



## Research article

# Follicular complex may predict reproductive tactics in siluriform fishes

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## ABSTRACT

In this study, we evaluated the morphology and morphometry of the layers that make up the follicular complex surrounding mature oocytes in the six fish species *Auchenipterichthys longimanus*, *Ageneiosus ucayalensis*, *Hypophthalmus marginatus*, *Baryancistrus xanthellus*, *Panaqolus tankei* and *Peckoltia oligospila*, belonging to the order Siluriformes, which inhabit the Amazon basin. On the basis of the morphology and thickness of the layers of the follicular complex, the species were divided into two groups: 1- *A. longimanus*, *A. Ucayalensis* and *H. marginatus* and 2 – *B. xanthellus*, *P. tankei* and *P. oligospila*. The total thickness of the layers that make up the follicular complex showed a difference between type III and IV oocytes for all species of each group. Differences in the theca layer, follicular cells and zona radiata between species and between groups were submitted to statistical analysis. Morphologically, group 1 showed columnar follicular cells and thin zona radiata. Meanwhile, group 2 displayed a layer of cuboidal-shaped follicular cells layer and thicker zona radiata. These differences may be related to the environment and reproductive behaviors, as group 1 migrates without parental care and has eggs that are generally smaller and abundant. While group 2, represented by loricariidae, inhabit lotic environments, have reproductive tactics of parental care and eggs that are generally large and in small numbers. Therefore, we can infer that the follicular complex in mature oocytes can predict the reproductive tactics of the species.

## 1. Introduction

Teleost fish have different reproductive tactics, for example, migration [1–3], different types of spawning [4–6], parental care that occurs in different ways such as the storage of fertilized eggs in the mouth [7–9], the construction of nests and the maintenance of continuous aeration of the eggs [10–12]. In females, mature oocytes indicate some reproductive tactics such as the reproductive period [13,14] and type of spawning [15,16], and the animal's fecundity can be estimated [5,17,18].

The follicular complex comprises the mature oocyte, zona radiata (Zr), follicular (F) and theca (Tc) layers present in the fish ovary [19]. In recent years, the follicular complex has been studied with a focus on morphological description [15,20,21], in the chemical composition of the layers [22,23] and in the process of oocyte atresia during gonadal maturation [24,25]. The structures of the

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follicular complex stand out for their intrinsic interaction with oocytes and may be related to different habitats and reproductive behaviors. For example, the zona radiata is a complex extracellular matrix, and its thickness is related to the protection of eggs against abrasion [26]. In this sense, it is important to analyze the involvement of the follicular complex in different reproductive tactics or behavior used by different fish species.

The order Siluriformes has a wide geographic distribution with representatives on all continents, but the highest percentage of freshwater Siluriformes is concentrated in South America [27]. This order is the second highest in diversity of freshwater species that have different body morphology, feeding habits and reproductive strategies [28,29]. Accordingly, we highlight the families Auchenipteridae (*Auchenipterichthys longimanus* Guenther, 1864 and *Ageneiosus ucayalensis* Castelnau, 1855.), Pimelodidae (*Hypophthalmus marginatus* Valenciennes, 1840) and Loricariidae (*Baryancistrus xantheilus* Rapp Py-Daniel et al., 2011, *Panaqolus tankei* Cramer & Sousa and *Peckoltia oligospila* Günther 1864) that have particularities in terms of reproductive ecology, feeding habits and the environments where they occur.

*Auchenipterichthys longimanus* is a seed disperser along rivers [30], including endangered plants such as the tree *Virola surinamensis* [31]. However, *A. ucayalensis* is carnivorous and exerts direct and indirect effects on the aquatic biota being a fishing resource used to feed the Amazonian communities [32]. On the other hand, *H. marginatus* shows reproductive migration [33] like other species of catfish in the same family [34]. It has a wide geographic distribution that goes from the Amazon basin to Suriname, which is important for artisanal, subsistence fishing [35].

Meanwhile, loricariidae are sedentary, show low fecundity, and have large eggs, which indicates parental care [36–38]. The representatives of this family are highly valued in the world aquarium trade, where the species are widely sold because of their exuberant color pattern [39]. *P. tankei* has a wide geographic distribution in South America [40], where it features split spawning and low fertility [17]. *P. oligospila* has a mottled brown coloration [41], and it is highly exploited by ornamental fisheries as well as *B. xantheilus* in its juvenile phase. The latter is rheophilic, and its distribution is strongly linked to the rapids of the Xingu River [42]. In its adult phase, it can reach up to 29.6 cm, where it is an important item in the diet of riverside communities [43].

Although all the cited species have socioeconomic and ecological importance in the Amazon region, studies on the morphology of the follicular complex in these species are non-existent. Nonetheless, their description is important in understanding different reproductive strategies. Therefore, based on the hypothesis that the thickness and the morphological characteristics of the oocyte follicular complex in the species are related to an adaptation to the environment and predict parental care, the aim of this study was to analyze the morphology and morphometry of the follicular complex in mature oocytes in six fish species from the order Siluriformes and associate the morphological differences with the possible reproductive tactics of these species.

## 2. Materials and methods

### 2.1. Sample collection

The species were collected in the Amazon rivers (Xingu, Anapu, Tocantins, Guamá and Lago Bolonha) from 2012 to 2018 using different fishing gear (mesh net, longline, diving). Thirty specimens of mature females were collected per species, namely *A. longimanus*, *A. ucayalensis*, *H. marginatus*, *B. xantheilus*, *P. tankei* and *P. oligospila*. The size of adult individuals is given in Table 1 as reported in the literature. Captured fish were anesthetized with benzocaine hydrochloride ( $0.1 \text{ g.L}^{-1}$ ) and euthanized with sodium pentobarbital solution (60–100 mg/kg) as seen in the literature [44–46]. Subsequently, gonads were removed by ventral incision. All animal experiments were approved by the National Council for Control of Animal Experimentation (CONCEA), were performed in accordance with approved guidelines, CEUA No. 5557211118 and follows the recommendations in the ARRIVE guidelines.

### 2.2. Light microscopy

Mature gonad fragments for all species were fixed in Bouin's solution for 24 h. The samples were then dehydrated in increasing concentrations of ethanol, cleared in xylene and infiltrated and embedded in paraffin [51]. Sections 5  $\mu\text{m}$  thick were cut and stained with hematoxylin and eosin (HE) and examined under a Carl Zeiss light microscope (AxioStar Plus 1169151).

### 2.3. Morphometry

Four mature females were used, and measurements were taken of the total thickness of the follicular complex, theca, follicular cells

**Table 1**  
Average size of species.

Species	Average adult size (mm)	Reference
<i>Auchenipterichthys longimanus</i>	191 mm	Freitas et al., 2016 [47]
<i>Ageneiosus ucayalensis</i>	218 mm	Oliveira et al., 2017 [48]
<i>Hypophthalmus marginatus</i>	462,8 mm	Hainfellner et al., 2019 [49]
<i>Baryancistrus xantheilus</i>	297 mm	Giarrizzo et al., 2015 [43]
<i>Panaqolus tankei</i>	98.3 mm	Mendes et al., 2018 [17]
<i>Peckoltia oligospila</i>	131,5 mm	Santos et al., 2020 [50]

and the zona radiata of 100 type III and IV oocytes per species of each group, totaling 1200 oocytes analyzed. The classification of oocyte development was adapted from the literature [21]: oocyte III was characterized by a central nucleus, acidophilic cytoplasm containing yolk globules and cortical alveoli at the periphery of the cytoplasm; the mature oocyte or oocyte IV was characterized by a larger cytoplasm filled with yolk globules and cortical alveoli located under the plasma membrane, with the nucleus migrated to the cell periphery. These cells accounted for the greater thickness of the follicular complex.

Only cells that contained a nucleus were measured. Serial sections were cut, and the slides were evaluated under a photomicroscope with the NIS-elements BR software (4.00.07-bit), and measurements were made at  $100\times$  magnification. Measurements were performed along a line superimposed on the follicular complex at four different points of the cell.

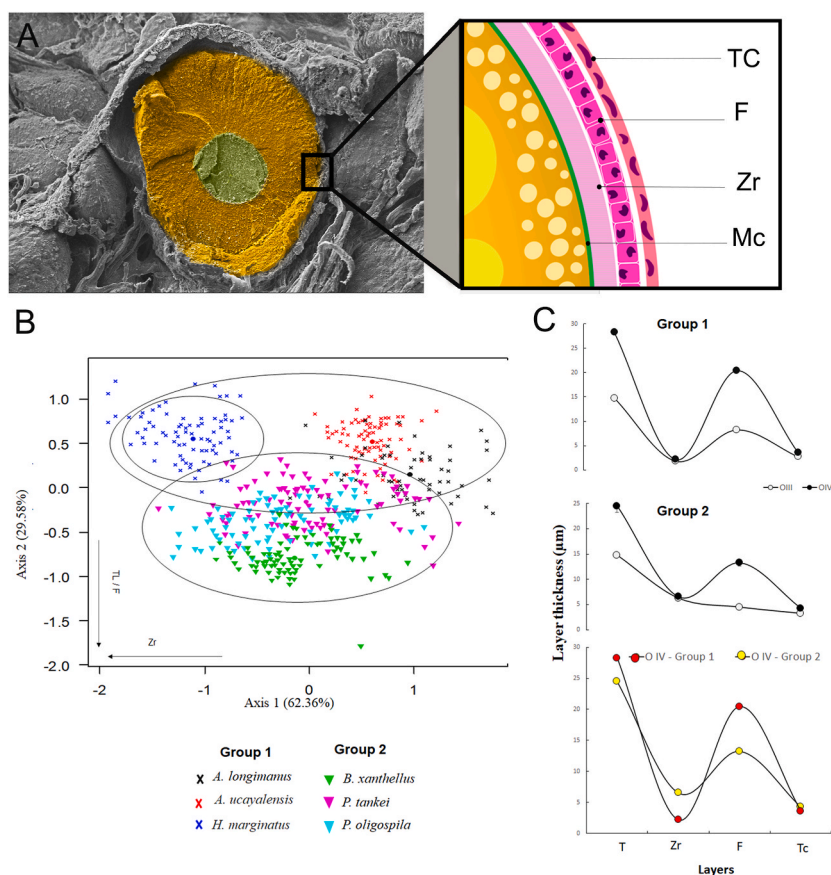
#### 2.4. Statistical analysis

The data were plotted and evaluated for the statistical assumptions of normality and homoscedasticity using the Shapiro-Wilk test, and as the assumptions were not met, non-parametric tests were used to determine statistical significance. The Mann-Whitney  $U$  test was used ( $P < 0.05$ ) to evaluate possible significant differences in layer thickness (total, theca, follicular cells and zona radiata) of type III and IV oocytes between groups, we checked for possible significant differences in the thickness of the layers of the follicular complex in the six species using the Kruskal-Wallis (H) test ( $P < 0.05$ ) with Nemenyi *post hoc* test.

Principal coordinate analysis (PCoA) was used to assess possible differences in layer thickness morphology in relation to species group, i.e., whether species are grouped differently [52]. As the morphological parameters were measured in the same units, the data were log-transformed, and the distance matrix was prepared using Euclidean distance. Permutation analysis (Permanova) was applied to assess whether the variation observed in the graph was significant [53]. All analyses were performed using the Core Team Program (2016).

#### 2.5. Scanning electron microscopy (SEM)

Fragments of ovaries were fixed in Karnovsky's solution (4% paraformaldehyde, 2% glutaraldehyde in 0.1 M sodium cacodylate



**Fig. 1.** Graphic representation of the mean thickness of the follicular complex. (A) Schematic drawing of the follicular complex. (B) Principal coordinate analysis (PCoA) showing the morphological variables of the siluriform species. (C) Mean thickness of type III and IV oocytes in groups and among groups. Abbreviations: T: total layer, Tc: theca layer, F: follicular cell, Zr: zona radiata and Mc: cytoplasmic membrane.

buffer, pH 7.4) for 24 h. After fixation, the fragments were washed in 0.1 M sodium cacodylate buffer, pH 7.4 and post-fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer, pH 7.4 for 2 h. For SEM analysis, the specimens were dehydrated in a graded ethanol series (30–100%) and critical-point dried using CO<sub>2</sub>. Fragments of ovaries were mounted on stubs, coated with gold and examined using a LEO 1430 and TESCAN Mira3 scanning electron microscope.

### 3. Results

#### 3.1. Morphometry of follicular complex

The species analyzed showed a different mean thickness for the layers that make up the follicular envelope in the mature oocyte (Fig. 1A). In the total layer's thickness there were significant differences among *A. longimanus*, *A. ucayalensis* and *H. marginatus* ( $H = 185.06$ ;  $D. F = 5$ ;  $p < 0.05$ ). Regarding the theca layer, there were significant differences only in *H. marginatus* compared to the other species ( $H = 54.63$ ;  $D. F = 5$ ;  $p < 0.05$ ). In the follicular cell layer, a significant difference was observed among *H. marginatus* and all species in the study and between *A. ucayalensis* and *P. tankei* ( $H = 133.15$ ;  $D. F = 5$ ;  $p < 0.05$ ). In the zona radiata layer, difference was not observed among *A. longimanus*, *A. ucayalensis* and *H. marginatus*, where these species had the lowest means with significant differences in relation to the other species and also *P. tankei*, which differed from all species ( $H = 189.40$ ;  $D. F = 5$ ;  $p < 0.05$ ) (Table 2).

PcoA showed that there were significant differences (PERMANOVA: Pseudo  $F = 281.51$ ,  $p = 0.001$ ) among species in relation to the thickness of the different layers (zona radiata, follicular and theca layers), in which this variation was 91.94% explained in the first two axes (Fig. 3A). The first axis corresponded to 62.36% of the data variation, where the variables that most negatively contributed to the formation of this axis were the total thickness of the layers and thickness of follicular cell layer. The second axis corresponded to 29.58% of the variation, where the variable that most contributed to this variation was the zona radiata. Through PcoA, we observed the separation into two groups, with group 1 formed by *A. longimanus*, *A. ucayalensis* and a subgroup within this group formed only by *H. marginatus* and group 2 formed by the species *B. xanthellus*, *P. tankei* and *P. oligospila* (Fig. 1B).

In group 1, comparing the total thickness of the follicular complex between type III and IV oocytes, significant differences were evidenced ( $U = 11.97$ ;  $p < 0.05$ ); layer thickness was smaller for type III oocytes (14.75  $\mu\text{m}$ ) and greater for type IV oocytes (28.55  $\mu\text{m}$ ). In type III oocytes, the layers of zona radiata, follicular cells and theca had a mean thickness of 1.84, 8.24 and 2.73  $\mu\text{m}$ , respectively. Meanwhile, in type IV oocytes, there was an increase in thickness in the zona radiata with 2.18  $\mu\text{m}$ , follicular cells with 20.44  $\mu\text{m}$  and theca layers, with 3.60  $\mu\text{m}$ . In both oocytes, the greatest thickness was observed in the follicular cell layer and the smallest in the zona radiata layer. There were significant differences between layers of oocyte III and IV, zona radiata ( $U = 4.43$ ;  $p < 0.05$ ), follicular cells ( $U = 11.72$ ;  $p < 0.05$ ) and theca ( $U = 6.72$ ;  $p < 0.05$ ) (Fig. 1C).

In group 2, the total thickness of the follicular complex also showed significant differences ( $U = 18.01$ ;  $p < 0.05$ ) between oocyte III (14.75  $\mu\text{m}$ ) and IV (24.50  $\mu\text{m}$ ). Type III oocytes showed thicker layers when compared to group 1, with a mean thickness of 6.24, 4.46 and 3.21  $\mu\text{m}$  for the zona radiata, follicular cells and theca, respectively, while type IV oocytes showed 6.54, 13.26 and 4.26  $\mu\text{m}$  for the zona radiata, follicular cells and theca, respectively. The highest value was found in the zona radiata layer in type III oocyte and in the type IV follicular cell layer. There was a significant difference in the zona radiata layer between the oocyte types ( $U = 2.12$ ;  $p < 0.05$ ); follicular cells ( $U = 19.00$ ;  $p < 0.05$ ) and theca ( $U = 7.96$ ;  $p < 0.05$ ) (Fig. 1C).

We analyzed the type IV oocytes (oocyte ready for spawning) between the two groups and found that the total thickness of the follicular complex for group 1 was 28.25  $\mu\text{m}$ , compared to 24.50  $\mu\text{m}$  for group 2, but there was no significant difference ( $U = 1.59$ ;  $p > 0.05$ ). The theca and zona radiata layers were larger in group 2 (Theca: 4.26  $\mu\text{m}$ ; Zona radiata: 6.54  $\mu\text{m}$ ) compared to group 1 (Theca: 3.60  $\mu\text{m}$ ; Zona radiata: 2.18  $\mu\text{m}$ ). However, the follicular cell layer was thicker in group 1 (20.44  $\mu\text{m}$ ) than in group 2 (13.26  $\mu\text{m}$ ). There were significant differences for all values observed in the layers, namely zona radiata, follicular cells and theca ( $U = -20.45$ ;  $U = 5.78$ ;  $U = -3.80$ ;  $p < 0.05$ , respectively) (Fig. 1C).

#### 3.2. Morphology of follicular complex

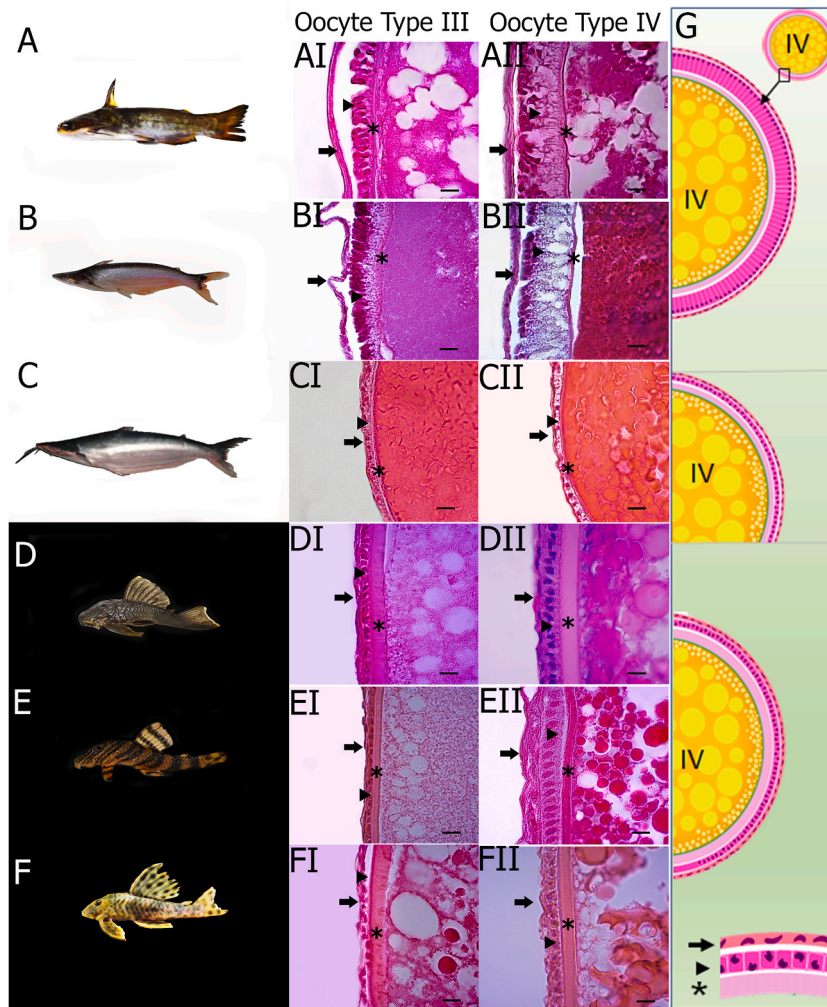
The follicular complex in the six species (Fig. 2A–F) showed morphological differences between oocyte type III and IV (Fig. 2 AI–FII). In type IV oocytes, the different characteristics of the layers were more evident between species (Fig. 2 G).

In group 1, the theca layer of type III and IV oocytes (Fig. 3 A) was thin, formed by squamous cells (Fig. 2 AI – CII). In type IV oocytes, there is an increase in theca thickness (Fig. 2 AII – CII). SEM showed the basement membrane and connective fibers for *A. longimanus* and *A. ucayalensis* (Fig. 3 B, C). However, *H. marginatus* displayed a thinner theca layer when compared to the other

**Table 2**

Means and standard deviations of the layers that make up the follicular complex among the siluriform species.

Species	Total thickness ( $\mu\text{m}$ )	Theca thickness ( $\mu\text{m}$ )	Follicular thickness ( $\mu\text{m}$ )	Zona radiata thickness ( $\mu\text{m}$ )
<i>Auchenipterichthys longimanus</i>	43.23 $\pm$ 13.02	4.49 $\pm$ 1.18	34.03 $\pm$ 13.03	2.54 $\pm$ 0.94
<i>Ageneiosus ucayalensis</i>	29.78 $\pm$ 5.72	3.84 $\pm$ 1.3	22.97 $\pm$ 5.00	1.67 $\pm$ 0.63
<i>Hypophthalmus marginatus</i>	10 $\pm$ 2.22	2.3 $\pm$ 1.18	5.38 $\pm$ 1.54	2.13 $\pm$ 0.72
<i>Baryancistrus xanthellus</i>	26.26 $\pm$ 6.11	4.69 $\pm$ 1.61	12.64 $\pm$ 4.94	8.65 $\pm$ 1.805
<i>Panaqolus tankei</i>	25.93 $\pm$ 8.69	4.08 $\pm$ 1.42	15.25 $\pm$ 9.27	4.31 $\pm$ 1.05
<i>Peckoltia oligospila</i>	27.26 $\pm$ 6.74	5.89 $\pm$ 1.58	8.58 $\pm$ 5.64	5.89 $\pm$ 1.58



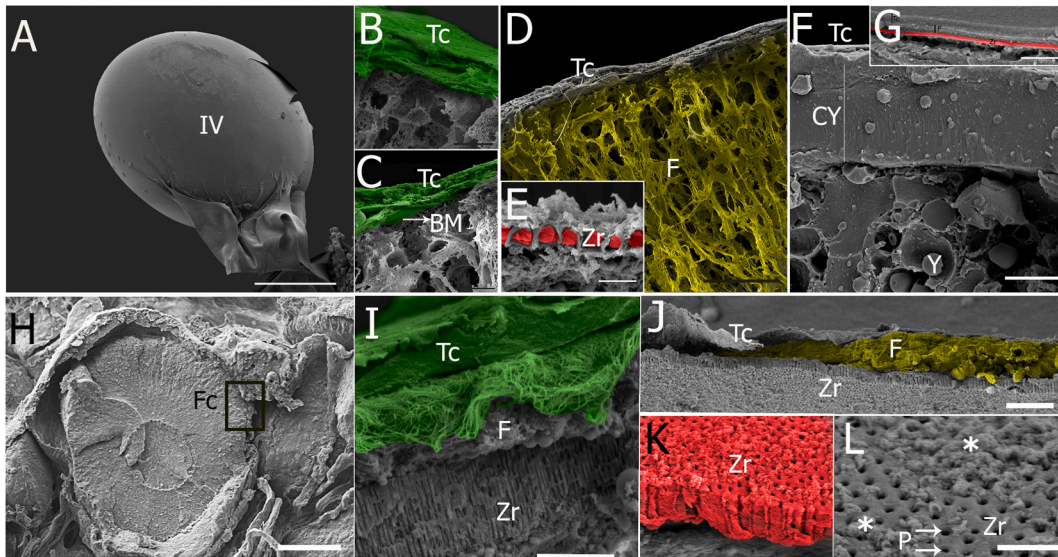
**Fig. 2.** Morphology of the follicular complex in type III and IV oocytes in six species of the order Siluriformes. (A-AII) *Auchenipterichthys longimanus* (B-BII) *Ageneiosus ucayalensis* (C-CII) *Hypophthalmus marginatus* (D-DII) *Baryancistrus xanthellus* (E-EII) *Panaqolus tankei* (F-F<sup>II</sup>) and *Peckoltia oligospila* (G). Schematic drawing of the follicular complex of type IV oocytes of the species. Scale bar: AI – FII: 100  $\mu$ m Abbreviations: IV: type IV oocyte, arrow: theca layer, arrowhead: follicular cell, asterisk: zona radiata.

species, where it was possible to observe a thick layer formed by yolk globules compacted near the cytoplasmic membrane of the oocyte (Fig. 3 F). The follicular cell layer in type III and IV oocytes in *A. longimanus* and *A. ucayalensis* showed a columnar shape with the nucleus located in the basal pole of the cell (Fig. 2 AI - BII and 3D). Meanwhile, in *H. marginatus*, they showed a cuboidal shape with a globose nucleus (Fig. 2 CI, CII), where the SEM showed a thin layer with poorly defined cell boundaries (Fig. 3G). The zona radiata in type III oocytes was thinner and more homogeneous, however, in type IV oocytes, it was thicker, displaying channels or pores connecting the oocyte and follicular cell for all species (Fig. 2 AII - CII). In *A. longimanus*, cytoplasmic projections were very evident inside these channels (Fig. 3 E). However, in *H. marginatus*, the zona radiata was continuous and homogeneous (Fig. 3G).

In group 2, the theca layer of type III and IV oocytes (Fig. 3H) was thin, formed by squamous cells (Fig. 2 DI - FII). In *P. oligospila*, the theca layer displayed an extensive fibrillar network (Fig. 3I). The follicular cell layers in types III and IV oocytes showed a cuboidal shape and distinct cell boundaries, especially in type IV oocytes for all species (Fig. 2 DII - FII and 3J). The zona radiata in type III oocytes showed connecting channels or pores (Fig. 2 DI - FI); these structures increased in type IV oocytes (Fig. 2 DII - FII). SEM demonstrated pores with an amorphous material inside in *B. xanthellus* oocytes (Fig. 3J-L).

#### 4. Discussion

This study characterized the morphology of the follicular complex in the six Siluriformes species that were selected because of their socioeconomic and ecological importance [31,32,35]. Some species are exploited because of their economic importance, as is the case of *H. marginatus* [54,49], and species of the loriciariidae family including *H. zebra* [39,55,56], the last through the ornamental fish



**Fig. 3.** Scanning electron microscopy of mature oocytes in species. (A) mature oocyte. (B, C) theca layer consisting of connective fibers and basement membrane. (D) columnar follicular cell layer, (E) zona radiata with cytoplasmic projections inside. (F) cross-section of oocyte showing yolk globules (G) follicular complex. (H) mature oocyte, (I, J) cross-section of oocyte showing theca layer consisting of connective fibers, follicular cell and zona radiata. (K–L) zona radiata showing pores and amorphous material. A, F and G: *H. marginatus*, B: *A. ucayalensis*, C, D and E: *A. longimanus*, H and I: *P. oligospila*, J–L: *B. xanthellus*. Scale bar: A: 200  $\mu\text{m}$ ; B, C, I and K: 5  $\mu\text{m}$ ; D, H and J: 20  $\mu\text{m}$ ; E: 1  $\mu\text{m}$ ; F: 10  $\mu\text{m}$ ; G and L: 2  $\mu\text{m}$ . Abbreviations. - IV: type IV oocyte, Fc: follicular complex, Tc: theca layer, F: follicular cell, Zr: zona radiata, P: pores, Y: compacted yolk globules, asterisk: amorphous material. Illustrative colors.

trade, thereby putting it in the category of endangered species of extinction [57]. Therefore, studies on reproduction provide data to aid in the induced reproduction and captive breeding of species of economic interest. Through PCoA, it was possible to observe the presence of two distinct groups, group 1 formed by the species *A. longimanus*, *A. ucayalensis* and *H. marginatus*, and group 2 formed by the species *B. xantellus*, *P. tankei* and *P. oligospila*. The species showed a significant increase in thickness in all layers that make up the follicular complex in type IV oocytes compared to type III oocytes, where there was variable thickness depending on the species. These data confirm the pattern that occurs in Siluriformes in relation to oogenesis, where the main events occur in the secondary growth phase of the oocyte, such as thickening of the layers and the total increase in cell volume [4,21,58].

The theca layer of the type IV oocyte of *H. marginatus* showed significant differences in the pattern of thickness and morphology between the species in the present study, unlike what is observed among teleost where there are no major morphological variations in this layer; its appearance is observed in the early stages of oogenesis, being one of the first layers of the follicular complex to form [59–62]. Additionally, an extensive fibrillar network was observed in the theca layer in *A. ucayalensis* and *A. longimanus* (group 1) and *P. oligospila* (group 2). We believe that this fibrillar network assists in the remodeling of the theca layer during oocyte development. Since the cells that make up the theca layer are steroidogenic, they aid in the development of the oocyte and are surrounded by type IV collagen [21,63,64].

In group 1, the thickness of the follicular cell layer in type IV oocytes increased the total thickness of the follicular complex, but it was not significantly different between groups. However, there were significant differences among species. It is known that follicular cells are responsible for the conversion of hormones, mainly  $17\beta$ -estradiol which helps in the maintenance of oocytes throughout ovarian development [24,65]. In *A. ucayalensis* and *A. longimanus*, the columnar shape of the follicular cells is similar to that observed in *Trachelyopterus galeatus* [4] and *Schizodon knerii* [20]. Similar values for layer thickness have been observed in species of other families such as *Lophiosilurus alexandri* [66] and *Bryconops affinis* [67]. During the reproductive cycle of these species, more than one spawning peak has been observed [68–70]. Possibly, in the present study, follicular cells were metabolically active, favoring an increase in cell volume and a rapid maturation of oocytes.

In group 2, the zona radiata of oocyte IV was thicker, with similar results being observed in representatives of the family Loricariidae as in *Ancistrus dolichopterus*, *Ancistrus dolichopterus* [71], and *Loricariichthys* sp., *Loricariichthys platymetopon* and *Loricaria* sp. [26]. These species display parental care, where the males carry the eggs on their abdomen until the larvae hatch, also noted in *P. tankei* which exhibits parental care [17]. *Baryancistrus xanthellus*, an endemic species that inhabits environments characterized by stream rapids, showed amorphous material inside the channels of the zona radiata and greater thickness of this structure. Similar characteristics were demonstrated in *H. zebra*, an endemic loricariidae that inhabits stream rapids rivers and shows parental care [21]. The morphology and thickness of the zona radiata can reflect adaptations to different environments where the eggs are deposited [72]; this structure acts in the protection of eggs and their adhesion to different substrates [26]. Accordingly, we can infer a relationship between the zona radiata and the environment, where the latter determines the characteristics and greater thickness of the zona radiata, besides these species displaying parental care.

Meanwhile, a thin zona radiata was observed in *H. marginatus*, which shows reproductive migration without any specialization. Although there are no reports of *A. longimanus* and *A. ucayalensis* showing reproductive migration, *A. longimanus* has a greater ability to move during feeding periods [73]. Migratory species have eggs with specific characteristics such as free and weakly adhesive eggs and even thinner zona radiata [74]. Thus, we can suggest that the smaller thickness of the zona radiata in species of group 1 can indicate spawning with free eggs and possible migration.

In this study, the morphological and morphometric analyses of the follicular complex, mainly in type IV oocytes, in the species *A. longimanus*, *A. ucayalensis*, *H. marginatus*, *B. xanthellus*, *P. tankei* and *P. oligospila* were different. These differences occurred particularly in the thickness of the zona radiata and of the follicular cells, which allowed the establishment of two groups, group 1 (*A. longimanus*, *A. ucayalensis* and *H. marginatus*) which showed a thin zona radiata and variation in the thickness of the follicular cells and group 2 (*B. xanthellus*, *P. tankei* and *P. oligospila*) which exhibited increased thickness both in the zona radiata and the follicular cells. Therefore, the follicular complex in mature oocytes can predict a reproductive tactic. Group 1, by exhibiting reproductive migration and not having parental care, does not require a structural investment in the follicular complex. On the other hand, group 2, by having eggs with high adhesiveness that guarantees parental care, the species invests energy to guarantee the structural integrity of the follicular complex.

Reproductive strategies in tropical fish are influenced by abiotic, endocrine, neuroendocrine factors, among others, that establish relationships between the environment and the way of life of organisms [75–77]. Although this study has limitations regarding endocrinology and neuroendocrinology, it shows in detail the existing morphological differences in the follicular complex of six species that have different reproductive habits. Therefore, it serves as a basis for understanding the numerous reproductive strategies in neotropical fish.

#### Author contribution statement

Ivana Kerly da Silva Viana: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Maria Auxiliadora P. Ferreira; Rossineide M. Rocha: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Yanne A. Mendes: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Breno Richard M. Silva; Liziane A. B. Gonçalves: Analyzed and interpreted the data; Wrote the paper.

#### Data availability statement

Data included in article/supplementary material/referenced in article.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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