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DNA Barcoding Reveals Widespread Co-Occurrence of Other Chub Species in the Range of the Endemic Catalan Chub, *Squalius laietanus*

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Abstract: The Catalan chub, *Squalius laietanus*, was described in 2007 and has recently reached Vulnerable status on the IUCN Red List of Threatened Species. The assigned range for this species includes the lower course of the Ebro River in Spain and northwards to the Tech, Têt, Agly, and Massane Rivers in Southeastern France. In this study, 246 chub specimens caught at 15 sites in the nine main river basins of the Spanish range were barcoded by sequencing a 624 bp fragment of the mitochondrial COI gene. *Squalius laietanus* haplotypes were found in 68% of the fish caught. In a tributary of the Ebro River, all specimens (6%) were identified as the Tagus chub, *S. pyrenaicus*, another endemic Iberian chub species. The remaining fish (26%) had a haplotype typical of the European chub, *S. cephalus*, populations outside Spain. This haplotype was present in six sites in four basins, being particularly abundant in the Ebro River. Considering that *Squalius* species can hybridize with other native and non-native cyprinid species, the potential co-occurrence of several species in the same site or basin raises concerns about the real extent of an exclusive range for the Catalan chub and the integrity of its native gene pools throughout its range.

Keywords: conservation genetics; cytochrome c oxidase subunit I; mitochondrial markers; *Squalius cephalus*; *Squalius laietanus*

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1. Introduction

Freshwater habitats and their species have been suffering the greatest biodiversity losses in recent times. The latest Living Planet Index [1] reports that there has been an 85% decline in freshwater vertebrate fauna since 1970. In Europe, the Mediterranean basin has long been recognized as one of the global "biodiversity hotspots" because of the concentration of a high number of endemic species in this region [2]. However, Mediterranean freshwater fish species are among the groups most threatened by pollution, water abstraction, habitat fragmentation, the introduction of exotic species, overfishing, and the repopulation of non-native stocks [3,4], and 56% of this freshwater fauna is classified as threatened by the International Union for the Conservation of Nature (IUCN) [5]. In the Iberian Peninsula, the formation of large endorheic basins during the Miocene contributed to the current richness of its native freshwater fish species [6–8]. In the last 30 years, molecular markers have contributed to the identification of endemic cyprinid species in the Iberian Peninsula [7,9]. For instance, the sequencing of some mitochondrial DNA (mtDNA) genes helped clarify the evolutionary relationships between the native Iberian *Barbus* species and assign some of them to the genus *Luciobarbus* [10].

At the end of the 20th century, only three species of chub (formerly in the genus *Leuciscus* and now in the genus *Squalius*) were thought to inhabit the Iberian Peninsula, namely, the two endemic species *S. pyrenaicus* and *S. carolitertii* as well as *S. cephalus*, widely distributed throughout Europe. Soon after, DNA-sequencing analyses suggested there was high divergence between populations within these three lineages, leading to the description of several new endemic chub species: *S. aradensis* and *S. torgalensis* [11], *S. valentinus* and *S. malacitanus* [12], *S. castellanus* [13], and, most recently, *S. gaditanus* and *S. tartessicus* [14]. Indeed, DNA-sequencing analyses suggest that the diversity of the *Squalius* genus in the Iberian Peninsula is still underestimated [8]. Concerning the North-eastern Iberian chub populations identified as the European chub (*S. cephalus*), the sequencing of the mitochondrial cytochrome b gene (cyt b) revealed a close relationship with Greek chub populations rather than with the hydrographically closer Central European ones [15]. Further morphological analyses provided conclusive evidence towards the recognition of these populations as a distinct new endemic species: the Catalan chub, *S. laietanus*. Thus, *S. laietanus* can be differentiated from other *Squalius* species in Europe by the presence of a subinferior mouth with a projecting upper lip and flank scales edged with a band of black pigmentation [16]. Phylogenetic analyses based on the sequencing of mitochondrial (cytochrome oxidase c subunit I, COI, and cyt b) and nuclear genes (subunit I of the recombination activating gene, RAG1, and ribosomal protein S7, RPS7) confirmed there was a close evolutionary relationship between *S. laietanus* and *S. orpheus*, the latter being a species found in Greece and Turkey [17,18]. These studies clearly distinguished *S. laietanus* from *S. cephalus* and showed a long-time evolutionary divergence from all of the other previously described endemic *Squalius* species in the Iberian Peninsula.

The distribution of the Catalan chub in Spain spans from the lower course of the Ebro River and its main regional tributary, the Segre River, northwards to the French border. Suitable habitats are found in most of the short regional coastal rivers and in the Tech, Têt, Massane, and Agly rivers in Southern France [16,18,19]. However, populations of this species are small and fragmented and have been declining since 1990, mainly due to pollution, habitat degradation, and competition with non-native species [20]. As climate change is likely to prolong periods of low rainfall and drought in the Mediterranean region [21], endemic species with restricted distribution ranges, such as *S. laietanus*, will be more vulnerable to extinction [22]. Since 2011, *S. laietanus* has been listed as vulnerable on the Spanish Red List of Threatened Species [23], and in 2024, the species also achieved this status on the IUCN Red List of Threatened Species. However, this IUCN assessment still recognizes some data uncertainties regarding population sizes and the current distribution of this species.

One of the most important concerns was the observation of chub specimens with a combination of morphological features diagnostic of both *S. laietanus* and *S. cephalus* in the French Agly and Tech Rivers [18]. Regarding the Iberian Peninsula, the European chub, *S. cephalus*, is currently considered a non-native species [24], but its presence within the area of distribution of *S. laietanus* is suspected [20]. Given the framework of the previously documented presence of *S. cephalus* within the range of *S. laietanus* in France [18,19], the aim of the present study was to characterize the chub species present in the main Catalan rivers in the distribution range of *S. laietanus*, from the Muga to the Ebro River, using the mitochondrial gene COI. If the results confirmed the presence of the European chub, this finding would open the door to possible hybridization events that would raise important concerns regarding the future maintenance of native *S. laietanus* populations in these river basins.

2. Materials and Methods

2.1. Study Area and Sampling

In 2021, a total of 246 chub samples were collected for the Inland Fisheries Services of the Autonomous Government of Catalonia at 15 different sites (Table 1, Figure 1) in the nine main river basins in Catalonia, from the Ebro River and northwards to the Muga River. The captured chubs were anesthetized on site with clove oil to collect a ventral fin clip from each one. After recovering from anesthesia, the fish were released back into the river at the site of capture. In addition, 22 fin clips from chub specimens collected at two sites in the Moselle River in Mersch (Luxembourg) and morphologically identified as *S. cephalus* were kindly provided by Dr. Frankie Thielen (Foundation Natur & Ëmwelt) and used as reference samples for this non-native species in Spain. All tissue samples were preserved in 70% ethanol and stored at −40 °C until DNA extraction using the Chelex-proteinase K procedure [25].

Table 1. Sampling sites for each Catalan river basin included in this study.

Basin	**River**	**Site**	**Code**	**N**
Muga	Muga	Cabanes	MMC	16
	Muga	Albanyà	MMA	16
Fluvià	Fluvià	Vilert	FFV	14
	Borró	Argelaguer	FBB	16
Ter	Terri	Sant Andreu del Terri	TTG	16
Tordera	Tordera	Hostalric	TTH	16
Besòs	Ripoll	Ripollet	BRR	16
Llobregat	Anoia	La Fortesa	LAA	16
	Marganell	Marganell	LMA	16
	Rajadell	Manresa	LRA	16
Gaià	Gaià	Aiguamúrcia	GGA	16
Francolí	Francolí	Valls	FRV	26
Ebro	Ebro	Benissanet	EEB	16
	Canaletes	Horta de Sant Joan	ECH	14
	Segre	Lleida	ESL	16

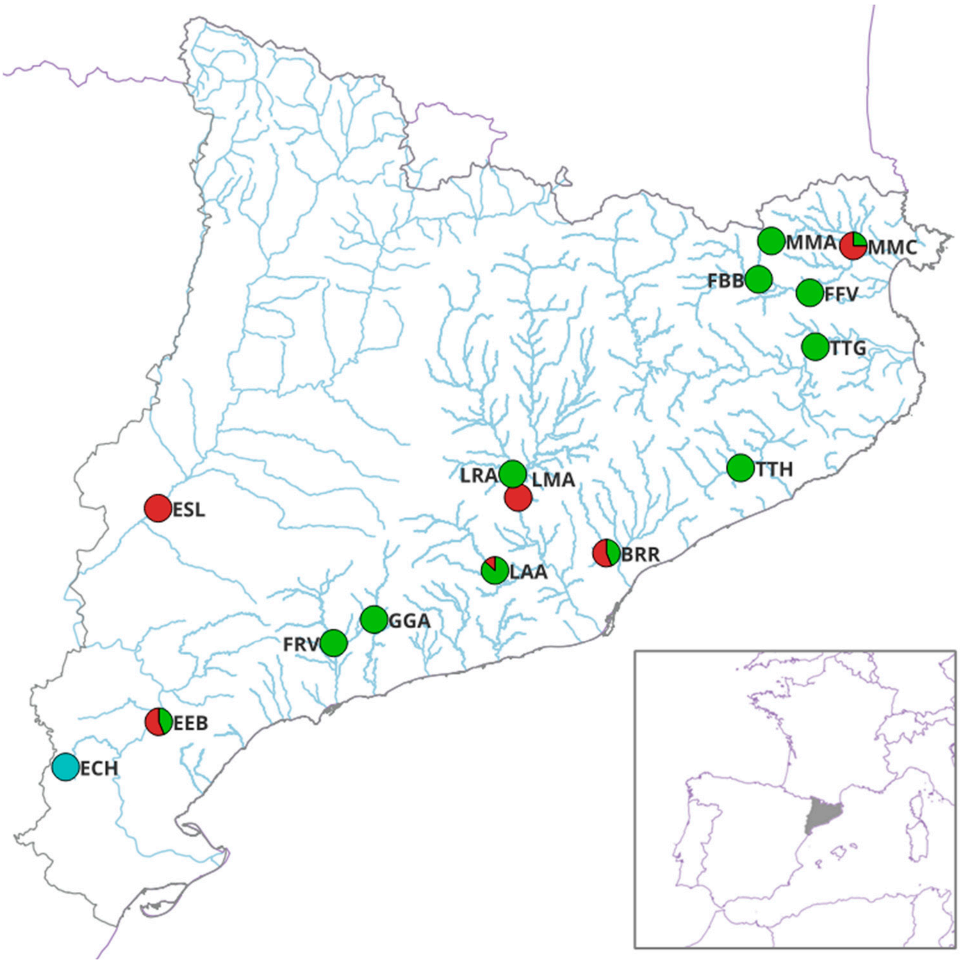


Figure 1. Geographic sites of this study. Site codes are listed in Table 1. The frequencies of each mitochondrial species found are represented by pie charts, where green indicates *S. laietanus*, red represents *S. cephalus*, and blue denotes *S. pyrenaicus*. Map was created with QGIS 3.36.0.

2.2. Molecular Identification of the Collected Fish

To determine the putative species of each captured chub, a fragment of the mitochondrial gene COI was analyzed. Because of its low diversity within species but significant divergence between them, sequences of this gene have been widely used as a DNA barcode for fish species identification [26,27]. In the Iberian Peninsula, the availability of COI sequences from public libraries such as GenBank or FISH-BOL covered 98.1% of the 76 native fish species and 30 of the non-native fish species, including all native chub species in the genus *Squalius* [28]. The COI fragment was amplified via PCR according to the conditions described by Perea et al. [17]. The final volume of the PCR mix was 25 µL, containing 1 × GotTaq® Hot-Start Colorless Master Mix (Promega), 0.2 µM of the primers FishF1 and FishR1 [29], and 1 µL of extracted DNA. PCR reactions were performed in a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA). The amplification products were verified on 1.5% agarose gels stained with GelRed Nucleic Acid Stain (Biotium, Fremon, CA, USA) and visualized using the GD–1000 Axygen® Gel Documentation System (Axygen, Union City, CA, USA). The resulting PCR products were purified and sequenced by Macrogen (Madrid, Spain). We used the aforementioned primers to sequence the molecular marker. In the first batch, 10 samples were randomly selected for sequencing validation using both forward and reverse primers. This subset showed no mismatches for a fragment of 624 bp between the forward and reverse sequence results of the PCR amplicon. Thus, this fragment was used for species identification of all remaining samples sequenced with the FishR1 primer only.

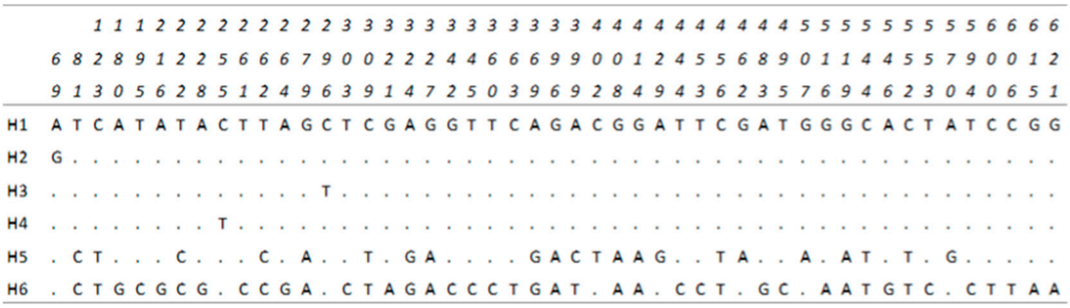
2.3. Sequence Data Analysis

The obtained sequences were aligned using BioEdit [30]. DAMBE7 [31] was used to identify distinct haplotypes among the sequences. Assignment of the detected haplotypes to previously described chub species was achieved using GenBank-derived reference sequences of *S. laietanus* from the Ebro basin in Spain and the Tech and Massane basins in France and other Iberian chub species (*S. aradensis*, *S. carolitertii*, *S. malacitanus*, *S. pyrenaicus*, *S. torgalensis*, and *S. valentinus*), along with chub species distributed across the Mediterranean basin, from Italy to Greece (Table S1), due to previous studies suggesting there are phylogenetic relationships between the Catalan chub and chub lineages outside Spain [15,17]. Species belonging to the Cyprinidae family were used as an outgroup. Phylogenetic analyses were conducted using the GTR + I + G substitution model, as suggested by the Akaike Information Criterion through JModelTest 2.1.10[32]. A Maximum Likelihood Estimation (MLE) phylogenetic tree was constructed in MEGA11 [33] using 1000 bootstrap replicates.

3. Results

3.1. Haplotypes in Wild Populations and Their Phylogenetic Relationships

A total of 243 out of the 246 analyzed specimens yielded high-quality sequencing results; the remaining 3 were excluded from further analysis. The alignment of the 624 bp of COI resulted in the identification of six distinct haplotypes (H1 to H6, with GenBank accession numbers PQ764027–PQ764032), differentiated by 49 variable sites (Figure 2). Phylogenetic reconstruction using these haplotypes (Figure 3) showed that four haplotypes, H1 to H4, clustered together with all *S. laietanus* sequences from GenBank and were therefore assigned to this species. These four haplotypes are highly conserved, differing from one another by only a single nucleotide position. The *S. laietanus* sequences were phylogenetically close to sequences of *S. orpheus*. The haplotype H5 was observed in all our sequenced European chub specimens from the Moselle River basin and, as expected, clustered with other *S. cephalus* sequences retrieved from GenBank. The lineage including *S. cephalus* is clearly distinct from the one related to the Catalan chub. Finally, haplotype H6 clustered with sequences from *S. pyrenaicus* in a different lineage that grouped other native chub species from the Iberian Peninsula.



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	6	8	2	8	9	1	2	2	5	6	6	6	7	9	0	0	2	2	2	4	4	6	6	6	9	9	0	0	1	2	4	5	5	6	8	9	0	1	1	4	4	5	5	7	9	0	0	1	2
	9	1	3	0	5	6	2	8	5	1	2	4	9	6	3	9	1	4	7	2	5	0	3	9	6	9	2	8	4	9	4	3	6	2	3	5	7	6	9	4	6	2	3	0	4	0	6	5	1
H1	A	T	C	A	T	A	T	A	C	T	T	A	G	C	T	C	G	A	G	G	T	T	C	A	G	A	C	G	G	A	T	T	C	G	A	T	G	G	G	C	A	C	T	A	T	C	C	G	G
H2	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H3	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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H5	.	C	T	.	.	.	C	.	.	.	C	.	A	.	.	T	.	G	A	.	.	.	.	G	A	C	T	A	A	G	.	.	T	A	.	.	A	.	A	T	.	T	.	G	.	.	.	.	.
H6	.	C	T	G	C	G	C	G	.	C	C	G	A	.	C	T	A	G	A	C	C	C	T	G	A	T	.	A	A	.	C	C	T	.	G	C	.	A	A	T	G	T	C	.	C	T	T	A	A

Figure 2. Variable nucleotide positions for the COI gene among the haplotypes observed in this study.

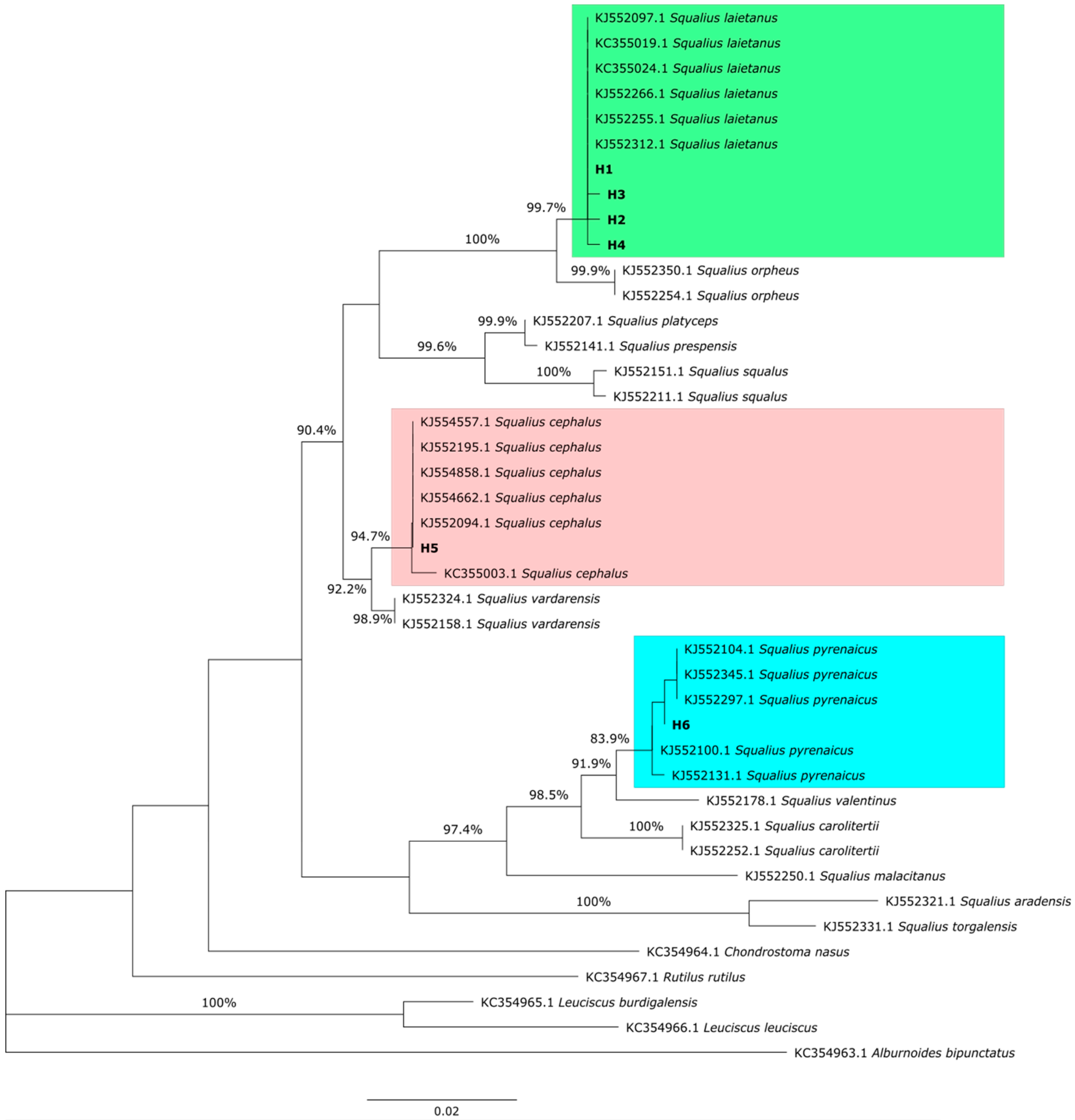

Figure 3. Maximum likelihood phylogenetic tree of the six haplotypes described in this study (in bold) and reference sequences from GenBank. Bootstrap values above 80% are shown. Colors indicate the lineage for *S. laietanus* (green), *S. cephalus* (red), and *S. pyrenaicus* (blue).

3.2. Haplotype Distribution Throughout Catalan Basins

The most prevalent haplotype was H1, observed in 148 out of 243 specimens (60.9%) and across 11 of the 15 sites studied in Catalonia. This haplotype distribution confirmed the presence of the mitochondrial lineage of *S. laietanus* in all the studied basins (Figure 1, Table 2). In the Muga River basin, the northernmost area within the study region, all individuals sampled from the upper stream site at Albanyà (MMA) shared the H2 haplotype of *S. laietanus*, the only studied Catalan chub population that clearly differed from the rest. H3 was found in one *S. laietanus* specimen collected from the Francolí River, and H4 was obtained in a single individual from the Tordera River. All the remaining individuals from these two sites showed the haplotype H1. H5, corresponding to *S. cephalus*, was identified at six sites, representing 26.3% of the chub specimens. These sites, where the mitochondrial lineage of the European chub was identified, are situated within four river basins: the Muga, Besòs, Llobregat, and Ebro basins. In the last basin, there were more specimens with the H5 haplotype than those that were assigned to *S. laietanus*. All individuals sampled at the LMA site in the Llobregat River basin also displayed the H5 haplotype. Finally, H6, belonging to *S. pyrenaicus*, was identified in all the individuals sampled from Canaletes River, a tributary of the Ebro River.

Table 2. Haplotype abundances for the study sites. N: number of individuals sequenced. Site codes are listed in Table 1.

Territory	Basin	Site Code	N	*S. laietanus*				*S. cephalus*	*S. pyrenaicus*
				H1	**H2**	**H3**	**H4**	**H5**	**H6**
Catalan basins	Muga	MMC	16	4				12	
		MMA	15		15				
	Fluvià	FFV	14	14					
		FBB	16	16					
	Ter	TTG	16	16					
	Tordera	TTH	15	14		1			
	Besòs	BRR	16	7				9	
	Llobregat	LAA	15	13				2	
		LMA	16					16	
		LRA	16	16					
	Gaià	GGA	16	16					
	Francolí	FRV	26	25			1		
	Ebro	EEB	16	7				9	
		ECH	14						14
		ESL	16					16	
Luxembourg	Moselle		22					22	

4. Discussion

This study represents the first extensive analysis on the distribution of the mitochondrial lineage of the Catalan chub (*S. laietanus*) in the core of its distribution range. This analysis includes the main river systems of this region. Using the COI gene sequences as a barcode, we obtained results demonstrating the presence of the mitochondrial lineage of this species in all the basins studied. Nevertheless, at some sites, we detected the presence of the mitochondrial lineage of the European chub (*S. cephalus*), for which the corresponding specimens displayed the haplotype H5, the same haplotype that was found in the specimens from the Moselle River in Luxembourg. This haplotype was shared by more than 25% of the specimens collected in this study (Table 2), and it was distributed across 6 of the 15 (40%) study sites in Catalonia. These sites were in some of the most important regional river basins, i.e., the Muga, the Besòs, the Llobregat, and the Ebro, which altogether cover almost half of the native range of *S. laietanus*. In a single site from the Ebro basin, we detected the H6 haplotype, typifying the Iberian-endemic Tagus chub, *S. pyrenaicus*. This species was already reported in a site in the Matarraña River, another close tributary of the Ebro in this area [15]. The origin of these Tagus chub populations in the Ebro River basin is uncertain. As indicated by the presence of *S. cephalus* haplotypes in the range of *S. squalus* in the Po basin in Switzerland [34], the observation of the Tagus chub in the Ebro basin could represent recent human-mediated translocations from geographically close tributaries of the Tagus basin or river captures. Anglers were involved in the introduction of the bleak (*Alburnus alburnus*) into the Ebro in 1992 and in the rapid spread of this species to other major Iberian rivers [35]. In fact, the information available at the Spanish Ministry of Ecological Transition on the distribution of the Tagus chub (*S. pyrenaicus*) suggests that its presence in the Matarraña River of the Ebro basin could be due to an artificial introduction [36]. River captures (where one river, primarily through the erosion of its headwaters, reaches the flow of a neighboring one) have occurred since the end of the Pliocene and have involved some tributaries of the Ebro basin and the upper reaches of rivers on the southern side of the Iberian chain, that is, in the distribution area of *S. pyrenaicus* [37,38]. It was unexpected that neither of the two Ebro tributaries sampled in this study (Segre and Canaletes rivers) contained *S. laietanus* haplotypes because according to Doadrio [23], the distribution of *S. laietanus* within the Ebro River is confined to its lower course, mainly in the Matarraña and Segre rivers.

The European chub (*S. cephalus*) was reported to exist throughout the whole Ebro basin before the description of *S. laietanus* as a new species. Since then, the European chub has been considered non-native in the Iberian Peninsula [24], but its reported high abundance in this study may open a door to the native occurrence of the European chub in this basin. Nevertheless, in contrast to the four COI haplotypes detected for *S. laietanus* in our study, only a single *S. cephalus* haplotype (H5) was observed among the four river basins where this species was detected in Catalan rivers. This haplotype was shared with all the specimens from distant sites in the Moselle River in Luxembourg and with sequences retrieved from GenBank from sites in the French basins of the Agly and Massane rivers, which are considered to be within the range of *S. laietanus* [16,18]. Therefore, the origin of *S. cephalus* in the study area may be more related to translocation for recreational fishing, as it is difficult for anglers to distinguish between these two chub species. However, we must keep in mind that the COI gene has been widely used as a DNA barcode due to its low sequence diversity within species but significant divergence between them [26], and therefore it might not be suitable for resolving the origins of these European chub populations. In fact, our H5 haplotype matches the GenBank sequences described for the European chub in many other rivers in France, Germany, Switzerland, and Austria [34,39,40]. Sequences at more variable mtDNA regions can be more effective for determining the origins of the European and Tagus chub populations introduced in the range of the Catalan chub. In our study region, for instance, the cyt b gene was utilized to trace the source of the introduced American mosquitofish, *Gambusia holbrooki* [41], and the mitochondrial control region to depict the phylogenetic relationships between brown trout (*Salmo trutta*) populations in Spain and those present in the rest of Europe.[42].

As mentioned above, four haplotypes were observed for *S. laietanus*. Haplotype H1 probably represents the ancestral haplotype of the Catalan chub due to its higher frequency, greater distribution, and closer identity to H5 of *S. cephalus* (94.5%) and *S. pyrenaicus* (93.4%). Haplotypes H3 and H4 were each observed in a single specimen, but H2 was shared by the fifteen specimens caught at the MMA site in the Muga River. The fact that a variant of the mtDNA COI gene, commonly used as a barcode because of its low intraspecific diversity, appeared fixed in one site probably reflects the historical isolation of Catalan chub populations among and within these rivers and a strong genetic drift associated with small effective sizes as a result of the historically irregular availability of fresh water in Mediterranean ecosystems and, more recently, human alterations to these habitats and fish communities [43]. Detailed information on the sizes of local populations of *S. laietanus* in Catalan rivers is scarce. In the Tordera River, population sizes ranged from an average of 7.24 ± 5.5 fish ha^{-1} in intermittent stretches to 111 ± 53.5 fish ha^{-1} in perennial stretches [44,45]. Abundances reported in other regional rivers are often lower (see Figure S4A in Appendix A of the Supplementary Materials of the paper by Murphy et al. [46]). In addition, reductions in chub occurrence in the study catchments ranged from 27% [43] to 69% [47] at the turn of the 21st century. The huge reduction in the total size of the Catalan chub induced by these threats in recent years led to its reassignment to the Vulnerable category of the IUCN Red List in 2024. Ongoing climate change will also stress these populations by increasing the intermittency of water flow in these Mediterranean rivers [44,45,48,49]. Worse conditions and lower gonadal weight have been reported for this species in intermittent streams [50], and pollution can reduce the number of vitellogenic oocytes in females and result in smaller seminiferous tubule diameters in males [51]. The Catalan chub is another example of how many vertebrate species described since 1990 have become endangered shortly after being described [52]. In fact, all *Squalius* species from the Iberian Peninsula described since 1990 are now listed in the threatened category of the IUCN Red List, with the exception of *S. gaditanus* and *S. tartessicus*, which were just described in 2023 [14] and have not yet been assessed.

Of additional concern for the Catalan chub and maybe for the other Iberian endemic chub species is its hybridization with phylogenetically related species. Our analysis showed the co-occurrence of *S. cephalus* and *S. laietanus* haplotypes in several of the study sites. Mitochondrial markers, such as COI, are maternally inherited and consequently cannot be employed to detect hybrid individuals when used without other sources of information, but using nuclear markers, Rose et al. [19] confirmed there was hybridization between Catalan and European chub in Southeastern French rivers, wherein COI gene sequencing revealed both species. Molecular methods have been used to verify hybridization between *S. alburnoides* specimens and other native and non-native (*A. alburnus*) cyprinids in the Guadalquivir River basin of the southeastern Iberian Peninsula [53]. Hybridization could lead to the dilution of the native gene pools of the Catalan chub; for example, the singular H2 haplotype was fixed in the MMA site of the Muga River, but a few kilometers downstream at the MMC site, more than 50% of the individuals displayed the H5 haplotype of *S. cephalus*. Certainly, *S. laietanus* was the only mitochondrial species detected in the Fluvià, Ter, Tordera, Gaià, and Francolí basins, but the presence of the European chub should not be completely excluded since, in this study, only a single site was revised for these basins, except for the Fluvià. Nevertheless, the lowland areas of the Muga, Fluvià, and Ter rivers are located in a plain with a complex network of irrigation channels, marshes, and lagoons that are interconnected, especially during floods, a factor that contributed to the successful invasion of these three basins by the Eastern mosquitofish *Gambusia hoolbrooki* [54].

In conclusion, this study provides the most detailed genetic survey of the distribution of the endemic species *S. laietanus* in Catalan river basins to date. This species was present in all the main regional river basins studied, but up to 25% of the collected chub specimens showed the mitochondrial lineage of *S. cephalus*. The potential co-occurrence of *S. laietanus* and *S. cephalus* in several study locations raises concerns about potential hybridization between them and thus the genetic integrity of *S. laietanus* populations in the basins studied. Further research is needed, including using both new locations and tributaries within the studied rivers, to refine the range of this species and the genetic population structure within and between rivers. The use of nuclear markers is required to confirm and quantify hybridization. Research addressing these questions will provide a better understanding of the observed pattern of mitochondrial variation, which is critical for managing and preserving Catalan chub populations. Until this understanding is obtained, a conservative strategy that minimizes and stabilizes the potential spread of the European chub is warranted. One possible strategy could be the designation of genetic refuges for the Catalan chub in the studied river sections for which there is no evidence of the existence of the European chub. This strategy has been successfully implemented for the management of the regional brown trout fisheries threatened by releases of non-native stocks [55,56]. The primary aim of these genetic refuges for chub conservation should be the prohibition of chub releases and translocations. As observed for brown trout, the acceptance of genetic refuges by anglers will be a pivotal factor in ensuring the conservation of the Catalan chub. It is therefore essential to educate anglers on the significance of preserving the genetic integrity of Catalan chub populations within the context of native biodiversity. Moreover, anglers should be engaged in the design of refuge areas. These refuges should not be exclusively associated with the prohibition of angling; rather, they should encompass strategies such as reducing captures or promoting catch-and-release practices.

Supplementary Materials: The following supporting information can be downloaded at www.mdpi.com/xxx/s1. Table S1: List of accession numbers of COI sequences included in the phylogenetic analysis and their respective countries of origin and species assignments.

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