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MORFOLOGIA DA CONCHA E DO APARELHO COPULADOR MASCULINO DE POMACEA (PERRY, 1810): UMA ANÁLISE CONSIDERANDO VARIAÇÕES CROMÁTICAS

MORPHOLOGY OF THE SHELL AND THE MALE COPULATORY APPARATUS OF *POMACEA* (PERRY, 1810): AN ANALYSIS CONSIDERING CHROMATIC VARIATIONS

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Resumo: Ampullariidea é uma família de moluscos de água doce amplamente distribuída em áreas tropicais. São animais de grande importância ecológica nos ecossistemas em que estão inseridos, sendo comumente registrados como vetores de doenças, pragas rurais e potenciais invasores. A delimitação taxonômica de seus representantes ainda é vaga, pelo fato de que a maioria das identificações de espécies são apenas com a forma da concha. Neste trabalho são realizadas análises integrativas de observações conquiológicas e anatômicas do órgão copulador masculino de ampularídeos com três diferentes padrões na coloração da concha, assim classificados como branco, escuro e listrado. Todos os dados coletados foram submetidos à análise estatística no software Past. A análise conquiológica não sugeriu grandes diferenças anatômicas entre as espécies com diferentes padrões de coloração da concha, assim como não há diferenças estatisticamente significativas entre os dados morfométricos dos órgãos copuladores, indicando que as espécies examinadas representam a mesma espécie. No entanto, estudos histológicos e moleculares são necessários para confirmar essa indicação. As observações morfológicas do órgão copulador masculino indicam que os espécimes examinados pertencem ao gênero *Pomacea* (PERRY, 1810) quando comparados com o material da literatura.

Palavras-chave: Amazônia. Molusco. Conquiliologia. Ampullariidea. Água doce.

Abstract: Ampullariidea is a family of freshwater molluscs, which is widely distributed in tropical areas. They are animals of great ecological importance in the ecosystems in which they are inserted, being commonly registered as disease vectors, rural pests and potential invaders. Their representatives taxonomic delimitation is still vague, due to the fact that most species identifications are only based on the shape of the shell. In this work, integrative analysis of conchiological and anatomical observations of the male copulatory organ of ampullarids are carried out with three different patterns in the shell color, so classified as white, dark and striped. All data collected were subjected to statistical analysis in the Past software. The Conchiological analysis did not suggest big anatomical differences among the species with different shell color patterns, as well as there aren´t statistically significant differences among the morphometric data of copulatory organs, indicating that the species examined represent the same species. However, histological and molecular studies are necessary to confirm this indication. The morphological observations of male copulator organ indicate that the examined specimens belong to the Pomacea genus when compared with the literature material.

Keywords: Amazon. Mollusc. Conchiology. Ampullariidea. Freshwater.

1. INTRODUCTION

Ampullariidae is a diverse family of continental water gastropod molluscs with a wide distribution in tropical zones (Rosa, 2006). These animals, popularly known as apple snails, have remarkable physiological and anatomical characteristics that for many years have captivated interest from inside and outside science. They have globose shells that may or may not have band patterns. The color of the shells usually varies from dull green to brown, although there are specimens that vary from black to brown or from gray to white, yellow, blue and green. They are amphibious animals, which allows them to explore both terrestrial and aquatic environments, having two structures for breathing: gills on the right side of the body and a lung. The eyes are shown in eyestalk and the operculum is usually chitinous and typically circular.

Ampullariidae appeared during Gondwana, in what is now Africa, presenting two divergent monophyletic lineages since the supercontinent separation, called the Old World clade and the New World clade (Hayes et al., 2009). There isn´t a satisfactorily comprehensive taxonomic review for family since the 20th century. However, recent studies of phylogenetical relations based on molecular data of Ampullariidae settled down rescuing the family as a monophyletic group (Hayes et al., 2009). Molecular works have also contributed to sustain the family as monophyly, helping to clarify internal relationships (Cazzaniga, 2002). They are currently grouped into nine genus, with Pomacea being the most known worldwide (Watanabe, 2014), for its wide distribution and mainly for its invasive potential in rice growing areas, where they cause serious damage to these crops (Lowe et al., 2000).

Although the Ampullariidae classification has advances, the family's systematics are still confuse and hard to understand, with many taxonomic problems. Hayes et al. (2012) defines the taxonomic situation as “galloping”, so complicated is the reality of the literature in Ampullaridae. Great part of the taxonomic problems involving family representatives are the result of misidentifications (Lowe et al., 2000). For the New World clade there are at least 300 species group names available and in the neotropics, few species have been correctly named, because even if the general morphology of Ampullariidae is restricted, there are species that present many conchological, ontogenetic and ecophenotypic variations (Hayes et al., 2012). For this reason, it is very likely that new researches will show a considerable reduction into number of species in family. Other fact that contributes to the confused scene of Ampullariidea's systematic is that much of identifications are carried out by conchology only, and its limits are overbearing among the species, defined by individual opinions that are hardly shared among the authors (Cazzaniga, 2002).

In Brazil and in the Amazon context, the situation does not change so much, on the contrary, there is a great deficit of knowledge about the Amazon malacofauna if compared to other regions of Brazil (Bonetto, 1967; Simone, 2006). Pain (1960) has already described the situation of Amazonian Ampullariidae as “desperate confusion”. This affirmation deserves attention mainly because these animals are one of the main components of diversity in freshwater environments, having an important connection among the aquatic and terrestrial food chains (Donnay & Beissinger, 1993; Ebenman & Jonsson, 2005).

As said about the similarity of the shells in Ampullaridae, many works have been developed with them over the years in the search for differences that can be able to diagnose precisely the species, but most of them brought the conclusion that such differences are mere modifications possibly derived from the environment and they are not enough to support a correct identification (Strobel,1876). This was the case with d'Orbigny (1840), who in his great field experience, defined that the changes were so many that “it would be possible to be sure of finding a different species after walking a hundred steps” (original in French) (Cazzaniga, 2002).

Getting the evidence about the insufficiency of conchological characters in the identifications of Ampullariidae, works with morphology and internal anatomy started to stand out and show the most convincing information. In his work on taxonomy and morphology Pomacea lineata, Thiengo (1987) briefly describes the main works in the Ampullariidae mollusc anatomy area and emphasizes the use of internal morphology as an auxiliary tool in identifications, in order to avoid the index fragmentation caused by a recognition based only on the morphology of the shell. In this same work, Thiengo also concludes that the male reproductive system can show diagnostic characters of species, a conclusion that cannot include only Pomacea lineata.

Talking about the ecological importance of Ampullariidae, especially in freshwater environments, the lack of malacological studies in the Amazon and the confused situation of identifying the group, this work aimed to include conchological and anatomical studies in order to better diagnose Ampullariidae individuals with different color patterns that happen on Maracana beach, in the city of Santarem, in the Lower Amazon region.

2. METHODOLOGY

There were collected 21 individual members at Maracana beach, urban area of Santarem city, located on the right bank of the lower Tapajos river, Pará state (Figure 1). The collections were carried out under authorization at SISBIO nº 49255-1.

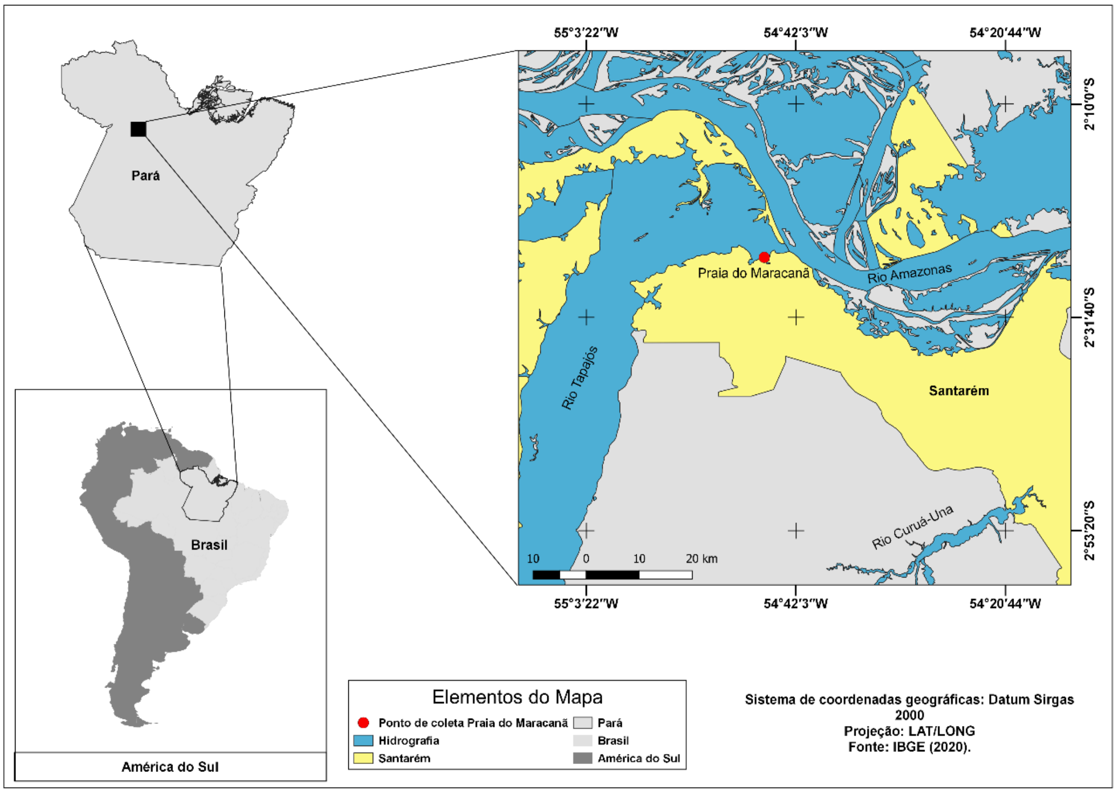

Figura 1. Map of the collection site.

The animals were sorted and stored in 70% alcohol in the ichthyological collection at Federal University of Para West. In the laboratory, there were collected morphometric dice from the shell with 21 specimens before dissection of the animal, since Ampullariidea generally does not present sexual dimorphism (Thiengo, 1987). The recognized measures were collected with the aid of a digital caliper and they are listed below (Table 1), following the landmarks (Figure 2).

Tabela 1. Morphometric variables.

Shell morphometric variable (mm)	Abbreviation
Total height of the shell	Atc
Total width of the shell	Ltc
First lap height	Apv
Second lap height	Asv
Third lap height	Atc
First lap width	Lpv
Second lap width	Lsv
Third lap width	Ltc
Columellar shaft size	Tec
Operculum height	Ao
Operculum width	Lo
Copulatory organ morphometric variables (mm)	**Abbreviation**
Animal size	Ta
Penis sheath length	Cbp
Penis sheath width	Lbp
Penis length	Cp
Width of penis	Lp
Penis bag size	Tbp

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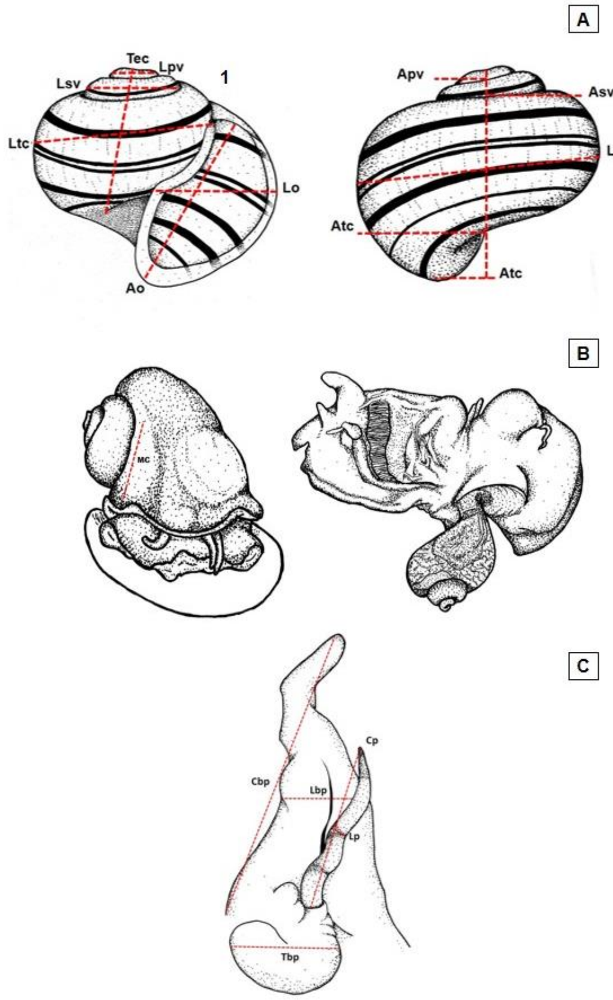

Figura 2. (A) Schematic representation showing the reference points of the morphometric variables collected from the shell; (B) schematic representation of the animal without the shell and the cut site (in red) in the columellar muscle region; (C) Schematic representation showing the reference points of the variables considered in the copulator apparatus.

After collecting the conchological data, the animals were removed from the shell, measured with a digital caliper and dissected with scalpel support. Only males were separated, a total of 18 individuals, to watch the reproductive structure. The cut was made in the region of the columellar muscle, in order to view all over the pallial cavity (Figure 2). The animals were subsequently stained with lugol for collection of the following morphometric data from the copulatory organ (Table 1, Figure 2). All measures were taken in mm with the aid of a millimeter lens attached to a glass.

The morphometric data of the shell and the copulatory organ were submitted to a Principal Component Analysis (PCA) in the model suggested by Gotelli & Ellison (2011), which consists on assigning colors and symbols to the points of each group, allowing the identification of possible distinctions among them. The Broken Stick criterion was also used to the main components analysis, in addition to a PERMANOVA to monitor each PCA. A linear and multiple regression test was also performed using male copulatory organ morphometric data with the purpose of identifying relations between the variables. All statistical analyzes were performed with Past software (Hammer et al. 2001).

There were added observations about general morphology of molluscs sampled in this study, which is the basis for taxonomic identification through comparisons with the material found in the literature.

3. RESULTS

Specimens of Ampullariidae collected at Maracanã beach had three distinct colors between them (Figure 3), classified here: white (A), striped (B) and dark (C). The result of descriptive statistics referring to each color pattern with the shell and the copulatory organ morphometric data is listed in table 2.

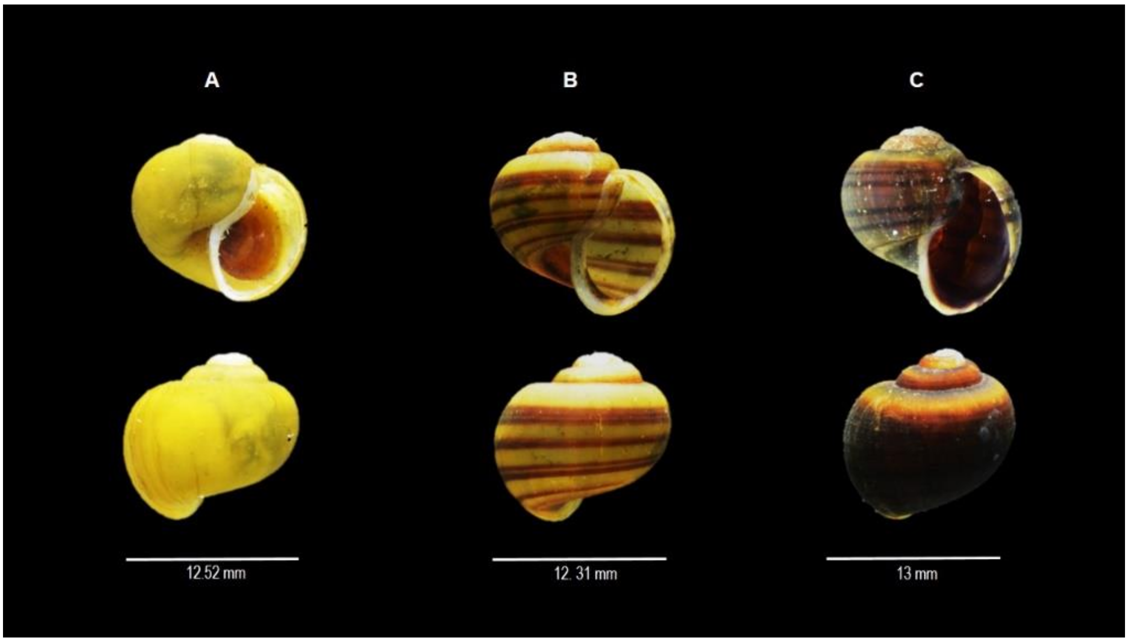

Figura 3. Colorful pattern of Ampullariidae collected at Maracanã beach

Tabela 2. Results of descriptive statistics with the three shell color patterns for the variables Atc (total shell height) and Ltc (total shell width) of the variables Cbp (penis sheath length) and Cp (penis length) of the copulatory organ

Dark	Atc	Ltc	Striped	Atc	Ltc	White	Atc	Ltc
Min	10,14	10,9	**Min**	8,16	8,3	**Min**	9,75	9,78
Max	13,67	13,19	**Max**	13,98	13,21	**Max**	11,1	11,35

Amplitude	3,53	2,29	**Amplitude**	5,82	4,91	**Amplitude**	1,35	1,57
Mean	11,90	11,93	**Mean**	10,32	10,42	**Mean**	10,53	10,54
Standard deviation	1,20	0,67	**Standard deviation**	2,03	1,74	**Standard deviation**	0,56	0,64
Variance	1,45	0,46	**Variance**	4,15	3,05	**Variance**	0,32	0,41
Dark	**Cbp**	**Cp**	**Striped**	**Cbp**	**Cp**	**White**	**Cbp**	**Cp**
Min	2,6	2	**Min**	2	0,5	**Min**	1,5	1,6
Max	10	3,4	**Max**	6,89	10	**Max**	7,5	11
Amplitude	7,4	1,4	**Amplitude**	4,89	9,5	**Amplitude**	6	9,4
Mean	6,18	5,28	**Mean**	5,41	4,5	**Mean**	5,4	5,88
Standard deviation	3,42	2,97	**Standard deviation**	2,81	3,28	**Standard deviation**	2,63	3,96
Variance	11,72	8,86	**Variance**	7,94	10,8	**Variance**	6,94	15,73

The conchological morphometric data when submitted to PCA demonstrated a seeming difference between the dark shell to the other two shell color patterns (Figure 4). With the PCA scree plot tool, we can see component 1 accounting for more than 90% of the data variation (Figure 4), with the variables Atc and Ltc being the most representative of the component (Table 3). The result obtained with the PCA was corroborated by PERMANOVA result, which, despite having presented a p-value = 0.07 for differences between the three shell morphotypes, presented $p < 0.05$ for dark shells in relation to white and striped shells.

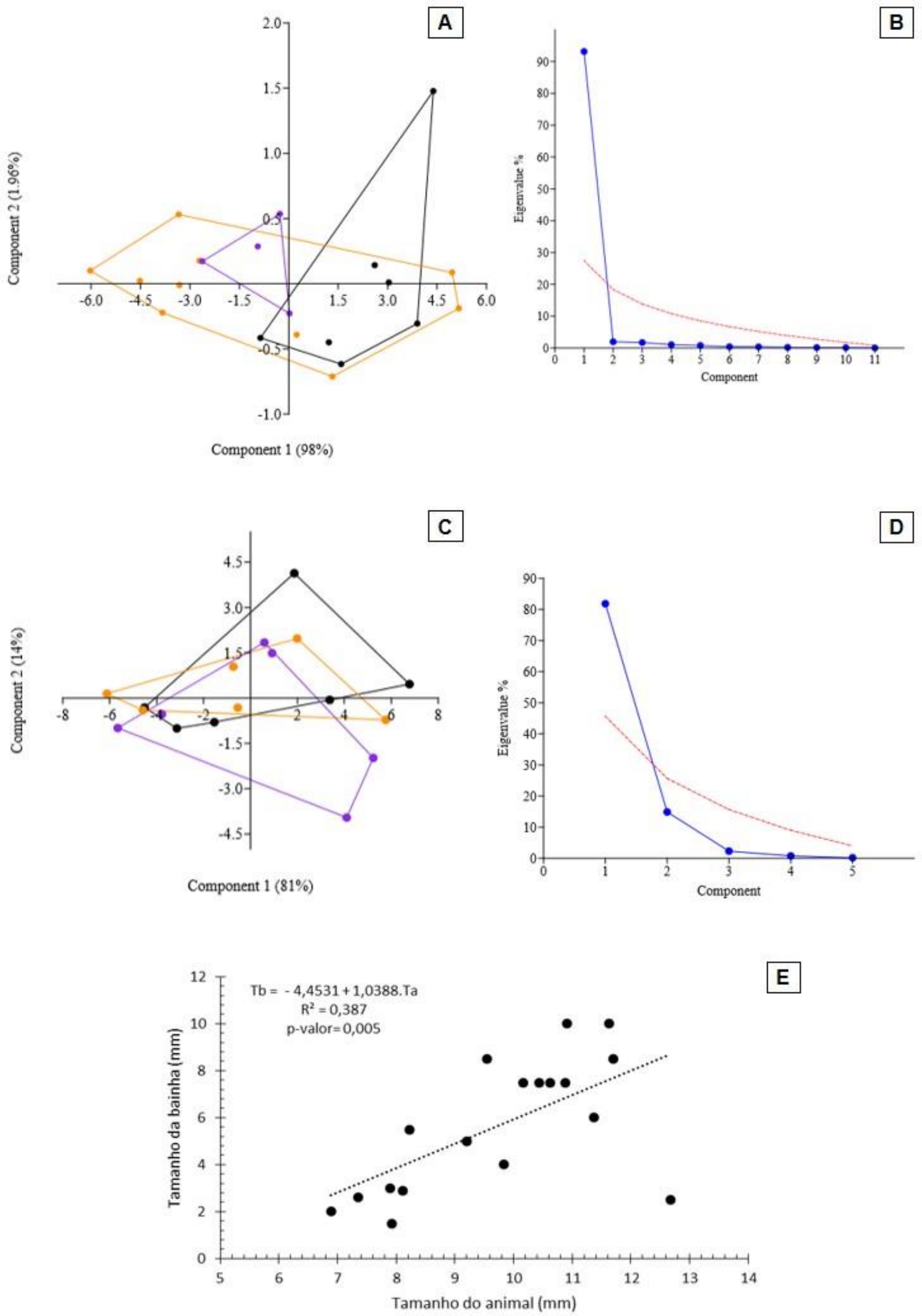

Figura 4. (A) Result of principal component analysis of the shell morphometric data of Ampullariidae collected at Maracanã beach. Symbols in purple, orange, and black represent the white, striped, and dark shell morphs, respectively; (B) Scree plot demonstrating the contribution of each major component to the entire PCA with shell morphometric data; (C) PCA result with data collected from the male copulator apparatus; (D) Scree plot demonstrating the contribution of each main component to the entire PCA with the morphometric data of the copulator apparatus; (E) Linear regression test result. (Tb) sheath size, (Ta) animal size.

Tabela 3. Result of the contribution of each variable in the first three components of the PCA with the morphometric data of the shell.

Variables	Component 1	Component 2	Component 3
Ltc	0.425	-0.633	0.063
Atc	0.504	0.559	-0.506
Apv	0.045	0.155	0.106
Asv	0.064	-0.115	0.084
Atv	0.209	0.357	0.654
Lpv	0.115	0.036	0.258
Lsv	0.210	0.027	0.218
Ltv	0.386	-0.312	-0.242
Tec	0.335	-0.046	0.330
Ao	0.352	0.010	-0.080
Lo	0.265	0.133	0.059

Regarding PCA performed with male copulatory organ morphometric data, the morphs are seen overlapping in the graph (Figure 4), indicating that the structures in general do not present great differences. The PCA scree plot with male copulatory organ morphometric data demonstrate that component 1 explained most of the variation in data (Figure 4), with component 1 having the variable Cp and Cbp as the most representative (Table 4).

Variables	Component 1	Component 2
Cbp	0.6149	0.7473
Lbp	0.1554	0.1247
Cp	0.7563	-0.6486
Lp	0.0480	0.0202
Tbp	0.1526	0.0699

Tabela 4. Result of the contribution of each variable in the first three components of PCA with the morphometric data of the copulatory apparatus.

The PERMANOVA result with copulatory organ morphometric data confirms the PCA result, presenting a p-value of 0.897, and do not show significant values in comparisons between the color patterns.

Overall, the copulatory organ macro anatomy resembles those described for the genus Pomacea in Bravo's work (2011) on genus morphology and histology. The penis has a cylindrical shape, with a filiform characteristic, with samples having a longer and thinner penis or a shorter and thinner one (Figure 5).

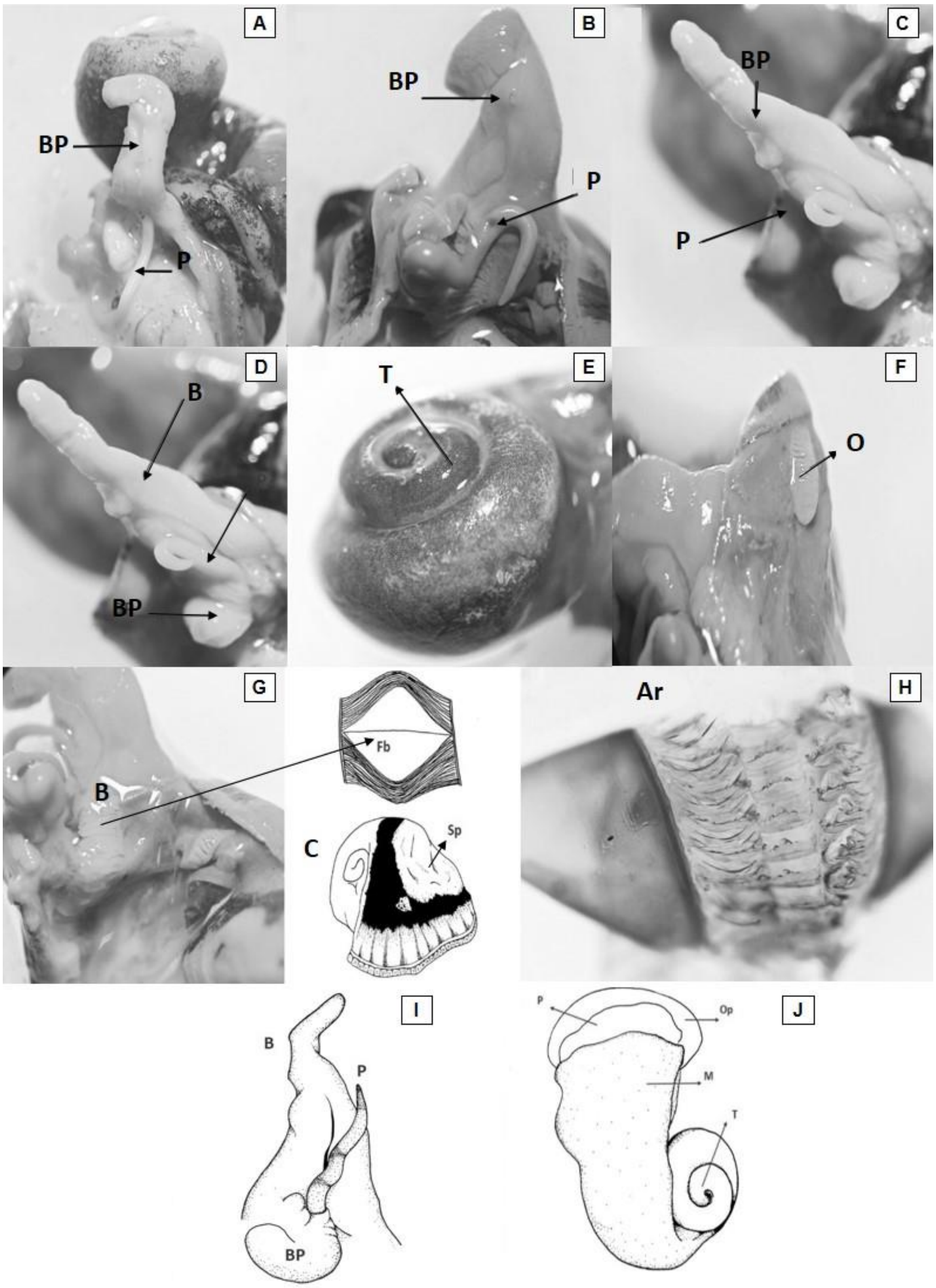

Figura 5. (A, B, C) Photographs of the male copulator apparatus and its variations. 1x. (BP) Penis sheath, (P) penis; (D) Photograph of the copulatory apparatus. In red (B) Penis sheath, (BP) Penis pouch and (P) Penis; (E) Photograph of the testicle (T). 2x; (F) Photograph of the pallial cavity with emphasis on the ophradium (O). 3.2x; (G) Photograph of the location of the gills. 2.5x. Schematic illustration of the shape of the gills and mantle with its pallial cavity closed pointing to the lung sac. (B) gills, (Fb) gill shape, (Sp) lung sac; (H) Photograph of the radular

apparatus (Ar). 5x; (I) Schematic illustration of the structures of the copulatory apparatus; (J) General illustration of the animal's structure locating the position of the testis. (P) Foot, (Op) operculum, (M) mantle, (T) testicle.

In this work, it was viewed that the sheath growing had small variations in size (Figure 5). The linear regression test for copulatory organ morphometric data shows a positive relation between the penis sheath and the animal size (Figure 5), and the multiple regression test confirms this result, indicating the value of F = 1, 16 and the p-value = 0.005 (Table 5).

Tabela 5. Multiple regression test result. (Tb) sheath size, (Ta) animal size.

N= 18	**Regression summary of the dependent variable Tb. R = ,62209426 R²= ,38700127 Adjusted R² = ,34868885 F(1,16) =10,101 p<,00584 Standard error: 2,2778**					
	Beta	**Beta standard error**	**B**	**Beta standard error**	**T16**	**p-valor**
Intercept			-4,4531	3,2290	-1,3790	0,1868
Ta	0,6220	0,1957	1,0387	0,3268	3,1782	0,0058

Talking about the other structures of the male copulatory organ, the penis pouch was well developed, with an oval shape, making it possible to see cleanly the coiled penis inside the pouch (Figure 5). The testicles, for the three shell colors, were located in the first three turns of the spiral, filling a cream-colored portion.

On the other hand, the respiratory system is created by gills and a pulmonary sac (Figure 5). With the gills made up of triangular-shaped lamellae located in the mantle cavity, which can be found just below the penis sheath (Figure 5), and the lung sac in the left part of the pallial cavity. They have an osphradium that is located into face of the mantle, on the left portion of the pallial cavity, with a piriform shape (Figure 5). The radicular organ was of the taenioglossate type (Figure 5), with an elongated central tooth that was wider than longer and a main trapezoidal cusp.

4. DISCUSSION

Colorful pattern

Ampullariidae molluscs usually have uncounted streak complexes and pigment of neural origin. Basically, a neural area into the mantle encodes the needful information for shell growth and for pigment deposition. This neural area can be influenced by environment disturbances that causes changes in the shell color patterns (Boettiger et al., 2009). It is important to say that the specimens of different shell color patterns were collected in the same environment, therefore, they were under the influence of the same environmental conditions.

According to Boettiger et al. (2009), differences in shell color pattern may not result from dramatic anatomical differences. This means that it is possible to find Ampullariidae molluscs with very different color patterns, but with very similar shell morphology. This may be the reality of the specimens collected at Maracana beach with the white and striped color patterns, since the animals, despite the distinct color pattern, do not appear to have great morphological differences in the shell. This was not true for dark shell color pattern, as the permanova result indicated that there are differences in the dark shell morphometry compared to white or striped shells.

It is important to understand that even though dark shells have differences in relation to others, this evaluation needs to follow other standards that not only conchological conformation, as such variation can be intraspecific. Species such as P. nobilis (Reeve, 1856), P. erythrostoma (Reeve, 1856) and P. haemastoma (Reeve, 1856) are classified in literature as belonging to the same clade, and may only represent variations of the same species (Ramírez et al., 2020). We think that the same case of variation can be the one viewed in the dark color pattern.

Male copulatory organ

Regarding the PCA result with male copulatory organ data, the observation of structure similarity between the different color patterns may suggest that they are not distinct species, although the conformity of the dark shell shows significant differences in relation to the others. This is why the male sexual organ can present diagnostic characters of species (Thiengo, 1987). It is possible then that the ampullariids from Maracana beach that have three shell color patterns (white, striped and dark) belong to the same species, which highlights the need for morphological, histological or molecular studies during the identification of Ampullariidae species, being impossible to use only shell for such activity.

Overall, the copulatory organ’s macro anatomy resembles those described for genus Pomacea at Bravo's work (2011) on genus morphology and histology. The penis had similar characteristics to descriptions of Pomacea lineata's samples studied by Thiengo (1987). The testicles were arranged in the first three turns of the spiral, as in most Pomacea. The results with variations in the sheath length can have explanations in the ontogenetic stage or in the animal's feeding. Sheath length being affected by low feed levels has been seen in mating and spawning work with Pomacea canaliculata (Estoy et al., 2002). The penis sheath is an important structure during reproduction as it can ease penetration (Bravo, 2011).

The Pomacea genus (Perry, 1810).

Molluscs of Pomacea genus are fresh, dioecious, internally fertilized and highly fecund (Melo, 2015). As having great ecological importance, these animals are good indicators of environmental quality and for many years they were used in the biological control of Planorbidae molluscs, mainly because of the vector species of schistosomiasis (Mesquita, 1982). They are animals that usually inhabit different aquatic environments, with a preference for slower water and gentle current (Bravo, 2011). Due to its high fecundity, these animals also became pests in rice plantations and other crops (Cowie, 2002), so that it has representatives in the list of the 100 worst invasive species (Lowe et al., 2000; Hayes et al., 2008).

Unlike other Ampullariidea, Pomacea is not seen forming a monophyletic clade (Hayes et al., 2009), this reality is due to the lack of a complete and comprehensive taxonomic treatment, in addition to the difficulties of identification by conchological characters (Cowie & Thiengo, 2003). Pomacea has several species included in a subgroup called the canaliculata complex, this group include species that were mostly misidentified as P. canaliculata, and the most of this group's species occurring in the Amazon basin (Hayes et al., 2009).

This work's contributions reinforce the need for more morphological studies with Ampullariidae molluscs from the Amazon, especially the Pomacea genus, bringing useful information that can be applied to genus and family systematic. At the local level, this research adds more in-depth information on animals biology, which can contribute to a greater success on informations about identification of Amazonian ampullariids, because when it is given identifications it is possible to ease further studies with: since by contributing to the identifications it is possible to facilitate further studies with:

cultivation, reproductive improvement, biomonitoring and information for foreign specialists dealing with Pomacea ampullariideos invasions.

5. CONCLUSION

The Ampullariidae molluscs collected on Maracana beach, although different colors, do not differ anatomically in their shell, excepting the dark shell pattern that appears to be possible intraspecific variation, since there are no differences into male copulatory organ, suggesting that they are not different species. However, this saying can only be validated through further histological and molecular studies.

Based on male copulatory organ macroanatomic observations and other general structures, the specimens can be identified as belonging to the Pomacea genus (Perry, 1810).

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