

Will the Italian endemic gudgeon, *Gobio benacensis*, survive the interaction with the invasive introduced *Gobio gobio*?

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A b s t r a c t. The genus *Gobio* in Italy was represented by the endemic species *G. benacensis*. The original distribution of this species was the Padano-Venetian district, but since a long time it was introduced in central Italy. Introductions of alien species to Italy during the last 10 years brought the sudden introduction of the Danubian *G. gobio*. Genetic and morphological analyses revealed the extensive presence of *G. gobio*, which rapidly colonised several rivers in Italy causing the progressive decline of *G. benacensis*, which now should be considered as an endangered species. Among examined populations those found in the Tagliamento River and transplanted in the Ombrone River represent genetic reservoirs of this species which will probably disappear in northern Italy.

Key words: Italy, *Gobio*, endemic species, morphology, genetics, conservation

Introduction

Gobio benacensis (Pollini, 1816) is an endemic species in Italy. Its original distribution included the Padano-Venetian district, from Isonzo River in the north to the River Marecchia in the south (Bianco & Taraborelli 1986, Bianco 1991, Bianco 1994). The species was also introduced in several river basins such as the Arno, the Tiber and the Ombrone in central Italy (Bianco 1994) (Fig. 1). More recently, due to the intensification of fish stockings with fish sometimes of Transalpine origin, several cyprinid species appeared in Italy, for example the Iberian *Barbus graellsii* Steindachner, 1866, Danubian lineage of chub, *Leuciscus cephalus* (Linnaeus, 1758), and now also *Leuciscus idus* (Linnaeus, 1758) (on fishes kindly provided by Thomas Busatto), while others increased their range, e.g. *Pseudorasbora parva* (Temminck et Schlegel, 1846), *Rhodeus sericeus* (Pallas, 1776). As result of several collecting trips carried out in Italy, an abnormal increase in populations of gudgeon was observed, especially in the rivers Meletta (Po river basin) and Assino (Tiber river basin). These populations include large individuals (up to 106 mm SL) never found in *G. benacensis* populations, where the adults may reach about 70–80 mm SL (Bianco & Taraborelli 1986, Bănărescu et al. 1999) (Fig. 2). Compared to *G. gobio*, *G. benacensis* is a less invasive and a more discrete species which moderately colonises the running water of the *Barbus* zone.

The aim of this contribution is to identify these populations either on morphological and genetic basis with some consideration on several related species.

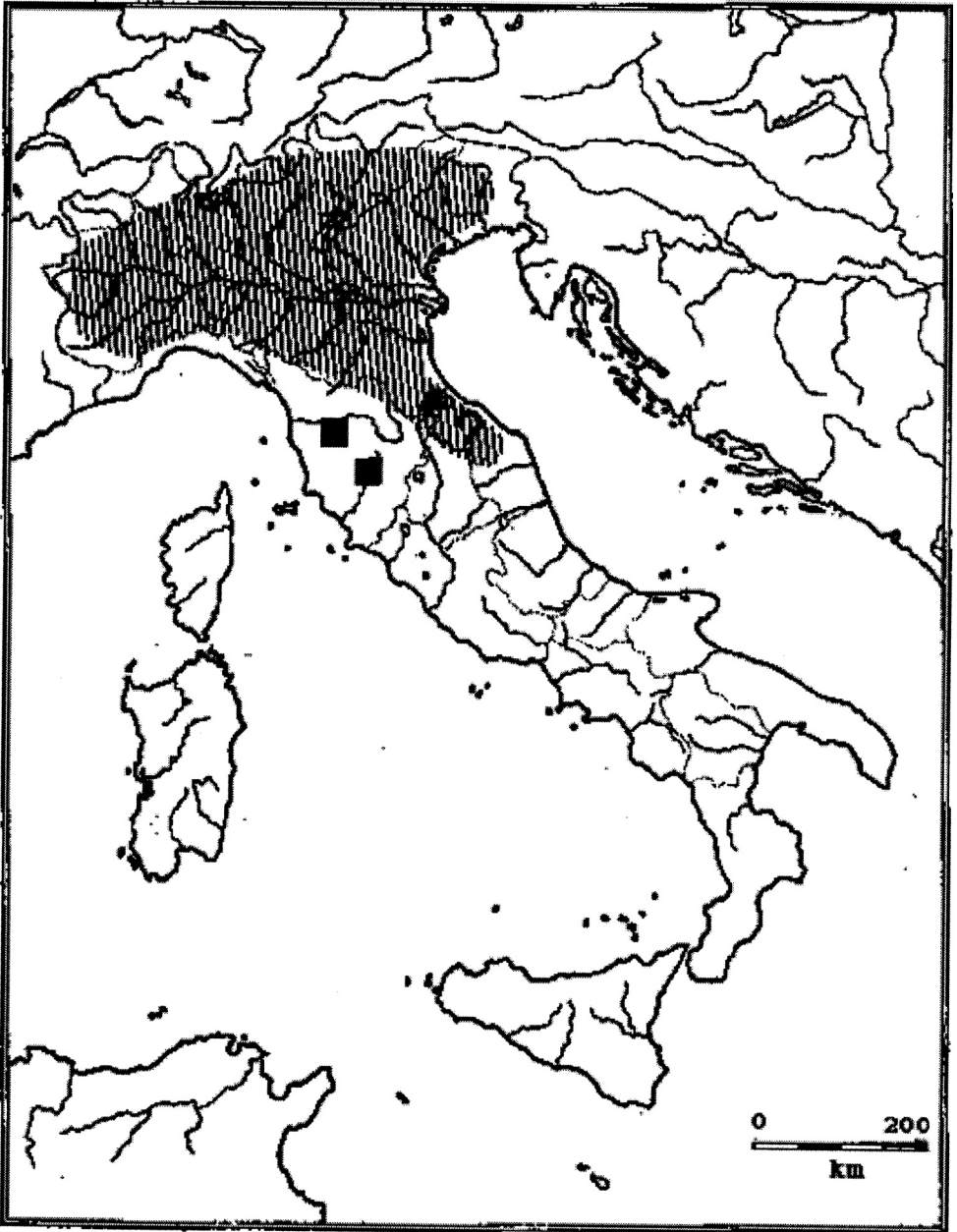


Fig. 1. Distribution of *G. benacensis* in Italy 1990. Squares are for introduced populations.

Materials and Methods

38 gudgeon specimens, collected from five different sites (Fig. 3) were used for the morphological analyses. 22 specimens were used for genetic analyses. Among the morphological characters the identification of *G. benacensis* from others species of the genus *Gobio* was

the number of scales between the origin of anal fin and the vent, which are about 2 or 3 (rarely 4) in *G. benacensis* and 4 to 8 in *G. gobio* (Linnaeus, 1758) and others *Gobio* species (Fig. 4) (Bianco & Taraborelli 1986). This feature characterised also the difference between *Romanogobio parvus* Naseka et Freyhof, 2004, and *R. ciscaucasicus* Berg, 1932 (Naseka & Freyhof 2004). The species cannot be easily identified from others characters (Bianco & Taraborelli 1986). So, consequently, we used this single character to discriminate *G. benacensis* from *G. gobio*.

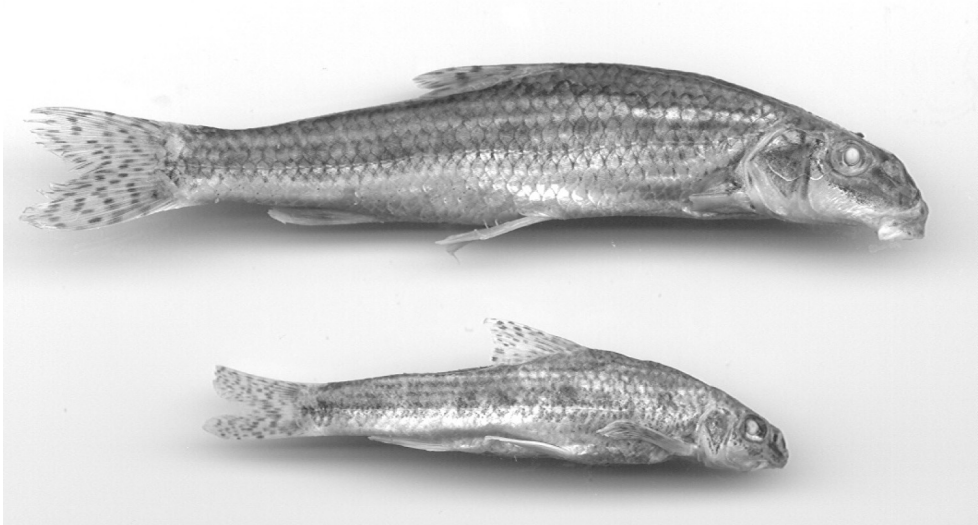


Fig. 2. *G. gobio* (above), 106 mm SL from the R. Meletta, and *G. benacensis* (below) 64 mm SL, from the R. Tagliamento.

For genetic analysis, we used the Polymerase Chain Reaction (PCR) and DNA sequencing to identify at the specific level 22 specimens belonging to genus *Gobio* sampled in five different rivers (Fig. 3). We chose to use the mitochondrial cytochrome *b* gene (*cytb*) because it allows the identification of morphologically similar species of cyprinids (Zardoya & Doadrio 1998).

Total DNA was extracted from a pectoral fin dissected from living specimens and fixed in 95% ethyl alcohol. The surface of fins was cleaned with sterile water and subjected to 20 min of UV irradiation; DNA was extracted using the Easy-DNA extraction kit from Invitrogen (Carlsbad, CA). We used primers Glu-F and Cytb-R (Zardoya & Doadrio 1998) to amplify and sequence a 342 bp fragment of the mitochondrial region coding for the *cytb* gene. PCR and sequencing conditions were as in Bianco & Ketmaier (2001). Strands were sequenced in both directions and analysed using the program Sequencher 3.1.1 (Gene Codes Corporation, Ann Arbor, MI) and aligned by eye. Sequences were submitted to GenBank (Accession numbers AY641521-AY641525). Aligned sequences were analysed by the Maximum Parsimony (MP; heuristic searches, ACCTRAN character-state optimisation, 100 random stepwise addition, TBR branch-swapping algorithm) (Farris 1970) and by Neighbor-Joining (NJ) (Saitou & Nei 1987). NJ analyses were carried out on Tamura & Nei (1993) distances (corrected with an empirically determined gamma parameter estimated via Maximum Likelihood; $\alpha = 0.248$). The bootstrap method (Felsenstein

1985) was employed to test the robustness of phylogenetic hypotheses (1000 replicates). Competing phylogenetic hypotheses were tested using the *Templeton* test (1983) and the two-tailed Wilcoxon rank-sum test (*Larson* 1994). All these analyses were carried

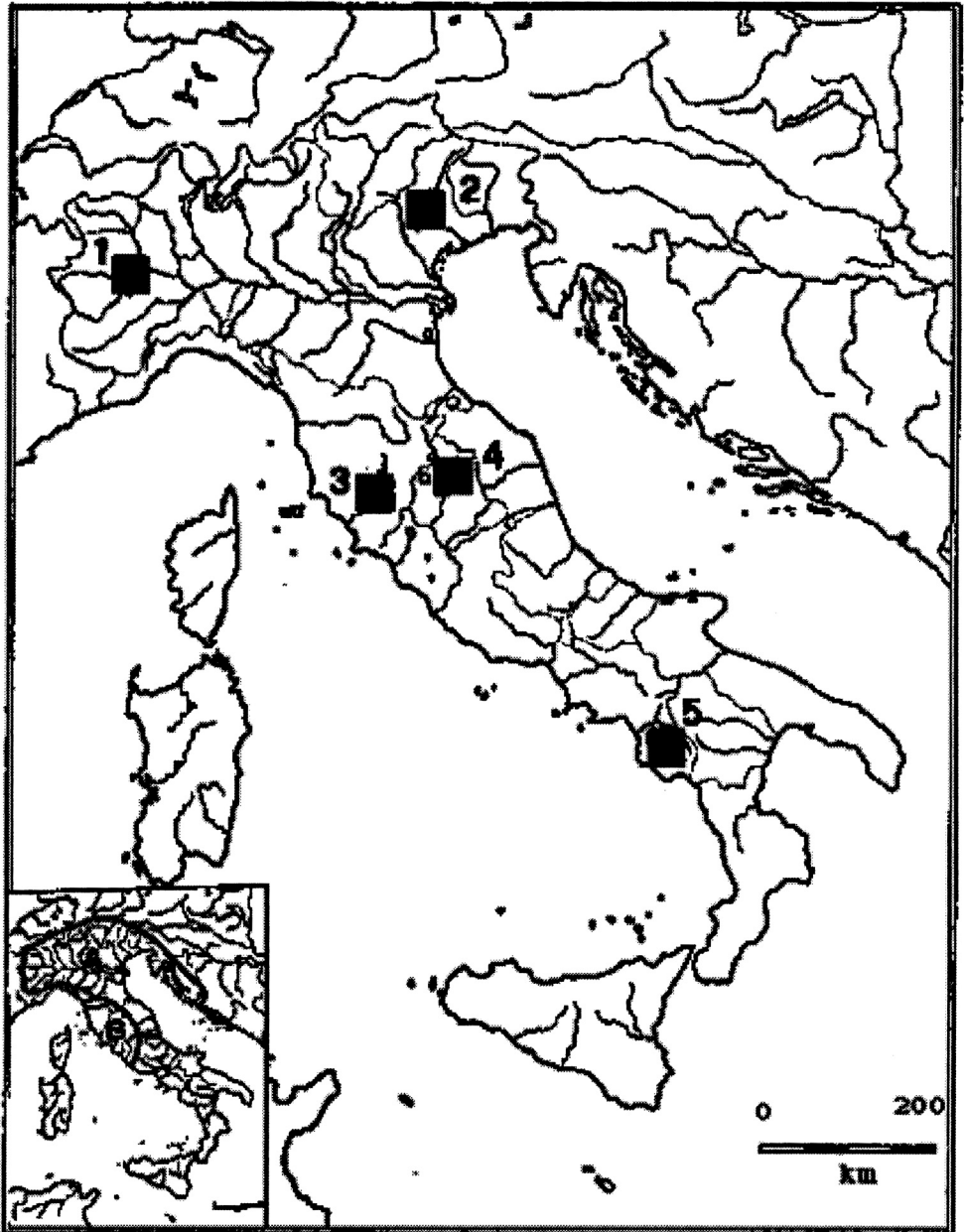


Fig. 3. Localities of recently collected material used for genetic and morphologic analyses (the codes for each population are presented in parentheses) 1) R. Meletta (MEL); 2) F. Tagliamento (TAG); 3) R. Ombrone (OMB); 4) R. Assino (ASS) and 5) R. Badolato (BAD). Inset: boundaries of A Padano-Venetic (were *G. benacensis* was native) and B Tuscan-Latium (were *G. benacensis* was introduced in the past) districts of freshwater fish distribution.

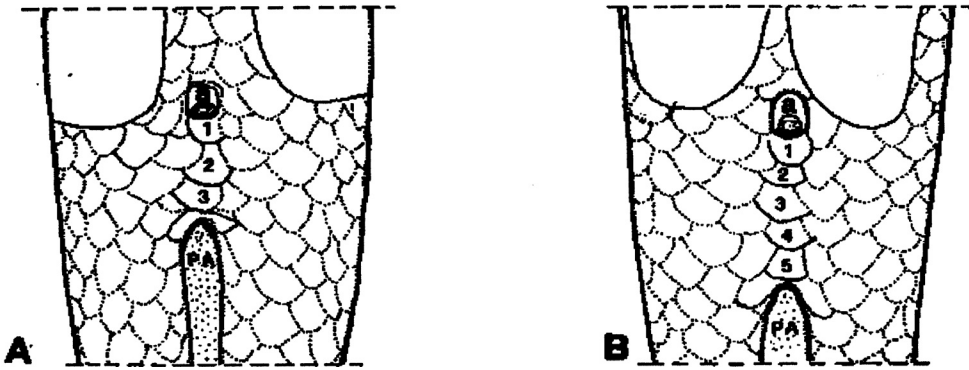


Fig. 4. The discriminant character between *G. benacensis* (A) and *G. gobio* (B): the numbers of scales between the anus (a) and the anterior origin of the anal fin (PA) are 2–3 in *G. benacensis* and 4–8 in *G. gobio*.

out with PAUP* 4.0β10 (S w o f f o r d 2002). To clarify the taxonomic status of the Italian populations of gudgeon, we included in all phylogenetic analyses sequences of *G. gobio* from the river Rhone, France (code RHO; GenBank n° Y10452), and *G. lozanoi* Doadrio et Madeira, 2004 from the river Tajo, Spain: a species recently described from Spain, previously identified as *G. gobio* (GenBank n° AF045996) (D o a d r i o & M a d e i r a 2004). We also included in the analyses sequences of *G. balcanicus* Dimovski et Grupche, 1977 (GenBank n° AF090750), and two species of the genus *Romanogobio*: *R. banarescui* Dimovski et Grupche, 1974 (GenBank n° AF090751) and *R. ciscaucasicus* Berg, 1932 (GenBank n° AF095607). *Pseudorasbora parva* (GenBank Accession n° AF051873) was used as the out-group.

The specimens examined in this contribution are preserved in the fish collection of the Institution of the first author (PGB).

Results and Discussion

According to the examination and comparison of the diagnostic morphological character only two of the examined populations should be considered as true *G. benacensis* (TAG and OMB), while the others belong to *G. gobio* (Table 1). Genetic data are summarised in Fig.5, which shows MP and NJ trees (we found only one haplotype per population, therefore we used a single sequence in the phylogenetic analyses). MP and NJ trees are not statistically different according to the Templeton and Wilcoxon rank-sum tests ($p = 0.365$). Species of the genus *Gobio* form a moderately supported monophyletic clade in the MP tree (68% of bootstrap support); in the NJ tree species of the genus *Romanogobio*, recently rehabilitated by N a s e k a (1996) and N a s e k a & F r e y h o f (2004), are embedded within *Gobio*, but their position in the tree is not statistically supported (52% of bootstrap support). Examined specimens from the rivers Meletta (MEL), Assino (ASS) and Badolato (BAD) share the same haplotype with the *G. gobio* from the river Rhone (RHO) and are quite close to *G. lozanoi* from the Iberian peninsula (SPA). All these populations always form a monophyletic clade, with *G. balcanicus* placed basal. TAG and OMB are the closest relatives to each other (100% and 99% bootstrap support in MP and NJ analyses, respectively) and represent two extant populations of *G. benacensis*. This species is clearly differentiated on genetic grounds from *G. gobio*. Indeed, the

Table 1. Number of scales between the anus and the origin of anal fin (N sq a-A) in five Italian populations of the genus *Gobio* examined for morphological and genetic analyses.

River code	n	SL (mm) Range	N sq a-A Range
Meletta (MEL)	6	62–106	5–7
Tagliamento (TAG)	14	34–74	2–3
Assino (ASS)	7	74–102	4–5
Ombrone (OMB)	5	36–71	2–3
Badolato (BAD)	6	54–86	4–6

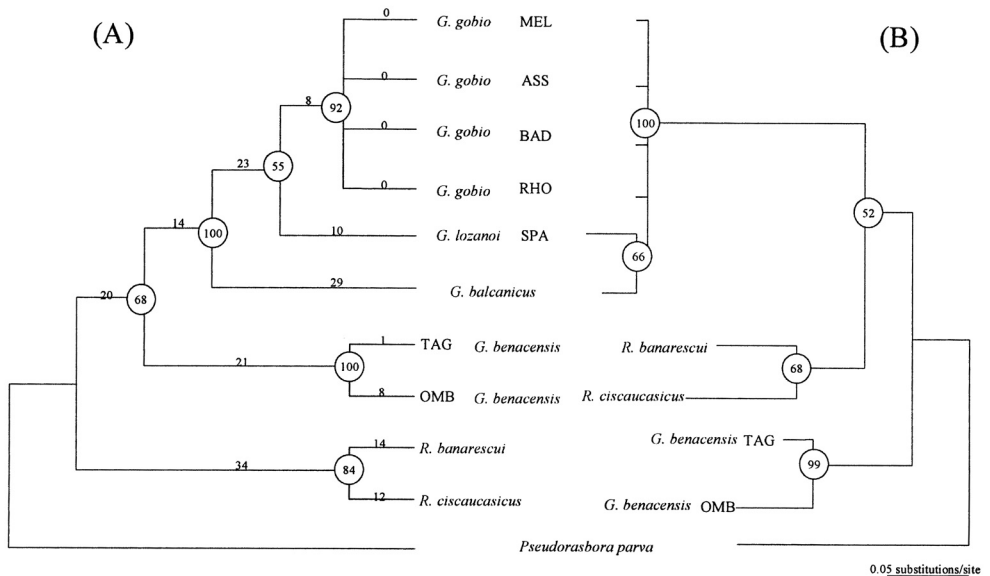


Fig. 5. Evolutionary relationships between populations and species included in the study. Numbers at nodes are bootstrap percentages over 1000 replicates (only percentages greater than 50% are shown). A) Maximum Parsimony tree (MP; TL = 194; CI = 0.812; 43.8% and 16.1% of 342 characters were variable and parsimony informative on all positions, respectively). Numbers above branches are the branch lengths. B) Neighbor-Joining (NJ) tree built on DTetN values.

analysis of genetic distance values (data not shown) agrees with tree topologies and with our taxonomic conclusions. The DTetN value among the two putative *G. benacensis* populations and all the *G. gobio* populations included in the study is 0.237 ± 0.001 , higher than the value we detected between *R. banarescui* and *R. ciscaucasicus* (DTetN = 0.115).

G. benacensis and *G. gobio* in Italy can be easily identified by the examination of the number of scales between the vent and the anterior origin of the anal fin. In addition, *G. benacensis* is a more discrete species, forming small communities as compared with *G. gobio* which in Italy tends to be an invasive species. Finally, the Italian gudgeon is a smaller species as it may reach about 80 mm standard length, whereas *G. gobio* may grow up to 130 mm standard length (Bianco & Taraborelli 1986, Bianco 1994, Pizzul et al. 1993, Bănărescu et al. 1999, present data).

Conclusion

Since 1994, when studies on *G. benacensis* were performed (Bianco & Taraborelli 1986, Bianco 1994, Bianco 1994, 1995), all the Italian populations belonged to the endemic *G. benacensis*, which was transplanted also outside its original range.

At present, as result of hidden introductions, among the examined populations, *G. gobio* was found in three basins out of the five investigated: River Meletta in northern Italy, River Assino in central and River Badolato in southern Italy where gudgeon are invasive and tend to occupy the running waters of the *Barbus* zone. Probably this species will also interfere with other rheophilic cyprinids species, such as barbels and the minnow *Telestes muticellus* (Bonaparte, 1837).

Still pure populations of *G. benacensis* survive at least in River Tagliamento near the town of San Vito al Tagliamento and in Ombrone river where the Italian gudgeon was introduced probably before the year 1983 and where it forms quite scarce populations.

An extensive survey of all populations of gudgeon in Italy, coupled with a larger amount of DNA sequences, are needed in order to outline the present distribution of *G. benacensis*, to test for possible hybridisation events between the latter species and *G. gobio* and to adopt all the conservative measurements to save this endemic, now critically endangered species.

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