drostr, *D. rostriformis*; Drostr_bugen, *D. r. bugensis*.

included, along with the individuals collected in Lake Garda, in the same clade (Figure 3). It is worth highlighting that all the sequences of *D. polymorpha* recorded in the large and deep lakes south of the Alps (Garda, Como and Iseo; Quaglia et al. 2008) were all included in the corresponding clade, well separated from that of *D. bugensis*. Similarly, *Dreissena p. anatolica*, was considered endemic to Turkish lakes, whereas *D. blanci* and *D. carinata* were collected in Greece and in the Balkan Peninsula (Stepien et al. 2013).

Discussion

Taxonomic attribution

The taxonomy of the *Dreissena* species from the Ponto-Caspian region is still object of debate (Stepien et al. 2013; Voroshilova 2016; Wesselingh et al. 2019). As a result, many researchers have used different names, i.e., *D. bugensis*, *D. rostriformis* (*D. grimmii*), or *D. r. bugensis* to indicate the

individuals of quagga mussel identified in the European continent and North America (e.g., Figure 3). The overlapping of names originated also from the lack of clear genetic distinction using the widespread mtDNA COI gene (Stepien et al. 2013). *Dreissena bugensis* and *D. rostriformis* have different geographic origin, the former being endemic to the estuaries of the northern Black Sea and introduced in western and central Europe and North America (May et al. 2006), and the latter endemic of the Caspian Sea; moreover, the recent *D. rostriformis* Caspian species should be treated different from individuals identified from Pliocene deposits of Crimea in the Black Sea Basin, and the name *Dreissena grimmi* (Andrusov, 1890) should be applied instead (see Wesselingh et al. 2019 for a thorough account). Therefore, considering the geographic separation of the two species and the non overlapping ecological tolerances (Rosenberg and Ludyanskiy 1994), *D. bugensis* and *D. grimmi* (*D. rostriformis*) should be considered as viable species (Rosenberg and Ludyanskiy 1994; Wesselingh et al. 2019; Van de Velde et al. 2020).

The discrimination between the two main molluscs invaders zebra mussel and quagga mussel based on morphological characters can be affected by errors. For example, in a study carried out in the German Danube system, Beggel et al. (2015) reported that, using only shell morphology, 17.5% of *D. bugensis* and *D. polymorpha* were assigned to the wrong species, suggesting that dreissenid mussels should be always genetically verified.

It is worth observing that the individuals collected in Lake Garda showed a mtDNA COI gene sequence coincident with the Q1 haplotype described by Marescaux et al. (2016). This haplotype showed a wide distribution encompassing the North America Great Lakes area, Western Europe, the Pontic region and Eastern Europe, and for this reason it was considered to represent the ancestral haplotype (Marescaux et al. 2016) (Table 1). Inferences on a more precise origin of the invading population would require additional approaches including, besides mitochondrial diversity, analyses of nuclear genetic variability.

Lake Garda as a hot spot for invasions

The discovery of the quagga mussel in 2022 is the last of a long series of reports of non-indigenous species introduced into Lake Garda. In the review by Ciutti and Cappelletti (2017), at least 42 different NIS were reported, including 15 invertebrates, 23 fish, 3 macrophytes and 1 macroalga.

Among molluscs, the most apparent introduction in Lake Garda was that of *D. polymorpha* at the end of the 1960s (Giusti and Oppi 1973); since then, the zebra mussel colonized several other water bodies throughout Italy. After the 1990s, other non-indigenous molluscs were identified in Lake Garda, i.e., the Asian clam *Corbicula fluminea* (Müller, 1774) in 2000; *Corbicula fluminalis* (Müller, 1774) in 2008; *Corbicula leana* Prime, 1867 and *Corbicula largillierti* (Philippi, 1844) in the second half of 2000s; and

Sinanodonta woodiana (Lea, 1834) in 2009 (Cappelletti et al. 2009; Ciutti and Cappelletti 2009; Lopez-Soriano et al. 2018). For a few of these, Lake Garda represented the first point of introduction in Italy, i.e., *D. polymorpha*, *C. fluminalis* and *C. largilliertii*. In general, the spread of these species occurs when they are attached to boats, carried in ballast water or aquarium trade, used as bait, or transported by water currents. In Lake Garda it was hypothesized that the introductions were due to recreational boating.

As for the quagga mussel (and the zebra mussel), shipping was considered one of the main primary routes of introduction into North America and Central and Western Europe (Orlova et al. 2005b). Nevertheless, lacking the direct connection of rivers, streams or canals with other water bodies colonized by *D. bugensis* on the northern side of the Alps (including the northern perialpine region), a more probable cause of introduction of the quagga mussel into Lake Garda may be due, as in the case of the previous molluscs' introductions, by unintentional overland transport by recreational boating and fishing gear. Transport can involve shells attached to the hull, or even larval conveyance surviving in small water volumes inside the boats and equipment (Choi et al. 2013; Dalton and Cottrell 2013). As one of the most popular tourist activities in Lake Garda and in the other large lakes south of the Alps, recreational boating could play a significant role in the further spread of *D. bugensis* in other Italian lakes. This mode of transport was considered instrumental in explaining the impressive large-distance movement of quagga mussel from the Great Lakes region to southwestern USA states (Wong and Gerstenberger 2011). Nevertheless, considering the potential transport of dreissenids shells over large areas (De Ventura et al. 2016), a precise identification of the origin of the new population discovered in Lake Garda, i.e., from central Europe or the northern perialpine region, cannot be assessed precisely.

Invasion pattern

The identification of quagga mussel in the two main basins of Lake Garda allowed hypothesizing a complete colonization of this new NIS in the whole lake. Nevertheless, the size of the shells collected so far was quite small (< 17 mm), suggesting a recent introduction of this population in the lake. For example, Bij de Vaate (2010) suggested that individuals collected in the Rhine with length > 18 mm were at least 1 year old. Individuals collected in the Rhine delta in The Netherlands with shell lengths reaching the maximum size of 24.6 mm were considered to be at least 2 years old (Molloy et al. 2007). Four years after the colonization of the Volga River, the population of quagga mussel included all the size classes up to the largest individuals with shells measuring 30 mm (Orlova et al. 2005a). However, considering the limited sampled area, a reliable estimate of the colonization timing will require further observations extended in other areas and depths of the lake.

Although based on an experimental setting focused on the identification of microbial communities in pelagic and biofilm habitats, the analysis of the bulk and environmental DNA collected in 2019 in 37 lakes and 22 rivers in the Alps (Salmaso et al. 2022) allowed to identify sequences of 18S rRNA genes attributable to *D. bugensis* only in the lakes Geneva and Bourget, but not in Lake Garda (Kurmayer et al. 2021).

The introduction of quagga mussel is often accompanied by a decline in zebra mussel populations (Orlova et al. 2005b; Ram et al. 2012). In the littoral zone of Lake Constance, the replacement of zebra mussels by quagga mussels was very fast, occurring in around four years, from 2016 to 2019 (Haltiner et al. 2022). The higher competitive ability of *D. bugensis* has been attributed to substantially higher growth rates and wider growth ranges, allowing it to reach adult size earlier and providing greater flexibility in fluctuating environments (Metz 2018). Moreover, veligers of the quagga mussel have been observed all year round, whereas the zebra mussel veligers have only been found in summer (Gerstenberger et al. 2011; Haltiner et al. 2022), therefore giving to the former a wider period of time to find suitable substrata to colonize. Additionally, compared to zebra mussel, quagga mussel can colonize deeper substrata, down to around 130 m (Mills et al. 1993) and even down to 251 m (Haltiner et al. 2022).

After the first introduction of *D. polymorpha* in Italy at the end of the 1960s, Lake Garda confirmed its pivotal role as a southern Alpine corridor for the introduction of non-indigenous species previously established at the northern border of the Alps. Considering its high colonization rate and compared with other invasion patterns observed in Europe and North America, it can be assumed that *D. bugensis* will soon establish itself with dominant populations throughout Lake Garda. At the same time, it can also be assumed that it will soon spread to other Italian water bodies.

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Authors' contribution

N.S., A.B.: research conceptualization. A.B.: sampling campaign. A.B., M.P., N.S.: molecular analyses. N.S.: data analysis, draft preparation, and review. A.B., C.C., F.C., M.P., N.S.: data interpretation and review of the manuscript.

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