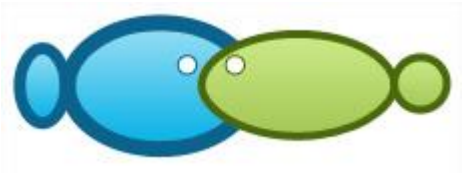

DNA barcoding of *Brycon henni* (Characiformes: Characidae) in the Yotoco, Sonso, Guabas and Sabaletas rivers of Colombia

Edilma Vélez-Sinisterra, Diana López-Alvarez, Carlos A. Jaramillo Cruz, Jaime E. Muñoz Florez

Facultad de Cienccias Agropecuarias, Universidad Nacional de Colombia, Palmira, Valle del Cauca, Colombia. Corresponding author: D. López-Alvarez, dilopezal@unal.edu.co

Abstract. *Brycon henni* (Sabaleta) is an endemic fish of the water basins of the Cauca, Magdalena, San Juan, and Patia rivers. This fish is captured due to its high nutritional value. However, its habitats are contaminated due to anthropic action, which has led to a considerable reduction in the natural populations of this fish species. In this study, 40 caudal fin samples of *B. henni* were collected in the Yotoco, Sonso, Guabas, and Sabaletas rivers basins of the Department of Valle del Cauca, Colombia. DNA extraction and amplification of the RAG1 nuclear region and 16S and COI mitochondrial genes analyses were performed. This research aimed to molecularly identify the species *Brycon henni*, as well as determine haplotypes associated with the study river basins using DNA barcoding. The results identified four species, namely *Brycon henni, Brycon* sp.*, Bryconamericus caucanus,* and *Creagrutus* sp. Furthermore, 22 haplotypes were found for the COI and RAG1 gene regions and seven haplotypes for the 16S gene region.
Key Words: 16S, COI, haplotypes*,* RAG1, sabaleta*.*

Introduction. Colombia has suitable conditions for the development of fishing activities, since it has diverse thermal floors with stable climates year-round, a great variety of water systems, adequate surfaces for plant crops and animal culture in fresh or saltwater, as well as a broad fluvial system and high biodiversity (Esquivel et al 2014).

Fishermen and fishing in Colombia have a negative impact on aquatic habitats due to tourism, excessive fishing, environmental contamination, and others. Furthermore, human settlements near rivers do not have good living conditions since they represent the lowest social strata (Moreno 2018).

The Cauca river basin, located from 950 m asl, has a high diversity of fish compared with other regions at the same elevation. This basin displays a high level of endemism due to the isolation caused by the geographic valley of the Cauca river (Maldonado-Ocampo et al 2005). In Colombia, the construction of water reservoirs in the Magdalena-Cauca river basin has modified freshwater ecosystems, especially affecting fish species and their migration-reproduction processes, which, in turn, influence commercial and artisanal fishing (Jiménez-Segura et al 2014). Therefore, human populations near rivers suffer negative effects on their economy and food security since they rely on fishing activities (Tognelli et al 2016).

As a result of the negative effects of anthropic actions on global biological diversity, natural resource management efforts (use, exploitation, conservation, and restoration) have focused on recording biodiversity, so the population can become aware of its function and use in a sustainable development (Chandra & Idrisova 2011). In this regard, DNA barcoding is an important tool for experts who study species that are difficult to identify based on morphology (Kerr et al 2007). This technique can be applied in food security, biogeography, biodiversity studies, unidentified species, and invasive species (Gong et al 2018). Furthermore, DNA barcoding is relevant in estimating the biological diversity and determining its preservation; it is also efficient in characterizing

different species and solving identification challenges for conventional taxonomy (Akram et al 2017).

Brycon henni, commonly known as "sabaleta", is an autochthonous species of Colombia and is important in the diet of human communities living near rivers. Furthermore, it is of interest to fishermen, since it displays an aggressive behavior during fishing, and due to its organoleptic characteristics (Montoya-Lopez et al 2006). This species is not present in any risk category proposed by the Red book of freshwater fish of Colombia (Mojica et al 2012) or the red list of threatened species of the International Union for Conservation of Nature (Tognelli et al 2016). Nevertheless, sabaleta inhabits waters with high dissolved oxygen levels (between 7-10 mg L^{-1}) and is susceptible to reduced levels of dissolved oxygen (Builes & Urán 1974; Botero-Botero & Ramírez-Castro 2011; Zapata-Restrepo et al 2017). Similarly, river contamination causes losses in the number of individuals of this species (Hurtado-Alarcón et al 2011).

It is necessary to characterize the biological diversity of *Brycon henni* in different water basins of Valle del Cauca, using DNA barcoding, due to the importance of this species as a food resource for human populations near rivers. The main objective of this study was to identify the genetic diversity of groups within the species *Brycon henni* in the Sonso, Yotoco, Guabas, and Sabaletas rivers, based on RAG1 nuclear gene and 16S rRNA and COI mitochondrial gene sequences.

Material and Method

Description of the study sites. Between 2014 and 2015, 40 sabaleta samples were collected from the water basins of Valle del Cauca, Colombia, specifically, in the municipalities of Guacari (Sonso river) located at coordinates 3°48'21.8"N and 76°17'29.3"W, with an elevation of 992 m a.s.l.; Yotoco, located at 3°52'18.7"N and 76°24'11.6"W, with an elevation of 1017 m a.s.l.; Ginebra (Guabas river) located at coordinates 3°45'56"N and 76°14'4.5"W, with an elevation of 1160 m a.s.l.; and Buenaventura (Sabaletas) located at 3°45'9.5"N and 76°57'13.6"W, with an elevation of 53 m a.s.l. (Figure 1). Sampling consisted mainly of collecting samples of caudal fin fragments; some animals were bought from local fishermen and conserved in plastic containers with 90% alcohol. The samples were transported in a portable plastic refrigerator and stored at 4°C, in the Molecular Biology Laboratory of Universidad Nacional de Colombia Palmira campus.

DNA extraction. Genomic DNA was extracted from the caudal fin samples using the phenol:chloroform:isoamyl alcohol protocol proposed by Bardakci & Skibinski (1994). The extracted DNA was conserved at -80°C. DNA quality and quantity were analyzed on a COLIBRI spectrophotometer for microvolumes (Titertek-Berthold ®). Additionally, DNA quality was visually assessed through 1% agarose gel electrophoresis, in 0.5% TBE running buffer at 100 volts for 30 minutes. The genomic DNA was visualized using GelRed DNA dye in a Cleaver View (Cleaver Scientific Ltd) transilluminator with ultraviolet light at a wavelength of 254/312 nanometers.

PCR conditions and sequencing. A fragment of approximately 650 bp of the COI mitochondrial gene region was amplified using primers FishF1 and FishR1 (Hubert et al 2008). The PCR reaction was performed in a final volume of 25 µL that contained 8.9 µL mili-Q water, 2.5 µL 10X $(NH_4)_2SO_4$ Buffer, 2.5 µL 25 mM $MgCl_2$, 4 µL 20 mM dNTPs, 1 µL of each 10 mM primer, 0.1 µL 5 U/µL Taq Pol, 1 µL 5X BSA, 3 µL 10% Trehalose, and 1 µL DNA at concentrations ranging from 20 to 2000 ng μL^{-1}. The thermal cycling conditions for the COI gene region comprised initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 40 s, annealing at 52°C for 70 s, extension at 72°C for 1 min, and a final extension cycle at 72°C for 10 min (Hubert et al 2008).

For the 16S rRNA gene region, a fragment of 584 bp was amplified using primers 16Sa-L and 16Sa-L (Palumbi 1996). The PCR reaction was performed in a final volume of 25 µL that contained 8.9 µL mili-Q water, 2.5 µL 10X $(NH_4)_2SO_4$ Buffer, 2.5 µL 25 mM

$MgCl_2$, 4 µL 20 mM dNTPs, 1 µL of each 10 mM primer, 0.1 µL 5 U/µL Taq Pol, 1 µL 5X BSA, 3 µL 10% Trehalose, and 1 µL DNA at concentrations ranging from 20 to 2000 ng μL^{-1}. The thermal cycling conditions for the 16S gene region involved initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 52°C for 45 s, extension at 72°C for 1 min, and a final extension cycle at 72°C for 10 min (Palumbi 1996).

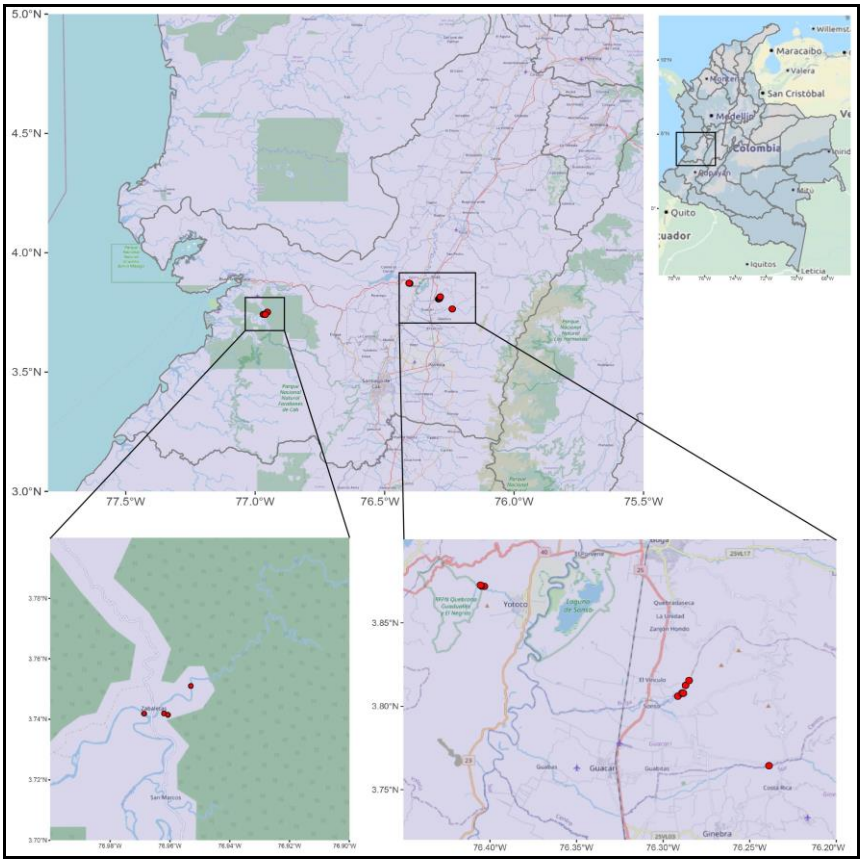

Figure 1. Map of the geographic locations of the collected samples.

Finally, a fragment of approximately 1492 bp was amplified for the RAG1 gene region through a nested PCR using external primers 2510F and 4090R in a final PCR reaction volume of 25 µL that contained 7.9 µL mili-Q water, 2.5 µL 10X KCl Buffer, 3.5 µl 25 mM $MgCl_2$, 4 µL 20 mM dNTPs, 1 µL of each 10 mM primer, 0.1 µL 5 U/µL Taq Pol, 1 µL 5X BSA, and 4 µL DNA at concentrations ranging from 20 to 2000 ng µL (Li & Ortí 2007). The thermal cycling conditions were an initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 2 min, and a final extension cycle at 72°C for 10 min. The second amplification of the nested PCR was performed using internal primers 2535F and 4078R in a final PCR reaction volume of 50 µL that contained 21.8 µL mili-Q water, 5 µL 10X KCl Buffer, 5 µL 25 mM $MgCl_2$, 8 µL 20 mM dNTPs, 2 µL of each 10 mM primer, 0.2 µL 5 U/µL Taq Pol, 2 µL 5X BSA, and 4 µL purified PCR product from the first PCR (Li & Ortí 2007). The thermal cycling conditions were an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 40 s, annealing at 45°C for 40 s, extension at 72°C for 1 min and 40 s, and a final extension cycle at 72°C for 10 min.

All of the PCR reactions were performed on PeQSTAR (PeQLAB) and Multigene optimax (LABNET) thermocyclers. PCR amplification was assessed on 1.5% agarose gels using the protocol described by Schmitz & Riesner (2006). The mitochondrial gene fragments (COI and 16S) were sequenced by MACROGEN USA and the nuclear gene region (RAG1) was sequenced by BIONEER KR; both consisted of forward and reverse sequencing using the PCR primers (Table 1).

Table 1

Primers used to amplify fragments of the mitochondrial and nuclear genes

Marker	*Primer*	*Primer sequence (5'-3')*	*Reference*
16S	16Sa-L	5'-ACGCCTGTTTATCAAAAACAT-3'	Palumbi 1996
	16Sb-H	5'-CCGGTCTGAACTCAGATCACGT-3'	
COI	FishF1	5'-TCAACCAACCACAAAGACATTGGCAC-3'	Hubert et al 2008
	FishR1	5'-TAGACTTCTGGTGGCCAAAGAAT-3'	
RAG1	2510F	5'-TGGCCATCCGGGTMAACAC-3'	Li & Ortí 2007
	4090R	5'-CTGAGTCCTTGTGAGCTTCCATRAAYT-3'	
RAG1	2535F	5'-AGCCAGTACCATAAGATGTA-3'	Li & Ortí 2007
	4078R	5'-TGAGCCTCCATGAACTTCTGAAGRTAYTT-3'	

Note: Y - C,T; R - A,G; M - A,C.

Data alignment. Geneious v8.05 software for Windows was used to generate separate alignment matrices for the COI, 16S, and RAG1 gene sequences. Furthermore, manual sequence editing was done to eliminate PCR primer sequences and bases added during the annealing process, and to improve the final alignment. Finally, a unique consensus sequence was created for each gene region from each individual.

Genetic distance analysis. The inter and intraspecific genetic distances of *B. henni, Brycon* sp*., Bryconamericus caucanus,* and *Creagrutus* sp*.* were calculated for each DNA barcode locus. Kimura 2-parameter (K2P) evolutionary model was implemented in Mega7 software (Kumar et al 2016) and used to estimate the evolutionary distance based on the number of nucleotide substitutions (Kimura 1980). All gap and missing data positions were discarded; furthermore, Neighbor-Joining (NJ) trees were constructed based on the sequence divergence estimated with K2P, with 10000 bootstrap replicas.

Haplotype network analysis. The number of haplotypes were estimated using TCS 1.21 software (Clement et al 2000) based on statistic parsimony analysis. Additionally, phylogenetic networks were constructed from the DNA sequence alignments for the COI, 16S, and RAG1 gene regions, based on 95% confidence intervals, and missing data were treated as a fifth state (Clement et al 2000).

Phylogenetic analysis. Phylogenetic analyses were conducted separately for the sequenced samples belonging to *B. henni*, *Brycon* sp*., B. caucanus,* and *Creagrutus* sp*.* The trees were rooted because the species are distant. The best nucleotide substitution models for each gene region were determined using jModelTest 2.1.10 based on the hierarchical likelihood ratio test and Akaike information criterion. Bayesian inference was applied using parameters nst=5 and rates=gamma, while the other parameters were calculated by the algorithm (Darriba et al 2012).

Results and Discussion. Forty samples, belonging to *B. henni* (n=23), *Brycon* sp*.* (n=10), *B. caucanus* (n=2), *Creagrutus* sp*.* (n=5), were collected in Guacarí (Sonso), Yotoco, Buenaventura (Sabaletas), and Ginebra (Guabas) geographical regions. COI (n=39, 100%), 16S (n=39, 97.5%), and RAG1 (n=33; 94.3%) gene regions were amplified and sequenced.

The Basic Local Alignment Search Tool (BLAST) results showed an average identity of 96.67% and an E-value of zero. For *B. henni*, the alignments showed high similarity; for instance, the sequences from the population in the Sonso river showed identities and coverages of 100% and the sequences from populations in the Yotoco and Guabas rivers demonstrated identities and coverages of 99%. Moreover, in the Guabas river, two individuals belonging to the species *B. caucanus* were identified, and, in the Yotoco river, five individuals of the species *Creagrutus* sp*.* were determined. Accordingly, these two species were included as outgroups to achieve a more complete analysis (Table 2). Finally, ten specimens were included from each geographical zone.

Tables 3, 4, and 5 show that the taxonomic identifications based on the BLAST alignment results are unclear for the population in the Sabaletas river, Buenaventura, since the sequences aligned to different species. Consequently, the specimens were

assigned to *Brycon* sp. Table 3 shows a high match score with *Brycon argenteus* for the COI gene region; however, the coverage was 98% and the identity was 97.38%.

Table 2

BLAST alignment results for the sequences from the studied populations

Population	*Blast hit*	*Identity (%)*	*Coverage (%)*	*E-value*	*Score*	*Accession*
Sonso	*Brycon henni*	100	100	0	1162	KP027535.1
Guabas	*Brycon henni*	99	99	0	1152	KP027535.1
Guabas	*Bryconamericus caucanus*	99	79	0	954	KF210045.1
Yotoco	*Brycon henni*	99	99	0	1219	KP027535.1
Yotoco	*Creagrutus* sp.	90	99	0	852	HM144046.1
Sabaletas*	*Brycon* sp.					

Note: * - all regions showed different identities and coverages at this site.

Table 3

BLAST alignment results of the COI gene region for samples from the Sabaletas river, Buenaventura

Description	*Max score*	*Query coverage*	*E value*	*Identity (%)*	*Accession*
Brycon argenteus voucher stri-3896 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1103	98%	0.0	97.38	MG936800.1
Brycon argenteus voucher stri-4548 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1101	98%	0.0	97.37	MG936798.1
Brycon argenteus voucher stri-4061 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1098	98%	0.0	97.22	MG936799.1
Brycon argenteus voucher stri-7594 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1096	98%	0.0	97.22	MG936797.1
Brycon petrosus voucher stri-16207 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1092	98%	0.0	97.07	MG936812.1
Brycon petrosus voucher stri-7407 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1092	98%	0.0	97.07	MG936811.1
Brycon argenteus voucher stri-11804 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1092	98%	0.0	97.07	MG936796.1
Brycon argenteus voucher stri-3708 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1092	98%	0.0	97.07	MG936795.1
Brycon obscurus voucher stri-1340 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1086	98%	0.0	96.91	MG936810.1
Brycon argenteus voucher stri-11266 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1086	98%	0.0	96.91	MG936794.1
Brycon sp. nov. Acla stri-1722 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1081	98%	0.0	96.76	MG936816.1
Brycon obscurus voucher stri-1385 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitocondrial	1081	98%	0.0	96.76	MG936807.1
Brycon obscurus voucher AM-27 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1075	98%	0.0	96.60	MG936808.1
Brycon sp. nov. Acla stri-3856 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1068	97%	0.0	96.87	MG936815.1
Brycon henni voucher YTR-32 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	948	99%	0.0	92.81	MK355252.1

Table 4 shows the low alignment scores against *B. henni* for the 16S gene region, despite having 100% coverage and an average identity of 96.54%. These results demonstrate that the samples from the Sabaletas river do not belong to this species.

Table 4

BLAST alignment results of the 16S gene region for samples from the Sabaletas river, Buenaventura

Description	Max Score	Query Coverage	E value	% Identity	Accession
Brycon henni voucher Bhen-UNAL-001 mitochondrion, complete genome	955	100%	0.0	96.54	KP027535.1
Brycon petrosus voucher LBP2750-18504 16S ribosomal RNA gene, partial sequence; mitocondrial	955	100%	0.0	96.71	KF780001.1
Brycon hilarii voucher LBP2766-17634 16S ribosomal RNA gene, partial sequence; mitocondrial	946	100%	0.0	96.37	KF779984.1
Brycon henni voucher LBP2857-18984 16S ribosomal RNA gene, partial sequence; mitocondrial	941	100%	0.0	96.19	KF779982.1
Chilobrycon deuterodon voucher LBP9334-45002 16S ribosomal RNA gene, partial sequence; mitocondrial	902	100%	0.0	94.99	KF780004.1
Chilobrycon deuterodon voucher LBP9334-45001 16S ribosomal RNA gene, partial sequence; mitocondrial	900	100%	0.0	94.99	KF780003.1
Brycon aff. atrocaudatus KTA-2014 voucher LBP1356-17096 16S ribosomal RNA gene, partial sequence; mitochondrial	889	100%	0.0	94.65	KF779971.1
Pseudocorynopoma heterandria voucher LBP2862-18570 16S large subunit ribosomal RNA gene, partial sequence; mitocondrial	841	100%	0.0	92.96	HQ171268.1
Lophiobrycon weitzmani voucher LBP1225-38090 16S large subunit ribosomal RNA gene, partial sequence; mitochondrial	824	100%	0.0	92.45	HQ171411.1
Mimagoniates rheocharis voucher MCP 28770, caixa II-53 16S ribosomal RNA gene, partial sequence; mitocondrial	824	98%	0.0	92.72	FJ748993.1
Mimagoniates lateralis voucher AMNH233402 16S large subunit ribosomal RNA gene, partial sequence; mitocondrial	822	100%	0.0	92.28	AY788051.1
Brycon chagrensis voucher LBP2749-18510 16S ribosomal RNA gene, partial sequence; mitocondrial	821	100%	0.0	92.29	KF779972.1
Mimagoniates lateralis voucher UFRGS 8998, Tec 710 16S ribosomal RNA gene, partial sequence; mitocondrial	819	98%	0.0	92.55	FJ748998.1
Planaltina sp. k CO-2018 voucher LBP18902 75270 16S ribosomal RNA gene, partial sequence; mitocondrial	813	100%	0.0	92.08	KY671259.1
Planaltina sp. k CO-2018 voucher LBP18902 75269 16S ribosomal RNA gene, partial sequence; mitocondrial	813	100%	0.0	92.08	KY671258.1
Corynopoma riisei isolate S418 16S ribosomal RNA gene, partial sequence; mitocondrial	813	96%	0.0	93.05	KF209792.1

The RAG1 sequence alignments showed the highest match score to *B. petrosus*; however, the average coverage was 90% and the identity was 99.37%. Therefore, *B. petrosus* was not assigned as homologous species (Table 5).

Table 5

BLAST alignment results of the RAG1 gene region for samples from the Sabaletas river, Buenaventura

Description	Max score	Query coverage	E value	Identity (%)	Accession
Brycon petrosus voucher LBP2750-18504 recombination activating protein 1 (RAG1) gene, partial cds	2294	90%	0.0	99.37	KF780109.1
Brycon sp. UFRGS11377 recombination activating protein 1 (rag1) gene, partial cds	2231	97%	0.0	96.26	KF210893.1
Brycon henni voucher LBP2857-18984 recombination activating protein 1 (RAG1) gene, partial cds	2228	90%	0.0	98.42	KF780097.1
Brycon vermelha voucher LBP9066-42508 recombination activating protein 1 (RAG1) gene, partial cds	2202	87%	0.0	99.18	KF780110.1
Chilobrycon deuterodon voucher LBP9334-45002 recombination activating protein 1 (RAG1) gene, partial cds	2183	87%	0.0	98.94	KF780111.1
Brycon pesu voucher OS 18361 PE10072 recombination activating protein 1 (Rag1) gene, partial cds	2152	98%	0.0	94.85	KX086967.1
Brycon nattereri voucher LBP8101-37541 recombination activating protein 1 (RAG1) gene, partial cds	2067	90%	0.0	96.13	KF780103.1
Brycon opalinus voucher LBP6306-29349 recombination activating protein 1 (RAG1) gene, partial cds	2050	90%	0.0	95.90	KF780106.1
Brycon opalinus voucher LBP6303-29001 recombination activating protein 1 (RAG1) gene, partial cds	2045	90%	0.0	95.82	KF780105.1
Brycon orbignyanus voucher LBP82746-18004 recombination activating protein 1 (RAG1) gene, partial cds	2034	90%	0.0	95.66	KF780104.1
Brycon amazonicus voucher LBP2187-15567 recombination activating protein 1 (RAG1) gene, partial cds	2028	90%	0.0	95.58	KF780085.1
Hepsetus odoe voucher LBP7527-35373 recombination activating protein 1 (RAG1) gene, partial cds	2028	90%	0.0	95.58	HQ289288.1
Brycon amazonicus voucher LBP2187-15565 recombination activating protein 1 (RAG1) gene, partial cds	2028	90%	0.0	95.58	HQ289158.1
Brycon orthotaenia voucher LBP249-4215 recombination activating protein 1 (RAG1) gene, partial cds	2023	90%	0.0	95.50	KF780107.1
Brycon ferox voucher LBP8100-37529 recombination activating protein 1 (RAG1) gene, partial cds	2023	90%	0.0	95.50	KF780095.1

The Kimura 2-parameter model indicated high interspecific genetic divergence and low intraspecific divergence. The samples classified as *Brycon* sp. showed the highest intraspecific genetic distance (COI=1.2%, 16S=0.44%, and RAG1=0.31%). Regarding interspecific divergence, the highest distance was found between *Brycon* sp. and *Creagrutus sp.* (10.37%) for the COI gene region, and the lowest was obtained between *B. henni* and *Brycon* sp. (3.38%) (Table 6). Moreover, in the 16S region, the highest divergence was observed between *Brycon* sp. and *Creagrutus* sp. (3.6%) and the lowest was between *B. henni* and *Brycon* sp. (1.12%) (Table 6).

The RAG1 nuclear region showed a similar tendency compared to the two mitochondrial markers. For instance, low interspecific and high intraspecific distances were found; specifically, 0.18% for *B. henni*, 0.31% for *Brycon* sp., 1.16% for *B. caucanus*, and 0.21% for *Creagrutus* sp. The highest interspecific distance was observed between *Brycon* sp. and *Creagrutus* sp. (4.59%), and the lowest was between *B. henni* and *Brycon* sp. (0.71%) (Table 6).

Table 6
Sequence variation and discrimination power of the three gene regions for DNA barcoding

Items	*Regions*		
	COI	*16S*	*RAG1*
Number of species	4	4	4
Number of aligned sequences	657	584	1404
% amplification success	97.5	100	87.5
% sequencing success	100	97.5	94.3
% successfully identified species (*B. henni/Brycon* sp./*B. caucanus/Creagrutus* sp.)	100	100	100
% variable nucleotide positions	25.11	10.27	13.82
% Diagnostic nucleotide sites (Parsimony-informative site)	24.50	10.10	10.32
Average interspecific distance (Min. - Max.)	0.0859 (0.0 - 0.2203)	0.0835 (0.0 - 0.0797)	0.0319 (0.0 - 0.0964)
Average intraspecific distance for *B. henni* (Min. - Max.)	0.004(0.0- 0.0046)	0.000 (0.0-0.0)	0.0018 (0.0 – 0.0085)
Average intraspecific distance for *Brycon* sp. (Min. - Max.)	0.0120 (0.0- 0.0249)	0.0044 (0.0 -0.0087)	0.0031 (0.0 – 0.0068)
Average intraspecific distance for *Bryconamericus caucanus* (Min. - Max.)	0.0031 (0.0-0.0031)	0.0017 (0.0 – 0.0017)	0.0116 (0.0 – 0.0116)
Average intraspecific distance for *Creagrutus* sp. (Min. - Max.)	0.00 (0.0-0.00)	0.00 (0.0-0.00)	0.0021 (0.0 – 0.0034)
Average interspecific distance between *B. henni - B. caucanus.* (Min. - Max.)	0.0361 (0.0 - 0.2127)	0.0121 (0.0- 0.0797)	0.0171 (0.0- 0.0960)
Average interspecific distance between *B. henni - Creagrutus* sp. (Min. - Max.)	0.0677 (0.0 - 0.2073)	0.0225 (0.0-0.0739)	0.0264 (0.0 – 0.0913)
Average interspecific distance between *B. henni- Brycon* sp. (Min. - Max.)	0.0338 (0.0 - 0.0806)	0.0112 (0.0- 0.0326)	0.0071 (0.0 – 0.0212)
Average interspecific distance between *Brycon* sp. - *B. caucanus.* (Min. - Max.)	0.0781 (0.0 - 0.2203)	0.0290 (0.0- 0.0835)	0.0346 (0.0 – 0.0964)
Average interspecific distance between *Brycon* sp. - *Creagrutus* sp. (Min. - Max.)	0.1037 (0.0 - 0.2008)	0.0360 (0.0 – 0.0719)	0.0459 (0.0 – 0.0951)

DNA sequencing of the COI region generated 657 base pairs, including 165 (25.1%) variable sites (Table 6) and 22 haplotypes identified from 39 sequences (Table 7). The haplotype network comprised four haplotype groups separated by 20, 20, and 40 mutational steps, respectively. This mitochondrial region showed the highest number of variable sites. The most representative group was haplogroup 1, which was composed of *B. henni* (n=16, 72.73%), followed by haplogroup 2 with *Brycon* sp. (n=3, 13.64), haplogroup 3 with *Bryconamericus caucanus* (n=2; 9.1%), and haplogroup 4 with *Creagrutus* sp. (n=1; 4.5%).

The first haplogroup showed three haplotypes (h1, h2, and h16), which were a combination of individuals from Sonso, Guabas, and Yotoco, while the remaining were individual haplotypes. The second haplogroup comprised two haplotypes (h18 and h19) and an individual haplotype (h17) in individuals from Sabaletas, Buenaventura. The third haplogroup contained two individual haplotypes (h20 and h21), while the fourth haplogroup comprised a single haplotype (h22) (Table 7 and Figure 2a).

Table 7

List of haplotypes of *Brycon henni*, *Bryconamericus caucanus*, *Brycon* sp., and *Creagrutus* sp. obtained from the statistical parsimony network analyses (TCS) for partial COI, 16S, and RAG1 gene sequences

COI LOCUS		
Haplotype	*Number*	*Individual*
h1-Bhenni	4	Bhenni11 Bhenni14 Bhenni37 Bhenni39
h2-Bhenni	3	Bhenni10 Bhenni2 Bhenni40
h3-Bhenni	1	Bhenni7
h4-Bhenni	1	Bhenni38
h5-Bhenni	1	Bhenni1
h6-Bhenni	1	Bhenni9
h7-Bhenni	1	Bhenni17
h8-Bhenni	1	Bhenni18
h9-Bhenni	1	Bhenni31
h10-Bhenni	1	Bhenni19
h11-Bhenni	1	Bhenni8
h12-Bhenni	1	Bhenni4
h13-Bhenni	1	Bhenni6
h14-Bhenni	1	Bhenni5
h15-Bhenni	1	Bhenni12
h16-Bhenni	3	Bhenni16 Bhenni20 Bhenni3
h17-Brycon	1	Brycon25
h18-Brycon	5	Brycon21 Brycon26 Brycon27 Brycon29 Brycon30
h19-Brycon	3	Brycon22 Brycon23 Brycon24
h20-Bcauca	1	Bcauca13
h21-Bcauca	1	Bcauca15
h22-Creag	5	Creag32 Creag33 Creag34 Creag35 Creag36
16S LOCUS		
Haplotype	Number	Individual
h1-Bhenni	22	Bhenni1 Brycon10 Bhenni11 Bhenni12 Bhenni14 Bhenni16 Bhenni17 Bhenni19 Bhenni2 Bhenni20 Bhenni3 Bhenni31 Bhenni37 Bhenni38 Bhenni39 Bhenni4 Bhenni40 Bhenni5 Bhenni6 Bhenni7 Bhenni8 Bhenni9
h2-Bhenni	1	Bhenni18
h3-Brycon	6	Brycon21 Brycon25 Brycon26 Brycon27 Brycon28 Brycon30
h4-Brycon	3	Brycon22 Brycon23 Brycon24
h5-Bcauca	1	Bcauca15
h6- Bcauca	1	Bcauca13
h7-Creag	5	Creag32 Creag33 Creag34 Creag35 Creag36
LOCUS RAG1		
Haplotype	Number	Individual
h1-Bhenni	8	Bhenni12 Bhenni1 Bhenni20 Bhenni2 Bhenni37 Bhenni39 Bhenni5 Bhenni8
h2-Bhenni	1	Bhenni10
h3-Bhenni	2	Bhenni18 Bhenni19
h4-Bhenni	1	Bhenni31
h5-Bhenni	4	Bhenni11 Bhenni14 Bhenni16 Bhenni3
h6-Bhenni	1	Bhenni40
h7-Bhenni	1	Bhenni17
h8-Bhenni	1	Bhenni7
h9-Brycon	1	Brycon25
h10-Brycon	1	Brycon24
h11-Brycon	1	Brycon26
h12-Brycon	1	Brycon28
h13-Brycon	1	Brycon23
h14-Brycon	1	Brycon30
h15-Brycon	1	Brycon21
h16-Brycon	1	Brycon22
h17-Bcauca	1	Bcauca13
h18-Bcauca	1	Bcauca15
h19-Creag	1	Creag32
h20-Creag	1	Creag34
h21-Creag	1	Creag35
h22-Creag	1	Creag36

The 16S gene fragment spanned 584 base pairs, including 60 variable sites (Table 6). The haplotype network showed a similar structure to the COI region despite having a fewer number of haplotypes (seven haplotypes), derived from 39 sequences (Table 7). Furthermore, the same four haplotypes described previously were separated by 14, 7, and 14 mutational steps. This marker is the most conserved among the three studied here, since it shows the lowest percentage of variable sites. The most representative haplogroup was haplogroup 1 *B. henni* (n=2, 28.57%), which comprised mainly haplotype 1 (h1) from Sonso, Guabas, and Yotoco, as well as the individual haplotype 2 (h2). Furthermore, haplogroup 2 *Brycon* sp. (n=2, 28.57%) comprised two haplotypes (h3 and h4) represented by individuals from Sabaletas, Buenaventura; haplogroup 3 *B. caucanus* (n=2, 28.57%) was composed of two individual haplotypes (h5 and h6), and haplotype 4 *Creagrutus* sp. (n=1; 14.29%) contained one haplotype (h7) (Table 7 and Figure 2b).

Finally, the RAG1 region spanned 1404 base pairs, including 194 (13.82%) variable nucleotides (Table 6). It displayed 22 haplotypes derived from 33 sequences (Table 7) and four haplogroups separated by 15, 107, and 52 mutational steps. This region shows a similar structure compared to the mitochondrial regions; for instance, the first haplogroup is represented by *B. henni* (n=8, 36.36%) with three haplotypes (h1, h3, and h5); specifically, haplotype h1 is observed in a combination of individuals from Guabas, Sonso, and Yotoco, while the remaining haplotypes are monophyletic (h2, h4, h6, h7, and h8). The second haplogroup belongs to *Brycon* sp. (n=8; 36.36%) and comprises individual haplotypes from Sabaletas, Buenaventura (h9, h10, h11, h12, h13, h14, h15, and h16). The third haplogroup *B. caucanus* (n=2; 9.1%) is composed of individual haplotypes (h17 and h18) and, finally, the fourth haplogroup *Creagrutus* sp. (n=4; 18.2%) comprises individual haplotypes (h19, h20, h21, and h22) (Table 7 and Figure 2c).

The most variable markers were COI and RAG1, which displayed 22 different haplotypes each (Table 7). The Neighbour-joining dendrogram obtained for the COI mitochondrial region showed two large groups. The first subgroup included individuals of *B. henni* from three nearby zones, namely Guacari, Sonso river (violet), Ginebra, Guabas river (green), and the Yotoco river (blue), indicating gene flow across these populations. The second subgroup comprised individuals of *Brycon* sp. collected at Sabaletas river, Buenaventura (red), with a bootstrap support of 92.05%. The outgroup or second group has a bootstrap support of 100 and includes individuals of *B. caucanus* (green) collected at Guabas and, finally, the fourth clade comprises individuals of *Creagrutus* sp. (blue) from Yotoco (Figure 3a).

The dendrogram reconstructed from 16S gene sequences showed two clearly defined groups. Particularly, the first group shows a clear division between *B. henni* - *Brycon* sp. and *B. henni* - *Creagrutus* sp. (Figure 3b). Similarly, the dendrogram derived from RAG1 nuclear sequences confirmed the two previously described groups and showed the greatest resolution for *B. henni* and *Creagrutus* sp., as well as a close relationship between *B. henni* and *Brycon* sp. in the first group (Figure 3c).

Similar structures were observed for the dendrogram estimated from the three gene fragments and the molecular analysis of *B. henni*, which demonstrated two groups; the first group displayed two subgroups composed of the species *B. henni* - representing individuals from Yotoco, Sonso, and Guabas - and *Brycon* sp. specimens collected at Sabaletas, Buenaventura. Finally, the second group comprised the outgroups that contained two subgroups belonging to *B. caucanus* from the Guabas river and *Creagrutus* sp. collected from the Yotoco river (Figure 3d).

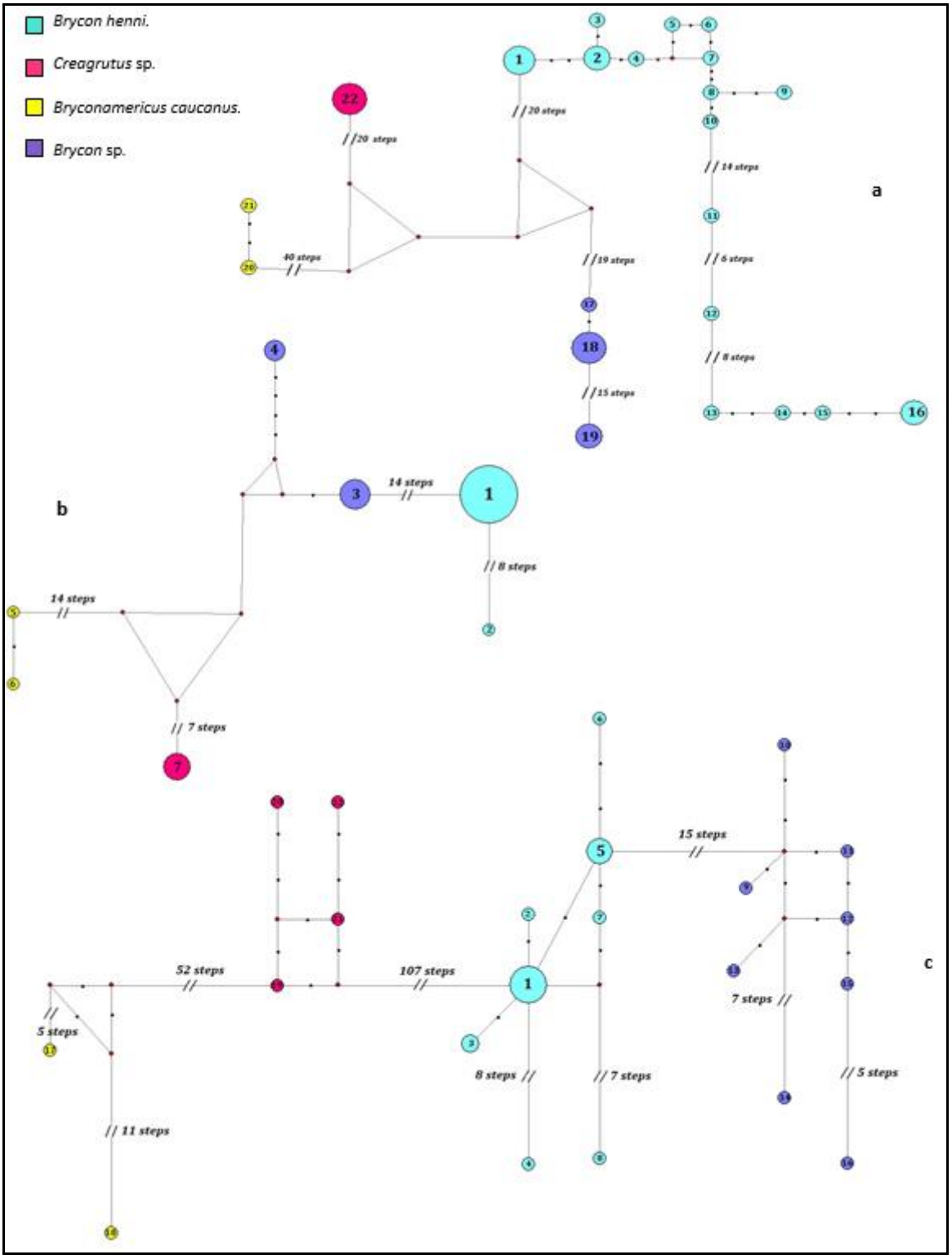

Figure 2. Haplotype network for: a - COI; b - 16S; c - RAG1 gene regions.

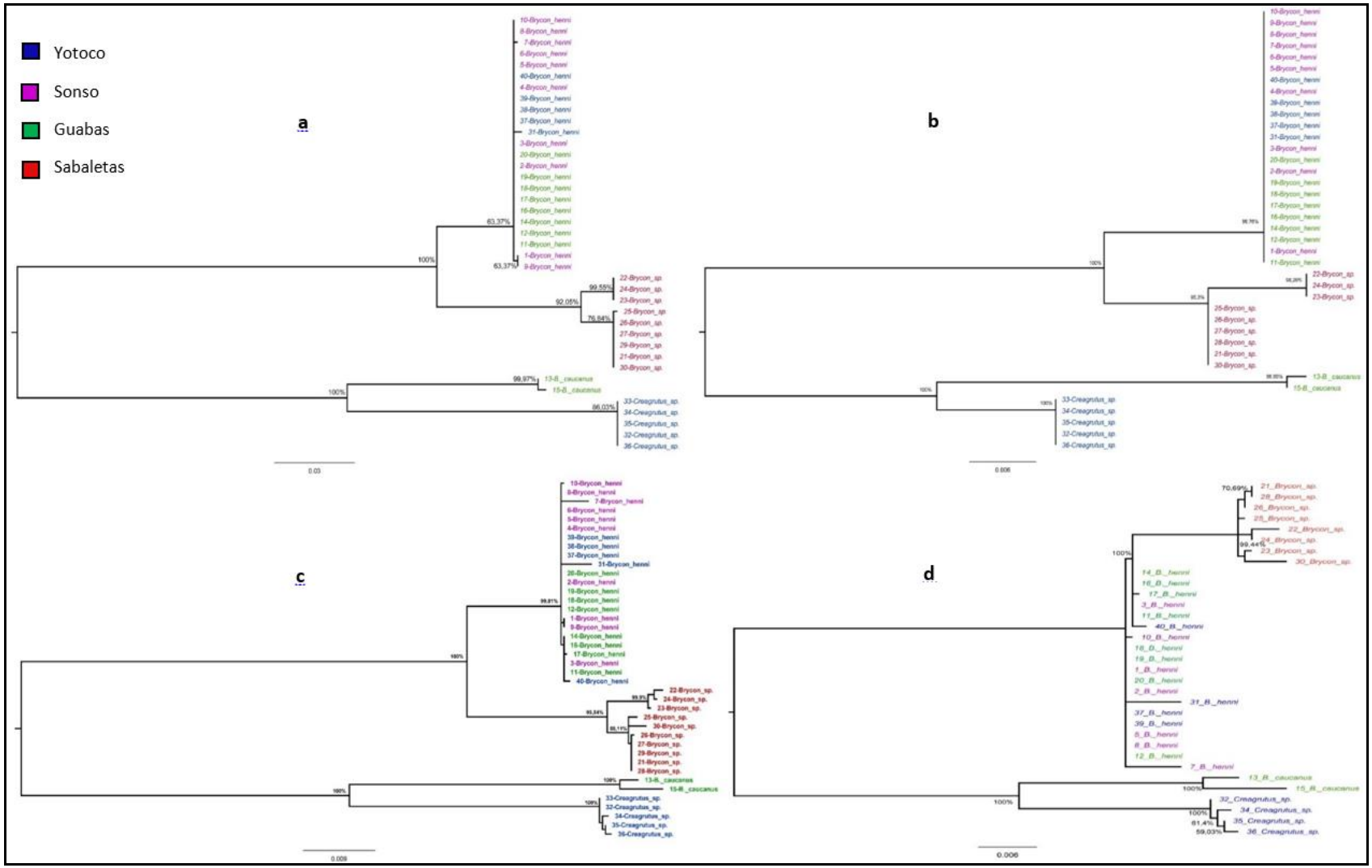

Figure 3. Trees constructed using Neighbor-Joining and distances (Kimura 1980) based on: a - COI; b - 16S; c - RAG1 gene sequences; d - combined data (COI-16S-RAG1).

Colombia spans the Pacific, Caribbean, Orinoco, and Amazon basins. Approximately 70% of the population lives in the Caribbean basin, particularly, near the Cauca and Magdalena river basins, which are the most important (Gualdron-Duran 2016). The individuals studied here were collected from three tributaries of the Cauca river, namely the Sonso and Guabas rivers in the right flank and the Yotoco river in the left flank. Additionally, samples were collected from the Sabaletas river, which is a tributary of the Anchicaya river located in the municipality of Buenaventura in the Pacific basin.

This study relied on a DNA barcode reported by the FISH-BOL community for the cytochrome C oxidase (COI) subunit I gene, since this gene has been used among 31000 known fish species, of which 98% marine species and 93% freshwater species can be discriminated through DNA barcoding (Ward 2012). Landínez-García et al (2014) reported the complete mitochondrial genome sequence of *B. henni*, which spans 16885 bp.

A phylogenetic analysis of the genera *Brycon* and *Henochilus* based on the 16S gene showed that *Brycon* is paraphiletic. *Henochilus* is a sister group to *B. ferox* and *B. insignis*, and the most basal species included in the analysis were the trans-Andean species *B. henni, B. petrosus*, and *B. chagrensis* (Hilsdorf et al 2008). In another study on molecular phylogenetics using the COI gene to study the fish belonging to the Midas cichlidae complex (*Amphilophus citrinellus*, *Amphilophus labiatus*, *Amphilophus zaliosus*) in lakes and lagoons in Nicaragua, Páiz-Medina & Huete-Pérez (2012) determined the presence of several species of cichlidae family, which were discriminated by this gene. However, additional genes were required to establish the relationships between these species.

This research was based on the COI and 16S mitochondrial regions; however, the COI region was most informative since it displayed 100% sequencing success, greater polymorphism and more informative haplotype networks compared with the other gene regions. Therefore, this study confirms the use of this marker as a DNA barcode, which has also been used in several fish studies. For example, Mohanty et al (2015) demonstrated that the COI gene is more useful than the 16S rRNA gene for DNA barcoding of Indian carps (*Labeo rohita, Catla catla, Cirrhinus mrigala, Labeo fimbriatus, Labeo bata* and *Cirrhinus reba*), reporting the use of this marker to identify cultivable carp species, while the 16S rRNA markers did not achieve this level of identification due to low genetic divergence. In Taiwan, Bingpeng et al (2018) used DNA barcoding (COI) to identify fish species, reporting high efficiencies. In Brazil, a new species of *Hyphessobrycon* was identified in the high basins of the Munim and Preguiças rivers through DNA barcoding (Guimarães et al 2018). In the marine waters in China, Zhang, et al (2013) identified and discriminated eight species of four subgenera of the genus *Sebastes*. On the other hand, a phylogenetic analysis of the 16S, COI, MYH6 genes and intron S72 demonstrated the monophyly of *Bryconinae* species from southeastern Brazil (Travenzoli et al 2015). In a DNA barcoding study conducted on rosy tetras (*Hyphessobrycon rosaceus*) and related species (Characiformes: Characidae: *Hyphessobrycon*) in the Brazilian Amazon basin, a complete diagnosis was not achieved; however, distinct evolutionary units were discovered in certain species of *Hyphessobrycon*, as well as haplotype exchange across species (Castro Paz et al 2014). Finally, a molecular phylogenetic analysis in Siluriformes using the COI gene proved that this marker was highly effective to evaluate the evolutionary relationship of some species (Indu et al 2012).

In this study, the RAG1 nuclear region displayed the lowest amplification and sequencing percentages (87.5% and 94.3%, respectively). Furthermore, the nested PCR required more protocol steps and materials. The haplotype network showed a higher number of haplotypes compared with the COI gene, but displayed differences regarding the structure of the groups. This marker was also useful to confirm the identity of the individuals (*Byrcon* sp.) collected from the Sabaletas river, based on a BLAST search. This demonstrated the use of the nuclear RAG1 gene region in fish identification. Bufalino & Mayden (2010) describe an analysis of RAG1 and S7 nuclear DNA sequences to assess phylogenetic relationships in *Phoxinus* in North America and demonstrated a high degree of topological variability similar to other studies based on mitocondrial DNA.

Sullivan et al (2006) conducted a phylogenetic analysis on catfishes (Teleostei: Siluriformes) using RAG1 and RAG2 nuclear gene sequences, which confirmed the monophyly of Siluriformes. South American Loricarioidei are identified as a sister group to other catfishes, which are divided into Diplomystidae and Siluroidei. Overall, these reports indicate that this molecular marker is effective in reconstructing phylogenetic relationships.

Several studies demonstrate the importance of using two types of regions (i.e., mitochondrial and nuclear). For instance, a study conducted by Barbosa et al (2014) on species of the *Stellifer* group of weakfishes (Sciaenidae, Perciformes) of the southwest Atlantic presented a complete molecular phylogeny of the genus *Stellifer* based on mitochondrial (16S rRNA and COI) and nuclear (rodopsin, TMO-4C4, RAG1) genes. Furthermore, the analysis showed close relationships among the taxa, as well as two distinct lineages of *Stellifer*. Likewise, Cramer et al(2011) conducted a molecular phylogenetic analysis on Neoplecostominae and Hypoptopomatinae based on COI, RAG1, RAG2, and F-Reticulon 4 genes, finding that Hypoptopomatini, Otothyrini, and Neoplecostominae and other species-rich genera were not monophyletic. Moreover, Oliveira et al (2011) studied the phylogenetic relationships in the family Characidae based on mitochondrial (16S rRNA, Cytb) and nuclear (Myh6, RAG1, RAG2) genes, contributing a novel phylogenetic framework for a special, morphologically diverse group of freshwater fish of ecological importance and evolutionary significance across the Neotropical region and parts of Africa.

The haplotype network showed genetic dissimilarity between populations of *Brycon* sp*.* collected in the Sabaletas river and populations of *B. henni* from the Yotoco, Guabas, and Sonso rivers. Additionally, outgroups belonging to *B. caucanus* and *Creagrutus* sp*.* were also observed. Recent studies on *Brycon*, such as the one by Arruda et al (2019), showed that haplotype differences are found when there are also geographic differences between populations. Accordingly, these studies confirmed that molecular distances between haplotype pairs from different water basins are much greater than those between haplotypes from the same region.

The phylogenetic analysis based on Neighbor-Joining for the mitochondrial (COI and 16S) and nuclear (RAG1) regions showed two large groups. The first group contained two clades, namely a *B. henni* clade (according to the BLAST alignments) composed of individuals collected in the Sonso, Yotoco, and Guabas rivers, and a second clade that corresponded to *Brycon* sp*.*, which grouped specimens collected in the Sabaletas river. The BLAST alignment results for the 16S, COI, and RAG1 sequences from the Sabaletas river specimens were inconclusive, so a species-level identification was not possible. Moreover, the second group comprised two clades belonging to *B. caucanus* and *Creagrutus* sp*.* collected from the Guabas and Yotoco rivers, respectively. These species were included as outgroups. The results found here are similar to those reported by Abe et al (2014), who found that Bryconidae is composed of five main clades, including *Brycon*, *Chilobrycon*, *Henochilus*, and *Salminus*. Furthermore, their results pointed out a possible ancient invasion of Central America to explain the occurrence of *Brycon* in that region, which suggests a taxonomic review of these groups. Similarly, Ashikaga et al (2015) indicated that *Brycon orbignyanus*, an endangered species, was structured into different subpopulations in La Plata river basin. Also, in areas with the best environmental conditions, the species showed subgroups with the highest rates of genetic variability. Likewise, Pereira et al (2013) correctly identified 252 freshwater Neotropical fish species using DNA barcoding and reported that 14 species showed K2P intraspecific distances that exceeded 2%. Thus, two to six subgroups were observed in the Neighbor-Joining dendrogram.

Conclusions. We demonstrate the efficiency of DNA barcoding based on COI, 16S, and RAG1 gene regions for the identification of *Brycon henni*, *Brycon* sp*.*, *Bryconamericus caucanus*, and *Creagrutus* sp*.* The phylogenetic and haplotype network analyses of *B. henni* from three nearby geographical regions, namely the Sonso, Yotoco, and Guabas rivers, indicated continuous gene flow across the individuals. Furthermore, the specimens from the Sabaletas river, Buenaventura, identified as *Brycon* sp*.,* are different from the

individuals from the Yotoco, Guabas, and Sonso rivers, belonging to *B. henni.* Moreover, *B. caucanus* from the Guabas river and *Creagrutus* sp*.* from the Yotoco river were established as outgroups. Finally, the COI and RAG1 gene regions showed high haplotypic diversity, indicated by 22 haplotypes, although the two regions displayed different structures.

Acknowledgements. This work was funded by COLCIENCIAS (MINCIENCIAS) through Grant Number 645 Programa de Jóvenes Investigadores 2014. The authors thank everyone who assisted us in sample collection (Álvaro Domínguez, Adriana Rueda, Fernando Montenegro, Manuel Esteban Bordón, Iván Manuel López, Andi Pamela Mendoza, Joaquín Alonso Marín Caicedo, Marino Arango Escobar, Oscar Arango Gómez). They also thank Jorge Mario Londoño for the valuable suggestions made at the beginning of the study. We would also like to thank Andrea Gonzalez for translating the manuscript.

Conflict of Interest. The authors declare that there is no conflict of interest.

References

Abe K. T., Mariguela T. C., Avelino G. S., Foresti F., Oliveira C., 2014 Systematic and historical biogeography of the Bryconidae (Ostariophysi: Characiformes) suggesting a new rearrangement of its genera and an old origin of Mesoamerican ichthyofauna. BMC Evolutionary Biology 14(1):152, 15 p.

Akram S., Arshan K. M. L., Abdul J. H., 2017 DNA barcoding and phylogenetic analysis of five ascidians (Phlebobranchia) distributed in Gulf of Mannar, India. Mitochondrial DNA Part A: DNA Mapping, Sequencing, and Analysis 29(4):581-586.

Arruda P. S., Ferreira D. C., Oliveira C., Venere P. C., 2019 DNA barcoding reveals high levels of divergence among mitochondrial lineages of *Brycon* (Characiformes, Bryconidae). Genes 10(9:639, 12 p.

Ashikaga F. Y., Orsi M. L., Oliveira C., Senhorini J. A., Foresti F., 2015 The endangered species *Brycon orbignyanus*: genetic analysis and definition of priority areas for conservation. Environmental Biology of Fishes 98:1845-1855.

Barbosa A. J. B., Sampaio I., Schneider H., Santos S., 2014 Molecular phylogeny of weakfish species of the Stellifer group (Sciaenidae, Perciformes) of the western South Atlantic based on mitochondrial and nuclear data. PloS ONE 9(7):e102250, 8 p.

Bardakci F., Skibinski D. O., 1994 Application of the RAPD technique in tilapia fish: species and subspecies identification. Heredity 73(2):117-123.

Bingpeng X., Heshan L., Zhilan Z., Chunguang W., Yanguo W., Jianjun W., 2018 DNA barcoding for identification of fish species in the Taiwan Strait. PLoS ONE 13(6):e0198109, 13 p.

Botero-Botero A., Ramírez-Castro H., 2011 [Trophic ecology of *Brycon henni* (Pisces: Characidae) in the Portugal de Piedras river, upper Cauca basin, Colombia]. Revista MVZ Córdoba 16(1):2349–2355. [In Spanish].

Bufalino A. P., Mayden R. L., 2010 Molecular phylogenetics of North American phoxinins (Actinopterygii: Cypriniformes: Leuciscidae) based on RAG1 and S7 nuclear DNA sequence data. Molecular Phylogenetics and Evolution 55(1):274-283.

Builes J., Urán A., 1974 [Study of the sexual cycle of the sabaleta *Brycon henni* Eigenmann. Its behavior and artificial fertilization]. Actualidades Biológicas 7(3):2-12. [In Spanish].

Castro Paz F. P., da Silva Batista J., Rebelo Porto J. I., 2014 DNA barcodes of rosy tetras and allied species (Characiformes: Characidae: *Hyphessobrycon*) from the Brazilian Amazon basin. PLoS ONE 9(5):e98603, 8 p.

Chandra A., Idrisova A., 2011 Convention on Biological Diversity: a review of national challenges and opportunities for implementation. Biodiversity and Conservation 20(14):3295-3316.

Clement M., Posada D., Crandall K. A., 2000 TCS: a computer program to estimate gene genealogies. Molecular Ecology 9(10):1657-1659.

Cramer C. A., Bonatto S. L., Reis R. E., 2011 Molecular phylogeny of the Neoplecostominae and Hypoptopomatinae (Siluriformes: Loricariidae) using multiple genes. Molecular Phylogenetics and Evolution 59(1):43-52.
Darriba D., Taboada G. L., Doallo R., Posada D., 2012 jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9(8):772-772.
Esquivel M. A., Merino M., Restrepo J., Narváez A., Polo C., Plata J., Puentes V., 2014 [State of fisheries and aquaculture in Colombia]. Autoridad Nacional de Acuicultura y Pesca, AUNAP, 34 p. [In Spanish].
Gong S., Ding Y., Wang Y., Jiang G., Zhu C., 2018 Advances in DNA barcoding of toxic marine organisms. International Journal of Molecular Sciences 19(10):2931, 18 p.
Gualdrón Durán L. E., 2016 [Evaluation of the water quality of Colombian rivers using physicochemical and biological parameters]. Revista Dinámica Ambiental 1:83-102. [In Spanish].
Guimarães E. C., De Brito P. S., Feitosa L. M., Carvalho-Costa L. F., Ottoni F. P., 2018 A new species of *Hyphessobrycon* Durbin from northeastern Brazil: evidence from morphological data and DNA barcoding (Characiformes, Characidae). ZooKeys 765:79-101.
Hilsdorf A. W. S., Oliveira C., Thadeo de Lima F. C., Matsumoto C. K., 2008 A phylogenetic analysis of *Brycon* and *Henochilus* (Characiformes, Characidae, Bryconinae) based on the mitochondrial gene 16S rRNA. Genetics and Molecular Biology 31:366-371.
Hubert N., Hanner R., Holm E., Mandrak N. E., Taylor E., Burridge M., Watkinson D., Dumont P., Curry A., Bentzen P., Zhang J., April J., Bernatchez L., 2008 Identifying Canadian freshwater fishes through DNA barcodes. PLoS One 3(6):e2490, 8 p.
Hurtado-Alarcón J. C., Mancera-Rodríguez N. J., Saldamando-Benjumea C. I., 2011 [Genetic variability of *Brycon henni* (Characiformes: Characidae) in the middle basin of the Nare and Guatapé rivers, Río Magdalena system, Colombia]. Revista de Biología Tropical 59(1):269-282. [In Spanish].
Indu M., Ambili T. R., Manimekalan A., 2012 *In sillico* analysis of the molecular phylogeny of Siluriformes inferred from mitochondrial *CO1* gene. International Journal of Advanced Life Sciences 5(1):71-78.
Jiménez-Segura L. F., Restrepo-Santamaría D., López-Casas S., Delgado J., Valderrama M., Álvarez J., Gómez D., 2014 [Ichthyofauna and development of the hydroelectric sector in the Magdalena-Cauca river basin, Colombia]. Biota Colombiana 15(2):3-25. [In Spanish].
Kerr K. C. R., Stoeckle M. Y., Dove C. J., Weigt L. A., Francis C. M., Hebert P. D. N., 2007 Comprehensive DNA barcode coverage of North American birds. Molecular Ecology Notes 7(4):535-543.
Kimura M., 1980 A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16(2):111-120.
Kumar S., Stecher G., Tamura K., 2016 MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7):1870-1874.
Landínez-García R. M., Alzate J. F., Márquez E. J., 2014 Complete mitogenome of the neotropical fish *Brycon henni*, Eigenmann 1913 (Characiformes, Bryconidae). Mitochondrial DNA Part A 27(3):2259-2260.
Li C., Ortí G., 2007 Molecular phylogeny of Clupeiformes (Actinopterygii) inferred from nuclear and mitochondrial DNA sequences. Molecular Phylogenetics and Evolution 44(1):386-398.
Maldonado-Ocampo J. A., Ortega-Lara A., Usma Oviedo J. S., Galvis Vergara G., Villa-Navarro F. A., Vásquez Gamboa L., Prada-Pedreros S., Ardila Rodríguez C., Calle V. J. C., 2005 [Fish from the Andes of Colombia]. Humboldt Institute, Colombia, 346 p. [In Spanish].
Mohanty M., Jayasankar P., Sahoo L., Das P., 2015 A comparative study of COI and 16S rRNA genes for DNA barcoding of cultivable carps in India. Mitochondrial DNA 26(1):79-87.

Mojica J. I., Usma J. S., Álvarez-León R., Lasso, C. A. (Eds). (2012). *Libro rojo de peces dulceacuícolas de Colombia 2012.* Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Instituto de Ciencias Naturales de la Universidad Nacional de Colombia, WWF Colombia y Universidad de Manizales, 319 p.
Montoya-López A. F., Carrillo L. M., Olivera Ángel M., 2006 [Some biological and captive aspects of the sabaleta *Brycon henni* Eigenmann, 1913 (Pisces: Characidae)]. Revista Colombiana de Ciencias Pecuarias 19(2):180-186. [In Spanish].
Moreno L. T., 2018 [Fishing and artisanal fishermen in Colombia]. PEGADA - A Revista Da Geografia Do Trabalho 19(2):343-377. [In Spanish].
Oliveira C., Avelino G. S., Abe K. T., Mariguela T. C., Benine R. C., Ortí G., Vari R. P., Corrêa e Castro R. M., 2011 Phylogenetic relationships within the speciose family Characidae (Teleostei: Ostariophysi: Characiformes) based on multilocus analysis and extensive ingroup sampling. BMC Evolutionary Biology 11(1):275, 25 p.
Páiz-Medina L., Huete-Pérez J., 2012 [Molecular phylogenetics of fish of the Midas Cichlidae Complex that inhabit lakes and lagoons in Nicaragua, using the COI gene]. Encuentro 93:101-116. [In Spanish].
Palumbi, 1996 Nucleic acids II: The Polymerase Chain Reaction. In: Molecular systematics. 2nd Edition. Hillis D. M., Moritz C., Mable B. K. (eds), Sinauer, pp. 205-247.
Pereira L. H. G., Hanner R., Foresti F., Oliveira C., 2013 Can DNA barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna? BMC Genetics 14(1):20, 14 p.
Schmitz A., Riesner D., 2006 Purification of nucleic acids by selective precipitation with polyethylene glycol 6000. Analytical Biochemistry 354(2):311-313.
Sullivan J. P., Lundberg J. G., Hardman M., 2006 A phylogenetic analysis of the major groups of catfishes (Teleostei: Siluriformes) using rag1 and rag2 nuclear gene sequences. Molecular Phylogenetics and Evolution 41(3):636-662.
Tognelli M. F., Lasso C. A., Bota-Sierra C. A., Jiménez-Segura L. F., Cox N. A., 2016 [Conservation status and distribution of freshwater biodiversity in the tropical Andes]. IUCN, 199 p. [In Spanish].
Travenzoli N. M., Silva P. C., Santos U., Zanuncio J. C., Oliveira C., Dergam J. A., 2015 Cytogenetic and molecular data demonstrate that the Bryconinae (Ostariophysi, Bryconidae) species from southeastern Brazil form a phylogenetic and phylogeographic unit. Plos ONE 10(9):e0137843, 18 p.
Ward R. D., 2012 FISH-BOL, a case study for DNA barcodes. In: DNA barcodes. Methods in molecular biology (methods and protocols). Kress W., Erickson D. (eds), Humana Press, vol. 858, pp. 423-439.
Zapata-Restrepo L. M., Orozco-Jiménez L. Y., Rueda-Cardona M., Echavarría S. L., Mena-Moreno N., Palacio-Baena J. A., 2017 [Genotoxic assessment of Rio Grande water (Antioquia, Colombia) by frequency of micronucleated erythrocytes of *Brycon henni* (Characiformes: Characidae)]. Revista de Biología Tropical 65(1):405-414. [In Spanish].
Zhang H., Zhang Y., Zhang Z., Gao T., 2013 DNA barcodes of eight species in genus *Sebastes*. Biochemical Systematics and Ecology 48:45-50.

Received: 09 February 2021. Accepted: 17 February 2021. Published online: 19 August 2021.
Authors:
Edilma Vélez-Sinisterra, Facultad de Ciencias Agropecuarias, Universidad Nacional de Colombia, 43600 Palmira, Valle del Cauca, Colombia, e-mail: edvelezsi@unal.edu.co
Diana López Alvarez, Facultad de Ciencias Agropecuarias, Universidad Nacional de Colombia, 43600 Palmira, Valle del Cauca, Colombia, e-mail: dilopezal@unal.edu.co
Carlos Alberto Jaramillo Cruz, Facultad de Ciencias Agropecuarias, Universidad Nacional de Colombia, 43600 Palmira, Valle del Cauca, Colombia, e-mail: cajaramillocr@unal.edu.co
Jaime Eduardo Muñoz Florez, Facultad de Ciencias Agropecuarias, Universidad Nacional de Colombia, 43600 Palmira, Valle del Cauca, Colombia, e-mail: jemunozf@unal.edu.co

How to cite this article:
Vélez-Sinisterra E., López-Alvarez D., Jaramillo Cruz C. A., Muñoz Florez J. E., 2021 DNA barcoding of *Brycon henni* (Characiformes: Characidae) in the Yotoco, Sonso, Guabas and Sabaletas rivers of Colombia. AACL Bioflux 14(4):2211-2228.