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Occurrence of Four Freshwater Stingrays (Chondrichthyes: Potamotrygoninae) in the Uatumã River Basin, Amazon Region: A Field Study

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Abstract: The Amazon region has the largest diversity of freshwater stingrays; however, there are still places where information about this group is scarce. The present work aims to record the occurrence of freshwater stingrays in the Uatumã river basin, Amazonas, Brazil. For this, collections were carried out in three municipalities bathed by the Uatumã River, Presidente Figueiredo, Itapiranga, and São Sebastião do Uatumã. The rays were collected through fishing using a hand net and, after capture, were identified through the pattern of the dorsal and ventral color of the disc, the absence or presence of the labial groove, and the organization of the row of spines on the tail, and were also measured, weighed, and sexed. In total, 69 specimens of Potamotrygonines were captured, 59 of which were from the genus *Potamotrygon* and ten individuals from the genus *Paratrygon*. The present study provides preliminary information on the characteristics of freshwater stingray species that may occur in the Uatumã River. Furthermore, new research is necessary to improve the identification of specimens to determine the diversity and elucidate aspects related to the biology of elasmobranchs in the Uatumã River Basin, Brazil.

Keywords: biodiversity; geographic distribution; Potamotrigoníneos; Amazonian; stingrays; Brazil

Key Contribution: Add knowledge to characterize the freshwater stingray population in the Uatumã River, Amazon region, Brazil, contributing to increasing knowledge regarding the geographic distribution of freshwater stingrays.

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1. Introduction

Stingrays of the subfamily Potamotrygoninae are the only taxon within the subclass of Elasmobranchii adapted to survive and reproduce exclusively in freshwater environments. They are endemic to the rivers of South America and are found in almost all countries, except for rivers in Chile, where Brazil stands out for having more richness [1]. Currently, the group is composed of 45 species divided into four genera: the genus *Paratrygon* (Duméril, 1865), which is considered a species complex, with ten species; *Plesiotrygon* (Rosa, Castelo, Thorson, 1987) and *Heliotrygon* (De Carvalho & Lovejoy, 2011), with two species each; and *Potamotrygon* (Garman, 1877), which is the most diverse genus with 31 valid species [2,3].

Some species present high phenotypic plasticity and a wide distribution in the Amazon basin, and they can be found in all types of water (black, clear, white, and intermediate types), such as South American freshwater stingrays *Potamotrygon motoro* (Müller & Henle, 1841) and Smooth back river stingray *Potamotrygon orbignyi* (Castelnau, 1855), other species

have specialized physiological mechanisms to interact with the environment where they live, and therefore have a geographical distribution limited to water courses, such as the species Cururu ray *Potamotrygon wallacei* (Carvalho, Rosa and Araújo, 2016), which is endemic to the Rio Negro basin, and the White-blot ched river stingray *Potamotrygon leopoldi* (Castex & Castello, 1970) native to the Xingu river [4–8].

Stingrays can be found in different habitats and substrates, with their occurrence recorded in both lentic and lotic habitats, as well as rocky, sandy, muddy, or leaf litter bottoms [9,10]. However, despite the wide variety of habitats, stingrays have a preference for specific areas; the species *Paratrygon aiereba* has a preference for areas with sandy substrates; on the other hand, *Potamotrygon motoro* can live in intermediate areas between igapós and beaches, with muddy bottoms [11].

Elasmobranchs are predators in almost all environments where they live and play an essential role in the energy flow between trophic levels [12]. Regarding reproductive aspects in Amazonian elasmobranchs, the seasonal hydrological cycle of river floods and droughts influences reproduction [13] but, in general, they have similar biological characteristics to marine elasmobranchs, such as low fecundity, slow growth, great longevity, complex reproduction patterns, and late sexual maturation; these aspects result in low rates of population renewal and greater vulnerability to overfishing and environmental changes [14–16]. Furthermore, constant accidents and injuries involving stingray stingers have driven the practice of harmful fishing, which consists of capturing animals for mutilation purposes by removing the tail or sacrificing it [17,18].

In recent years, there have been significant advances in studies of freshwater stingrays, including studies on contaminants [19], the determination of stingray meat using low-cost analytical tools [20], blood physiological assessment in a natural environment [21–23], and blood parasite records [24,25]. Still, despite these studies, most Potamotrygonines are listed as Data Deficient on the IUCN List of Threatened Species due to a lack of information on population rates and geographic dispersion [26]. This lack of information occurs because these are regions of difficult access and complexity, where the transport of people and cargo are mainly carried out via waterways [27]; among these locations is the Uatumã River basin, Amazonas, Brazil.

The Uatumã River is part of the complex hydrographic network of the Amazon basin that covers an area of 611,200 km^2, which is equivalent to 57% of the Brazilian territory [28]. The Uatumã River is a tributary of the Amazon River, and its total area is around 70,600 km^2; its sources are located in the Precambrian shield of the Guianas, from which it transports black, acidic waters with a low amount of sediment and nutrients [28]. Uatumã is a case of how managing natural resources can significantly impact the ecosystem, as the Balbina Hydroelectric Plant (BHP) was installed on this river between 1983 and 1987, which flooded an area of more than 3000 km² of forests [29]. Of the Uatumã basin area, around 18,862 km^2 represents the contribution basin of the HPP [28]. Due to the scarcity of information on the presence of elasmobranchs in the Uatumã River, the present work aims to record the occurrence of freshwater stingrays in the Uatumã River basin to expand knowledge about their geographic distribution. More specifically, it aims to (i) determine the diversity and biometrics of captured freshwater stingrays; (ii) assess developmental stage; and (iii) identify intraspecific phenotypic variability.

2. Materials and Methods

This research was carried out upon release from the Biodiversity Authorization and Information System (SISBIO) following Normative Instruction Ordinance ICMBio n°748/2022. Number: 76127-4 and by the Ethics Committee on the Use of Animals of the Federal Institute of Education, Science and Technology of Amazonas (2019/010.02.0905). This study was developed following the regulations of the ethical principles in animal experimentation considered by the National Animal Control Council Animal Experimentation—CONCEA.

The collections took place in the Uatumã River basin, the northeastern region of the State of Amazonas, Brazil, with points in the municipalities of Presidente Figueiredo (PF),

Itapiranga (ITA), and São Sebastião do Uatumã (SSU) (Figure 1). The collections followed the direction of the river that goes from north to south, starting in Presidente Figueiredo, then Itapiranga, and finally, São Sebastião do Uatumã, which was the closest collection point to the mouth of the Uatumã River, on the Amazon River. In Presidente Figueiredo, collections took place in an area that was impacted by the construction of the Balbina Hydroelectric Plant.

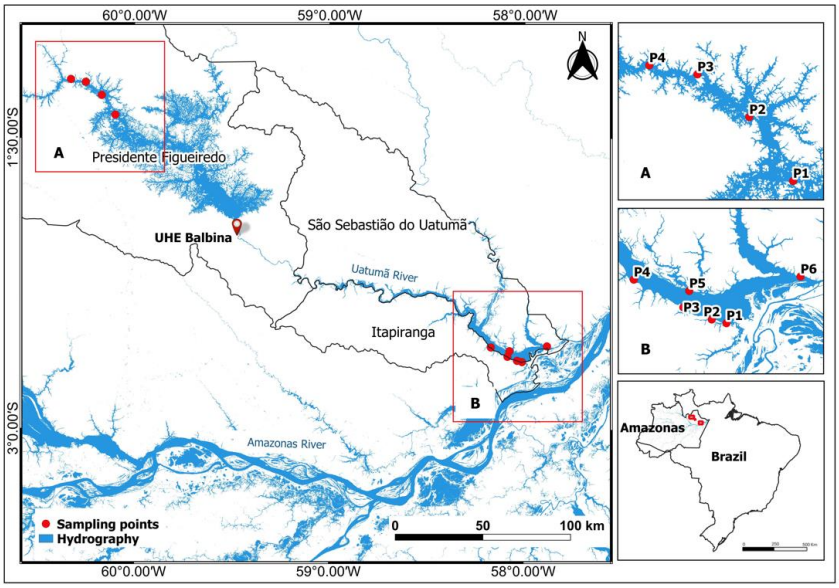

Figure 1. Geographic location of the Uatumã River with an indication of sampling points in the municipalities of Presidente Figueiredo, Itapiranga, and São Sebastião do Uatumã, a northeastern region of Amazonas, Brazil.

The capture of stingrays occurred through night fishing with a hand net, made of micro mesh with a 20 × 20 mm mesh (Figure 2), at a depth of 0.5 to 1.5 m, following the recommendations of Oliveira et al. [30]. Stingrays were collected in November 2022 (dry), May 2023 (flood), and September 2023 (dry).

Figure 2. *Potamotrygon scobina* captured with a hand net being introduced into an immersion bath with eugenol, Uatumã River, northeastern Amazonas, Brazil.

After capture, to avoid possible accidents with stingers, the rays were anesthetized through immersion baths in plastic pockets with eugenol (200 $\mu L \cdot L^{-1}$) and then mechanical containment of the stinger was performed using forceps; subsequently, procedures were carried out to obtain biometric data, the disc width—DW and total length—TL were measured using a measuring tape (Figure 3), and the weight was checked using a portable scale.

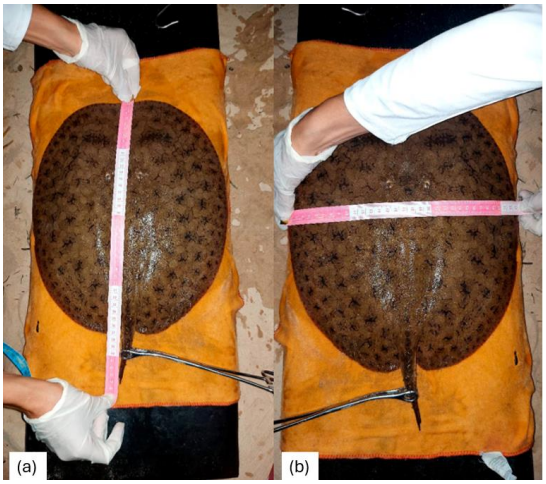

Figure 3. Measurement of biometric parameters in *Paratrygon* spp. collected in the Uatumã River basin, northeastern Amazonas, Brazil. (**a**) Measurement of total length; (**b**) measurement of disc width.

The developmental stage of the captured stingrays was determined from the values recorded for the disc width—DW,; for the *Potamotrygon motoro* stingray, the recommendations of Araújo [31] were followed; for *Potamotrygon scobina* and *Potamotrygon orbignyi*, the recommendations of Acosta-Santos et al. [32] and Lasso et al. were followed [33], respectively; for specimens of the genus *Paratrygon*, data from the species *Paratrygon aiereba* described by Araújo [34] were used.

For identification, the key proposed by Rosa and Carvalho [35] was used, verifying five characteristics: (1) disc shape; (2) pattern of the ventral color of the disc; (3) dorsal color pattern of the disc; (4) absence or presence of the labial groove; (5) distribution pattern of spines on the tail. In addition, sexing was also carried out based on observation of the presence or absence of claspers.

The biometric data were organized in the statistical program R, which was evaluated based on mean and standard deviation.

3. Results and Discussion

3.1. Diversity and Biometric

In total, 69 specimens of Potamotrygonines were captured, 59 of which were from the genus *Potamotrygon* and 10 individuals from the genus *Paratrygon*. Of the 59 individuals of the genus *Potamotrygon*, 44 were of the species *P. orbignyi*, 12 *P. motoro,* and 3 *P. scobina* (Figure 4).

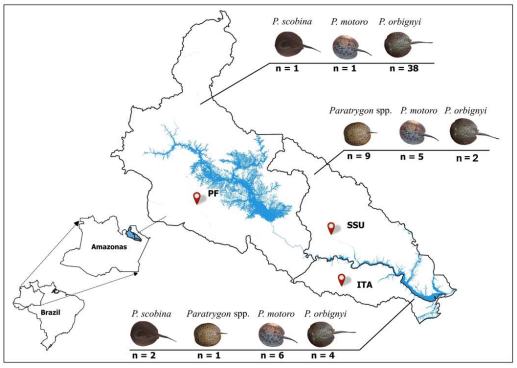

Figure 4. Indication of stingrays captured in the municipalities of Presidente Figueiredo (PF), São Sebastião do Uatumã (SSU), and Itapiranga (ITA) in the Uatumã River basin, northeastern region of Amazonas, Brazil.

Data for biometric parameter values and developmental stages are found in Table 1. All neonatal individuals were captured during the high water season of the Uatumã River

in May. This fact may be related to the influence of the hydrological period of Amazon rivers on the reproduction of stingrays [13]. It is essential to highlight that, in this collection, only neonatal individuals were captured, and no individuals were found in other stages of development. In addition to being related to reproduction, this may also be related to the increase in the volume of water, the periodic expansion of the stingrays' habitat, and the reduction in transparency, which causes low visibility and makes capture difficult [36,37]. All other specimens captured at different stages of development (juveniles, subadults, and adults) were collected during the Uatumã River's dry period between September and November. At this time of year, the volume of water is smaller, the habitat is reduced, and there is an increase in transparency, making capturing these individuals easier [4,17]. The total length of the neonate of *P. motoro* captured at point ITA was not determined, as it was captured without the tail, demonstrating the presence of harmful fishing.

Table 1. Values of biometric parameters of stingrays captured in the Uatumã River basin, northeastern Amazonas region, Brazil.

Locality	Species	Development Stage	Sex	n	DW (cm)	TL (cm)	Weight (g)
PF	*P. orbignyi*	Young	M F	18 15	19.0 ± 2.69 20.0 ± 2.27	33 ± 2.79 34 ± 3.69	384 ± 101.9 420 ± 139.7
		Adult	M F	2 3	24.0 ± 0.0 24.0 ± 0.0	38.5 ± 0.70 42.0 ± 1.52	625.0 ± 7.07 683.0 ± 110.1
	P. motoro	Young	M	1	21.0	32.0	570.0
	P. scobina	Adult	M	1	23.0	44.0	470.0
ITA	*P. orbignyi*	Neonate Young	F F	1 3	7.5 24.7 ± 3.5	13.0 45.5 ± 3.5	270.0 660.0 ± 266.3
	P. motoro	Neonate	M	1	8.0	-	310.0
		Young	M F	1 3	24 21.7 ± 3.05	43 41 ± 9.64	530 433.5 ± 179.2
		Adult	M	1	41.5	51.0	570.0
	P. scobina	Neonate Adult	F M	1 1	11.0 24.8	21.5 47.0	590.0 480.0
	Paratrygon spp.	Subadult	M	1	45.0	57.0	3100.0
SSU	*P. orbignyi*	Young	F	2	20.0 ± 4.2	37.0 ± 4.2	310.0 ± 169.7
	P. motoro	Young	M F	2 3	23 ± 1.0 31 ± 2.82	47.3 ± 6.07 53.05 ± 13.4	436.6 ± 49.3 1075 ± 233.3
	Paratrygon spp.	Young	M F	2 6	23 ± 1.41 23.21 ± 1.54	40.75 ± 12.3 45 ± 8.83	435 ± 35.35 425 ± 103.2
		Subadult	F	1	33.0	44.0	1330.0

n: Number of samples, DW: disc width, TL: total length.

3.2. Diagnosis of Potamotrygon orbignyi (Castelnau, 1855)

The most common freshwater stingray in the Uatumã River basin was *P. orbignyi*, with the highest incidence in the portion located in the Presidente Figueiredo municipality. This portion of the Uatumã River was impacted by the construction of the Balbina hydroelectric plant, and the flooded vegetation is still decomposing, compromising water quality in the region [38]. The occurrence of *P. orbignyi* in the hydrographic areas impacted by hydroelectric plants was also recorded for the Parnaíba, Tocantins, and Araguaia river basins, highlighting the high adaptability of *P. orbignyi* [39,40].

A total of 38 specimens of *P. orbignyi* were captured at the site. Although this species presents high polychromatism in the dorsal region of the disc [40], all individuals presented the same reticulated pattern (Figure 5a,b). This may have occurred due to the construction of the hydroelectric plant, which intensified the impact of population fragmentation within and between tributaries, resulting in a reduction in genetic diversity and the loss of color variability [39]. The captured species presented the typical pattern of spines on the tail,

organized in a row, with a well-developed labial groove, and the majority presented a rounded spot of variable size and color in the ventral region (black and dark gray) in the portion located between and below the slits.

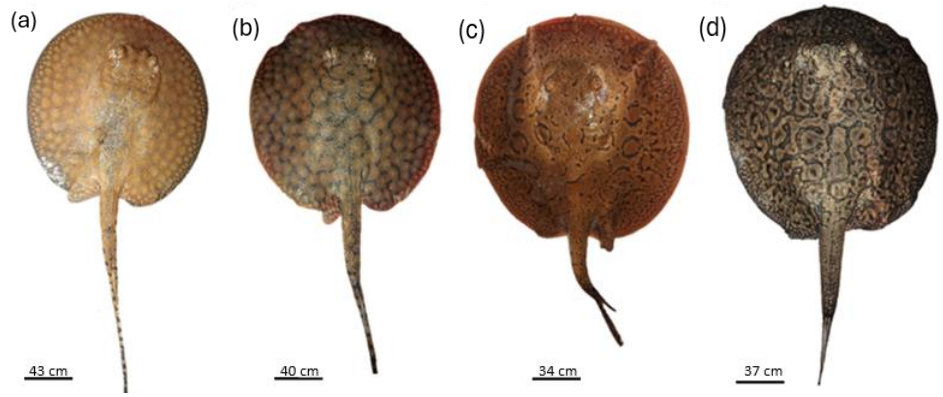

Figure 5. Specimens of *P. orbignyi* were collected in the Uatumã River, northeastern Amazonas, Brazil. (**a**,**b**) *P. orbignyi* young with a reticulated color pattern; (**c**) *P. orbignyi* young with a brownish background; (**d**) *P. orbignyi* young with a black background.

Other factors that may explain the abundance of *P. orbignyi* are the effect of the transformation of the river, a lotic environment into a lentic climate, as well as the increase in the flooded area, which consequently increased the biomass of invertebrates, such as insects, favoring the diet of *P. orbignyi*. *P. orbignyi* is predominantly insectivorous [40–42].

In other stretches of the Uatumã River, the occurrence of *P. orbignyi* was lower. Still, in addition to presenting the reticulated dorsal color pattern (Figure 5a,b), species with other patterns, such as a brownish background a black background (Figure 5d), were also found (Figure 5c).

3.3. Diagnosis of Potamotrygon motoro (Müller & Henle, 1841)

The *P. motoro* stingray occurred at all the points analyzed in this study, but unlike *P. orbignyi*, *P. motoro* showed a higher incidence in the area outside the influence of the Balbina hydroelectric plant, with only one specimen captured in this region. The specimen presented a variation in the color pattern not found at other points in this study, with small, bicolored ocelli with a yellow center and black outer ring arranged on a dark brown background and without the presence of ocelli on the tail (Figure 6b).

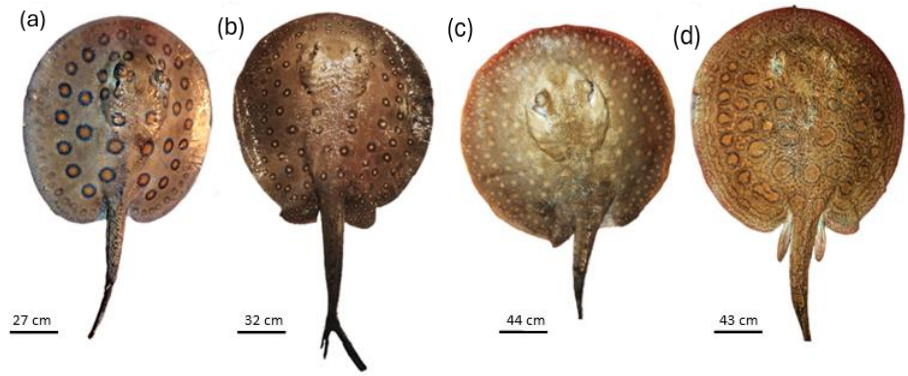

Figure 6. Specimens of *Potamotrygon motoro* collected in the Uatumã River basin, northeastern Amazonas, Brazil. (**a**) *P. motoro* young with tricolor ocelli captured in ITA and SSU; (**b**) *P. motoro* young with bicolor ocelli captured in PF; (**c**) *P. motoro* young with yellow ocelli captured in ITA and SSU; (**d**) *P. motoro* young with a less common pattern for the species, collected at the point in ITA.

In total, four different patterns were found in the color of *P. motoro* in the Uatumã River, and the pattern present in stingrays a and b (Figure 6a,b) is the most widespread and comprehensive in the Amazon basin region [43].

Patterns a and c were found both at the point in ITA and the point in SSU. Pattern a is formed by large, tricolor eyespots with a yellow and orange background and a black outer ring arranged on a brown background, while pattern c is formed by yellowish ocelli arranged on a light brown background (Figure 6a,c). The pattern of stingray d (Figure 6d) is less common for this species, being found in more restricted areas. Only one specimen with this pattern was collected at the point in ITA and, to date, this pattern has only been recorded in three regions, two in Peru and one in Brazil on the Tarauacá River in the state of Acre [43]. All the captured specimens had a circular disc, poorly developed labial furrow, and a single row of spines on the tail.

3.4. Diagnosis of Potamotrygon scobina (Garman, 1913)

P. scobina was the species found least frequently; its occurrence was recorded at two collection points, PF and ITA (Figure 3), with two specimens at the ITA point and one at the PF point. The specimens from the ITA point presented a brownish disc with numerous clustered light spots distributed throughout the disc and tail with a single row of spines (Figure 7a).

Figure 7. Specimens of *Potamotrygon scobina* collected in the Uatumã River basin, northeastern Amazonas, Brazil. (**a**) Specimen adult collected in ITA; (**b**) specimen adult collected in PF.

The PF specimen had a brownish disc with small, light eyespots and a tail with spines arranged irregularly at the base and organized in a single row on the rest of the tail in addition to spots spread throughout the tail (Figure 7b).

All the captured individuals had a subcircular disc with a more prominent central portion, a robust tail up to the base of the stinger that became thinner from that point onwards, a poorly developed labial groove, a poorly exposed pelvic fin, and a light ventral region, showing a white color.

3.5. Diagnosis of Paratrygon spp. (Dúmeril, 1865)

Until recently, the genus Paratrygon was believed to be the only monotypic genus in the family Potamotrygonidae, with only the species *Paratrygon aiereba* [2].

Although *P. aiereba* is one of the first species of freshwater stingrays described, there is still no extensive and specific literature about it [3]. The discussion about gender diversity had already been addressed by Rosa [44], Rosa et al. [10], and Carvalho et al. [5], but the species was only dismembered by Loboda [2] after carrying out a taxonomic and morphological review of the genus, which resulted in eight more species.

However, although it is currently known that *Paratrygon aiereba* is a complex species, the characteristics of the species are not yet well elucidated, which is why individuals of *Paratrygon* are referred to only by the generic epithet in the present work.

In the Uatumã River, three color patterns were found for *Paratrygon*, with patterns b and c (Figure 8b,c) being the most similar and predominant. For pattern a (Figure 8a), only one specimen was found at point SSU. In Figure 8, stingray d is the standard of stingray c at another stage of development, with stingray c being a young individual and stingray d a subadult.

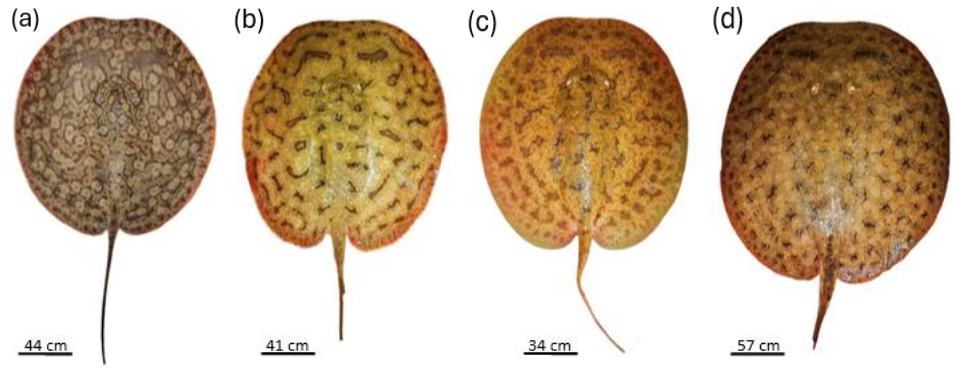

Figure 8. Specimens of the genus *Paratrygon* captured in the Uatumã river basin, northeastern Amazonas, Brazil. (**a**) Specimen young captured at the SSU point; (**b**) specimen young collected in ITA and SSU; (**c**) specimen young collected in ITA and SSU; (**d**) subadult specimen collected in ITA and SSU.

All the captured specimens presented characteristics already described in the literature: an absence of labial groove; slightly concave shaped disc; a long, thin tail without the presence of spines in young individuals and subadult individuals; a short, robust tail with long spines organized irregularly throughout the tail.

All the captured individuals of the *Paratrygon* spp. were captured in areas with a sandy substrate, corroborating what Oliveira described [11] about the preference of these stingrays for beaches. This preference may be related to the absence of *Paratrygon* spp. at the PF point.

4. Conclusions

In general, the present work adds knowledge about the characterization of the stingray population in the Uatumã River region and the Amazon region, contributing to an increasing collection of knowledge related to the geographic distribution of freshwater elasmobranchs.

Although only four species were collected, the high degree of polychromatism found in the color pattern of the rays was notable since the color was related to adaptation processes related to the characteristics of the species, such as eating habits, hunting, and escaping from threats [45]. Furthermore, the high rate of young individuals collected reflects the capture method used in this study.

This study provides preliminary information on the characteristics of freshwater stingray species that may occur in the Uatumã River. Furthermore, new research is necessary to improve the identification of specimens to determine the diversity and elucidate aspects related to the biology of elasmobranchs present in the Uatumã River Basin.

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Institutional Review Board Statement: This study was conducted following the Declaration of Helsinki and approved by the Ethics Committee on the Use of Animals of the Federal Institute of Education, Science and Technology of Amazonas (2019/010.02.0905).

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