



Biogeography of Iberian freshwater fishes revisited: the roles of historical versus contemporary constraints

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ABSTRACT

Aim The question of how much of the shared geographical distribution of biota is due to environmental vs. historical constraints remains unanswered. The aim of this paper is to disentangle the contribution of historical vs. contemporary factors to the distribution of freshwater fish species. In addition, it illustrates how quantifying the contribution of each type of factor improves the classification of biogeographical provinces.

Location Iberian Peninsula, south-western Europe (c. 581,000 km²).

Methods We used the most comprehensive data on native fish distributions for the Iberian Peninsula, compiled from Portuguese and Spanish sources on a 20-km grid-cell resolution. Overall, 58 species were analysed after being categorized into three groups according to their ability to disperse through saltwater: (1) species strictly intolerant of saltwater (primary species); (2) species partially tolerant of saltwater, making limited incursions into saltwaters (secondary species); and (3) saltwater-tolerant species that migrate back and forth from sea to freshwaters or have invaded freshwaters recently (peripheral species). Distance-based multivariate analyses were used to test the role of historical (basin formation) vs. contemporary environmental (climate) conditions in explaining current patterns of native fish assemblage composition. Cluster analyses were performed to explore species co-occurrence patterns and redefine biogeographical provinces based on the distributions of fishes.

Results River basin boundaries were better at segregating species composition for all species groups than contemporary climate variables. This historical signal was especially evident for primary and secondary freshwater fishes. Eleven biogeographical provinces were delineated. Basins flowing to the Atlantic Ocean north of the Tagus Basin and those flowing to the Mediterranean Sea north of the Mijares Basin were the most dissimilar group. Primary and secondary freshwater species had higher province fidelity than peripheral species.

Main conclusions The results support the hypothesis that historical factors exert greater constraints on native freshwater fish assemblages in the Iberian Peninsula than do current environmental factors. After examining patterns of assemblage variation across space, as evidenced by the biogeographical provinces, we discuss the likely dispersal and speciation events that underlie these patterns.

Keywords

Assemblage composition, basin boundaries, biogeography, climate, dispersal, environment, freshwater fish, history, Iberian Peninsula.

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INTRODUCTION

A common assumption in ecological research is that organisms sharing the same geographical location also share the same current environmental constraints and at least some of the same evolutionary history. The question is how much of this shared geographical distribution is due to biotic responses to the current environmental conditions vs. historical changes in these conditions. Here, we use distributions of native Iberian freshwater fishes to examine the roles of historical and contemporary factors in determining patterns of fish assemblage composition in the Iberian Peninsula. To our knowledge, this is the first time that both types of factors – historical and contemporary – have been examined on an equal footing in Iberian biogeographical studies (but see Bonada *et al.*, 2005; Araújo *et al.*, 2008).

Barriers to dispersal often constrain distributions of species and strongly influence spatial patterns of assemblage composition. Barriers such as long-term basin boundaries are often considered more relevant to contemporary geographical patterns of freshwater fishes than are current environmental conditions (e.g. Hugueny & Lévêque, 1994; Yap, 2002; Smith & Bermingham, 2005). This view contrasts with that of assemblage organization in terrestrial and marine ecosystems, where current climate is often considered the main determinant of the geographical distributions of species (e.g. Chust *et al.*, 2006; Burgmer *et al.*, 2007).

The constrained dispersal of freshwater fishes among aquatic environments permits strong inferences about the roles of historical vs. contemporary factors, species dispersal and species responses to environmental changes. In particular, we would expect the influence of terrestrial barriers and hydrological connections to differ between species strictly restricted to freshwater and species that might disperse through marine environments, including those with current occasional or frequent incursions into saltwater. The Iberian Peninsula provides an excellent case study for comparing the contributions of historical vs. current environmental factors to spatial patterns of fish assemblages due to the long-term existence of basin divides, many endemic species and a well-known evolutionary history of fishes for the area.

Historical vs. contemporary factors

The contrasting roles of history and the modern environment in structuring biotic assemblages have spurred long debates (e.g. Davis & Scholtz, 2001; Bonada *et al.*, 2005; Svenning & Skov, 2005; Tedesco *et al.*, 2005). There is a growing consensus that both types of factor play important roles, but it is difficult to tease apart their respective contributions, in part because contemporary and historical factors tend to covary (Araújo *et al.*, 2008). Analysing patterns of species assemblages at macroecological scales might shed some light on this debate by revealing the extent to which current species distributions are at equilibrium with their environment and/

or are constrained by historical factors. It is said that species distributions are at equilibrium with their current environment if they occur in all suitable areas and are absent from unsuitable ones (Hutchinson, 1957). One approach to assessing the degree of equilibrium of species with the present environment is to measure patterns of covariation between species assemblages and current environmental factors (see Araújo & Pearson, 2005, and references therein). It assumes that if species are at equilibrium with the environment, then covariation between assemblage composition and environment will be high. In contrast, if the covariation is weak it suggests non-equilibrium with the environment, possibly due to historical and/or ecological factors, such as limited dispersal ability.

Contemporary environmental barriers, such as climate, and historical dispersal barriers, such as drainage boundaries, are likely to have influenced Iberian freshwater fish assemblages in multiple ways. Barriers with low permeability to fish dispersal possibly induced vicariant differentiation between species, and permeability might have increased via new pathways created by environmental changes or through hydrological reconfigurations, such as river capture or river entry into estuaries. In particular, we would expect the influence of barriers to differ between species that are strictly restricted to freshwater and species that are tolerant of salt water if the latter use an additional dispersal pathway through the marine environment.

Indeed, the rise of physical barriers to fish dispersal accompanying the formation of river basins (in the Pliocene–Pleistocene, 2.5–1.8 Ma; Calvo *et al.*, 1993) is expected to rank highly among the key historical events affecting contemporary Iberian freshwater fish distributions. These barriers shaped ‘biogeographical islands’, with drainage reconfigurations occurring only sporadically (Calvo *et al.*, 1993). In addition, it is likely that previous speciation occurred following the formation of Iberian endorheic (closed) basins, which produced a system of inland lakes (in the Upper Miocene, 11–5 Ma; López-Martínez, 1989; Doadrio, 2002; Doadrio & Carmona, 2004; Robalo *et al.*, 2006). Subsequent sporadic events, such as the creation of new dispersal routes due to downstream confluences of adjacent river systems formed during drops in sea level (in the Pleistocene, *c.* 120 ka), might also be relevant (Machordom & Doadrio, 2001; Doadrio & Carmona, 2003; Sousa-Santos *et al.*, 2007).

Biogeographical provinces

Contemporary biogeographical provinces, defined by species assemblages, can be used to explore and understand patterns of similarity of species occurrences and the likely processes of dispersal, speciation and extinction among species and across landscapes. For example, if two provinces harbour distinct or endemic species one could hypothesize that the provinces’ boundaries acted as strong barriers, which might have favoured allopatric speciation. If these provinces also harbour widespread species, one might hypothesize that the provinces’

boundaries became more permeable after speciation. Patterns of freshwater fish distributions have been used to help delineate biogeographical provinces and to examine hypotheses regarding probable speciation events as well as post-speciation dispersal in North America (Hocutt & Wiley, 1986), Lower Mesoamerica (Smith & Bermingham, 2005), South America (Hubert & Renno, 2006), West Africa (Hugueny & Lévêque, 1994), Europe (Reyjol *et al.*, 2007), Asia (Yap, 2002) and across the world (Lévêque *et al.*, 2008). However, the delineation of such provinces has typically been based on species similarities among river basins or stream catchments without previous tests of the relevance of such units of analysis on current species assemblage patterns.

The importance of historical basin delineation as a main driver of fish assemblage composition in the Iberian Peninsula has been reported in several studies. Some authors found concordance between Iberian freshwater species assemblages and basin delineation (Arévalo, 1929; Hernando, 1990; Hernando & Soriguer, 1992; Vargas *et al.*, 1998; Mesquita *et al.*, 2007), whereas others found concordance between species distributions and current latitudinal patterns based on either Cyprinidae or all primary species (Lozano, 1952; Almaça, 1978; Doadrio, 1988). However, the role of current environmental conditions in shaping fish assemblage composition remains largely unexplored. Indeed, previous studies examining the role of contemporary environments have been limited to specific regions of the peninsula (e.g. Carmona *et al.*, 1999; Filipe *et al.*, 2002; Magalhães *et al.*, 2002).

Here, we examine relationships between assemblages of Iberian native freshwater fishes and sets of historical and contemporary environmental variables. In particular, we ask whether dispersal barriers among basins are a more accurate predictor of current patterns of native fish assemblage composition than contemporary environmental factors. Subsequently, we ask whether the identification of the most constraining factor might help us explore and comprehend occurrence patterns across space (Table 1). We first analyse the influence of the geographical structure of river basins vs. current climate on local native species composition. We do this by separating groups of species with distinct tolerance of salinity to assess the effects of marine and terrestrial barriers on fish distribution. Finally, in the light of our findings, we redefine biogeographical provinces and examine occurrence patterns and the permeability of dispersal barriers.

MATERIALS AND METHODS

Study area

The Iberian Peninsula is in south-western Europe and comprises approximately 581,000 km² (Fig. 1). Its climate is varied, including desert, Mediterranean, alpine and Atlantic areas (Rivas-Martínez, 1987). Current environmental conditions produce several types of watercourses, ranging from Mediterranean streams with harsh cycles of flooding and drying to more mesic streams with continuous flow. The geological and faunal evolutionary history of the Iberian Peninsula is complex. Iberian streams have been isolated from Central Europe since the rise of the Pyrenees Mountains during the Tertiary (80–20 Ma), and were only partially covered by ice during the Quaternary glaciations. This is reflected in the large number of species that exist there compared with northern Europe (both endemics and non-endemics), a pattern analogous to other European Mediterranean peninsulas (Griffiths, 2006; Smith & Darwall, 2006).

Data sources

We built the species database by merging information from 1990 to 2008 on freshwater fish distributions from Portuguese and Spanish sources. We excluded non-native species to allow a clearer interpretation of the influences of evolutionary history vs. current environment. Species occurrence records were compiled from three main sources: (1) electrofishing samples compiled for the Portuguese Red Data Book (Rogado *et al.*, 2005); (2) electrofishing samples designed to sample watercourses with limited previous collection efforts by the first author; and (3) data published in the Spanish Atlas and Red Data Book based on a 10-km grid cell (Doadrio, 2002). This grid was used as the unit of analysis for the database since it is the coarsest sampling grain among data sources and it ensures inclusion of at least one electrofishing sample per cell. Data aggregation for each grid cell was performed with the ARCMAP 9.1 summarization tool (ESRI, 2001). The database contains 64 native fish species, based on Iberian specialists' taxonomic synthesis of phylogenetic studies summarized in the Portuguese and Spanish Red Data Books (Doadrio, 2002; Rogado *et al.*, 2005) and on subsequent species descriptions (Doadrio & Elvira, 2007; Doadrio *et al.*, 2007; Gante *et al.*,

Table 1 Mechanisms potentially driving contemporary patterns of fish assemblage composition in the Iberian Peninsula.

Forcing factors	Events	Processes	Predictions
Long-term river basin divides	River basin formation	Allopatric speciation due to impassable barriers	Areas of distinct basins harbour differentiated and endemic species
Current environmental variation	Current climate	Species distributions constrained by physiological limitations	Areas of distinct environments harbour differentiated and endemic species
Recent punctuated hydrological connections	For example, headwater captures, basin mouth connections	Post-speciation dispersal	Closely related species or the same species occur in neighbouring provinces linked by current or historical dispersal routes

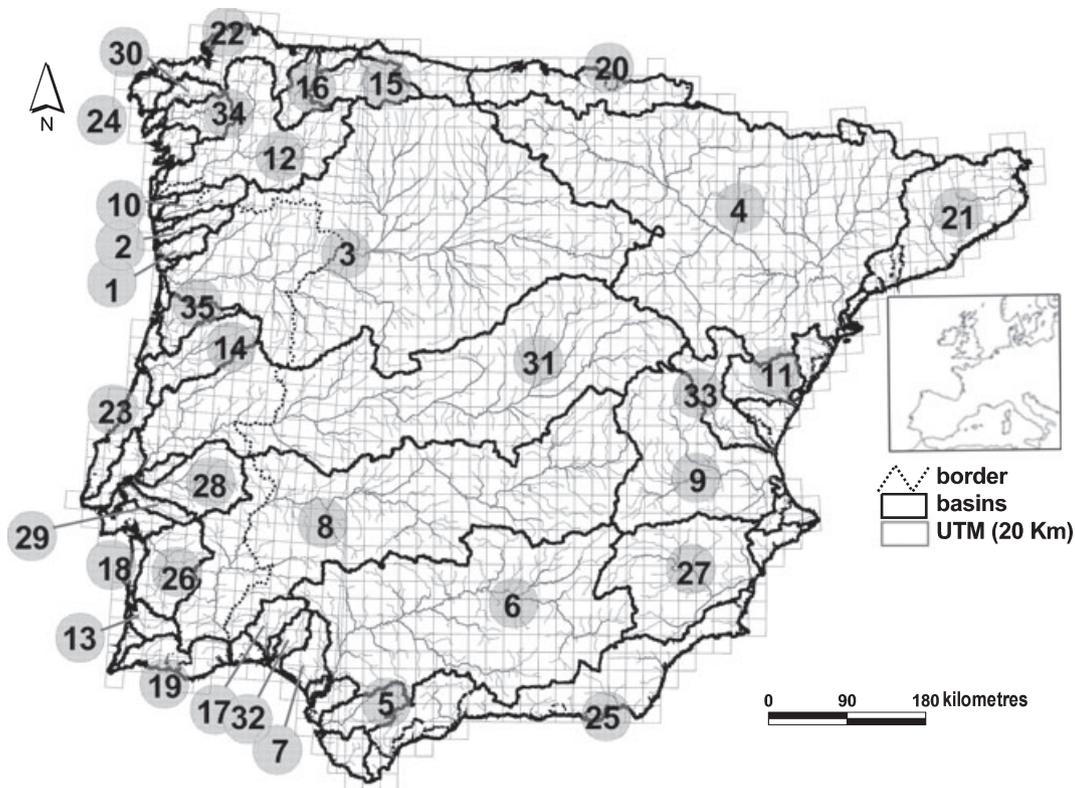


Figure 1 The 35 hydrological basins of the Iberian Peninsula, located in south-western Europe: 1, Ave; 2, Cávado; 3, Douro; 4, Ebro; 5, Guadalete; 6, Guadalquivir; 7, Guadiana; 8, Guadiana; 9, Júcar; 10, Lima; 11, Mijares; 12, Minho; 13, Mira; 14, Mondego; 15, Narcea; 16, Navea; 17, Odiel; 18, Alentejo coastal basins; 19, Algarve coastal basins; 20, Cantabria and Basque coastal basins; 21, Catalunya and northern València coastal basins; 22, Galicia and Asturias coastal basins; 23, Extremadura coastal basins; 24, Northern coastal basins; 25, Southern València, Extremadura and Castilha coastal basins (designated from now on as Medit. basins); 26, Sado; 27, Segura; 28, Sorraia; 29, St. Estevão; 30, Tambre; 31, Tagus; 32, Tinto; 33, Túrria; 34, Uila; 35, Vouga. Main watercourses, 20 × 20 km Universal Transverse Mercator (UTM) grid cells and borders of Portugal and Spain are also represented.

2007). We treated *Lampetra fluviatilis* (Linnaeus, 1758) and *Lampetra planeri* (Bloch, 1784) as a single entity due to the likelihood that they are different ecotypes of a single species (Rogado *et al.*, 2005; Espanhol *et al.*, 2007).

Species occurrence data were validated by the following procedure: (1) recent species records not previously documented in a river basin (i.e. the entire area drained by a river and its tributaries) were identified as possible translocations and excluded, and (2) questionable identifications of specimens, when available, were confirmed at the Natural History Museums of Lisbon and Madrid. Species for which information about their distributions was somehow limited were excluded from our analysis, while rare species were retained to minimize the chances of overlooking distinctive but sparsely represented communities. The 58 species used in our analyses are listed in Appendix S1 in Supporting Information, as are the reasons for excluding the six species previously listed. Since oceans can be a major barrier to fish dispersal, species were classified according to physiological saltwater tolerance (*sensu* Darlington, 1957). Forty species are strictly intolerant of saltwater (i.e. primary species), five can tolerate limited incursions into saltwaters (i.e. secondary) and 13 are saltwater tolerant (i.e. peripheral). The latter species

migrate back and forth from the sea to freshwaters or have invaded freshwaters recently.

Data were resampled in 20-km grid cells to avoid computational limitations that would arise when using smaller grid cells. This procedure also reduced the effects of false species absences and of merging distinct (but not quantified) sampling efforts among collections. The final dataset comprised 1620 grid cells after excluding coastal cells containing less than 50% land (Fig. 1). For convenience, we assumed that observed species absences in grid cells were real. Across all 58 species, occurrences covered 1457 grid cells (i.e. 89.9%).

Climate variables and basin limits were built from digital cartography with free internet access. Climate data included 19 variables related to thermal and hydrological characteristics known to constrain freshwater fish distributions (Matthews, 1998). Climate variables were based on records from 1950 to 2000 at 1-km grid cell resolution (Hijmans *et al.*, 2005, 2007; see Appendix S2). Here, mean values were attributed to each grid cell in ArcMAP 9.1 (ESRI, 2001). To be consistent with current topography, river basin boundaries (as defined previously) were generated based on a 90-m resolution digital terrain model (DTM of CGIAR-CSI, 2005) in ArcMAP 9.1

(ESRI, 2001) using both the Catchment Polygon Processing and Drainage Point Processing tools (ESRI, 2005). The 290 Iberian river basins obtained when using a minimum basin area of 0.4 km² were considered as potentially suitable areas to support freshwater fishes, according to the terrain validation we performed for a subset of basins. Afterwards, the small adjacent basins were merged with the basic geographical tools. The 35 basins obtained were considered an appropriate match for the spatial resolution of the grid cells used (basin area range 1300–97,673 km²; see Fig. 1).

Data analysis

Data analysis was designed to tease apart the relative contributions of historical (i.e. basin delineation) vs. contemporary environmental factors (i.e. climate variables). Because species with different capabilities of dispersing through saltwater may display different levels of association with river basins, analyses were conducted using four matrices of distinct species groups: (1) primary (40 species × 388 samples), (2) secondary (5 × 100), (3) peripheral (13 × 450), and (4) all species (58 × 1457). Since dispersal of organisms is contingent on proximity and the existence of pathways between locations, we expect nearby locations to have more similar species composition than distant ones (Whittaker, 1975; Wilkinson & Edds, 2001). Here we considered that the spatial structure of fish species composition – naturally clumped at this scale – might be a consequence of the ecological processes under investigation, which should not be removed. Using the multivariate nonparametric distance-based methods (Smith *et al.*, 1990; Clarke & Gorley, 2001) commonly used in biogeographical studies (e.g. Stickle & Hughes, 2000; Araújo *et al.*, 2004) allowed us to incorporate the spatial structure of the data and avoid the well-documented statistical problems that occur when using standard statistical tests on spatially autocorrelated data (see Legendre, 1993). The effect of the spatial structure of the data on the results obtained was tested by a second-order spatial autocorrelation analysis based on a geographical distances matrix (Legendre, 1993).

Analysis 1: To distinguish influences of historical vs. contemporary factors

To examine the relative influence of river basin and current climate on patterns of assemblage composition among native fish species, three analytical steps were performed. First, to assess which combination of climatic variables best explains spatial variation in fish assemblages, we applied a heuristic procedure termed BVSTEP (Clarke & Warwick, 1994) to select the ‘best’ subset of environmental variables that maximizes the correlation between each group of species and the environment. This step was performed instead of testing the Iberian climate zones defined by a cluster analysis because it enables us to minimize the possibility that the climatic variables used do not determine the occurrences of species for each species group considered. The procedure starts with the construction of one

pairwise similarity matrix for species occurrences and another similarity matrix for climate variables. Regarding species occurrences, distances between grid cells were calculated with the Bray–Curtis index (Bray & Curtis, 1957) because it minimizes the effect of false absences by ignoring null values shared between assemblages. Regarding climate variables, to calculate climatic differences between grid cells we used Euclidean distances after standardizing variables to zero mean and unit variance. The BVSTEP routine starts by identifying the climate variable most strongly correlated with variation in species assemblages (Spearman’s coefficient), then adding the best remaining variables via forward selection until the significance of the coefficient improves by less than 0.001 (Clarke & Warwick, 1994). In each BVSTEP analysis we performed five restarts, with initial species subsets chosen at random to assess the sensitivity of BVSTEP to the initial conditions. Each run used *c.* 50% of the variables.

Second, to allow statistical comparisons of climate and basin factors, we clustered climate variables previously identified by BVSTEP by forcing the production of 35 climate zones to match the number of river basins. The *k*-means non-hierarchical clustering method used performs an iterative relocation procedure for a given number of clusters, where the objects are moved to minimize within-cluster variability and maximize between-cluster variability until the most significant ANOVA results are obtained. To ensure the consistency and distinctiveness of the clusters obtained, the distance of each sample from the respective cluster centre, as well as the Euclidean distances between clusters, were checked. This method has been considered appropriate to classify environmental data despite the arbitrary divisions it imposes (see Fuller *et al.*, 2007, and references therein).

Finally, to test differences in assemblage composition between climate zones and basins, we used nonparametric analysis of similarity (ANOSIM) for each species matrix. This test operates directly on a pairwise distance matrix without the assumption of multivariate normality (Clarke, 1993). It compares ranked similarities of the matrix between and within groups selected a priori by calculating the global *R* statistic, and performs a randomization routine where the empirical distribution of the global *R* under a null model is generated to estimate significance (Clarke, 1993). Global *R* ranges between –1 and +1, where 0 indicates completely random grouping. Here, climate zones and basins were the groups previously defined and tested independently for significant differences in each species distance matrix. All ANOSIM tests involved 999 random group reassignments.

Analysis 2: To characterize biogeographical provinces

To delineate biogeographical provinces, occurrence data for the 58 species were aggregated by the factor that best explained variation in assemblage composition, i.e. the river basins or climate domains (see Analysis 1), and a 58 species × 35 units of analysis matrix was obtained. Then, a three-step procedure was adopted. First, a dissimilarity matrix between the selected

35 units of analysis was calculated using the Bray–Curtis measure. Hierarchical cluster analysis of the dissimilarity matrix was then performed with the unweighted pair-group method using arithmetic averages (UPGMA; Sneath & Sokal, 1973). Second, we mapped alternative solutions for province delineation by varying the dissimilarity cut-off in the clustering dendrogram. The adopted cut-off was the one that maximized dissimilarity while still preserving spatial contiguity of the clusters (i.e. selecting the maximum number of clusters with adjoining units of analysis). Third, we again used the ANOSIM global *R* statistic, now to test if between-province differences were significantly different from within-province differences (see Analysis 1 for procedure details).

To test how much of the similarity of the delineated provinces is due to spatial contiguity, we tested the second-order spatial autocorrelation by performing partial Mantel tests using three matrices: matrix 1 of geographical distance between the paired units of analysis, where '1' indicates adjoining areas and '2' indicates areas with no common boundary; matrix 2 of biogeographical membership, where '0' was assigned for units of analysis belonging to the same province and '1' otherwise; and matrix 3 of assemblage similarity based on the original 58×35 matrix. The partial Mantel test allowed us to control the effect of the spatial structure of the data by examining the hypothesis that fish similarities (matrix 3) and province membership (matrix 2) are strongly related even when the effect of geographical distance between units of analysis is removed (matrix 1) (Hugueny & L  v  que, 1994; Smith & Bermingham, 2005). A total of 10,000 randomized resampling iterations were generated to estimate significance.

Furthermore, to explore patterns of fish species composition and likely dispersal we quantified average faunal similarities among provinces and identified which fish species contributed the most to that similarity by performing a similarity-breakdown analysis (SIMPER) on the 58×35 species matrix. Also, based on the average of Bray–Curtis similarities, both among assemblages and among species within each province, we calculated: (1) the proportion of area occupied by a species within each province – province constancy, *C* (Clarke & Warwick, 1994); and (2) the ratio of a species' province constancy to its constancy across the entire Iberian Peninsula – province fidelity, *F* (Ibrahim *et al.*, 1972). Constancy varies between 0 and 1, reaching 1 if the species is detected among all units of analysis; a fidelity value < 1 indicates that a species is less widespread within the province than across the Iberian Peninsula overall, whereas a value of > 1 indicates that a species is more widespread in the considered province than across the peninsula overall (for a similar approach see Newall & Magnuson, 1999; Wells *et al.*, 2002).

For hierarchical cluster analyses, ANOSIM, BVSTEP and SIMPER routines we used PRIMER version 5.2.0 software (Clarke & Gorley, 2001); for non-hierarchical cluster analysis we used SPSS version 15.0 (SPSS Inc., 2006). Partial Mantel tests were performed using ZT version 1.0 software (Bonnet & Van de Peer, 2002).

RESULTS

Historical vs. contemporary factors

When examining fish distributions with regard to current climate, we found that variables reflecting extreme climatic seasonality correlated significantly with assemblage composition, especially for primary and secondary species (Table 2). For primary species, precipitation and temperature summer extremes, winter temperature and precipitation seasonality were strong significant correlates of species occurrence, while for secondary species, winter temperature and precipitation showed a stronger correlation. For peripheral species, annual and summer precipitations were the strongest correlates, and the climate zones obtained were less spatially clustered than the ones obtained for the remnant species groups (for maps of the climate zones see Appendix S3).

When analysing species composition with regard to current climate and basin delineation we found that both sets of variables were significantly associated with the four groups of species considered (Table 3). The degree of association with current climate and with basin divides was greater for primary species (do not disperse through saltwater) and for all species combined (i.e. greater ANOSIM global *R* statistics). Assemblages of secondary species (occasionally disperse through saltwater) were less discriminated by both climate and basin

Table 2 Environmental variables selected by BVSTEP (i.e. variables that maximize correlation with species assemblage variation at $P < 0.001$).

Variables	Species groups			
	Primary	Secondary	Peripheral	All
Mean temperature of wettest quarter	1	1		1
Mean temperature of driest quarter	2			2
Precipitation of driest month	3			3
Precipitation seasonality	4			4
Precipitation of driest quarter	5			5
Precipitation of warmest quarter	6		2	6
Mean temperature of coldest quarter		2		
Precipitation of wettest month		3		
Precipitation of coldest quarter		4		
Annual precipitation			1	
Correlation coefficient (r_s)	0.519	0.481	0.073	0.474

Cell numbers represent variable rankings (starting at the first variable selected). For species groups and variable details see Appendices S1 and S2.

Table 3 One-way ANOSIM global R statistics for species composition. Climatic zones and basin memberships were used (independently) as factors.

Species groups	Current climate	Basins
Primary	0.578**	0.802**
Secondary	0.306**	0.628**
Peripheral	0.096**	0.124*
All	0.559**	0.807**

For species details see Appendix S1.

Significant at: * $P < 0.05$, ** $P < 0.001$.

divides. Both factors discriminated peripheral species assemblages (live mostly in saltwater) even less well. Comparing both factors tested, basin divides were consistently better than current climate at segregating species composition for all species groups.

For all species combined, both results on climate variables selected and on the degree of association with climate and basin divides closely resemble those for the primary species group, which has the highest number of species (Tables 2 and 3).

Biogeographical provinces of freshwater fishes

Given that river basin delineation was strongly associated with fish assemblages of all species groups, river basins were used as units of analysis to delineate biogeographical provinces. In the river basins' dendrogram, spatial contiguity of basins was lost for more than 11 clusters, corresponding to the cut-off value of 47% Bray–Curtis dissimilarity (Fig. 2). These 11 provinces differed significantly in species composition (ANOSIM global

$R = 0.778$, $P < 0.001$). Atlantic basins north of the Tagus and Mediterranean basins north of the Mijares were the most dissimilar group of basins (named the North-west, Tambre, Navea-Narcea, North, and North-east provinces; Figs 2 & 3). The Mediterranean small coastal basins were the next most dissimilar (provinces of Túrria-Mijares and Central-east; Figs 2 & 3). Three of the 11 provinces comprised solely a small coastal basin: the Tambre, Mira and North basins. Among these, the Mira Basin was the most dissimilar, being defined as a province when only four clusters were recognized.

Interbasin similarity in fish assemblages and province membership was significantly related even after removing the effect of geographical distance (partial Mantel test, $R = 0.411$, $P = 0.0001$). This pattern indicates that the delineation of biogeographical provinces is robust even when the second-order spatial autocorrelation is removed.

Regarding the provinces' ichthyofaunal characterization, average similarity among provinces for all species occurrences ranged between 94.12 in the Túrria-Mijares Province and 63.84 in the Central-east Province (Table 4). The maximum contribution of a single species to those values was 33.33% in the Tinto-Odiel Province, which also had the fewest species contributing to the average similarity (three species contributed 80% of the average similarity); the North-east Province had the most contributing species (13 species contributed 80% of the average similarity). Overall, peripheral species contributed most to the average similarity within provinces and across the Iberian Peninsula. Accordingly, primary and secondary species occurred more frequently within a province than across the entire Iberian Peninsula (i.e. they had higher values of fidelity; Table 4). Species with the least province fidelity across the Iberian Peninsula, which occurred in almost all provinces,

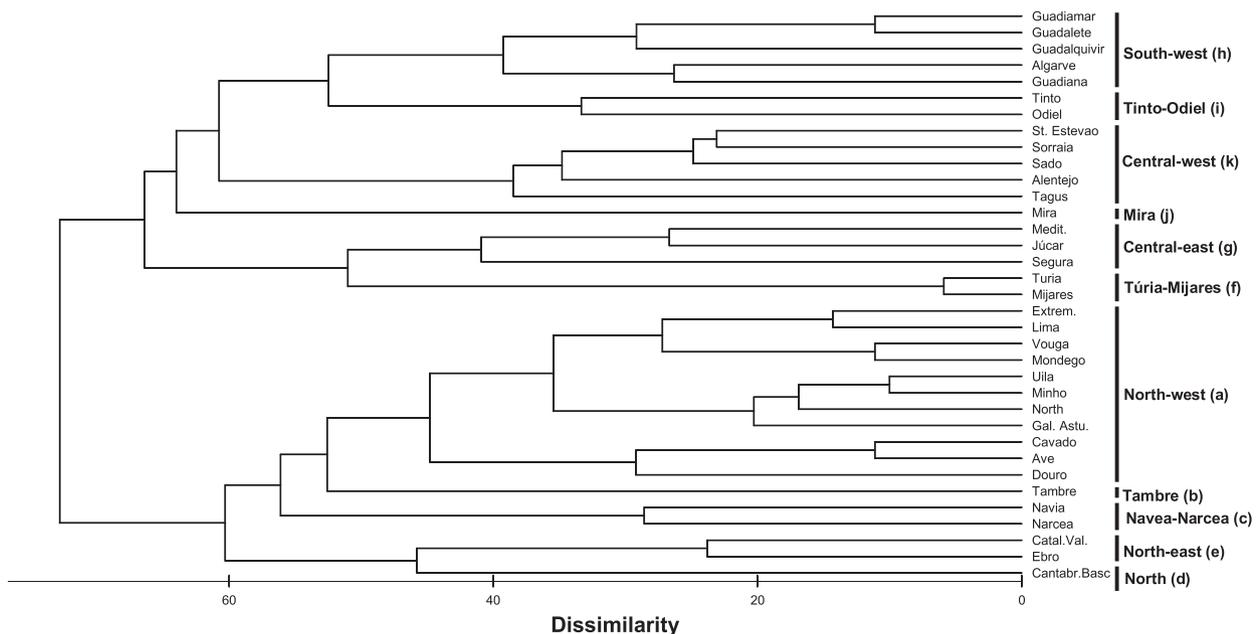


Figure 2 Dendrogram of the overall dissimilarity for the 58 species of freshwater fish among the 35 basins built by the unweighted pair-group method using arithmetic averages (UPGMA) based on the Bray–Curtis dissimilarity square matrix. The axis represents Bray–Curtis dissimilarity values; province membership of each basin is indicated by its name and code.



Figure 3 The 11 biogeographical provinces delimited by overall similarity of freshwater fish assemblages among basins and preserving geographical contiguity: a, North-west; b, Tambre; c, Navea-Narcea; d, North; e, North-east; f, Túrria-Mijares; g, Central-east; h, South-west; i, Tinto-Odiel; j, Mira; k, Central-west.

were *Anguilla anguilla* (Linnaeus, 1758), *Cobitis paludica* (de Buen, 1930) and *Achondrostoma arcasii* (Steindachner, 1866). The most widespread species ($C > 0.6$) were *A. anguilla*, *C. paludica* and *Petromyzon marinus* Linnaeus, 1758. The 22 least frequently occurring species ($C < 0.1$) were primary, except the anadromous *Acipenser sturio* Linnaeus, 1758 and the secondary *Cottus gobio* Linnaeus, 1758. There were 18 species unique to a province (31.0% of all species considered). The South-west Province contains the greatest number (six) of these endemic species (i.e. 23.1% of Iberian species), suggesting that basin boundaries have especially constrained freshwater fish assemblages in this region.

DISCUSSION

River basin boundaries emerge as the main determinant of Iberian fish distributions when tested against current climate, especially for species restricted to freshwaters (primary). The results point to a non-equilibrium state of assemblages with contemporary environmental conditions, contingent on a species' ability to disperse through time via hydrological connections. The primacy of historical factors over the current environment also applies to European amphibians and reptiles (Araújo *et al.*, 2008), but was not observed for freshwater caddisflies of the south-eastern Iberian Peninsula, which might reflect the high dispersal capacity of caddisflies or the possible confounding effects of historical and environmental variables (Bonada *et al.*, 2005).

Also, by using data at a grid-cell scale, we clearly show that river basin boundaries are appropriate units of analysis in biogeographical studies of freshwater fishes, at least more than current climate. River basins have been adopted as units of analysis in previous studies, but without being properly tested with finer-resolution datasets against other alternatives (e.g. Hugué, 1990).

The diminished role of river basin boundaries in determining distributions of peripheral species, thriving mainly in saltwater, relative to primary and secondary species, is probably due to the dispersal of fish along coastlines, which decreases the isolation of populations. Indeed a reduced genetic differentiation of peripheral species between basins has been recorded (Lucas *et al.*, 2001), which together with our results is evidence for the likely relevance of dispersion processes through saltwaters. On the other hand, our results show that distributions of secondary species seem more shaped by basin boundaries than those of peripheral species, which suggests that secondary species are less able to disperse through coastal seas, despite their tolerance of salinity. Additional support for this hypothesis comes from our results on the lower constancy of secondary species across the Iberian Peninsula. Similar patterns have been described in other regions of the world (Hugué, 1990; Smith & Bermingham, 2005).

Quantifying the relevance of historical factors such as river basin boundaries provided new insights into the spatial patterns of Iberian freshwater fishes. Our analysis of similarity in species composition among basins and the delineation of biogeographical provinces provided useful information on likely speciation and dispersal mechanisms. In particular, basin boundaries seem to be responsible for the high rates of Iberian endemism observed for primary and secondary species, which is not surprising since basin boundaries can limit dispersal, even for some terrestrial animals (e.g. Lobo *et al.*, 2006). In provinces currently harbouring many endemic species, it is likely that multiple additional factors (such as past climate and/or local isolation processes) acted synergistically and allowed them to become centres of speciation (see the molecular studies of Zardoya & Doadrio, 1999; Mesquita *et al.*, 2007). For example, in the South-west Province, distinct speciation events probably occurred for *Aphanius baeticus* (Doadrio, Carmona &

Table 4 SIMPER analysis for each province.

North-west (a)						
S	26					
Sunique	3					
N	11					
AvSim	64.53					
Species	C	F	AvSim	%	Cum%	
<i>Anguilla anguilla</i>	1.00	1.06	8.30	12.86	12.86	
<i>Platichthys flesus</i>	1.00	1.85	8.30	12.86	25.72	
<i>Squalius caroliteritii</i>	0.91	3.14	6.52	10.11	35.83	
<i>Pseudochondrostoma duriense</i>	0.82	2.41	5.57	8.63	44.47	
<i>Petromyzon marinus</i>	0.82	1.24	5.33	8.25	52.72	
<i>Cobitis paludica</i>	0.73	0.95	4.22	6.54	59.25	
<i>Achondrostoma oligolepis</i>	0.73	2.81	4.06	6.30	65.55	
<i>Barbus bocagei</i>	0.73	2.15	4.06	6.30	71.85	
<i>Gasterosteus gymmurus</i>	0.73	1.70	4.04	6.25	78.10	
<i>Achondrostoma arcasii</i>	0.64	1.39	3.41	5.28	83.30	
North-east (e)						
S	26					
Sunique	2					
N	2					
AvSim	76.19					
Species	C	F	AvSim	%	Cum%	
<i>Phoxinus phoxinus</i>	1.00	5.00	4.76	6.25	6.25	
<i>Squalius laietanus</i>	1.00	16.67	4.76	6.25	12.50	
<i>Barbus graellsii</i>	1.00	11.11	4.76	6.25	18.75	
<i>Barbus haasi</i>	1.00	9.09	4.76	6.25	25.00	
<i>Salarias fluviatilis</i>	1.00	5.88	4.76	6.25	31.25	
<i>Valencia hispanica</i>	1.00	11.11	4.76	6.25	37.50	
<i>Cobitis paludica</i>	1.00	1.30	4.76	6.25	43.75	
<i>Achondrostoma arcasii</i>	1.00	2.17	4.76	6.25	50.00	
<i>Anguilla anguilla</i>	1.00	1.06	4.76	6.25	56.25	
<i>Atherina boyeri</i>	1.00	1.96	4.76	6.25	62.50	
<i>Alosa fallax</i>	1.00	2.50	4.76	6.25	68.75	
<i>Gasterosteus gymmurus</i>	1.00	2.33	4.76	6.25	75.00	
<i>Liza ramada</i>	1.00	2.94	4.76	6.25	81.25	
South-west (h)						
S	26					
Sunique	6					
N	5					
AvSim	66.88					
Species	C	F	AvSim	%	Cum%	
<i>Pseudochondrostoma willkommii</i>	1.00	5.00	7.36	11.00	11.00	
<i>Squalius pyrenaicus</i>	1.00	1.96	7.36	11.00	22.00	
<i>Barbus sclateri</i>	1.00	3.23	7.36	11.00	33.00	
<i>Iberochondrostoma lemmingii</i>	1.00	3.85	7.36	11.00	44.00	
<i>Cobitis paludica</i>	1.00	1.30	7.36	11.00	55.00	
<i>Anguilla anguilla</i>	1.00	1.06	7.36	11.00	66.00	
<i>Petromyzon marinus</i>	1.00	1.52	7.36	11.00	77.00	
<i>Aphanius baeticus</i>	0.80	7.27	4.67	6.98	83.98	

Table 4 Continued.

Central-east (g)						
S	19					
Sunique	2					
N	3					
AvSim	63.84					
Species	C	F	AvSim	%	Cum%	
<i>Squalius pyrenaicus</i>	1.00	1.96	8.28	12.97	12.97	
<i>Barbus guiraonis</i>	1.00	5.00	8.28	12.97	25.95	
<i>Anguilla anguilla</i>	1.00	1.06	8.28	12.97	38.92	
<i>Atherina boyeri</i>	1.00	1.96	8.28	12.97	51.90	
<i>Syngnathus abaster</i>	1.00	9.09	8.28	12.97	64.87	
<i>Aphanius iberus</i>	1.00	5.88	8.28	12.97	77.85	
<i>Barbus sclateri</i>	0.67	2.16	3.03	4.75	82.59	
Túria-Mijares (f)						
S	9					
Sunique	1					
N	2					
AvSim	94.12					
Species	C	F	AvSim	%	Cum%	
<i>Parachondrostoma turiense</i>	1.00	16.67	11.76	12.50	12.50	
<i>Squalius pyrenaicus</i>	1.00	1.96	11.76	12.50	25.00	
<i>Squalius valentinus</i>	1.00	7.14	11.76	12.50	37.50	
<i>Barbus guiraonis</i>	1.00	5.00	11.76	12.50	50.00	
<i>Barbus haasi</i>	1.00	9.09	11.76	12.50	62.50	
<i>Cobitis paludica</i>	1.00	1.30	11.76	12.50	75.00	
<i>Achondrostoma arcasii</i>	1.00	2.17	11.76	12.50	87.50	
Navea-Narcea (c)						
S	9					
Sunique	0					
N	2					
AvSim	71.43					
Species	C	F	AvSim	%	Cum%	
<i>Phoxinus phoxinus</i>	1.00	5.00	14.29	20.00	20.00	
<i>Pseudochondrostoma duriense</i>	1.00	2.94	14.29	20.00	40.00	
<i>Alosa alosa</i>	1.00	2.50	14.29	20.00	60.00	
<i>Anguilla anguilla</i>	1.00	1.06	14.29	20.00	80.00	
Tinto-Odiel (i)						
S	6					
Sunique	0					
N	2					
Similarity	66.67					
Species	C	F	AvSim	%	Cum%	
<i>Squalius pyrenaicus</i>	1.00	1.96	22.22	33.33	33.33	
<i>Barbus sclateri</i>	1.00	3.23	22.22	33.33	66.67	
<i>Iberochondrostoma lemmingii</i>	1.00	3.85	22.22	33.33	100.00	

Table 4 Continued.

Central-west (k)					
S	28				
Sunique	1				
N	5				
Similarity	66.21				
Species	C	F	AvSim	%	Cum%
<i>Pseudochondrostoma polylepis</i>	1.00	5.00	7.01	10.59	10.59
<i>Squalius alburnoides</i> complex	1.00	2.70	7.01	10.59	21.18
<i>Squalius pyrenaicus</i>	1.00	1.96	7.01	10.59	31.76
<i>Iberochondrostoma lusitanicum</i>	1.00	7.14	7.01	10.59	42.35
<i>Cobitis paludica</i>	1.00	1.30	7.01	10.59	52.94
<i>Anguilla anguilla</i>	1.00	1.06	7.01	10.59	63.53
<i>Barbus bocagei</i>	0.80	2.35	4.17	6.30	69.83
<i>Lampetra</i> (<i>L. fluviatilis</i> and <i>L. planeri</i>)	0.80	4.00	4.17	6.30	76.12
<i>Petromyzon marinus</i>	0.80	1.21	3.93	5.93	82.06

For each province, species richness (S), number of species restricted to the province (Sunique), number of basins analysed (N) and AvSim are presented. Three provinces were not subjected to SIMPER since they comprised only one basin. Species contributing up to 80% of the species assemblage similarity for each province are listed. Species constancy (C), fidelity (F), average similarity (AvSim) and its percentage (%) and cumulative percentage (Cum%) are also shown. For species details see Appendix S1.

Fernández-Delgado, 2002), *Anaocypris hispanica* (Steindachner, 1866), *Iberochondrostoma oretanum* (Doadrio & Carmona, 2003), *Squalius aradensis* (Coelho, Bogutskaya, Rodrigues & Collares-Pereira, 1998), and *Squalius palaciosi* (Doadrio, 1980). These species are thought to have evolved via allopatric speciation from widespread species not particularly constrained by dispersal barriers.

Basin boundaries seem to have exhibited punctuated permeability through: (1) intermittent connections of freshwaters due to hydrological reorganization associated with climate change and/or orogeny events (e.g. river capture); or (2) river-mouth freshwater pathways, most probably formed during the lowering of sea levels (e.g. during the Messinian salinity crisis). Recent phylogenetic studies in other regions mention the biogeographical importance of past rearrangements of drainage networks (Waters & Wallis, 2000; McGlashan & Hughes, 2001; Burrige *et al.*, 2007). For the Iberian Peninsula, genetic studies are shedding light on the role of punctuated connectivity among basins, such as river captures and other hydrological rearrangements (see Mesquita *et al.*, 2005; Sousa-Santos *et al.*, 2007), and downstream confluences of adjacent river systems due to sea-level fluctuations (see Mesquita *et al.*, 2007). Province distinctiveness may also reflect remnant influences of the historical formation of endorheic basin divides prior to current basin boundaries. For example, the Central-west Province was previously partially occupied by the Tagus-Sado Endorheic Basin (Calvo *et al.*, 1993), which may still account for some similarity among current species assemblages (see Carmona *et al.*, 2002). It is clear from these considerations that it will be instructive to assess the influence of other historical factors to better understand the biogeographical processes at finer spatial scales.

The 11 provinces defined here represent the first biogeographical approach for the Iberian Peninsula that considers

both primary and non-primary fish species, and that incorporates recent taxonomic advances. These provinces reflect an evident distinctiveness of fish assemblages in some small coastal basins and strong latitudinal and Atlantic–Mediterranean gradients, both prominent in previous studies based on Cyprinidae or all primary fishes (Arévalo, 1929; Lozano, 1952; Almaça, 1978; Doadrio, 1988; Hernando, 1990; Hernando & Soriguer, 1992; Soriguer *et al.*, 2005) and those based on both primary fishes and amphibians (Vargas *et al.*, 1998). Our provinces are also similar to previous Iberian regionalizations based on non-fish fauna (e.g. García-Barros *et al.*, 2002), but are distinct from provinces delineated for pteridophyte flora (e.g. Márquez *et al.*, 2001). Given that biogeographical provinces are not necessarily similar for different groups of organisms (see Rabeni & Doisy, 2000; Abell *et al.*, 2008), we recommend developing regions independently for distinctive taxonomic groups rather than assuming that one group adequately represents biogeographical patterns for all biota (see Procheş, 2005; Rodrigues & Brooks, 2007).

Our results show that it is not necessary to invoke contemporary environmental variables to understand current patterns of assemblage variation of Iberian freshwater fish. However, this does not imply that climate is not important or that other factors, such as direct human interference, do not constrain species distributions at local or regional levels. The fact that isolation emerged as a major constraint on the composition of fish assemblages could be interpreted as a sign that global environmental changes might not be important in shaping the future distributions of species. This clearly would be a misleading interpretation. First, there is no evidence that climate does not affect local distributions of species. Our results show that different sets of species occur in different river basins, highlighting the relevance of terrestrial barriers to dispersal, but this does not disprove the hypothesis that

climate constrains the distributions of species, and the probable great impacts of global environmental changes on such fauna limited to aquatic environments for dispersal and persistence. Indeed, the correlation of distinct climate variables with distinct species groups points to the necessity of exploring the ecological processes involved, namely during past climatic changes (see Tedesco *et al.*, 2005). A strong test of the hypothesis that climate constrains the distributions of species would involve laboratory or ecosystem experiments using translocations of species from one river basin to another, although this latter option might have potentially large impacts and be unsuitable on practical and ethical grounds. Second, environmental changes are not limited to the direct effects of climate on species distributions. In particular, the long history of human settlement in the Iberian Peninsula has altered landscapes, created anthropogenic barriers such as dams and reservoirs, caused translocations of fish between watersheds, and consequently led to local declines of native species and increased distribution and abundance of non-native species (see Clavero & García-Berthou, 2006).

The current global rate of homogenization of fish faunas (Olden, 2006) suggests that environmental effects will eventually overwhelm the effects of historical processes, and that biological interactions with non-native species may strongly influence distributions of native fishes (Hoeinghaus *et al.*, 2007). This scenario might be exacerbated by the massive impacts on fish distributions predicted by Portugal's plan to build 10 new hydroelectric dams (Paterson *et al.*, 2008). These are topics for future research (e.g. reanalysing our dataset under the assumption that all rare species go extinct) since a proper understanding of the role of these factors can be useful for predicting and mitigating the effects on biological assemblages. However, despite the future likelihood of pervasive anthropogenic change, our main conclusion remains intact; i.e. isolation of basins in space and time provides a parsimonious explanation for today's geographical patterns of variation in freshwater fish assemblages.

Here, we point out as a promising topic for future research the relative importance of possible dispersal pathways created by climate-driven sea-level fluctuations in the past as a model for understanding the dispersal consequences of sea-level fluctuations associated with current climate change. In addition, explicitly linking our approach to phylogeographical data might provide missing historical information that would help to understand current regional patterns of assemblage composition (Brown & Lomolino, 2000). As a way to strengthen hypothesis testing, additional efforts to quantify uncertainties should be made, such as the adequacy of typical fish sampling efforts (particularly concerning rare species; see Cao *et al.*, 2002) and the influence of using land-based grid cells for understanding patterns of freshwater biodiversity.

CONCLUSIONS

The long-term basin boundaries of the Iberian Peninsula (since the Pliocene–Pleistocene) represent major constraints on

current fish assemblage composition. In other words, current climate does not need to be invoked to explain existing biogeographical patterns. As expected, peripheral freshwater species, which frequently inhabit saltwater, are less constrained by basin divides than primary or secondary species. Our analyses also revealed that permeability varies considerably among river basins, most likely due to intermittent basin connectivity in the past. Further investigation is needed to clarify the patterns of basin permeability. We also showed that basins are appropriate units of analysis for delimiting biogeographical provinces for Iberian fishes, since basins integrate the shared evolutionary history and current hydrological connectivity of freshwater assemblages.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Freshwater fish species analysed for the Iberian Peninsula and classified according to Darlington (1957).

Appendix S2 Contemporary climate variables examined as potential correlates of fish distributions.

Appendix S3 Contemporary climate zones (of 35 predefined clusters) that maximize the correlation with occurrences of the four species groups.

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BIOSKETCH

This study is part of **Ana Filipa Filipe**'s PhD project, focused on understanding the patterns and processes of freshwater fish distribution and diversity in the Iberian Peninsula (<http://ffishgul.fc.ul.pt>). Her main research interests encompass macroecology, in particular freshwater biodiversity and its relation with the environment, past events and anthropogenic stressors.

Author contributions: A.F.F., M.B.A., P.L.A. and M.J.C-P. conceived the ideas; A.F.F. and I.D. gathered the data; A.F.F. produced the database; A.F.F. and M.B.A. analysed the data; A.F.F. led the writing with inputs from the remaining authors.

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