

The increasing spread of the European barbel in the Italian large lowland rivers is threatening the native species

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ABSTRACT

Freshwater ecosystems are heavily altered by human activities, with anthropic introductions of non-native species substantially contributing to their biotic degradation. The invasion by alien species can alter ecosystem balances with direct and indirect impacts across different levels of biotic organization. The number of invasive alien species is particularly high in Mediterranean fresh waters including rivers draining in the northern Adriatic basins. Here, the Padanian barbel *Barbus plebejus* endemic to the Padano-Venetian district, is threatened by the introduction of the European barbel *Barbus barbus* via competition and introgressive hybridization. In this study, we genetically characterised using the partial mitochondrial DNA Cytochrome b gene, barbels present in two main tributaries of the Po River, the lower Ticino and Oglio rivers where the two species are suspected to co-occur. Since the two river sections are highly regulated and present severe hydrological alterations caused by dams and other hydraulic structures, the aim of the study was to provide information on the barbel populations composition in these systems to foster the implementation of more suitable management plans aimed at the conservation of the native species. A total of 86 barbel have been analysed (50 in the Ticino River and 36 in the Oglio River) corresponding to 13 haplotypes of which 8 were phylogenetically attributed to *B. barbus* and five to *B. plebejus*. The high haplotypic diversity and the unimodal trend evidenced by the mismatch distribution analysis for *B. barbus* haplotypes support a potential demographic expansion. Altogether, these results highlighted that *B. barbus* is progressively expanding in northern Italy while posing a serious risk for the conservation of the native *B. plebejus*, stressing the need to actively focus ecosystem and fisheries management regulations to stop *B. barbus* from spreading further throughout northern Italy.

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INTRODUCTION

On a global basis, freshwater habitats are rich sources of biodiversity (Strayer and Dudgeon, 2010), but they are also hotspots for human exploitation and modification (Monroe *et al.*, 2009). The general progressive degradation of aquatic ecosystems in recent years has led to an impoverishment of the autochthonous fish fauna, being the introduction of invasive alien species one of the most important perturbation factors of anthropic origin (Jackson *et al.*, 2017; Mollot *et al.*, 2017). The invasion by alien species alters ecosystem balances with direct and indirect impacts on native species (Didham *et al.*, 2005; Cucherousset and Olden, 2011; Hermoso and Clavero, 2011; Simberloff *et al.*, 2013).

In particular, fish translocation and stocking activities, two widely used management measures, are progressively recognized detrimental to native ichthyofauna in the receiving fresh waters (Cucherousset *et al.*, 2020). Indeed, if the ecological niche of the invasive alien species overlaps with that of the native species (Strayer, 2006), and particularly if the two species are taxonomically related, the possibility of forming hybrid individuals is high, with the consequent loss of genetic integrity of the autochthonous populations (Hänfling *et al.*, 2005; Hayden *et al.*, 2010; Meraner *et al.*,

2013; Geiger *et al.*, 2016; Zaccara *et al.*, 2021) and potential alteration of their functional role (De Santis *et al.*, 2021). Moreover, the presence of trophic interactions between the invader and the native species can lead to increased predation and competition pressure (David *et al.*, 2017; Jackson *et al.*, 2017). The foraging behaviours of the invader can modify the habitat characteristics through ecological engineering (Mollet *et al.*, 2017), as well as transmitting novel pathogens to the native species (Sheath *et al.*, 2015). Consequently, invasive alien fish represent a considerable global challenge, requiring effective management and regulation (Pimentel *et al.*, 2000; Dlugosch and Parker, 2008; Estoup and Guillemaud, 2010).

In Mediterranean rivers there are more than 200 endemic freshwater fish species, of which more than half have been reported as being under threat (Smith and Darwall, 2006). In particular, in the northern Adriatic basins, endemic species belonging to the Cyprinidae family are severely impacted by the introduction of non-native species besides by other disturbances such as the hydrologic alteration caused by dams impounding river sections and regulating flows (Bianco, 1995; Smith and Darwall, 2006; Bianco, 2014). Padanian barbel *Barbus plebejus* Bonaparte, 1839 is among these threatened species. It is endemic to the Padano-Venetian district and has been introduced to several rivers that drain into the Tyrrhenian Sea, specifically in the Tuscano-Latium district (Bianco, 2003). It has a fluvio-lacustrine ecophenotype characterised by a relatively large body size (total length up to 60 cm) and preferring habitats of large lowland rivers with consistent, moderate flows (Tsigenopoulos *et al.*, 1999; Kottelat and Freyhof, 2007; Buonerba *et al.*, 2015). Considered a species with moderate flexibility of adaptation, it is however affected to a great extent by anthropogenic impacts, such as hydrological alterations through the construction of dams, canalizations, gravel mining and sediment flushing (Freyhof *et al.*, 2020), as well as by genetic pollution due to introductions of allochthonous species (Zerunian, 2003; Zaccara *et al.*, 2014; Geiger *et al.*, 2016; Zaccara *et al.*, 2021). In the Habitat Directive 92/43/EEC (Annexes II and V) (European Union, 1992) it is listed among the “animal species of interest whose conservation requires the designation of special protected areas”; in the Italian Red List it is evaluated as vulnerable due to a significant decrease of its population in the last decade because of the presence of allochthonous species, mainly of the European barbel *Barbus barbus* Linnaeus, 1758, responsible for the high fragmentation of its home-range due to its relatively fast spread (IUCN, 2022).

As a result of fish stocking operations, the European barbel has repeatedly been introduced into many Italian rivers since mid-90s (Bianco, 1995). Since then, invasive populations have quickly grown, notably in the lowland rivers within northern Adriatic basins (mainly the Po

River basin) where its preferred habitats are common (Meraner *et al.*, 2013). The ecological niche of *B. barbus* highly overlaps with the ecological niche of *B. plebejus* (*i.e.*, fluvio-lacustrine ecophenotype (Kottelat and Freyhof, 2007)), favouring widespread introgressive hybridization (Meraner *et al.*, 2013; Zaccara *et al.*, 2014; Buonerba *et al.*, 2015). It is possible to discriminate morphologically the two species of barbel from the number of scales along the lateral line and from the shape and microstructure of the dorsal fin, but this discrimination can be unreliable due to the variability of these traits within the same species (Bianco, 2003; Kottelat and Freyhof, 2007; Quadroni *et al.*, 2023a). Moreover, the occurrence of hybridisation further hinders the morphological discrimination (Tsigenopoulos *et al.*, 2002; Gante *et al.*, 2008; Buonerba *et al.*, 2015) making the use of molecular techniques necessary (Zaccara *et al.*, 2021).

The lower Ticino and Oglio rivers, two main tributaries of the Po River, are characterised by habitats in which the endemic *B. plebejus* and the invasive *B. barbus* can co-occur, as already detected for the lower Adda River (Zaccara *et al.*, 2014). Both rivers are highly regulated with a severe hydrological alteration caused by dams and other hydraulic structures. This determines habitat impoverishing that can contribute to exacerbate the invasion processes by *B. barbus* (Hobbs and Huenneke, 1992), which may also be favoured by the defragmentation projects implemented in the last decade in order to re-establish the longitudinal connectivity of these rivers (Puzzi *et al.*, 2017; Zaccara *et al.*, 2021).

The aim of this study was thus to genetically characterise barbel specimens in the two mentioned rivers using the partial mitochondrial DNA Cytochrome b gene, in order to add knowledge on the *B. barbus* presence in the Po River basin for the implementation of more suitable management plans aimed at the conservation of the native species.

METHODS

Study area

Both Ticino and Oglio rivers are left-side tributaries of the Po River, the longest Italian river (Fig. 1). Their lowland part originates from sub-alpine lakes, Lake Maggiore and Lake Iseo, respectively. The lower Oglio is 156 km long and its watershed area is 3840 km², while the lower Ticino is 110 km long and its watershed area is 8172 km².

The lower Oglio is included in the Oglio Sud Regional Park while the lower Ticino, which flows at the border between two Italian regions, is included in two regional parks, *i.e.*, the Lombard Park of the Ticino Valley and the Management Body of the Protected Areas of Ticino and Lake Maggiore.

Both rivers are impacted by various pressures. They have been altered from their pristine status because of multiple water uses (mainly for agriculture) and the construction of hydropower plants, dams, and other hydraulic structures. The altered flow regime is one of the major causes of habitat heterogeneity loss. The streamflow of the two rivers are regulated by the Miorina and Sarnico dams, respectively. These dams regulate the lake outflows for flood protection and to satisfy the multiple needs of agriculture, hydropower production, tourism, and environmental flow, according to well-defined general rules. To maintain stable water levels in the lakes, water is mainly retained during non-irrigation periods and released during summer to meet agricultural needs (Salmaso *et al.*, 2016; Racchetti *et al.*, 2019).

Most water abstraction structures are located in the

upper part of both rivers. In the lower Ticino, water diversion is mostly carried out 7 km downstream from the Miorina Dam, at the Panperduto Dam, where approximately 60% of the mean annual discharge is diverted for agriculture and hydropower. In the first 20 km, the lower Oglio hosts six run of the river hydropower plants that creates a sequence of lentic and lotic stretches; the remaining part of the river suffers mainly from water abstraction for agricultural uses. In both rivers, the environmental flow is a seasonally modulated minimum flow that varies depending on the reach, with approximately 5% of the annual mean discharge being the lowest allowed flow (Laini *et al.*, 2018; Salmaso *et al.*, 2021).

In recent years both rivers, as well as the Po River, have been subjected to defragmentation plans within conservation projects for the recovery of the ecological cor-

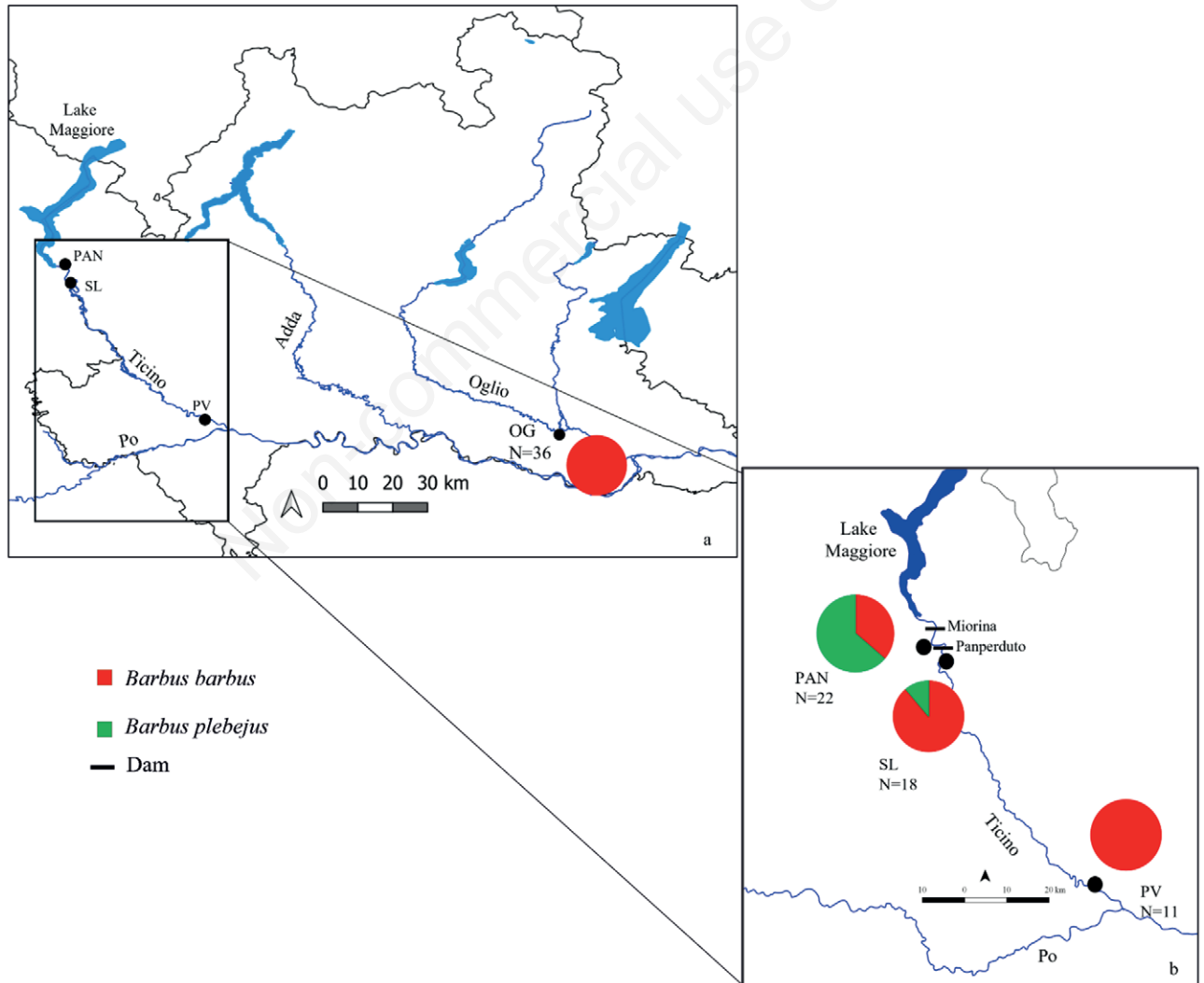


Fig. 1. Sampling sites at a) Oglio River (OG) and b) Ticino River (PAN, SL, PV) and the two main dams (Miorina and Panperduto dams). At each site the number of successfully analysed individuals is detailed (see Tab. 1). Pie charts show haplogroup frequency of *Barbus plebejus* (green) and *Barbus barbuis* (red).

ridors with the construction of fish passages associated to the existing dams and weir removal. The pathway from Lake Maggiore to the Adriatic Sea was completely re-established in 2017 (Puzzi *et al.*, 2017).

Fish sampling

In total 94 fish were sampled for the genetic analyses using backpack electric fishing. Specifically, in the Oglio River, 36 individuals were collected during October 2015 and September 2016 at Calvatone, around 90 km downstream from Lake Iseo, close to the confluence with the Po River; along the Ticino River three sites were sampled between 2020 and 2022: 25 individuals were collected in the fish passage at the Panperduto Dam (PAN) during April and May 2021, 20 individuals around 2 km downstream, at Somma Lombardo (SL) during May 2022, and 13 individuals further downstream, at Pavia (PV) during July 2020, almost at the confluence with the Po River (Fig. 1).

Following their capture, the fish were anaesthetised (MS-222) and measured (fork length, nearest mm), and tissue samples (anal fin clips) were taken from each individual, preserved in 95% ethanol and then stored at 4°C prior to DNA extraction. Following their recovery to normal behaviour, the fish were released to their approximate location of capture.

Genetic analyses

Extraction of genomic DNA was completed through a proteinase K digestion followed by sodium chloride extraction and ethanol precipitation (Aljanabi and Martinez, 1997). The genetic analyses then focused on the sequencing of the partial mitochondrial DNA Cytochrome *b* gene (*cyt b*) through the specific primer pair L15267 and H1646 (Briolay *et al.*, 1998). Polymerase chain reaction (PCR) amplifications were performed with Multiplex PCR kit (Qiagen), in 10 µL reaction volume containing approximately 10 ng of template DNA and 0.2 µM primer pair. Thermal cycling was performed as follows: denaturation of 15 min at 95°C, followed by 35 cycles of 94°C for 30 s, 90 sec at 55°C of annealing temperature and the extension step at 72°C for 90 s, the final elongation was at 72°C for 10 min. PCR products were purified using the EuroSAP kit (PCR Enzymatic clean-up kit-Euroclone) and sequenced at Macrogen Inc.

The new haplotypes of *cyt b* were deposited in GenBank database under the accession numbers: OQ676372-OQ676376.

Sequences were manually aligned using BioEdit v. 7.1.3.0 (Hall, 1999) to eliminate ambiguities and to check polymorphic sites. DNA polymorphism indices, like haplotype diversity (H) and nucleotide diversity (π), were calculated using DnaPS v. 5 (Librado and Rozas, 2009). In addition to the sequences obtained here, 50 sequences

from GenBank database (Tab. S1) have been added to the dataset. These sequences are representative of both fluvi-lacustrine and reophilic ecophenotypes of *Barbus* species. In particular, all the sequences belonging to *B. barbus* and *B. plebejus* ever described in northern Italy have been added. The phylogenetic analyses were performed using GTR (Lanave *et al.*, 1984) model of sequences evolution as estimated with jModeltest v. 2.1.10 (Darriba *et al.*, 2012) and implementing two different optimality criteria: Bayesian analysis (BI) and maximum likelihood (ML). The BI analysis was performed using Mr Bayes v. 3.2 (Ronquist *et al.*, 2012) with a Markov Chain Monte Carlo algorithm (MCMC): four simultaneous and independent Markov chains from random trees were started and run for 1,000,000 generations, with the first 25,000 generations (2500 trees) discarded as the burn-in ($p < 0.01$). The ML analysis was performed through Garli v. 1.0 software (Zwickl, 2006) and PAUP v. 4.0b10 software (Swofford, 2003). Phylogenetic trees were visualised using FigTree v. 1.4.3 (Rambaut, 2016) and rooted using *Barbus comizo* (Steindachner, 1864) sequence (Tab. S1). To analyse the genealogical relationships within *B. barbus* and *B. plebejus* sequences, Minimum Spanning Networks (MSNs) were built on the same multiple alignment through a statistical parsimony criterion as available in TCS v. 1 (Clement *et al.*, 2000) using a confidence level of $\geq 95\%$ (14 mutational steps).

Historic demographic expansion was then investigated for *B. barbus* population in the Oglio River and Ticino River (all sampling sites combined) by analysing frequency distributions of pairwise differences (mismatch distribution analysis) using DnaPS v. 5 (Librado and Rozas, 2009).

RESULTS

Eighty-six partials mitochondrial *cyt b* sequences (844 bp in length) were obtained from 94 individuals of the sampled *Barbus*, sequencing of eight samples failed (Tab. 1). Sequence alignment produced 13 haplotypes, of which five were unique. The phylogenetic analyses suggested that eight haplotypes fell within the *B. barbus* cluster, and five within the *B. plebejus* cluster (Fig. 2; Tab. 1). All eight haplotypes already present in the public database GenBank, have been already described in northern Italy (Tables 1 and S1). Two new described haplotypes fell within *B. plebejus* cluster, PI01 and PI02, and were retrieved at site Panperduto (PAN) and Somma Lombardo (SL), respectively, and three fell within *B. barbus* cluster: Ba01 found at PAN, whilst Ba02 and Ba03 in the Oglio River (Fig. 2; Tab. 1). Considering the upstream-downstream gradient within the Ticino River, it is possible to observe a change in the population composition. The most upstream site (PAN) is mostly represented by *B. plebejus*

individuals (64%), decreasing percentage moving downstream, being 11% at the second site (SL), up to complete replacement with *B. barbus* further downstream, at site

PV (Fig. 1; Tab. 1). The Oglio River was composed only by *B. barbus* individuals. In the Ticino River, both species showed medium-high value of haplotype diversity (H),

Tab. 1. Haplotype distribution and genetic information based on *cyt b* mtDNA partial gene sequences (844 bp length). The population (ID), number of analysed specimens (N) and genetic diversity values (haplotype, H, and percentage of nucleotide, $\pi\%$, diversity) are indicated.

ID	N	<i>Barbus barbus</i>									<i>Barbus plebejus</i>							
		BAR1	PK-451	Bb01	BAR3	Bb05	Ba01*	Ba02*	Ba03*	H±SD	$\pi\%±SD$	Bp01	Bp11	Bp15	PI01*	PI02*	H±SD	$\pi\%±SD$
PAN	22	5		1	1		1			0.64±0.18	0.2±0.01	11	1	1	1		0.40±0.16	0.1±0.1
SL	18	9			5	2				0.61±0.008	0.1±0.01	1				1	1±0.25	0.12±0.1
PV	11	8	2							0.36±0.16	0.1±0.01						-	-
OG	36	27	1		5			2	1	0.43±0.10	0.06±0.02						-	-
Tot	86	49	3	1	11	2	1	2	1	0.49±0.07	0.01±0.02	12	1	1	1	1	0.45±0.151	0.1±0.2

PAN, Panperduto Dam; SL, Somma Lombardo; PV, Pavia; OG, Oglio River; *unique haplotypes, GenBank Acc. No. OQ676372-OQ676376.

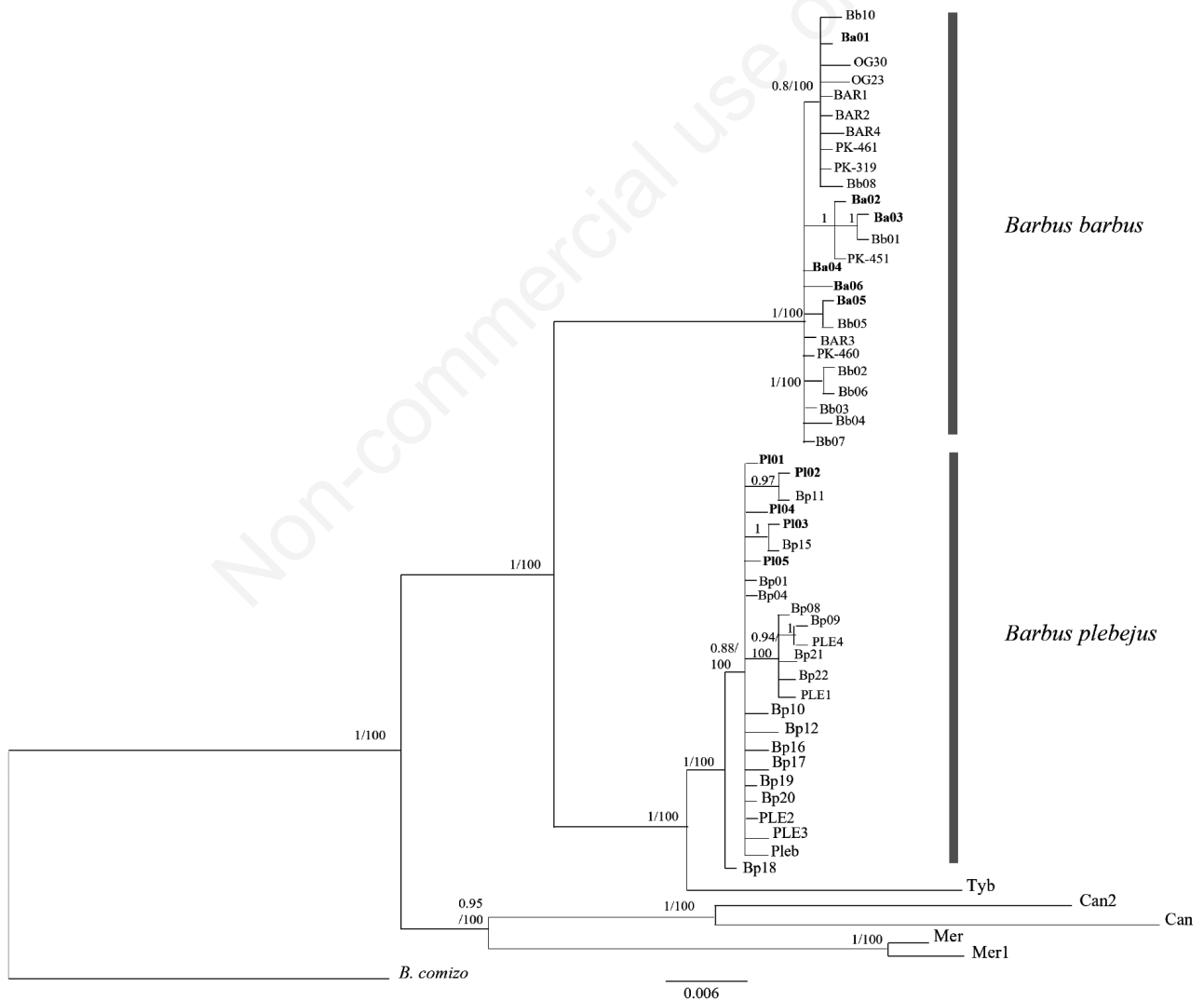


Fig. 2. Bayesian tree for *Barbus* sp. based on mitochondrial *cyt b* partial gene (844 bp). Statistical support for clades was expressed as percentage of bootstrap (1000 replicates) and as posterior probabilities values; only significant node supports ($\geq 70\%$) are displayed. Trees were rooted using *Barbus comizo* (see Tab. S1).

and medium value of percentage of nucleotide diversity ($\pi\%$), except for the most downstream site, PV, which yielded a lower haplotype diversity comparable to the haplotype diversity detected in the Oglio River (Tab. 1). The Minimum Spanning Networks (MSNs) gave a “radial pattern” around the two most widespread haplotypes (BAR1 and BAR3) for *B. barbus* (Fig. 3). The most frequent *B. barbus* haplotypes (BAR1, BAR3 and PK-451) overlapped to haplotypes previously described in northern Italy (Fig. 3; Tab. S1), originated from the Danube River (Kotlík *et al.*, 2004). Haplotype Bb05 and Bb01, previously described in the Po River basin (Meraner *et al.*, 2013), are connected to the second widespread haplotype BAR3 (Fig. 3). The two unique haplotypes, Ba02 and Ba03, are separated by one mutational step from the most widespread haplotype BAR1, whilst the unique haplotype

Ba01 is separated by one mutational step from haplotype BAR3 (Fig. 3). The MSNs for *B. plebejus* (Fig. 3) mainly showed a radial pattern around the most widespread haplotype (Bp01) widely described in the Brenta River, Adda River, Adige River and in general in the Po River basin (Meraner *et al.*, 2010; Zaccara *et al.*, 2014). The demographic analysis of both Ticino and Oglio River populations of the European barbel based on the distribution of haplotypes differences showed a unimodal trend (Fig. 4).

DISCUSSION

The management and regulation of invasive alien species can be strongly informed by their invasion genetics (Hänfling, 2007). Information on the introduction his-

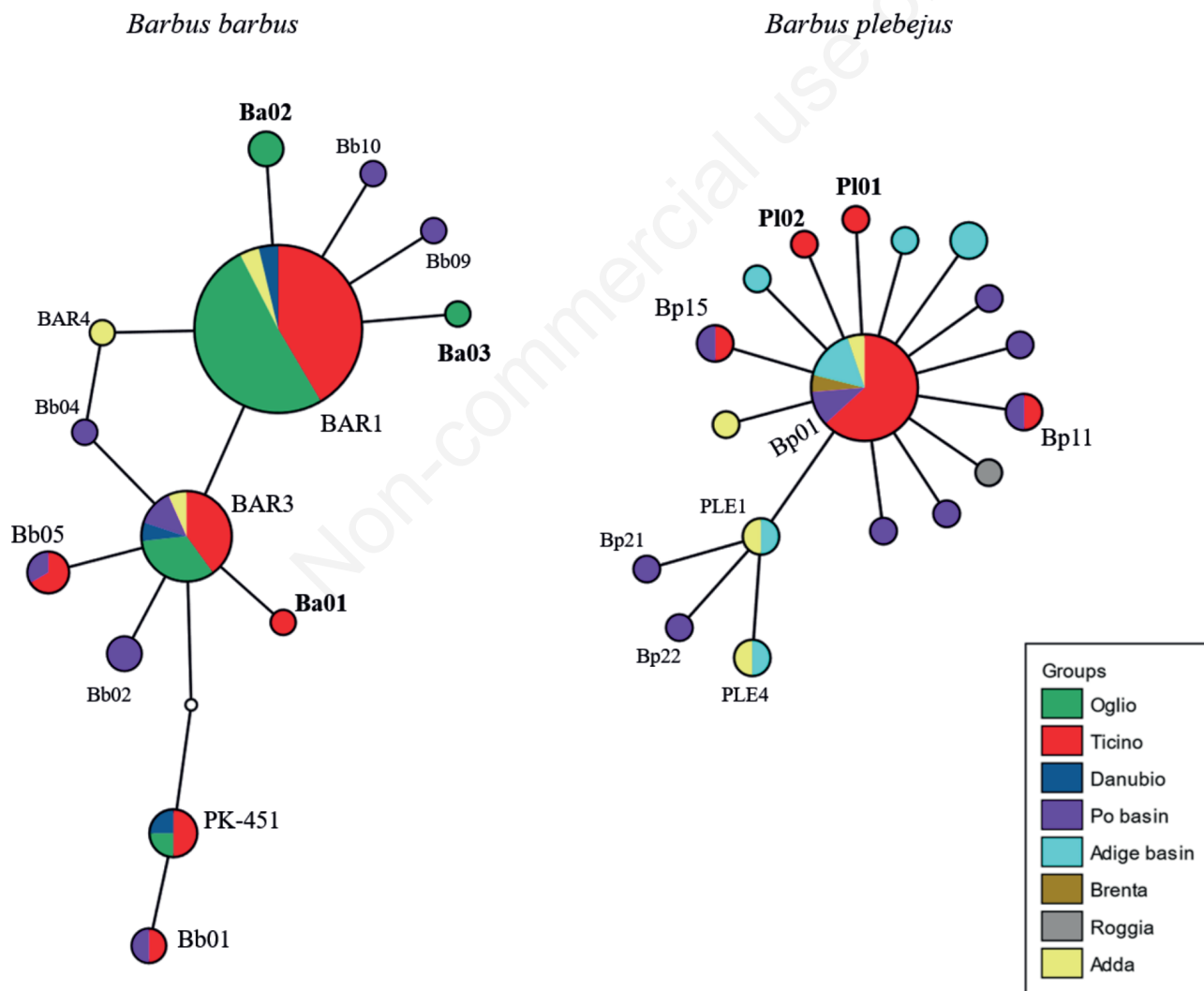


Fig. 3. Minimum spanning networks of *Barbus barbus* and *Barbus plebejus* based on mitochondrial *cyt b* partial gene (844 bp) and GenBank selected sequences (see Tab. S1). Each circle represents one haplotype, and the sizes of circles are proportional to the number of individuals sharing the same haplotype. White small circles represent missing haplotypes (mutational steps).

tory of the invader, its biogeographic source, population connectivity, and co-occurrence with other species in both the native and invasive range can add knowledge on its genetic diversity in the invasive range, how this diversity varies spatially, and help to identify the introduction pathways (Lawson Handley *et al.*, 2011; Bock *et al.*, 2015; Hardouin *et al.*, 2018). Moreover, in the case of many cyprinids such as barbels, the invasion process is facilitated by hybridization between the invader and the native species, with a consequent loss of native genetic diversity and locally adapted genotypes (Scribner *et al.*, 2000; Zaccara *et al.*, 2021).

Previous studies highlighted the presence of the European barbel in the Po River basin (Meraner *et al.*, 2013; Zaccara *et al.*, 2014; Buonerba *et al.*, 2015), providing evidence of its relevant spread with a risk of local extinction of the native species in the Italian large lowland rivers. Indeed, the results of this study confirmed the presence of this invader in both lower Ticino and Oglio rivers, updating the current status of *B. barbus* invasion. Specifically, the lower Ticino showed a compromised population, with an increasing presence of the invasive alien species moving downstream towards the confluence with the Po River. On the contrary, the genetic variability of *B. barbus* increased moving upstream with levels comparable to what observed in the Adda River (haplotype diversity range 0.65–0.87) (Zaccara *et al.*, 2014). The values of nucleotide diversity were instead lower by an order of magnitude (Adda River π between 0.016 and 0.020; Ticino River π between 0.001 and 0.002) (Zaccara *et al.*, 2014), suggesting a more recent differentiation. The lowland stretch of the Oglio River was instead composed only by *B. barbus* individuals, likewise

the most downstream site of the Ticino River, both close to the confluence with the Po River.

The gene genealogy of native *B. plebejus* provided a star phylogeny pattern suggesting a starburst radiation from one widespread, and largely distributed in northern Italy, haplotype (Bp02) (Meraner *et al.*, 2013; Zaccara *et al.*, 2014; Buonerba *et al.*, 2015). This might reflect a recent size expansion from a few numbers of founders (Avice, 2000), as previously proposed for the Adda River, where a significant demographic expansion after recent bottleneck was suggested (Zaccara *et al.*, 2014). Indeed, studies inferring the phylogeography of other European fish species have mostly documented episodes of bottlenecks during Pleistocene glaciations (Bernatchez, 2001; Kotlík and Berrebi, 2001). Thus, the genetic pattern of *B. plebejus* might be interpreted as consequence of the post-glacial colonization of the Padano-Venetian district following the last glacial maximum (Bianco, 1995). However, this expansion is perturbed by the introduction of *B. barbus*. The *B. barbus* gene genealogy evoked a radial pattern around two widespread haplotypes, originally found in the Danube River (Kotlík *et al.*, 2004), probably a consequence of stocking activities from a donor population that originated in this river. However, the introduction of the European barbel into Italian waters has been reported since mid-90s (Bianco, 1995). The combination of relatively high values of haplotypes diversity (H) and low values of nucleotide diversity (π) found in both Ticino and Oglio rivers supports the hypothesis of a recent expansion of *B. barbus* starting from a low number of founders (Grant and Bowen, 1998) (*i.e.*, stocking activities from a donor population) for both river populations.

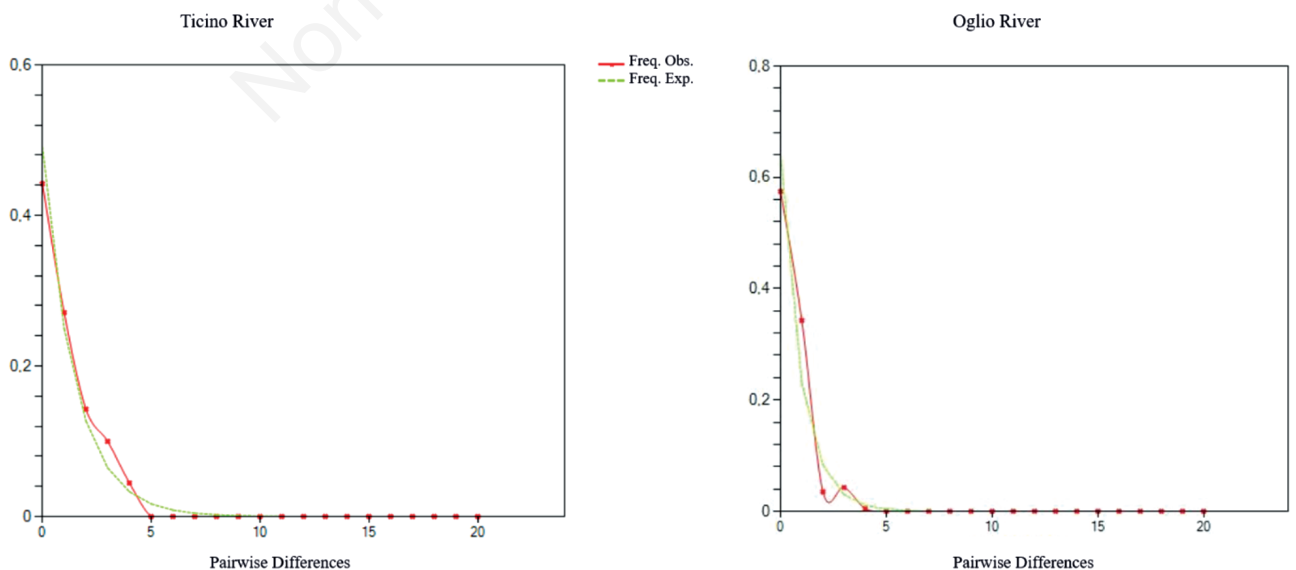


Fig. 4. Mismatch analysis based on nucleotide differences and frequencies among haplotypes based on mitochondrial *cyt b* partial gene (844 bp) of *Barbus barbus* individuals in Ticino River and Oglio River.

Indeed, this hypothesis was further supported by a uni-modal trend inferred through mismatch distribution analysis, corresponding to an expanding population. The current study thus showed that *B. barbatus* is expanding, probably favoured by clear waters, with fast currents and high concentration of oxygen, and by low impacted riverbeds (Britton and Pegg, 2011).

Both species can be affected by the hydrological alteration of the regulated Ticino and Oglio rivers, mainly by the minimum flows which in recent years have been exacerbated by the climate change (Salmaso *et al.*, 2021). Their habitat requirements vary with development; for *B. barbatus*, areas in the littoral zone with minimal flow are important for larvae, riffle areas for juveniles, and mid-channel habitats for adults (Britton and Pegg, 2011). Similarly, the habitat availability for both juveniles and adults of *B. plebejus* increases with increasing values of minimum flows (from 6% to 11% of mean annual natural flow), and only adults prefer high flows (around 100 m³/s) in the river reach of the lower Ticino characterized by the highest hydrological alteration (SL, few kilometres below the Panperduto Dam) (Quadroni *et al.*, 2023b). It is therefore unlikely that hydrological alterations could have favoured one species over the other. On the contrary, the recovery of river connectivity within the Po River basin (Puzzi *et al.*, 2017) has certainly favoured the expansion of the invasive alien barbel. Britton and Pegg (2011) reported restoration of river connectivity along with of habitat heterogeneity as a necessary management measure for the conservation of *B. barbatus*. The construction of the so-called “ecological webs” has thus a negative B-side related to the spread of allochthonous species. Indeed, in Italian watercourses where some reaches are inaccessible to *B. barbatus* due to structures in the river preventing their upstream movement, pure autochthonous barbel populations still persist (Zaccara *et al.*, 2021).

CONCLUSIONS

The combination of introduced *B. barbatus* and anthropogenic disturbances has substantial repercussions for the endemic *B. plebejus*. Whilst it is important to actively focus on tightening fishing and fisheries management regulations to stop *B. barbatus* from spreading further in the investigated area and throughout Italy (Zaccara *et al.*, 2019a; Zaccara *et al.*, 2019b), it is also fundamental to search for, isolate, culture and restock with purebred *B. plebejus* Italian rivers within the Padano-Venetian district and, at the same time, restoring the riverine habitats suitable for this species by implementing further mitigation measures against hydro-morphological alterations.

In this study, the analysis concentrated only on the mitochondrial DNA (uniparental inheritance) did not allow to detect hybridization between *B. plebejus* and *B. barbatus*,

but the occurrence of this phenomenon where the mitochondrial lineages of the two species co-existed (*i.e.*, Ticino sites) is highly probable. Thus, it would be also interesting to increase the knowledge on the hybrid lineages which can have a higher adaptive capacity than the native parental genotypes to altered environmental conditions that are driven by anthropogenic exploitation of the freshwater resources (Oziolor *et al.*, 2019; De Santis *et al.*, 2021).

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