Journal of Fish Biology (2001) **59** (Supplement A), 190–208
doi:10.1006/jfbi.2001.1766, available online at http://www.idealibrary.com on IDEAL®

Anthropogenic changes in the freshwater fish fauna of Italy, with reference to the central region and *Barbus graellsii*, a newly established alien species of Iberian origin

P. G. Bianco*‡ and V. Ketmaier†

**Department of Zoology, University Federico II, Via Mezzocannone 8, 80134 Naples, Italy and †Department of Animal and Human Biology, University ' La Sapienza ', Viale dell'Università 32, 00100 Rome, Italy*

In a survey of the west-central Italian rivers Ombrone, Fiora, Albegna and Bruna, among established exotic species, the Iberian barbel *Barbus graellsii* was recorded in Italian fresh waters for the first time. Morphological identification was supported by comparison of cytochrome b sequences with those from related barbel species. Other exotics of particular note were *Barbus barbus*, *Pseudorasbora parva* and *Leuciscus cephalus*, together with the Padano-Venetian *Chondrostoma genei* and *Padogobius bonelli*. Native species still present included *Leuciscus lucumonis*, *Telestes muticellus*, *Rutilus rubilio* and *Padogobius nigricans*, but were now more restricted to upper reaches and smaller watercourses. The deleterious effect of alien species on native forms is discussed with particular reference to probable competition between *C. genei* and *L. lucumonis*, and between the two gobies. The checklist of species introduced to the fresh waters of Italy is now updated to 34. For the Italian freshwater fish fauna in general, the conservation status of three native species (*Acipenser naccari*, *Salmo marmoratus* and *Knipowitschia punctatissima*) has been improved but five species are believed to be at risk (*Salmo carpio*, *L. lucumonis*, *Scardinius scardafa*, *Gobio benacensis* and *P. nigricans*) and four anadromous species (*Petromyzon marinus*, *Lampetra fluviatilis*, *Acipenser sturio* and *Huso huso*) no longer breed in Italian fresh waters. The processes of change in the composition of the Italian freshwater fish fauna as a whole may be summarized as successively ' padanization ', ' danubization ' and now ' globalization '.

Key words: freshwater fishes; Italy; alien species; translocation; molecular taxonomy; *Barbus graellsii*.

INTRODUCTION

Italy has been a country long interested in the introduction of alien freshwater fishes. The first of these probably occurred during the Roman period or earlier, and several species traditionally considered as native could be of non-native origin in view of their bioecological and biogeographical features (Bianco, 1998*a*).

At present the major centres providing species for stocking public waters are located in northern Italy and supply ' white fishes ' (a mixture of species, mostly chubs and barbels, but with several others included accidentally) (Bianco, 1990*a*, 1998*a*; Melotti, 1994). It has been calculated that, in recent times, these farms have imported *c.* 100 t every week of such fishes from outside Italy, mostly eastern Europe (Ielardi, 1998). This fishes are bought and officially released by the provincial authorities in every river. Following these practices, such species

‡Author to whom correspondence should be addressed. Tel.: +39 81 790 3349; fax: +39 81 790 3342; email: gibianco@unina.it

0022–1112/01/59A190+19 $35.00/0

as the European *Barbus barbus* (L.), recorded in Italy in 1994 (Bianco, 1995*a*) and the Asiatic *Pseudorasbora parva* (Temminck & Schlegel), found in Italy in 1987 (Sala & Spampanato, 1991), have reached in a few years an almost pan-Italian distribution. The introduction and establishment is now reported of another alien species, the Iberian barbel *Barbus graellsii* Steindachner (Bianco, 1998*b*), identified by both morphological and sequencing criteria.

In the last two decades such methodology, driven by molecular biology, has been successfully applied to solve several systematic problems with European freshwater fishes (Durand *et al.*, 2000). This species has been found during surveys of an area including the rivers Bruna, Ombrone, Albegna and Fiora from Grosseto and Siena Provinces in western central Italy. The Iberian barbel was found with the Danubian *B. barbus* and the native *Barbus tyberinus* Bonaparte. The Provincial Administration of Grosseto has intensively stocked the water bodies of its territory with white fishes for at least 20–25 years. In 1996, for instance, 1500 kg of barbels were released at 11 sites on the Ombrone, Fiora, Albegna and Orcia, with material from several fish farms, with extensive stockings of ' non-barbel ' species (pike *Esox lucus* L., trout *Salmo trutta* L., chub *Leuciscus cephalus* (L.), carp *Cyprinus carpio* L., tench *Tinca tinca* (L.)) (G. Giarola, pers. comm.). The Iberian barbel is thought to have been introduced in this way.

About three other alien species have become established in the last five years. Such introductions have resulted in a radical change in the composition of the Italian freshwater fish fauna with the local extinction or decline of native populations. A balance between alien and native fishes in many water bodies is still to be reached.

MATERIALS AND METHODS

The Rivers Ombrone, Fiora, Albegna and Bruna were surveyed on several occasions during 1994–1995 (Bianco, 1995*b*) and also in 2000 as part of a provincial programme for the reintroduction of the otter *Lutra lutra* (L.). About 25 different localities were surveyed at least twice, in spring-summer and autumn-winter respectively. Fishes were collected by electrofishing. Each specimen was measured (total length, L_T), weighed, some scales collected for age determination, and then released. Only a few specimens, especially those of the alien species, were killed for morphological, biological and genetic analyses. Two small specimens of *B. tyberinus* and of the putative Iberian *Barbus*, and three specimens of *B. barbus*, were fixed immediately in ethyl alcohol for DNA analysis. The mitochondrial cytochrome b gene (cytb) was chosen because of its usefulness for distinguishing morphologically similar species of cyprinids (Zardoya & Doadrio, 1998). Further details of extraction methods and sequencing for species identification are given in Appendix I. For the morphological comparisons preserved specimens were used of several Iberian *Barbus*.

The total quantity of fish collected in the three basins permitted the description of local communities by category ' native ', ' translocated ' (to the study area from northern Italy), and ' exotic ' (from outside Italy), number of species, number of individuals, and biomass. Twelve sites were selected to show the influence of alien species on native assemblages in different biotopes, namely, six sites from the middle and low courses (sites 1, 2, 3 and 10, 11, 12, Table I) of main rivers, and six from the upper reaches or brooks and rivulets (sites 3, 4, 5 and 7, 8, 9, Table I). Since the species *Leuciscus cephalus* (L.) in the study has originated from three different sources (native, transplanted from northern Italy, and introduced from the Danube area), this species has been included in each

TABLE I. Twelve selected collecting localities within the study area and number of fish species belonging to native, Padano-Venetian (Padanic) and extra-Italian (exotic) categories. *Barbus graellsii* was found in localities 1, 2 and 3

Origins	Collecting localities					
	1. River Ombrone, Grosseto	2. River. Albegna, Albinia	3. River Albegna, Canino	4. Lanzo Brook, Cinigiano	5. Upper River Albegna, Arcidosso	6. Upper River Fiora, Fiora
Native	4	4	4	7	5	4
Padanic	6	3	2	3	1	1
Exotic	5	6	3	0	0	0

Origins	Collecting localities					
	7. La Gonna Brook, Monticiano	8. Upper River Farma, Roccastrada	9. Upper River Merse, San Galgano	10. River Ombrone, Buonconvento	11. River Ombrone, Montalcino	12. River Merse, S. Lorenzo
Native	6	7	6	4	5	5
Padanic	2	5	5	7	7	8
Exotic	1	0	2	3	4	6

category and the number of fish and the biomass divided by two. The chub populations found in lower courses of rivers, together with the Iberian barbel (sites 1, 2 and 3, Table I), have been considered to be all of Danubian origins since they have modally eight branched rays in the anal fin which is typical for Danubian chub.

RESULTS

A list of species collected in the study area with their areas of original distribution and populations trends is given in Table II. The following notes concern the Iberian barbel, other exotic species, Padano-Venetian species established in the area, and native species.

IBERIAN BARBEL

Three species of *Barbus* were found in the waters surveyed, the introduced Danubian *B. barbus*, the native *B. tyberinus*, and a third species which is now identified as the Iberian barbel *Barbus graellsii* Steindachner, an endemic species of the River Ebro in Spain, and belonging to the '*Messinobarbus*' group characterized by the prominent nuptial tubercles on the snout in adult males (Bianco, 1998*b*) (Fig. 1). A comparisons of morphological features of several *Barbus* species (Table III) supports identification of the Iberian barbel and the present material corresponds well with the description and illustration given by Doadrio *et al.* (1991) for *B. graellsii*. It is believed to have been introduced to the study area by stocking of fish from northern Italian distributors.

Comparison of cytochrome b sequences derived from the putative Iberian barbel in the present work with GenBank sequences for several *Barbus* taxa (Table III), showed a highest identity score (99%) with that of *Barbus graellsii* (Accession Number AF045973) All other Iberian species examined had smaller identity scores, ranging from 95 to 97%, and the lowest values were those found in comparisons with *B. tyberinus* and *B. barbus* (89% each).

Among the three *Barbus* species found at the study sites, *B. graellsii* cannot be misidentified because of its uniformly silvery colouration, larger scales and ink black peritoneal membrane, in contrast to the greyish peritoneum in the other species. Gill rakers of *B. graellsii* are also much more numerous than in *B. barbus* and *B. tyberinus*. Another diagnostic character is the presence of large nuptial tubercles on the snout of adult males (Fig. 1) (absent or only incipient in immature males). *Barbus barbus* has a well developed and strongly denticulate dorsal fin spine, whereas in *B. graellsii* and the native *B. tyberinus* this spine is normally smooth and only in few cases is the proximal third of the ray ossified and weakly serrated.

Adults and young of *B. graellsii* were collected in April and November 2000. The April sample comprised adults in full spawning condition with females of 0·85–0·92 kg and 340–390 mm L_T and males of 0·22–0·84 kg and 230–340 mm L_T. The females had eggs at different stages of development which may indicate multiple spawning. Ripe eggs were *c.* 2·0 mm in diameter. Males had the snout, the head and each scale of the body covered by nuptial tubercles. The gonadosomatic indices for two females were 9·6 and 10·5%, similar values to those found in six mature males, which ranged from 8·0 to 9·2%.

TABLE II. Species collected in the study area with their origins and trend of populations (Bianco, 1995*b* and unpubl. data); Nat, native; PV, Padaric; Ex, exotic

Species	River basins				Origins			Occurrence and status
	Ombrone	Albegna	Fiora	Bruna	Nat	PV	Ex	
Anguillidae								
Anguilla anguilla (L.)	×	×	×	×	×			Native, stocked
Esocidae								
Esox lucius L.	×					×		Locally abundant, stocked
Salmonidae								
Salmo trutta L.	×	×	×	×		×	×	Intensively stocked
Cyprinidae								
Cyprinus carpio L.	×	×	×				×	Stocked yearly
Carassius auratus (L.)	×	×	×				×	Locally invasive, stocked
Rutilus aula (Bonaparte)	×					×		Occasional, stocked unintentionally
Rutilus rubilio (Bonaparte)	×	×	×	×	×			Progressive decline
Tinca tinca (L.)	×	×	×				×	Stocked yearly
Leuciscus cephalus (L.)	×	×	×	×	×	×	×	Invasive, stocked
Leuciscus lucumonis Bianco	×				×			Progressive decline, localized
Telestes muticellus (Bonaparte)	×	×	×	×	×			Locally, well structured
Scardinius erythrophthalmus (L.)	×					×		Occasional, stocked unintentionally
Alburnus arborella De Filippi	×							Occasional, stocked unintentionally
Chondrostoma genei (Bonaparte)	×	×	×			×		Stocked unintentionally, invasive
Barbus tyberinus Bonaparte	×	×	×	×	×			Local extinction, declining
Barbus barbus (L.)	×	×	×				×	Stocked, frequent
Barbus graellsii Steindachner	×	×	×				×	Stocked, invasive
Gobio benacensis Pollini	×			×		×		Occasional, stocked unintentionally
Pseudorasbora parva (T.L.S.)	×	×	×				×	Stocked unintentionally, invasive

TABLE II. *Continued*

Species	River basins				Origins			Occurrence and status
	Ombrone	Albegna	Fiora	Bruna	Nat	PV	Ex	
Cobitidae								
Cobitis bilineata Canestrini	×					×		Localized, stocked unintentionally
Sabanejewia larvata (De Filippi)	×					×		Localized, stocked unintentionally
Ictaluridae								
Ictalurus melas (Rafinesque)	×						×	Occasional, stocked?
Centrarchidae								
Lepomis gibbosus (L.)	×	×	×				×	Occasional, stocked unintentionally
Gobiidae								
Padogobius nigricans (Canestrini)	×				×			Declining, locally extinct
Padogobius bonellii (Bonaparte)	×					×		Expanding

FIG. 1. Lateral view of the head of an adult male *Barbus graellsii*, 240 mm L_T, from the River Ombrone showing horny tubercles on snout.

OTHER EXOTIC SPECIES

Barbus barbus

In the study area this exotic species of *Barbus*, already reported for Italy (Bianco, 1995*a*) was collected with *B. graellsii* in the low reaches of the rivers. Most were medium sized specimens without signs of spawning, and were introduced probably during one of the more recent stockings.

Pseudorasbora parva

This is recorded for the first time from Grosseto Province. It is now established in the middle and lower courses of the Ombrone, Albegna and Fiora. The species was found at a wide size range and is locally very abundant. The species is already known in the upper parts of the Ombrone (Loro & Bortot, 1998) and Arno (Nocita & Vanni, 1999).

Leuciscus cephalus

According to Durand *et al.* (2000), Mediterranean populations of chub belong to a different genetic lineage from those of central Europe. In morphology, the Mediterranean populations of chub (Italy, Croatia, Western Greece) show modally nine branched rays in the anal fin compared with the modal eight rays of the Danube populations (Bianco, 1990*b*). The number of branched anal rays in the chub populations of the lower courses of the rivers studied was found to be modally eight in comparison with the modal value of nine found in the upper reaches and in other native Italian populations. This character seems to have taxonomic value being independent of ecological parameters although in Italy a

TABLE III. Morphological and genetic comparison of putative *Barbus graellsii* (as *Barbus* sp.) from Rivers Ombrone and Albegna, Tuscany, Italy, four species of '*Messinobarbus*' group of Iberian species and *B. tyberinus* and *B. barbus* from Tuscan rivers. IZA, collection of Università di l'Aguila, Italy

Characters	*Barbus* sp. River Ombrone and Albegna IZA 0154 $n=11$	*B. graellsii* River Ebro, Spain IZA 01145 $n=7$	*B. microcephalus* River Guadiana, Portugal IZA 0053 $n=4$	*B. steindachneri* River Guadiana, Spain IZA 00194 $n=9$	*B. comizo* River Guadiana Spain IZA 00144 $n=7$	*B. tyberinus* River Ombrone IZA 0096 $n=12$	*B .barbus* River Albegna IZA 00176 $n=11$
Morphology							
Horny tubercles in adult males	Well developed	Well developed*	Well developed*	Well developed*	Well developed*	Small to absent	Small to absent
Body colouration	Silvery Black	Silvery Black	Silvery Black	Silvery Black	Silvery Black	Spotted Grayish	Spotted Grayish
Peritoneal membrane							
Serration on 1st dorsal ray	Fine to absent	Fine to absent	Strong	Strong	Strong	Fine to absent	Strong
Number of lateral line scales (means in parentheses)	45–49 (47·2)	46–50 (47·6)	48–51 (49·3)	47–51 (49·1)	46–49 (47·7)	53–58 (56·4)	54–59 (56·8)
Total gill rakers (means in parentheses)	17–22 (19·3)	19–21 (20·2)	14–16 (15·0)	14–17 (15·3)	18–22 (20·6)	8–12 (9·4)	10–13 (11·8)
Genetics							
GenBank accession number	AF397299	AF045973	AF045971	AF045968	AF04597	AF397300	AF397298
Percentage of genetic identity with *Barbus* sp. from Ombrone	—	99%	97%	96%	95%	89%	89%

*Doadrio *et al.* (1991) and C. Almaca, pers. com.

slight negative geographic variation has been found from north to south (P. G. Bianco, pers. obs.). However, present results would confirm the introduction of the Danubian lineage of chub into this area of central Italy.

TRANSLOCATED PADANO-VENETIAN SPECIES

Chondrostoma genei *(Bonaparte)*

A mostly riverine invasive species which is now present everywhere and abundantly so in several localities on the middle courses of the rivers. It has also entered small streams and rivulets and seems to be a direct competitor with *Leuciscus lucumonis* Bianco and *Rutilus rubilio* (Bonaparte), two rheophiline species which nowadays survive mostly in small riverine biotopes.

Padogobius bonelli *(Bonaparte)*

Another invading species, this goby at one locality (site 4, Table I) is dominant with respect to the endemic *Padogobius nigricans* (Canestrini) (*c.* 80% of the sample). The two species are in competition and in central Italy the introduction of *P. bonelli* appears to have caused the progressive decline and local extinction of *P. nigricans* (Zerunian & Taddei, 1998).

NATIVE SPECIES

Leuciscus lucumonis

This cyprinid was found only in the Ombrone basin. It has disappeared from the main basin and has been found at only four localities (upper River Farma, La Gonna Brook, Lanzo Brook and River Gretano) with reduced populations. Competition with *C. genei* seems to be the main reason of its progressive decline. A similar situation was found in the Tiber in central Italy (Mearelli *et al.*, 1996).

Telestes muticellus *(Bonaparte)*

Populations still remain in the middle and upper parts of the main rivers but especially in small streams. It is the most frequent native species also in the upper reaches of the Tiber basin (Mearelli *et al.*, 1996).

Rutilus rubilio

This species is quite scarce and did not form consistent schools as in other rivers of the Tuscany-Latium district (Arno and Tiber). The populations from the study area are smaller than in other localities and considered as draft populations (Bianco & Taraborelli, 1986).

Padogobius nigricans

The introduction of *P. bonelli*, a direct competitor, will probably cause the extinction of this species.

EFFECTS OF ALIEN SPECIES ON NATIVE FISH ASSEMBLAGES

Local fish assemblages were studied in detail at 12 localities representing a variety of biotopes, out of 25 visited during the surveys. Introduced species tended to localize in the low courses of the main rivers Fiora, Ombrone and Albegna, while native species were more frequent in the upper reaches of main basins and in small tributaries. In the lower courses of rivers (site 1, 2, 3, Table I, Fig. 2) and the main Ombrone and Merse (sites 10-11-12, Table I,

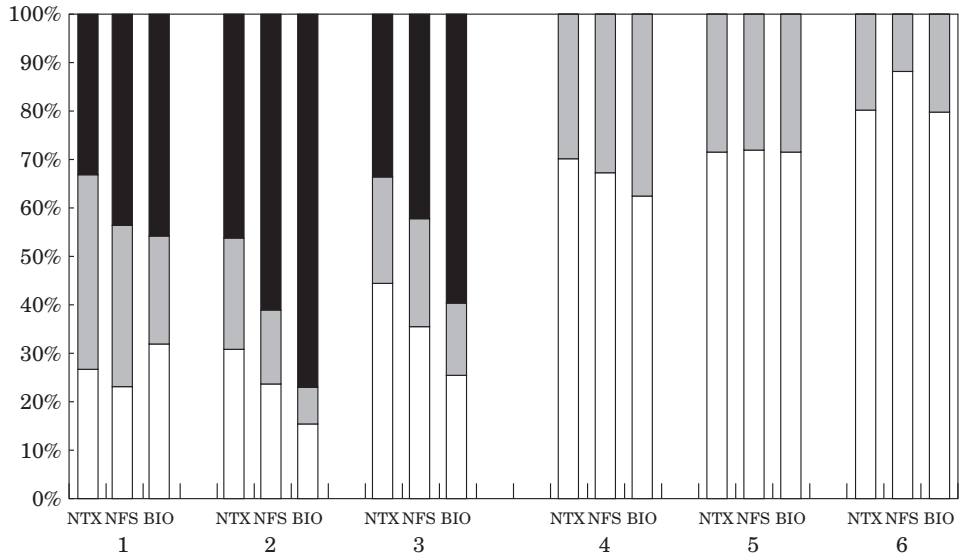

FIG. 2. Percentage composition of fish assemblages at six localities (sites 1–6 from Table I) of Grosseto Province. NTX, Number of species; NFS, number of fishes; BIO, biomass of different categories: □, native ▨, translocated from Padano-Venetian district; ■ exotic, introduced from outside Italy.

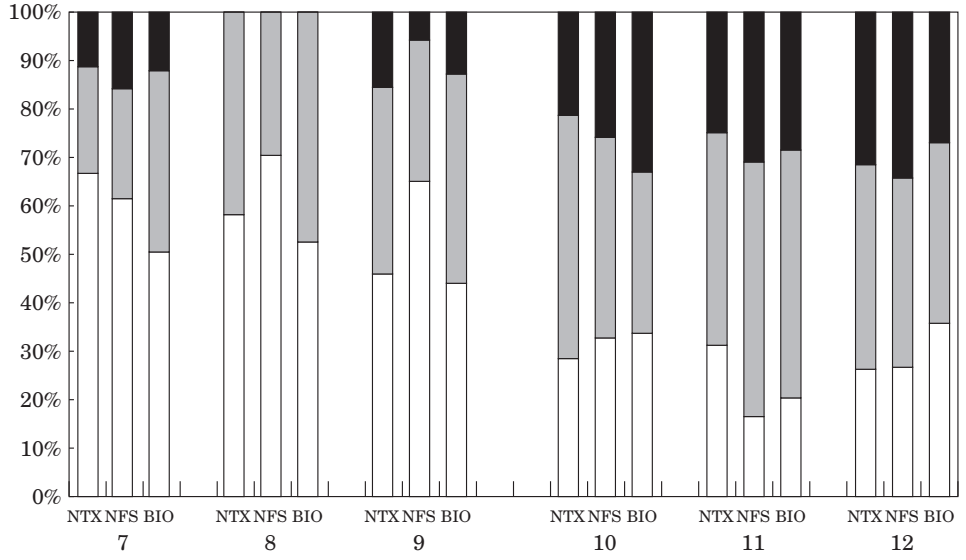

FIG. 3. Percentage composition of fish assemblages at six localities (sites 7–12 from Table I) of Siena Province, Ombrone drainage. Abbreviations as Fig. 2.

Fig. 3), the percentage of native fishes, either as number of species, number of fishes, or biomass, ranged from 18% (biomass at site 3) to 42% (at site 1). The native species were better represented at sites 7, 8 and 9 and were dominant in sites 4, 5 and 6 which were the smaller rheophilic biotopes, with up to 63% of total biomass and up to 90% of number of fishes at site 6. The tendency of native

TABLE IV. Updated check list of exotic species established in public waters in Italy

Species	Area of establishment	Year of naturalization and references
Salmonidae		
Salvelinus fontinalis (Mitchill)	All Italy	1891, Sommani (1969); Gandolfi *et al.* (1991)
S. namaycush (Walbaum)	Northern Italy several localities	1995, A. Mojetta (pers. com.)
Oncorhynchus mykiss (Walbaum)	River Savena, Northern Italy	1995, Rizzoli *et al.* (1998)
Coregonus wartmanni (Bloch)	Lakes of Northern and Central Italy	1861, De Filippi (1861); Gandolfi *et al.* (1991)
C. macrophthalmus Nüsslin	Lakes of Northern Italy	1940, Sommani (1969); Gandolfi *et al.* (1991)
Cyprinidae		
Rutilus pigus (Lacepède)	Upper Adriatic river drainages	Roman Period, Middle Ages—Gandolfi *et al.* (1991)
R. rutilus L.	Upper Adriatic river drainages. Expanding in NE Italy	1989, Delmastro & Balma (1991); T. Busatto, pers. com.
Pachychilon pictum (Heckel & Kner)	River Serchio, central Italy	1989, Delmastro & Balma (1990)
Abramis brama (L.)	NE Italy	1985, Marconato *et al.* (1986)
Blicca bjoerkna (L.)	NE Italy	1993, Confortini *et al.* (1993)
Telestes. agassizi (Valenciennes)	River Isonzo, NE Italy	1962, Bianco (1995*c*)
Chondrostoma nasus (L.)	River Isonzo, NE Italy	1960, Povz (1983)
Rhodeus amarus Bloch	Padano-Venetian	1988, Confortini (1990), Loro *et al.* (1994)
Pseudorasbora parva (Temminck & Schlegel)	Northern Italy; expanding in central Italy	1988, Sala & Spampanato (1991); Confortini (1998); Nocita & Vanni (1999); Loro & Bortot (1990); present data.
Barbus barbus (L.)	Expanding in Northern and Central Italy	1995, Bianco (1995*a*); M. Lorenzoni, pers. com.; present data.
B. petenyi (Heckel)	River Isonzo, NE Italy	1995, Bianco (1995*a*)
Barbus graellsii Steindachner	Rivers Fiora, Ombrone, Albegna in central Italy	Present data
Gobio gobio (L.)	At least Eastern and western northern Italy	Present data
Cyprinus carpio L.	All Italy	Roman Period Balon (1969)
Carassius auratus (L.)	All Italy	17th century (?) Bianco (1998*b*)
Tinca tinca (L.)	All Italy	17th century (?) Bianco (1998*b*)

TABLE IV. *Continued*

Species	Area of establishment	Year of naturalization and references
Cobitidae		
Misgurnus fossilis (L.)	River Ticino NW Italy	1998, Groppali (1999)
Ictaluridae		
Ictalurus melas Rafinesque	All Italy	1906, Cavallini (1933), Gandolfi *et al.* (1991)
I. nebulosus (Lesveur)	All Italy	1906, Cavallini (1933)
I. punctatus (Rafinesque)	River Oglio and Pavia Province in Northern Italy	1986? Gandolfi *et al.* (1991), Bernini *et al.* (1996)
Siluridae		
Silurus glanis L.	Expanding upriver in the Po basin. Established in Lake Garda	1956, Manfredi (1957), Sicuro *et al.* (1998), Confortini (1998)
Gadidae		
Lota lota (L.)	Northern Italy	1800(?), Pavesi (1881), Gandolfi *et al.* (1991)
Atherinidae		
Odonthestes bonariensis Valenciennes	Probably extinct	1974, Ferrero (1981), L. Tancioni (pers. com.)
Poecilidae		
Gambusia holbrooki Girard	All Italy	1922, Grassi (1923), Gandolfi *et al.* (1991)
Percidae		
Perca fluviatilis L.	All Italy	1860(?), Pavesi (1896), Gandolfi *et al.* (1991)
Gymnocephalus cernuus (L.)	Expanding in NE Italy	1985, Chiara (1986), Loro *et al.* (1994)
Stizosteidon lucioperca (L.)	Expanding in Northern and Central Italy	1900, Supino (1930), Gandolfi *et al.* (1991), Giovinazzo *et al.* (1999)
Centrachidae		
Micropterus salmoides (Lacepède)	All Italy	1897, Supino (1930), Gandolfi *et al.* (1991)
Lepomis gibbosus (L.)	All Italy	1900, Supino (1930), Gandolfi *et al.* (1991)

species to use rheophilic biotopes was observed also in the upper basin of the Tiber in Central Italy (Mearelli *et al.*, 1996).

UPDATED CHECKLIST OF INTRODUCED SPECIES IN ITALY

Several additional introduced species must now be added to the list given by Bianco (1998*a*) and an updated checklist of 34 species now established in Italian fresh waters is given in Table IV. Native species comprise 44 taxa, making a total freshwater fish diversity of 78 species.

Among such introductions, Rizzoli *et al.* (1998) reported the reproduction of *Oncorhynchus mykiss* (Walbaum) in the River Savena, northern Italy, while Freyhof (1998) reported the establishment in Lake Trasimeno of *Pomatoschistus canestrinii* (Ninni) a small, typically brackish water goby, which, while not an exotic, is otherwise endemic to northern Adriatic lagoons. The weather loach *Misgurnus fossilis* (L.) was already well established by 1998 in several canals of the Ticino basin and is the subject of intensive fishing for food by local fishermen. The origin of this introduction is unknown (Groppali, 1999). Another established exotic is *Gobio gobio* (L.). During two surveys in 1998, in the River Bormida (Po basin, north-western Italy) and in the Brenta (north-eastern Italy) respectively, large populations of gudgeon were observed (P. G. Bianco, pers. data). Their morphology revealed them to be *G. gobio*, by possession of two or three scales between the anus and anal fin origin as against five to seven scales found in *Gobio benacensis* (Pollini) (Bianco & Taraborelli, 1986). This invasive occurrence of *G. gobio* was never realized before since *G. benacensis* was formerly considered a localized and quite infrequent species (Delmastro, 1986; G. Delmastro, pers. comm.). The spreading of *G. gobio* may well bring about the extinction of *G. benacensis*. However, since *G. benacensis* has in turn been accidentally introduced into several rivers of central and southern Italy (Bianco, 1994), these new populations will probably become an available genetic reservoir for the species.

CONSERVATION STATUS OF NATIVE SPECIES

Drastic protective measures must be undertaken to ensure the safety of at least three freshwater fish species in Italy. These are *Salmo carpio* L., endemic to Lake Garda and nearly extinct (Confortini, 1998), *L. lucumonis*, an obligate riverine species which shows a mosaic distribution in rivulets of the Arno, Serchio, Ombrone and Tiber basins (present data; Bianco, 1995*b*; Mearelli *et al.*, 1996), and *Scardinius scardafa* (Bonaparte), a rehabilitated species which now lives at a single locality, the Lake of Scanno within its original Tuscany-Latium range (Ketmaier *et al.*, 2001). In the case of *L. lucumonis*, restricted biotopes such as streams and brooks are at great risk of destruction and very susceptible to local human demand for water. They can become dry in a very short time with the extinction of this species (Bianco, 1994). Indeeed, the type locality of *L. lecumonis*, a brook tributary of the Ombrone, dried up about 10 years ago. In the light of present findings, two other species might also be considered as endangered, *G. benacensis* and *P. nigricans* (Zerunian & Taddei, 1998), as the result of introduction of congeneric species.

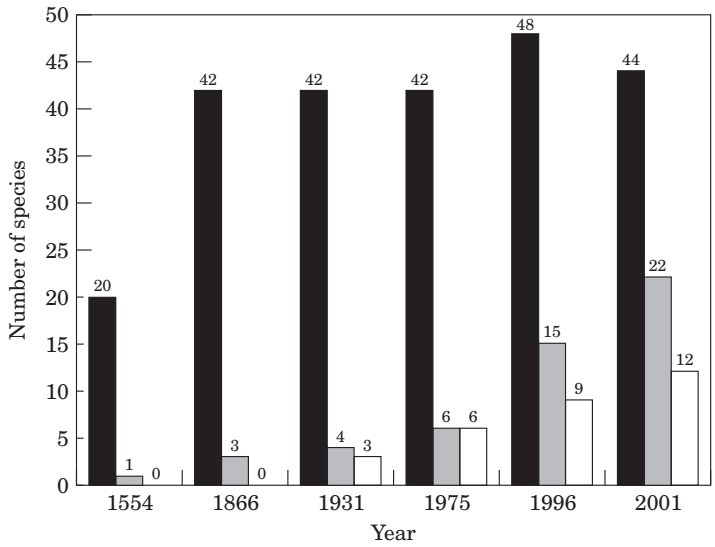

FIG. 4. Trends in composition of recognized native and exotic freshwater fish species in Italy from 1556 up to 2001 (updated from Bianco, 1998*a*). ■, Native species; ▨, exotic species of European origin; □, exotic species of extra-European origin.

However, the conservation status of some species has changed for the better in the last decade. The sturgeon *Acipenser naccari* Bonaparte, previuosly considered as endangered, has been successfully reproduced in captivity and restocked (Arlati *et al.*, 1988; Mortorio, 1996) and could be regarded as safe. Similarly, *Salmo marmoratus* Cuvier, through stocking practices (Sicuro *et al.*, 1996), is now saved. The goby *Knipowitschia punctatissima* (Canestrini), which previously was considered very rare, has now been found to be common in the Padano-Venetia district (Marconato *et al.*, 1990; Loro *et al.*, 1994; E. Marconato, pers. comm.). In contrast, four anadromous species, the lampreys *Petromyzon marinus* L. and *Lampetra fluviatilis* (L.) and the sturgeons *Acipenser sturio* L. and *Huso huso* (L.), which over the past 15–30 years have not been recorded (Marconato *et al.*, 1990; Bernini *et al.*, 1996; Bonfigli & Landini, 1998; Massidda *et al.*, 1998; present study) must now be considered as reproductively extinct in Italian fresh waters.

The pattern of change in the Italian freshwater fish fauna since the sixteenth century is illustrated in Fig. 4 (data from Bianco, 1998*a*) where increasing recognition of native diversity has been accompanied by an expanding input of exotic species but offset by the extinctions noted above.

CONCLUSIONS

In Italy a rapid evolution of freshwater fish communities is in progress. In the past 30 years there have been processes of ‘ padanization ’, with transplantation of native species from north to central Italy, followed by ‘ danubization ’, with introduction of Danubian species throughout Italy. The current process could be termed ‘ globalization ’ with the establishment of Iberian, Albanian, eastern Asian and North American elements. There is even a report of the establishment

of an African *Clarias* species in the Arno basin (T. Busatto, pers. comm.). The present updated list of introduced species indicates 34 in 2001, and several, such as *Silurus glanis* L., *P. parva* and *Rhodeus amarus* Bloch, are expanding in distribution. Contrary to this tendency for increase in diversity, four native migratory species (two lampreys and two sturgeons) no longer breeding in Italian rivers.

In the present work, the Iberian barbel *B. graellsii* is recorded for the first time in Italy and has been found to have become an established species in at least three rivers of central western Italy where it predominates together with chub, *L. cephalus*, of Danubian origin. Translocation of invasive species as *C. genei* and *P. bonelli*, from northern Italy, as well as the introduction of exotic species, appear to be principal causes in the reduction of native species especially in the main and lower reaches of rivers. Native species have been found to occur nowadays in more riverine habits, and in central Italy small brooks or the upper courses of the main rivers appear to act as refugia.

References

Altschul, S. F., Gish, W., Miller, W., Myers, E. & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology* **215,** 403–410.

Arlati, G., Bronzi, P., Colombo, L. & Giovannini, G. (1988). Induzione della riproduzione nello storione italiano (*Acipenser naccarii*) allevato in cattività. *Rivista Italiana di Acquicoltura* **23,** 94–96.

Balon, E. (1969). Studies on the wild carp *Cyprinus carpio*. I. New opinion concerning the origins of the carp. *Prace Laboratoria Rybarstva a Hydrobiolologie* **2,** 99–120.

Bernini, F., Bisogni, L., Nardi, P. A. & Soria, P. (1996). Artificializzazione del popolamento ittico: l'esempio delle acque pavesi. In *Atti 5° Convegno Associazione Italiana Itiologi Acqua Dolce*, pp. 333–338. Vicenza: Amministrazione Provinciale, Assessorato alla Pesca.

Bianco, P. G. (1990*a*). Vanishing freshwatwer fish in Italy. *Journal of Fish Biology* **37**(Suppl. A), 235–237.

Bianco, P. G. (1990*b*). Potential role of the palaeohistory of the Mediterranean and Paratethys basin on the early dispersal of Europe-Mediterranean freshwater fishes. *Ichthyological Exploration of Freshwaters* **1,** 167–184.

Bianco, P. G. (1994). L'ittiofauna continentale dell'Appennino umbro-marchigiano, barriera semipermeabile allo scambio di componenti primarie tra gli opposti versanti dell'Italia centrale. *Biogeographia* **17,** 427–485.

Bianco, P. G. (1995*a*). A revision of Italian species of Barbus. *Ichthyological Exploration of Freshwaters* **6,** 305–324.

Bianco, P. G. (1995*b*). Studio sull'ittiofauna, la lontra e l'ambiente nelle valli del Farma e del Merse. In *Allegato 2: Indagine Sull'ittiofauna e i Decapodi*, pp. 1–39. Istituto di Ecologia Applicata, Amministrazione Provinciale di Siena.

Bianco, P. G. (1995*c*). Factors affecting the distribution of fishes especially in Italy. *Cybium* **19,** 241–259.

Bianco, P. G. (1998*a*). Freshwater fish transfers in Italy: history, local modification of fish composition, and a prediction on the future of native populations. In *Stocking and Introductions of Fishes* (Cowx, J., ed.), pp. 165–197. Oxford: Fishing News Books.

Bianco, P. G. (1998*b*). Diversity of Barbinae fishes in southern Europe with description of a new genus and a new species. *Italian Journal of Zoology* **65**(Suppl.), 125–136.

Bianco, P. G. & Taraborelli, T. (1986). *Gobio gobio benacensis* (Pollini, 1816) sottospecie valida per l'Italia (Pisces, Cyprinidae). *Bollettino del Museo Civico di Storia Naturale di Verona* **11**(1984), 525–536.

Bonfigli, R. & Landini, W. (1998). Alcune considerazioni sulla distribuzione di specie eurialine nel fiume Vara (Sp). In *Atti 6° Convegno Associazione Italiana Itiologi Acqua Dolce*, pp. 50–56. Provincia di La Spezia, Regione Liguria.

Briolay, J., Galtier, N., Brito, R. M. & Bouvet, Y. (1998). Molecular phylogeny of Cyprinidae inferred from cytochrome b DNA sequences. *Molecular Phylogenetics and Evolution* **8,** 435–442.

Cavallini, F. (1933). Il pesce gatto *Ameiurus catus* nelle acque del Ticino. *Natura, Milano* **24,** 23–26.

Chiara, G. (1986). Prima segnlazione di *Acerina* (*Gymnocephalus*) *cernua* L. (Osteichthyes, Perciformes) per l'Italia. *Quaderni Ente Tutela per la Pesca, Udine* **13,** 59–60.

Confortini, I. (1990). Presenza del rodeo amaro *Rhodeus sericeus* (Pallas, 1776), nel fiume Menago (Provincia di Verona) (Pisces, Cyprinidae). *Bollettino del Museo Civico di Storia Naturale di Verona* **16,** 329–332.

Confortini, I. (1998). Evolution of fish community of the Lake Garda: present and past situation. In *Atti 6° Convegno Associazione Italiana Itiologi Acqua Dolce*, pp. 77–86. Regione Liguria, Provincia di La Spezia.

Confortini, I., Maio, G., Marconato, E., Salviati, S., Dell'Antonio, L., Bertolo, A. & Perini, V. (1993). Presenza della *Blicca bjoernka* (L.) nel lago di Ledro (Provincia di Trento) (Pisces, Cyprinidae). *Annali dei Musei Civici, Rovereto* **9,** 289–294.

De Filippi, F. (1861). Rapporto sull'introduzione in Italia del *Coregonus wartmanni* e del *Salmo umbla. Archivio di Zoologia, Anatomia e Fisiologia, Modena* **1,** 27–31.

Delmastro, G. B. (1986). *I Pesci del Bacino del Po*. Milan: CLESAV.

Delmastro, G. B. & Balma, G. B. (1990). Primo reperto per l'Italia e acclimatazione del ciprinide endemico balcanico *Pachychilon pictum* (Heckel & Kner, 1858) nel fiume Serchio in Toscana (Osteichthyes: Cyprinidae). *Bollettino del Museo Regionale Scienze Naturali di Torino* **8,** 411–416.

Delmastro, G. B. & Balma, G. B. (1991). Nota sulla comparsa del ciprinide transalpino *Rutilus rutilus* (Linnaeus, 1758) nelle acque piemontesi. *Rivista di Idrobiologia* **29,** 163–167.

Doadrio, I., Elvira, B. & Bernat, Y. (1991). *Peces Continentals Espanoles. Colleccion Técnica*. Madrid: ICONA-CSIC.

Durand, J. D., Unlu, E., Doadrio, I., Pipoyan, S. & Templeton, A. R. (2000). Origin, radiation, dispersion and allopatric hybridization in the chub *Leuciscus cephalus. Proceedings of the Royal Society of London B* **267,** 1687–1697.

Ferrero, L. (1981). Acclimatization experiment and introduction of an Argentinian ichthyo species. *Quarterly Newsletter European Mariculture Society* **21,** 93–95.

Freyhof, J. (1998). First record of *Pomatoschistus canestrinii* (Ninni, 1883) in Lake Trasimeno. *Rivista di Idrobiologia* **37,** 107–108.

Gandolfi, G., Zerunian, S., Torricelli, P. & Marconato, A. (1991). *I Pesci delle Acque Interne Italiane*. Roma: Istituto Poligrafico e Zecca dello Stato.

Gilles, A., Lecointre, G., Faure, E., Chappaz, R. & Brun, G. (1998). Mitochondrial phylogeny of European cyprinids: implications for their systematics, reticulate evolution and colonization time. *Molecular Phylogenetics and Evolution* **10,** 132–143.

Giovinazzo, G., Carosi, A., Lorenzoni, M., Petesse, M. L. & Mearelli, M. (1999). Growth of *Stizosteidon lucioperca* (L.) in the middle reach of the River Tiber. *Quaderni ETP* **28,** 169–173.

Grassi, B. (1923). Acclimatazione della *Gambusia* in Italia. *Atti Accademia dei Lincei* **32,** 544–568.

Groppali, R. (1999). Prima segnalazione di naturalizzazione del cobite di stagno *Misgurnus fossilis* (L.) in Italia. *Quaderni ETP* **28,** 227–228.

Ielardi, G. (1998). Acque dolci, sorprese amare. *Oasis* **14,** 92–97.

Ketmaier, V., Bianco, P. G., Cobolli, M. & De Mattheais, E. (2001). Genetic differentiation and biogeography in the genus *Scardinius* (Bonaparte, 1837) (Cyprinidae) based on allozyme data. *Zoologica Scripta*, in press.

Loro, R. & Bortot, N. (1998). Comunità ittiche dell'Ombrone: valutazioni ecologiche per la stesura di un piano Faunistico. In *Atti 6° Convegno Associazione Italiana Itiologi Acqua Dolce*, pp. 202–211. Regione Liguria, Provincia di La Spezia.

Loro, R., Zanetti, M. & Turin, P. (1994). *Carta Ittica della Provincia di Treviso*. Provincia di Treviso: Assessorato Caccia Pesca Ecologia.

Manfredi, P. (1957). Cattura di un *Silurus glanis* nell'Adda presso Lecco. *Natura* **48,** 28–30.

Marconato, A., Salviati, S., Maio, G. & Marconato, E. (1986). *La Distribuzione dell'ittiofauna nella Provincia di Vicenza*. Provincia di Vicenza, Assessorato Pesca.

Marconato, E., Salviati, S., Maio, G. & Marconato, A. (1990). *La Fauna Ittica della Provincia di Padova*. Padova: Provincia di Padova, Assessorato alla Pesca.

Massidda, P., Sabatini, A., Davini, M. A., Conti, G., Loddo, G. & Cau, A. (1998). Nuovi dati sulla distribuziobne dell'ittiofauna d'acqua dolce in Sardegna. In *Atti 6° Convegno Associazione Italiana Itiologi Acqua Dolce*, pp. 239–246. Regione Liguria, Provincia di La Spezia.

Mearelli, M., Carosi, A., Giovinazzo, G., Lorenzoni, M., Petesse, M. L. & Santucci, A. (1996). Relazione tra ordine dei corsi d'acqua e popolamenti ittici nel bacini idrografico dell'alto Tevere. In *Atti 5° Convegno Associazione Italiana Itiologi Acqua Dolce*, pp. 277–294. Vienza: Amministrazione Provinciale, Assessorato alla Pesca.

Melotti, P. (1994). *Indagine Sullo Stato dall'Acquacoltura nelle Acque Interne*. Verona: Associazione Piscicultori Italiani.

Mortorio L. (1996). L'allevamento degli storioni. Doctoral Thesis, University of Milan.

Nocita, A. & Vanni, S. (1999). Cataloghi del Museo di Storia Naturale dell'Università di Firenze. XIX. *Actynopterigi, Cypriniformes. Atti. Società Toscana di Scienze Naturali* **106,** 115–130.

Pavesi, P. (1881). L'ultima sementa di pesci nei nostri laghi. *Rendiconti del Istituto Lombardo* **14,** 3–12.

Pavesi, P. (1896). La distribuzione dei pesci in Lombardia. *Premiata Tipografia Fusi* Pavia.

Povz, M. (1983). Change in area of two species from the genus *Chondrostoma* (Pisces) in Slovenia. *Bioloski Vestnik Ljubljana* **31,** 45–52.

Rizzoli, M., Falconi, R. & Zaccanti, F. (1998). Preliminary observation on an acclimatized population of Oncorhynchus mykiss in Northern Appennin (Bologna, Italy). In *Atti 6° Convegno Associazione Italiana Itiologi Acqua Dolce*, pp. 317–322. Regione Liguria, Provincia di La Spezia.

Sala, L. & Spampanato, A. (1991). Prima segnalazione di *Pseudorasbora parva* in acque interne Italiane. *Rivista di Idrobiologia* **29,** 461–467.

Sicuro, B., Forneris, G. & Pascale, M. (1996). Il popolamento ittico dei fiumi piemontesi. In *Atti 5° Convegno Associazione Italiana Itiologi Acqua Dolce*, pp. 247–258. Vicenza: Amministrazione Provinciale, Assessorato alla Pesca.

Sommani, E. (1969). Variazioni apportate all'ittiofauna dall'attività dell'uomo. *Bollettino di Pesca Piscicultura e Idrobiologia* **23**(1967), 149–166.

Supino, F. (1930). Considerazioni generali sui pesci d'acqua dolce d'Italia. *Natura* **21,** 78–96.

Zardoya, R. & Doadrio, I. (1998). Phylogenetic relationships of Iberian cyprinids: systematic and biogeographical implications. *Proceedings of the Royal Society of London* **265,** 1365–1372.

Zerunian, S. & Taddei, A. R. (1998). Competition between native species and introduced species: the stream goby and the Padanian goby in the Amaseno river (Osteichthyes, Gobiidae). In *Atti 6° Convegno Associazione Italiana Itiologi Acqua Dolce*, pp. 443–450. Regione Liguria, Provincia di La Spezia.

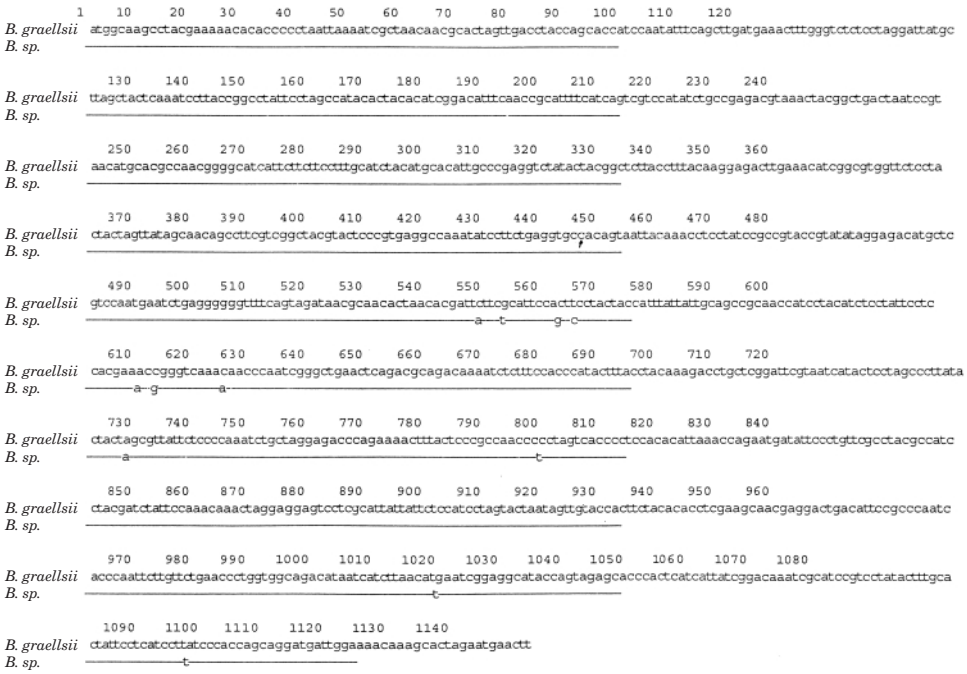

FIG. 5. Alignment of sequence retrieved from Gen Bank (*Barbus graellsii*: AF045973) and that of putative *B. graellsii* (as *B.* sp), representing almost identical nucleotides.

APPENDIX I
MOLECULAR IDENTIFICATION OF *BARBUS GRAELLSII*

Total DNA was extracted from a pectoral fin from fresh material of each of the three sympatric *Barbus* species, *B. barbus*, *B. tyberinus* and the putative *B. graellsii*, and fixed in 95% ethyl alcohol The fin surface was washed with sterile water and subjected to 20 min of UV irradiation; DNA was extracted using the Easy-DNA extraction kit from Invitrogen (Carlsbad, CA). A combination of two sets of primers (Glu-F; Cytb-R; Cytb-F; Thr-R) (Zardoya & Doadrio, 1998) were used to amplify two contiguous and overlapping fragments (660 and 521 bp respectively) covering the entire cytochrome b gene (1140 bp). Double stranded amplifications were performed in a solution containing 10 mM Tris-HCl (pH 8·8), 50 mM KCl, 1·5 mM $MgCl_2$, each dNTP at 2·5 mM, 200 ng for each primer, 10–100 ng of genomic DNA and 1 unit of AmpliTaq (Perkin-Elmer-Cetus). After a 2 min denaturation step (94° C), each PCR cycle consisted of denaturation for 1 min at 94° C, annealing for 1 min at 48° C and extension for 1 min and 30 s at 72° C. This cycle was repeated 40 times and followed by a 2 min incubation step at 72° C. PCR fragments were purified using the GenEluteTM PCR DNA Purification Kit (Sigma). Sequences were determined with an ABI 373A automated sequencer (Applied Biosystems) following the manufacturer's protocols. Strands were sequenced in both directions and analysed using the programme Sequencher 3.1.1 (Gene Codes Corporation, Ann Arbor, MI). Sequences were submitted to GenBank (Accession of numbers, AF397298 and AF397300).

GenBank was searched for sequence similarity using nucleotide-nucleotide BLAST (Basic Local Alignment Search Tool). This is a set of similarity search programmes, which use a heuristic algorithm, designed to explore all available sequences (Altschul *et al.*, 1990). The sequences, retrieved from GenBank for several Iberian species (*B. graellsii* Steindachner: AF045973; *B. microcephalus* Almaca: AF045971; *B. steindachneri* Almaca: AF045968; *B. comizo* Steindachner: AF045967) were aligned and sequences were obtained from *B. tyberinus*, *B. barbus*, and the putative *B. graellsii*. A percentage of genetic identity as implemented in BLAST (Altschul *et al.*, 1990) was calculated between the taxa. Results showed that the sequence corresponding to Accession Number AF045973, belonging to *B. graellsii*, had the highest identity score (99%) with the sequence submitted from the putative *B. graellsii*. Alignment of the two sequences is shown in Fig. 5. Eleven transitional differences (TS) were found between the two species; as expected third codon positions are most variable (seven TS, 63·6%), first and second codon positions equally accounting for the other TS. The pattern of nucleotide substitution (Briolay *et al.*, 1998; Gilles *et al.*, 1998) as well as the high score of identity between the two sequences led to the conclusion that the specimen under review belonged to the species *B. graellsii*, a species endemic to the River Ebro in Spain, an identification which supported that from morphology described and illustrated by Doadrio *et al.* (1991).