




Mitochondrial genetic variation of endemic stingray *Potamotrygon magdalenae* (Myliobatiformes: Potamotrygonidae) in the middle Magdalena River basin, Colombia

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Potamotrygon magdalenae is the only freshwater stingray species reported to be endemic to Colombia, inhabiting the Magdalena–Cauca, San Jorge, Atrato, and Catatumbo River basins. While aspects of its ecology, reproduction, and biochemistry are well understood, its population genetics remain underexplored, particularly given the various threats it faces. To assess the population structure of *P. magdalenae*, we analyzed mitochondrial DNA sequences of the MT-CYB gene from 60 individuals sampled across three swamps in the middle Magdalena River basin. Our analysis of 693-bp sequences revealed 24 variable sites across 22 haplotypes ($hd = 0.83277$, $\pi = 0.00246$). Significant gene flow was observed between the swamps, supporting the hypothesis that *P. magdalenae* constitutes a single panmictic population ($\Phi_{ST} = -0.0083$, $p = 0.68035$). Tajima's D test ($D = -1.62$, $p < 0.05$) and Fu's F_s -test ($F_s = -4.59$, $p < 0.02$) indicate a recent population expansion following a bottleneck event, likely due to habitat degradation, despite the limitations of molecular marker. No genetic differentiation was found across the 200 km study area, and genetic estimates were comparable to those of other fish species endemic to the Magdalena River.

Keywords: Cytochrome B, Freshwater stingray, Haplotype, Panmixia, Population genetics.

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Potamotrygon magdalenae es la única especie de raya de agua dulce reportada como endémica de Colombia, habitando las cuencas de los ríos Magdalena-Cauca, San Jorge, Atrato y Catatumbo. Si bien se comprenden aspectos ecológicos, reproductivos y bioquímico, su genética poblacional ha sido poco estudiada, especialmente dado las distintas amenazas que enfrenta. Para evaluar la estructura poblacional de *P. magdalenae*, analizamos secuencias de ADN mitocondrial del gen MT-CYB de 60 individuos muestreados en tres ciénagas de la cuenca media del río Magdalena. Nuestro análisis de secuencias de 693 pb reveló 24 sitios variables en 22 haplotipos ($hd = 0.83277$, $\pi = 0.00246$). Se observó un flujo génico significativo entre las ciénagas, lo que apoya la hipótesis de que *P. magdalenae* constituye una única población panmíctica ($\Phi_{ST} = -0.0083$, $p = 0.68035$). Las pruebas de neutralidad de Tajima ($D = -1.62$, $p < 0.05$) y Fu ($F_s = -4.59$, $p < 0.02$) indican una reciente expansión poblacional tras un evento de cuellos de botella, probablemente debido a la degradación del hábitat de la especie, a pesar de las limitaciones del marcador. No se encontró diferenciación genética en el área de estudio de 200 km, y las estimaciones genéticas fueron comparables a las de otras especies de peces endémicas del río Magdalena.

Palabras clave: Citocromo B, Genética de poblaciones, Haplotipo, Panmixia, Raya de agua dulce.

INTRODUCTION

The family Potamotrygonidae (Neotropical stingrays) comprises around 40 species of fish, including both marine and freshwater organisms (Loboda *et al.*, 2021; Fricke *et al.*, 2024). Of all these, the subfamily Potamotrygoninae stands out, whose origin and diversification as the only living lineage of freshwater-adapted elasmobranchs within this family have been linked to historical marine incursions in northern South America (Lovejoy *et al.*, 1998; 2006; Bloom, Lovejoy, 2011; Fontenelle *et al.*, 2021).

Potamotrygon magdalenae (Duméril, 1865) is the only stingray species endemic to Colombia, inhabits both lentic, and lotic systems and has been recorded in the Atrato, Magdalena, Cauca, San Jorge, and Catatumbo rivers (Lasso *et al.*, 2013). It typically occurs in shallow, turbid waters with muddy substrates (Dahl, 1971; Lasso *et al.*, 2013). Its diet consists primarily of aquatic macroinvertebrates, particularly larval-stage insects of the order Diptera (Mejía-Falla *et al.*, 2016; Márquez-Velásquez *et al.*, 2019). Several aspects of its reproductive biology have been described (Pedreros-Sierra, Ramírez-Pinilla, 2015; Pedreros-Sierra *et al.*, 2016; Anaya-López, Ramírez-Pinilla, 2017; Lizcano-Gutiérrez, Ramírez-Pinilla, 2022), along with studies on its ecological and biochemical aspects (Singer, Ballantyne, 1989; Ramos-Socha, Grijalba-Bendeck, 2011; Márquez-Velásquez *et al.*, 2019; Pérez-Rojas *et al.*, 2022).

However, few studies have addressed the population genetics of this species, particularly in relation to other *Potamotrygon* species (García *et al.*, 2016; Batista-Morales *et al.*, 2017; Renza-Millán *et al.*, 2019; Rizo-Fuentes *et al.*, 2021). Regarding the assessment of the population genetic component of other indigenous fish species

distributed within the Magdalena River basin, such as striped catfish (*Pseudoplatystoma magdaleniatum*) and bocachico (*Prochilodus magdalenae*), significant levels of genetic variation have been documented utilizing microsatellite molecular markers and mitochondrial gene segments (Aguirre-Pabón *et al.*, 2013; Berdugo, Narváez, 2014; Landínez-García *et al.*, 2020; García-Castro *et al.*, 2021).

As is the case with other elasmobranchs, *P. magdalenae* exhibits slow reproductive cycles and low offspring numbers, leading to limited population growth (Araújo *et al.*, 2004; Mejía-Falla *et al.*, 2009). The species faces a high risk of extinction due to several threats, including overfishing, habitat degradation and destruction, commercial fishing practices, and mining activities (Lasso *et al.*, 2013). Given this information, the International Union for Conservation of Nature (IUCN) reports a declining trend in population size, leading to the classification of the species as Near Threatened (NT). If populations continue to decline, the species could become endangered (IUCN, 2025). The limited understanding of biological aspects may result in an underestimation of the extent to which these organisms are threatened. A similar classification was observed in the Red Book of Freshwater Fishes of Colombia, where the species is classified as NT at the national level (Mojica *et al.*, 2012). Conversely, the National Plan of Action for the Conservation and Management of Sharks, Rays, and Chimeras in Colombia (PAN-Colombia) emphasizes the formulation and implementation of strategies to safeguard these species, given their high-priority status (Caldas *et al.*, 2010).

To gain an understanding of the population dynamics of the endemic Colombian stingray, *P. magdalenae*, the present study aimed to examine aspects of its population genetics based on maternal inheritance. We collected samples from three swamps in the Magdalena River sub-basins. This study utilizes mitochondrial DNA sequences to analyze genetic diversity, population structure, and historical demographic events.

MATERIAL AND METHODS

Study area and fish sampling. This study analyzed the distribution of *P. magdalenae* across three key swamps: the Zapatosa complex, Simití, and Paredes (Fig. 1). We assessed samples obtained from 20 individuals in each swamp. Zapatosa and Paredes swamps showed the maximum straight-line distance (199 km), while Simití and Paredes had the shortest (60 km). The surveyed swamps are primarily surrounded by tropical dry forests (García, Dister, 1990; Cruz *et al.*, 2009), except for Simití, which lies within tropical rainforests (Vilardy *et al.*, 2014).

Fishing techniques, such as trammel nets and cast nets, were employed to capture individuals (Gama *et al.*, 2013). We supplemented our collection with specimens contributed by residents. A total of 60 specimens of *P. magdalenae* were meticulously identified, according to the guides of Maldonado-Ocampo *et al.* (2005) and Lasso *et al.* (2011). Blood samples of 1 mL were collected from each individual from the caudal artery using syringes and then deposited into tubes containing an equal volume of absolute ethanol. After drawing blood samples, we photographed and marked all captured individuals with plastic bands to prevent resampling during potential recapture events, then released them back into the swamps. We preserved these samples at 4 °C and stored them in the Laboratorio de Genética y Biotecnología (LGyB-UIS) at the Universidad Industrial de Santander.

DNA extraction and PCR amplification. The DNA extraction protocol involved treating each sample with a 10% SDS solution and Proteinase K before nucleic acid extraction was carried out (Green, Sambrook, 2012), with adjustments made from LgyB-UIS, which includes an additional wash with phenol:chloroform:isoamyl alcohol (25:24:1).

Before amplifying the cytochrome b (MT-CYB) fragments through Polymerase Chain Reaction (PCR), the concentration and quality were assessed using a Nanodrop 2000 spectrophotometer (Thermo Scientific, USA). The runs were then carried out on a 1% agarose gel to verify the integrity of the DNA. We diluted all samples to 100 ng/ μ L for optimal PCR performance.

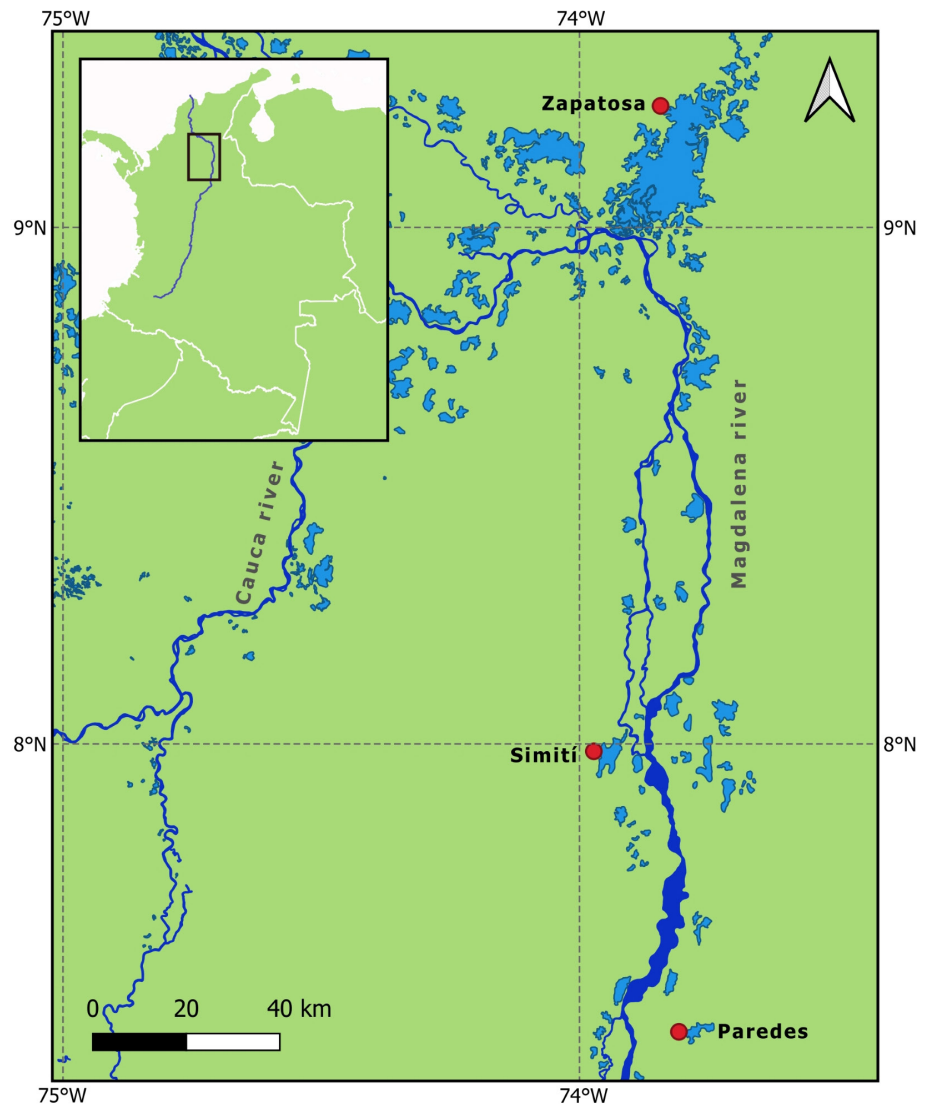


FIGURE 1 | Map of the sampling area for *Potamotrygon magdalenae* in the middle Magdalena River basin in northeastern Colombia. Swamps are shown in lighter blue, and the main stream of rivers Magdalena and Cauca are shown in darker blue. The red circles indicate the assessed swamps: the Zapatoso swamp complex ($09^{\circ}15'00.82''\text{N}$ $73^{\circ}48'29.08''\text{W}$), the Simiti swamp ($07^{\circ}57'19.07''\text{N}$ $73^{\circ}57'05.33''\text{W}$), and the Paredes swamp ($07^{\circ}26'52.60''\text{N}$ $73^{\circ}47'34.93''\text{W}$). The WGS84 system was used.

We amplified the mitochondrial MT-CYB gene using the primers and standardized conditions described by López-Ardila *et al.* (2022). A 750 bp band was visualized and verified by 1% agarose gel electrophoresis using an EZ-Vision® intercalating agent (Amresco) and a GeneRuler 1 kb DNA Ladder (Thermo Scientific, USA).

The amplicons were subsequently purified and subjected to sequencing at the Servicio de Secuenciación y Análisis Molecular (SSiGMol) at the Universidad Nacional de Colombia. This service utilizes BigDye Terminator v. 3.1 (Applied Biosystems) technology. We deposited the MT-CYB sequences in GenBank under accessions PP261281 to PP261302.

Population genetic analysis. MT-CYB gene fragments were visualized and manually edited using CodonCode Aligner v. 1.2.2 (CodonCode Co.). We retained sequences with Phred scores greater than 30 (Ewing *et al.*, 1998; Shi *et al.*, 2016) for analysis. Subsequently, the sequences were aligned using Mega v. 10.1.8 software (Kumar *et al.*, 2018), employing the default Muscle algorithm parameters (Edgar, 2004). Haplotype diversity (h_d), nucleotide diversity (π), and the identification of polymorphic sites were determined using DnaSP v. 6.12.03 (Rozas *et al.*, 2017). We visualized DNA sequence variations through median-joining haplotype networks (Leigh, Bryant, 2015; PopART v. 10.1.0.0).

Genetic differentiation between population pairs (Φ_{ST}) and gene flow estimation (Nm) were analyzed to assess the genetic structure of *P. magdalenae* across the sampled swamps. We first tested the metapopulation genetic structure hypothesis by performing an analysis of molecular variance (AMOVA) across three sampling locations. We additionally tested the isolation-by-distance hypothesis using a Mantel test. To evaluate the demographic history, neutrality tests were performed, including Tajima's D (Tajima, 1989) and Fu's F_s (Fu, 1997). We visualized differences in genotyped haplotype distributions through Mismatch Distribution analysis in Arlequin v. 3.5 (Excoffier, Lischer, 2010).

We used JModelTest v. 2 (Darriba *et al.*, 2012) to determine the best-fit nucleotide substitution model for MT-CYB sequences. We constructed a Bayesian Skyline Plot (BSP) in BEAST v. 2.5 (Bouckaert *et al.*, 2019) with 100 million generations to assess contemporary population expansion in *P. magdalenae*. We visualized this assessment using Tracer v. 1.7.1 was present in the studied waters with a frequency of 26%. POMACb3 and POMACb5 (each with a 3% frequency) were shared exclusively between Zapatoso and Paredes. Additionally, we identified 15 locality-excluding (private) haplotypes, each occurring at a frequency of 0.0166% within a single swamp (Tab. 1). The haplotype network showed no local clustering, indicating gene exchange between individuals from different swamps (Fig. 2).

The haplotype network revealed POMACb7 and POMACb4 in all three swamps, with POMACb3 and POMACb5 present in both the Zapatoso and Paredes swamps. Of the 15 individual haplotypes, seven were reported in the Simití swamp, five in the Zapatoso swamp, and three in the Paredes swamp (Fig. 2).

Based on the MT-CYB sequences obtained, the haplotype diversity (h_d) ranged from 0.67895 (Paredes) to 0.90526 (Zapatoso) with a mean value of 0.83277 ± 0.036 , while the nucleotide diversity (π) oscillated between 0.00162 (Paredes) and 0.00334 (Zapatoso) with a mean value of 0.00246 ± 0.001547 (Tab. 2).

TABLE 1 | Haplotypes and haplotypic frequency based on a fragment of the MT-CYB gene from *Potamotrygon magdalenae*. The mitogenome NC_036041 was used as a reference, located at the top of the table in bold. Hap = haplotypes; f (sd) = haplotypic frequency (standard deviation); Z = Zapatosá; S = Simití; P = Paredes; Acc = Accession number in the GenBank. The Position refers to the specific cite inside of gene in which nucleotide variability is encountered. *Nomenclature used in the network in Fig. 2.

Haplotype (nomenclature*)	Position																						f	sd	Swamp		
	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1					
	4	4	4	4	4	4	4	4	4	4	4	5	5	5	5	5	5	5	5	5	5	5					
	6	6	6	6	6	7	7	7	8	8	8	9	0	1	1	1	1	2	2	2	2	2				3	3
	3	3	4	7	9	1	2	4	6	7	9	5	6	2	2	3	5	0	2	5	6	9				0	1
1	7	7	2	5	1	5	7	7	7	7	1	2	1	5	3	8	9	4	0	1	7	2	4				
G	C	A	A	A	G	A	T	A	A	C	T	A	T	T	C	C	T	C	G	A	A	A	A				
POMACb1 (H1)	.	G	.	.	.	A	.	C	.	G	0.0166	0.0166	Z
POMACb2 (H2)	C	T	0.0333	0.0233	Z
POMACb3 (H3)	A	.	C	.	.	.	C	0.0333	0.0233	Z, P
POMACb4 (H4)	A	.	C	0.2666	0.0575	Z, S, P
POMACb5 (H5)	.	G	.	.	.	A	.	C	0.0333	0.0233	Z, P
POMACb6 (H6)	.	.	.	T	.	A	.	C	G	0.0166	0.0166	Z
POMACb7 (H7)	C	0.3166	0.0605	Z, S, P
POMACb8 (H8)	A	A	.	C	0.0333	0.0233	Z
POMACb9 (H9)	A	.	C	.	.	.	C	G	0.0166	0.0166	Z
POMACb10 (H10)	.	G	G	C	0.0166	0.0166	Z
POMACb11 (H11)	T	A	G	C	G	G	G	.	.	0.0166	0.0166	Z
POMACb12 (H12)	C	G	.	.	0.0166	0.0166	S
POMACb13 (H13)	.	G	C	0.0333	0.0233	S
POMACb14 (H14)	A	.	C	.	.	T	C	C	.	0.0166	0.0166	S
POMACb15 (H15)	C	A	0.0166	0.0166	S
POMACb16 (H16)	A	.	C	A	0.0166	0.0166	S
POMACb17 (H17)	0.0166	0.0166	S
POMACb18 (H18)	.	.	.	T	.	A	.	C	0.0166	0.0166	S
POMACb19 (H19)	A	.	C	C	.	T	0.0166	0.0166	S
POMACb20 (H20)	A	.	C	T	0.0166	0.0166	P
POMACb21 (H21)	A	.	C	C	0.0166	0.0166	P
POMACb22 (H22)	A	.	C	T	C	0.0166	0.0166	P

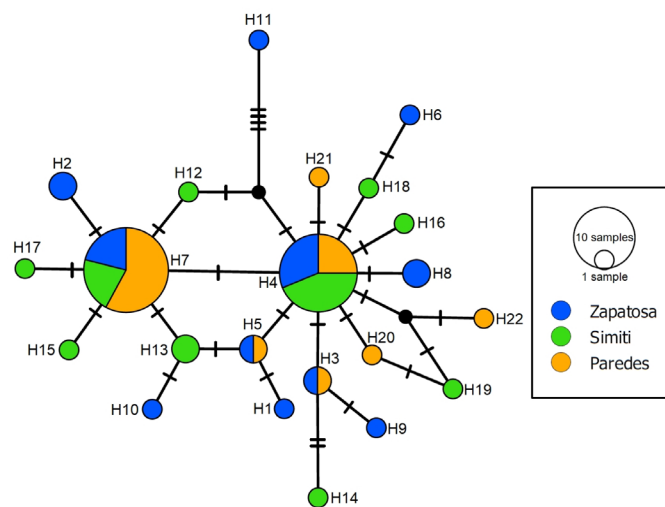


FIGURE 2 | Haplotype network based on partial sequences of the MT-CYB gene of *Potamotrygon magdalenae*. Black circles indicate unsampled haplotypes within the study, and cross lines represent mutational changes between haplotypes.

Gene flow and genetic differentiation. Gene flow (Nm) of females in *P. magdalenae*, estimates ranged from 40 (Zapatoso-Paredes) to ∞ (Simití-Zapatoso/Simití-Paredes), indicating high migration rates. Pairwise Φ_{ST} values were low (Simití-Zapatoso: -0.014; Simití-Paredes: -0.026; Zapatoso-Paredes: 0.012) (Tab. 3). AMOVA attributed 95% of *P. magdalenae* genetic variation to differences within swamps. Together with these obtained values of genetic differentiation, the haplotype network presented in Fig. 2 does not show a concentration of haplotypes with in swamp. The Mantel test revealed a positive correlation between genetic and geographic distance, although it does not support the isolation by distance hypothesis in the Magdalena stingray ($R^2 = 0.9268$; $p = 0.190$).

Neutrality tests and mismatch analysis support recent population expansion in *P. magdalenae*. Tajima’s D and Fu’s F_s values were significantly negative (Tab. 2; $p < 0.02$), and the unimodal mismatch distribution (Fig. 3A) matched expectations for expansion. The Bayesian skyline plot (Fig. 3B) estimated a gradual increase in effective population size over the last 1,500 years, corroborating this trend.

TABLE 2 | Haplotype and nucleotide diversity by locality from MT-CYB gene sequences assessed in *Potamotrygon magdalenae*. Genetic diversity values with their respective standard deviations. n = number of sequences, s = polymorphic sites, H = number of haplotypes, hd = haplotype diversity, π = nucleotide diversity, D = Tajima’s neutrality test, F_s = Fu neutrality test. *Significant values - p value < 0.05. **Significant values - p value < 0.02.

Swamp	n	s	H	hd	π	D	F_s
Zapatoso	20	15	11	0.90526 ± 0.0441	0.00334 ± 0.002130	-1.669*	-5.241**
Simití	20	12	10	0.85263 ± 0.0630	0.00247 ± 0.001679	-1.774*	-5.419**
Paredes	20	7	7	0.67895 ± 0.1024	0.00162 ± 0.001228	-1.412	-3.122**
General	60	24	22	0.83277 ± 0.036	0.00246 ± 0.001547	-1.621*	-4.594**

TABLE 3 | Gene flow and genetic differentiation from partial sequences of the MT-CYB gene of *Potamotrygon magdalenae*. Above the diagonal: paired values of Nm ; below the diagonal: paired values of genetic differentiation (Φ_{ST}). On the diagonal, the values of Tajima’s D and Fu’s F_s neutrality tests. * $p < 0.05$; ** $p < 0.02$.

Swamp	Zapatoso	Simití	Paredes
Zapatoso	D = -1.66903* Fs = -5.24085**	∞	39.8
Simití	-0.01419	D = -1.77436* Fs = -5.41853**	∞
Paredes	0.01240	-0.02593	D = -1.41981 Fs = -3.12201**

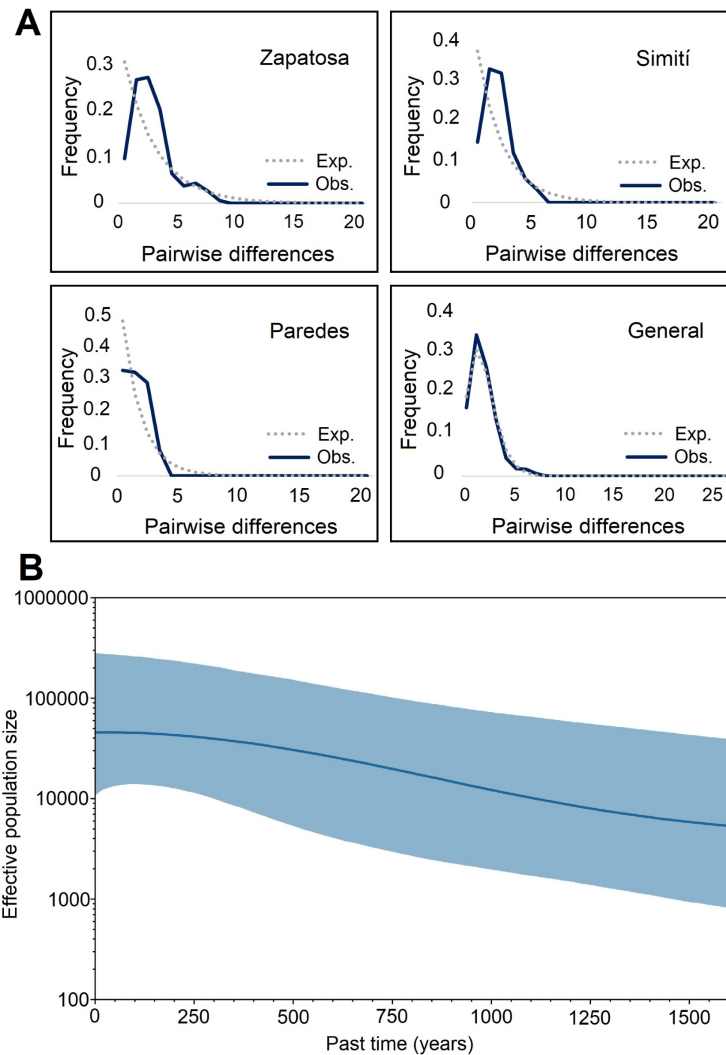


FIGURE 3 | Demographic history of *Potamotrygon magdalenae* using partial MT-CYB gene sequences. **A.** Distribution of nucleotide differences between haplotype pairs. X-axis: paired nucleotide differences, Y-axis: difference frequency. **B.** Bayesian Skyline Plot analysis. X-axis: effective population size, Y-axis: time in years. The solid blue line represents the mean, and the blue shaded area indicates the 95% highest posterior density.

DISCUSSION

This study expands current knowledge on the population genetics of the Colombian freshwater stingray, *Potamotrygon magdalenae*, using 693 bp of the mitochondrial gene MT-CYB sequences. Our findings expand the limited genetic knowledge of *P. magdalenae*, a species that has been previously addressed in only four studies (Torres *et al.*, 2022).

The genetic parameters estimated in this research indicate that *P. magdalenae* exhibits high haplotype diversity and low nucleotide diversity. This characteristic pattern, consistent with the findings of Hilsdorf, Hallerman (2017) for Neotropical fishes, suggests a recent population expansion following a historical bottleneck event.

This demographic bottleneck could reflect a combination of natural factors (Aguirre *et al.*, 2021), overfishing (Restrepo *et al.*, 2021), water pollution (Gómez-Martínez *et al.*, 2024), and abortions of pregnant females (Lizcano-Gutiérrez, Ramírez-Pinilla, 2022), as well as historical processes that have shaped the species population. However, it is important to investigate how anthropogenic processes may impact the species and the Magdalena River ecosystem in the future. Pollution and contamination represent threats to aquatic biodiversity, particularly to ichthyofauna (Gallo-Vélez *et al.*, 2022), which further exacerbates the challenges faced by *P. magdalenae*.

This high haplotype diversity and low nucleotide diversity patterns align with findings reported for other potamotrygonid stingrays across northern South America, *i.e.*, *Paratrygon aiereba* (Frederico *et al.*, 2012; Rizo-Fuentes *et al.*, 2021) and multiple *Potamotrygon* species (*P. motoro*, Renza-Millán *et al.*, 2019; *P. orbignyi*, Batista-Morales *et al.*, 2017; *P. scobina*, Toffoli, 2006).

Ramírez-Hernández (2018) reported significantly lower genetic diversity ($h = 0.47 \pm 0.59$; $\pi = 0.001 \pm 0.0002$) using 549-bp MT-COI sequences in *P. magdalenae* (Tab. 2). This discrepancy from the pattern described previously may reflect sampling dissimilarity between studies and unequal sample distribution across localities, as well as properties intrinsic to the marker (Fratini *et al.*, 2016). For studies assessing the same population, it is recommended to use between 10 and 20 samples to obtain reliable estimates of genetic structure (Grünwald *et al.*, 2017).

In *P. magdalenae*, the gene sequence analysis of MT-CYB revealed a latitudinal gradient, where genetic diversity decreased from north to south, with peak diversity observed in the northern Zapatos swamp (Fig. 1; Tab. 2). This spatial pattern supports the hypothesis proposed by Lovejoy *et al.* (1998) regarding the incursion of marine stingrays into northern South American freshwater systems during the Miocene. According to this hypothesis, marine-derived elasmobranchs underwent adaptive freshwater colonization through introgressive processes (Smith, 1992). The widespread distribution of the POMACb7 haplotype (C14747T), which shows minimal sequence variation across all sampled swamps, suggests it may represent the ancestral lineage of Magdalena basin populations. The prevalence and conservation of this haplotype across the species' range will indicate long-term stability in the face of historical environmental changes.

Studies of genetic diversity in other Magdalena River endemic fishes, such as *Prochilodus magdalenae* (Landínez-García *et al.*, 2020; García-Castro *et al.*, 2021) and *Pseudoplatystoma magdaleniatum* (Aguirre-Pabón *et al.*, 2013), *P. magdalenae* exhibits high genetic diversity maintained through the river's role as a migratory corridor (García-Castro *et al.*, 2024). In this sense, the N_m estimated and AMOVA results (95% within-swamp variation; Tab. 3) support the idea of the Magdalena River as a migratory corridor for *P. magdalenae*, in addition to favoring the idea of panmixia along the 200 km of the study area, despite the non-significant isolation-by-distance pattern (Mantel test: $R^2 = 0.93$, $p = 0.19$) (Diniz-Filho *et al.*, 2013). These findings contrast with the MT-COI-based study by Ramírez-Hernández (2018), which did not report any geographic structure.

Key factors likely contribute to this genetic homogeneity, such as life history traits (Toffoli, 2006; Ramírez-Hernández, 2018), historical factors (Frederico *et al.*, 2012; Batista-Morales *et al.*, 2017; Renza-Millán *et al.*, 2019), and anthropogenic impacts (Lasso *et al.*, 2016), all of which could be responsible for the absence of genetic structure in *P. magdalenae*, as confirmed by the haplotype network. In addition to shared haplotypes,

POMACb4 and POMACb7 are found in all swamps, while POMACb3 and POMACb5 are detected in two swamps, indicating female-mediated gene flow even across different swamps. This pattern of gene flow suggests that while private haplotypes may exist, they do not restrict overall dispersal within the population. These results contrast with those of Ramírez-Hernández (2018), who identified many private haplotypes.

Different evidence suggests recent population expansion in *P. magdalenae*. Genetic diversity metrics (Tab. 2), neutrality tests (Tab. 3), a star-like haplotype network topology (Fig. 2), and demographic reconstructions, including a unimodal mismatch distribution and a Bayesian Skyline Plot showing an expansion over approximately 1,500 years (Fig. 3) (Drummond *et al.*, 2005). We also emphasize that these signals should be interpreted with caution, and that a multilocus approach would be advisable to overcome the potential influence of factors such as sample size and differences in gene-specific evolutionary rates, which may reflect distinct evolutionary histories (Zamora-Mejías *et al.*, 2022). Our findings provide evidence in support of the population expansion approach, also noted for the MT-COI gene (Ramírez-Hernández, 2018). This same trend has been observed in other endemic fish species from the Magdalena River (Aguirre *et al.*, 2013).

The results led to the conclusion based on its maternal inheritance of MT-CYB that *P. magdalenae*: i) has not shown significant genetic differentiation and have high contemporary gene flow; ii) post-bottleneck expansion is supported by Bayesian Skyline Plot (≈ 1500 -year) and iii) parallels genetics and demographic patterns compared to other Magdalena River migratory fishes (*Prochilodus magdalenae*, *Pseudoplatystoma magdaleniatum*). The validation of these primers, coupled with evidence of panmictic gene flow, establishes molecular tools to monitor population dynamics and genetics in endemic stingrays.

This study provides further insights into the genetic and demographic structure of *Potamotrygon magdalenae*, offering valuable guidance for conservation strategies within the Magdalena River basin. While mitochondrial markers enable the detection of maternal lineages and recent demographic events, their uniparental inheritance and limited genomic scope constrain the ability to reconstruct a population's genetic history fully. To overcome these limitations, future research should integrate nuclear and male parental inherited markers using a multilocus approach and extend sampling efforts to include poorly characterized portions of the Magdalena and Atrato rivers, particularly in the southern and northern basin areas influenced by anthropogenic interventions. Such expanded analyses would help clarify and complement our understanding of population structure and gene flow, considering the distinct evolutionary dynamics revealed by each marker type.

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