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Genomic Diversity and Species Boundaries of the Chilean Silversides Fishes (Atheriniformes, Atherinopsidae)

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Abstract: Silverside fishes in Chile, abundant in marine and freshwater habitats, are classified in two genera: *Odontesthes* and *Basilichthys*. Both genera have widespread distributions across southern South America, with marine origins. Despite extensive information on Chilean freshwater silversides and their overlapping distributions along a latitudinal gradient, their taxonomy and diversification remain contentious. This study examines the diversity of *Chilean silversides* using RADseq genomic data from 78 *Odontesthes* and 60 *Basilichthys* individuals, covering most of their range. The phylogenetic and structural analyses of approximately 20,000 SNPs reveal some geographic variation but indicate no differentiation between *Odontesthes mauleanum* and *O. brevianalis*. The genus *Basilichthys*, in contrast, presents a disjunct distribution, with populations in coastal rivers of Peru (*B. semotilus*) that are separated from Chilean populations by the Atacama Desert. Chilean *Basilichthys*, traditionally classified as *B. microlepidotus* and *B. australis* until 2012, also show no genetic differentiation consistent with species boundaries but exhibit latitudinal differences consistent with isolation by distance. The contrasting patterns of genetic differentiation exhibited by species of these genera may be explained by the more frequent exchange with marine species for *Odontesthes* that do not occur in *Basilichthys*, in addition to the recent geological history of glaciations affecting the southern range of their distribution.

Keywords: RADseq; population structure; latitudinal gradient

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1. Introduction

The freshwater fish fauna of Chile, due to its geographical isolation, exhibits notable differences compared to other American regions, characterized by low richness and a high level of endemism. Some species are restricted to two or three river basins [1–3]. Over the past 10 million years, the Chilean landscape has undergone significant changes due to geological and climatic processes [4], shaping the topography, ecosystems, and distribution of the vegetation distribution in different regions of the country. The gradual and continuous formation of the Andes Mountain range has been a key factor in this process, leading to the formation of valleys, mountains, and volcanoes in the region. This mountain range has significantly influenced climatic patterns and the distribution of water resources in Chile. In the northern area, the dry and arid climate gave rise to the formation

of the Atacama Desert, considered one of the driest deserts in the world. However, there have also been climatic fluctuations and interglacial episodes with wetter periods, allowing for the existence of temporary vegetation and water bodies in the region. In the southern region of Chile, Patagonia has witnessed dramatic changes in the extent of glaciers and vegetation patterns over the last 10 million years. These periods have been marked by glacier advances and retreats, leaving a significant imprint on the landscape, as evidenced by rivers fed by melting ice and the formation of lakes, providing new habitats [5,6]. Glacier-related phenomena directly influence the distribution of genetic diversity through population size reductions (resulting in genetic bottlenecks) and the displacement of entire populations and indirectly through their effects on landscapes, such as the rearrangement of hydrographic basins [7]. The river hydrology and geology may have influenced the evolution of the relatively small body sizes of most native fish species and the ability to ascend streams above 1000 m in altitude, except for those found in systems of the high Andean lands [1].

The chilean silverside is represented in the region by the family Atherinopsidae, tribe Sorgentinini. Two genera of this family are found in Chile, *Odontesthes* Evermann & Kendall 1906, which has protractile maxillaries, and *Basilichthys* Girard 1855, without a protractile mouth, which separates it clearly from the other genus [8]. Both taxa are widely distributed in the region and have been associated with a marine origin due to their high salt tolerance. Information on species of these genera is abundant; however, the taxonomy, distribution, evolution, and diversification processes in this group are still controversial. *Basilichthys* is restricted to the western slopes of the Andes Range in Chile and Peru, while *Odontesthes* has marine and freshwater species that inhabit both Pacific and Atlantic slopes and coastal areas. Previous phylogenetic analyses and ancestral habitat inference have suggested that these genera originated in a euryhaline habitat around 22 Ma [9]. In contrast, Hughes et al. [10] suggested that the ancestral distribution area for the same ancestor was the Pacific Ocean. More generally, some studies suggest that marine-to-freshwater habitat transitions might have accelerated the evolutionary rates of diversification in many fish lineages [11–13], giving rise to the classical theory called "Ecological Opportunity" [14,15]. This theory attempts to explain the high diversity of freshwater fish species existing today based on the idea that habitat changes promote speciation, morphological variation, and resource partitioning, thus giving rise to further diversification or adaptive radiations. Changes in habitat styles and distribution areas seem to play an important role in the evolution of the Sorgentinini tribe [9,10,16,17].

Two freshwater species of the *Odontesthes* genus live in Chile [18]: *Odontesthes mauleanum* (Steindachner 1896) is commonly found in Andean lakes and deep rivers from the Maule River basin in the north to Lake Llanquihue in the south [8]. In lakes, this species feeds mainly on zooplankton, while in rivers it usually feeds on benthic macroinvertebrates [19]. Like other Atherinids it has rapid growth, reaching 60–100 mm in the first year of life; growth predictions show a total length of 30 to 40 cm at eight years. The second species that lives in Chile is *Odontesthes brevianalis* (Günther 1880), a silverside that lives in estuarine and coastal river systems in Chile from La Serena in the north to Chiloé Island in the south [8], eating small mollusks and zooplankton. It has been reported to reach up to 40 cm in total length at four year [18]. Mature specimens have been found in spring, but detailed information on their reproduction is still lacking [20]. The morphological distinction between these two species in the field is problematic—the only diagnostic trait is the presence of scales that are noticeably crenulate along the side of the body (*O. mauleanum*) or only on the caudal peduncle (*O. brevianalis*) [8]. An earlier molecular study based on mitochondrial DNA (mtDNA) and RADseq data [10] reported no segregation of mtDNA haplotypes between these two species and some differentiation among populations

based on RADseq data but with uncertain support for species boundaries due to limited taxon sampling.

Currently, two species of the genus *Basilichthys* are recognized in freshwater habitats on the western versant of the Andes in Chile: *B. microlepidotus* (Jenyns 1841) and *B. semotilus* (Cope 1874). The distribution of *B. semotilus* ranges from the Reque River in Perú to the Loa River in Northern Chile, whereas *B. microlepidotus* is found from Central Chile (Huasco River) south to Chiloé Island [8]. Previously, a third species was recognized (*B. australis* Eigenmann 1928) for populations located to the south of the Aconcagua basin, but Veliz et al. [21] showed that this species and *B. microlepidotus* share the most common mitochondrial haplotypes. Morphologically, these two species also are hard to distinguish based on the presumed diagnostic characteristics, such as the number of scales along the lateral line or the number of rays in the fins. For these reasons, *B. microlepidotus* is considered the valid species based on priority and *B. australis*, a junior synonym [21]. As with most atherinids, *B. microlepidotus* grows rapidly, reaching around 12 cm in total length in the first year of life, and begins to reproduce in the first year [22,23]. It reaches a maximum total length of 30 cm. Spawning begins in spring when the water temperature reaches 15 °C and continues throughout the spring and summer months. Maturation begins at one year of age. It feeds on benthos and is a predator of aquatic macroinvertebrates [24].

The silversides, *Odontesthes brevianalis*, *O. mauleanum*, and *Basilichthys microlepidotus*, are some of Chile's endemic species currently classified as Vulnerable on the Red List of Threatened Species [25] due to the presence of invasive species and habitat fragmentation [18,26]. However, information on these species remains scarce, and population analyses are still lacking. Defining the taxonomic boundaries of a species can have significant repercussions not only for science but also for conservation strategies, especially for threatened species, as demonstrated based on these silversides in Chile. Taxonomic controversies hamper the effective implementation of conservation actions, such as population management and habitat restoration. This confusion can hinder the accurate identification of at-risk populations and the assessment of their conservation status. Therefore, addressing these taxonomic barriers is crucial to strengthen the relevance of conservation biology studies, thereby ensuring appropriate and effective management strategies for the protection of vulnerable species of commercial interest. The silverside, in particular, has been found to be affected by pollution, driven by land use, industrial operations, and urban growth, in Chilean freshwater ecosystems [27].

Hence, the objective of this study is to analyze the diversity within and among species of *Chilean silversides* using genomic data (RADseq) to better characterize the genetic boundaries between species. Our first aim is to investigate the genetic differentiation between the species *O. brevianalis* and *O. mauleanum*, which are very difficult to distinguish morphologically. The second aim is to confirm the lack of differentiation between *B. australis* and *B. microlepidotus*. These analyses include an assessment of the population structure for all species.

2. Materials and Methods

2.1. Study Area and Sample Collection

Samples were collected by seining between 2004 and 2007, with permission from the National Authority granted to EMH, and immediately killed via an overdose of MS-222, with fin clips preserved in 95% ethanol for DNA extraction. For this study, we analyzed 79 individuals of *Odontesthes* collected from 11 localities and 60 individuals of *Basilichthys* from 19 localities spanning their distributional range in Chile (Figure 1 and Figure S1, Table S1). The initial assignment of individuals of *Odontesthes* to a species was based on the locality: estuarine or coastal fishes were assigned to *O. brevianalis*, while individuals from lakes and deep rivers were assigned to *O. mauleanum* [8]. Outgroup taxa (*O. regia*) were

obtained from marine coastal sites. For the genus *Basilichthys*, we assigned individuals into North and South groups based on the collection locality, while intermediate localities in the Aconcagua and Maipo River basins where the distribution of *B. microlepidotus* "north" and *B. microlepidotus* "south" overlapped were initially labeled as *Basilichthys* sp. Outgroup taxa (*O. semotilus*) were obtained from the Loa River.

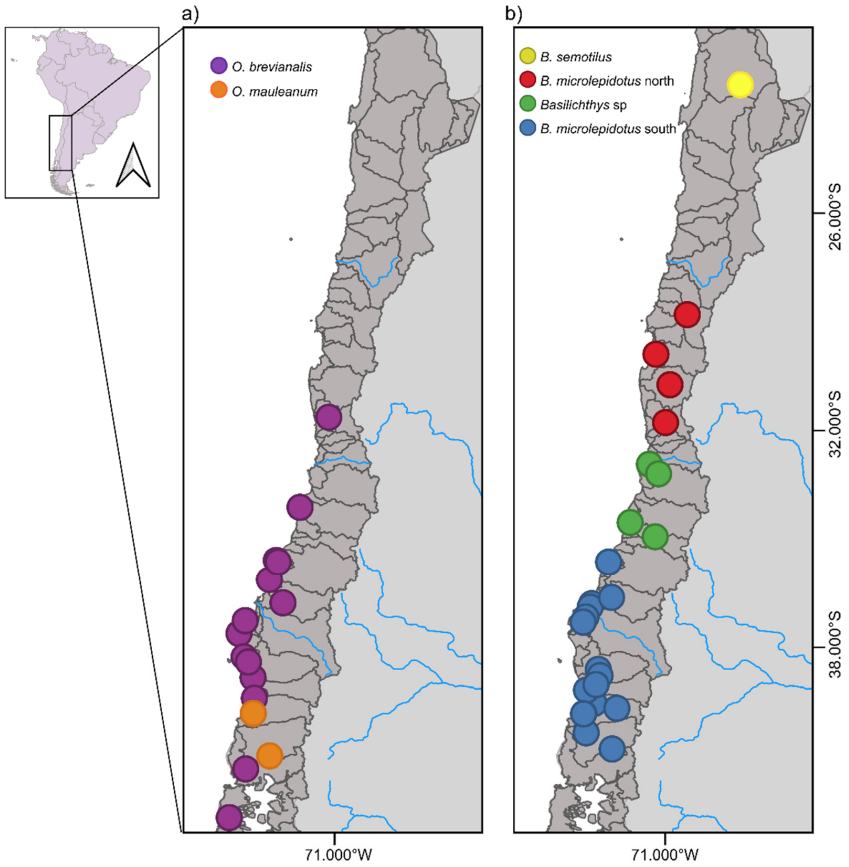


Figure 1. Map showing the sample localities of each species in Chile. (**a**) *O. brevianalis* n = 52, *O. mauleanum* n = 27. (**b**) *B. semotilus* n = 4; *B. microlepidotus* north n = 7; *B.* sp n = 10; and. *B. microlepidotus* south n = 39.

2.2. ddRAD Sequences

Genomic DNA was extracted in 96-well-plate format on a Gene Prep (Autogen, Holliston, MA, USA), following manufacturer's instructions, at the Laboratory of Analytic Biology at the National Museum of Natural History (Washington, DC, USA). Genomic libraries were generated following the original ddRAD sequencing protocol [28] at the University of Virginia Sequencing and Genomics Core Facility (Virginia, VA, USA), modified to use *Mse*I and *Pst*I restriction enzymes and a 350–550 bp size selection to be compatible with previous ddRADseq data generated for *Odontesthes* [10]. Paired-end libraries were sequenced on two lanes of an Illumina HiSeq 4000 at the University of Chicago Genomics Facility (Chicago, IL, USA). Sequences were demultiplexed, quality filtered, and assembled into ddRAD loci using ipyrad v. 0.7.30 [29] using the following settings: assembly method de novo, 5 as Max low quality base calls, 0.02 as Max # SNPs per locus, 5 as max low-quality base, 0.5 of Max heterozygous sites per locus, 6 of mindepth_majrule, and 0.85 of clust_threshold. We used four different datasets (Table 1). The first includes two species of *Odontesthes*: *O. brevianalis* y *O. mauleanum* and an outgroup that was composed of *O. regia*, n = 9. The second includes only individuals of *O. brevianalis* and *O. mauleanum*. The third dataset includes *B. semotilus*, *B. microlepidotus* (north and south), and *Basilichthys* sp. And the fourth scheme comprised the third dataset without *B. semotilus* (Table 1). We performed

additional filtering on datasets 2 and 4 (Table 1) with VCFtools v.0.1.16 [30], adjusting for missing data (–max-missing 0.75 for *Odontesthes* and 0.98 for *Basilichthys*) because some analyses are more sensitive to missing data.

Table 1. Summary of the RADseq datasets used in the downstream analysis. *n*: number of individuals; min. samples per loci: minimum number of samples per loci; Par. Inf.: parsimony informative sites; Sing. sites: singletons sites; Dist. patt: distinct patterns.

Dataset	*n* (min. Samples Per Loci)	Species (*n*)	Loci	SNPs	Missing Sites (%)	Fit Model BIC	Par. Inf.	Sing. Sites	Dist. Patt.	SNPs VCF	Re-Construction
1	86 (40)	*O. brevianalis* (52); *O. mauleanum* (27) + *O. regia* (7)	12,009	26,110	46.56	TVMe + ASC + R3	8545	3766	12,130	610	IQTree (Figure 2a)
2	79 (40)	*O. brevianalis* (52); *O. mauleanum* (27)	14,338	22,025	43.28	TVM + F + ASC + R3	4361	2635	6957	227	SplitsTree (Figure 3a), LEA and DAPC (Figure 4a), PCA (Figure 5a), BFD (Table 2)
3	60 (54)	*B. semotilus* (4); *B. microlepidotus* north (7); *B. microlepidotus* south (39); *B.* sp. (10)	6843	16,155	7.69	TVM + F + ASC + R2	8417	1279	6606	987	IQTree (Figure 2b)
4	56 (49)	*B. microlepidotus* north (7); *B. microlepidotus* south (39); *B.* sp. (10)	13,680	25,596	9.53	TVM + F + ASC + R3	6793	3404	10,111	1198	SplitsTree (Figure 3b), LEA and DAPC (Figure 4b), PCA (Figure 5b), BFD (Table 2)

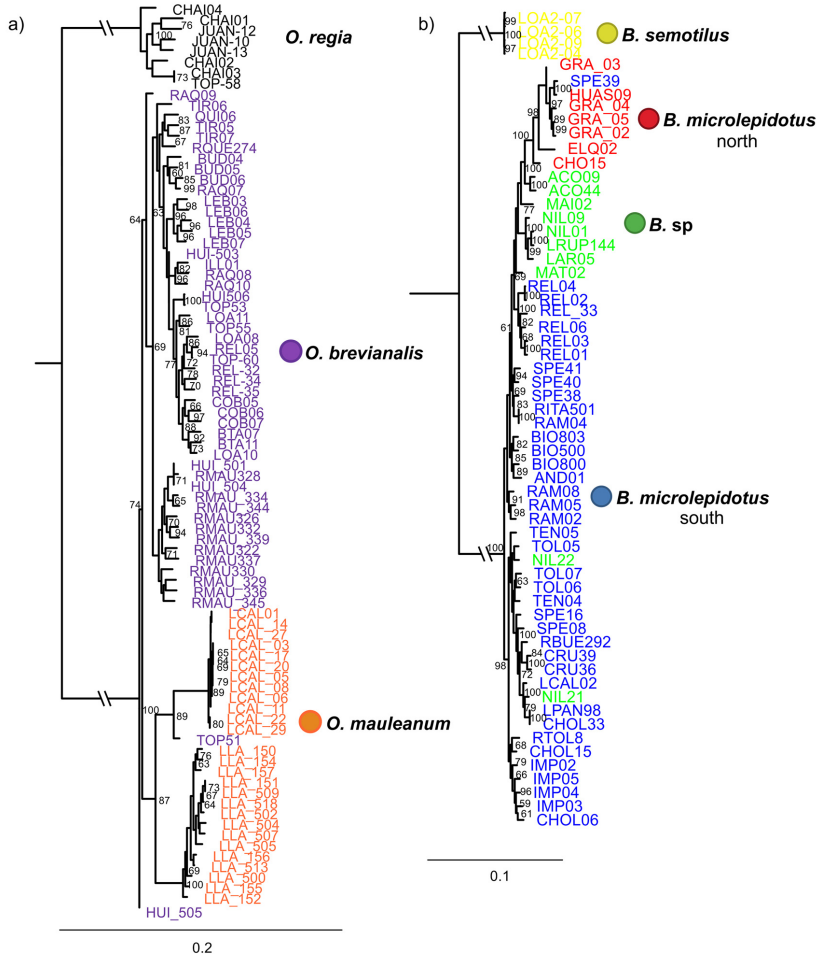


Figure 2. Maximum likelihood trees: (**a**) for *Odontesthes brevianalis* and *O. mauleanum* based on 86 individuals and 26,110 SNPs; (**b**) for the genus *Basilichthys* based on 60 individuals and 16,155 SNPs. The UFBoot values are shown in the nodes excluding those less than 60.

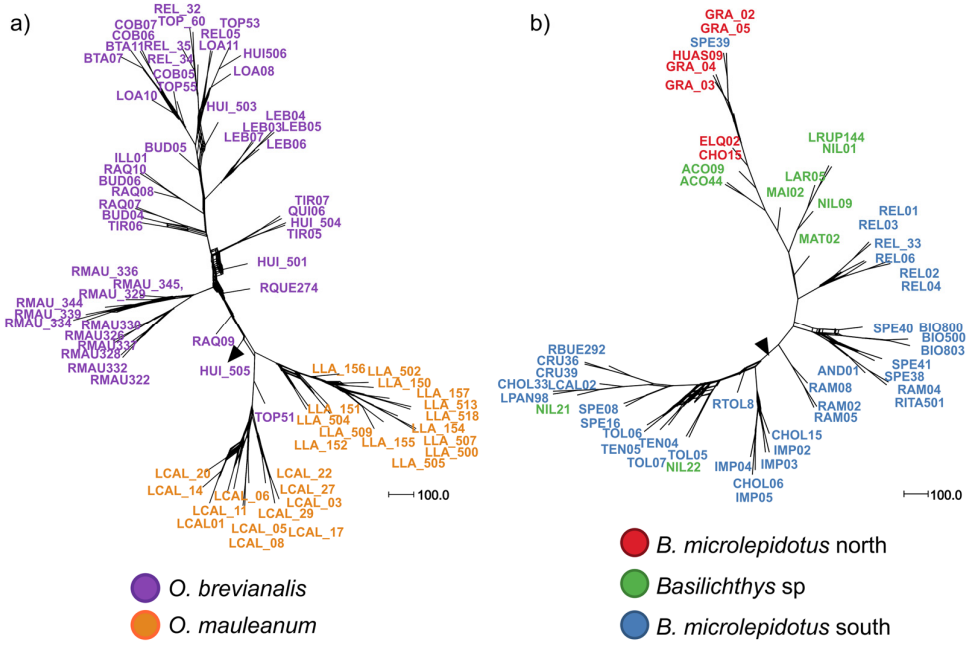


Figure 3. Phylogenetic networks. (**a**) *Odontesthes brevianalis* and *O. mauleanum*; (**b**) *Basilichthys microlepidotus* north, *B. microlepidotus* south, and *Basilichthys* sp. Arrowheads show the putative root for each network according to the phylogenetic results shown in Figure 2.

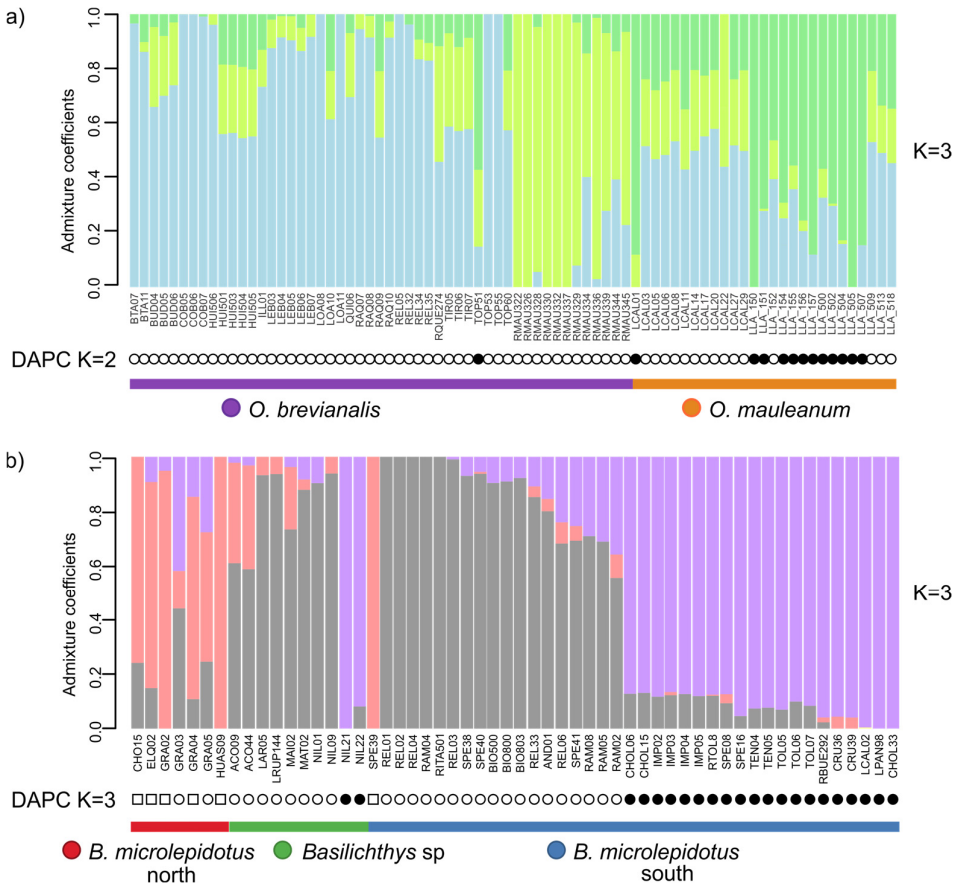


Figure 4. Structure and DAPC. (**a**) *Odontesthes brevianalis* and *O. mauleanum*; (**b**) *Basilichthys microlepidotus* north, *B. microlepidotus* south, and *Basilichthys* sp.

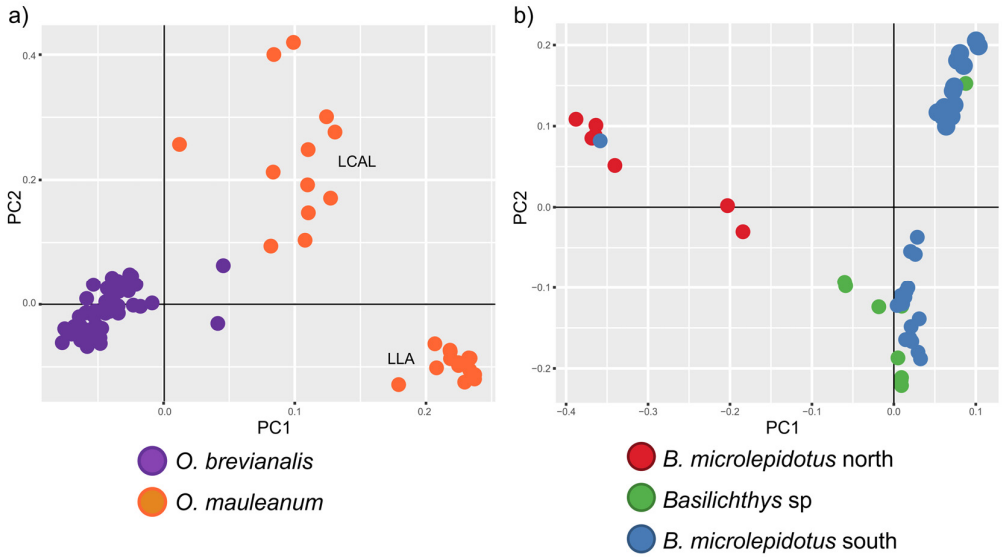


Figure 5. PCA for species. (**a**) *Odontesthes brevianalis* and *O. mauleanum*; (**b**) *Basilichthys microlepidotus* north, *B. microlepidotus* south, and *Basilichthys* sp.

Table 2. BDF results. Stepping stone sampling results for six species delimitation models. O. brev: *Odontesthes brevianalis*; O. mau: *O. mauleanum*; B. south: *Basilichthys microlepidotus* south; B. north: *B. microlepidotus* north; DAPC: Principal Component Discriminant Analysis; K: cluster; STR: Structure Analysis; MLE: marginal likelihood estimation; BF: Bayes factor.

Genus	*n* Indiv	*n* Species	Model	MLE	BF	2 × ln (BF)	Rank
Odontesthes	65	2	O. reg + O.mau/O.brev	−799.19			1
		3	O. reg + O. mau + O. brev	−2089.368	2580.357	15.711	2
		2	O. brev + O. mau	−3755.274	-	-	3
	79	2	DAPC: K1 + K2	−570.828	6368.891	17.518	2
		3	STR: K1 + K2 + K3	−503.57	6503.407	17.56	1
Basilichthys	59	2	B. semo + B. micro/B. sp	−14,164.594	-	-	4
		4	B. semo + B. micro north + B. micro south + B. sp	−13,630.528	1068.1311	13.957	3
		4	B. semo + DAPC: K1 + K2 + K3	−13,321.713	1685.761	14.859	2
		4	B. semo + STR: K1+ K2 + K3	−13,104.867	2119.453	15.318	1
	56	3	B. north + B. south + B. sp	−24,156.608	-	-	3
		3	DAPC: K1 + K2 + K3	−23,440.831	1431.553	14.533	2
		3	STR: K1 + K2 + K3	−23,047.263	2218.691	15.409	1

2.3. Data Analyses

Phylogenetic reconstructions were run using datasets 1 and 3: *Odontesthes* + outgroup and all *Basilichthys* species.

Concatenated ddRAD loci were analyzed based on Maximum Likelihood using IQ-Tree [31]. We selected the best-fitting model with ModelFinder [32] and generated 1000 UF-Boot replicates to assess branch support [33]. The ASC option was used to correct for an

ascertainment bias in the likelihood calculations, given that SNP assemblies consist only of variable sites.

All subsequent analyses were carried out on datasets without an outgroup for *Odontesthes* (dataset 2) and without *B. semotilus* for the genus *Basilichthys* (dataset 4). A phylogenetic network was estimated using SplitsTree4 v.4.17.1 [34] with the best-fitting model and the Neighbour-Net algorithm, and statistical support for branches was calculated using 1000 replicates.

Additional downstream analyses were conducted in R [35]. To evaluate and identify structures or subgroups within a species or group of species, we performed a structure analysis using the LEA 3.20.0 package [36] with the "snmf" function, which estimates the optimal number of groups based on the criterion of entropy. The cross-entropy criterion is based on the prediction of masked genotypes to evaluate the fit of a model with K populations. This criterion helps to choose the number of ancestral populations. A lower cross entropy value means better performance in terms of predictability. For this analysis, we tested from k = 2 to k = 10 groups.

Principal Component Discriminant Analysis (DAPC) was implemented in the Adegenet v.2.0 package [37]. This method does not make assumptions about population models but instead defines synthetic variables for which genetic variation is maximized between groups of individuals (K) and minimized within groups. For this analysis, we transformed the genepop file into a genind object with the "genepop_to_genind" function. Then, the "find.clusters" function was used to evaluate K-means ranging from 1 to 10, and the best supported number of clusters was identified with the Bayesian Information Criterion (BIC).

To eliminate potentially atypical SNPs that do not follow the assumption of neutrality in the population structure analyses, we performed a Principal Component Analysis (PCA) with the pcadapt package [38] from the VCF file obtained from ipyrad. Before performing the PCA, test statistics and *p* values were calculated based on the correlations between the SNPs and the first K principal components (PCs). Through this study, we analyzed how our individuals were distributed on a plane.

To assess the role of geographical distance on genetic structuration, we used the Mantel test [39] with the ade4 package [40]. For this end, we built a matrix of genetic distance by localities, with the hierfstat package [41], and a matrix of geographical distance. This was performed only for the populations of interest, datasets 2 and 4. We also constructed genetic distance matrices considering outgroups, *O. regia* for *Odontesthes* and *B. semotilus* for *Basilichthys* (datasets 1 and 3), in order to verify how distant these species were.

To explicitly assess species boundaries, we used Bayes Factors Delimitation (BFD; [42]) implemented in the SNAPP template of BEAST 2 [43]. This approach allows for the simultaneous comparison of models with different numbers of species and varying sample assignments to species. To achieve this, we conducted two separate analyses for each genus. First, we included outgroups (datasets 1 and 3) to validate the correct species delimitation by considering species with no taxonomic uncertainty. The second analysis focused exclusively on the species under study (datasets 2 and 4), where we evaluated several models varying between two and three species. These models were based on the following: (a) field-identified species, (b) DAPC groupings, and (c) structure groupings (see below). For each dataset, additional filtering was performed by removing missing data using VCFtools v.0.1.16 [30] (Table 1 "SNPs VCF"). This assembly was converted to NEXUS binary format in PGDSpider v.2.1.1.5 [44]. To estimate the marginal likelihood of each species delimitation model, we conducted five independent path sampling analyses ($\alpha = 0.3$) using 40 steps with an MCMC length of 300,000 generations and a pre-burnin of 30,000. We ranked the models by their average marginal likelihoods (MLEs) and calculated the Bayes Factors as BF = 2 × (MLE1–MLE0), where MLE0 corresponds to model A and

MLE1 to each alternative model [45]. A positive BF value indicates support in favor of model 1, while a negative BF value indicates support in favor of model 0. The strength of support for a model was obtained through the BF model selection statistic (calculated as $2 \times \ln$ (BF)), and assessed in the following manner: $0 \leq 2 \times \ln(BF) \leq 2$ reflects weak support for model 1; $2 \leq 2 \times \ln(BF) \leq 6$ reflects positive support; $6 \leq 2 \times \ln(BF) \leq 10$ reflects strong support; and $2 \times \ln(BF) \geq 10$ reflects decisive support [45].

Hughes et al. [10] recorded a recent mitochondrial capture of the common marine haplotypes of *O. regia* and *O. smitti* in a freshwater population from Lake Llanquihue of *O. mauleanum*, suggesting one or more instances of marine invasions or hybridization among species. To test the putative effect of such hybridization on our results based on a nuclear genomic signal, we repeated the phylogenetic reconstructions and the network and the structure analyses without this population (Figures S2–S4).

3. Results

Data Analyses

Table 1 shows the different datasets used for our analyses, with the number of individuals, species, total SNPs, number of loci, missing data, and different parameters taken from IQ-TREE such as the following: parsimony informative sites, singletons sites and distinct patterns.

The phylogenetic tree for the species *O. brevianalis* and *O. mauleanum* was rooted with *O. regia*. The best nucleotide substitution model that fit the data was TVMe + ASC + R3 according to BIC (Table 1). The reciprocal monophyly of *O. brevianalis* and *O. mauleanum* was not supported in our results (Figure 2a) due to a few misplaced samples from the localities TOP and HUI. The phylogenetic results for *Basilichthys* (tree with *Odontesthes* as the outgroup is shown in Figure S5) show that individuals identified as *B. microlepidotus* north and south and *Basilichthys* sp. formed a single clade but following a pattern corresponding to the latitude (Figure 2b), which is consistent with the geographical distribution of individuals (Figure 1b and Figure S1b).

To analyze genetic relationships on a finer scale, phylogenetic networks were generated based on the individuals of *O. brevianalis* and *O. mauleanum* (Figure 3a) and *B. microlepidotus* north, *B. microlepidotus* south, and *Basilichthys* sp. (Figure 3b). The results were consistent with the phylogenetic analyses (Figure 2), without a well-marked separation by species.

The structure analysis for *O. brevianalis* and *O. mauleanum* (Figure 4a, see also Figure S6a) supported three genetic groups: one cluster was represented by the specimens identified as *O. brevianalis* from RMAU (Maullin River) that were different from the rest of *O. brevianalis*; a second cluster was represented by individuals of *O.mauleanum* from LLA (Llanquihue Lake); a third cluster grouped individuals of *O. brevianalis* from all other localities. Individuals from LCAL (Calafquen Lake) had mixed ancestry combining the three clusters. The DAPC analysis supported two different clusters, one with the majority of *O. mauleanum* with the LLA label (Figure 4a), similar to the second cluster supported by the structure mentioned above.

Both the structure analysis and the DAPC for *Basilichthys* divided the dataset into three clusters (Figure 4b): the first was composed of most of the *Basilichthys* sp. and half of the *B. microlepidotus* south (grey color for structure and white circles in DAPC, see also Figure S6b); the second cluster grouped most *B. microlepidotus* north individuals and one individual of *B. microlepidotus* south (pink color in structure and white squares in DAPC) and, finally, a cluster with the remaining *B. microlepidotus* south, as well as two individuals of Basilichthys sp with an NIL label (from the Nilahue River).

PCA results agreed with previous analyses (Figure 5). For *Basilichthys* individuals (Figure 5b), *Basilichthys* sp. were closer to *B. microlepidotus* south than to *B. microlepidotus* north.

The Mantel test resulted in no significant correlation between genetic and geographic distances for the *Odontesthes* species ($p = 0.193$). However, a significant result was obtained for *Basilichthys* ($p = 0.003$), indicating isolation by distance among pairs of localities. The genetic distances (Table S2) observed between *O. regia* and all the localities of *O. mauleanum* and *O. brevianalis* were FST = 0.8, whereas the average of the genetic distance among all the localities of *O. mauleanum* and *O. brevianalis* was around FST = 0.38. For *Basilichthys*, the genetic distances between *B. semotilus* and all the localities of *B. microlepidotus* (both north and south) and *Basilichthys* sp. were above FST = 0.9, while the average distance among these localities (without *B. semotilus*) was FST = 0.44.

The results for the BDF* models, for *Odontesthes* and *Basilichthys*, are summarized in Table 2. For both *Odontesthes* and *Basilichthys* (with and without *B. semotilus*), the models based on the structure were the best, which considers that the three groups given by the structure analysis were the top-ranked model (highest MLE value).

4. Discussion

In previous studies, it has been seen that several species belonging to the genera *Odontesthes* and *Basilichthys* are difficult to distinguish both at the morphological and genetic levels [10,21]. That is why in this work we have analyzed these two sister genera throughout Chile using genomic data.

4.1. Odontesthes

Our results indicate that *Odontesthes mauleanum* and *O. brevianalis* do not form reciprocal monophyletic clades, which would be expected under traditional species-delimitation frameworks. In contrast, a clear differentiation with the marine species *O. regia* was observed, both in the genealogical analysis (Figure 2) and in the BDF test (Table 2) when *O. regia* was included. Structure and BDF analyses that did not include *O. regia* supported three clusters for samples of *O. mauleanum* and *O. brevianalis*, but these clusters did not coincide with nominal species boundaries (Figure 4a). Therefore, they are considered a signal of the population structure. Genealogical analyses also distinguished individuals from three localities: RMAU (from the Maullin River), LCAL (from the Calafquen Lake locality), and LLA (from the Llanquihue Lake) (Figures 2a and 3a). The structure analysis revealed a distinct grouping (Figure 4a), where individuals from LCAL shared genetic affinities with individuals identified as *O. brevianalis*. Meanwhile, the RMAU and LLA populations formed separate clusters. Not surprisingly, the BDF analysis (Table 2) showed that the grouping obtained based on the structure best defines the boundaries of the *Odontesthes* individuals under study.

DAPC results (Figure 4a) revealed only two clusters, with the main differentiation driven by the majority of LLA individuals. The Mantel test was not significant, primarily due to the high genetic distances (Table S3) observed in the LCAL population despite its small geographic separation. Collectively, these genomic data suggest that the separation between *O. brevianalis* and *O. mauleanum* is not supported, highlighting the complex population structure within these putative (nominal) species.

Hughes et al. [10] provided evidence of recent mitochondrial introgression, where marine haplotypes characteristic of *O. regia* were detected in a freshwater population of *O. mauleanum* from the Lake Llanquihue (LLA). Their biological data led them to propose that this phenomenon might have been caused by mitochondrial capture after a marine incursion into freshwater environments, resulting in a temporary secondary contact zone

between *O. mauleanum* and *O. regia*. These marine mitochondrial haplotypes appear to be widespread in Lake Llanquihue, a significant water body in Chile's Los Lagos District, situated around 22 km north of the Reloncaví Sound. The marine incursion into freshwater environments by *O. regia* and the temporary secondary contact with the LLA population may result in the genomic differentiation of this population with respect to others (which have not undergone hybridization with *O. regia*).

Lake Llanquihue has likely experienced glacial coverage by the Llanquihue Glacier on at least three occasions over the past 70,000 years, with the most recent glaciation concluding roughly 14,000 years ago [46,47]. Interestingly, the origins of the introgressed haplotypes may extend further back in time, potentially associated with the end of the colder Patagonian glaciation around 700,000 years ago or the larger Patagonian glaciation approximately 1.2 million years ago [48]. However, there is no concrete geological evidence supporting marine incursions during these glacial periods. Nevertheless, it is possible that some *O. regia* individuals may have colonized new areas (dispersal) or invaded new ecological regimes (resource abundance) in less saline environments. Silversides have great adaptability to different salinities. For example, a stable population of *O. argentinensis*, which is the marine species most widely distributed in the Atlantic Ocean, has been observed living inside the Mar Chiquita Lagoon (where the water is mixo-oligohaline) [17–50]. Genomic studies have shown that this population of *O. argentinensis* is capable of hybridizing with *O. bonariensis* (a freshwater species) when salinities are suitable for both species. This colonization of less saline bodies of water may also have occurred with *O. regia* individuals in Lake Llanquihue, thus facilitating secondary contact with the population of *O. mauleanum* that lives in the LLA.

Perhaps these secondary contacts with other species were more frequent than expected and caused gene mixing more than once. This could explain why the Mantel test was not significant for the populations of the *O. mauleanum* and *O. brevianalis* groups, and therefore, no isolation by distance was observed. Some of the populations (LLA, LCAL, RMAU) are very close geographically but are very different at the genomic level.

Despite these putative secondary contacts, the two closely related parental species (*O. regia* and *O. mauleanum*/*O. brevianalis*) are well differentiated morphologically and all individuals analyzed in this study were unambiguously assigned to either group based on genetic data analyses (Figure 2a and Figure S1). The highly supported reciprocal monophyly and a high FST value suggest that the hybridization rate between these groups is not sufficient to alter the genomic identity.

4.2. Basilichthys

Based on the results of the species tree for *Basilichthys* and the calculated FST values, we confirm the clear separation between *B. semotilus* and *B. microlepidotus* (Figure 2b and Figure S5). These findings are consistent with previous research based on nuclear and mitochondrial markers [9]. This evidence reinforces the taxonomic distinction between these species and supports the validity of the classification proposed thus far [21]. Unfortunately, the number of *B. semotilus* specimens analyzed in this study was very low compared to the species' extensive distribution range. Expanding the sampling to northern basins, including those in Peru, could reveal even greater diversity within the genus. Between the populations of *B. semotilus* and other *Basilichthys* species lies the Atacama Desert, a significant barrier to gene flow. The Loa River, where the *B. semotilus* specimens were collected, was historically considered "empty" of fish [51,52]. However, it is now known to be inhabited by *B. semotilus* and *Trichomycterus punctulatus* [2].

The results within *B. microlepidotus* from the network analysis, structure, DAPC, and PCA indicate some genetic differentiation among individuals, corresponding to their

geographical locations, particularly the latitude. The genealogical reconstruction (Figure 2b) and network analysis (Figure 3b) show that individuals identified as *B. microlepidotus* (north) consistently cluster at the opposite end from *B. microlepidotus* (south), with *Basilichthys* sp. positioned between them—a pattern consistent with the sampling map (Figure 1b). Thus, the genealogical relationships among individuals reflect the geographical proximity of the sampled localities. This result aligns with the significant Mantel test findings. Both the structure and DAPC analyses divided the data into three clusters, almost perfectly matching the field identification (*B. microlepidotus* north, *Basilichthys* sp. + some *B. microlepidotus* south, and the southernmost *B. microlepidotus* north). Regarding the BDF analysis for *Basilichthys*, it was also evident that the grouping obtained based on the structure best defines the boundaries of the individuals under study. These findings suggest that *B. microlepidotus* has undergone complex evolutionary and adaptive processes, with migration and dispersal playing critical roles. Despite the significant distances between some sampling sites, which belong to rivers that independently flow into the sea, *B. microlepidotus* appears to exhibit a stepping-stone distribution. As a freshwater species, it raises the question of how dispersal between basins occurs. Previous studies suggest that individuals may use marine routes for such movements [53].

On the other hand, *B. microlepidotus* also faces threats from invasive species, such as salmonids, and habitat fragmentation. However, its adaptability and genetic diversity provide some resilience against environmental challenges [54,55]. The species' ability to thrive in disturbed environments [56–60] may be a key strategy for its future survival. These findings underscore the importance of conserving *B. microlepidotus* populations, particularly in areas with high genetic diversity and more vulnerable populations.

5. Conclusions

The ichthyofauna of Chile is characterized by having a low richness, compared to other South American regions; this is due to its isolation caused by the Andes Mountain chain, which also gives it a high degree of endemism. Due to this high endemism, better knowledge at the population level of the species that inhabit this country is necessary to develop effective conservation plans and a new conservation classification status.

We have corroborated the synonymy of *B. microlepidotus* (valid species) with *B. australis*, and our results also suggest possible synonymy between *O. brevianalis* and *O. mauleanum*, which further reduces the number of species for this isolated region of the world. This genetic structure of these species is possibly the product of factors such as migration and natural selection. The geographic distribution and distance between populations seem to play an important role in the genetic differentiation of *Basilichthys* species but not for *Odontesthes* where secondary contact with other species was observed.

The results of this work contribute to the knowledge of the evolution and genetic diversity in the genus *Basilichthys* and the Chilean species of the genus *Odontesthes*, about which there is little information. In addition, they provide relevant information for the conservation of these species that are in a Vulnerable state of conservation. However, more research is needed to better understand the factors driving the genetic differentiation of silverside populations. Future research using more integrative approaches could be very useful for decision-making, such as the following: (i) comparing geographically separated populations from different habitat types (fjords, rivers, lakes, ocean); (ii) combining genetics with oceanographic/hydrological models that simulate currents and larval dispersal; (iii) and relating genetic differences to environmental variables (salinity, temperature, oxygen, pH, pollution) using genotype–environment association models (Redundancy Analysis–RDA, Latent Factor Mixed Models–LFMM).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d17050347/s1, Figure S1. Map of sampling points with labels of individuals. (a) *Odontesthes*; (b) *Basilichthys*; Figure S2. Maximum Likelihood *Odontesthes* tree without LLA (*O. mauleanum* of Llanquihue in Chile) individuals. Estimated under the TVMe + ASC + R2 model in IQ-Tree, with 1000 ultrafast bootstrap replicates; Figure S3. Phylogenetic network of *Odontesthes* tree without LLA (*O. mauleanum* of Llanquihue in Chile) individuals; Figure S4. Structure analysis of *Odontesthes* tree without LLA (*O. mauleanum* of Llanquihue in Chile) individuals; Figure S5. Maximum Likelihood *Basilichthys* tree with *Odontesthes* species as the outgroup. Estimated under the HKY + F + R3 model in IQ-Tree, with 1000 ultrafast bootstrap replicates; Figure S6. Map of sampling points colored by structure groups. (a) *Odontesthes*; (b) *Basilichthys*; Table S1. Table of water bodies under study with geographical and physicochemical characteristics. PPMA: maximum annual rainfall; QMA: maximum annual flow. Table S2. Matrix of genetic distances between pairs of localities for *Odontesthes*. (A) Including *O. brevianalis*, *O. mauleanum*, and *O. regia*; (B) including only localities of *O. brevianalis* and *O. mauleanum*. The highest values are highlighted in yellow; Table S3. Matrix of genetic distances between pairs of localities for *Basilichthys*. (A) Including localities of *B. microlepidotus* (north and south), *Basilichthys* sp., and *B. semotilus*; (B) including only localities of *B. microlepidotus* (north and south) and *Basilichthys* sp. The highest values are highlighted in yellow.

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References

1. Vila, I.; Fuentes, L.; Contreras, M. Peces Límnicos de Chile. *Boletín Mueso Nac. Hist. Nat. Chile* **1999**, *48*, 61–75. [CrossRef]
2. Dyer, B.S. Revisión Sistemática De Los Pejerreyes De Chile (Teleostei, Atheriniforme). *Estud. Oceanol.* **2000**, *19*, 99–127.
3. Habit, E.; Dyer, B.S.; Vila, I. Estado de Conocimiento de Los Peces Dulceacuícolas de Chile. *Gayana* **2006**, *70*, 100–112. [CrossRef]
4. Ramos, V. The Birth of Southern South America. *Am. Sci.* **1989**, *77*, 444–450.
5. Hulton, N.R.J.; Purves, R.S.; McCulloch, R.D.; Sugden, D.E.; Bentley, M.J. The Last Glacial Maximum and Deglaciation in Southern South America. *Quat. Sci. Rev.* **2002**, *21*, 233–241. [CrossRef]
6. Ruzzante, D.E.; Walde, S.J.; Cussac, V.E.; Dalebout, M.L.; Seibert, J.; Ortubay, S.; Habit, E. Phylogeography of the Percichthyidae (Pisces) in Patagonia: Roles of Orogeny, Glaciation, and Volcanism. *Mol. Ecol.* **2006**, *15*, 2949–2968. [CrossRef]
7. Zemlak, T.S.; Habit, E.M.; Walde, S.J.; Carrea, C.; Ruzzante, D.E. Surviving Historical Patagonian Landscapes and Climate: Molecular Insights from *Galaxias maculatus*. *BMC Evol. Biol.* **2010**, *10*, 67. [CrossRef]
8. Dyer, B.S. Systematic Revision of the South American Silversides (Teleostei, Atheriniformes). *Biocell* **2006**, *30*, 69–88.
9. Campanella, D.; Hughes, L.C.; Unmack, P.J.; Bloom, D.D.; Piller, K.R.; Ortí, G. Multi-Locus Fossil-Calibrated Phylogeny of Atheriniformes (Teleostei, Ovalentaria). *Mol. Phylogenet. Evol.* **2015**, *86*, 8–23. [CrossRef]

10. Hughes, L.C.; Cardoso, Y.P.; Sommer, J.A.; Cifuentes, R.; Cuello, M.; Somoza, G.M.; González-Castro, M.; Malabarba, L.R.; Cussac, V.; Habit, E.M.; et al. Biogeography, Habitat Transitions and Hybridization in a Radiation of South American Silverside Fishes Revealed by Mitochondrial and Genomic RAD Data. *Mol. Ecol.* **2020**, *29*, 738–751. [CrossRef]
11. Betancur-R, R.; Ortí, G.; Stein, A.M.; Marceniuk, A.P.; Alexander Pyron, R. Apparent Signal of Competition Limiting Diversification after Ecological Transitions from Marine to Freshwater Habitats. *Ecol. Lett.* **2012**, *15*, 822–830. [CrossRef] [PubMed]
12. Betancur-R, R.; Ortí, G.; Pyron, R.A. Fossil-Based Comparative Analyses Reveal Ancient Marine Ancestry Erased by Extinction in Ray-Finned Fishes. *Ecol. Lett.* **2015**, *18*, 441–450. [CrossRef] [PubMed]
13. Meier, J.I.; Stelkens, R.B.; Joyce, D.A.; Mwaiko, S.; Phiri, N.; Schliewen, U.K.; Selz, O.M.; Wagner, C.E.; Katongo, C.; Seehausen, O. The Coincidence of Ecological Opportunity with Hybridization Explains Rapid Adaptive Radiation in Lake Mweru Cichlid Fishes. *Nat. Commun.* **2019**, *10*, 5391. [CrossRef] [PubMed]
14. Ricklefs, R.E.; Schluter, D. Species Diversity: Regional and Historical Influences. In *Species Diversity in Ecological Communities: Historical and Geographical Perspectives*; Ricklefs, E.E., Schluter, D., Eds.; The University of Chicago Press: Chicago, IL, USA, 1993; pp. 350–363.
15. Losos, J.B. Adaptive Radiation, Ecological Opportunity, and Evolutionary Determinism: American Society of Naturalists E. O. Wilson Award Address. *Am. Nat.* **2010**, *175*, 623–639. [CrossRef]
16. Hughes, L.C.; Somoza, G.M.; Nguyen, B.N.; Bernot, J.P.; González-Castro, M.; Díaz de Astarloa, J.M.; Ortí, G. Transcriptomic Differentiation Underlying Marine-to-Freshwater Transitions in the South American Silversides *Odontesthes argentinensis* and *O. bonariensis* (Atheriniformes). *Ecol. Evol.* **2017**, *7*, 5258–5268. [CrossRef]
17. González-Castro, M.; Cardoso, Y.P.; Hughes, L.C.; Ortí, G. Hybridization Is Strongly Constrained by Salinity during Secondary Contact between Silverside Fishes (Odontesthes, Atheriniformes). *Heredity* **2022**, *129*, 233–243. [CrossRef]
18. Vila, I.; Habit, E. Current Situation of the Fish Fauna in the Mediterranean Region of Andean River Systems in Chile. *Fishes Mediterr. Environ.* **2015**, *2015*, 1–19. [CrossRef]
19. Klink, A.; Eckmann, R. Age and Growth, Feeding Habits, and Reproduction of *Cauque mauleanum* (Steindachner 1896) (Pisces: Atherinidae) in Southern Chile. *Stud. Neotrop. Fauna Environ.* **1985**, *20*, 239–249. [CrossRef]
20. Vargas, C.; Contreras, M.; Vila, I. Edad y Crecimiento de *Odontesthes brevianalis* (Günther 1880) En La Laguna Conchalí (31°53′; 71°32′), Chile. *Gayana* **2002**, *66*, 199–202. [CrossRef]
21. Véliz, D.; Catalán, L.; Pardo, R.; Acuña, P.; Díaz, A.; Poulin, E.; Vila, I. The Genus *Basilichthys* (Teleostei: Atherinopsidae) Revisited along Its Chilean Distribution Range (21° to 40° S) Using Variation in Morphology and MtDNA. *Rev. Chil. Hist. Nat.* **2012**, *85*, 49–59. [CrossRef]
22. Comte, S.; Vila, I. Modalidad Re- Productiva de *Basilichthys microlepidotus* (Jenyns) En El Río Choapa. (Pisces: Atherinidae). *An. Mus. Hist. Nat. Valparaíso* **1987**, *18*, 85–94.
23. Comte, S.; Vila, I. Spawning of *Basilichthys microlepidotus* (Jenyns). *J. Fish Biol.* **1992**, *41*, 971–981. [CrossRef]
24. Bahamondes, I.; Soto, D.; Vila, I. Hábitos Alimentarios de Las Espe—Cies de Atherinidae Del Embalse Rapel. *Medio Ambient.* **1979**, *4*, 3–18.
25. IUCN. The IUCN Red List of Threatened Species. Version 2024-1. Available online: https://www.iucnredlist.org (accessed on 28 June 2024).
26. Vila, I.; Veloso, A.; Schlatter, R.; Ramirez, C. *Macrófitas y Vertebrados de Los Sistemas Límnicos de Chile*; Editorial Universitaria: Santiago, Chile, 2006.
27. Cortés-Miranda, J.; Veliz, D.; Rico, C.; Vega-Retter, C. Copy number variations in response to chronic pollution: Basilichthys microlepidotus in central Chile. *Neotrop. Ichthyol.* **2025**, *23*, e240083. [CrossRef]
28. Peterson, B.K.; Weber, J.N.; Kay, E.H.; Fisher, H.S.; Hoekstra, H.E. Double Digest RADseq: An Inexpensive Method for de Novo SNP Discovery and Genotyping in Model and Non-Model Species. *PLoS ONE* **2012**, *7*, e37135. [CrossRef]
29. Eaton, D.A.R. PyRAD: Assembly of de Novo RADseq Loci for Phylogenetic Analyses. *Bioinformatics* **2014**, *30*, 1844–1849. [CrossRef]
30. Danecek, P.; Auton, A.; Abecasis, G.; Albers, C.A.; Banks, E.; Depristo, M.A.; Handsaker, R.E.; Lunter, G.; Marth, G.T.; Sherry, S.T.; et al. The Variant Call Format and VCFtools. *Bioinformatics* **2011**, *27*, 2156–2158. [CrossRef]
31. Nguyen, L.T.; Schmidt, H.A.; Von Haeseler, A.; Minh, B.Q. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274. [CrossRef]
32. Kalyaanamoorthy, S.; Minh, B.Q.; Wong, T.K.F.; Von Haeseler, A.; Jermiin, L.S. ModelFinder: Fast Model Selection for Accurate Phylogenetic Estimates. *Nat. Methods* **2017**, *14*, 587–589. [CrossRef]
33. Hoang, D.T.; Chernomor, O.; Von Haeseler, A.; Minh, B.Q.; Vinh, L.S. UFBoot2: Improving the Ultrafast Bootstrap Approximation. *Mol. Biol. Evol.* **2018**, *35*, 518–522. [CrossRef]
34. Huson, D.H.; Bryant, D. Application of Phylogenetic Networks in Evolutionary Studies. *Mol. Biol. Evol.* **2006**, *23*, 254–267. [CrossRef] [PubMed]

35. R Core Team. R: A Language and Environment for Statistical Computing 2023. Available online: https://www.R-project.org. (accessed on 28 March 2025).
36. Frichot, E.; François, O. LEA: An R Package for Landscape and Ecological Association Studies. *Methods Ecol. Evol.* **2015**, *6*, 925–929. [CrossRef]
37. Jombart, T. Adegenet: A R Package for the Multivariate Analysis of Genetic Markers. *Bioinformatics* **2008**, *24*, 1403–1405. [CrossRef]
38. Luu, K.; Bazin, E.; Blum, M.G.B. Pcadapt: An R Package to Perform Genome Scans for Selection Based on Principal Component Analysis. *Mol. Ecol. Resour.* **2017**, *17*, 67–77. [CrossRef]
39. Mantel, N. The Detection of Disease Clustering and a Generalized Regression Approach. *Cancer Res.* **1967**, *27*, 209–220. [CrossRef]
40. Dray, S.; Dufour, A.B. The Ade4 Package: Implementing the Duality Diagram for Ecologists. *J. Stat. Softw.* **2007**, *22*, 1–20. [CrossRef]
41. Goudet, J. HIERFSTAT, a Package for R to Compute and Test Hierarchical F-Statistics. *Mol. Ecol. Notes* **2005**, *5*, 184–186. [CrossRef]
42. Leache, A.D.; Fujita, M.K. Bayesian Species Delimitation in West African Forest Geckos (*Hemidactylus fasciatus*). *Proc. R. Soc. B Biol. Sci.* **2010**, *277*, 3071–3077. [CrossRef]
43. Bouckaert, R.; Vaughan, T.G.; Barido-Sottani, J.; Duchêne, S.; Fourment, M.; Gavryushkina, A.; Heled, J.; Jones, G.; Kühnert, D.; De Maio, N.; et al. BEAST 2.5: An Advanced Software Platform for Bayesian Evolutionary Analysis. *PLoS Comput. Biol.* **2019**, *15*, e1006650. [CrossRef]
44. Lischer, H.E.L.; Excoffier, L. PGDSpider: An Automated Data Conversion Tool for Connecting Population Genetics and Genomics Programs. *Bioinformatics* **2012**, *28*, 298–299. [CrossRef]
45. Kass, R.; Raftery, A. Bayes Factors. *J. Am. Stat. Assoc.* **1995**, *90*, 773–795. [CrossRef]
46. Clapperton, C.M. The Quaternary Glaciation of Chile: A Review. *Rev. Chil. Hist. Nat.* **1994**, *67*, 369–383.
47. Harrison, S.; Glasser, N.F. The Pleistocene Glaciations of Chile. In *Quarternary Glaciations—Extent and Chronology*; Ehlers, J., Gibbard, P.L., Hughes, P.D., Eds.; Elsevier B.V. Hera: Amsterdam, The Netherlands, 2011; pp. 739–756.
48. Ruzzante, D.E.; Walde, S.J.; Gosse, J.C.; Cussac, V.E.; Habit, E.; Zemlak, T.S.; Adams, E.D.M. Climate Control on Ancestral Population Dynamics: Insight from Patagonian Fish Phylogeography. *Mol. Ecol.* **2008**, *17*, 2234–2244. [CrossRef] [PubMed]
49. González-Castro, M.; Díaz De Astarloa, J.M.; Cousseau, M.B.; Figueroa, D.E.; Delpiani, S.M.; Bruno, D.O.; Guzzoni, J.M.; Blasina, G.E.; Deli Antoni, M.Y. Fish Composition in a South-Western Atlantic Temperate Coastal Lagoon: Spatial temporal Variation and Relationships with Environmental Variables. *J. Mar. Biol. Assoc. UK* **2009**, *89*, 593–604. [CrossRef]
50. González-Castro, M.; Rosso, J.J.; Mabragana, E.; Diaz De Astarloa, J.M. Surfing among Species, Populations and Morphotypes: Inferring Boundaries between Two Species of New World Silversides (Atherinopsidae). *Comptes Rendus—Biol.* **2016**, *339*, 10–23. [CrossRef]
51. Ringuelet, R.A. Zoogeografía y Ecología de Los Peces de Aguas Continentales de La Argentina y Consideraciones Sobre Las Áreas Ictiológicas de América Del Sur. *Ecosur* **1975**, *2*, 3.
52. Arratia, G.; Peñafort, M.B.; Menu-Marque, S. Peces de La Región Sureste de Los Andes y Sus Probables Relaciones Biogeográficas Actuales. *Deserta* **1983**, *7*, 7.
53. Quezada-Romegialli, C.; Fuentes, M.; Véliz, D. Comparative Population Genetics of *Basilichthys microlepidotus* (Atheriniformes: Atherinopsidae) and *Trichomycterus areolatus* (Siluriformes: Trichomycteridae) in North Central Chile. *Environ. Biol. Fishes* **2010**, *89*, 173–186. [CrossRef]
54. Habit, E.; Parra, O.; Valdovinos, C. Ictiofauna De Un Sistema Fluvial Receptor De Aguas Servidas: Respuestas a Una Nueva Planta De Tratamiento (Rio Quilque, Chile Central). *Gayana* **2005**, *69*, 94–103. [CrossRef]
55. Pardo, R.; Vila, I.; Capella, J.J. Competitive Interaction between Introduced Rainbow Trout and Native Silverside in a Chilean Stream. *Environ. Biol. Fishes* **2009**, *86*, 353–359. [CrossRef]
56. Cortes-Miranda, J.; Véliz, D.; Flores-Prado, L.; Sallaberry, M.; Vega-Retter, C. Genetic Diversity and Origin of a Fish Population Recently Colonizing a Reservoir: The Case of *Basilichthys microlepidotus*, Central Chile. *Popul. Ecol.* **2022**, *64*, 227–237. [CrossRef]
57. Vega-Retter, C.; Rojas-Hernández, N.; Cortés-Miranda, J.; Véliz, D.; Rico, C. Genome Scans Reveal Signals of Selection Associated with Pollution in Fish Populations of *Basilichthys microlepidotus*, an Endemic Species of Chile. *Sci. Rep.* **2024**, *14*, 15727. [CrossRef] [PubMed]
58. Vega-Retter, C.; Vila, I.; Véliz, D. Signatures of Directional and Balancing Selection in the Silverside *Basilichthys microlepidotus* (Teleostei: Atherinopsidae) Inhabiting a Polluted River. *Evol. Biol.* **2015**, *42*, 156–168. [CrossRef]

59. Vega-Retter, C.; Rojas-Hernandez, N.; Vila, I.; Espejo, R.; Loyola, D.E.; Copaja, S.; Briones, M.; Nolte, A.W.; Véliz, D. Differential Gene Expression Revealed with RNA-Seq and Parallel Genotype Selection of the Ornithine Decarboxylase Gene in Fish Inhabiting Polluted Areas. *Sci. Rep.* **2018**, *8*, 4820. [CrossRef]
60. Rojas-Hernandez, N.; Véliz, D.; Vega-Retter, C. Selection of Suitable Reference Genes for Gene Expression Analysis in Gills and Liver of Fish under Field Pollution Conditions. *Sci. Rep.* **2019**, *9*, 3459. [CrossRef] [PubMed]

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