

Development of microsatellite loci and population genetics in the bumblebee catfish species *Pseudopimelodus atricaudus* and *Pseudopimelodus magnus* (Siluriformes: Pseudopimelodidae)

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The Neotropical freshwater catfish *Pseudopimelodus atricaudus* and *Pseudopimelodus magnus* are two recently discovered species endemic to the Colombian Magdalena-Cauca River basin. In this study, a set of 13 microsatellite loci were developed by using next generation sequence technology to assess the genetic diversity and population structure in *P. atricaudus* and test for cross-species amplification in *P. magnus*. Both species exhibited high genetic diversity (*P. atricaudus*: Na: 9.000 – 9.769 alleles/locus, Ho: 0.760 – 0.804, H_E: 0.804 – 0.840; *P. magnus*: Na: 12.8 – 5.4 alleles/locus, Ho: 0.638 – 0.683, H_E: 0.747 – 0.755) compared to the mean levels of genetic diversity reported for Neotropical Siluriformes, and lack of genetic differentiation among sampling sites within the Cauca River (*P. atricaudus*: F'_{ST} = 0.013 – 0.017, P > 0.05, D'_{est} = -0.004 – 0.023, P > 0.05; *P. magnus*: F'_{ST} = 0.031, P = 0.055; D'_{est} = 0.045, P = 0.058). This work is the first insight on the diversity and the population genetics of species of the family Pseudopimelodidae and provides a framework to further population genetic and conservation analyses needed in this poorly studied family at the microevolutionary level.

Keywords: Colombia, Freshwater fish, Gene flow, Genetic diversity, Magdalena-Cauca basin.

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Los bagres neotropicales *Pseudopimelodus atricaudus* y *Pseudopimelodus magnus* son dos especies recientemente descubiertas, endémicas de la cuenca Magdalena-Cauca en Colombia. En este estudio, se desarrollaron 13 loci microsatélites usando tecnología de secuenciación de próxima generación para evaluar la diversidad genética y la estructura poblacional de *P. atricaudus* y evaluar su amplificación cruzada en *P. magnus*. Ambas especies exhibieron altos valores de diversidad genética (*P. atricaudus*: N_a : 9.000 – 9.769 alelos/locus, H_o : 0.760 – 0.804, H_e : 0.804 – 0.840; *P. magnus*: N_a : 12.8 – 5.4 alelos/locus, H_o : 0.638 – 0.683, H_e : 0.747 – 0.755) comparados con los valores promedios de diversidad genética reportados para Siluriformes neotropicales, y ausencia de estructura genética entre los sitios analizados (*P. atricaudus*: F'_{ST} = 0.013 – 0.017, $P > 0.05$, D'_{est} = -0.004 – 0.023, $P > 0.05$; *P. magnus*: F'_{ST} = 0.031, $P = 0.055$; D'_{est} = 0.045, $P = 0.058$). Este trabajo representa la primera aproximación a la diversidad y genética poblacional de especies de la familia Pseudopimelodidae y proporciona un marco de referencia para futuros estudios genético-poblacionales y de conservación, requeridos en esta familia de bagres poco estudiada en el nivel microevolutivo.

Palabras clave: Colombia, Cuenca Magdalena-Cauca, Diversidad genética, Flujo génico, Pez dulceacuícola.

INTRODUCTION

The family Pseudopimelodidae (formerly assigned to Pimelodidae) is a Neotropical family that comprises seven genera and 50 valid species with a wide distribution in South America (Fricke *et al.*, 2020). *Pseudopimelodus* Bleeker, 1858, one of the six genera recorded in Colombia (DoNascimento *et al.*, 2017), contains large and medium-sized species in a distribution range from the Atrato River in Colombia to the La Plata River in Argentina (Shibatta, 2003). Only two members of this genus have been recorded in commercial and artisanal fisheries from different Colombian basins: *Pseudopimelodus bufonius* (Valenciennes, 1840) from Atrato, Orinoco and Amazon river basins and *Pseudopimelodus schultzi* (Dahl, 1955) from Atrato-Sinú and Magdalena-Cauca river basins (DoNascimento *et al.*, 2017). However, a recent phylogeographic study based on the mitochondrial *cox1* and the nuclear *rag2* genes showed that *Pseudopimelodus* comprises at least five divergent lineages in Colombia (Rangel-Medrano *et al.*, 2020): lineage 1 (Magdalena River and Cauca River lower sectors and the San Jorge River); lineage 2 (Amazon River hydrographic zone); lineage 3 (Orinoco River hydrographic zone); lineage 4 (Atrato River and Sinú River); lineage 5 (Magdalena River upper sector and, Cauca River upper and middle sectors). Based on these results, further osteological and external morphological analyses allowed the description of two new *Pseudopimelodus* species in Colombia: *Pseudopimelodus atricaudus* (lineage 1) Restrepo-Gómez, Rangel-Medrano, Márquez & Ortega-Lara, 2020 and *Pseudopimelodus magnus* (lineage 5) Restrepo-Gómez, Rangel-Medrano, Márquez & Ortega-Lara, 2020.

Currently, there is a gap in our knowledge of the biology, diversity, and genetic structure of members of the genus *Pseudopimelodus*, especially for the recently discovered species, which could hinder the development and implementation of effective management and

conservation programs. In this context, prior knowledge about genetic variability is crucial for the proper population management of these two species (Hilsdorf, Hallerman, 2017). In the current literature, *P. atricaudus* and *P. magnus* have been identified as *P. schultzi* and categorized as least concern in the IUCN Red List of Threatened Species (<https://www.iucnredlist.org/>; Mesa-Salazar, Sanchez-Duarte, 2016), mainly because it was thought that nominal *P. schultzi* had a wide distribution range. However, this species has been categorized as Near Threatened (NT) in the Colombian red list of freshwater fishes because of habitat degradation and pollution (Mojica *et al.*, 2012). In addition, *P. magnus* can reach a large size, and plausibly could be harvested as an alternative fishery resource and consequently, might bear higher fishing pressure than the other species previously identified as *P. schultzi*. Therefore, it is important to characterize the genetic diversity of natural populations of these two species to reassess its current conservation status.

Population genetic studies are absent for all members of Pseudopimelodidae. Indeed, only one study developed and characterized a set of 13 microsatellite loci in *Microglanis cottoides* (Boulenger, 1891) and most of them were successfully cross-amplified in other Pseudopimelodid species (Souza-Shibatta *et al.*, 2013). In contrast, some genetic studies have been conducted in the phylogenetically related family Pimelodidae showing gene flow and genetic structure. For example, using polymorphic microsatellite analyses, Ribolli *et al.* (2012) detected lack of genetic structure in *Pimelodus maculatus* Lacepède, 1803, from the upper Uruguay River. In addition, lack of genetic differentiation was observed in wild populations of *Pseudoplatystoma corruscans* (Spix & Agassiz, 1829) from the São Francisco River (Dantas *et al.*, 2013) and within the Paraguay basins in Brazil (Prado *et al.*, 2018). This later study also detected gene flow in *Pseudoplatystoma reticulatum* Eigenmann & Eigenmann, 1889 from Paraguay basin in Brazil. Additionally, other studies in the genus *Pseudoplatystoma* Bleeker, 1862, and *Steindachneridion* Eigenmann & Eigenmann, 1919, have shown genetic structure likely explained by homing behavior, geographical accidents, disconnections among rivers, isolation by distance, or short-distance migration range (Sekine *et al.*, 2002; Abreu *et al.*, 2009; Pereira *et al.*, 2009; Carvalho *et al.*, 2012; Telles *et al.*, 2014; Fonseca *et al.*, 2017; Prado *et al.*, 2018).

Considering the geomorphological and topographic characteristics of the middle sector of the Cauca canyon in Colombia (Landínez-García, Márquez, 2016), *Pseudopimelodus* species inhabiting upstream and downstream of the rapids may exhibit population genetic structure. To tests this hypothesis, we developed a set of 13 microsatellite loci using next generation sequence technology in *P. atricaudus* and tested for cross-amplification in two populations of *P. magnus* from Magdalena-Cauca basin. The information obtained in this study, regarding genetic variability and extent of genetic structure, will help providing useful information for conservation and management programs aiming to protect natural populations of these bumblebee catfish species in Colombia.

MATERIAL AND METHODS

Sampling. A total of 123 ethanol preserved muscle tissue samples of *Pseudopimelodus* (*P. atricaudus*: 58; *P. magnus*: 65) previously studied by Rangel-Medrano *et al.* (2020) were submitted to microsatellite analyses. Collection information, voucher numbers

and GenBank accessions for all sequences obtained were previously provided by Rangel-Medrano *et al.* (2020). Samples came from the middle and lower sections of the Cauca River (S1-S8) previously described by Landínez-García, Márquez (2016) (Fig. 1). Collection sites were located upstream and downstream in the Cauca River canyon, which is the steepest margin of the Antioqueño Plateau in the northern portion of the Central Cordillera (Restrepo-Moreno *et al.*, 2009) and marks the boundary between the middle and lower sectors of the Cauca River. This landform has been considered a geographic barrier for many fish species (Dahl, 1971) and is the site of the largest hydropower project in Colombia (Ituango). Collection sites downstream the Cauca River canyon, are in sectors where the Cauca River flows through lower slopes and meanders through a more ample floodplain.

Microsatellite loci development. Total genomic DNA was extracted from muscle tissue of one individual of *P. atricaudus* collected in the Cauca River lower sector by using the DNeasy Blood & Tissue (Qiagen, Germany) and ~5 ng/μL of the extracted DNA was employed to prepare a genomic library that was subsequently sequenced by using the pyrosequencing process in a 454 FLX next generation sequence platform

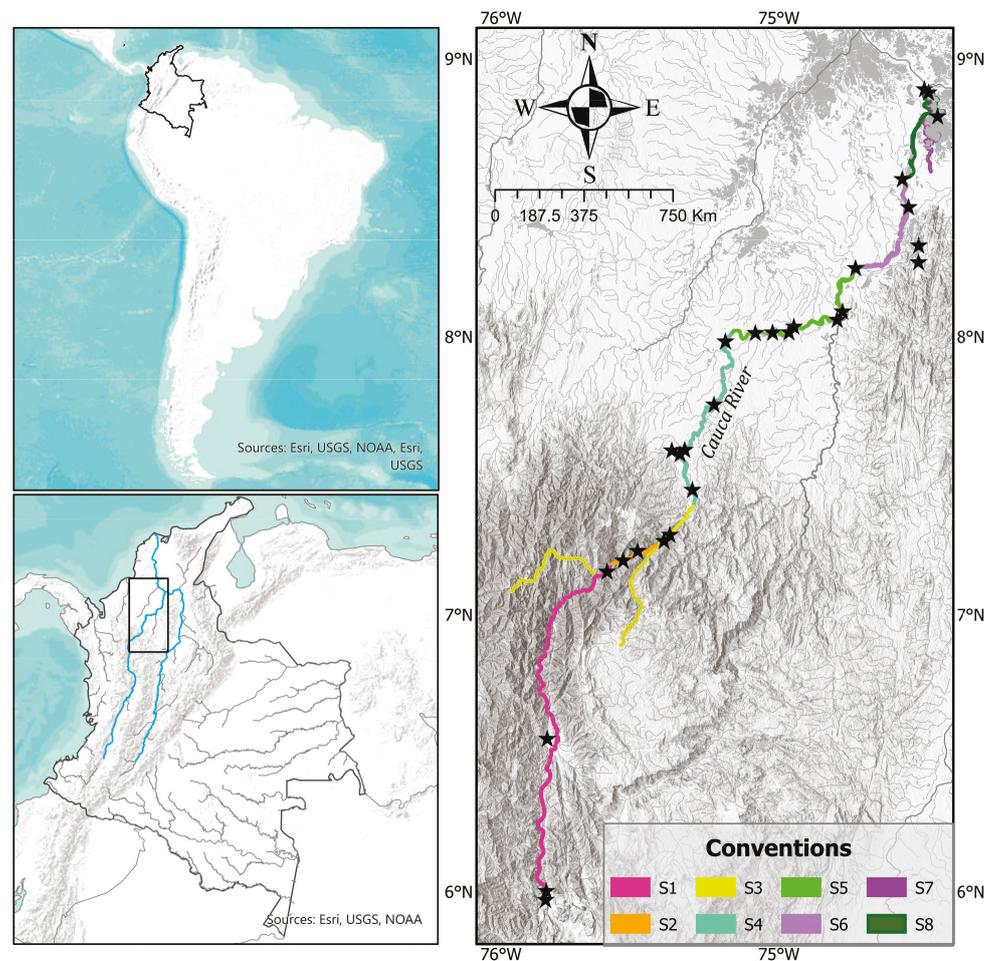


FIGURE 1 | Sampling sites of *Pseudopimelodus magnus* and *P. atricaudus* in the middle and lower sectors of the Cauca River.

(Roche Applied Science) following Landínez-García *et al.* (2016). Then, we used the methodology described by Landínez-García, Márquez (2018) for cleaning of nuclear genome reads, primer design, electronic polymerase chain reaction (ePCR), polymorphism analysis, validation and amplification condition optimization for 30 microsatellite loci.

In brief, the software PAL_FINDER v0.02.03 (Castoe *et al.*, 2010) was employed to detect and extract potential amplifiable loci (PAL) corresponding to reads containing perfect tri-, tetra- and pentanucleotide microsatellites loci. Next, the software PRIMER3 v.2.0 (Rozen, Skaletsky, 2000) was used for primer design in the flanking regions of each PAL setting a set of criteria as described in Castoe *et al.* (2010) such as GC content >30%; melting temperatures 58–65°C with a maximum 2°C difference between paired primers, the last two 3' nucleotides were G or C and an average amplicon size of at least 100 bp. All other software settings were left at their default values. Finally, an electronic PCR (ePCR; Rotmistrovsky *et al.*, 2004) was conducted to verify the correct alignment of each pair of primers with the corresponding DNA template.

A total of 30 microsatellite loci were selected to assess the level polymorphism in 15 samples of *P. atricaudus* from Cauca River based on standard PCR conditions (Sambrook, Russell, 2001). Amplicons were separated in 8% polyacrylamide gels, ran at 120 volts for 40 min using a Mini Protean® Tetra vertical electrophoresis cell (Bio-Rad Laboratories, Inc.) and visualized by silver staining. Furthermore, primers that showed consistent amplification, PCR products of 100–400 bp and ability to detect heterozygotes were selected for subsequent diversity and population structure analyses in *P. atricaudus* and tested for cross-amplification in *P. magnus*.

A set of 13 of 15 polymorphic loci were selected based on the above-mentioned criteria and used to assess the levels of genetic diversity and population structure in all collected samples of *P. atricaudus* and *P. magnus*. For this purpose, the 5' end of each forward primer was labeled with either fluorescent dye FAM6, VIC, NED or PET (Applied Biosystems). Polymerase chain reactions were conducted using a final volume of 10 µL with ~30 ng/µL of genomic DNA, 1X of PCR buffer, 2.5 mM of MgCl₂, 0.4 mM of dNTPs mix (Invitrogen™), 1 pmol/µL of each primer and 0.04 U/µL of Platinum *Taq* DNA Polymerase (Invitrogen™). Thermal conditions consisted of an initial denaturation step of 95°C during 3 min, followed by 30 cycles of 94°C for 30 s, an annealing temperature of 54–56°C for 20 s and an extension step of 72°C for 18 s. An automated DNA sequencer ABI 3730 XL were used to separate all PCR products using the GeneScan™ 500 LIZ® as internal size standard. Finally, allelic scoring was conducted with the program GeneMapper v4.0 (Applied Biosystems). Each dataset was checked for potential genotyping errors including null alleles, allele dropout and scoring of stutter peaks by using the software Micro-Checker v2.2.052 (Van Oosterhout *et al.*, 2004). The software CERVUS v3.0.7 (www.fieldgenetics.com) was employed to determine the polymorphic information content for each locus.

Genetic diversity and population structure analyses. Before performing diversity and genetic structure intraspecific analyses, all 123 samples of *Pseudopimelodus* and 10 loci that showed consistent amplification in both species were used to explore patterns

of genotype distribution across all river sections analyzed. Structuring pattern was compared with barcoding results previously found by Rangel-Medrano *et al.* (2020) for confidently identifying both species. To this end, a Bayesian clustering method was implemented in software STRUCTURE v2.3.4 (Pritchard *et al.*, 2000). The number of populations (K) was estimated using the admixture ancestry model, the LOCPRIOR option and correlated allele frequencies. Chain parameters included 20 independent runs using a burn-in of 50000 iterations followed by 500000 Markov chain Monte-Carlo (MCMC) chains assuming K=1 to K=7. The web-based software STRUCTURESELECTOR (Li, Liu, 2018) was used to calculate the most likely number of K based on the ΔK and $\text{LnP}(K)$ methods previously described by Evanno *et al.* (2005) and the choose K algorithm (Raj *et al.*, 2014). In addition, since uneven sampling often leads to wrong inferences on hierarchical structure and biased estimates of the true number of subpopulations, STRUCTURESELECTOR was also used to calculate the estimators MedMedK, MedMeaK, MaxMedK and MaxMeaK (Puechmaille, 2016). Finally, the integrated software CLUMPAK (Kopelman *et al.*, 2015) was used to generate a graphical representation of the results.

Furthermore, STRUCTURE analyses were also conducted to examine intraspecific population structure in samples of *P. atricaudus* and *P. magnus*, using 13 and 10 loci, respectively. In addition, the degree of population genetic differentiation was calculated by the standardized statistics D'_{est} (Meirmans, Hedrick, 2011) and F'_{st} (Meirmans, 2006) and Analysis of Molecular Variance (AMOVA) using the software GenAlex v6.503. Finally, in order to explore alternative grouping patterns, the ADEGENET package (Jombart, 2008) implemented in R (www.r-project.org) was used to perform a Discriminant Analysis of Principal Components using all genotypes of both species.

Once all genotypes of *P. atricaudus* and *P. magnus* were confidently identified, genetic diversity analyses were conducted independently for both species. Genetic diversity indices such as number of alleles per locus (N_a), observed (H_o) and expected (H_e) heterozygosity were calculated using the software GenAlex v6.502 (Peakall, Smouse, 2012). Estimation of population specific inbreeding coefficient (F_{is}), linkage disequilibrium among the pairs of loci and departures from Hardy-Weinberg expectations was performed by using a Markov chain-based approximations method in the software Arlequin v3.5.2.2 (Excoffier, Lischer, 2010). Finally, we adjusted the statistical significance for multiple comparisons by applying sequential Bonferroni correction (Holm, 1979; Rice, 1989).

RESULTS

Microsatellite development. A total of 13 of 30 microsatellite loci evaluated were polymorphic, showed consistent amplification and absence of null alleles or genotyping errors in *Pseudopimelodus atricaudus* (Tab. 1). The number of alleles ranged from 5 (Patr03 and Patr11) to 17 (Patr05) with a mean of 8.769 alleles/locus and an average H_o of 0.750. In addition, all loci met the Hardy-Weinberg and linkage assumptions. The PIC values ranged from 0.525 (Patr12) and 0.895 (Patr14) with an average of 0.803 (Tab. 1). Cross-amplification of these 13 microsatellite loci in *Pseudopimelodus magnus* showed successful amplification in 10 loci.

TABLE 1 | List of microsatellite loci developed for *Pseudopimelodus atricaudus*. The asterisk indicates lack of amplification in samples of *Pseudopimelodus magnus*. Na: number alleles per locus; H_o and H_e : observed and expected heterozygosity, respectively; PIC: polymorphism information content; P: statistical significance for tests of departure from Hardy-Weinberg equilibrium; F_{IS} : inbreeding coefficient; $P(F_{IS})$: statistical significance for F_{IS} .

Locus	Repeat motif	Forward (F) and reverse (R) sequence 5'-3'	Size Range (bp)	Na	Ho	He	PIC	P	F_{IS}	$P(F_{IS})$
Patr01	(ATATC)n	F: TCTCTATTGCCCTATGGTGGG	273-353	9	0.933	0.867	0.847	0.570	-0.080	0.889
		R: CATACATGCAGCACACTGGC								
Patr02	(TCTCC)n	F: TCACTTTAAGAGAGGAAGACCCG	95-140	7	0.667	0.738	0.714	0.090	0.100	0.342
		R: GTGACAGTGGTTGGGTGAGC								
Patr03*	(ATCTT)n	F: TTGTGTTATCCCCTTGCTGG	144-179	5	0.714	0.786	0.759	0.678	0.094	0.363
		R: TCCCAGTTACCAGTTCATTTC								
Patr04*	(ATTTT)n	F: GAAGTGTACGGGCACTGAGC	140-215	10	0.800	0.915	0.877	0.548	0.130	0.149
		R: TCACTGAATTAACGTGCAGCC								
Patr05	(ATCT)n	F: GGAACAGCTGTGTAGGTGAAGC	183-311	17	1.000	0.940	0.892	0.825	-0.066	1.000
		R: CCTTAGTGTCTCTAAAGAGATCGG								
Patr06	(ATCT)n	F: GAATGGCACAGACAGAAAGGC	129-213	10	0.800	0.897	0.884	0.204	0.111	0.197
		R: TGCACGAATTTCTCTGGGG								
Patr07	(ATCT)n	F: GCATCTCATCCATACTTGAGC	216-268	10	0.800	0.908	0.881	0.324	0.080	0.308
		R: GCATAACCATTTCTGCTCTGG								
Patr08	(ATCT)n	F: TGAATGAGTAATGCCACTGCC	220-268	8	0.786	0.852	0.851	0.862	0.123	0.156
		R: ACTGGTAACCTTCCGGTCCC								
Patr09	(ATCT)n	F: AAGGTGAGGTGAGATCTGTCAGG	125-189	7	0.733	0.782	0.772	0.406	0.064	0.415
		R: CCTCACCTTCAGATAAACATTCC								
Patr10	(AAAG)n	F: GAGACCATGTGACACTCATCC	157-269	9	0.600	0.674	0.713	0.592	0.113	0.264
		R: AGAGAACTGACGTTCCGCC								
Patr11	(AATG)n	F: ATTCTCCGTTGCTCTCAGC	93-149	5	0.467	0.409	0.825	1.000	-0.146	1.000
		R: CACTGCTTCAGTCTCATGTTCG								
Patr12*	(ATCT)n	F: CCCAGAGAACACAGAATAAACCC	141-197	8	0.733	0.832	0.525	0.716	0.123	0.211
		R: AAAATATTTGCAGAAATAAGAGGGG								
Patr13	(ATCT)n	F: AGCGTAATGGTGCCATAGCC	199-251	9	0.733	0.878	0.850	0.195	0.170	0.081
		R: CGCACTTAGAGGCGATTACC								

Genetic structure. Genetic structure analysis using pooled samples of *Pseudopimelodus atricaudus* and *P. magnus* showed $K=2$ as the most likely number of clusters as indicated by ΔK (Mean $\text{LnP}(K)$: -4991.64; Fig. 2A) and Puechmaille statistics (MedMed $K=2$, MedMea $K=2$). Geographical distribution of genotypes revealed that *P. atricaudus* occurs predominantly in Cauca River lower sector, although one genotype of this species was found in the Cauca River middle sector (Fig. 2A). Conversely, *P. magnus* was present predominantly in Cauca River middle sector, although some genotypes were found coexisting with *P. atricaudus* in Cauca River S4 (Fig. 2A).

Genetic structure analysis at intraspecific level in both species (Figs. 2B–C), showed $K=2$ as the most likely number of populations, however, all samples of *P. atricaudus* (Mean $\text{LnP}(K)$: -2489.26) and *P. magnus* (Mean $\text{LnP}(K)$: -2489.26) were assigned to the same specific genetic cluster, suggesting that they correspond to a single genetic stock as confirmed by Puechmaille estimators (MedMed $K=1$; MedMean $K=1$). For *P. atricaudus*, this result is concordant with the pairwise comparisons using the standardized F'_{ST}

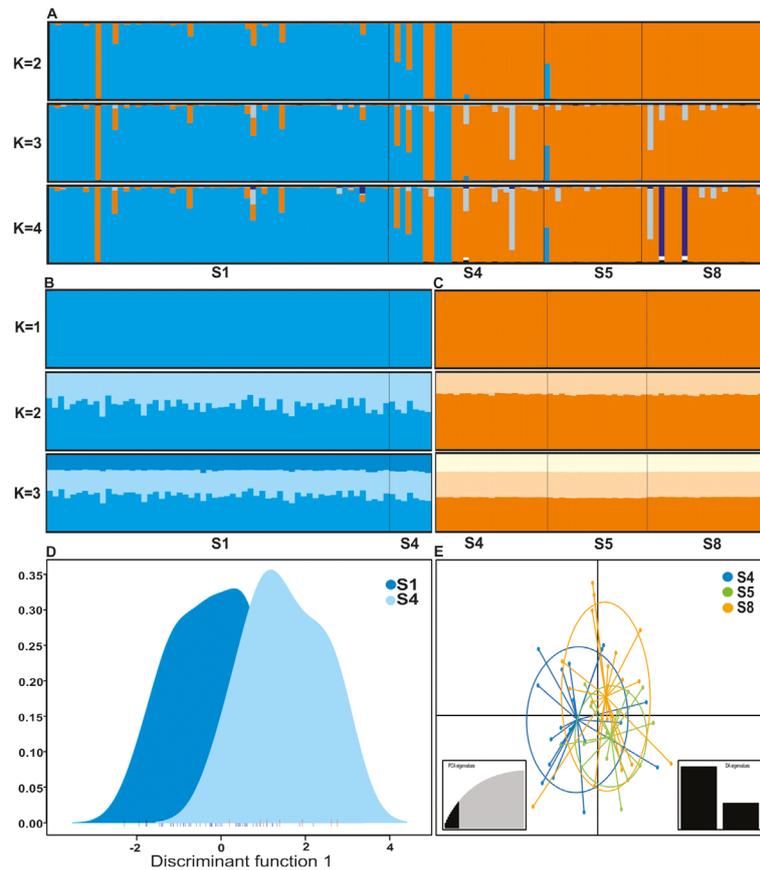


FIGURE 2 | Population structure suggested by STRUCTURE (A-C) and the Discriminant Analysis of the Principal Components (D, E) for *Pseudopimelodus magnus* (A, B, D), and *Pseudopimelodus atricaudus* (A, C, E). S1: sector 1 of the middle Cauca River; S4, S5, and S8: sectors 4, 5 and 8 of the lower Cauca River, respectively.

and D'_{est} statistics and Discriminant analysis of Principal Components, which detected lack of significant genetic differentiation among the sampling sites (Fig. 2E): S4-S5 ($F'_{ST}=0.017$, $P=0.308$; $D'_{est}=0.023$, $P=0.305$), S4-S8 ($F'_{ST}=0.013$, $P=0.856$; $D'_{est}=-0.004$, $P=0.857$) and S5-S8 ($F'_{ST}=0.015$, $P=0.861$; $D'_{est}=0.011$, $P=0.862$). Similarly, for *P. magnus*, STRUCTURE analysis is consistent with the results of standardized F'_{ST} and D'_{est} statistics ($F'_{ST}=0.031$, $P=0.055$; $D'_{est}=0.045$, $P=0.058$) and Discriminant Analysis of Principal Components (Fig. 2D).

Genetic diversity. For *Pseudopimelodus atricaudus* (Tab. 2), all 13 microsatellite loci produced a total of 373 different alleles ranging from 4 (Patr11) to 18 (Patr05) alleles per locus. Levels of genetic diversity were higher in S4 (Na: 9.923; H_O : 0.804, $H_E=0.840$) followed by S8 (Na: 9.769; $H_O=0.798$; $H_E=0.825$) and S5 (Na: 9.000; $H_O=0.760$; $H_E=0.804$). All loci satisfied Hardy-Weinberg and Linkage assumptions after sequential Bonferroni correction. Furthermore, values of population specific inbreeding coefficient were not significant in all sections evaluated for this species in the Cauca River (F_{IS} : S4=0.023, $P=0.245$; S5=0.035; $P=0.158$, S8=0.011, $P=0.355$).

In *Pseudopimelodus magnus* (Tab. 3), a total of 182 different alleles were detected ranging from 25 (Patr05) to 3 (Patr11). Levels of genetic diversity were higher in Cauca River S1 (Na: 12.800; $H_o=0.683$; $H_e=0.755$) than in S4 (Na: 5.400; $H_o=0.638$; $H_e=0.747$). Furthermore, all loci satisfied Linkage and Hardy-Weinberg equilibria although 6 of 10 loci analyzed in Cauca River S1 showed departures from Hardy-Weinberg expectations after sequential Bonferroni correction. Finally, samples of both sites analyzed exhibited significant values of F_{IS} (Cauca River S1 $F_{IS}=0.064$, $P=0.000$; Cauca River S4 $F_{IS}=0.132$; $P=0.033$).

TABLE 2 | Genetic diversity per locus and across loci of *Pseudopimelodus atricaudus* in three sites of the Cauca River. S4, S5 and S8: sectors 4, 5 and 8 of the lower Cauca River, respectively; N: Sampling size; Na: number alleles per locus; H_o and H_e : observed and expected heterozygosity, respectively; P: statistical significance for tests of departure from Hardy-Weinberg equilibrium; F_{IS} : inbreeding coefficient; $P(F_{IS})$: statistical significance for F_{IS} .

Locus	S4 (N= 19)						S5 (N= 17)						S8 (N= 20)					
	Na	H_o	H_e	P	F_{IS}	$P(F_{IS})$	Na	H_o	H_e	P	F_{IS}	$P(F_{IS})$	Na	H_o	H_e	P	F_{IS}	$P(F_{IS})$
Patr01	10	0.895	0.852	0.233	-0.052	0.826	9	0.941	0.884	0.578	-0.067	0.860	9	0.900	0.887	0.324	-0.015	0.692
Patr02	8	0.500	0.827	0.001	0.402	0.000	7	0.688	0.730	0.057	0.060	0.441	7	0.650	0.688	0.009	0.057	0.424
Patr03	6	0.611	0.754	0.060	0.194	0.108	5	0.625	0.796	0.193	0.221	0.082	8	0.706	0.859	0.012	0.183	0.058
Patr04	8	0.842	0.879	0.190	0.043	0.414	10	0.824	0.913	0.707	0.100	0.168	12	0.850	0.890	0.103	0.046	0.368
Patr05	16	0.947	0.882	0.295	-0.076	0.939	18	1.000	0.925	0.961	-0.084	1.000	17	0.842	0.905	0.356	0.071	0.278
Patr06	13	0.944	0.927	0.281	-0.019	0.752	10	0.824	0.898	0.378	0.086	0.267	14	0.947	0.899	0.064	-0.055	0.888
Patr07	11	0.842	0.876	0.632	0.040	0.451	11	0.824	0.916	0.379	0.104	0.172	12	0.850	0.919	0.562	0.077	0.244
Patr08	10	0.941	0.888	0.782	-0.062	0.879	8	0.800	0.857	0.916	0.069	0.359	8	0.889	0.873	0.347	-0.019	0.679
Patr09	10	0.684	0.838	0.129	0.188	0.050	8	0.765	0.783	0.581	0.023	0.550	9	0.750	0.795	0.142	0.058	0.363
Patr10	10	0.737	0.784	0.069	0.061	0.388	9	0.647	0.679	0.670	0.049	0.502	8	0.789	0.700	0.924	-0.132	0.954
Patr11	7	0.778	0.667	0.529	-0.172	0.926	5	0.412	0.367	1.000	-0.126	1.000	4	0.550	0.586	0.252	0.063	0.453
Patr12	10	0.833	0.867	0.725	0.040	0.442	8	0.765	0.841	0.868	0.094	0.265	9	0.800	0.862	0.084	0.073	0.296
Patr13	10	0.895	0.883	0.246	-0.013	0.698	9	0.765	0.863	0.302	0.117	0.195	10	0.850	0.867	0.053	0.020	0.536
Across loci	9.923	0.804	0.840	0.012	0.023	0.245	9.000	0.760	0.804	0.817	0.035	0.158	9.769	0.798	0.825	0.001	0.011	0.355

TABLE 3 | Genetic diversity per locus and across loci of *Pseudopimelodus magnus* in two sites of the Cauca River. S1 and S4: sectors 1 and 4 of the middle and lower Cauca River, respectively; N: Sampling size; Na: number alleles per locus; H_o and H_e : observed and expected heterozygosity, respectively; P: statistical significance for tests of departure from Hardy-Weinberg equilibrium; F_{IS} : inbreeding coefficient; $P(F_{IS})$: statistical significance for F_{IS} .

Locus	S1 (N= 58)						S4 (N= 7)					
	Na	H_o	H_e	P	F_{IS}	$P(F_{IS})$	Na	H_o	H_e	P	F_{IS}	$P(F_{IS})$
Patr01	14	0.828	0.891	0.147	0.072	0.087	6	0.714	0.857	0.194	0.178	0.288
Patr02	9	0.776	0.656	0.000	-0.185	0.988	5	0.571	0.670	0.117	0.158	0.361
Patr05	25	0.839	0.897	0.023	0.065	0.096	7	0.714	0.868	0.102	0.189	0.234
Patr06	17	0.860	0.911	0.068	0.057	0.142	7	1.000	0.879	0.758	-0.154	1.000
Patr07	9	0.707	0.827	0.029	0.146	0.024	5	0.571	0.769	0.296	0.273	0.160
Patr08	14	0.839	0.868	0.311	0.033	0.277	6	0.857	0.791	0.874	-0.091	0.870
Patr09	10	0.839	0.800	0.889	-0.050	0.839	5	0.571	0.725	0.548	0.226	0.274
Patr10	11	0.370	0.546	0.000	0.324	0.000	4	0.429	0.495	0.435	0.143	0.446
Patr11	6	0.259	0.492	0.000	0.477	0.000	3	0.286	0.615	0.104	0.556	0.055
Patr13	13	0.517	0.658	0.008	0.216	0.002	6	0.667	0.803	0.165	0.184	0.309
Across loci	12.800	0.683	0.755	< 0.001	0.064	0.000	5.400	0.638	0.747	0.164	0.132	0.033

DISCUSSION

Microsatellite development. The present study developed a set of 13 polymorphic microsatellite loci to assess genetic diversity and population structure of the endemic bumblebee catfish species *Pseudopimelodus atricaudus*. This is the first study reporting the development of microsatellite markers in the genus *Pseudopimelodus* and the second in the family Pseudopimelodidae. PIC values ranged from 0.525 to 0.895 with an average of 0.803 which indicates that these loci are highly informative (Botstein *et al.*, 1980). Although two additional loci showed good PIC values in the analyzed specimens, they were not considered in further analysis because of inconsistencies in the amplifications. However, 13 loci were used to confidently assess levels of genetic diversity in *P. atricaudus* and are, therefore, appropriate for further population genetic analyses in this species.

Furthermore, only 10 out of 13 microsatellite loci developed for *P. atricaudus* were successfully cross-amplified in *P. magnus*, since three loci failed to amplify in several individuals of this species, producing a high proportion of missing data. As described for the use of heterologous microsatellite loci in other taxa with distant phylogenetic relationships (Dakin, Avise, 2004), this differential amplification success may result from the high genetic divergence previously detected by nuclear and mitochondrial gene sequences (Rangel-Medrano *et al.*, 2020). Despite this limitation, these 10 loci allowed us to estimate the levels of gene flow and diversity of *P. magnus* in the sampled sites.

Genetic structure and diversity. In concordance with previous phylogeographic studies (Rangel-Medrano *et al.*, 2020), the Bayesian Structure analysis revealed two genetic clusters corroborating the predominance of *Pseudopimelodus magnus* and *P. atricaudus* in the middle and lower sections of Cauca River, respectively. This finding supports the hypothesis that the Cauca River canyon represents a significant barrier to dispersion. However, this study revealed the presence of genotypes of *P. atricaudus* and *P. magnus* in S4, suggesting the coexistence of both species in that sector of the river. Although not documented so far, the coexistence of these species may be facilitated by occasional downstream leakage of larvae of *P. magnus* from upper and middle sectors of the Cauca River. In addition, we also detected one genotype of *P. atricaudus* in Cauca River S1, however, the events that allowed the presence of this individual in the Cauca River middle sector are not yet clear. Moreover, our results showed that *P. atricaudus* and *P. magnus* are reproductively isolated as we found no sign of genetic admixture between them. However, to make a more robust assessment of this result, additional studies are encouraged using more genetic markers and a more exhaustive sampling across the Magdalena–Cauca River basin. Furthermore, additional studies are needed to determine if these species are reproductively isolated through mechanical or behavioral barriers, which are key components of speciation.

Specifically, for *Pseudopimelodus atricaudus*, all three methods to assess levels of population genetic structure revealed genetic homogeneity across sampling sites, not supporting our hypothesis of genetic structure. Similar results were found in *P. magnus* using the software STRUCTURE and the statistics D'_{est} and F'_{st} , although Discriminant Analysis of Principal Components showed a subtle genetic differentiation in samples from S1 and S4, likely caused by uneven sampling between these two localities. These

findings are not surprising considering the middle-distance migratory range described for *P. magnus* and *P. atricaudus*, however, we encourage further genetic structure analyses including additional sampling sites of these species within the Magdalena-Cauca River to have a better understanding of the genetic structure of these species. In addition, genetic structure results obtained in this study may not be comparable with those obtained in members of the phylogenetically related family Pimelodidae, mainly due to marked differences in their life cycles. For instance, it has been reported that members of Pseudopimelodidae are solitary bottom-dwelling species with low vagility or sedentary habits (Esguícero, Arcifa, 2010) while some pimelodids such as *Pimelodus maculatus* and species of *Pseudoplatystoma* exhibit gregarious behavior and high migratory capacity (Carolsfeld *et al.*, 2003; Sabinson *et al.*, 2014).

Moreover, since all markers used in this study corresponded to tetra and pentanucleotide loci, average number of alleles per locus identified for *P. atricaudus* and *P. magnus* are not comparable with values detected in *M. cottoides* in which most of the loci analyzed included dinucleotide motifs in 24 individuals (Souza-Shibatta *et al.*, 2013). However, average values per site of observed and expected heterozygosities detected (see Tabs. 2–3) were higher than those found in that *cis*-Andean species (Souza-Shibatta *et al.*, 2013) and other phylogenetically related species of the family Pimelodidae, such as *Brachyplatystoma rousseauxii* (Castelnaud, 1855) (Batista, Alves-Gomes, 2006), *P. corruscans* and *P. reticulatum* (Vaini *et al.*, 2016; Prado *et al.*, 2017). Nevertheless, these values of genetic diversity were similar to those found in the pimelodid species *P. maculatus* (Paiva, Kalapothakis, 2008; Ribolli *et al.*, 2012). Overall, the results obtained in the present study showed that *P. atricaudus* and *P. magnus* exhibit a high genetic diversity compared to the global mean levels of genetic diversity reported for Neotropical Siluriformes ($H_E: 0.609 \pm 0.210$; Hilsdorf, Hallerman, 2017).

Since *P. atricaudus* is not considered an important fishery resource in the Magdalena-Cauca River basin, we suggest that the high genetic diversity detected in this species may be related to the low fishing pressure across its distribution range. Moreover, although we found good levels of genetic diversity in *P. magnus*, technical problems with the analysis suggest that species-specific primers must be designed to make a more precise assessment and for future monitoring of the genetic diversity of this species. This is important because *P. magnus* is currently being exploited for fish markets and local consumption given its large size, and so will be more likely to suffer a decrease in its genetic variability. In addition to fishing pressure, it is well known that river damming, as in the case of Ituango hydropower project in the Cauca River middle sector, is an important factor of freshwater habitat alteration due to the magnitude of freshwater ecosystem disturbances during and after dam construction (Nilsson *et al.*, 2005; Liermann *et al.*, 2012). The information obtained in this study constitute an important starting point for the formulation of future conservation and management policies and can be used to supplement conventional conservation assessments for these bumblebee catfish species in the Magdalena-Cauca River basin.

This study is the first report of a set of microsatellite loci for species of the genus *Pseudopimelodus* and the second within the family Pseudopimelodidae. Results obtained here showed that populations of the two new species *P. atricaudus* and *P. magnus* exhibit lack of genetic differentiation among sampling sites within the Cauca River and high levels of genetic diversity. However, given the deep genetic divergence detected

between these two species, new diversity and genetic differentiation analyses are encouraged using species specific markers for *P. magnus* to avoid potential technical problems associated with genotyping.

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AUTHOR'S CONTRIBUTION

José David Rangel-Medrano: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing-original draft, Writing-review and editing.

Edna Judith Márquez: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing-original draft, Writing-review and editing.

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COMPETING INTERESTS

The authors declare no competing interests.

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