# Molecular systematics of the freshwater stingrays (Myliobatiformes: Potamotrygonidae) of the Amazon, Orinoco, Magdalena, Esequibo, Caribe and Maracaibo basins (Colombia- Venezuela): evidence from mitochondrial genes

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## Abstract

Freshwater stingrays from the family Potamotrygonidae have a restricted distribution to the freshwater systems of South America. Lack of adequate information about the taxonomic and evolutionary relationships, ecology, biology and distribution of several species belonging to this family makes them vulnerable to anthropic activities, including commercial exploitation for the ornamental fish market. Samples were collected from the main river basins in Colombia and Venezuela (Amazon, Orinoco, Magdalena, Esequibo, Caribe and Maracaibo) for four genera and seven species of the family (*Heliotrygon gomesi, Paratrygon aiereba, Plesiotrygon iwamae, Potamotrygon motoro, Potamotrygon yepezi, Potamotrygon schroederi, Potamotrygon magdalenae*), and some unidentified species. Molecular markers Cytochrome Oxidase subunit I, Cytochrome *b* and ATPase subunit 6 were amplified and sequenced. Maximum likelihood and Bayesian Inference analysis were performed to obtain topologies for each marker and for a concatenated dataset including the three genes. Small dataset may compromise some methods estimations of sequence divergence in the ATP6 marker. Monophyly of the four genera in the Potamotrygonidae family was confirmed and phylogenetic relations among member of the *Potamotrygon* genus were not clearly resolved. However, results obtained with the molecular marker Cyt*b* appear to offer a good starting point to differentiate among genera and species as a tool that could be used for fast molecular identification (Barcode). The application of this gene as a barcode could provide a useful tool than can be applied for Management and regulation of extraction practices for these genera. Sequencing of complete mitochondrial genomes would be the next step for testing evolutionary hypothesis among these genera. Population studies should be undertaken for *Potamotrygon magdalenae*, *Paratrygon aiereba* and *Potamotrygon motoro* to establish possible population differentiation among basins, information that is key for delineating conservation and management plans for these species.

**Keywords:** Potamotrygonidae, molecular systematics, Colombia, Venezuela, conservation.

## 1. Introduction

Freshwater stingrays (Potamotrygonidae) have a restricted distribution to the freshwater systems of South America (Lovejoy, 1996). These rays are distributed in the following

principal basins and sub basins: Orinoco, Maracaibo, Magdalena, Atrato, Essequibo, Oyapok, Maroni, Corantijn, Amazonas, Río Negro, Guaporé, Tapajós, Parnaiba, Cuiyabá, Paraná, Paraguay, Uruguay and Río de La Plata (Rosa, 1985). This family includes four currently valid genera: *Plesiotrygon, Paratrygon, Heliotrygon* and *Potamotrygon* (Carvalho, 2001; Ishijara and Taniuchi, 1995; De Carvalho and Lovejoy, 2011). *Paratrygon* is the only monotypic genus in the family, while two species are classified in each of the genera *Plesiotrygon* and *Heliotrygon.* Between 18 and 20 species have been described for the genus *Potamotrygon* (Araujo *et al*., 2004; De Carvalho *et al*., 2011; De Carvalho and Lovejoy, 2011; De Carvalho and Ragno, 2011). In Colombia there are about ten reported species for the family distributed in the Orinoco, Amazon, Magdalena, Caribe and Maracaibo basins (Rosa *et al*., 2010; Maldonado-Ocampo *et al*., 2008).

This group shares the biological characteristics of other elasmobranches. They have internal fertilization, low fecundity and slow growth (Araújo *et al*., 2004; Mejía-Falla *et al*., 2008). Their reproductive cycle depends on the changes of the hydrological cycle in their habitat, but by being restricted to freshwater environments, they are subject to changing conditions. Such changes can be caused by low or high water seasons, increment of pollution and habitat destruction (Charvet-Almeida *et al*., 2002; Charvet-Almeida *et al*., 2005). Little is known about the components of the life history of Potamotrygonidae (Charvet-Almeida *et al*., 2002). This lack of adequate information about the majority of the species in this family makes evaluations of their role in the ecosystem and management a difficult task (Charvet-Almeida *et al*., 2002). All of this makes this group of species more vulnerable than their marine counterparts, especially to anthropic activities, including commercial exploitation (Araújo *et al*., 2004).

Freshwater stingrays exploitation for the ornamental fish market is a common activity in several South American countries (Araújo *et al*., 2004). In Colombia, this market represents a

high income source to the local economy in various regions of the country (Falla and Poveda, 2008); its main fish extraction point is the Orinoquia region, representing more than 76% of the total ornamental fish extracted from the rivers of the country (Falla and Poveda, 2008). Fish are traded in the national and international markets, being Potamotrygonidae one of the most exported families of fish and having high commercial value (Mancera-Rodríguez and Álvarez-León, 2008). The impact of this activity on the ecosystem and the extraction of wild fish from their natural habitat have not been evaluated and are still unknown. The total number of extracted stingrays by species is hard to measure, since the records may not be accurate, they do not include the number of stingrays that die during fishing activities and transport, and due to difficulties in the identification based in basic morphology and coloration, the records for captures for each species may not be accurate either (Mancera-Rodríguez and Álvarez-León, 2008).

Since the species of the family Potamotrygonidae are vulnerable to commercial exploitation, its conservation is a priority that should be supported by thorough scientific information. One aspect complicating the possibility of obtaining important information regarding the life history and abundance of these rays is the lack of clarity regarding their taxonomy and systematic relationships (Araújo *et al*., 2004). The identification of species belonging to this family is usually done using morphological approaches, such as measurements of multiple characters from the skeleton, like the mandibular arch and hyomandibular bone, and muscles, like the ventral muscles of the cranial region, as well as studying their embryology, lateral line systems and physiology (Lovejoy, 1996). Due to the polychromatic patterns in their coloration a fast, easy and accurate morphological identification has not been possible, and could result in misidentifications of species in the group (Araújo *et al*., 2004).

Molecular identification, or "molecular barcodes" have been applied for several Elasmobranchii groups as an important tool for conservation (Ward *et al*., 2008; Ward *et al*.,

2009; Hebert *et al*., 2003a; Hebert *et al*., 2003b;Caballero *et al*., 2012). However, the standard barcode marker, the mitochondrial gene Cytochrome Oxidase subunit I (COI) has not provided clear identification advantages for Potamotrygonidae (Toffoli *et al*., 2008). Some of the issues found when using COI for this family include problems assigning individuals to species (Buhay, 2009). The assignment is critically dependent on sufficient population sampling of the group studied and it has lacked power for discovering and assigning Potamotrygonidae species using conventional analysis methods (Toffoli *et al*., 2008). Other studies have shown relative success when using different mitochondrial markers in Potamotrygonidae, for example the mitochondrial gene Cytochrome *b* (Cyt*b*) (Dunn *et al*., 2003; Lovejoy *et al*., 1998). For these reasons it is important to test additional molecular markers in order to find possibly better "molecular barcodes" for this group and also test them o a higher number of individuals from different geographic distributions, to be able to obtain information that once combined with ecological and morphological data, allows for taxonomic and systematic clarification for a high number of species in this family (Toffoli *et al*., 2008).

Acquiring information that allows for successful species identification is necessary to allow establishment of control and capture regulation measures in the extraction and trade of the stingray species of interest (Caldas *et al*., 2010), including molecular data for generating practical tools to solve relations and perform identifications within organisms (Edwards, 2009; Yan and Rannala, 2012; Baker, 2008). The aim of this study is framed in the "Plan de Acción Nacional de Tiburones", PAN-Tiburones, Colombia (Caldas *et al*., 2010), which establish the need for include molecular tools that can contribute to the conservation (e. g. IUCN conservation status) and management plans (e.g. fishing quotas) for this Elasmobranchii group. We aim to reconstruct a first approximation of the systematic of the group by analyses of three mitochondrial markers: Cytochrome Oxidase subunit I (COI),

Cytochrome *b* (Cyt*b*) and ATPase subunit 6 (ATP6) from freshwater stingrays obtained from several rivers in Colombia and Venezuela, giving an estimate of the separation of clades in the studied species.

## 2. Material and Methods

### *2.1 Sample collection and DNA extraction*

A total of 119 samples were included in this study, fifty tissue samples were obtained from specimens in La Salle Natural History Museum (MHNLS Venezuela). Sixty specimens were collected from different river basins and had tissue extracted, with collaboration from Instituto de Investigación de Recursos Biológicos Alexander von Humboldt (IAvH, Colombia). Nine specimens were provided by Omacha Foundation (Colombia). Geographical locations are shown in Table 1 and Figure 1. Samples from Colombia were labeled according to the basins, sub-basins and additional collecting points. First the Orinoco basin was divided in sub-basins and one collecting point: Inirida (INI), Orotoy (ORO), Casanare (CAS), Bita (BIT) sub-basins and the collection center in Puerto Carreño (PCA); The Caribe basin represented as Atrato (ATR) sub-basin; The Amazon basin was divided in Caqueta (CAQ) and Putumayo (PUT) sub-basins; The Magdalena basin (MAG) was not divided and the Maracaibo basin is represented by the Catatumbo (CAT) sub-basin; We also included samples from the ornamental fish market in Bogota (BOG). Samples from Venezuela were labeled as follows, Orinoco basin: Orinoco River (ORI), Caura (CAU) sub-basin and the Orinoco River Delta (DEL); Maracaibo basin (MAR) and Esequibo basin represented by the Cuyuni (CUY) sub-basin.

All tissue samples were preserved in ethanol at 70% and stored at 4°C. Total DNA was extracted using the QIAGEN DNeasy tissue kits and the extraction protocol using Chelex® resine100 (Walsh *et al*., 1991).

*2.2 PCR Amplification and sequencing*

DNA amplification via polymerase chain reaction (PCR) was performed following this protocol for the three mitochondrial genes: 30.5µL reaction volume containing 0.016U (0.1µL) Taq polymerase (Biolase), 0.33mM of each primer (1µL), 0.49mM dNTPs (0.3µL), 2.87mM MgCl2 (3µL), 0.98x Buffer (3µL), Bovine Serum Albumin (2µL) and ddH2O DNAse and RNAse free (19.6µL). A fragment of approximately 600 bp of the mitochondrial Cytochrome *b* (Cytb) was amplified using the primers CB2 5'-TGAAACTGACCATGACACTAA-3' and GLUDG 5'-TGAAACTGACCATGACACTAA-3' described by Kocher *et al*. (1989). The temperature profile was as follows: 2 minutes of initial denaturation at 94°C followed by 34 cycles of denaturation at 94°C for 1 minute, annealing at 57°C for 45 s, extension at 72°C for 40s and a final extension at 72°C for 10 minutes.

Fragments of approximately 800 pb of the ATPase 6 gene were amplified using the primer pair PotaATPf3_Lys (5'-GTAAAACGACGGCCAGTGTCCAGCATTAGCCTTTTAAGC-3') and PotaATPr3 primers (5'-CTGGGGTCAACTATRTGATATG-3'). Fragments of approximately 800 bp from the mitochondrial gene COI were amplified with the primers COIf.1 (5-GTAAAACGACGGCCAGTCTTAACACAACWTTCTTTGACCC-3´) and COIr.3 (5´- ACGTTTTGATGCRAAKGCYTCTC -3´). The sequences for the two primer pairs (ATP6 and COI) were kindly provided by Daniel Toffoli. These two mitochondrial genes used the same PCR conditions as for Cyt*b*, but changing the annealing temperature to 53ºC (COI) and 57ºC (ATPase). The PCR products were evaluated in a 1% agarose gel. Successful PCR products were cleaned using PEG (Polyethyleneglycol) (Kawai *et al*., 1980). Sequencing reactions were performed using the BigDye Terminator v.3.1 Cycle Sequencing Chemistry (Applied Biosystems). Sequencing was performed in both directions in an

automated ABI 3500 DNA sequencer (Applied Biosystems) at Universidad de los Andes and Universidad Nacional, Colombia.

*2.3 Data Analysis*

Sequences from the mitochondrial genes COI, ATPase 6 and Cyt*b* were edited and aligned manually using the software Geneious v 4.7 (Drummond *et al*. 2009). Forward and reverse sequences were confirmed by alignment with each other and a consensus sequence was obtained. Four different data sets were constructed: the first one contained 97 COI sequences, the second one contained 89 Cyt*b* sequences generated in this study plus seven additional sequences previously published by De Carvalho & Lovejoy (2011) and Lovejoy *et al*. (1998) (Table S1); the third one included 26 ATP6 sequences and the fourth and last one included a concatenated alignment of 14 shared sequences from samples shared among the first three data sets. Sequences from the Chupare stingray (*Himantura schmardae)* were used as outgroups for all analyses (Accession: JN184062).

The optimal substitution model for each data set was selected by the Akaike Information Criterion (AIC) using ModelTest v3.7 (Posada and Crandall 1998) and PAUP* v4.0b10 (Swofford 2003). For Maximum Likelihood RAxML-HPC BlackBox v7.3.2 (Stamatakis, 2006) was used to perform 200-replicate bootstrap analyses on each data set using the CIPRES Portal (Miller *et al*., 2010). Bayesian inference (BI) was performed in MrBayes v3.1 (Huelsenbeck and Ronquist 2001) using three hot and one cold chains, 10 M generations were run with a 15000 generation burn-in, sampling every 1,000 generations. The convergence of all Bayesian analyses was observed and assessed in Tracer v1.5 (Drummond and Rambaut, 2007). Branch supports from ML and BI were mapped in the branches, with bootstrap values from 0-100 that indicate the percentage value of the interior branches produced by the resampling procedure and the subsequent tree reconstruction of a 1000

repetitions. Also the posterior probabilities ranging between 0-1 for BI, that are probabilities of the branches determined given a prior probability, likelihood function, and data.

The Shimodaira-Hasegawa test (SH test) (Shimodaira and Hasegawa, 1999) was implemented in PAUP, in order to compare the evolutionary hypotheses of each obtained topology for each dataset analyzed, using PAUP 4.0b10 (Swofford 2003). This analysis was performed for the comparison of BI to ML topologies. The same test was used to compare the ML topologies from each gene to the topology of the three concatenated genes, using the same samples. This was done in order to prove if there were significant differences between topologies generated for independent datasets when compared to the concatenated dataset.

A ‘‘stemminess” analysis was implemented for obtaining an estimate of the amount of phylogenetic signal contributed by the internal branches (Caballero *et al*., 2008; Longhorn *et al*., 2010). This was done in PAUP 4.0b10, and NJ trees were built with uncorrected distance estimates (minimum evolution, ME). This analysis was performed using only the 15 samples of the concatenated dataset in the NJ trees for COI, Cyt*b* and ATP6. Internal branch lengths were summed and stemminess was determined as the sum of internal branch lengths/ME score, for assessing the contribution of internal branch lengths, higher stemminess values indicating a greater degree of phylogenetic information in the tree generated for each particular dataset (Caballero *et al*., 2008; Longhorn *et al*., 2010).

**3. Results**

Phylogenies for each dataset were generated under the models of evolution defined by the Akaike information criterion in ModelTest (Posada and Crandall 1998) and were as follows: for COI the SYM+G model, for Cyt*b* the GTR+G model and for ATP6 the TVM+G model. For the concatenated dataset a partitioned matrix including these models was used. For each gene two topologies were obtained with the ML and BI methods, showing the phylogenetic

relations between the four genera of the family and confirming their monophyly with high supports (bootstrap support values (BS) for ML and posterior probability values (PP) for BI). Relationships among species varied according to the gene topology but the majority of groupings were shared by the four topologies.

*3.1 SH test and Stemminess analysis*

The results from the SH test showed no significant difference in the topologies generated by ML and BI for the four datasets independently (Table 2). The SH test comparison between the topologies generated for each gene and the topology of the concatenated genes showed differences between the concatenated topology and the topologies for COI and Cyt*b*, but no significant difference with the ATP6 topology (Table 3).

The stemminess analysis indicated that in the NJ topologies, of the 15 same samples used for the concatenated tree, ATP6 gene showed a higher proportion of character changes attributed to internal branches relative to those on terminal branches. The NJ trees for COI, Cyt*b* and the concatenated tree showed similar stemminess values (Table 4), meaning that the three genes contain what seems to be useful phylogenetic signal for phylogenetic analysis for this family.

*3.2 COI phylogeny*

For the complete dataset of the COI gene the BI topology is shown with posterior probabilities and with the bootstrap support values from the ML analysis (Figure 2).

Several groups showed high supports from both methods, main groups or clades were labeled with letters from A to G. For example, the entire genus *Paratrygon* was grouped together (B), but the only sample of *Heliotrygon* sp. (A) obtained in this study clustered with it. In *Paratrygon* samples belonging to basins directly connected to the Orinoco River grouped

together with high support (B1); the *Paratrygon* sample shown as an external group from the other samples (B2) belongs to Caqueta River, in the Amazon Basin, and this positioning had a high support in both ML and BI.

In the *Potamotrygon* genus the number of species sampled in this study was higher. *P. yepezi* samples grouped together (E) without showing separation by collection localities. This species was positioned as the more basal species from this genus, while the other internal groups were separated in four clearly defined clades; the first clade (G), included samples from the *motoro* complex. *P. motoro* was identified in this study as a species complex since samples from a variety of collection localities and also unidentified samples grouped together in this clade. *P. motoro* samples were only separated from one group by collection localities (G1) that included all the Orinoco Delta samples from the genus *Potamotrygon*.

The third internal clade (F) included samples from the Magdalena and Atrato Rivers, considered both as *P. magdalenae* given its distribution (Charvet-Almeida and Pinto de Almeida, 2009). Samples from the Atrato River (F2) were separated from samples from Magdalena River (F1), showing a possible separation by geographical collection localities. This clade had high BS and PP supports in both ML and BI reconstructions (95/1).

The fourth clade (D) included samples from *P. schroederi* grouped with unidentified samples from different collection localities (Puerto Carreño, Caura River and Casanare River) that possibly belong to this species.

*3.3 Cytb phylogeny*

The dataset for Cyt*b* had a smaller number of samples than that used for the COI phylogenetic reconstruction but had a more complete coverage of the genera belonging to the family Potamotrygonidae. The BI reconstruction is shown with PP supports and with the

support values from ML bootstrap analysis (Fig3). In these reconstructions, seven samples previously published by De Carvalho & Lovejoy (2011) and Lovejoy *et al*. (1998) were included, since they provided and additional frame of reference for delimiting groups.

In this phylogenetic reconstruction the clades for the four genera showed high branch supports splitting them as monophyletic groups. *Heliotrygon* was the most basal genus (A), followed by *Paratrygon* (B), leaving *Plesiotrygon* (C) as the sister group of *Potamotrygon*. The genus *Paratrygon* in this topology also showed a splitting, with high branch supports (92/0.99), of the samples by collecting points. In this reconstruction the number of samples of this genus was higher, splitting samples from Putumayo River, Caqueta River and the sample AF110629 (from the Amazon (B2)), from all samples from collection localities points that belonged to basins directly connected to the Orinoco River (B1).

For the genus *Potamotrygon* the same clades observed in the COI phylogenies were found (D, F, E and G), but the topology was somewhat different. *P. yepezi* (E) was not defined as a basal species, but was still grouped inside the *Potamotrygon* genus (H). *P. schroederi* was positioned next to two unidentified samples from the Orinoco River that possibly belong to the same species, this clade also positions next to some unidentified samples from different collection localities (D), including the Caura River, Casanare River, Orotoy River, Puerto Carreño and the fish market in Bogotá.

The *P. magdalenae* samples from the Magdalena River were grouped with samples from Atrato River (F) showing again a possible geographic subspecies or even species split. The *motoro* complex grouped inside a clade defined by sample AF110626 (a *P. motoro* sequence from Amazon River). Inside this clade (G) the only supported delimitations were the *P. motoro* samples from Putumayo and Caqueta Rivers and the *P. motoro* samples from Puerto Carreño, and Inirida and Caura rivers. This was different from the COI topology, showing

possible different separations among the motoro complex based on geographic collection localities. The separation between the Amazon and Orinoco basins in the middle Miocene may be the reason for the patterns found in the tree, assuming that the Casiquiare River is geographical barrier for these species.

*3.4 ATP6 phylogeny and concatenated tree*

The dataset of the ATP6 gene included less samples than the ones for the two previous genes. The BI topology is shown with PP supports and with the support values from ML bootstrap analysis (Fig4). The concatenated tree was made with BI and ML, mapping both supports on the BI topology; it contains the sequences shared in common by the three previous topologies for a total of 15 samples (Fig5).

In both reconstructions the monophyly of *Paratrygon* was supported. In the ATP6 phylogeny, the genus *Paratrygon* was positioned basal in the family (B), divided again by geographical collection localities related to the Amazon (B2) and Orinoco (B1) basins. The genus *Plesiotrygon* was not clearly supported as the sister group of the genus *Potamotrygon.* In the concatenated tree the genus *Paratrygon* was also separated by the same collection localities (B1 and B2). The genus *Plesiotrygon* was not included in this reconstruction due to lack of successful amplification of particular samples, so it was not possible to confirm monophyly from *Potamotrygon* in this topology. The genus *Potamotrygon* was divided in the same three clades in both the ATP6 and the concatenated tree reconstructions (G, E and D). Among these clades no clear or supported separation according to geographical collecting points was observed.

**4. Discussion**

Freshwater stingrays have diversified in the river systems of South America, many of which run through Colombia and Venezuela (Rosa, 1985). However for the majority of species little is known about their ecology, existing population sizes and about the evolutionary patterns that led to their current distribution (Hubert and Renno 2006; Araújo *et al*., 2004). The systematic of this Elasmobranchii group is still unresolved and the pressure that fisheries and other anthropic activities exert on them is hard to measure (Mancera-Rodríguez and Álvarez-León, 2008). The evaluation of the molecular systematics of Potamotrygonidae using the mitochondrial markers COI, Cyt*b* and ATP6, provides us with an initial approach to understand how this group has diversified is in the rivers systems from Colombia and Venezuela.

The mitochondrial markers used for this study showed a good phylogenetic signal overall (over 50% stemminess values), but the phylogenetic reconstruction from the concatenated dataset was rejected by two of the three datasets (COI and Cyt*b*). Reconstruction from COI and Cyt*b* shared high support values in the main defined groups, arguably giving the best topologies of the four constructed trees, the higher sampling in these trees influence positively the resolution of the trees (Burleigh and Matthews, 2004; Zhang *et al*., 2010). Multilocus data can be used to estimate the species tree, but it can converge on an incorrect estimate when an anomalous gene tree is generated or when it is sensitive to sampling variation for small numbers of loci, on the other hand the gene trees can give information regarding the processes that have shaped organismal genome (Degnan and Rosenberg, 2009). We compared the concatenated tree with the gene trees to apply a correct approach for analyzing the information provided by the topologies. The gene Cyt*b* has proved to be more effective for resolving relationships among divergent lineages, such as rainbow fishes, sharks and killifishes (Zhu *et al*., 1994; Martin et al., 1992; Garcia *et al*., 2000), reinforcing the assumption that it may be one of the constructed topologies with higher resolution.

The SH test did not show significant differences between the ATP6 topology and the concatenated topology. This may be due to ATP6 having the highest stemminess value, with a 67% contribution of internal branch lengths to the total minimum-evolution score. This result provides information regarding this gene fragment as the one having the highest phylogenetic signal of the four topologies (Longhorn *et al*., 2010). For measuring the phylogenetic signal a small dataset was used and this may overestimate the percentage of invariable sites and affect the estimates of substitution parameters (Burleigh and Matthews, 2004). Given that nucleotide substitution models are designed to make corrections in the distance estimates (Bos and Posada, 2005), the effect of the small dataset may compromise some methods used to correct sequence divergence for multiple substitutions (Marshall and Baker, 1998), including the model used to generate the ATP6 topology. One caveat of our study is that it was not possible to obtain the expected number of sequences for the ATP6 gene sequences due to DNA degradation that lowered the quality of the sequences and therefore the sampling for each gene had different sample sizes. Also, gene trees and the concatenated or "species" tree needs improvement by addition of samples; the model calculation process must be reappraised for the ATP6 gene to avoid bias by underestimation of sequence divergence as well as possible effects of selection on this gene. It has been suggested that the family Potamotrygonidae is a divergent lineage that has provided evidence of recent radiation (Toffoli *et al*., 2008). Taking this into consideration, it is possible that in this family the ATP6 model is underestimating the sequence divergence between recently divergent taxa, but to confirm this it is necessary to have complete genetic data (i.e. full mitogenomes) so that the effects of one gene on the overall topology can be measured (Duchêne *et al*., 2011).

Even with the small datasets in some of the four trees, they shared almost all the clades in the topologies. The molecular data obtained of the family Potamotrygonidae supports the

previous studies by establishing the genus *Plesiotrygon* as the sister clade of *Potamotrygon*, and *Paratrygon* as the sister clade of the *Plesiotrygon-Potamotrygon* group (Marques, 2000; Toffoli *et al.,* 2008). The genus *Heliotrygon* is established as the basal monophyletic clade of Potamotrygonidae, the COI tree has a sample labeled as *Heliotrygon* from the Orinoco River and it groups with *Paratrygon*, the phylogenetic tree showed good branch supports in the *Paratrygon* clade indicating two possibilities, one that it was possibly misidentified, agreeing with the reported distribution of the genus *Heliotrygon* in the Amazon basin (De Carvalho and Lovejoy, 2011), and two that this sample belongs to an unreported species of *Heliotrygon* from the Orinoco were the clade cannot be solved because more samples are needed.

Potamotrygon is the more diversified genus of the family (Rosa, 1985), the short branches and the polytomies found in the phylogenetic trees are signal that this genus may be going through a process of recent radiation (Toffoli *et al*, 2008). Among member of this genus the relationships between species were not clear. *P. yepezi* showed no separation or misidentifications and with COI is set as the basal clade of *Potamotrygon*, but in the *Cytb* reconstruction it showed an unresolved relation in the same branch. This result suggests that the evolutionary process of these species cannot be assed with these molecular markers alone, and that complete mitochondrial genetic data and nuclear information, that can give us the evolutionary history of Potamotrygonidae, is needed (Bernardi *et al*., 1993; Duchêne *et al*., 2011).

The complex *motoro* included a high number of unidentified samples that belong possibly to the *P. motoro* species. In this species, coloration and morphological similarity can cause misidentifications (Araújo *et al*., 2004). A complete morphological identification of the individuals is needed to identify the species that are clustering in the *motoro* complex.

The species *P. schroederi* is clearly separated from the other species, there are unidentified samples as a sister clade from *P. schroederi*. The complete morphological identification of the individuals in the sister clade will provide, in future studies, a good reference for the sister species of *P. schroederi*.

The species *P. magdalenae* showed geographic separation from the collection localities in both the COI and Cyt*b* reconstructions, showing samples from the Atrato River grouping together as a sister clade from the Magdalena River samples. This separation suggests possible population isolation and differentiation. To further understand this suggested population differentiation, a genetic population study can contribute to the estimation of population units and divergence times among *Potamotrygon* species (Crandall *et al.*, 2000). The isolation and differentiation of species in the Magdalena, Atrato and Maracaibo basins may provide an interesting example of how paleogeological processes may have shaped the evolutionary history of Potamotrygonidae (Machado-Allison, 2008), knowing that in the middle Miocene, about 11 MYA, the eastern mountain range of the Andes uplifted and limited the connection between the Magdalena basin and the western region of the continent and that around the inferior Miocene-Pliocene the Atrato basin was created from the closure of the Panama isthmus (Lundberg *et al.*,1988).

Between the *Potamotrygon motoro* (with Cyt*b*) and *Paratrygon aiereba* (with COI, Cyt*b* and ATP6), samples there is a clear separation which is related to geographical collection localities. Samples from the Orinoco basin are separated from the ones collected in rivers belonging to the Amazon basin. This separation showed a high support by the topology for *Paratrygon* and *Potamotrygon motoro* clades, even if for *Potamotrygon motoro* this separation did not show the highest branch support values. These results suggest a possible separation without constant gene flow between basins (Posada and Crandall, 2001) and may be related to paleogeography processes. The central mountain range of the Andes (100 Mya)

formed a marginal basin that separated this region from the rest of the eastern continental platform, the basin river mouth drained in the Caribbean around the region that we call today Lake Maracaibo, harboring aquatic fauna from rivers and estuaries (Lundberg *et al.,* 1994). When the central mountain range of los Andes uplifted, the marginal basin formed the riverbed of the Paleo-Orinoco River that drained where today we find the lake Maracaibo basin and the endemic species *P. yepezi*. Between the Paleocene and Miocene periods, the Paleo-Amazon River change its riverbed in direction to the Pacific Ocean generating a big wetland called the Pebas Lake which allowed the fauna diversification in new niches, this wetland overflowed towards the Caribbean forming the Paleo Amazon-Orinoco system in which several species shared the same riverine system including species from Potamotrygonidae (Galvis *et al.,* 2007; Lovejoy *et al.,* 1998). The separation of this system occurred around 10-11 Mya, when the Eastern mountain range of the Andes was uplifted causing an Orinoco-Amazonas vicariance event, sedimentations from the erosion in the Andes caused the Orinoco to gradually shift east towards its current position (Lundberg *et al.* 1998), separating the two main basins in north South America, separation that influenced the fauna found in today river systems. The Amazon and Orinoco basins in the present time are only connected as follows: Amazon basin- Rio Negro basin- the arm of the Casiquiare River- Orinoco basin. The Casiquiare River can serve as both an opportunity for gene flow for freshwater species or a barrier to it (Winemiller *et al*. 2008), in the case of *Paratrygon aiereba* and *Potamotrygon motoro* we suggest that it acts as a barrier separating different evolutionary units. Samples from the Casiquiare corridor and Rio Negro are needed to test this via. The effect of this possible barrier in *Paratrygon aiereba* and *Potamotrygon motoro* can be a determinant factor for determining possible species or subspecies differentiation among these groups and possible changes to their taxonomic status. Studies aiming to further

clarify these differentiation processes are urgently needed to provide key information required to delineate conservation plans in all the countries that share these basins.

The species *Potamotrygon motoro* was described in the Parana basin (Rosa, 1985), given the geographical distance between the "*P. motoro*" that was sampled in this study, and the Parana basin, it is possible that the similarity between the two organisms comes only from the chromatic patterns, sampling from both basins and geographical points between them are necessary to confirm this difference.

## 5. Conclusions and recommendations

The family Potamotrygonidae is a diverse group of Elasmobranchii that still has unresolved phylogenetic relationships. This study was an initial step to try to clarify the phylogenetic relationships among genera and species from this family distributed in northern South America, particularly in rivers of Colombia and Venezuela. To further investigate and solve relationships among member of this family, a study of complete mitochondrial genomes, as well as sequencing of nuclear markers is urgently needed. To define an "ideal" molecular marker for the identification of species the effects of one gene on the overall topology must be measured, but according to past studies and the results of this study, one such molecular marker could be the Cyt*b*. This gene provides a good reference for identification of most of the species for a fast molecular identification. The genera in the family Potamotrygonidae can be differentiated with fast molecular analysis; molecular methods like barcoding can be used in conservation efforts applied to regulating extraction of individual by genus. Population studies should be undertaken for *Potamotrygon magdalenae*, *Paratrygon aiereba* and *Potamotrygon motoro* to establish separation between basins, confirm population differentiation and clarify distribution and taxonomic status of individuals classified in these species in order to obtain useful information to establish and develop national and regional

conservation plans for these genera. Steps to follow includes increasing the number of samples of each species, and extending the collection points for a higher sampling, to the point that the interconnecting basins and the main basins are proportionally represented in the sampling.

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**Figures**

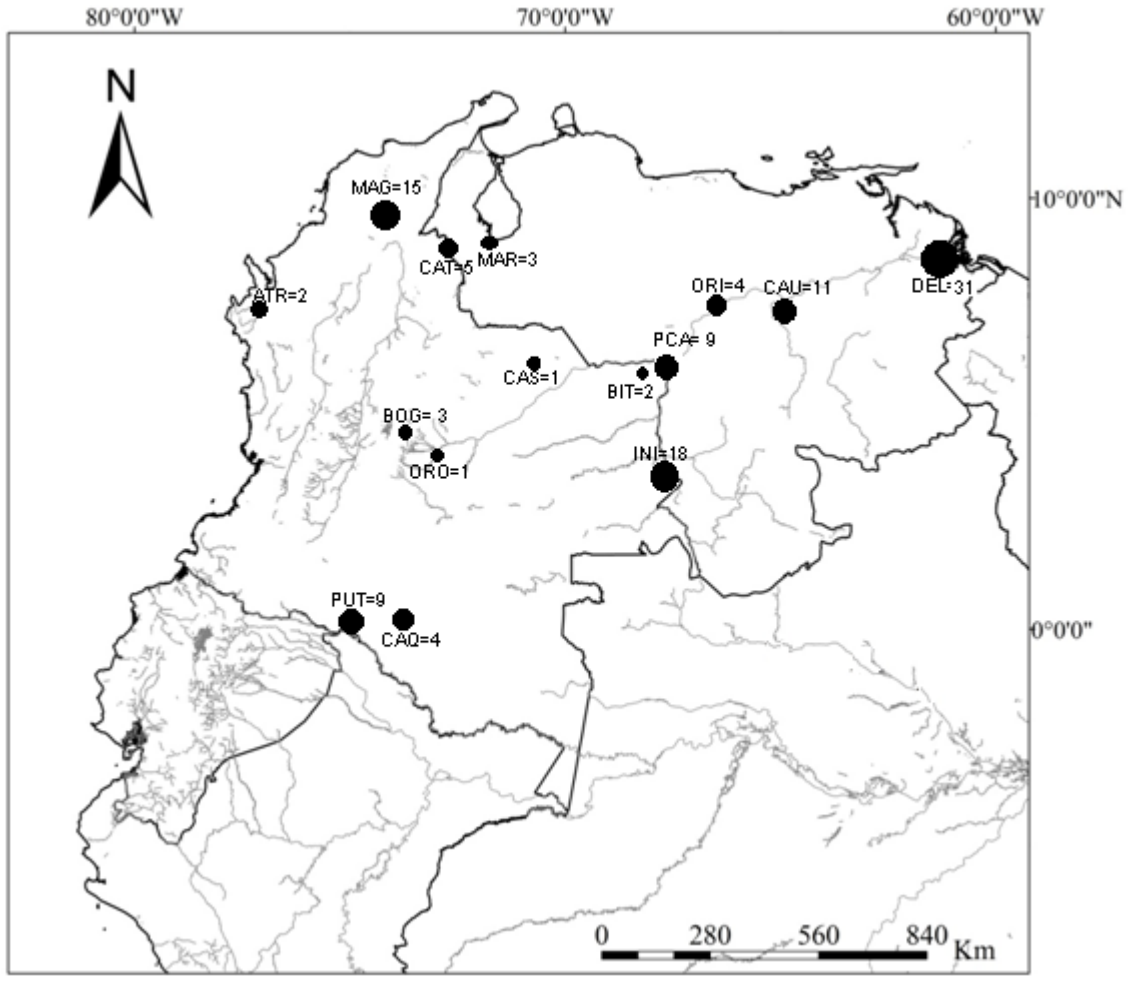


**Figure 1.** Map showing sample locations and sampling sizes for each collection locality.

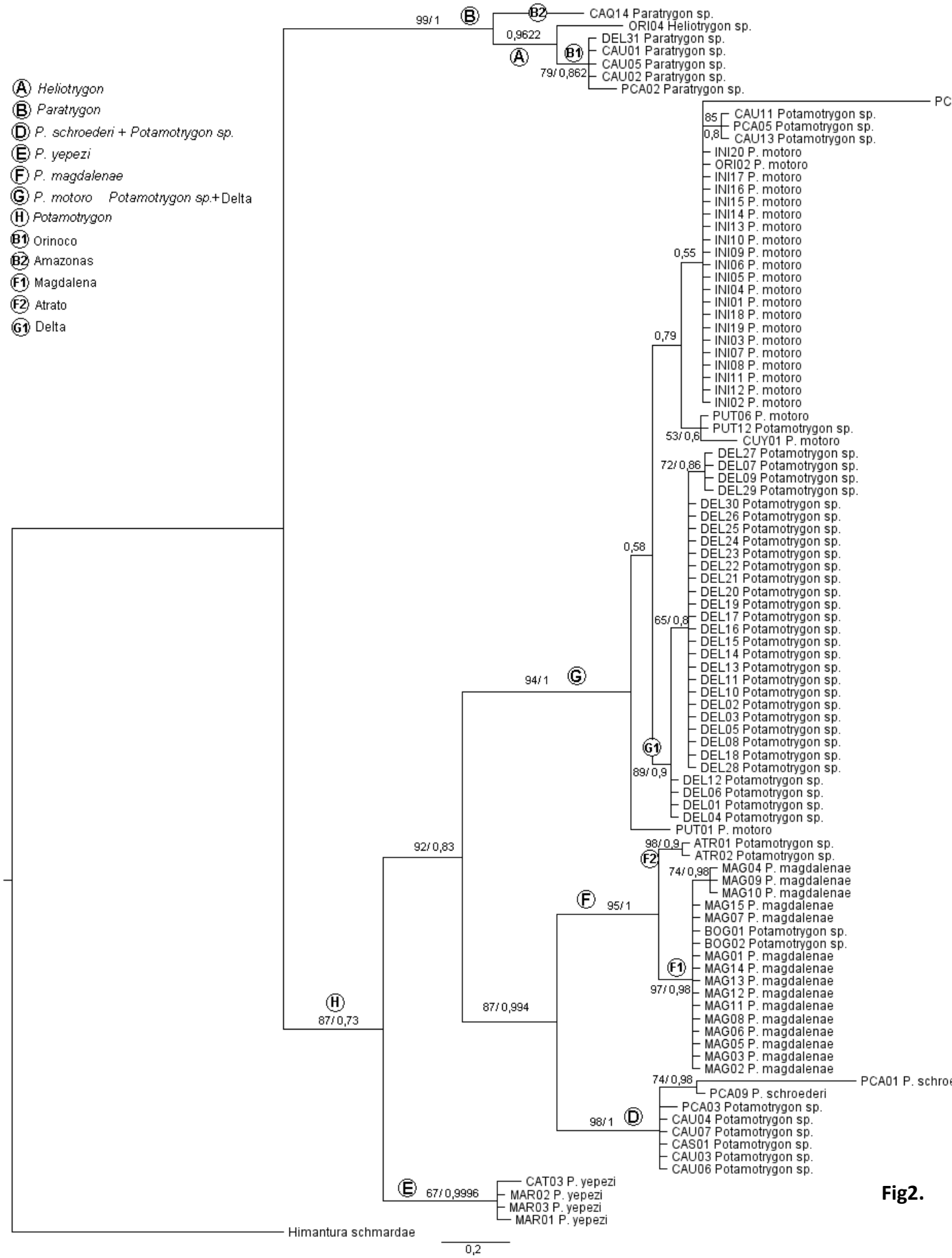
Ⓐ Heliotrygon
Ⓑ Paratrygon
Ⓓ P. schroederi + Potamotrygon sp.
Ⓔ P. yepezi
Ⓕ P. magdalenae
Ⓖ P. motoro Potamotrygon sp.+Delta
Ⓗ Potamotrygon
Ⓑ1 Orinoco
Ⓑ2 Amazonas
Ⓕ1 Magdalena
Ⓕ2 Atrato
Ⓖ1 Delta
99/1
0,9622
79/0,862
CAQ14 Paratrygon sp.
ORI04 Heliotrygon sp.
DEL31 Paratrygon sp.
CAU01 Paratrygon sp.
CAU05 Paratrygon sp.
CAU02 Paratrygon sp.
PCA02 Paratrygon sp.
85
0,8
CAU11 Potamotrygon sp.
PCA05 Potamotrygon sp.
CAU13 Potamotrygon sp.
INI20 P. motoro
ORI02 P. motoro
INI17 P. motoro
INI16 P. motoro
INI15 P. motoro
INI14 P. motoro
INI13 P. motoro
INI10 P. motoro
0,55
INI09 P. motoro
INI06 P. motoro
INI05 P. motoro
INI04 P. motoro
INI01 P. motoro
INI18 P. motoro
0,79
INI19 P. motoro
INI03 P. motoro
INI07 P. motoro
INI08 P. motoro
INI11 P. motoro
INI12 P. motoro
INI02 P. motoro
PUT06 P. motoro
PUT12 Potamotrygon sp.
53/0,6
CUY01 P. motoro
72/0,86
DEL27 Potamotrygon sp.
DEL07 Potamotrygon sp.
DEL09 Potamotrygon sp.
DEL29 Potamotrygon sp.
DEL30 Potamotrygon sp.
DEL26 Potamotrygon sp.
DEL25 Potamotrygon sp.
DEL24 Potamotrygon sp.
0,58
DEL23 Potamotrygon sp.
DEL22 Potamotrygon sp.
DEL21 Potamotrygon sp.
DEL20 Potamotrygon sp.
DEL19 Potamotrygon sp.
65/0,8
DEL17 Potamotrygon sp.
DEL16 Potamotrygon sp.
DEL15 Potamotrygon sp.
DEL14 Potamotrygon sp.
94/1
DEL13 Potamotrygon sp.
DEL11 Potamotrygon sp.
DEL10 Potamotrygon sp.
DEL02 Potamotrygon sp.
DEL03 Potamotrygon sp.
DEL05 Potamotrygon sp.
DEL08 Potamotrygon sp.
89/0,9
DEL18 Potamotrygon sp.
DEL28 Potamotrygon sp.
DEL12 Potamotrygon sp.
DEL06 Potamotrygon sp.
DEL01 Potamotrygon sp.
DEL04 Potamotrygon sp.
PUT01 P. motoro
92/0,83
98/0,9
ATR01 Potamotrygon sp.
ATR02 Potamotrygon sp.
74/0,98
MAG04 P. magdalenae
MAG09 P. magdalenae
MAG10 P. magdalenae
95/1
MAG15 P. magdalenae
MAG07 P. magdalenae
BOG01 Potamotrygon sp.
BOG02 Potamotrygon sp.
MAG01 P. magdalenae
MAG14 P. magdalenae
97/0,98
MAG13 P. magdalenae
MAG12 P. magdalenae
87/0,994
MAG11 P. magdalenae
MAG08 P. magdalenae
MAG06 P. magdalenae
MAG05 P. magdalenae
MAG03 P. magdalenae
MAG02 P. magdalenae
87/0,73
74/0,98
PCA01 P. schroe
PCA09 P. schroederi
PCA03 Potamotrygon sp.
98/1
CAU04 Potamotrygon sp.
CAU07 Potamotrygon sp.
CAS01 Potamotrygon sp.
CAU03 Potamotrygon sp.
CAU06 Potamotrygon sp.
67/0,9996
CAT03 P. yepezi
MAR02 P. yepezi
MAR03 P. yepezi
MAR01 P. yepezi
Himantura schmardae
0,2

**Fig2.**

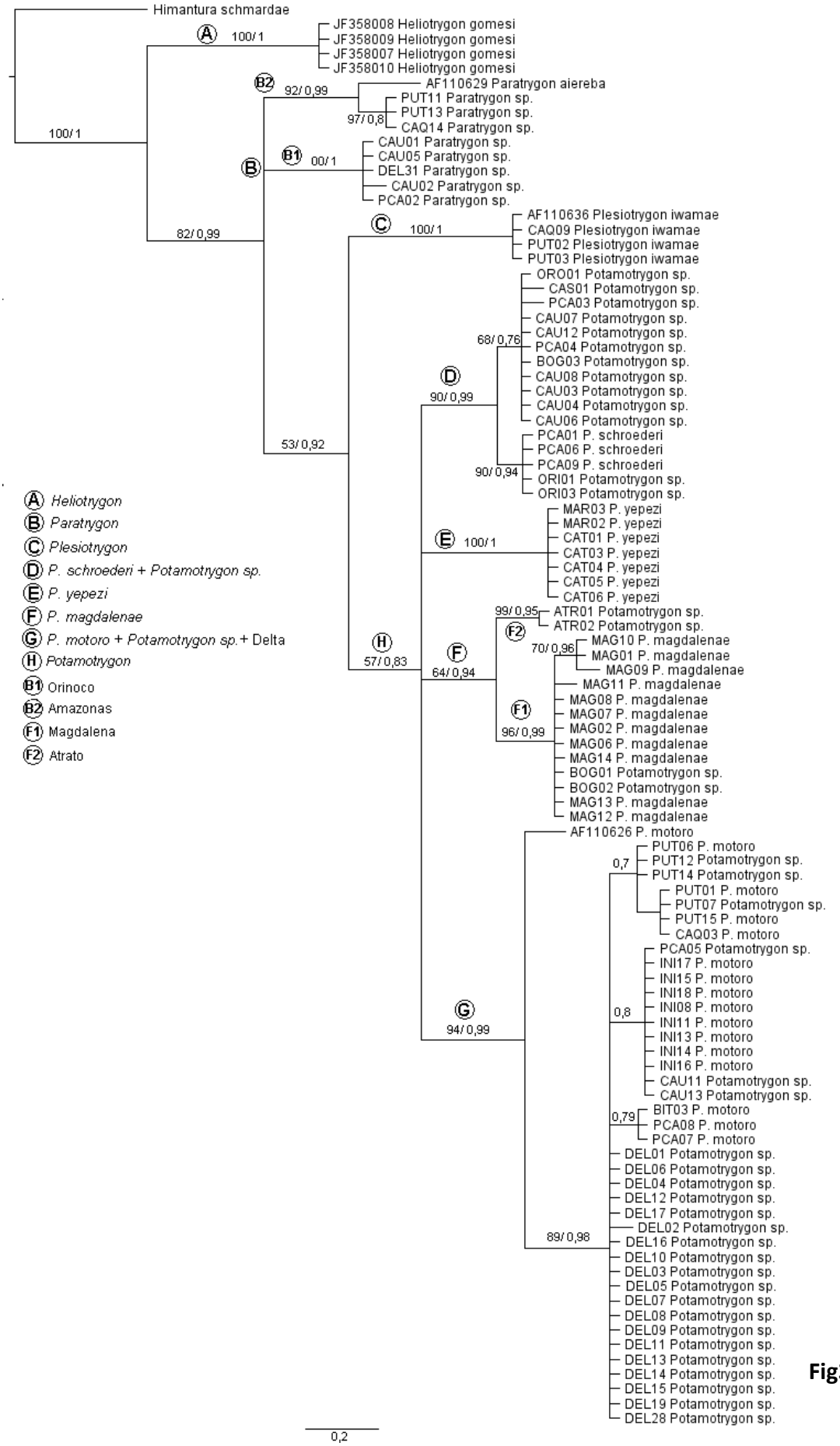
Himantura schmardae
Ⓐ 100/1
JF358008 Heliotrygon gomesi
JF358009 Heliotrygon gomesi
JF358007 Heliotrygon gomesi
JF358010 Heliotrygon gomesi
Ⓑ2
92/0,99
AF110629 Paratrygon aiereba
PUT11 Paratrygon sp.
97/0,8
PUT13 Paratrygon sp.
CAQ14 Paratrygon sp.
100/1
Ⓑ
Ⓑ1 00/1
CAU01 Paratrygon sp.
CAU05 Paratrygon sp.
DEL31 Paratrygon sp.
CAU02 Paratrygon sp.
PCA02 Paratrygon sp.
82/0,99
Ⓒ 100/1
AF110636 Plesiotrygon iwamae
CAQ09 Plesiotrygon iwamae
PUT02 Plesiotrygon iwamae
PUT03 Plesiotrygon iwamae
ORO01 Potamotrygon sp.
CAS01 Potamotrygon sp.
PCA03 Potamotrygon sp.
CAU07 Potamotrygon sp.
CAU12 Potamotrygon sp.
68/0,76
PCA04 Potamotrygon sp.
BOG03 Potamotrygon sp.
Ⓓ
90/0,99
CAU08 Potamotrygon sp.
CAU03 Potamotrygon sp.
CAU04 Potamotrygon sp.
CAU06 Potamotrygon sp.
53/0,92
PCA01 P. schroederi
PCA06 P. schroederi
90/0,94
PCA09 P. schroederi
ORI01 Potamotrygon sp.
ORI03 Potamotrygon sp.
MAR03 P. yepezi
MAR02 P. yepezi
Ⓔ 100/1
CAT01 P. yepezi
CAT03 P. yepezi
CAT04 P. yepezi
CAT05 P. yepezi
CAT06 P. yepezi
99/0,95
ATR01 Potamotrygon sp.
Ⓕ2
ATR02 Potamotrygon sp.
70/0,96
MAG10 P. magdalenae
MAG01 P. magdalenae
MAG09 P. magdalenae
Ⓗ
57/0,83
Ⓕ
64/0,94
MAG11 P. magdalenae
MAG08 P. magdalenae
Ⓕ1
MAG07 P. magdalenae
96/0,99
MAG02 P. magdalenae
MAG06 P. magdalenae
MAG14 P. magdalenae
BOG01 Potamotrygon sp.
BOG02 Potamotrygon sp.
MAG13 P. magdalenae
MAG12 P. magdalenae
AF110626 P. motoro
PUT06 P. motoro
0,7
PUT12 Potamotrygon sp.
PUT14 Potamotrygon sp.
PUT01 P. motoro
PUT07 Potamotrygon sp.
PUT15 P. motoro
CAQ03 P. motoro
PCA05 Potamotrygon sp.
INI17 P. motoro
INI15 P. motoro
INI18 P. motoro
INI08 P. motoro
0,8
INI11 P. motoro
INI13 P. motoro
INI14 P. motoro
INI16 P. motoro
CAU11 Potamotrygon sp.
CAU13 Potamotrygon sp.
Ⓖ
94/0,99
0,79
BIT03 P. motoro
PCA08 P. motoro
PCA07 P. motoro
DEL01 Potamotrygon sp.
DEL06 Potamotrygon sp.
DEL04 Potamotrygon sp.
DEL12 Potamotrygon sp.
DEL17 Potamotrygon sp.
DEL02 Potamotrygon sp.
89/0,98
DEL16 Potamotrygon sp.
DEL10 Potamotrygon sp.
DEL03 Potamotrygon sp.
DEL05 Potamotrygon sp.
DEL07 Potamotrygon sp.
DEL08 Potamotrygon sp.
DEL09 Potamotrygon sp.
DEL11 Potamotrygon sp.
DEL13 Potamotrygon sp.
DEL14 Potamotrygon sp.
DEL15 Potamotrygon sp.
DEL19 Potamotrygon sp.
DEL28 Potamotrygon sp.
Ⓐ Heliotrygon
Ⓑ Paratrygon
Ⓒ Plesiotrygon
Ⓓ P. schroederi + Potamotrygon sp.
Ⓔ P. yepezi
Ⓕ P. magdalenae
Ⓖ P. motoro + Potamotrygon sp.+ Delta
Ⓗ Potamotrygon
Ⓑ1 Orinoco
Ⓑ2 Amazonas
Ⓕ1 Magdalena
Ⓕ2 Atrato
0,2

**Fig3.**

**Figure 2.** Bayesian tree from analysis of COI sequence data using SYM+G substitution model, showing relationships of three genera and seven species from the family Potamotrygonidae. Circled letters indicate main branches group separations, letters with number indicate specific or related locality. Support values are given in the branches (ML/BI).

**Figure 3.** Bayesian tree from analysis of Cytb sequence data using GTR+G substitution model, showing relationships of three genera and seven species from the family Potamotrygonidae. Circled letters indicate main branches group separations, letters with number indicate specific or related locality. Support values are given in the branches (ML/BI).

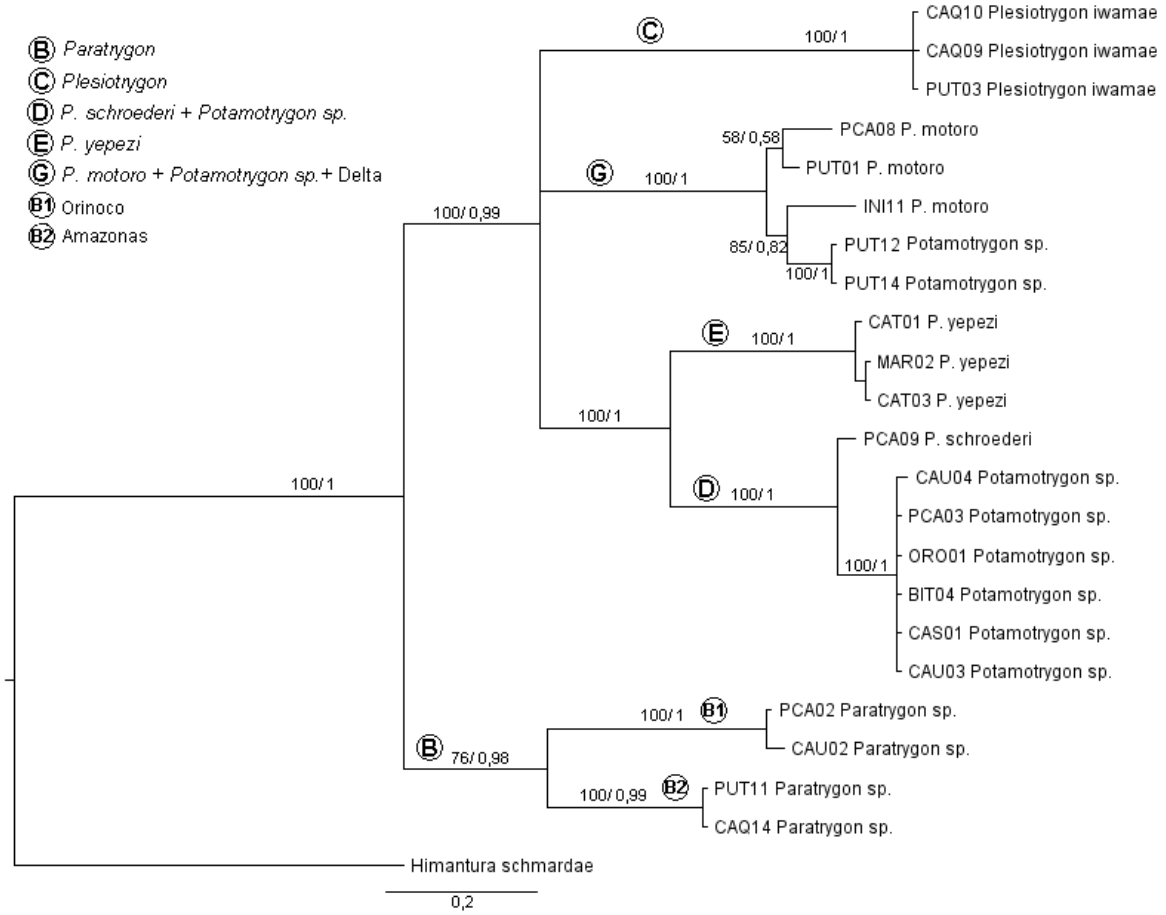


**Figure 4.** Bayesian tree from analysis of ATP6 sequence data using TVM+G substitution model, showing relationships of three genera and seven species from the family Potamotrygonidae. Circled letters indicate main branches group separations, circled letter G does not include Delta samples in this tree, letters with number indicate specific or related locality. Support values are given in the branches (ML/BI).

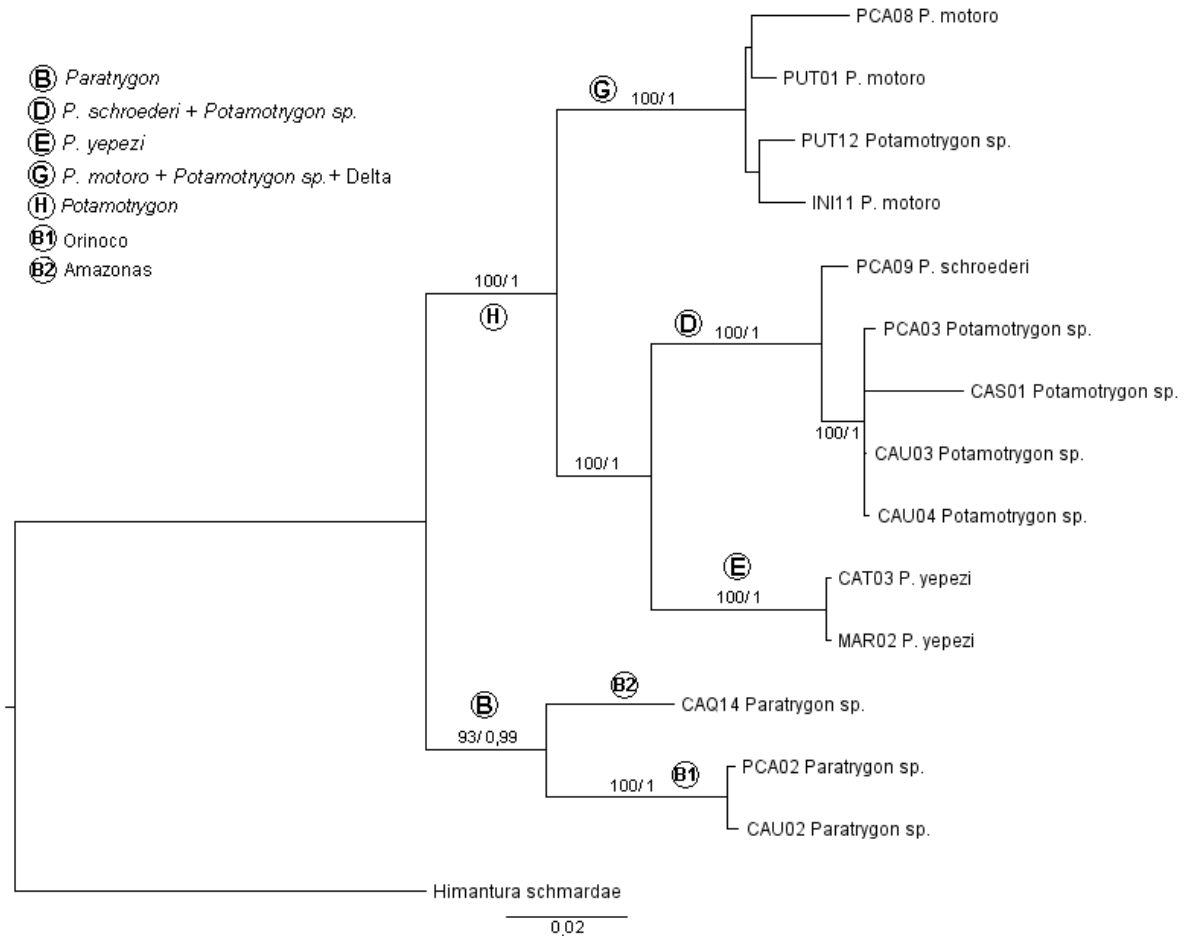


**Figure 5.** Bayesian tree from analysis of COI+Cytb+ATP6concatenated sequence data using substitution models corresponding to each data set in a partitioned alignment, showing relationships of three genera and seven species from the family Potamotrygonidae. Circled letters indicate main branches group separations, letter G does not include Delta samples in this tree, letters with number indicate specific or related locality. Support values are given in the branches (ML/BI).

## Tables

**Table 2.** Shimodaira-Hasegawa Test comparing Bayesian inference (BI) against Maximum Likelihood (ML) topologies, for each data set.

| **COI+Cytb+ATP6** | **-ln L** | **Diff -ln L** | **P** |
|---|---|---|---|
| BI | 5258.96551 | 0.00171 | 0.216 |
| ML | 5258.96381 | (best) | |
| **COI Tree** | **-ln L** | **Diff -ln L** | **P** |
| BI | 1678.24604 | 4.84858 | 0.214 |
| ML | 1673.39746 | (best) | |
| **ATP6 Tree** | **-ln L** | **Diff -ln L** | **P** |
| BI | 2520.13133 | 1.79419 | 0.183 |
| ML | 2518.33714 | (best) | |
| **Cyt*b* Tree** | **-ln L** | **Diff -ln L** | **P** |
| BI | 970.17010 | 9.47032 | 0.113 |
| ML | 960.69978 | (best) | |

**Table 3.** Shimodaira-Hasegawa Test comparing Maximum Likelihood (ML) topologies for each data set (COI, Cyt*b* and ATP6) against null hypothesis of the concatenated (COI+Cyt*b*+ATP6).

| **Gene** | **-ln L** | **Diff -ln L** | **P** |
|---|---|---|---|
| COI+Cyt*b*+ATP6 | 5258.96381 | (best) | |
| COI | 5284.0469 | 25.0789 | 0.017* |
| **Gene** | **-ln L** | **Diff -ln L** | **P** |
| COI+Cyt*b*+ATP6 | 5258.96381 | (best) | |
| ATP6 | 5258.96551 | 0.00171 | 0.216 |
| **Gene** | **-ln L** | **Diff -ln L** | **P** |
| COI+Cyt*b*+ATP6 | 5258.96381 | (best) | |
| Cytb | 5388.25137 | 29.28756 | 0.010* |

**Table 4.** Sum of internal branch lengths, minimum-evolution scores and stemminess values (sum of internal branch lengths/ME score, in percentage), calculated from Neighbor-joining trees (NJ) for each data set.

| **Dataset:** | **COI** | **Cyt*b*** | **ATP6** | **Cyt*b*+COI+ATP6** |
|---|---|---|---|---|
| Sum of internal branch lengths | 0,2103 | 0,206 | 0,2658 | 0,2328 |
| ME score | 0,3689 | 0,3524 | 0,3947 | 0,3931 |
| Stemminess value (%) | 57 | 58,5 | 67 | 59 |

**Table 1.** Sample codes with corresponding species, geographic location and collector/ Institute

| **Species** | **Sample code** | **Geographic location** | **Collector/ Institute** |
|---|---|---|---|
| *Potamotrygon schroederi* | PCA01 | Orinoco River, Puerto Carreño, Colombia | Fundacion Omacha |
| *Paratrygon aiereba* | PCA02 | Orinoco River, Puerto Carreño, Colombia | Fundacion Omacha |
| *Potamotrygon sp.* | PCA03 | Orinoco River, Puerto Carreño, Colombia | Fundacion Omacha |
| *Potamotrygon sp.* | PCA04 | Orinoco River, Puerto Carreño, Colombia | Fundacion Omacha |
| *Potamotrygon sp.* | PCA05 | Orinoco River, Puerto Carreño, Colombia | Fundacion Omacha |
| *Potamotrygon motoro* | PCA07 | Orinoco River, Puerto Carreño, Colombia | Fundacion Omacha |
| *Potamotrygon motoro* | PCA08 | Orinoco River, Puerto Carreño, Colombia | Fundacion Omacha |
| *Potamotrygon schroederi* | PCA09 | Orinoco River, Puerto Carreño, Colombia | Fundacion Omacha |
| *Potamotrygon sp.* | BOG01 | Ornamental market, Bogotá, Colombia | Garcia D. and Caballero S., U. Andes |
| *Potamotrygon sp.* | BOG02 | Ornamental market, Bogotá, Colombia | Garcia D. and Caballero S., U. Andes |
| *Potamotrygon sp.* | BOG03 | Ornamental market, Bogotá, Colombia | Garcia D. and Caballero S., U. Andes |
| *Potamotrygon magdalenae* | MAG01 | Magdalena River, Magdalena, Colombia | Lasso C. A., IAvH |
| *Potamotrygon yepezi* | MAR01 | Maracaibo River, Venezuela | Lasso C. A., IAvH |

| | | | |
|---|---|---|---|
| *Potamotrygon sp.* | ATR01 | Atrato River, Colombia | Lasso C. A., IAvH |
| *Potamotrygon sp.* | ATR02 | Atrato River, Colombia | Lasso C. A., IAvH |
| *Potamotrygon magdalenae* | MAG02 | Magdalena River, Magdalena, Colombia | Lasso C. A., IAvH |
| *Potamotrygon magdalenae* | MAG03 | Magdalena River, Magdalena, Colombia | Lasso C. A., IAvH |
| *Potamotrygon magdalenae* | MAG04 | Magdalena River, Magdalena, Colombia | Lasso C. A., IAvH |
| *Potamotrygon magdalenae* | MAG05 | Magdalena River, Magdalena, Colombia | Lasso C. A., IAvH |
| *Potamotrygon magdalenae* | MAG06 | Magdalena River, Magdalena, Colombia | Lasso C. A., IAvH |
| *Potamotrygon magdalenae* | MAG07 | Magdalena River, Magdalena, Colombia | Lasso C. A., IAvH |
| *Potamotrygon magdalenae* | MAG08 | Magdalena River, Magdalena, Colombia | Lasso C. A., IAvH |
| *Potamotrygon magdalenae* | MAG09 | Magdalena River, Magdalena, Colombia | Lasso C. A., IAvH |
| *Potamotrygon magdalenae* | MAG10 | Magdalena River, Magdalena, Colombia | Lasso C. A., IAvH |
| *Potamotrygon magdalenae* | MAG11 | Magdalena River, Magdalena, Colombia | Lasso C. A., IAvH |
| *Potamotrygon magdalenae* | MAG12 | Magdalena River, Magdalena, Colombia | Lasso C. A., IAvH |
| *Potamotrygon magdalenae* | MAG13 | Magdalena River, Magdalena, Colombia | Lasso C. A., IAvH |
| *Potamotrygon magdalenae* | MAG14 | Magdalena River, Magdalena, Colombia | Lasso C. A., IAvH |
| *Potamotrygon magdalenae* | MAG15 | Magdalena River, Magdalena, Colombia | Lasso C. A., IAvH |
| *Potamotrygon sp.* | DEL01 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL02 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL03 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL04 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL05 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL06 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL07 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL08 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL09 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL10 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL11 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL12 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL13 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |

| | | | |
|---|---|---|---|
| *Potamotrygon sp.* | DEL14 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL15 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL16 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL17 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL18 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL19 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Paratrygon aiereba* | CAU01 | Caura River, Venezuela | MHNLS |
| *Paratrygon aiereba* | CAU02 | Caura River, Venezuela | MHNLS |
| *Potamotrygon sp.* | CAU03 | Caura River, Venezuela | MHNLS |
| *Potamotrygon sp.* | CAU04 | Caura River, Venezuela | MHNLS |
| *Paratrygon aiereba* | CAU05 | Caura River, Venezuela | MHNLS |
| *Potamotrygon sp.* | CAU06 | Caura River, Venezuela | MHNLS |
| *Potamotrygon sp.* | CAU07 | Caura River, Venezuela | MHNLS |
| *Potamotrygon sp.* | CAU08 | Caura River, Venezuela | MHNLS |
| *Potamotrygon sp.* | CAU11 | Caura River, Venezuela | MHNLS |
| *Potamotrygon sp.* | CAU12 | Caura River, Venezuela | MHNLS |
| *Potamotrygon sp.* | CAU13 | Caura River, Venezuela | MHNLS |
| *Potamotrygon yepezi* | MAR02 | Maracaibo River, Venezuela | MHNLS |
| *Potamotrygon yepezi* | MAR03 | Maracaibo River, Venezuela | MHNLS |
| *Potamotrygon motoro* | ORI01 | Orinoco River, Venezuela | MHNLS |
| *Potamotrygon motoro* | ORI02 | Orinoco River, Venezuela | MHNLS |
| *Potamotrygon motoro* | INI01 | Inirida River, Inirida, Colombia | MHNLS |
| *Potamotrygon sp.* | ORI03 | Orinoco River, Venezuela | MHNLS |
| *Heliotrygon sp.* | ORI04 | Orinoco River, Venezuela | MHNLS |
| *Potamotrygon motoro* | CUY01 | Cuyuni River, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL20 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL21 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL22 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL23 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL24 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL25 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL26 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL27 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL28 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL29 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon sp.* | DEL30 | Orinoco River, Delta Orinoco, | MHNLS |

| | | | |
|---|---|---|---|
| | | Venezuela | |
| *Paratrygon aiereba* | DEL31 | Orinoco River, Delta Orinoco, Venezuela | MHNLS |
| *Potamotrygon motoro* | INI02 | Inirida River, Inirida, Colombia | Lasso C. A. and Sierra M. T., IAvH |
| *Potamotrygon motoro* | INI04 | Inirida River, Inirida, Colombia | Lasso C. A. and Sierra M. T., IAvH |
| *Potamotrygon motoro* | INI05 | Inirida River, Inirida, Colombia | Lasso C. A. and Sierra M. T., IAvH |
| *Potamotrygon motoro* | INI06 | Inirida River, Inirida, Colombia | Lasso C. A. and Sierra M. T., IAvH |
| *Potamotrygon motoro* | INI08 | Inirida River, Inirida, Colombia | Lasso C. A. and Sierra M. T., IAvH |
| *Potamotrygon motoro* | INI09 | Inirida River, Inirida, Colombia | Lasso C. A. and Sierra M. T., IAvH |
| *Potamotrygon motoro* | INI10 | Inirida River, Inirida, Colombia | Lasso C. A. and Sierra M. T., IAvH |
| *Potamotrygon motoro* | INI11 | Inirida River, Inirida, Colombia | Lasso C. A. and Sierra M. T., IAvH |
| *Potamotrygon motoro* | INI12 | Inirida River, Inirida, Colombia | Lasso C. A. and Sierra M. T., IAvH |
| *Potamotrygon motoro* | INI13 | Inirida River, Inirida, Colombia | Lasso C. A. and Sierra M. T., IAvH |
| *Potamotrygon motoro* | INI14 | Inirida River, Inirida, Colombia | Lasso C. A. and Sierra M. T., IAvH |
| *Potamotrygon motoro* | INI15 | Inirida River, Inirida, Colombia | Lasso C. A. and Sierra M. T., IAvH |
| *Potamotrygon motoro* | INI16 | Inirida River, Inirida, Colombia | Lasso C. A. and Sierra M. T., IAvH |
| *Potamotrygon motoro* | INI17 | Inirida River, Inirida, Colombia | Lasso C. A. and Sierra M. T., IAvH |
| *Potamotrygon motoro* | INI18 | Inirida River, Inirida, Colombia | Lasso C. A. and Sierra M. T., IAvH |
| *Potamotrygon motoro* | INI19 | Inirida River, Inirida, Colombia | Lasso C. A. and Sierra M. T., IAvH |
| *Potamotrygon motoro* | INI20 | Inirida River, Inirida, Colombia | Lasso C. A. and Sierra M. T., IAvH |
| *Potamotrygon yepezi* | CAT01 | Catatumbo River, Colombia | Lasso C. A., IAvH |
| *Potamotrygon yepezi* | CAT03 | Catatumbo River, Colombia | Lasso C. A., IAvH |
| *Potamotrygon yepezi* | CAT04 | Catatumbo River, Colombia | Lasso C. A., IAvH |
| *Potamotrygon yepezi* | CAT05 | Catatumbo River, Colombia | Lasso C. A., IAvH |
| *Potamotrygon yepezi* | CAT06 | Catatumbo River, Colombia | Lasso C. A., IAvH |
| *Potamotrygon motoro* | PUT01 | Putumayo River, Putumayo, Colombia | Gomez G. A., SINCHI |
| *Plesiotrygon iwamae* | PUT02 | Putumayo River, Putumayo, Colombia | Gomez G. A., SINCHI |
| *Plesiotrygon iwamae* | PUT03 | Putumayo River, Putumayo, Colombia | Gomez G. A., SINCHI |
| *Potamotrygon motoro* | PUT06 | Putumayo River, Putumayo, Colombia | Gomez G. A., SINCHI |
| *Potamotrygon sp.* | PUT07 | Putumayo River, Putumayo, Colombia | Gomez G. A., SINCHI |
| *Paratrygon aiereba* | PUT11 | Putumayo River, Putumayo, Colombia | Lasso C. A., Morales M., Gómez G. A., SINCHI IAvH |
| *Potamotrygon sp.* | PUT12 | Putumayo River, Putumayo, Colombia | Lasso C. A., Morales M., Gómez G. A., SINCHI IAvH |
| *Paratrygon aiereba* | PUT13 | Putumayo River, Putumayo, Colombia | Lasso C. A., Morales M., Gómez G. A., SINCHI IAvH |
| *Potamotrygon motoro* | PUT15 | Putumayo River, Putumayo, Colombia | Gomez G. A., SINCHI |
| *Potamotrygon motoro* | BIT03 | Bita River, Vichada, Colombia | Sánchez-Duarte P., IAvH |
| *Potamotrygon sp.* | BIT04 | Bita River, Vichada, Colombia | Sánchez-Duarte P., IAvH |
| *Potamotrygon sp.* | ORO01 | Orotoy River, Meta, Colombia | Lasso C. A., IAvH |
| *Potamotrygon motoro* | CAQ03 | Caqueta River, Putumayo, Colombia | Gomez G. A., SINCHI |
| *Plesiotrygon iwamae* | CAQ09 | Caqueta River, Putumayo, Colombia | Gomez G. A., SINCHI |
| *Plesiotrygon iwamae* | CAQ10 | Caqueta River, Putumayo, Colombia | Gomez G. A., SINCHI |
| *Paratrygon aiereba* | CAQ14 | Caqueta River, Putumayo, Colombia | Gomez G. A., SINCHI |
| *Potamotrygon sp.* | CAS01 | Casanare River, Casanare, Colombia | Lasso C. A., IAvH |

## Supplementary material

**Table S2.** Additional sequences obtained from genbank.

| Accession | Species | Source |
|---|---|---|
| AF110626 | *Potamotrygon motoro* | Lovejoy *et al*. (1998) |
| AF110629 | *Paratrygon aiereba* | Lovejoy *et al*. (1998) |
| AF110636 | *Plesiotrygon iwamae* | Lovejoy *et al*. (1998) |
| JF358007 | *Heliotrygon gomesi* | De Carvalho & Lovejoy (2011) |
| JF358008 | *Heliotrygon gomesi* | De Carvalho & Lovejoy (2011) |
| JF358009 | *Heliotrygon gomesi* | De Carvalho & Lovejoy (2011) |
| JF358010 | *Heliotrygon gomesi* | De Carvalho & Lovejoy (2011) |

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## 2. INFORMACIÓN GENERAL DEL TRABAJO DE GRADO

TÍTULO DEL TRABAJO DE GRADO:
Molecular systematics of the freshwater stingrays (Myliobatiformes: Potamotrygonidae) of the Amazon, Orinoco, Magdalena, Esequibo,Caribe and Maracaibo basins (Colombia- Venezuela): evidence from mitochondrial genes.

DESCRIPCIÓN FÍSICA
Número de páginas: 32
Ilustraciones:

MATERIAL ACOMPAÑANTE (Cantidad):
Casetes Audio:
Discos compactos:
Casetes Video:
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*RESUMEN DEL TRABAJO DE GRADO:

Freshwater stingrays from the family Potamotrygonidae have a restricted distribution to the freshwater systems of South America.Lack of adequate information about the taxonomic and evolutionary relationships, ecology, biology and distribution of several species belonging to this family makes them vulnerable to anthropic activities, including commercial exploitation for the ornamental fish market. Samples were collected from the main river basins in Colombia and Venezuela(Amazon, Orinoco, Magdalena, Esequibo, Caribe and Maracaibo) for four genera and seven species of the family (Heliotrygon gomesi, Paratrygon aiereba, Plesiotrygon iwamae, Potamotrygon motoro, Potamotrygon yepezi, Potamotrygon schroederi, Potamotrygon magdalenae), and some unidentified species. Molecular markers Cytochrome Oxidase subunit I, Cytochrome b and ATPase subunit 6 were amplified and sequenced. Maximum likelihood and Bayesian Inference analysis were performed to obtain topologies for each marker and for a concatenated dataset including the three genes. Small dataset may compromise some methods estimations of sequence divergence in the ATP6 marker. Monophyly of the four genera in the Potamotrygonidae family was confirmed and phylogenetic relations among member of thePotamotrygon genus were not clearly resolved. However, results obtained with the molecular marker Cytb appear to offer a good starting point to differentiate among genera and species as a tool that could be used for fast molecular identification (Barcode). The application of this gene as a barcode could provide a useful tool than can be applied for Management and regulation of extraction practices for these genera. Sequencing of complete mitochondrial genomes would be the next step for testing evolutionary hypothesis among these genera. Population studies should be undertaken forPotamotrygon magdalenae, Paratrygon aiereba and Potamotrygon motoro to establish possible population differentiation among basins, information that is key for delineating conservation and management plans for these species.

...

OBJETIVOS DEL TRABAJO DE GRADO:

...

METODOLOGÍA DEL TRABAJO DE GRADO:

...

CONCLUSIONES DEL TRABAJO DE GRADO:

*PALABRAS CLAVES (TEMAS) DEL TRABAJO DE GRADO:

Potamotrygonidae, molecular systematics, Colombia, Venezuela, conservation.

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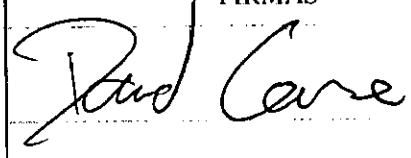
| David A. García V. |  |

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**Molecular systematics of the freshwater stingrays (Myliobatiformes: Potamotrygonidae) of the Amazon, Orinoco, Magdalena, Esequibo,Caribe and Maracaibo basins (Colombia- Venezuela): evidence from mitochondrial genes**

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