

Genetic characterization of *Gobio gobio* populations of the Iberian Peninsula based on cytochrome *b* sequences

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A b s t r a c t. The common gudgeon *Gobio gobio* (L.) has a great phenotypic plasticity being one of the most variable fish species in Europe. In the Iberian Peninsula it has been considered to be an introduced species. However, recent publications have considered that gudgeon populations from different basins of the Iberian Peninsula are native. In this work we tried to clarify the status of Iberian populations by means of molecular methods. Thirty-two specimens of common gudgeon from several localities placed in six different Iberian and South French basins (Duero, Ebro, Tajo, Nansa, Bidasoa and Nivelle) were analysed. We reconstructed the phylogenetic relationships between the Iberian gudgeon populations using the whole cytochrome *b* gene sequence (1141 bp). Moreover, one specimen of *G. gobio* from the Danube basin and two specimens of *Romanogobio* (*R. ciscaucasicus* and *R. uranoscopus*) were also included in the study. Our data confirmed the monophyly of the Iberian-French populations analysed and indicated a high genetic differentiation with respect to the common gudgeon populations from Central Europe. However, our results also indicated a very close genetic relationship among the populations of different Iberian basins, showing low genetic distances between them. The absence of population structure among the Iberian populations of *G. gobio* seems to be due to human activity.

Key words: cytochrome *b*, gudgeon, mtDNA, Iberian Peninsula

Introduction

The common gudgeon *Gobio gobio* (Linnaeus, 1758) is a Palaearctic species whose distribution area extends throughout most Europe and Central Asia, from the British Isles to the Japan Sea. The occurrence of this species in the northern part of the East Asia is difficult to determine because of taxonomic problems, mainly derived from the morphological plasticity of this species and its subspecies. It still remains to be determined whether the gudgeon occurring there belong to *G. gobio* or to another related species (Bř a n ě r e s c u 1999). The first report in the Iberian Peninsula was from the Duero basin in Spain

(L o z a n o – R e y 1919). It is considered that the first introductions in Spain were associated with the construction of the first rainbow trout hatcheries at the end of the 19th century: La Granja in the Duero river and Monasterio de Piedra in the Ebro river (L o z a n o – R e y 1935), where the common gudgeon was used as the mainly live-food of the cultured rainbow trout. Furthermore, its use as live-bait together with the increase in the sport fishing caused by the construction of the great reservoirs, has contributed to its expansion and rapid colonization of the entire Iberian geographical area over recent decades (D o a d r i o 2003). As a result, it is now found in almost all the main river basins, such as Ebro, Júcar, Turia, Guadalquivir,

Tajo, Duero and Cantabrian basins (D o a d r i o 1986, L o b o n – C e r v i a et al. 1991, D o a d r i o 2003).

However in the Bidasoa basin *G. gobio* was considered to be a native species and it appears listed as a vulnerable species in the Basque Country red-list (BOPV n° 140, 22 July 1996). Moreover, D o a d r i o (2003) considered the *G. gobio* populations of the Ebro and Bidasoa basins as native.

Up to now, only two studies have been carried out on the Iberian gudgeon populations. They were focused in the Duero basin, and both considered the allochthonous character of the gudgeon populations of this basin (C o e l h o 1981, L o b o n – C e r v i a et al. 1991). Based on biometrical studies and statistical analyses, C o e l h o (1981) tried to determine the subspecies of *G. gobio* living in the Portuguese rivers. Comparing her results with those obtained by other authors, C o e l h o (1981) found that all the Iberian gudgeons belonged to the nominal subspecies from Danubian area.

L o b o n – C e r v i a et al. (1991) found several differences between two common gudgeon populations of the Duero basin and between these two populations with regard to another one from the Ebro basin. The differences were based on intraspecific variations in life history, particularly in the growth rate, age and length at first maturity, and egg size. L o b o n – C e r v i a et al. (1991) concluded that these populations expressed a phenotypic plasticity imposed by the effect of contrasting environments.

The present study is the first attempt to clarify the genetic relationships and the status of Iberian common gudgeon populations on the basis of molecular characters. We used complete sequences of cytochrome *b* gene of several populations of common gudgeon from different Spanish basins in order to determine the relationships between Iberian and Danubian populations. The cytochrome *b* gene is widely used for phylogenetic analyses and is considered to be one of the most reliable mitochondrial markers for phylogeny (Z a r d o y a & M e y e r 1996).

Material and Methods

A total of 32 common gudgeon were collected by electrofishing in 22 different localities, 19 from different Iberian rivers, 2 from La Nivelle in France and the other from the Danube basin in the Czech Republic (Fig. 1). Two specimens of the genus *Romanogobio*: *R. uranoscopus* from the Danube basin (Romania) and *R. ciscaucasicus* (GenBank Accession No. AF095607) from the Caucasus in Russia, were also included in the analyses. Three different cyprinid species, *Barbus haasi*, *Pseudorasbora parva* and *Rutilus rutilus* were used as outgroups (GenBank Accession No. AF045976, Y10453, and Y10440, respectively) (Table 1).

Populations were collected in the wild. DNA was extracted from dorsal muscle, which was preserved in liquid nitrogen or 70% ethanol. Voucher specimens for these species were deposited in the collections of the Museo Nacional de Ciencias Naturales, Madrid, Spain. Total DNA extraction was performed according to the phenol/chloroform protocol extraction process.

The entire cytochrome *b* gene (1140 bp) was amplified by polymerase chain reaction (PCR) with the primers GluF 5'ACCACCGTTGTATTCAACTACAA3' and ThrR 5'ACCTCCGATCTTCGGATTACAAGACCG3' (M a c h o r d o m & D o a d r i o 2001). The amplification process was conducted as follows: 94°C (2 min), 35 cycles at 92°C (45 seg), 48°C (1 min 30 seg), 72°C (1min 45 seg), and a final extension at 72°C (3 min). PCR products were cloned using the pGEM-T vector (Promega) into *E. coli* JM109 and sequenced on an automated DNA sequencer (Applied Biosystem INC.). DNA sequences of both strands were obtained using M13 universal (forward and reverse) sequencing primers.

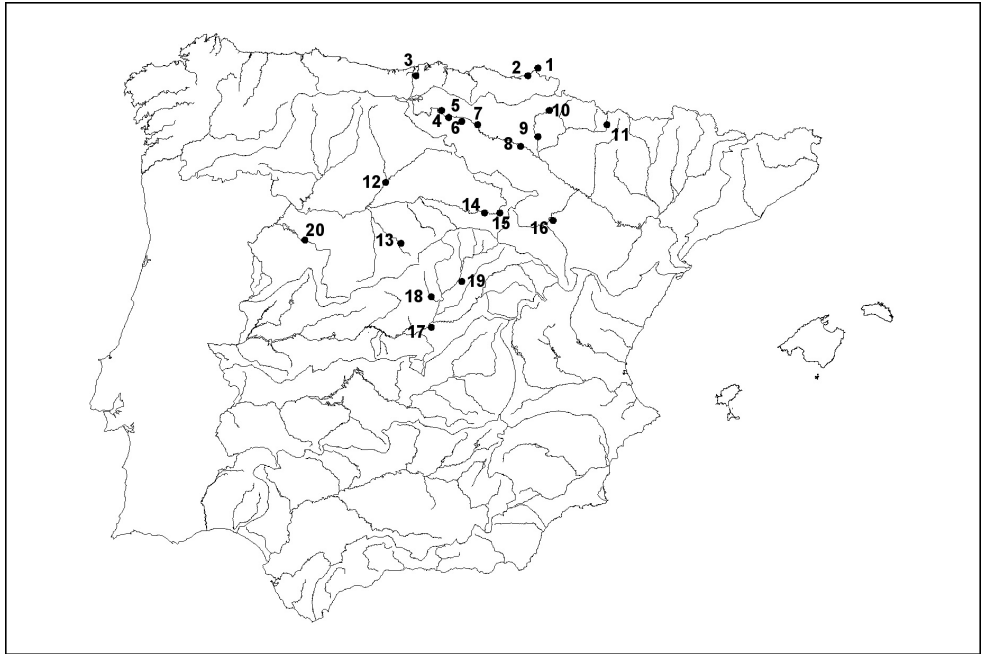


Fig. 1. Distribution of sampling localities and populations analysed. 1, Nivelle River (La Nivelle Basin); 2, Bidasoa River (Cantabrian Basin); 3, Nansa River (Cantabrian Basin); 4, Omecillo River (Ebro Basin); 5, Tumecillo River (Ebro Basin); 6, Zadorra River (Ebro Basin); 7, Ayuda River (Ebro basin); 8, Ega River (Ebro Basin); 9, Arga River (Ebro Basin); 10, Araquil River (Ebro Basin); 11, Esca River (Ebro Basin); 12, Pisuerga River (Duero Basin); Voltoya River (Duero Basin); 14, Izana River (Duero Basin); 15, Araviana River (Duero Basin); 16, Mesa River (Ebro Basin); 17, Cofio River (Tajo Basin); 18, Guadarrama River (Tajo Basin); 19, Lozoya River (Tajo River); 20, Tormes River (Duero Basin).

Phylogenetic analyses

CLUSTAL W version 1.6 (Thompson et al. 1994) was used for multiple alignment. Translation to proteins was also carried out to verify the alignments using the McClade program (Maddison & Maddison 1997). All codons positions were included in the phylogenetic analyses.

Saturation was analysed by plotting the absolute number of transitions and transversions against patristic values. Molecular phylogenetic analyses were performed according to principles of neighbor-joining (NJ), maximum-parsimony (MP), maximum-likelihood (ML), and Bayesian methods of phylogenetic inference (MB). Maximum-parsimony (MP) analyses [Paup* (Swofford 1999)] were performed with TBR branch swapping, MULPARS option, and 10 random stepwise additions using the heuristic search algorithm. For the MP analysis only minimal trees were retained and zero-length branches were collapsed. Different weighting matrices were employed to adjust for transitional saturation. Results were congruent for the different weightings and only results for Ti:Tv of 6:1 are shown. For NJ, ML and Bayesian inference analyses a hierarchical likelihood test (LRT) was performed using the program Model test 3.04 (Posada & Crandall 1998) to find the best model of evolution that fit our data. ML analyses were performed with quartet puzzling (Strimmer & Von Haeseler 1996) as implemented in PAUP* with 5.000 puzzling steps. The robustness of the inferred NJ, and MP trees was tested by bootstrapping (Felsenstein 1985) with 500 and 100 pseudoreplications, respectively. The

Bayesian inference was performed with MrBayes 3.0 (Huelsenbeck & Ronquist 2001) by simulating a markov chain for 1,000,000 cycles. For compilation of the Bayesian consensus topologies a “burning” of 100 trees was used.

Table 1. Specimens analyzed in this study and the sampling localities. ND, not done in this work.

Species and Code	Drainage system	Collection locality	Accession number
<i>Barbus haasi</i>	Ebro	Esca River	AF045976
<i>Rutilus rutilus</i>	Rhone	Saone River	Y10440
<i>Pseudorasbora parva</i>	Rhone	Rhone River	Y10453
<i>Gobio ciscaucasicus</i>	Kuma Basin	Kuma River	AF095607
<i>Gobio uranoscopus</i>	Danube	R. Valsan/Valsanesti (Romania)	AY426593
<i>Gobio gobio gobio</i>	Danube	Czech Republic	AY426592
<i>Gobio gobio</i> 01	ND	–	AF045996
<i>Gobio gobio</i> 02	Ebro	R. Omecillo/Espejo (Spain)	AY426562
<i>Gobio gobio</i> 03	Ebro	R. Tumeillo/Angosto (Spain)	AY426563
<i>Gobio gobio</i> 04	Ebro	R. Zadorra/Manzanos (Spain)	AY426564
<i>Gobio gobio</i> 05	Ebro	R. Ayuda/Berantevilla (Spain)	AY426565
<i>Gobio gobio</i> 06	La Nivelle	R. La Nivelle/Olabidea (Spain)	AY426566
<i>Gobio gobio</i> 07	La Nivelle	R. La Nivelle/Olabidea (Spain)	AY426567
<i>Gobio gobio</i> 08	La Nivelle	R. La Nivelle/Olabidea (Spain)	AY426568
<i>Gobio gobio</i> 10	La Nivelle	R. La Nivelle/Urte-Galea (France)	AY426569
<i>Gobio gobio</i> 11	Duero	R. Tormes/Salamanca (Spain)	AY426570
<i>Gobio gobio</i> 12	La Nivelle	R. La Nivelle/Olha (France)	AY426571
<i>Gobio gobio</i> 13	La Nivelle	R. La Nivelle/Olha (France)	AY426572
<i>Gobio gobio</i> 14	Duero	R. Nansa/Cantabria (Spain)	AY426573
<i>Gobio gobio</i> 15	Bidasoa	R. Bidasoa/Lesaka (Spain)	AY426574
<i>Gobio gobio</i> 16	Bidasoa	R. Bidasoa/Lesaka (Spain)	AY426575
<i>Gobio gobio</i> 17	Ebro	R. Ega/Zuñiga (Spain)	AY426576
<i>Gobio gobio</i> 18	Ebro	R. Ega/Zuñiga (Spain)	AY426577
<i>Gobio gobio</i> 19	Ebro	R. Eska/Burgui (Spain)	AY426578
<i>Gobio gobio</i> 20	Ebro	R. Eska/Burgui (Spain)	AY426579
<i>Gobio gobio</i> 21	Ebro	R. Arakil/Oskia (Spain)	AY426580
<i>Gobio gobio</i> 22	Ebro	R. Arakil/Oskia (Spain)	AY426581
<i>Gobio gobio</i> 23	Tajo	R. Guadarrama/Madrid (Spain)	AY426582
<i>Gobio gobio</i> 24	Tajo	R. Cofio/Madrid (Spain)	AY426583
<i>Gobio gobio</i> 25	Duero	Embalse de Aguilar/Palencia (Spain)	AY426584
<i>Gobio gobio</i> 26	Duero	R. Voltoya/Avila	AY426585
<i>Gobio gobio</i> 27	Ebro	R. Mesa/Zaragoza	AY426586
<i>Gobio gobio</i> 28	Ebro	R. Arga/Quinto Real	AY426587
<i>Gobio gobio</i> 29	Ebro	R. Arga/Quinto Real	AY426588
<i>Gobio gobio</i> 30	Duero	R. Izana/Soria	AY426589
<i>Gobio gobio</i> 31	Duero	R. Araviana/Soria	AY426590
<i>Gobio gobio</i> 32	Duero	R. Voltoya/Avila	AY426591

Results and Discussion

Among 1141 nucleotide sites, 530 were variable and 265 informative for parsimony. According to codon position, the most informative was the third position (217 parsimony informative

characters), followed by the first one (43 characters). Maximum parsimony results were based on a 6:1 Ti/Tv weight, following the empirically determined Ti/Tv ratio. MP analyses resulted in 30 most parsimonious trees of 754 steps with a 6:1 Ti/Tv weighting schemes (Consistence Index=0.747; Retention Index=0.684).

Low sequence divergence was found among the Iberian populations from different basins, being 0% in some cases [*G. gobio* from Arakil River (Ebro basin) and *G. gobio* from La Nivelles basin; *G. gobio* from the Tormes river (Duero basin) and *G. gobio* from the Nansa basin]. The mean percentage sequence divergence within the Iberian group was 0.9% [*G. gobio* from the Voltoya River (Duero basin) and *G. gobio* from the Nansa basin]. The highest sequence divergence among Iberian populations and French populations from La Nivelles basin was 1.2% [*G. gobio* from the Voltoya River (Duero basin) and *G. gobio* from La Nivelles basin]. Iberian-French populations constituted a different and independent group from the population of *G. gobio* analysed from Central Europe (Danube basin; Czech Republic), showing levels of divergence that varied from 5.5% to 5.9%.

The four analyses based on NJ, MP, ML and Bayesian inference yielded similar trees. Thus, the Bayesian tree was selected as representative of the phylogenetic relationship of the populations studied here (Fig. 2). The MP, NJ, ML and Bayesian analyses of the sequence data produced congruent topologies. The phylogeny recovered indicated in all cases a well-supported first dichotomy (bootstrap values 100, 81, and 95% in NJ, MP, and ML respectively), distinguishing the species of the genus *Romanogobio* (*R. ciscaucasicus* and *R. uranoscopus*) and *G. gobio* populations (Fig. 2).

In the first cluster the Iberian populations together with the Atlantic French populations from La Nivelles basin, constituted a unique clade. La Nivelles rises in the Iberian Peninsula and flows into the French-Atlantic Sea. In the same cluster, the sister group of the Iberian-French clade was formed by the specimens of *G. gobio* from the Danube basin (Czech Republic).

The relationships among the different Iberian populations of *G. gobio* were not well resolved, showing a very close genetic relationship between them. Iberian populations did not show a population structure like other freshwater native species from this region (Carmona et al. 2000, Machordom et al. 1995). Many of the specimens analysed from Atlantic basin (Bidasoa basin) were much closer to the Mediterranean specimens from the Ebro basin than to other specimens from the Atlantic basin (Nansa and La Nivelles basins). The mean genetic distance was shown to occur in gudgeon populations from the same basin (Duero basin). It seems to have been due to the human transference of specimens from one basin to another by translocation or by the use of common gudgeon as live bait. Low genetic distances occurring between the specimens from different basins (Ebro basin, Duero basin and Tajo basin) seem to be the consequence of such gudgeon transferences. The Iberian-South French clade is genetically different from Central Europe gudgeon populations. According to these results, analyses of *cyt-b* variation in *G. gobio* confirmed the existence of a phylogeographic discontinuity in genetic structure between Iberian and Central Europe populations. Iberian common gudgeon are genetically different from Central Europe gudgeon populations. Thus, the origin of these populations seems not to be from Central Europe. It could have an endemic origin from this area (Iberian Peninsula). However, an important argument to reject an autochthonous origin of *G. gobio* in the Iberian Peninsula was that Steindachner (1879) did not cite *G. gobio* in the Iberian Peninsula in his works of the 19th century. However, other species that had not being cited by this author, were found later and considered like endemic species from the Iberian Peninsula, such as the different species of the genus *Cobitis* (Bacescu 1962).

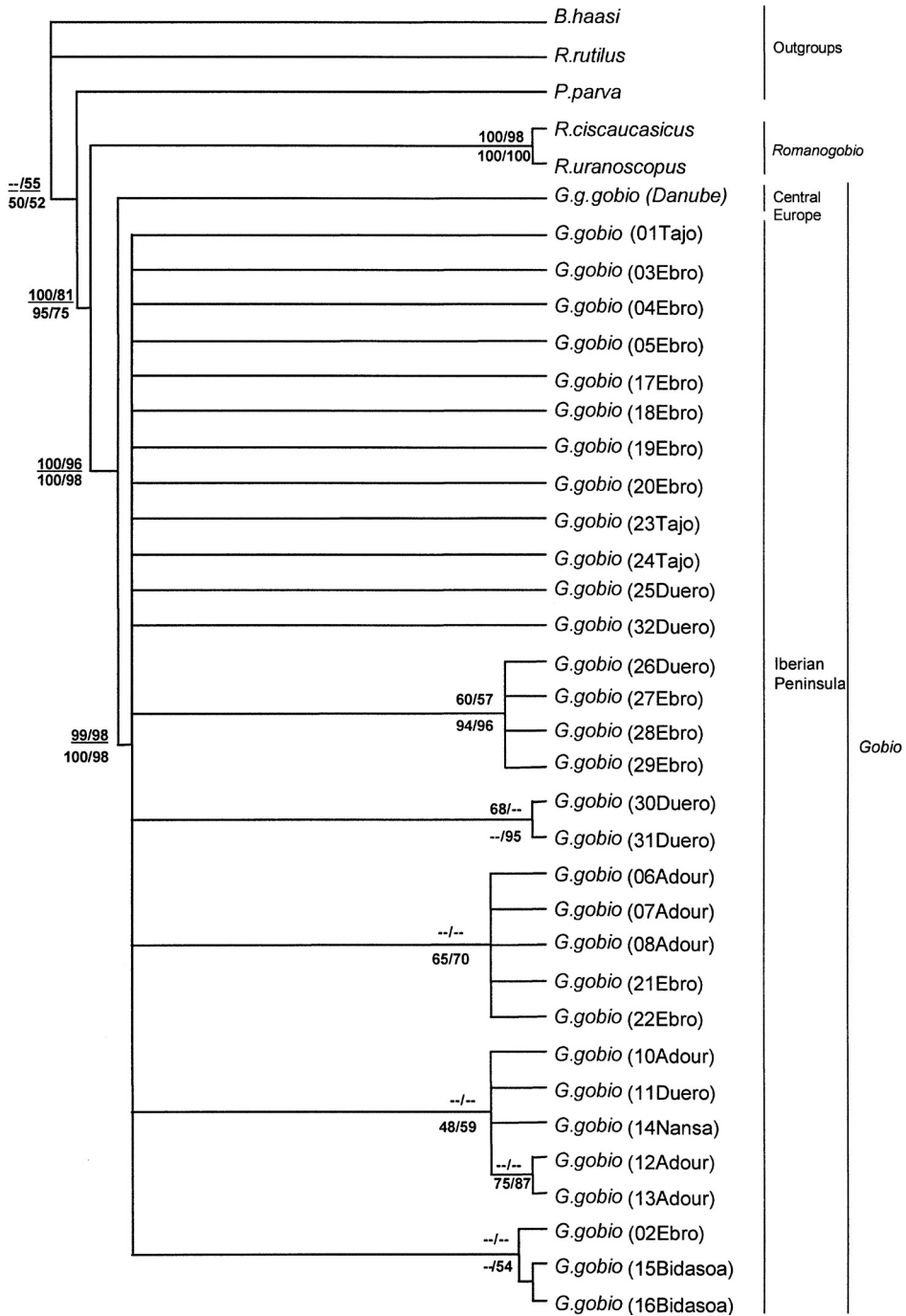


Fig. 2. Bayesian tree showing the phylogenetic relationships among the specimens analyzed, based on cytochrome *b* sequences. NJ/MP bootstrap values (500 and 100 replications, respectively) are indicated above branches: numbers below branches indicate those for ML (from quartet puzzling, 5000 replications) and Bayesian analysis (1,000,000 replicates). When a particular branch was not recovered by a certain method, two hyphens replace the corresponding bootstrap value

On the basis of these results, the possible native origin of Iberian gudgeon populations must be considered mainly due to the high genetic divergence between Iberian-South French and Central Europe populations. Nevertheless, because of the continuous transference of specimens among basins, we cannot conclude where is located the original basin(s) of native populations from which *G.gobio* specimens were translocated to the rest of Iberian basins.

Currently, we have started the analyses of several additional populations of *G.gobio* from North, Central and Southern Europe, including specimens from both Mediterranean and Atlantic drainages. In addition many samples belonging to other European and Caucasian species and subspecies of the genus *Gobio* will be include in the study with the aim to evaluate the phylogenetic relationships of Iberian *Gobio* populations with the rest of European taxa.

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