ORIGINAL ARTICLE

Freshwater Biology WILEY

Phylogenetic signal and evolutionary relationships among traits of inland fishes along elevational and longitudinal gradients

Carlos Cano-Barbacil[1] | Johannes Radinger[1,2] | Gaël Grenouillet[3] | Emili García-Berthou[1]

[1]GRECO, Institute of Aquatic Ecology, University of Girona, Girona, Spain

[2]Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany

[3]Laboratoire Evolution et Diversité Biologique (EDB), UMR5174, Université de Toulouse, CNRS, IRD, Université Toulouse III - Paul Sabatier, Toulouse, France

Correspondence
Carlos Cano-Barbacil, GRECO, Institute of Aquatic Ecology, University of Girona, Maria Aurèlia Capmany, 69, 17003 Girona, Spain.
Email: carlos.cano@udg.edu

Abstract

1. Understanding the main drivers of species distributions is one of the main goals of ecology. However, the relationships between traits and elevational and longitudinal distributions in inland fishes, as well as their underlying evolutionary processes, have been less investigated. Thus, we aimed to quantify and assess the relationships among several types of traits resulting from species' evolutionary histories by measuring their phylogenetic signal across inland fishes of the Iberian Peninsula. We also aimed to test for correlated evolution of these traits with elevation and stream size (i.e. stream order), to test whether a species' suite of traits and their elevational and longitudinal niche tend to evolve together.

2. We compiled data on 23 fish biological and ecological traits for 30 inland fish species present in the Iberian Peninsula. We quantified their phylogenetic signal using four complementary indices (Pagel's λ, Blomberg's K, and Abouheif's C_{mean} for continuous and $-D + 1$ for binary traits). We used both phylogenetic and non-phylogenetic methods to evaluate the relationship among traits and their relationship with elevation and stream order.

3. We found a significant phylogenetic signal for 65% of the studied traits. Phylogenetic signals were quite variable, but we did not detect clear differences between continuous and binary traits or among trait types (i.e. morphological, trophic, reproductive, and habitat use). Evolutionary models revealed that elevational and longitudinal distribution showed little evidence for directional trends of evolution. Hence, species elevational and longitudinal niches tend to resemble to those of the common ancestor.

4. Many fish traits were inter-correlated as revealed by phylogenetic methods, indicating correlated evolution of pairs of traits. For example, reproductive traits such as maximum longevity, fecundity and age at maturity tended to evolve together with fish maximum length. Consequently, certain orders of fish showed shared suites of traits.

5. Comparative methods revealed a significant positive relationship of parental care with elevation and stream order. By contrast, non-phylogenetic analyses and multivariate analyses indicated positive relationships between elevation and rheophily and lithophily, and a negative relationship between stream order and invertivory and rheophily.

6. Overall, our results point to a notable phylogenetic signal in many traits of inland fishes and a strong phylogenetic structure in their functional traits along their elevational and longitudinal gradients. Thereby, our results contribute to an improved understanding of species' adaptations to environmental changes with important conceptual and practical implications for minimising further species losses.

1 | INTRODUCTION

Understanding the main drivers of species distribution patterns and the mechanisms of coexistence is the central goal of ecology. Competition for resources and other ecological interactions often lead to the divergence of clades into multiple niches and the appearance of novel traits (Gilbert et al., 2018; Rüber et al., 1999). Over the course of evolution, some taxonomic groups accumulate morphological and ecological variation among their constituent species, others produce more similar species, and others can show parallel evolution (Rüber et al., 1999; Sidlauskas, 2008). However, closely related taxa tend to show similarities in many characteristics, including morphological, trophic, reproductive, behavioural, or ecological traits, due to common ancestry (Harvey & Pagel, 1991; Kamilar & Cooper, 2013). This phylogenetic relatedness can be measured by the *phylogenetic signal* (PS), defined by Blomberg and Garland (2002) as the "tendency for related species to resemble each other more than they resemble species drawn at random from the tree". Previous studies found that the PS varies substantially across trait types (Blomberg et al., 2003a; Freckleton et al., 2002; Kamilar & Cooper, 2013). Most but not all traits display significant PS, which tends to be strongest in morphological traits such as body size, intermediate in life-history and physiological traits, and low in behavioural traits (Blomberg et al., 2003a; Kamilar & Cooper, 2013). For instance, in primates, dietary traits and climatic niche were among the variables with lowest PS (Kamilar & Cooper, 2013). Comte et al. (2014) found that fish traits related to morphological attributes and trophic position showed stronger phylogenetic clustering than other reproductive and habitat use characteristics.

The retention of niche-related ecological traits over time, causing that closely related species are more ecologically similar than would be expected based on their phylogenetic relationships (Losos, 2008), is termed *phylogenetic niche conservatism* (PNC) and strong PS has often been interpreted as evidence of it (Wiens

et al., 2010). Some degree of PNC is likely in many species and its understanding is important to inform potential responses to global warming or species introductions in new areas (Wiens et al., 2010; Wiens & Graham, 2005). For instance, species with little tolerance to encompass the new environmental conditions and with strong PNC must either migrate or go extinct, while species with more evolutionarily labile traits could potentially adapt (Holt, 1990; Wiens et al., 2010). Nevertheless, strong PS can result from PNC or from Brownian motion (BM)-like evolutionary change (e.g. due to genetic drift or randomly fluctuating natural selection) (Losos, 2008; Wiens et al., 2010). So, PS is seen as a necessary but insufficient condition for PNC (Losos, 2008) and their relationship is complex (Revell et al., 2008; Wiens et al., 2010). So far, there is no universal test for PNC (Wiens et al., 2010) but a recent, promising approach is to compare the relative fit of different evolutionary models to the data, including the BM model and models of stasis or stabilising selection such as Ornstein–Uhlenbeck (OU) models (Kozak & Wiens, 2010; Losos, 2008; Wiens et al., 2010). The BM model assumes that the correlation structure among trait values is proportional to the extent of shared ancestry for pairs of species (Felsenstein, 1973), and works reasonably well as a model of trait evolution (Beaulieu et al., 2012). The OU models incorporate both selection and drift and are more general than pure drift models based on BM (Butler & King, 2004). They have been proved useful in a variety of contexts as they can capture the heterogeneity in the evolutionary process (Beaulieu et al., 2012; Pennell et al., 2015). In fact, several OU models with different degrees of complexity have been proposed, allowing to translate hypotheses regarding evolution in different selective regimes into explicit models (see Beaulieu et al., 2012; Butler & King, 2004; Hansen, 1997).

If traits have PS, it is often useful to apply phylogenetic methods (i.e. comparative methods), which have become a standard ecological tool in recent decades (Losos, 2008). When used in combination with trait-based approaches, phylogenetic analysis can

strengthen hypothesis testing and generate new insights (de Bello et al., 2015), as these methods account for the non-independence of species in statistical analyses due to shared evolutionary history (Felsenstein, 1985; Revell et al., 2008). For instance, it can prove useful to consider phylogeny when assessing evolutionary mechanisms underlying present trait-environment patterns (de Bello et al., 2015). However, non-phylogenetic analyses answer questions at different evolutionary scales (de Bello et al., 2015) and are also informative, particularly when well-resolved phylogenies are not available for study taxa (Losos, 1999). Comparing the results of comparative and non-phylogenetic analyses can also inform about the existence of PNC and thus, as a rule of thumb, it might be useful to apply both techniques to trait data (de Bello et al., 2015).

Elevational and longitudinal gradients (i.e. stream size or upstream–downstream) are well-studied in river ecosystems. Both spatial gradients covary and display variation in many environmental variables such as water temperature, stream flow, habitat features, and productivity (Jones et al., 2017; Vannote et al., 1980). They are also well known to shape fish communities, with changes in fish abundance, richness, species composition and traits (e.g. Cook et al., 2004). Several studies have already analysed the trait–environment relationships of freshwater fishes along these spatial gradients (Jones et al., 2017; Kennedy et al., 2003; Pease et al., 2012). For instance, some studies revealed that species from uppermost reaches have more fusiform bodies, larger egg sizes and longer spawning seasons, but smaller body sizes and smaller clutches than species from lower river reaches (Jones et al., 2017; Pease et al., 2012; Winemiller & Rose, 1992). Similarly, Kennedy et al. (2003) also revealed a significant intraspecific trait variability across the elevation gradient. However, few studies have addressed this issue from a phylogenetic perspective (Comte et al., 2014). Therefore, little is known about which traits or groups of traits (e.g., morphological, trophic, reproductive, and habitat-use traits) are the most conserved in inland fishes, and how their evolution correlates with their elevational and longitudinal distributions.

The Iberian Peninsula is well suited to study the evolutionary assembly of fish species and traits along spatial gradients because of its complex orography, diverse climate, and particular ichthyofauna. This region is a mountainous territory with a broad range of elevation rising from the sea level, over a large central plateau (Meseta Central) to the peaks of over 3000 m (e.g. Bayón & Vilà, 2019; Sabater et al., 2009). Moreover, Iberian freshwaters are inhabited by 68 native fish species, of which 41 are endemic and they have been subjected to very prolonged isolation and speciation processes (Doadrio, 2001), but they are also inhabited by 32 alien species, some of them widespread throughout the planet.

Our main objectives were: (1) to compare the PS of several morphological, trophic, habitat use, and reproductive traits in inland fishes (i.e. species from freshwater ecosystems, including diadromous fishes and a few marine species that enter rivers); and (2) to test for correlated evolution of these traits with elevational and longitudinal distribution (i.e. if traits and species niche tend to evolve together) under three models of niche evolution (i.e. BM, OU stasis,

and OU trend models). We hypothesised that the majority of traits would show PS (Johnson & Stinchcombe, 2007) but its magnitude would vary among trait types (i.e. morphological, trophic, reproductive, or habitat use). Specifically, we predicted that fish body size and other morphological traits would show higher PS than reproductive or habitat traits as in other taxonomic groups (Blomberg et al., 2003a; Comte et al., 2014; Kamilar & Cooper, 2013). Finally, we hypothesised that fish traits would display correlated evolution with elevational and longitudinal gradients, since the functional trait composition of fish assemblages is known to change across the river continuum (Pease et al., 2012).

2 | METHODS

2.1 | Trait dataset and swimming performance estimation

We obtained 23 fish biological and ecological traits (*traits* hereafter, see Table S1) data of inland fishes present in the Iberian Peninsula from two recently published databases (Cano-Barbacil, Radinger, Argudo, et al., 2020; Cano-Barbacil, Radinger, & García-Berthou, 2020), which contain trait information for the 100 inland fish species present in this territory. In total, we analysed 30 species (12 native and 18 alien species) that had complete data on traits (Cano-Barbacil, Radinger, Argudo, et al., 2020; Cano-Barbacil, Radinger, & García-Berthou, 2020). Of all traits considered, seven were described as continuous and 16 as binary variables. Traits considered were related to fish morphology ($n = 3$), trophic position ($n = 3$), reproduction ($n = 6$), and habitat use ($n = 11$) and were selected for their ecological relevance (i.e. features commonly used in trait-based approaches that are known to influence the reproductive success, individual survival and fitness; see Table S1 for further details). Specifically, species-specific critical swimming speed (U_{crit}), a measure of a species' swimming performance, was estimated from a recently compiled dataset of 196 experiments (Cano-Barbacil, Radinger, Argudo, et al., 2020) for all species considered in this work. U_{crit} is a standard experimental measure of prolonged swimming performance, which mediates fitness, survival and habitat selection of fish (Cano-Barbacil, Radinger, Argudo, et al., 2020; Plaut, 2001). We used random forests (RF) with the R-package 'party' (Hothorn et al., 2017) to estimate a standard U_{crit} value for each species (see results in Figure S1) while accounting for effects of experimental setups (water temperature, time step interval between velocity increments, and individual fish total length). RF were built based on optimal hyperparameters calculated using the R-package 'mlr' (Bischl et al., 2016), involving 550 trees with three variables randomly sampled at each split.

Finally, we calculated the relative mean elevational distribution (i.e. mean elevational distribution of each species divided by the maximum elevation of the Iberian Peninsula) and the mean longitudinal distribution (i.e. using Strahler's stream order; Strahler, 1957) for 28 out of the 30 inland fish species considered in this study (see also Figure 1 and Figure S2, and Table S2). These means were

calculated using the presence data available from the Instituto da Conservação da Natureza e das Florestas (Portugal) and the Global Biodiversity Information Facility (GBIF) (GBIF.org, 2019), with a grid resolution of 10 × 10 km UTMs (Universal Transverse Mercator, i.e. 100 km^2, $n = 6142$ total cells). The full ranges of elevation and stream orders studied were sampled fairly uniformly with respect to effort, as the majority of the occurrence data came from standardised samplings on which the fish atlases of Spain and Portugal are based (Doadrio, 2001; Doadrio et al., 2011; Ribeiro et al., 2007). Even though elevation is not a factor that directly determines fish distribution, it is considered a well suited proxy of important variables such as water flow velocity or temperature (see Figure S3) and has been previously used to evaluate and summarise inland fish distribution (Comte et al., 2014). For our dataset, elevation was weakly, but significantly correlated with stream order (see Figure S4). Specifically, we used the relative mean elevational distribution because using the absolute mean elevational distribution could lead to incorrect conclusions if species occupy different relative niches in different geographic regions (e.g. headwater species of the Iberian Peninsula might be lowland species in other regions).

2.2 | Phylogenetic signal in species' traits and elevational distribution

For the comparative analyses, we first obtained the phylogenetic tree of the studied Iberian species from a recent phylogeny of ray-finned fishes (Rabosky et al., 2018), using the function *fishtree_phylogeny* of the R-package *Fish Tree* (Chang et al., 2019). We then estimated the PS of single traits using different, complementary indices (Münkemüller et al., 2012). For continuous traits and elevational and longitudinal distribution, we used Pagel's λ (Pagel, 1999), Blomberg's K (Blomberg et al., 2003b), and Abouheif's C_{mean} (Abouheif, 1999) and their associated tests. Values of λ, K, and C_{mean} with larger deviations from zero all indicate stronger relationships between species traits and phylogeny (Münkemüller et al., 2012). Under a BM model, Pagel's λ and Blomberg's K are expected to be equal to 1, i.e. where trait evolution follows a random walk along the branches of the phylogeny (Münkemüller et al., 2012) and species inherit their features from ancestors but randomly and slowly vary at a constant rate through time (Comte et al., 2014). For both statistics, values of λ and K equal to 0 indicate that there is no PS in the studied trait, while values between 0 and 1 suggest some level of trait lability (Comte et al., 2014), i.e. change of traits during evolution. The upper limit of Pagel's λ is close to one, while Blomberg's K can take higher values that indicate stronger trait similarity among related species than expected under BM (Münkemüller et al., 2012). By contrast, Abouheif's C_{mean} is an autocorrelation index that is not based on any evolutionary model (Münkemüller et al., 2012), with larger deviations from zero indicating stronger PS. We used these three measures of PS because they are complementary since their performance depends on the underlying evolutionary model, sample size, and the possible existence of errors in the topology of the phylogeny (Münkemüller et al., 2012). For proper interpretation, we tested whenever possible if these estimates of PS were significantly different from 0 (p_0) and from 1 (p_1).

To measure the PS of binary traits we calculated the D statistic (Fritz & Purvis, 2010). D ranges within the interval ($-\infty$, ∞), with values lower than 1 indicating trait conservatism. To allow comparison with Blomberg's K statistic, D was transformed to
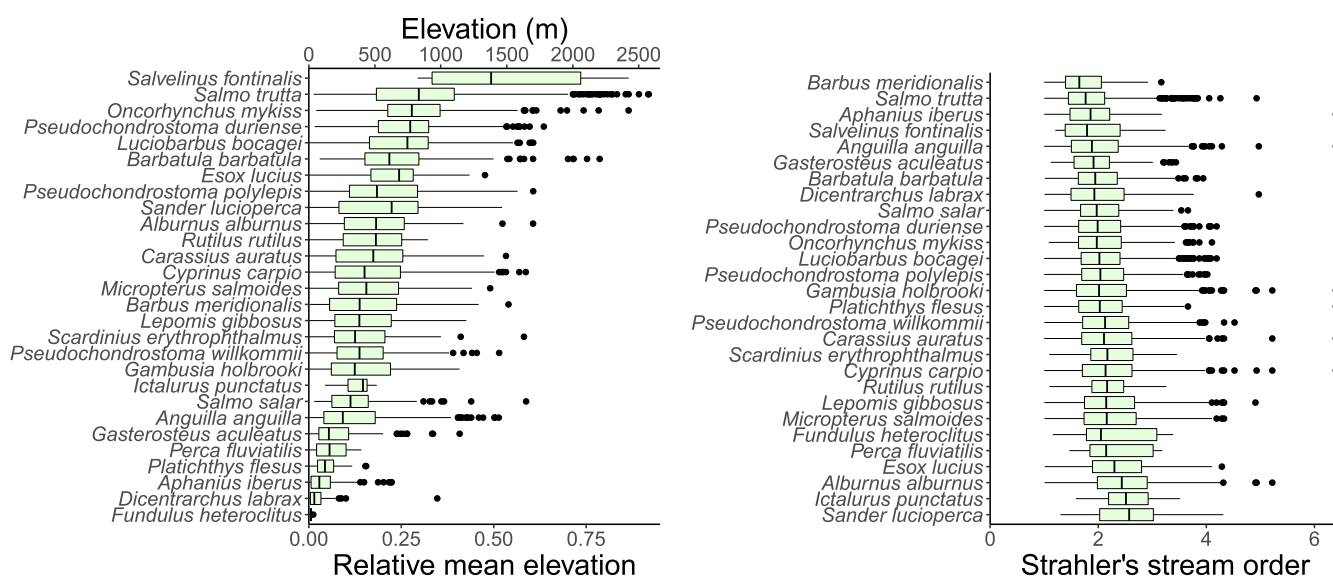

FIGURE 1 (a) Elevation and relative mean elevation, and (b) Strahler's stream order associated with the presences of 28 inland fish species in the Iberian Peninsula (see Table S2 for further details). Elevation and Strahler's stream order were calculated with a grid resolution of 10 × 10 km UTMs (Universal Transverse Mercator, i.e. 100 km^2, $n = 6142$ total cells). Relative mean elevation was calculated as the mean elevational distribution of each species divided by the maximum elevation of the Iberian Peninsula (3479 m)

−D + 1 (Goberna & Verdú, 2016), as an indicator of: (1) no significant signal (−D + 1 ~ 0); (2) traits more conserved than expected by chance but less than expected under BM (0 < −D + 1 < 1); (3) traits conserved as expected under BM (−D + 1 ~ 1); or (4) traits more conserved than expected under BM (−D + 1 > 1) (Fritz & Purvis, 2010). Pagel's λ and Blomberg's K were calculated with the R-package *phytools* (Revell, 2012) and the C_{mean} statistic with *adephylo* (Jombart et al., 2010). Prior to all statistical analyses, continuous trait data were $\log_{10}$-transformed to comply with the assumptions of parametric tests. D statistic was calculated with the R-package *caper* (Orme et al., 2018). As results using K and λ were very similar, and K also informs about trait variation that is more similar than expected under BM and is easily comparable to −D + 1 when using binary traits, we used K value for further analyses of continuous traits.

To analyse the effects of trait measurement scale (i.e. continuous or binary) and trait type (i.e. morphological, trophic, reproductive, and habitat use) on PS measure, we used a two-way ANOVA. In the ANOVA, we also calculated η^2, which is a measure of effect size based on the proportion of the total variance in the dependent variable that is associated with each individual factor or source of variation in the design (Richardson, 2011), and in the case of a single quantitative predictor is identical to the coefficient of determination r^2.

We used the BM model (Felsenstein, 1973) and two different OU (OU_{stasis} and OU_{trend}) models to evaluate how elevational and longitudinal distribution have evolved in fish. The OU_{stasis} model fits a random walk with a central tendency equal to the root value (Z_0) with an attraction strength proportional to the parameter α (Butler & King, 2004). The OU_{trend} model also includes an evolutionary trend, i.e. the optimum (θ) is not the same as the root value (Z_0), with a single optimum for all species (see Beaulieu et al., 2012; Blomberg et al., 2020 for more mathematical background of the BM and OU models). We applied the function *fitContinuous* of the package *geiger* (Pennell et al., 2014) to fit the BM model, and the *OUwie* function of the package *OUwie* for the OU models.

2.3 | Trait correlation with elevation and tests of correlated evolution

To identify the main fish life-history strategies and to visualise possible correlations among fish traits, we performed a principal coordinate analysis (PCoA), using the *wcmdscale* function of the *vegan* R-package (Oksanen et al., 2017) and log-transformation of continuous traits. To analyse if these ordination axes were related to elevation and stream order, we fitted two smooth surfaces using the *ordisurf* function of *vegan*. To test for PS of the set of traits, we also computed Pagel's λ, Blomberg's K and Abouheif's C_{mean} of these two axes.

To test for the relationship of species traits (as response variables) with mean elevation and stream order (as predictors), we performed phylogenetic generalised least squares (PGLS) using the

pgls function for continuous traits, and the *brunch* function for binary traits, both in the *caper* package (Orme et al., 2018). The maximum likelihood estimate of λ was incorporated as a parameter in the PGLS model for continuous traits, thus controlling for phylogenetic dependence in the data in a manner that is optimal for the data set (Freckleton et al., 2002). We also used generalised linear models (GLMs) without considering phylogenetic information (de Bello et al., 2015), with γ and binomial distributions for continuous and binary traits, respectively. In order to analyse and evaluate differences between non-phylogenetically and phylogenetically corrected analyses, we reconstructed the ancestral state of those significant traits using the *contMap* function in the *phytools* R-package (Revell, 2012, 2013).

Finally, to test for correlated evolution among fish traits, we performed PGLS using the *pgls* function for continuous traits and binary phylogenetic generalised linear mixed model with binomial error structure using the *binaryPGLMM* function of the *ape* package (Paradis & Schliep, 2019) for binary traits (Gilbert et al., 2018). For the non-phylogenetic analyses, we also used Pearson and Spearman correlation analyses, for continuous and binary traits, respectively. All statistical analyses and modelling tasks were performed with the software R, version 3.5.1 (R Development Core Team, 2019).

3 | RESULTS

3.1 | Phylogenetic signal in species' traits and elevational and longitudinal distribution

We found statistically significant PS in relative mean elevational distribution using Pagel's λ ($\lambda = 0.904$, $p_0 = 0.004$, $p_1 = 0.115$), Blomberg's K ($K = 0.538$, $p_0 = 0.035$, $p_1 = 0.123$) and Abouheif's C_{mean} ($C_{mean} = 0.343$, $p_0 = 0.007$). Moreover, results of evolutionary models on elevational distribution of inland fishes revealed that the OU_{stasis} model was preferable, with an AIC_c weight of 0.475 (Table 1). However, the BM model was just slightly worse, showing an AIC_c weight of 0.405, whereas the OU_{trend} model was less likely. The best model (i.e. OU_{stasis}) suggested that the current elevational distribution of fish species tended to resemble to that of the common ancestor, showing a stabilising selection around a fixed elevation root value ($Z_0 = 331.7$ m). The attraction strength to this root value, i.e. the strength of niche selection, was proportional to $\alpha = 0.007$. The rate of divergence, i.e. the rate of stochastic evolution away from the root value, was proportional to $\sigma^2 = 0.003$. By contrast, we did not find statistically significant PS in longitudinal distribution using Pagel's λ ($\lambda < 0.001$, $p_0 > 0.999$, $p_1 < 0.001$), Blomberg's K ($K = 0.337$, $p_0 = 0.064$, $p_1 = 0.006$), or Abouheif's C_{mean} ($C_{mean} = 0.071$, $p_0 = 0.266$). In addition, we found that the OU_{stasis} evolutionary model was preferable for longitudinal distribution, with an AIC_c weight of 0.786 (Table 1). This suggested that the evolutionary pattern of longitudinal distribution was similar compared with that of elevational distribution. It showed stabilising selection around fixed

TABLE 1 Results of three evolutionary models of niche evolution (Brownian motion, Ornstein–Uhlenbeck stasis, and Ornstein–Uhlenbeck trend models) to understand how elevational and longitudinal distributions have evolved in Iberian inland fish

Variable	Model	AIC_c	AIC_c weight	σ^2	Z_0	α	θ
Relative mean elevation	Brownian motion	23.362	0.405	0.002	353.0	–	–
	OU_{stasis}	23.044	0.475	0.003	331.7	0.007	–
	OU_{trend}	25.783	0.120	0.003	8.8	0.007	1033.5
Strahler's stream order	Brownian motion	−91.695	0.015	2.7×10^{-5}	2.146	–	–
	OU_{stasis}	−99.662	0.786	7.4×10^{-5}	2.154	0.025	–
	OU_{trend}	−96.922	0.200	7.4×10^{-5}	1.007	0.025	2.168

Note: The adjusted Akaike information criterion (AIC_c) and model weight are shown. σ^2 = rate of divergence (i.e. the rate at which taxa diverge from each other through time; it is unit dependent and cannot be compared); Z_0 = root value (i.e. starting value of the common ancestor of the species studied); α = attraction strength (i.e. strength of trait selection); θ = optimum (i.e. evolutionary optimal value); – indicates parameters not considered by the model.
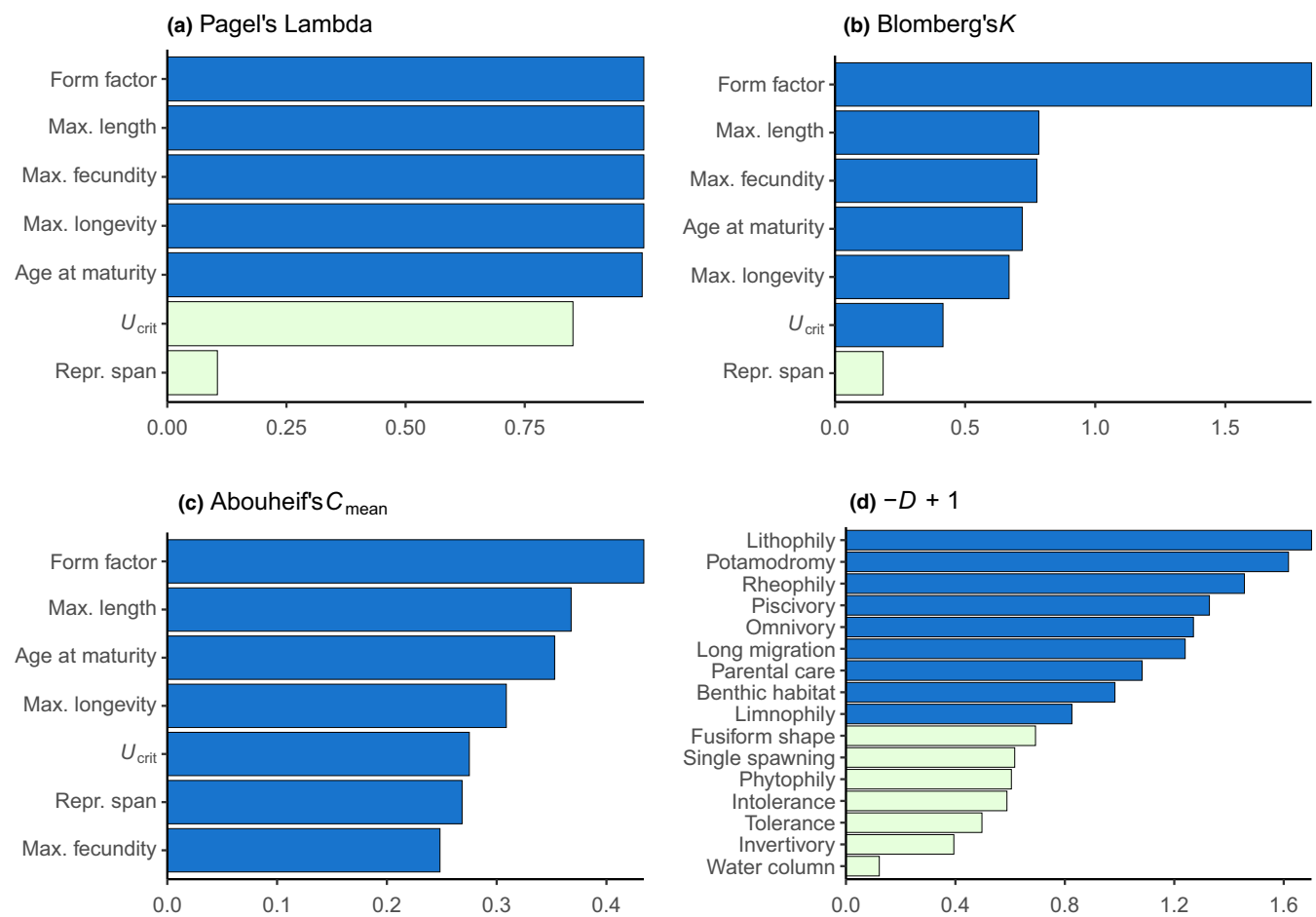

FIGURE 2 Phylogenetic signal in Iberian fish species traits using (a) Pagel's λ, (b) Blomberg's K, and (c) Abouheif's C_{mean} for continuous traits; and (d) −D + 1 for binary traits. Darker colour indicates traits with significant phylogenetic signal (i.e. $p_0 < 0.05$)

stream order root value ($Z_0 = 2.154$) with an attraction strength proportional to $\alpha = 0.025$, and a rate of divergence $\sigma^2 = 7.4.10^{-5}$.

Continuous species traits showed different levels of PS, with the three indices used yielding highly consistent results (Figure 2a–c). We also found clear PS in 5 and 6 out of 7 continuous traits using Pagel's λ and Blomberg's K, respectively (Figure 2a,b). By contrast,

reproductive span was not clearly related to phylogeny, as indicated by both measures ($\lambda = 0.105$, $p_0 = 0.764$; $K = 0.185$, $p_0 = 0.465$). Critical swimming speed (U_{crit}) showed clear PS using Blomberg's K ($K = 0.415$, $p_0 = 0.006$) but not with Pagel's λ ($\lambda = 0.851$, $p_0 = 0.172$). Blomberg's K varied widely across traits, most of them showing values between 0 and 1. Only *form factor* had $K > 1$, indicating that

this morphological trait is more phylogenetically conserved than expected under BM. Using Abouheif's C_{mean}, all seven traits exhibited significant levels of PS (Figure 2c). For example, species within salmonids, perciforms (e.g. *Micropterus salmoides*, *Sander lucioperca*, or *Dicentrarchus labrax*), and cypriniforms (*Cyprinus carpio* or *Abramis brama*) tended to show larger body sizes (i.e. maximum length), while the cyprinodontiforms (e.g. *Gambusia holbrooki* and *Aphanius iberus*) showed small body sizes (Figure 3). A similar pattern can be observed for form factor and fecundity, whereas this is less the case for reproductive span and U_{crit}, which are more labile traits.

Of the 16 considered binary traits, nine showed significant PS (Figure 2d). Seven traits (i.e. lithophily, potadromy, rheophily, piscivory, omnivory, long migration, and parental care) even had $-D + 1 > 1$, indicating that these traits are more conserved than expected under BM. Figure 4 shows how traits with a high PS have been conserved throughout the phylogeny. For instance, lithophilic spawning has been conserved in all salmonid species, all species of the genus *Pseudochondrostoma* and in two closely-related cyprinid species (*Luciobarbus bocagei* and *Barbus meridionalis*); and potadromy has been conserved only in some cypriniform species. However, PS did not clearly depend on trait type ($\eta^2 = 0.077$, $F_{3, 16} = 0.523$, $p = 0.673$), measurement scale ($\eta^2 = 0.016$, $F_{1, 16} = 0.329$, $p = 0.574$), or their interaction ($\eta^2 = 0.106$, $F_{1, 16} = 1.075$, $p = 0.365$; Figure S5).

3.2 | Trait correlation with elevation and tests of correlated evolution

The PCoA results showed that Iberian inland fishes vary in their trophic, morphological, habitat, and reproductive traits (Figure 5). The fitting of the smooth surface showed that changes in the fish traits were related to elevation (Figure 5a; $r^2_{adj} = 0.228$, $F_{3.11, 9} = 0.887$, $p = 0.048$) and stream order (Figure 5b; $r^2_{adj} = 0.379$, $F_{3.98, 9} = 0.887$, $p = 0.005$). The first principal axis summarised approximately 26.0% of overall variation in the trait dataset, and described an ecological and life-history gradient. Species with negative scores on the first PCoA axis were rheophilic, lithophilic, piscivorous, and large-sized species often migratory and with high swimming performance (i.e. higher U_{crit} values) and fewer spawning bouts (mostly salmoniforms and other upland species). By contrast, species with positive scores were lowland, limnophilic, phytophilic, tolerant species with large reproductive span and high parental care (Figure 5c). The second axis (15.8% of explained variation) distinguished among lowland species: with negative scores, large-sized, omnivorous species with late maturity and high fecundity and longevity (mostly lowland cyprinids); with positive scores, small, short-lived species with the opposite suite of traits (e.g. cyprinodontiforms; Figure 5c). Specifically, cypriniforms showed more diverse life-history strategies than other fish orders such as salmoniforms or cyprinodontiforms. Some cypriniforms are rheophilic, lithophilic, and with high swimming performance (e.g. *Barbus meridionalis*), whereas other species have high fecundity and water quality tolerance, and a phytophilic reproduction (e.g. *C. carpio*; Figure 5 and Figure S6).

The first ($\lambda = 0.999$, $p_0 < 0.001$; $K = 0.698$, $p_0 = 0.001$; $C_{mean} = 0.517$, $p_0 = 0.001$) and second axes ($\lambda = 0.552$, $p_0 = 0.015$; $K = 0.408$, $p_0 = 0.007$; $C_{mean} = 0.354$, $p_0 = 0.003$) of the PCoA also showed significant PS for the three statistics used, indicating that closely related species tended to exhibit a similar suite of fish life-history traits (Figure S7).

Using PGLS, we observed a significant positive relationship of parental care with both elevation and stream order (Table 2). However, in other PGLS models, a large amount of the variation of elevational distribution was explained by traits (e.g. potamodromy, benthic, and lithophily). Results obtained without considering phylogenetic information (i.e. GLMs) indicated several significant present-day relationships. For instance, we found a negative correlation between stream order and invertivory. Similarly, rheophily was positively related with elevation but negatively with stream order, whereas lithophily was also positively associated with elevation (Table 2). Ancestral state reconstruction of these four traits showed that their evolution followed a pattern of multiple independent character gains (and losses). We found five gains of the invertivory trait with one subsequent reversal (Figure S8a), three gains of parental with one subsequent reversal (Figure S8b), four gains of rheophily (Figure S8c), and three gains of lithophily (Figure S8d).

Moreover, using PGLS we found that several continuous traits were positively inter-correlated (e.g., maximum total length, maximum longevity, maximum fecundity, and age at maturity; Table S3). Reproductive span was negatively correlated with maximum longevity, age at maturity, and U_{crit} (Table S3). For binary traits, we found positive correlations between intolerance and invertivory, rheophily and lithophily, and limnophily and phytophily, and a negative correlation between lithophily and tolerance (Table S4). Using conventional correlation analyses, we found similar correlation patterns among fish traits (Figure S9 and S10).

4 | DISCUSSION

4.1 | Phylogenetic signal in species' traits and elevational and longitudinal distribution

In our study, we measured the PS of several morphological, trophic, habitat use, and reproductive traits in inland fishes and tested for correlated evolution of these traits with elevational distribution. In agreement with our first hypothesis, most of the studied traits showed significant PS. Form factor and maximum length were the continuous traits with the highest PS detected. This is in accordance with previous studies in fish and other species groups that also showed that morphological traits often have strong PS (Blomberg et al., 2003a; Kamilar & Cooper, 2013; Sternberg & Kennard, 2014). Although PS was quite variable, we did not find clear differences among types of traits, in contrast to previous works (Blomberg et al., 2003a; Kamilar & Cooper, 2013). These potential discrepancies might be due to different reasons. For instance, because of smaller differences in PS among trait types in freshwater fishes

FIGURE 3 Phylogenetic tree annotated with a matrix of continuous traits (circle size represents the standard deviate) associated with each species
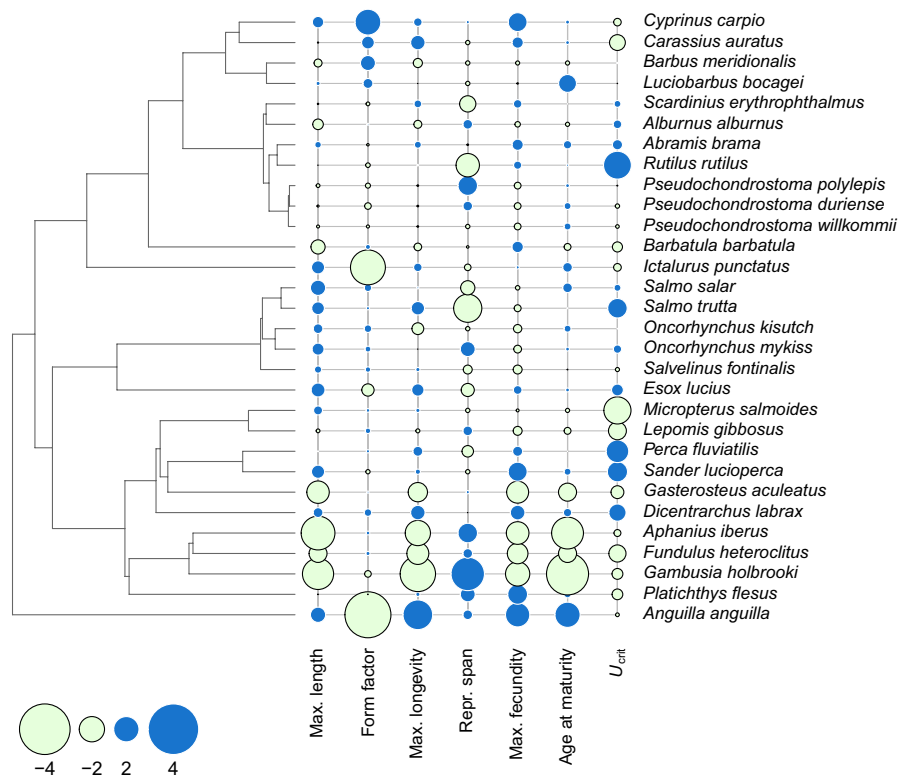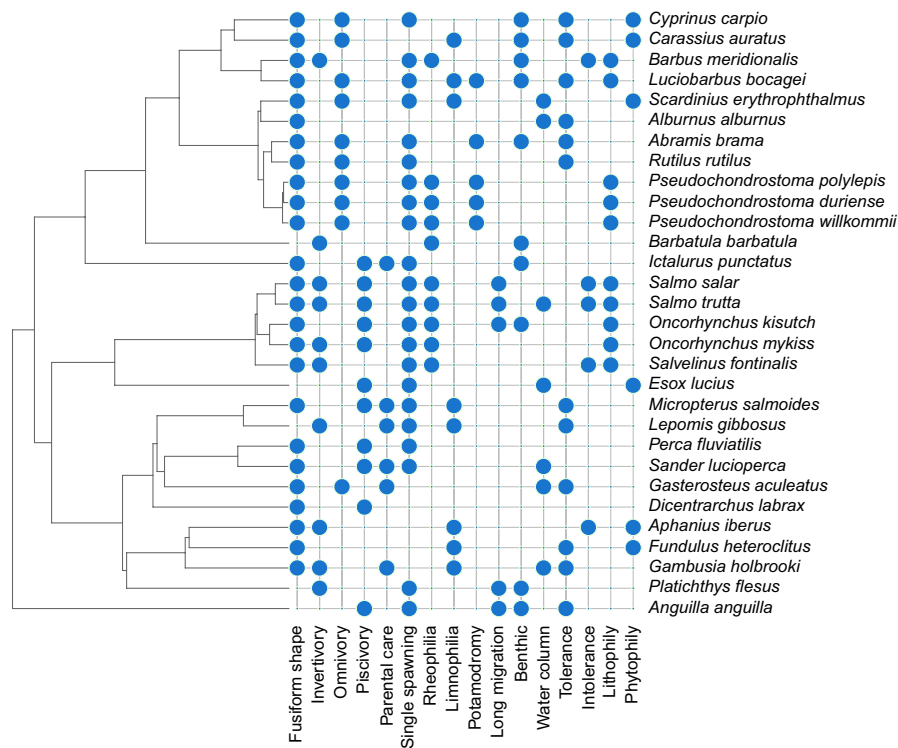

FIGURE 4 Phylogenetic tree annotated with a matrix of binary traits associated with each species

compared with other taxa, or because of low statistical power due to high PS variability combined with lesser data availability and lower diversity of traits in freshwater fishes. Regarding trophic traits, we found significant PS in two (omnivory and piscivory) of the three traits considered. Previous phylogenetic findings regarding trophic traits were rather inconsistent. For instance, Comte

et al. (2014) found that trophic position showed strong phylogenetic clustering in 32 European inland fishes, while Wagner et al. (2009) found an opposite pattern for 32 Tanganyikan cichlid species. The latter could be due to the rapid radiation observed in cichlid species accompanied by trophic diversification (Rüber et al., 1999). With respect to other traits, for example, we found that sensitivity to
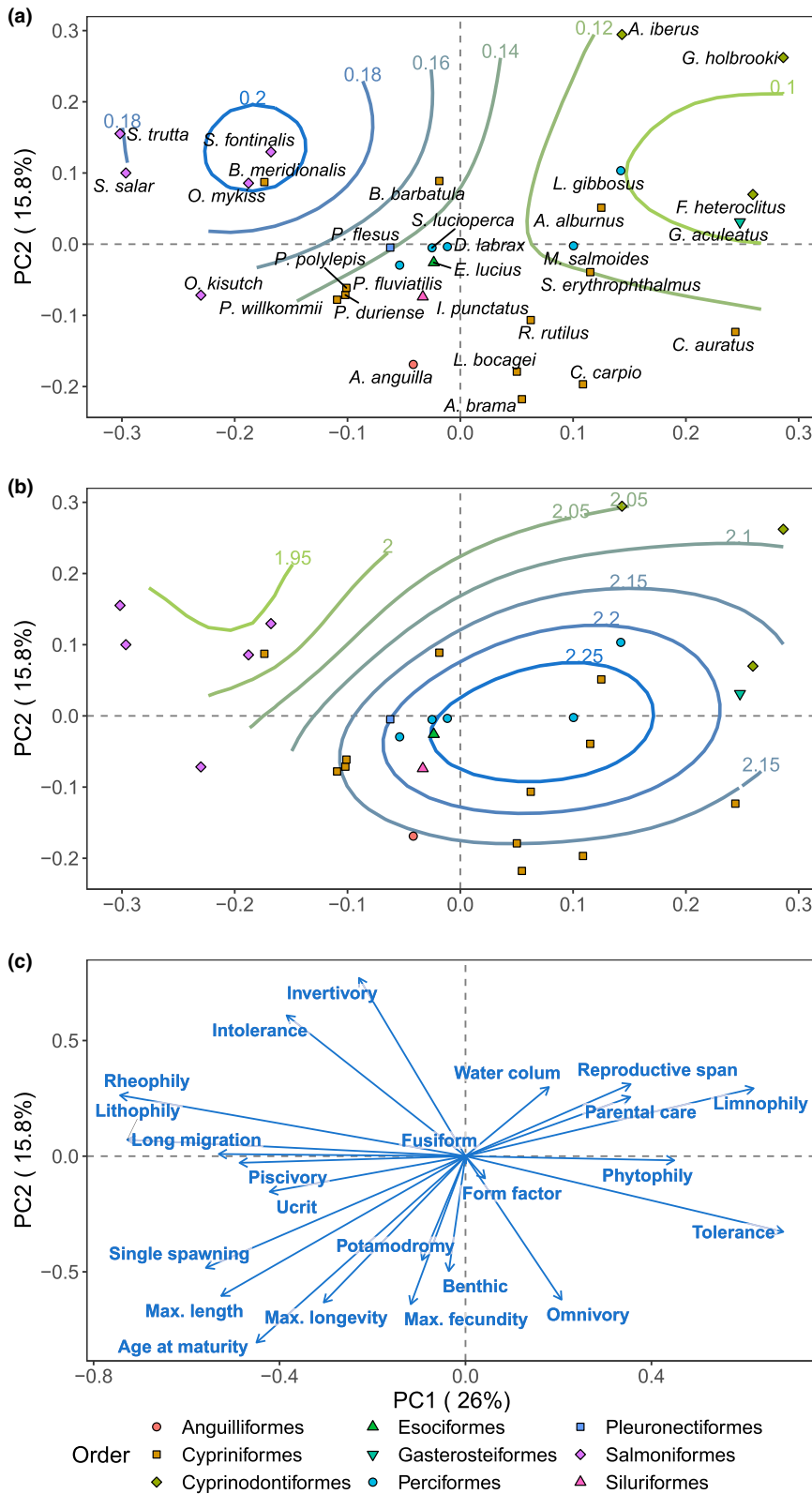
FIGURE 5 Principal coordinate analysis of the 23 biological and ecological traits for the fish species of the Iberian Peninsula. (a) Species scores with smooth response curves of relative mean elevation overlaid. Blue colours of the isopleths represent high elevation while green colours correspond to low elevations. (b) Species scores with smooth response curves of mean Strahler's stream order overlaid. Blue colours of the isopleths represent high stream orders while green colours correspond to low stream orders. (c) Eigenvector plot of the traits. The first axis distinguishes headwater species, with negative scores (i.e. rheophilic, lithophilic, piscivorous, and large-sized species with high swimming performance) from lowland species, with positive scores (i.e. limnophilic, phytophilic, and tolerant species). The second axis distinguishes among lowland species: with negative scores, large-sized, omnivorous species with late maturity and high fecundity and longevity; with positive scores, small, short-lived species with the opposite suite of traits

degradation of water quality and habitat (i.e. tolerance and intolerance traits) did not show clear PS. Similarly, a previous study on fish sensitivity to toxicants revealed that the PS was only significant for 24% of the chemicals analysed (Hylton et al., 2018). Finally, some reproductive traits such as parental care appeared to show also

strong PS, which is in agreement with previous findings (Sternberg & Kennard, 2014).

Results of our evolutionary analysis of elevational and longitudinal distribution revealed OU_{stasis} as the best performing model. However, in the case of elevational distribution, the parameter

TABLE 2 Relationship of fish traits with relative mean elevation and mean Strahler's stream order

Trait (and Type)	Measurement scale	Phylogenetic generalised least squares			Generalised linear models		
		r^2_{RME}	r^2_{SSO}	r^2	r^2_{RME}	r^2_{SSO}	r^2
Morphological							
Max. total length (cm)	Continuous	0.007	0.112.	0.112	0.057	0.013	0.070
Form factor	Continuous	0.089	0.003	0.089	0.001	0.051	0.052
Fusiform shape	Binary	0.147	0.068	0.147	0.004	4.5×10^{-5}	0.004
Trophic							
Invertivory	Binary	0.001	0.641.	0.650	0.010	**0.299***	0.309
Omnivory	Binary	0.015	0.004	0.021	0.003	2.8×10^{-3}	0.003
Piscivory	Binary	0.458	0.301	0.616	6.7×10^{-6}	0.029	0.029
Reproductive							
Max. longevity (years)	Continuous	0.003	7.3×10^{-5}	0.004	0.001	0.003	0.004
Reproductive span (months)	Continuous	0.065	0.002	0.069	0.066	0.010	0.076
Max. fecundity (number of eggs)	Continuous	2.1×10^{-4}	0.049	0.053	1.5×10^{-4}	0.063	0.063
Age at maturity (years)	Continuous	0.006	0.107.	0.135	0.008	1.0×10^{-5}	0.008
Parental care	Binary	**0.974***	**0.966***	0.991	0.011	0.130.	0.141
Single spawning	Binary	0.117	0.106	0.141	0.147.	0.002	0.149
Habitat use							
U_{crit} (cm/s)	Continuous	0.000	0.043	0.005	0.017	0.010	0.027
Rheophily	Binary	0.309	0.842.	0.849	**0.252***	**0.280***	0.532
Limnophily	Binary	0.145	0.060	0.169	0.045	0.003	0.048
Potamodromy	Binary	0.400	0.119	0.541	0.032	0.009	0.041
Long migration	Binary	0.470	0.768	0.786	0.029	0.142.	0.171
Benthic	Binary	0.624	0.325	0.635	0.005	0.011	0.017
Water column	Binary	0.361	0.019	0.366	0.014	0.046	0.060
Tolerance	Binary	3.5×10^{-4}	0.422.	0.425	0.023	0.023	0.046
Intolerance	Binary	0.131	0.863.	0.863	0.005	0.678.	0.683
Lithophily	Binary	0.676	0.226	0.920	**0.263***	0.253.	0.516
Phytophily	Binary	0.338	5.0×10^{-4}	0.338	0.052	0.021	0.073

Note: For phylogenetically corrected analyses, we used phylogenetic generalised least squares for continuous traits and the *brunch* function for binary traits, both from the *caper* package (Orme et al., 2018). For non-phylogenetically corrected analyses, we used generalised linear models with γ and binomial distributions for continuous and binary traits, respectively. Partial r^2 for each predictor and their *p* values expressed as asterisks (*≤0.05; . ≤0.1), and r^2 of the full model are shown. RME = relative mean elevation; SSO = Strahler's stream order. Note that predictors and continuous traits were log-transformed for the phylogenetic generalised least squares. Significant values are highlighted using bold format. The values showed in the table correspond to r^2.

α, which measures the strength of selection in OU models, was very close to zero, suggesting that the deterministic part of the OU model was negligible and that the model might be simplified to the BM model of pure drift (Butler & King, 2004). Thus, we can conclude that elevational and longitudinal distributions showed little evidence for directional trends of evolution and that the ecological niche of fish species tends to resemble to those of ancestors. Similarly, previous studies showed that the evolution of other traits such as trophic position or body size in diadromous and non-diadromous Clupeiformes fit much better to OU models than BM models (Bloom et al., 2018), as OU models can capture the heterogeneity in the evolutionary process and reflect the variability of

the trait value among species (Münkemüller et al., 2015; Pennell et al., 2015).

Evaluating trait lability and the relationship of traits with the environment is essential for managing biodiversity and minimising further species losses. Our results and further work could be helpful to understand the possible responses of inland fishes to environmental change. This study reinforces previous findings suggesting that in a scenario of ongoing climate change, the consequences on the inland ichthyofauna could be detrimental, especially in freshwater fishes with limited dispersal capacity (Markovic et al., 2014). Additionally, our results suggest that the majority of traits studied and the elevational niche could not be able to evolve as fast as the changes of the

environment, as revealed by the strong PS. Although there is some evidence of local adaptation to temperature changes in fishes (Jensen et al., 2008), our results suggest that fish species tend to retain their ancestral niche characteristics. Hence, those species inhabiting closer to their thermal limits would be likely to face increased extinction risk (Comte et al., 2014). Accordingly, Markovic et al. (2014), for example, suggested that eight European fish species are predicted to experience total range loss under future climate change.

4.2 | Trait correlation with elevation and tests of correlated evolution

Our results also indicate that several traits show correlated evolution, i.e. pairs of traits tend to evolve together because of processes such as natural selection or mutation. In addition, we found that the variation in some fish traits was correlated to their elevational and longitudinal distributions, especially when using non-phylogenetic methods. This suggests suites of traits that covary along the elevational and longitudinal river gradient (Vannote et al., 1980) and correlated evolution of traits less related to this environmental gradient. Some relationships are rather unsurprising and likely to be related to well-known co-occurring characteristics of riverine ecosystems (i.e. higher flow velocities are typically found in high elevation gravel-bed streams, while vegetated areas are more often associated with lowland lentic habitats). Thus, species inhabiting upstream stretches are typically rheophilic, lithophilic, and intolerant, whereas those from lower reaches are more frequently limnophilic, phytophilic, and tolerant (Belliard et al., 1999). Specifically, GLMs revealed positive relationships between elevation and traits such as rheophily and lithophily, and negative relationships between stream order and invertivory and rheophily. However, the relationship of these traits with elevation and stream order became non-significant after accounting for phylogenetic relatedness. This disagreement observed between phylogenetic and non-phylogenetic methods could result from small sample sizes, which might be insufficient to reject the null hypothesis after correcting for phylogenetic relatedness, as evidenced by the ancestral state reconstructions (i.e. few transitions between character states). However, it might also be due to the absence of an evolutionary relationship between elevation and stream order and these fish traits. In fact, we were not able to distinguish between both causes and, therefore, these results should be taken with due caution.

In agreement with previous studies (see Barneche et al., 2018; Blanck & Lamouroux, 2007), our results also showed evolutionary correlations among several reproductive traits (e.g. maximum longevity and fecundity, and age at maturity) and fish maximum length. For instance, PCoA revealed that salmoniform species showed large size, long longevity, and late maturity, which corresponds well to the periodic life-history strategy as defined by Winemiller and Rose (1992), while cyprinodontiforms species have opportunistic traits such as small body size, early sexual maturation, or continuous reproduction (Vila-Gispert et al., 2005; Winemiller & Rose, 1992).

The periodic strategy seems to be dominant in temperate and tropical lotic ecosystems, while the opportunistic strategy is more common in productive lowland habitats subjected to disturbances such as intermittent streams, ephemeral pools, or salt marshes (Winemiller, 2005). Cypriniforms are the most diverse order of the fish species studied, showing different strategies and a considerable morphological, physiological, and swimming performance diversity as seen in previous studies (Cano-Barbacil, Radinger, Argudo, et al., 2020; Cano-Barbacil, Radinger, García-Berthou, 2020; Howes, 1991).

As already explained, we consider the Iberian Peninsula as a good case study to evaluate the evolutionary process of fish traits along spatial gradients, due to strong elevational and climatic gradients and a particular fauna. Our study area is representative of rivers with relatively low fish richness but with many threatened endemisms and invasive species. Our results constitute a solid base on which to develop future studies, showing an overview of the fish trait lability and its evolutionary relationship with elevational and longitudinal distribution. However, we note that there are some limitations that might have affected our results, related to both methodological issues (e.g. small sample size due to low trait data availability of Iberian species) and uncertainties associated with trait data (e.g. low reliability of some categorical traits and lack of information on intraspecific variability; Cano-Barbacil, Radinger & García-Berthou, 2020) as already discussed.

5 | CONCLUSIONS

Overall, we found significant phylogenetic signal for 65% of the studied traits but no clear differences between continuous and binary, or among morphological, trophic, reproductive, and habitat-use traits. Evolutionary models revealed that elevational and longitudinal distributions showed little evidence for directional trends of evolution, and thus that the ecological niche tends to resemble that of the common ancestor. Phylogenetic methods showed that several traits such as maximum length and some reproductive traits are inter-correlated, reflecting that those traits have evolved together. We also found a significant positive relationship of parental care with elevation and stream order, using PGLS. However, we found a positive relationship between elevation and traits such as rheophily and lithophily, and a negative relationship between stream order and invertivory and rheophily when using non-phylogenetic methods. In sum, our study suggests that the well-known evolutionary relationship among fish species traits and distribution, and the strong phylogenetic signal observed for some features could have important implications for adaptation to global change, since many species would not be able to evolve and adapt to the new environmental conditions.

CONFLICT OF INTEREST

The authors certify that they have no affiliations with or involvement in any organisation or entity with a financial or non-financial interest in the subject matter or materials discussed in this manuscript.

AUTHOR CONTRIBUTIONS

C.C.B., J.R., and E.G.B. devised the study. C.C.B. compiled the data. Statistical analyses were carried out by C.C.B. with specific assistance from J.R., G.G., and E.G.B. C.C.B. wrote the original draft, and all authors commented on and contributed to revising the draft versions.

DATA AVAILABILITY STATEMENT

Data underlying our analyses are available via figshare (https://doi.org/10.6084/m9.figshare.14339168).

ORCID

Carlos Cano-Barbacil https://orcid.org/0000-0002-6482-5103
Johannes Radinger https://orcid.org/0000-0002-2637-9464
Gaël Grenouillet https://orcid.org/0000-0003-3588-3694
Emili García-Berthou https://orcid.org/0000-0001-8412-741X

REFERENCES

Abouheif, E. (1999). A method for testing the assumption of phylogenetic independence in comparative data. *Evolutionary Ecology Research*, *1*, 895–909. https://doi.org/10.1007/s11295-009-0238-5

Barneche, D. R., Robertson, D. R., White, C. R., & Marshall, D. J. (2018). Fish reproductive-energy output increases disproportionately with body size. *Science*, *360*, 642–645. https://doi.org/10.1126/science.aao6868

Bayón, Á., & Vilà, M. (2019). Horizon scanning to identify invasion risk of ornamental plants marketed in Spain. *NeoBiota*, *52*, 47–86. https://doi.org/10.3897/neobiota.52.38113

Beaulieu, J. M., Jhwueng, D. C., Boettiger, C., & O'Meara, B. C. (2012). Modeling stabilizing selection: Expanding the Ornstein-Uhlenbeck model of adaptive evolution. *Evolution*, *66*, 2369–2383. https://doi.org/10.1111/j.1558-5646.2012.01619.x

Belliard, J., Berrebi Dit Thomas, R., & Monnier, D. (1999). Fish communities and river alteration in the Seine Basin and nearby coastal streams. *Hydrobiologia*, *400*, 155–166. https://doi.org/10.1023/A:1003752215677

Bischl, B., Lang, M., Kotthoff, L., Schiffner, J., Richter, J., Studerus, E., Casalicchio, G., & Jones, Z. M. (2016). mlr: Machine learning in R. *The Journal of Machine Learning Research*, *17*, 1–5.

Blanck, A., & Lamouroux, N. (2007). Large-scale intraspecific variation in life-history traits of European freshwater fish. *Journal of Biogeography*, *34*, 862–875. https://doi.org/10.1111/j.1365-2699.2006.01654.x

Blomberg, S. P., & Garland, T. (2002). Tempo and mode in evolution: Phylogenetic inertia, adaptation and comparative methods. *Journal of Evolutionary Biology*, *15*, 899–910. https://doi.org/10.1046/j.1420-9101.2002.00472.x

Blomberg, S. P., Garland, T., & Ives, A. R. (2003a). Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution*, *57*, 717–745. https://doi.org/10.1111/j.0014-3820.2003.tb00285.x

Blomberg, S. P., Garland, T., & Ives, A. R. (2003b). Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution*, *57*, 717–745. https://doi.org/10.1111/j.0014-3820.2003.tb00285.x

Blomberg, S. P., Rathnayake, S. I., & Moreau, C. M. (2020). Beyond Brownian motion and the Ornstein-Uhlenbeck process: Stochastic diffusion models for the evolution of quantitative characters. *American Naturalist*, *195*, 145–165. https://doi.org/10.1086/706339

Bloom, D. D., Burns, M. D., & Schriever, T. A. (2018). Evolution of body size and trophic position in migratory fishes: A phylogenetic comparative analysis of Clupeiformes (anchovies, herring, shad and allies). *Biological Journal of the Linnean Society*, *125*, 302–314. https://doi.org/10.1093/BIOLINNEAN/BLY106

Butler, M. A., & King, A. A. (2004). Phylogenetic comparative analysis: A modeling approach for adaptive evolution. *American Naturalist*, *164*, 683–695. https://doi.org/10.1086/426002

Cano-Barbacil, C., Radinger, J., Argudo, M., Rubio-Gracia, F., Vila-Gispert, A., & García-Berthou, E. (2020). Key factors explaining critical swimming speed in freshwater fish: A review and statistical analysis for Iberian species. *Scientific Reports*, *10*, 18947. https://doi.org/10.1038/s41598-020-75974-x

Cano-Barbacil, C., Radinger, J., & García-Berthou, E. (2020). Reliability analysis of fish traits reveals discrepancies among databases. *Freshwater Biology*, *65*(5), 863–877. https://doi.org/10.1111/fwb.13469

Chang, J., Rabosky, D. L., Smith, S. A., & Alfaro, M. E. (2019). An r package and online resource for macroevolutionary studies using the ray-finned fish tree of life. *Methods in Ecology and Evolution*, *10*, 1118–1124. https://doi.org/10.1111/2041-210X.13182

Comte, L., Murienne, J., & Grenouillet, G. (2014). Species traits and phylogenetic conservatism of climate-induced range shifts in stream fishes. *Nature Communications*, *5*, 1–10. https://doi.org/10.1038/ncomms6053

Cook, R. R., Angermeier, P. L., Finn, D. S., Poff, N. L. R., & Krueger, K. L. (2004). Geographic variation in patterns of nestedness among local stream fish assemblages in Virginia. *Oecologia*, *140*, 639–649. https://doi.org/10.1007/s00442-004-1618-z

de Bello, F., Berg, M. P., Dias, A. T. C., Diniz-Filho, J. A. F., Götzenberger, L., Hortal, J., Ladle, R. J., & Lepš, J. (2015). On the need for phylogenetic 'corrections' in functional trait-based approaches. *Folia Geobotanica*, *50*, 349–357. https://doi.org/10.1007/s12224-015-9228-6

Doadrio I. (2001). Atlas y Libro Rojo de los Peces Continentales de España. Ministerio de Medio Ambiente.

Doadrio, I., Perea, S., Garzón-Heydt, P., & González, J. L. (2011). *Ictiofauna continental española. Bases para su seguimiento*. Ministerio de Medio Ambiente y Medio Rural y Marino, .

Felsenstein, J. (1973). Maximum likelihood estimation of evolutionary trees from continuous characters. *American Journal of Human Genetics*, *25*, 471–492.

Felsenstein, J. (1985). Phylogenies and the comparative method. *The American Naturalist*, *125*, 1–15. https://doi.org/10.1086/284325

Freckleton, R. P., Harvey, P. H., & Pagel, M. (2002). Phylogenetic analysis and comparative data: A test and review of evidence. *American Naturalist*, *160*, 712–726. https://doi.org/10.1086/343873

Fritz, S. A., & Purvis, A. (2010). Selectivity in mammalian extinction risk and threat types: A new measure of phylogenetic signal strength in binary traits. *Conservation Biology*, *24*, 1042–1051. https://doi.org/10.1111/j.1523-1739.2010.01455.x

GBIF.org (2019). GBIF Home Page.

Gilbert, K. J., Nitta, J. H., Talavera, G., & Pierce, N. E. (2018). Keeping an eye on coloration: Ecological correlates of the evolution of pitcher traits in the genus *Nepenthes* (Caryophyllales). *Biological Journal of the Linnean Society*, *123*, 321–337. https://doi.org/10.1093/biolinnean/blx142

Goberna, M., & Verdú, M. (2016). Predicting microbial traits with phylogenies. *ISME Journal*, *10*, 959–967. https://doi.org/10.1038/ismej.2015.171

Hansen, T. F. (1997). Stabilizing selection and the comparative analysis of adaptation. *Evolution*, *51*, 1341–1351. https://doi.org/10.1111/j.1558-5646.1997.tb01457.x

Harvey, P., & Pagel, M. D. (1991). *The comparative method in evolutionary biology*. Oxford University Press.

Holt, R. D. (1990). The microevolutionary consequences of climate change. *Trends in Ecology and Evolution*, *5*, 311–315. https://doi.org/10.1016/0169-5347(90)90088-U

Hothorn, T., Hornik, K., Strobl, C., & Zeileis, A. (2017). party: A laboratory for recursive partytioning. R package version 1.2-3

Howes, G. J. (1991). Systematics and biogeography: An overview. In I. J. Winfield, & J. S. Nelson (Eds.), *Cyprinid fishes: Systematics*, *biology and exploitation* (pp. 1–33). Chapman and Hall.

Hylton, A., Chiari, Y., Capellini, I., Barron, M. G., & Glaberman, S. (2018). Mixed phylogenetic signal in fish toxicity data across chemical classes. *Ecological Applications*, *28*, 605–611. https://doi.org/10.1002/eap.1698

Jensen, L. F., Hansen, M. M., Pertoldi, C., Holdensgaard, G., Mensberg, K. L. D., & Loeschcke, V. (2008). Local adaptation in brown trout early life-history traits: Implications for climate change adaptability. *Proceedings of the Royal Society B: Biological Sciences*, *275*, 2859–2868. https://doi.org/10.1098/rspb.2008.0870

Johnson, M. T. J., & Stinchcombe, J. R. (2007). An emerging synthesis between community ecology and evolutionary biology. *Trends in Ecology and Evolution*, *22*, 250–257. https://doi.org/10.1016/j.tree.2007.01.014

Jombart, T., Balloux, F., & Dray, S. (2010). adephylo: New tools for investigating the phylogenetic signal in biological traits. *Bioinformatics*, *26*, 1907–1909. https://doi.org/10.1093/bioinformatics/btq292

Jones, P. E., Augspurger, J., & Closs, G. P. (2017). Landscape-scale life-history gradients in New Zealand freshwater fish. *Freshwater Biology*, *62*, 570–581. https://doi.org/10.1111/fwb.12886

Kamilar, J. M., & Cooper, N. (2013). Phylogenetic signal in primate behaviour, ecology and life history. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*, 20120341. https://doi.org/10.1098/rstb.2012.0341

Kennedy, B. M., Peterson, D. P., & Fausch, K. D. (2003). Different life histories of brook trout populations invading mid-elevation and high-elevation cutthroat trout streams in Colorado. *Western North American Naturalist*, *63*, 215–223.

Kozak, K. H., & Wiens, J. J. (2010). Niche conservatism drives elevational diversity patterns in Appalachian salamanders. *American Naturalist*, *176*, 40–54. https://doi.org/10.1086/653031

Losos, J. B. (1999). Uncertainty in the reconstruction of ancestral character states and limitations on the use of phylogenetic comparative methods. *Animal Behaviour*, *58*, 1319–1324. https://doi.org/10.1006/anbe.1999.1261

Losos, J. B. (2008). Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. *Ecology Letters*, *11*, 995–1003. https://doi.org/10.1111/j.1461-0248.2008.01229.x

Markovic, D., Carrizo, S., Freyhof, J., Cid, N., Lengyel, S., Scholz, M., Kasperdius, H., & Darwall, W. (2014). Europe's freshwater biodiversity under climate change: Distribution shifts and conservation needs. *Diversity and Distributions*, *20*, 1097–1107. https://doi.org/10.1111/ddi.12232

Münkemüller, T., Boucher, F. C., Thuiller, W., & Lavergne, S. (2015). Phylogenetic niche conservatism – Common pitfalls and ways forward. *Functional Ecology*, *29*, 627–639. https://doi.org/10.1111/1365-2435.12388

Münkemüller, T., Lavergne, S., Bzeznik, B., Dray, S., Jombart, T., Schiffers, K., & Thuiller, W. (2012). How to measure and test phylogenetic signal. *Methods in Ecology and Evolution*, *3*, 743–756. https://doi.org/10.1111/j.2041-210X.2012.00196.x

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2017). vegan: Community ecology package. http://CRAN.R-project.org/package=vegan

Orme, C. D. L., Freckleton, R., Thomas, G., Petzold, T., Fritz, S., Isaac, N., Pearse, W. (2018). The caper package: comparative analysis of phylogenetics and evolution in R. 1–36.

Pagel, M. (1999). Inferring historical patterns of biological evolution. *Nature*, *401*, 877–884.

Paradis, E., & Schliep, K. (2019). ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, *35*, 526–528.

Pease, A. A., González-Díaz, A. A., Rodiles-Hernández, R., & Winemiller, K. O. (2012). Functional diversity and trait-environment relationships of stream fish assemblages in a large tropical catchment. *Freshwater Biology*, *57*, 1060–1075. https://doi.org/10.1111/j.1365-2427.2012.02768.x

Pennell, M. W., Eastman, J. M., Slater, G. J., Brown, J. W., Uyeda, J. C., FitzJohn, R. G., Alfaro, M. E., & Harmon, L. J. (2014). geiger v2.0: An expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. *Bioinformatics*, *30*, 2216–2218. https://doi.org/10.1093/bioinformatics/btu181

Pennell, M. W., Fitzjohn, R. G., Cornwell, W. K., & Harmon, L. J. (2015). Model adequacy and the macroevolution of angiosperm functional traits. *American Naturalist*, *186*, E33–E50. https://doi.org/10.1086/682022

Plaut, I. (2001). Critical swimming speed: Its ecological relevance. *Comparative Biochemistry and Physiology: A Molecular and Integrative Physiology*, *131*, 41–50. https://doi.org/10.1016/S1095-6433(01)00462-7

R Development Core Team (2019). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Rabosky, D. L., Chang, J., Title, P. O., Cowman, P. F., Sallan, L., Friedman, M., Kaschner, K., Garilao, C., Near, T. J., Coll, M., & Alfaro, M. E. (2018). An inverse latitudinal gradient in speciation rate for marine fishes. *Nature*, *559*, 392–395. https://doi.org/10.1038/s41586-018-0273-1

Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, *3*, 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x

Revell, L. J. (2013). Two new graphical methods for mapping trait evolution on phylogenies. *Methods in Ecology and Evolution*, *4*, 754–759. https://doi.org/10.1111/2041-210X.12066

Revell, L. J., Harmon, L. J., & Collar, D. C. (2008). Phylogenetic signal, evolutionary process, and rate. *Systematic Biology*, *57*, 591–601. https://doi.org/10.1080/10635150802302427

Ribeiro, F., Beldade, R., Dix, M., & Bochechas, J. (2007). Carta Piscícola Nacional

Richardson, J. T. E. (2011). Eta squared and partial eta squared as measures of effect size in educational research. *Educational Research Review*, *6*, 135–147. https://doi.org/10.1016/j.edurev.2010.12.001

Rüber, L., Verheyen, E., & Meyer, A. (1999). Replicated evolution of trophic specializations in an endemic cichlid fish lineage from Lake Tanganyika. *Proceedings of the National Academy of Sciences*

of the United States of America, *96*, 10230–10235. https://doi.org/10.1073/pnas.96.18.10230

Sabater, S., Feio, M. J., Graça, M. A. S., Muñoz, I., & Romaní, A. M. (2009). The Iberian Rivers. In K. Tockner, U. Uehlinger, & C. T. Robinson (Eds.), *Rivers of Europe* (pp. 113–149). Academic Press.

Sidlauskas, B. (2008). Continuous and arrested morphological diversification in sister clades of characiform fishes: A phylomorphospace approach. *Evolution*, *62*, 3135–3156. https://doi.org/10.1111/j.1558-5646.2008.00519.x

Sternberg, D., & Kennard, M. J. (2014). Phylogenetic effects on functional traits and life history strategies of Australian freshwater fish. *Ecography*, *37*, 54–64. https://doi.org/10.1111/j.1600-0587.2013.00362.x

Strahler, A. N. (1957). Quantitative analysis of watershed geomorphology, transactions of the american geophysical union. *Transactions, American Geophysical Union*, *38*, 913–920. https://doi.org/10.1029/TR038i006p00913

Vannote, R. L., Wayne, M. G., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, *37*, 130–137. https://doi.org/10.1139/f80-017

Vila-Gispert, A., Alcaraz, C., & García-Berthou, E. (2005). Life-history traits of invasive fish in small Mediterranean streams. *Biological Invasions*, *7*, 107–116. https://doi.org/10.1007/1-4020-3870-4_10

Wagner, C. E., McIntyre, P. B., Buels, K. S., Gilbert, D. M., & Michel, E. (2009). Diet predicts intestine length in Lake Tanganyika's cichlid fishes. *Functional Ecology*, *23*, 1122–1131. https://doi.org/10.1111/j.1365-2435.2009.01589.x

Wiens, J. J., Ackerly, D. D., Allen, A. P., Anacker, B. L., Buckley, L. B., Cornell, H. V., Damschen, E. I., Jonathan Davies, T., Grytnes, J.-A., Harrison, S. P., Hawkins, B. A., Holt, R. D., McCain, C. M., & Stephens, P. R. (2010). Niche conservatism as an emerging principle in ecology and conservation biology. *Ecology Letters*, *13*, 1310–1324. https://doi.org/10.1111/j.1461-0248.2010.01515.x

Wiens, J. J., & Graham, C. H. (2005). Niche conservatism: Integrating evolution, ecology, and conservation biology. *Annual Review of Ecology, Evolution, and Systematics*, *36*, 519–539. https://doi.org/10.1146/annurev.ecolsys.36.102803.095431

Winemiller, K. O. (2005). Life history strategies, population regulation, and implications for fisheries management. *Canadian Journal of Fisheries and Aquatic Sciences*, *62*, 872–885. https://doi.org/10.1139/f05-040

Winemiller, K. O., & Rose, K. A. (1992). Patterns of life-history diversification in North American fishes: Implications for population regulation. *Canadian Journal of Fisheries and Aquatic Sciences*, *49*, 2196–2218. https://doi.org/10.1139/f92-242

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.