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Genetic diversity and structure of *Brycon henni* in regulated and non-regulated water flow rivers of the Colombian Andes

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The sabaleta, *Brycon henni*, is a medium-size fish species found in the Andean range of the Magdalena-Cauca river basin in Colombia, where it is a fishery resource. Recruitment of sabaleta has affected construction of dams with unknown consequences on its genetic diversity and structure. Understanding the current genetic architecture in the dam-influenced areas compared to non-regulated water flow systems is crucial to diagnose the impact of human interventions and formulate proper management strategies. In this study, we evaluated the genetic structure and diversity of *B. henni* by using a set of microsatellites in individuals from 14 localities to identify the number of distinct genetic pools. We used various approaches to compare populations between regulated and non-regulated areas in the Magdalena-Cauca basin, and identified eleven well-differentiated and highly diverse population groups with marked genetic structures at local and regional levels. Genetic diversity of *B. henni* was very similar among non-regulated and dam-regulated water flow areas; however, one of the populations in non-regulated areas showed evidence of a founder effect associated with recent introductions. The effect of dams on the genetic structure of *B. henni* in the Magdalena-Cauca basin was negligible, probably due to their recent construction. Future reduction of genetic diversity and the loss of unique populations is expected due to the combined effect of geographic isolation and population declines. Thus, monitoring programs are necessary to detect changes in the genetic structure of fish populations to develop useful conservation actions.

KEYWORDS

Conservation, Dams, Magdalena-Cauca, Population genetics, Restocking

1 Introduction

The genetic structure of a population is shaped by historical processes in which evolutionary forces, the species' life history, and landscape features converge (Balloux and Lugon-Moulin, 2002). In freshwater systems, water network architecture and longitudinal fragmentation caused by waterfalls and rapids could imply ecological barriers that limit dispersal processes (Dias et al., 2013) which could lead to isolation as well as genetic and demographic differentiation (Phillipsen and Lytle, 2013). This limit of dispersal processes is particularly true in northern South America, in Colombia, where the Andes Mountains splits into three ranges separated by the valleys of the Magdalena and Cauca Rivers. Andean

topography and climate are highly diverse evidenced by the small spatial scales which lead to marked physical (Boschman, 2021) and ecological gradients (Benham and Witt, 2016). This ecological divergence promotes differentiation amongst populations, lineage diversification, and reproductive isolation (Frankham et al., 2012; Fjeldsa et al., 2012; Esquerré et al., 2019). In the Magdalena-Cauca river basin, this implies elevated levels of endemism (67.2%), where 158 (García-Alzate et al., 2021) of the 235 species reported in the basin are native (DoNascimiento et al., 2022).

The Magdalena-Cauca basin is an important center for economic development, where nearly 80% of the total population in Colombia is established. At least 50% of the nation's continental fishing programs are concentrated there (The Nature Conservancy, 2016), and it produces 90% of the Gross Domestic Product (GDP). These socio-economic conditions generate multiple pressures on the conservation of the fluvial network and on the aquatic fauna that have been described in multiple publications (Barletta et al., 2010; Patino and Estupinan-Suarez, 2016; Valencia-Rodríguez et al., 2022b). These anthropic pressures include overfishing, habitat fragmentation, and disturbance due to dams, residual water discharge, mining, and the introduction of exotic species (Patino and Estupinan-Suarez, 2016). Dams and dikes modify river flow and affect the interchange of material and energy with impacts on the composition and structure of populations (Ward et al., 2002). These physical barriers truncate migratory cycles and restrict dispersal among population groups (Earnest et al., 2014), leading to a decrease in the effective population size and the evolutionary potential (Brinker et al., 2018). Thus, the limited ability to respond to environmental changes could increase the risk of local extinctions (Liu et al., 2020). In some fishes, mainly migratory species, the interruption in the longitudinal connection of the water bodies generated by the construction of dams may cause strong fragmentation and structuring of populations over the course of several generations (Esguícero and Arcifa, 2010). In other cases, some hypothetical scenarios suggest a short-term homogenization followed by a long-term structuring in species that return to spawn in the stream where they were born (*homing behavior*) (Baggio et al., 2018).

The construction of dams to generate electricity in the Colombian Andes began in 1970. There are currently 25 dam producing over 100 MW of power and an undocumented number of smaller-scale dams (Angarita et al., 2018). Fragmentation due to the inclusion of the wall within the river channel, as well as other effects of dams on the composition of the ichthyofauna in the Magdalena-Cauca basin, have been described, but the impact on local fish populations remains to be systematically explored (Valencia-Rodríguez et al., 2022a; Martínez-Toro et al., 2022). In the Central mountain range, the landscape along the Porce River receives residual water discharge from the six-million-people city of Medellín, and its landscape has been highly transformed by agriculture, cattle farming, and urban, suburban, and rural expansion. In addition, two hydroelectric power plants in this river, built and operated by Empresas Públicas de Medellín, generate 1105 MW, which accounts for nearly 1% of the total installed hydroelectric capacity of Colombia.

Brycon henni Eigenmann, 1913, the focal species for this study, is an important fishery resource for local communities in Colombia Commercial fishing for *B. henni* is illegal throughout the country, as well as for recreational fishing in certain regions, (Santis et al., 2007). It occurs in lotic ecosystems with temperatures ranging from 18°C to 29°C (Builes and Uran, 1974) at altitudes between 300 m and 2400 m (GBIF, 2020) in clear, cold, and highly oxygenated waters (Builes and Uran, 1974; Hurtado-Alarcón et al., 2011; Restrepo-Escobar et al., 2016; Landínez-García and Márquez, 2020; Valencia-Rodríguez et al., 2021). In addition, this species has been reported as migratory for short distances (Builes and Urán, 1974; Lasso et al., 2010; Restrepo-Escobar et al., 2016), but its reproduction cycle is not entirely known. On the other hand, *homing behavior* has been suggested for this species (Landínez-García and Márquez, 2020). This recognition of birthplace usually results in selection of certain phenotypes and genotypes that are characteristic of a particular basin (de Campos Telles et al., 2011).

Despite the construction of several hydropower projects across the distribution range of *B. henni*, population genetic studies before such interventions have only been carried out in the Ituango area (Landínez-García and Marquez, 2020); thus, little evidence is available to contrast the historical and current population structure of *B. henni* in the dam-regulated water flow areas. In addition, a decrease in the number of captures of *B. henni* has been reported in the Porce II and III dams from 2011 through 2019 (Álvarez-Bustamante et al., 1969; Valencia-Rodríguez et al., 2022a), and implementation of stocking has been suggested as a measure to reduce environmental damage and guarantee food safety. However, these activities are expensive, complex, inefficient, and their impacts have not been sufficiently documented or monitored by environmental authorities (Mancera-Rodríguez, 2017). Furthermore, the impact of hybridization between historically isolated genetic stocks remains unknown.

Therefore, it is first necessary to understand the genetic structure and diversity of natural populations, recognize evolutionary and management units, and predict their adaptive potential to anthropogenic changes (Pfenninger et al., 2011). In this study, we evaluated the genetic structure and variability of *B. henni* along the Magdalena-Cauca basin in both dam-regulated and non-regulated water flow areas to provide evidence that will allow us to implement better conservation practices and prioritize specific areas for the conservation of this species.

2 Materials and methods

2.1 Ethical statement

This study was conducted with the recommendations and approval of the Animal Experimentation and Ethics Committee of the Universidad de Antioquia (CEEA). The protocol was reviewed and approved by CEEA on 14 November 2017, and the study was approved on 7 December 2017. Specimen collection was granted by License 0524 of the Ministry of Environment, 27 May 2014.

2.2 Study area

The Porce River is part of the Magdalena-Cauca river basin. It begins at 2660 masl and, after running 247 km northward, it joins the Nechí River at 170 masl, finally joining the Cauca River and then the Magdalena River before flowing into the Caribbean Sea. The Magdalena-Cauca basin has a bimodal rainfall pattern with elevation gradients that generate a wide diversity of landscapes and climatic conditions. This basin has produced most of the hydroelectric power generated over the past 40 years in Colombia (Jiménez-Segura et al., 2014).

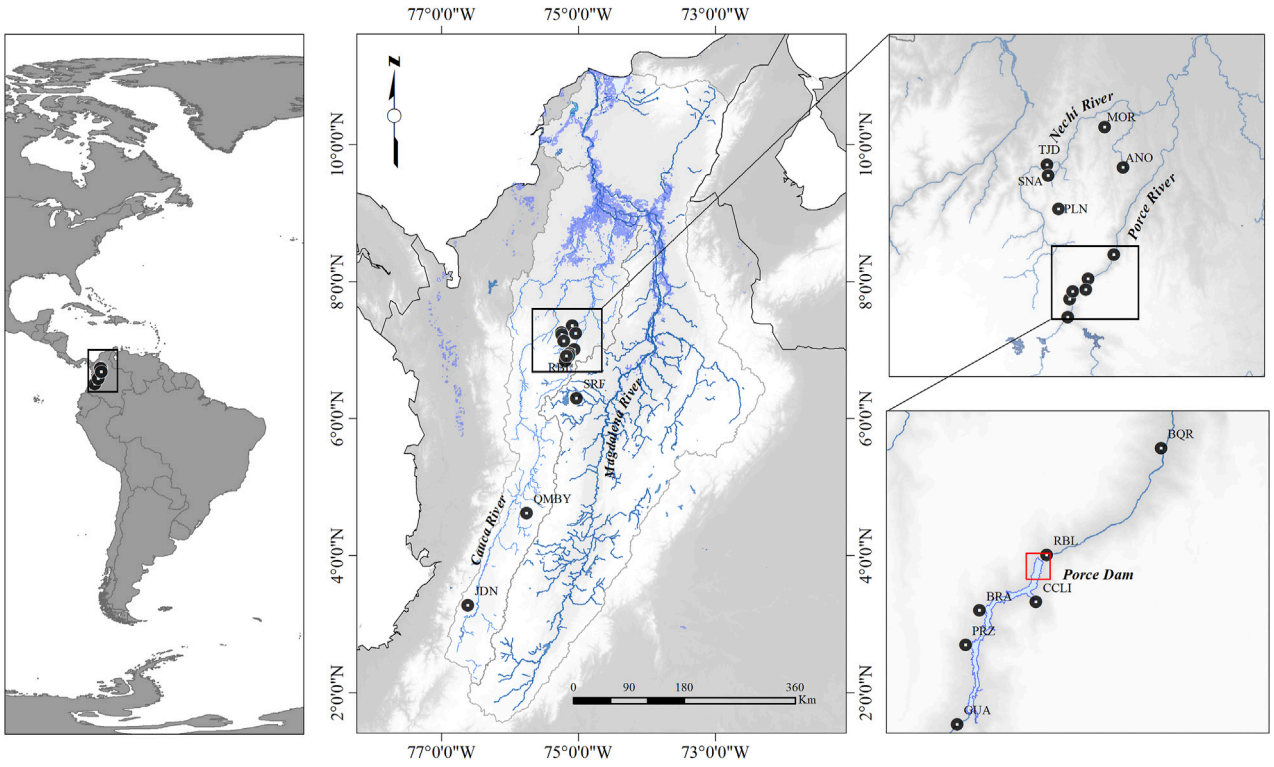

FIGURE 1
Sampling sites of *B. henni* populations. GUA – Guadalupe River, PRZ – Plan de Pérez Stream, BRA – Bramadora Stream, CCLI – Caracolí Stream, RBL – El Roble Stream, BQR – Boquerón Stream, TJD – La Tajada Stream, SNA – San Antonio Stream, PLN – La Plancha Stream, MOR – La Moreno Stream, ANO – Anorí River, JDN – Jordán River, QMBY – Quimbaya River, and SRF – Bizcocho River. The red lines in the figure represent the location of the wall in the Porce III Dam. The digital elevation model (SRTM, 1 arc-second) was obtained from the USGS Earth Explorer (HYPERLINK "https://earthexplorer.usgs.gov/"); the shape file of the dams and rivers was obtained from IGAC (https://geoportal.igac.gov.co/). All other products were produced by the authors and are not under copyright.

Samples were taken in 14 localities along the Magdalena-Cauca basin in 2019 (Figure 1). In the influence area of the Porce III Dam, the following six sampling sites were selected: GUA–Guadalupe River, PRZ–Plan de Pérez Stream, BRA–Bramadora Stream, CCLI–Caracolí Stream, RBL–El Roble Stream, BQR–Boquerón Stream. The three following sampling sites were selected along the Nechí River basin: TJD–La Tajada Stream, SNA–San Antonio Stream, PLN–La Plancha Stream. These two sampling sites were selected in the Anorí River subbasin: MOR–La Moreno Stream and ANO–Anorí River. One sampling site was selected in the upper Cauca River (JDN–Jordán River; one in the middle Cauca River (QMBY–Quimbaya River), and one in the middle section of the Magdalena River basin (SRF–Bizcocho River) (Figure 1; Table 1).

2.3 Sampling and DNA extraction

Due to the selectivity of the fish capture methods (e.g., body size), we used different fishing equipment and sampling efforts at each locality. In fast-flowing rivers and streams, we used cast nets with variable mesh sizes (0.5, 1.5, and 2 cm) And we also conducted captures using a portable electrofishing unit with 1 amp pulsed current (340 V, 1–2 A, CC) along the canal. The specimens captured were anesthetized with eugenol solution to reduce stress during manipulation (Javahery et al., 2012), and a portion of muscle (~0.5 cm^3) or a piece of the distal part of the caudal fin was taken. This tissue was stored in 96% ethanol. Three to four sacrificed individuals per locality were fixed in 4% formaldehyde and were stored in the Ichthyology Collection of the Universidad de Antioquia (CIUA–158); the list of localities and number of tissues collected can be found in Table 1. In some individuals tissue samples were taken from the caudal fins and were returned to the water body where they had been captured using a holding vessel to allow for their recovery and subsequent release. The species identification was based on a taxonomic key proposed by Maldonado-Ocampo et al. (2005) and the original description provided by Eigenmann (1913). Genomic DNA was extracted using a GeneJet Genomic DNA Purification kit (Thermo Scientific, Waltham, United States).

2.4 Mitochondrial DNA sequencing and microsatellite genotyping

A 550 pb fragment of the cytochrome oxidase 1 (*cox1*) mitochondrial gene was amplified and sequenced using the primers FishF1 (5′-TCAACCAACCACAAAGACATTGGCAC-3′) and FishR1 (5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′) proposed by Ward et al. (2005). Each PCR mix contained 1.0 µL of DNA, 1X Taq Buffer, 4 mM $MgCl_2$, 32 µM dNTPs, 100 µM of each primer, 1.2U of Taq Polymerase (Thermo

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TABLE 1 Sampling localities and voucher codes of individuals collected.

Abbreviation	Locality	Longitude	Latitude	Elev.	Number of samples	Collection codes
1.JDN	Cauca Basin, Jordán River	−76.61535	3.27088	1204	31	5386[a], 5386AA, 5386AB, 5386AC, 5386AD, 5386AE, 5386AF, 5386B, 5386C, 5386D, 5386E, 5386F, 5386G, 5386H, 5386I, 5386J, 5386K, 5386L, 5386M, 5386N, 5386O, 5386P, 5386Q, 5386R, 5386S, 5386T, 5386U, 5386W, 5386X, 5386Y, 5386Z
2.QMBY	Cauca Basin, Quimbaya River	−75.75777	4.61848	1270	32	5438[a], 5438AA, 5438AB, 5438AC, 5438AD, 5438AE, 5438AF, 5438AG, 5438B, 5438C, 5438D, 5438E, 5438F, 5438G, 5438H, 5438I, 5438J, 5438K, 5438L, 5438M, 5438N, 5438o, 5438P, 5438Q, 5438R, 5438S, 5438T, 5438U, 5438W, 5438X, 5438Y, 5438Z
3.SRF	Magdalena Basin, Bizcocho River	−75.02758	6.293204	984	18	5483[a], 5483B, 5483C, 5483D, 5483E, 5483F, 5483G, 5483H, 5483I, 5483J, 5483K, 5483L, 5483M, 5483N, 5483o, 5483P, 5483Q, 5483R
4.MOR	Cauca Basin, La Moreno Stream	−75.09068	7.35285	353	21	5320[a], 5320B, 5320C, 5320D, 5320E, 5320F, 5320G, 5320H, 5320I, 5320J, 5320K, 5320L, 5320M, 5320N, 5320O, 5320P, 5320Q, 5320R, 5320S, 5320T, 5320U
5.ANO	Cauca Basin Anorí River	−75.06046	7.18904	645	14	5281A, 5281B, 5281C, 5281D, 5281E, 5281F, 5281G, 5281H, 5281I, 5290A, 5290B, 5290C, 5290D, 5290E
6.TJD	Cauca Basin, La Tajada Stream	−75.24663	7.2511	570	21	5278A, 5278B, 5278C, 5278D, 5278E, 5278F, 5278G, 5278H, 5278I, 5278J, 5278K, 5278L, 5278M, 5278N, 5278O, 5278P, 5278Q, 5278R, 5278S, 5278T, 5278U
7.SAN	Cauca Basin, San Antonio Stream	−75.2429	7.22184	846	16	5274A, 5274B, 5274C, 5274D, 5274E, 5274F, 5274G, 5274H, 5274I, 5274J, 5274K, 5274L, 5274M, 5274N, 5274O, 5274P
8.PLN	Cauca Basin, La Plancha Stream	−75.21513	7.13148	705	9	5276A, 5276B, 5276C, 5276E, 5276F, 5276G, 5276H, 5276I, 5276J
9.GUA	Cauca Basin Guadalupe River, tributary to Porce III Dam	−75.190111	6.838694	705	37	5405A, 5405B, 5405C, 5405D, 5405E, 5405F, 5405G, 5405H, 5405I, 5405J, 5405K, 5405L, 5405M, 5405N, 5405O, 5405P, 5405Q, 5405R, 5405S, 5405T, 5405U, 5405W, 5419A, 5419B, 5419C, 5419D, 5419E, 5419F, 5419G, 5419H, 5419H, 5419I, 5419J, 5419K, 5419L, 5419M, 5419N, 5419O
10.PRZ	Cauca Basin, Plan de Perez Stream, tributary to Porce III Dam	−75.184917	6.887303	777	4	5335A, 5335B, 5335C, 5335D
11.BRA	Cauca Basin, La Bramadora Stream, tributary to Porce III Dam	−75.1763056	6.90847222	791	3	7551, 5418A, 5418B
12.CCLI	Cauca Basin, Caracolí Stream, tributary to Porce III	−75.141694	6.913583	725	6	5340, 5421, 7552, 9069, 9071, 9072
13.RBL	Cauca Basin, El Roble Stream, tributary to Porce III Dam	−75.135223	6.94243	548	17	5125, 5337A, 5337B, 5423A, 5423B, 5423C, 5423D, 5423E, 5423F, 5423G, 5423H, 5423I, 5423J, 5423K, 5423L, 5423M, 5423N
14.BQR	Cauca Basin, Boquerón Stream, tributary to Porce III Dam	−75.065192	7.007818	370	24	5406A, 5406B, 5406C, 5406D, 5406E, 5406F, 5406G, 5406H, 5406I, 5406J, 5406K, 5406L, 5420A, 5420B, 5420C, 5420D, 5420E, 5420F, 5420G, 5420H, 5420I, 5420J, 5420K, 5420L, 5420L

Scientific EP0406) in a final volume of 30 µL. The amplification started at 95°C for 2 min, followed by 35 cycles of 94°C for 30 s, 54°C for 30 s, 72°C for 1 min, and a final step at 72°C for 10 min. The amplicons were checked in 1% agarose electrophoresis, and both chains were then sequenced by Sanger technology in a commercial laboratory. Furthermore, two sequences of *B. henni* from the type locality (Dagua River), three sequences of *Brycon moorei* Steindachner 1878, and six sequences of *Brycon rubricauda* Steindachner 1879 were generated.

Ten specific microsatellite loci designed for *B. henni* (Landínez-García and Marquez, 2018) were amplified following a three-primer approach as defined by Blacket et al. (2012). These primers consist of a fluorescently labeled universal primer along with a pair of locus-specific primers, where the forward primer is modified with a 5' universal tail. Each reaction contained 2 µL of DNA, 1X Taq Buffer, 10 µM dNTPs, 10 µM forward primer, 10 µM reverse primer, 10 µM 10 µM 10 µM 10 µM labeled universal primer, 10 µL Taq Polymerase (Thermo Scientific EP0406) and variable concentrations of $MgCl_2$

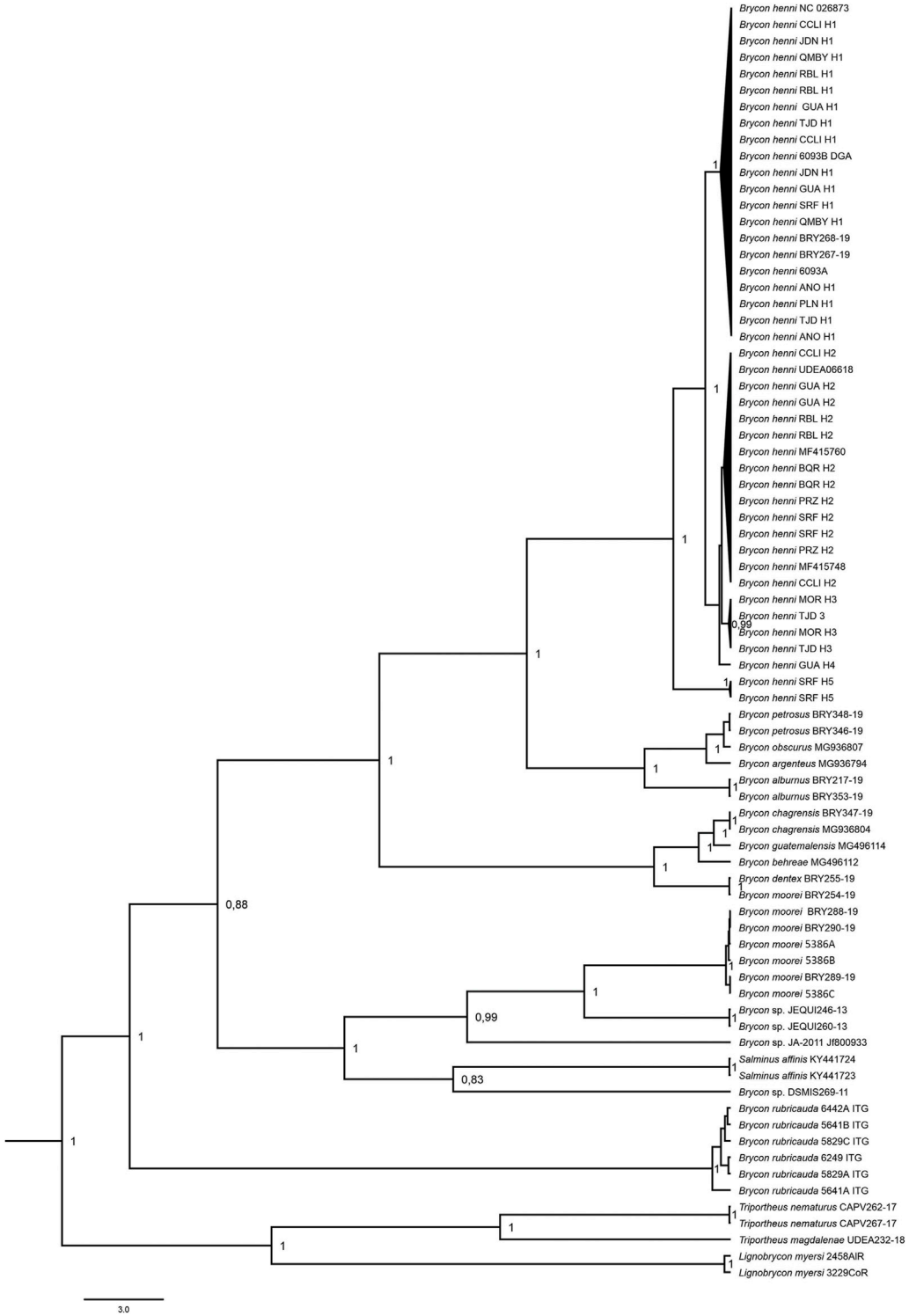

FIGURE 2
Bayesian phylogenetic tree of the cytochrome oxidase 1 (*cox1*) gene in *Brycon henni*.

(Supplementary Datasheet S1) in a final reaction volume of 15 μL. The amplification began at 95°C for 5 min, followed by 34 cycles of 94°C for 30 s, locus-specific alignment temperature (Supplementary Datasheet S1) for 45 s, and 72°C for 1 min with a final extension step at 72°C for 10 min. The amplicons were checked using 2% agarose electrophoresis and combined in three multiplexes to be run in an ABI 3130xl genetic analyzer using LIZ600 as size marker (Applied Biosystems, Foster City, CA, U.S.A.) The groups are as follows: G1: Bhe30, Bhe32, Bhe16; G2: Bhe47, Bhe34, Bhe27; and G3: Bhe26, Bhe05, Bhe20, Bhe39 (Supplementary Datasheet S1). Allele binning and assignment were done using Gene Mapper v.3.7 (Applied Biosystems, Waltham, Massachusetts, United States).

TABLE 2 Allelic range (Ra), Number of alleles per locus (Na), Polymorphism information content (PIC), (Frequency of Null Alleles (F(Null)) in *B. henni*.

Locus	Ra	Na	PIC	F(Null)
Bhe30	126–159	8	0.762	0.226
Bhe32	261–311	8	0.752	0.161
Bhe16	257–313	14	0.844	0.202
Bhe47	192–228	10	0.714	0.216
Bhe34	250–275	5	0.451	0.315
Bhe27	136–166	7	0.688	0.152
Bhe26	144–180	10	0.808	0.250
Bhe05	226–262	10	0.8	0.158
Bhe20	124–152	8	0.73	0.220
Bhe37	186–221	8	0.749	0.178

2.5 Cox1 analysis for taxonomic identification

Taxonomic validation was made from a reference database containing all the sequences for the Magdalena-Cauca species generated in this study (*B. henni*, *B. moorei*, and *B. rubricauda*) available in (CIUAB BOLD Systems). Additionally, we downloaded from BOLD System and Genbank other sequences from *B. henni*, *Salminus affinis* Steindachner 1880 (species that belong to the same family and co-occur in the same river basin), sequences of other Brycon Müller and Troschel, 1844 species from South America, and sequences of *Triportheus* Cope, 1872 and *Lignobrycon* Eigenmann and Myers, 1929, that belong to the Triportheidae family, recognized by Abe et al. (2014) as the sister group of Bryconidae (Supplementary Datasheet S2).

All sequences were aligned using the Muscle algorithm in Bioconductor (Edgar, 2004) and the haplotypes were generated in DnaSP v6 (Rozas, 2009). A Bayesian phylogenetic tree was constructed selecting the best-fit evolutionary model with ModelFinder (Kalyaanamoorthy et al., 2017) as implemented in the software IQ-tree2 (Minh et al., 2020), assuming a relaxed molecular clock model, a birth-death process as a tree prior, and random starting tree. A fossil calibration was applied in Triportheidae (*Lignobrycon* + *Triportheus*) using the age suggested by Abe et al. (2014). A Bayesian phylogenetic tree was run in CIPRES Science Gateway (BEAST on XSEDE) (Drummond and Rambaut, 2007) for 50 million generations sampling every 10,000 generations. TreeAnnotator v1.10.4 was used to summarize the maximum clade credibility tree, which was visualized in FigTree v1.4.3 (Rambaut, 2014).

2.6 Microsatellite analysis

2.6.1 Genetic diversity

The number of alleles per locus, the allele range, the polymorphism information content (PIC) and the frequency of null or F(Null) alleles were calculated using Cervus 3.0.7 (Kalinowaki et al., 2007). Linkage disequilibrium n (LD) between pairs of loci was calculated using with 10,000 permutations in Genpop (Rousset et al., 2017) in R (R Core Team, 2020). The effect of null alleles was tested in Micro-checker v2.2.1 (Van Oosterhout et al., 2004), and the Hardy-Weinberg Equilibrium (HWE) was evaluated per locus in each population using pegas (Pardis, 2010). In addition, to assess whether the sample size was sufficient to explore the total genetic variation of the species, a rarefaction analysis was conducted using PopGenKit (Rioux, 2015) with ten sample intervals and 1000 replicas for all samples.

For each locality, we evaluated: the Hardy-Weinberg Equilibrium (HWE), the total number of alleles per population (Na), percentage of polymorphic loci (%), allelic richness (Ar), observed heterozygosity (Ho), expected heterozygosity (He), inbreeding coefficient (Fis), and Fis 95% confidence interval in diveRsity package (Keenan, 2017). In addition, the values of Ho and He were compared amongst all population groups using a Kruskal-Wallis ANOVA and a Wilcoxon test for samples paired in R software (R Core Team, 2020). All *p*-values were adjusted using the BH procedure (Benjamini and Hochberg, 1995).

To evaluate genetic distances between localities, the pairwise Jost's D coefficient of genetic differentiation was calculated in DEMEtics with 1000 permutations (Jost, 2008; Gerlach et al., 2010). *p*-values were adjusted for the false discovery rate (FDR) using BH (Benjamini and Hochberg, 1995). These distances were plotted using heat maps and used to build a neighbor-joining tree in R (R Core Team, 2020).

2.6.2 Population structure

To know how many stocks are present in the dam influence area and because there are close sampling sites within distances of 30 km or less, where Landínez-García and Márquez (2020) identified two differentiated stocks, we conducted two types of analyses. First, we used STRUCTURE v. 2.3.4 (Pritchard et al., 2000) with 50 million Markov Chain Monte-Carlo (MCMC), 10% burn-in, K from 1 through 14 (number of localities), and 20 iterations for each K value. Those runs in STRUCTURE were analyzed in STRUCTURESELECTOR (Li and Liu, 2018) to calculate the optimum K using the Puechmaille methods (MedMeaK, MaxMeaK, MedMedK, and MaxMedK) (Puechmaille, 2016), the ΔK Evanno method (Evanno et al., 2005), and Ln Pr (X|K) (Pritchard et al., 2000). The second method was Discriminant Analysis of Principal Components (DAPC) (Jombart et al., 2010) implemented using the Adegenet package (Solymos et al., 2020). In contrast to STRUCTURE, HWE or independent segregation of the markers are not required (Jombart et al., 2010).

3 Results

3.1 Taxonomic identification

A total of 250 out of 253 samples were successfully genotyped for the *cox1* gene. The samples 5386C of JDN, 5281F of ANO, and 5483R of SRF failed due to limited DNA integrity. The 250 sequenced samples represented five haplotypes (H1-H5). H1 was present in nine of the 14 studied sites (JDN, QMBY, SRF, ANO, TJD, PLN, GUA, CCLI and RBL); H2 in SRF and in the dam surrounding area (GUA, PRZ, BRA, CCLI, RBL, BQR), H3 in MOR, TJD, SNA; H4 (GUA) and H5 (SRF) were found exclusively on a single site. Additionally, the studied sites showed the following haplotype distribution: JDN and QMBY (H1); SRF (H1, H2 and H5); ANO and PLN (H1); MOR and SNA (H3); TJD (H1 and H3); PRZ, BRA, BQR (H2), CCLI and RBL (H1 and H2), and GUA (H1, H2 and H4). As expected in the Bayesian tree, the *cox1*

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TABLE 3 Genetic diversity parameters of B. henni according to sample units. Individuals number (N), number of total alleles in each population (Na), percentage of polymorphic loci (%), Allelic richness (Ar), Observed heterozygosity (Ho), Expected heterozygosity (He), Hardy Weinberg Equilibrium, inbreeding coefficient (Fis), and 95% confidence interval. Values in bold denote statistical significance.

	N	Na	%	Ar	Ho	He	HWE	Fis	Fis_Low	Fis_High
JDN	31	39	43.61	2.39	0.53	0.49	0.39	−0.09	−0.17	0.01
QMBY	32	57	66.07	3.02	0.65	0.64	0.97	−0.01	−0.08	0.05
SRF	18	35	41.11	2.47	0.61	0.55	0.09	−0.11	−0.26	0.03
MOR	21	49	55.11	2.94	0.65	0.62	0.68	−0.05	−0.14	0.04
ANO	14	47	54.75	2.47	0.52	0.55	0.32	0.05	−0.09	0.18
TJD	21	45	51.18	2.87	0.63	0.63	0.92	0	−0.1	0.1
SNA	16	12	15.14	1.12	0.07	0.05	0.23	**−0.38**	**−0.74**	**−0.01**
PLN	9	19	22.36	1.62	0.32	0.27	0.23	−0.18	−0.41	0.01
GUA	37	48	55.57	2.74	0.6	0.59	0.7	−0.01	−0.08	0.06
PRZ	4	20	23.54	1.77	0.4	0.29	0.35	**−0.38**	**−0.68**	**−0.19**
BRA	3	25	29.22	2.1	0.5	0.46	0.76	−0.1	−0.77	0.14
CCLI	6	29	34.93	2.15	0.4	0.43	0.08	0.06	−0.19	0.27
RBL	17	40	47.43	2.52	0.54	0.54	0.91	0.01	−0.13	0.12
BQR	24	21	27.07	1.53	0.23	0.25	**0.01**	0.09	−0.07	0.23

haplotypes of the studied sites conformed a well-supported monophyletic cluster (Posterior probability: 1) with the reference mitogenome of *Brycon henni* (GenBank NC026873) and the individual from the type locality Dagua (6093B DGA), which confirms the taxonomic identity of the specimens analyzed in this study (Figure 2).

3.2 Quality of microsatellite data

A total of 88 alleles were identified combining all loci with an average of 8.8 alleles per locus, where locus *Bhe16* presented the highest number of alleles (14 alleles) and *Bhe35* the lowest number (5 alleles). The polymorphism information content (PIC) ranged from 0.45 (*Bhe34*) to 0.84 (*Bhe16*) with a mean of 0.73 (Table 2). The probability of null alleles and dropout ranged from 0.15 (*Bhe27*) to 0.31, where the *Bhe34* (0.31) marker presented the highest probability of null alleles (Table 2). No pair of loci presented linkage disequilibrium (Supplementary Datasheet S3). Only Bhe30 in BOQ is deviated from HWE (Supplementary Datasheet S4), and the rarefaction curves per locality showed that the number of alleles per locus in the localities PLN, PRZ, BRA, CCL, and SNA hardly reached the asymptote (Supplementary Datasheet S5B), which contrasts with an asymptotic behavior in the general rarefaction curve (Supplementary Datasheet S5A).

Table 2 Allelic range (Ra), Number of alleles per locus (Na), Polymorphism information content (PIC), (Frequency of Null Alleles (F (Null)) in *B. henni*.

3.3 Genetic diversity and structure

A greater number of alleles (A) was observed in QMBY, MOR, and GUA with 57, 49, and 48 alleles, respectively (Table 3), whereas the localities with the lowest values were PLN and SNA. A similar trend was observed in the proportion of polymorphic loci, where QMBY was the most diverse, followed by GUA. The greatest observed (Ho) and expected (He) heterozygosity were evidenced in QMBY, MOR, TJD, SRF, and GUA, whereas SNA, BQR, and PLN presented the lowest values (Table 3). The BQR population did not meet HWE. The inbreeding coefficients (Fis) were significantly lower than zero in SNA (−0.38) and PRZ (−0.38).

Individuals number (N), number of total alleles in each population (Na), percentage of polymorphic loci (%), Allelic richness (Ar), Observed heterozygosity (Ho), Expected heterozygosity (He), Hardy Weinberg Equilibrium, inbreeding coefficient (Fis), and 95% confidence interval.

The Kruskal-Wallis ANOVA among groups was significant for both Ho and He (p-value < 0.05). Regarding paired samples, the Wilcoxon test evidenced significant differences (p-value < 0.05) in Ho between BQR (Ho = 0.23, He = 0.25) and ANO, GUA, MOR, QMBY, RBL, SRF, and TJD; between SNA (Ho = 0.07, He = 0.05) and ANO, CCLI, GUA, JDN, MOR, QMBY, RBL, SRF, and TJD; and between PLN (Ho = 0.32, He = 0.27) and GUA, QMBY, SRF, and TJD (Table 4). The Jost's D pairwise genetic distances were not significant between GUA-BRA, GUA-CCLI, CCLI-BRA, RBL-BRA, RBL-CCLI and RBL-GUA (Figures 3A,B). Significant differences (p < 0.05) were found between all the other pairs. The greatest difference was found between JDN and PRZ (0.91). Bootstrap values in pairwise comparisons involving ANO, PRZ, and BRA were not calculated due to null alleles in ANO and low amounts of samples in PRZ and BRA.

ΔK, LnP(K) and Puechmaille in STRUCTURE yielded 3 and 11 as the most likely number of gene pools, respectively (Figure 4; Supplementary Datasheets S6, S7). In the first case (K3), a regional gradient is observed with a single stock in the south, another one in the dam-influenced area, and a third one in the northern localities

TABLE 4 Paired Wilcoxon test for observed (Ho) (lower diagonal) and expected (He) heterozygosities (upper diagonal). Values in bold denote statistical significance.

Ho/He	ANO	BQR	BRA	CCLI	GUA	JDN	MOR	PLN	PRZ	QMBY	RBL	SNA	SRF	TJD
ANO		**0.01**	0.52	0.48	0.37	0.77	0.10	**0.01**	0.10	0.16	0.83	**0.01**	0.66	0.20
BQR	**0.03**		0.05	0.10	**0.01**	0.07	**0.01**	0.91	0.98	**0.01**	**0.01**	**0.05**	**0.01**	**0.01**
BRA	0.95	0.23		0.98	0.10	1.00	0.06	0.07	0.23	0.07	0.19	**0.01**	0.24	0.08
CCLI	0.49	0.28	0.61		0.10	0.76	0.05	0.12	0.41	0.07	0.20	**0.01**	0.20	0.07
GUA	0.40	**0.02**	0.68	0.23		0.50	0.20	**0.01**	**0.05**	0.77	0.54	**0.01**	0.52	0.52
JDN	0.83	0.06	0.80	0.43	0.67		0.26	0.11	0.18	0.23	0.52	**0.01**	0.68	0.23
MOR	0.23	**0.03**	0.13	0.11	0.27	0.38		**0.02**	**0.04**	0.58	0.19	**0.01**	0.11	0.66
PLN	0.27	0.65	0.27	0.75	**0.03**	0.30	**0.03**		0.97	**0.01**	**0.01**	0.08	**0.01**	**0.01**
PRZ	0.51	0.70	0.65	0.95	0.32	0.60	0.32	0.93		**0.03**	0.10	0.06	0.12	**0.02**
QMBY	0.27	**0.01**	0.44	0.14	0.65	0.51	0.54	**0.02**	0.28		0.23	**0.01**	0.43	0.96
RBL	0.58	**0.02**	0.91	0.51	0.56	0.95	0.11	0.24	0.52	0.41		**0.01**	0.97	0.54
SNA	**0.01**	0.10	0.05	**0.03**	**0.01**	**0.02**	**0.01**	0.13	**0.09**	**0.01**	**0.02**		**0.01**	**0.01**
SRF	0.38	**0.02**	0.46	0.23	0.76	0.51	0.73	**0.05**	0.34	0.95	0.29	**0.01**		0.23
TJD	0.32	**0.01**	0.61	0.19	1.00	0.56	0.51	**0.04**	0.29	0.76	0.68	**0.01**	0.80	

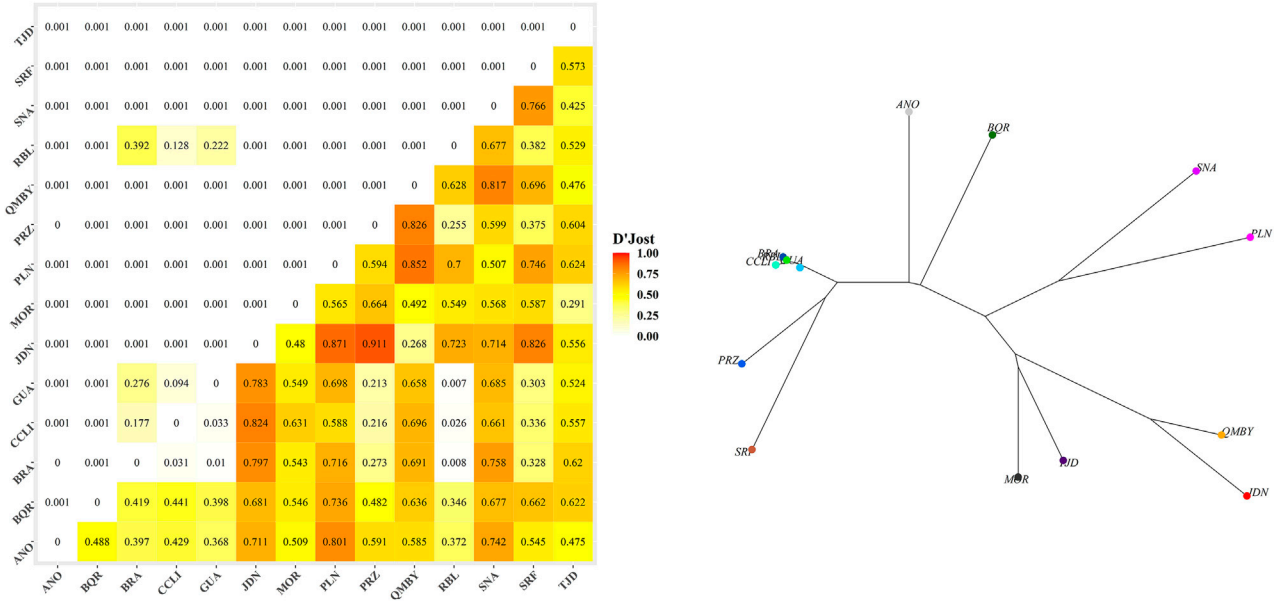

FIGURE 3
(A) Pairwise Jost's D genetic distances (below diagonal) and p-values (above diagonal). **(A)** Genetic distances. **(B)** Neighbor-joining tree based on genetic distances.

mixed with the two previous stocks. In (K11), however, the same number of stocks as localities are recovered, except GUA, BRA, CCLI, and RBL, which correspond to a unique genetic stock differentiated from PRZ found in the same complex of the dam intervention area (Figure 1). The DAPC analysis defining localities as *a priori* groups assigned most individuals to their original cluster, with a few exceptions in GUA, CCLI, and RBL (Figure 5A). The *Elbow* graph showed that the most likely number of clusters is 11 (Supplementary Datasheet S8). The assignment probability shows that in the dam area (GUA, PRZ, BRA, CCLI, and RBL) two stocks seem to converge (Group 6 and Group 2) (Supplementary Datasheet S9), thus generating 11 populations (Figure 5B). A likely number of 11 gene pools was obtained with both approaches, where the six localities in the dam-influenced area were assigned to three distinct

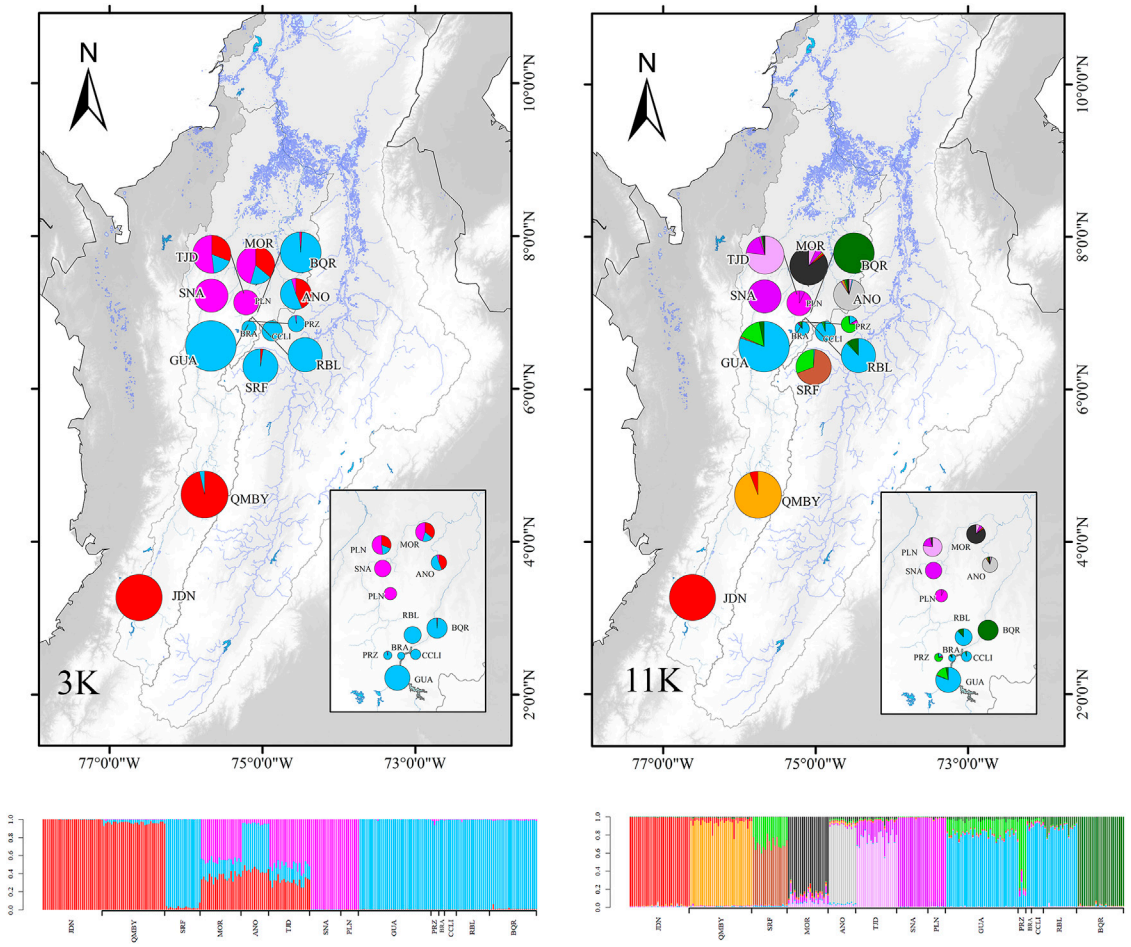

FIGURE 4
Genetic structure suggested by STRUCTURE with K = 3 and K = 11.

stocks; however, the assignment of few individuals to a given stock differs between these two approaches (Figure 4; Figure 5). Lastly, RBL is nested within the stock upstream of the dam, despite being located immediately downstream from the dam and geographically isolated from the rest (Figure 1).

4 Discussion

This study provides evidence of the genetic patterns of *B. henni* at fine and broad geographic scales. The taxonomic identity was efficiently validated with the *cox1* gene in both free-flowing rivers, and in systems fragmented by the Porce III Dam. Most localities evaluated in the system fragmented by this anthropic barrier exhibit high diversity without signs of inbreeding and without significant differences in relation to other diverse populations in free-flowing rivers. However, diversity varied along the Porce basin, where some free-flow areas have low genetic diversity. At the same time, discrete local populations were found along the entire basin except for some sites of the area of influence of the Porce III Dam, where three stocks were found in five localities, two of which were shared by localities upstream of the Porce III dam. In addition, at the regional level, three population stocks were evidenced in a latitudinal gradient.

The greatest diversity was registered in QMY, a free-flow area unaffected by hydroelectric projects. The number of alleles of most populations varies from 3.8 to 5.5, which is within the range described by Landínez-García and Márquez (2020). Likewise, the observed and expected heterozygosities of populations in our study were similar to those reported by Landínez-García and Márquez (2020) and Muñoz-A. et al. (2019), where He values ranged between 0.46–0.66 and 0.51–0.68, and Ho values between 0.43–0.64 and 0.24–0.43 respectively. However, the number of alleles per locus and both heterozygosity indexes were low in PLN, SNA, PRZ and BQR. Following Dlugosch and Parker (2008), PLN and SNA streams could be presenting a recent anthropogenic founder effect because of the translocation or introduction of the species in systems where they did not originally occur (Cardoso et al., 2009). Translocation is very common in South America (Povh et al., 2008), and may promote a founder effect due to a small population of origin. Translocation as an explanation for observed data is plausible because *B. henni* from SNA and PLN (H1) exhibit one of the most frequent haplotypes in the studied area. However, due to the lack of information of historical data, the heterozygote excess found could also be the result of a small

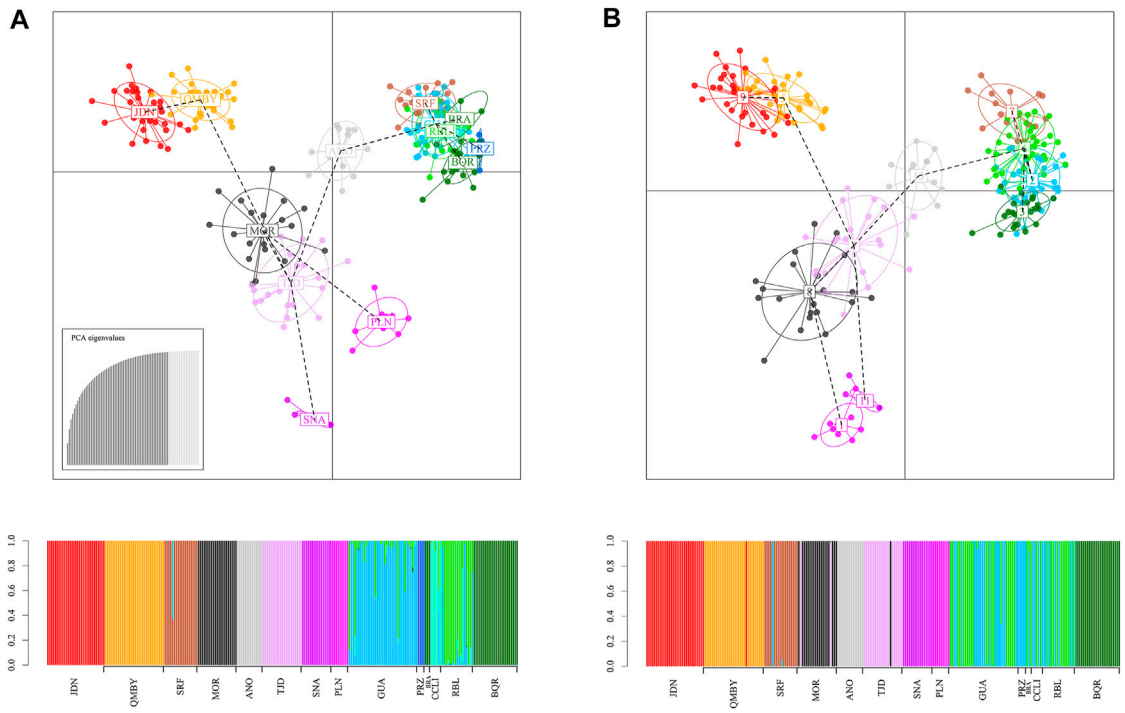

FIGURE 5
Genetic structure calculated using DAPC method. **(A)** Establishing *a priori* groups, **(B)** Without establishing *a priori* groups.

number of breeders due to severe genetic bottlenecks; in this case the allele frequencies in males and females may be different due to binomial sampling error, generating an excess of heterozygotes in the progeny relative to the proportion expected under Hardy-Weinberg Equilibrium (Robertson, 1965; Rasmussen, 1979). Consequently, further analyses with a greater sample are required to test these explanations, as well other biological causes such as overdominance selection or associative overdominance (Nei, 1987). This reasoning might also explain the heterozygosity excess observed in PRZ, but alternatively, this may be an artifact caused by the limited sampling size. Lastly, we do not know the reason for the low heterozygosity in BQR, but it could be explained by the low number of migrants, since it is downstream from the dam and disconnected from others potential tributaries.

The STRUCTURE analysis evidenced a regional structure with three stocks that runs south to north, where SRF, which is on the western side of the Central Mountain and is separated from the rest of localities by the mountain system, presents a high probability of belonging to the center stock. This hierarchical structure is also present in haplotype composition of the samples; for instance, the south stock (JDN, QMBY) is composed by H1, the center stock composed by H2 (GUA, PRZ, BRA, CCLI, RBL, BQR), although in some sites this haplotype co-occurs with H1 (GUA, CCLI, RBL); and the north stock exhibit alternatively H1 (ANO, PLN), H3 (MOR, SNA) or both haplotypes H1, H3 (TJD). SRF shares haplotypes with the second stock (H1, H2), although it also exhibits a low frequent haplotype (H5). Additionally, the microsatellite analysis revealed a spatial genetic substructure that explains the presence of 11 stocks in the 14 studied sites since two stocks were in more than one site (SNA-PLN; GUA-BRA-CCLI-RBL). The spatial structure found in this study supports the hypothesis previously proposed by Landínez-García and Márquez (2020) where *B. henni* populations make up genetic patches throughout their range of distribution. Moreover, in this study, nine out of ten microsatellite markers were highly informative with PIC values above 0.5 (Botstein, White, Skolnick, and Davis, 1980). Bhe34 turned out to be moderately informative, and none of the alleles presented linkage disequilibrium. Our results have a similar number of alleles to those reported by Landínez-García and Márquez (2020), which reinforces the evidence of the efficiency in the use of these markers and their replicability, mainly in monitoring the long-term diversity of the species.

In the area of influence of the Porce III dam, the RBL stream has been isolated for approximately 10 years from the localities upstream of the dam, i.e., GUA, PRZ, BRA and CCLI, but they all conform a single cluster, which makes sense in light of the proximity between these localities and the recent construction of the river dam. In fact, in the area of influence of the dam, the DAPC and STRUCTURE analyzes showed a recent mixture of two stocks which could have resulted from homogenization of population groups close to the dam, an effect suggested by Baggio et al. (2018). This homogenization result is feasible since the generation time of the species is eight to 9 months, estimated in its coefficient for the von Bertalanff model k~0.27 (GIUA, 2019), and approximately ten generations have passed since dam construction to the time of this study. However, it is also important to keep in mind that some factors, including the population dynamics and effective population size, among others, could overshadow the genetic fingerprint of the dam in local populations (Epps and Keyhobardi, 2015).

Considering that in this study GUA, BRA, CCL, PRZ, and RBL represent a group differentiated from the rest, it is important that it be treated as a unique stock independent from genetically differentiated populations in other regions of the Cauca River basin. Breeding of genetically different individuals could result in a decrease in local adaptability, which could lead to the non-survival of offspring in the natural environment (Melo et al., 2006). When considering maintaining the stock through captive breeding, it is important to avoid breeding between closely related individuals to prevent the inbreeding effect, which could affect the adaptive capacity and evolutionary potential of the species (Povh et al., 2008). We also acknowledge that very few captures were obtained in the PRZ stream, thus the result showing it representing a single stock should be regarded with caution. We recommend increasing the sampling effort in future studies.

This study reveals a possible effect of the construction of Porce III Dam on the local structure of the species since a process of homogenization in the dam's area of influence may be under way. Furthermore, we believe that events, such as translocations, have affected the genetic structure and diversity of the species in the basin. Overall, regarding the populations studied near the dam, there is still no significant sign of genetic erosion, demonstrated by positive and significant Fis. Lastly, since the genetic results obtained reflect events that occurred in past generations, we suggest studies on population genetics should be accompanied by ecological studies in order to measure the possible impact anthropic interference may have on the species (Fraser and Bernatchez, 2001; Povh et al., 2008). Since a future reduction of genetic diversity is expected due to the combined effect of geographic isolation and habitat degradation, we consider it pertinent to conduct future studies that evaluate: the quality of habitat available for species, the viability of reproduction in dam-affected areas, and what kind of biotics and abiotic factors determine the reproduction and stability of the populations in the basin Fjeldså et al., 2012.

Data availability statement

The datasets presented in this study can be found in Supplementary Material.

Ethics statement

The animal study was reviewed and approved by Ethics Committee on Animal Experimentation of the Universidad de Antioquia (CEEA).

Author contributions

JH-P, LJ-S, EM, and IS-C: Conceptualization, Design Analysis, Development methodology, Writing and Editing manuscript. JH-P and OC: Laboratory an Data Analysis JH-P: Fieldwork, Data Curation. LJ-S and IS-C: Attaining resources, Project Administration. IS-C, EM, and LJ-S: Project consultancy and design.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2023.1080028/full#supplementary-material

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