

## *Brycon gouldingi* (Teleostei, Characidae): aspects of the embryonic development in a new fish species with aquaculture potential

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### Summary

*Brycon gouldingi* is an endemic species from Tocantins-Araguaia basin, used as a food source by riverine communities and relevant to aquaculture. Information about the initial morphology of *B. gouldingi*, a recently described species, is absent. In the present study, we analysed the fertilization and the embryonic development of this species based on light and scanning electron microscopy. After collection of adult specimens in Mortes River – Mato Grosso, Brazil, adaptation to captivity and induced spawning at Buriti Fishculture, Nova Mutum – Mato Grosso, Brazil, in December 2007 and January 2008, samples were collected at pre-defined periods from egg extrusion up to larval hatching, which occurred at  $13.9 \pm 0.06$  h post-fertilization (hpf) in average. At the moment of extrusion, the eggs were slightly ovoid bearing a single micropyle per oocyte with a funnel-shaped micropyle canal and vestibule covered with longitudinal folds, typical of the genus *Brycon*. The embryonic development of *B. gouldingi* was characterized by six stages with distinct features: zygote (from fertilization up to formation of egg-cell); cleavage (cell divisions resulting in blastomeres, including the morula phase); blastula (several embryonic cells in a cup shape, without distinction of cell boundaries); gastrula (cell movement); histogenesis/organogenesis (formation of tissues and organs); and hatching (larval chorion rupture). Right after hatching, the larvae presented neither swimming abilities nor visual accuracy, and the digestive tract was undifferentiated. The present study is the first report on biological features of embryogenesis in *B. gouldingi*, providing relevant information to several approaches, mainly related to taxonomy, ecology, conservation and captive rearing of this new *Brycon* species.

Keywords: *Brycon*, Eggs, Embryos, Microscopy, Ultrastructure

### Introduction

Characidae is the largest and the most complex family within Characiformes, comprising the highest number of species in relation to the other families in this order

(Howes, 1982; Nakatani *et al.*, 2001) and it includes a great number of subfamilies with quite distinctive features. The subfamily Bryconinae encompasses several medium- to large-sized species, widespread throughout South and Central America (Britski *et al.*, 1999).

Fish species of the genus *Brycon* are severely threatened by dams and deforestation along river margins once they are rheophilic, undergoing reproductive upstream migration, and feed upon alloctone sources such as fruits and seeds (Castagnolli, 1992). According to IBAMA (2009), the genus *Brycon* represents many species in the List of Threatened Aquatic Species. These are: *Brycon devillei* and *Brycon insignis*, both known as piabanha; *Brycon nattereri*, popularly referred to as pirapitinga; *Brycon opalinus*, usually known as pirapitinga or pirapitinga-do-sul; *Brycon orbignyanus*, the piracanjuba, picaranjuva or bracanjuba; and *Brycon vermelha*, known as vermelha.

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According to Junk & Nunes de Mello (1990), the construction of dams causes a huge impact leading to losses of both fauna and flora, including some completely or relatively unknown species such as *Brycon gouldingi*, studied in the present work.

This species was recently described by Lima (2004) as an endemic fish from Tocantins-Araguaia River Basin that feeds mainly on fruits and insects, inhabiting tropical benthopelagic freshwater environments. There are no available reports about this species, although it has been used as a food source by local communities for a long time and raised empirically in captivity by some fish farmers (personal communication).

Morphologically, this species is characterized by the presence of a wide fifth supra-orbital bone, several narrow longitudinal and curving stripes throughout the body, dark pectoral and pelvic fins, a distinctive V-shaped spot in the peduncle and caudal fin, and 66–82 scales in the lateral line (Lima, 2004).

A successful rearing of a fish species depends on knowledge about its early biology, once it provides essential information to accomplish a mass laboratory production (Matkovic *et al.*, 1985). The embryonic process begins when the oocyte is fertilized by the spermatozoon via micropyle, and it basically focuses on the reorganization of egg elements. In order to be understood, embryonic studies should be combined with topological analyses of fertilized eggs (Depêche & Billard, 1994).

In Brazil, there are reports about these aspects in species like *Rhamdia hilarii* (Godinho *et al.*, 1978), *Prochilodus lineatus* (Castellani *et al.*, 1994), *Collosoma macropomum* (Albuquerque *et al.*, 1994; Ribeiro *et al.*, 1995), *Piaractus mesopotamicus* (Ribeiro *et al.*, 1995), *Pseudoplatystoma corruscans*, (Cardoso *et al.*, 1995), *Brycon cephalus* (Lopes *et al.*, 1995; Romagosa *et al.*, 2001), *Pimelodus maculatus* (Luz *et al.*, 2001), *Brycon orbignyanus* (Nakatani *et al.*, 2001) and *Brycon insignis* (Andrade-Talmelli *et al.*, 2001).

Besides these important reports, other works have been recently carried out in *Brycon orbignyanus* (Maciel, 2006; Ganeco *et al.*, 2008), *Prochilodus lineatus* (Ninhaus-Silveira *et al.*, 2006), *Brycon orthotaenia*, *Leporinus obtusidens*, *Prochilodus argenteus*, *Salminus brasiliensis* (Nakaghi *et al.*, 2006; Sampaio, 2006), the hybrid *Pseudoplatystoma corruscans* × *Pseudoplatystoma fasciatum* (Faustino *et al.*, 2007), *Brycon amazonicus* (Neumann, 2008), *Pseudoplatystoma corruscans* (Landines *et al.*, 2003; Marques *et al.*, 2008) and *Zungaro jahu* (Marques, 2008).

Because of the importance of basic studies on the embryonic development of fish, particularly to phylogenetics, biology, and production, combined with the lack of reports in *B. gouldingi*, the goal of the present work was to provide information

about the structure and ultrastructure of oocytes and the embryonic development of this species of aquaculture potential through induced reproduction of adult specimens.

## Material and methods

Ten collections were performed in December 2007 and January 2008, at Buriti fish farm, Nova Mutum, in the state of Mato Grosso, Brazil, after induced reproduction of specimens of *B. gouldingi* according to the methodology described by Woynarovich & Horváth (1983). In the 10 selected females, the concentration of the first hypophysis extract inoculation was 0.5 mg/kg, and the second one was applied 10 h later in a proportion of 5.0 mg/kg. Single shots at 1.0 mg/kg were applied in males. Fertilization took place in dry conditions by adding the spermatozoa onto the eggs. Then, water was added to the mixture to promote the egg's hydration. Afterwards, these eggs were gently rinsed in water to remove any excess semen and each set was individually placed in 200-l conical glass fiber incubators, with water renewal of 6 l s<sup>-1</sup> and at a temperature of 26°C.

The samples were collected at the moment of extrusion, fertilization (time zero), 10, 20, and 30 s post-fertilization, each minute up to 10 min, each 5 min up to 45 min, and then each hour up to larval hatching. The samples were fixed in modified Karnovsky's solution (2.5% glutaraldehyde and 2.5% paraformaldehyde) for 24 h. Afterwards, they were washed and transferred into 0.1 M sodium cacodylate buffer (pH 7.4) and stored at low temperature.

This material was transported to the Faculty of Agricultural and Veterinary Sciences at São Paulo State University (FCAV/UNESP), Jaboticabal campus – SP. The sample analysis using light microscopy was carried out in the Histology and Embryology Laboratory at the Morphology and Animal Physiology Department, whereas the analyses by scanning electron microscopy were performed in the Electron Microscopy Laboratory.

## Light microscopy

The selected samples were dehydrated for 24 h in alcohol 80% and for 30 min each in 95% and 100% alcohol. Then, they were immersed for 4 h in a pre-embedding GMA solution (glycol methacrylate) + 100% alcohol at a ratio of 1:1, and 16 h in the embedding GMA solution for further inclusion in histomolds. The samples were placed in a drier at 50°C for 24 h. Semi-serial 2-µm thick sections were obtained using a LEICA RM2255 microtome with tungsten carbide blades. The first five cuts

were discarded and the others were stained with hematoxylin–eosin (HE) (Tolosa *et al.*, 2003). Analysis and photodocumentation were carried out in a Leica DM 5000 B photomicroscope coupled with Leica Application Suite (LAS) software.

### Scanning electron microscopy

The samples were selected and post-fixed in 1% osmium tetroxide for 2 h, and washed again in the same buffer. They were subsequently dehydrated in a graded series of ethanol at 30, 50, 70, 80, 90 and 95% plus three baths at 100% for 7 min each. Then, they were dried to the critical point in a liquid CO<sub>2</sub> drier (BAL-TEC), mounted in copper grid, metallized with gold (DENTON VACUM DESK II) and electron-micrographed under a scanning electron microscope (JEOL-JSM 5410).

## Results

In the present study, we used the term ‘egg’ to refer to the stages from fertilization up to the end of gastrulation, when the formation of the embryonic axis takes place and it is then referred as ‘embryo’. The term ‘larva’ was used to the moment of hatching. The word ‘oocyte’ indicates the feminine gamete, prior to fertilization.

The oocytes of *B. gouldingi* were slightly egg shaped with a mean diameter of  $1.13 \pm 0.06$  mm and a greyish-green colouration at the moment of extrusion. A single micropyle (region through which a spermatozoon can enter) was visualized in each oocyte (Fig. 1A), being composed of a micropyle canal and a vestibule (depression) with longitudinal folds on the walls (Fig. 1B). It was possible to detect several cortical alveoli of different sizes at the peripheral region of oocytes (cortical cytoplasm) as well as the presence of chorion (protective oocyte membrane) (Fig. 1C,D).

The embryonic development of *B. gouldingi* in the 10 collections (from fertilization up to larval hatching) has taken, in average, 13.9 h at  $26.4 \pm 1.12^\circ\text{C}$ . During this period, we observed six stages: zygote, cleavage (including the morula phase), blastula, gastrula, histogenesis/organogenesis and hatching, with particular features as follows:

### Zygote stage

This stage comprised the moment of fertilization up to the formation of the zygote (or egg cell), with the distinction between the animal and vegetative poles. At time zero (when spermatozoa and oocytes were mixed for fertilization), several spermatozoa were observed over the vestibule towards the micropyle canal

(Fig. 2A) up to 1 min after fertilization (mpf) (Fig. 2B). The fertilization cone was seen blocking the entrance of the micropyle canal within 30 s post-fertilization (spf) (Fig. 2C). This evidence reveals that several eggs had been fertilized and protected from polyspermy by the formation of such cones, which trespass the micropyle canal and resemble a spherical structure.

Within 1 min and 30 s after fertilization, the spermatozoa were no longer detectable and the eggs assumed a flattened aspect because of the cytoplasm movements towards the animal pole, suggesting that the spermatozoa had already fertilized the oocytes at this moment.

At 3 mpf, the eggs were remarkably flattened as a result of the cytoplasm movements (Fig. 2D) that defined the animal and vegetative poles. Furthermore, the cortical alveoli were more concentrated in the vegetative pole than in the animal pole (Fig. 2E,F). This feature allowed us inferring that the cortical reaction (alveoli rupture) in *B. gouldingi* has started in the region of the animal pole following up to the vegetative pole, also avoiding the polyspermy. At 5 mpf, these alveoli were unnoticeable and, at 10 mpf, the differentiation between both animal and vegetative poles were more conspicuous (Fig. 2G), being totally differentiated at 45 mpf, thus forming the egg cell (Fig. 2H).

### Cleavage stage

This stage has taken place between 45 mpf and 1 h post-fertilization (hpf) at the animal pole, which has firstly divided into two blastomeres (embryonic cells) that further split into four blastomeres, and then successively up to 32 cells, while the number of small cells became increasingly higher (data not shown).

### Morula phase

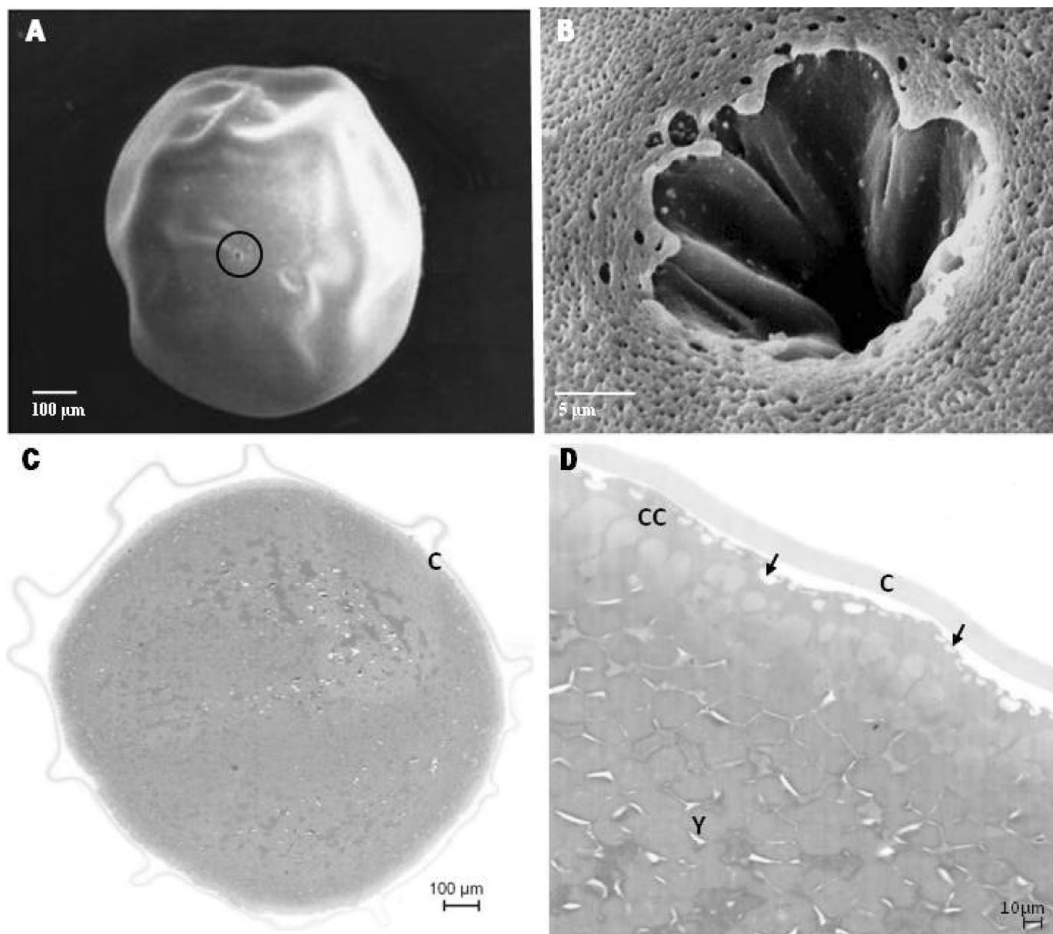
At 2 hpf, the successive cell divisions (typical of cleavage stage) yielded overlapped layers of blastomeres (more than 64 blastomeres) arranged in a ‘half-berry’ shape, characterizing the morula phase (data not shown).

### Blastula stage

The blastula stage was visualized at 3 hpf, when the blastoderm region (embryonic cells) and periblast became clear. This stage extended from the moment that the animal pole presented a cup shape, without identification of embryonic cell boundaries, up to the beginning of the cell movement (data not shown).

### Gastrula stage

This stage was characterized by the cell movement, being observed in the present study between 4 and 7 hpf. Figure 3A,B shows the blastoderm (embryonic



**Figure 1** Scanning electron micrographs (A,B) and photomicrographs (C,D) of oocytes of *B. gouldingi*. Extrusion: (A) Oocyte showing the micropyle (circle); (B) Detail of oocyte micropyle: funnel-shaped with longitudinal folds; (C) Oocyte with presence of chorion (C); (D) Detail of the oocyte with yolk (Y), cortical cytoplasm (CC) filled with cortical alveoli (→) and chorion (C).

cells) and periblast at the beginning of the cell movement. This first movement is called epiboly, in which the blastoderm cells encompass the egg. After the blastoderm cells involve nearly half the egg, the second movement got started, representing the involution of the most superficial cells (Fig. 3C). Such movement, named involution, led to a thickness of the egg margins, known as germ ring (Fig. 3D). The involution movement proceeded towards the animal pole, whereas the epiboly undergone the opposite direction (towards the vegetative pole), forming two layers the epiblast (outer layer) and the hypoblast (inner layer), culminating with the formation of the yolk plug (a portion of the yolk that remains uncovered by the embryonic cells) (Fig. 3E,F). Cell division was intense during this stage (Fig. 3D).

### Histogenesis/organogenesis stage

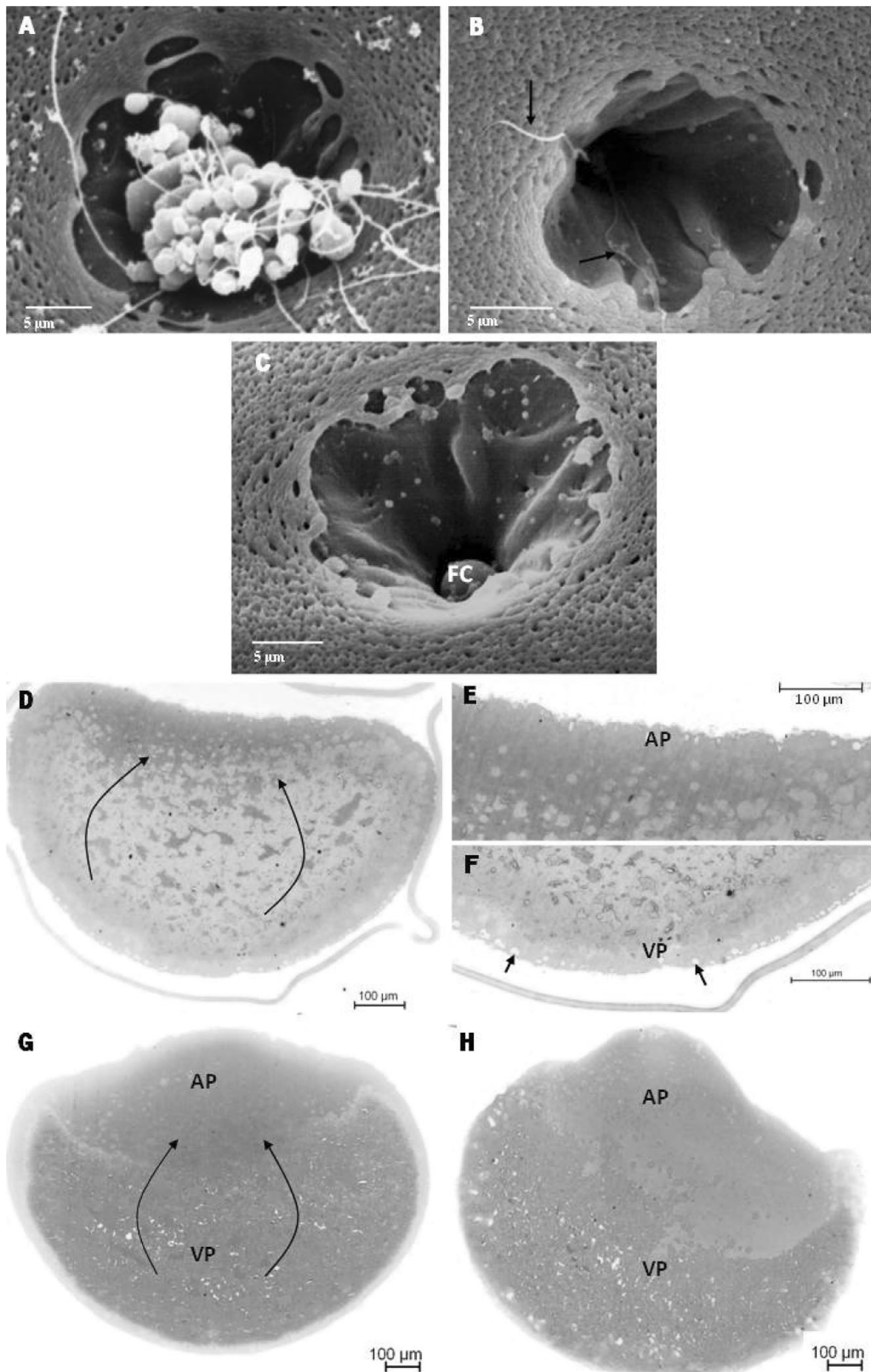
The formation of the embryo axis and the differentiation of cephalic and caudal region were observed at 8 hpf (Fig. 4A,B), characterizing the

organogenesis/histogenesis stage. The neural tube was evident in the cephalic region (Fig. 4B). The first somites could be noticed at 9 hpf (Fig. 4C–E) as well as the notochord (Fig. 4D). The optic vesicle was visualized at 10 hpf (Fig. 4G) and, at 11 hpf, tail detachment and embryo elongation through the cephalic–caudal axis were detected (Fig. 4H) along with the beginning of the differentiation of miomeres.

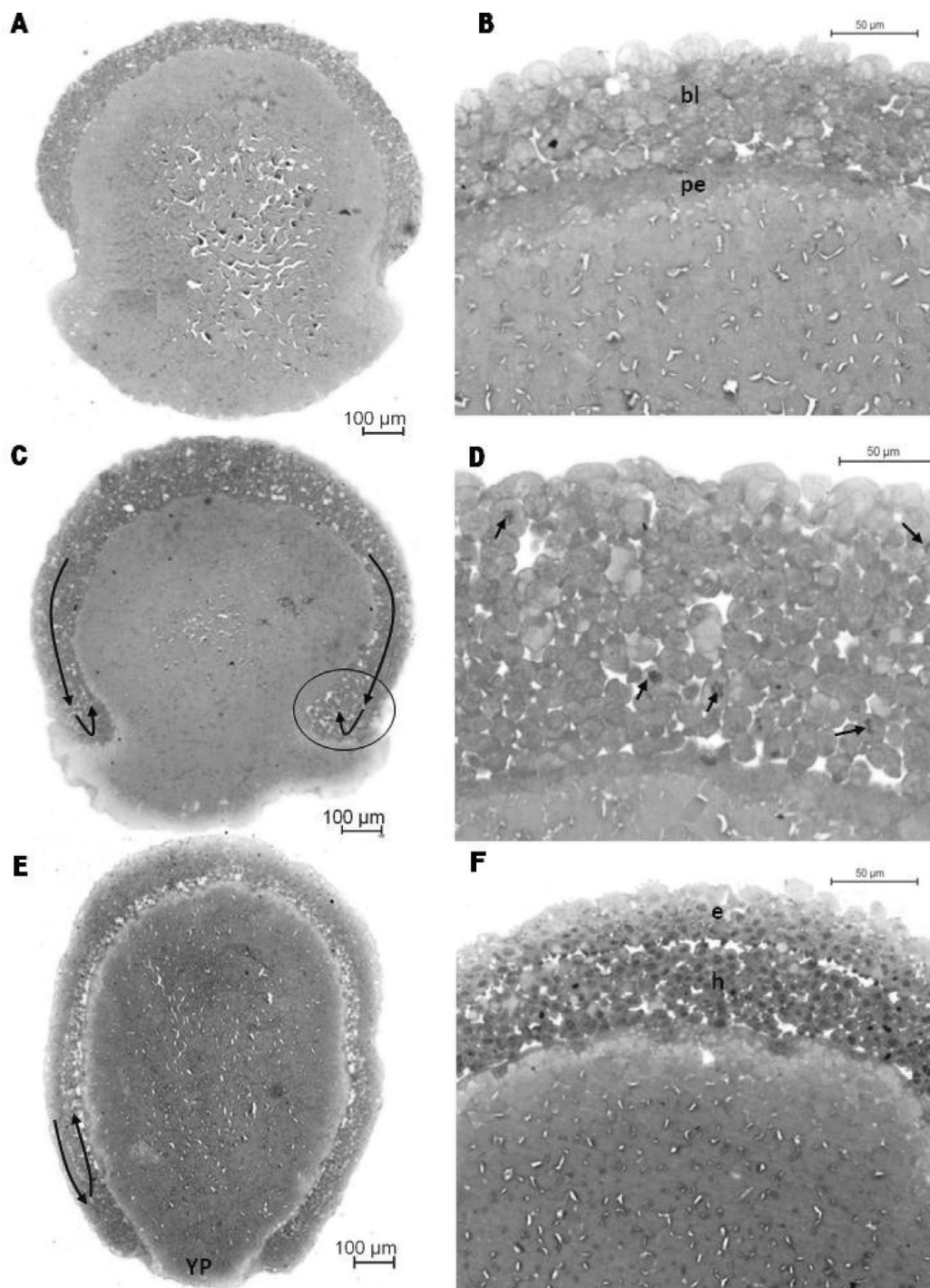
At 12 hpf, the olfactory placodes started developing (Fig. 5A,B), as well as the central nervous system (Fig. 5C) and the otic vesicle (Fig. 5C,E), besides the visualization of the notochord throughout a large extension of the embryo (Fig. 5D). At this moment, the optic cup started involving the crystalline primordium (Fig. 5F).

At 13 hpf, a rudimentary heart was noticeable (Fig. 5G) and a tegument-like epithelium extended continually right in front of the yolk sac (Fig. 5H), signaling the site where the mouth primordium is going to develop. The crystalline vesicle was completely involved by the optic cup (Fig. 5H). At this





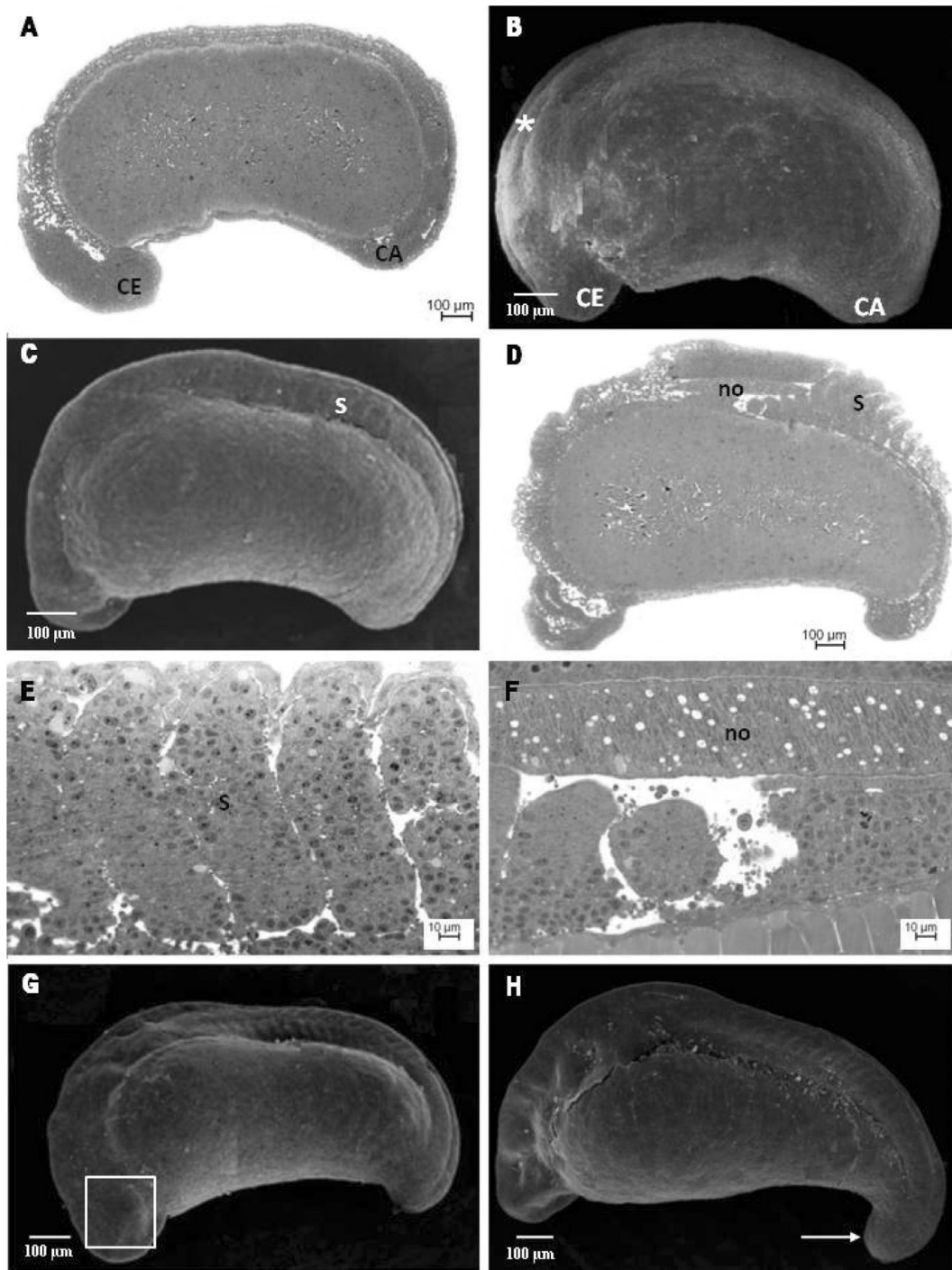
**Figure 2** Scanning electron micrographs (A–C) and photomicrographs (D–H) of eggs of *B. gouldingi*. (A) Fertilization (time zero) – spermatozoa on the micropyle vestibule. (B) 1 min post fertilization (mpf) spermatozoa in the micropyle vestibule. (C) 30 s post fertilization (spf) formation of the fertilization cone (FC). (D) 3 mpf: Beginning of cytoplasm movement (arrows) to define the animal and vegetative poles. (E) 3 mpf: Detail of the animal pole (AP). (F) 3 mpf: Detail of vegetative pole (VP) with cortical alveoli (→). (G) 10 mpf: cytoplasm movement defining the animal (AP) and the vegetative pole (VP). (H) 45 mpf: complete formation of animal (AP) and vegetative poles (VP).



**Figure 3** Photomicrographs of eggs of *B. gouldingi*. (A) Beginning of epiboly. (B) Beginning of epiboly with blastoderm (bl) and periblast (pe) in detail. (C) 6 hpf: Epiboly (↓) and involution (↑) movements with formation of the germ ring (circle). (D) 6 hpf: Detail of several embryonic cells undergoing mitosis (→). (E) 7 hpf: Gastrula stage highlighting the epiboly (↓) and involution (↑) movements in embryonic cells and formation of yolk plug (YP). (F) 7 hpf: Late gastrula stage with details of the formation of both layers: epiblast (e) and hypoblast (h).

moment, we also detected the development of central nervous system, forming the three primary vesicles: anterior brain (prosencephalon), medium brain (mesencephalon) and posterior brain (rhombencephalon).

The local of cerebellum formation was also observed (anterior region of rhombencephalon), as well as the epiphysis, derived from the posterior region of prosencephalon (Fig. 5G).



**Figure 4** Scanning electron micrographs (B,C,G,H) and photomicrographs (A,C,E,F) of embryos of *B. gouldingi*. (A,B) 8 hpf: Formation of the embryonic axis with differentiation of cephalic (CE) and caudal (CA) regions and presence of neural tube (\*). (C,D) 9 hpf: Observation of the first somites (S) and notochord (no). (E) 9 hpf: Detail of somites (S). (F) 9 hpf: Detail of notochord (no). (G) 10 hpf: Appearance of optic vesicle (inbox). (H) 11 hpf: Detachment of caudal region (arrow).

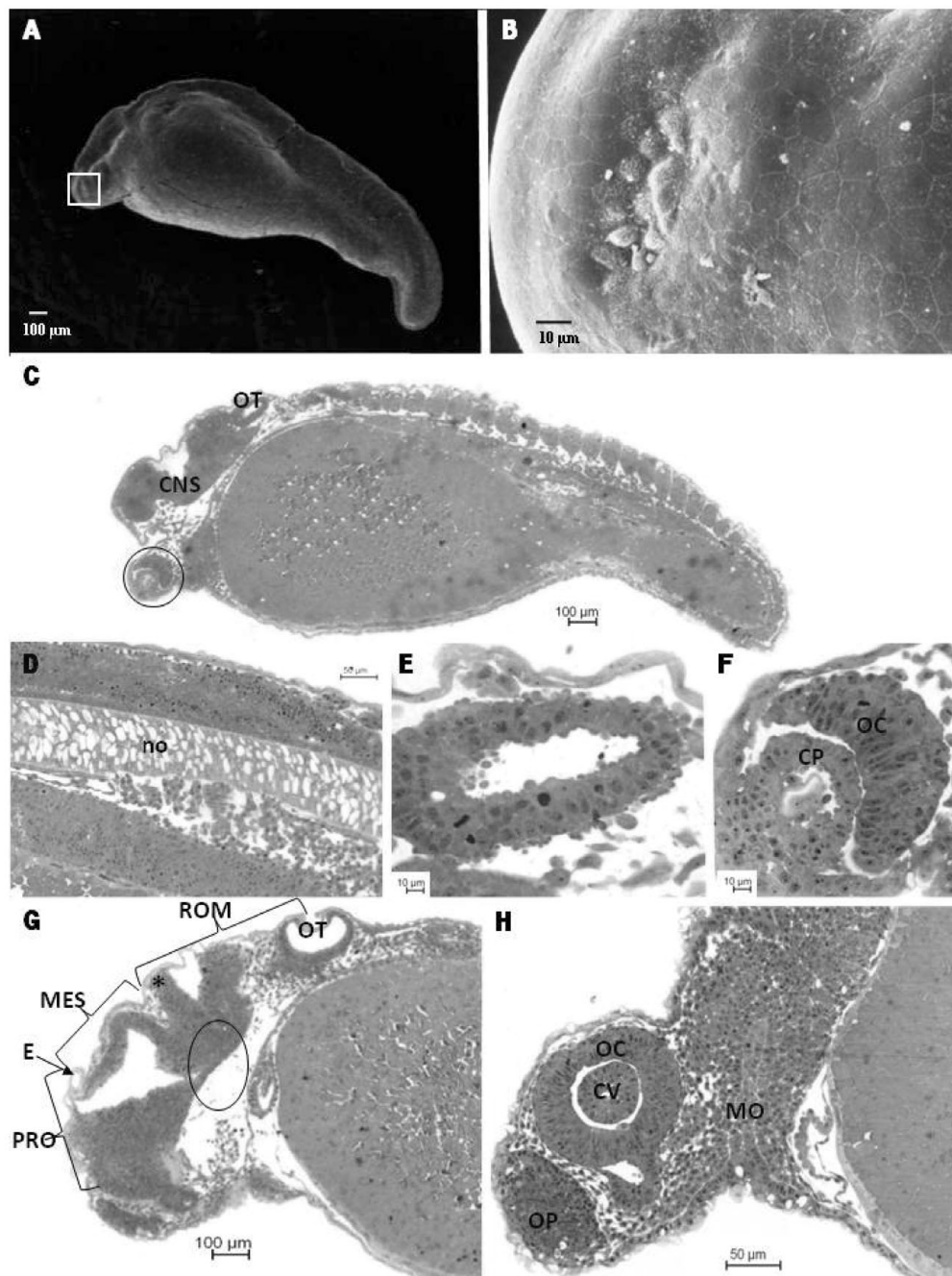
### Larval hatching stage

The larval hatching took place at 14 hpf and, at this moment, the larvae presented the head attached to the anterior portion of the yolk sac, a distended posture (Fig. 6A,B) and no fins, except for a embryonic fin

surrounding the entire caudal region (Fig. 6A). In the anterior dorsal region, the central nervous system was under development, being observed as a neural tube yet (Fig. 6C,F).

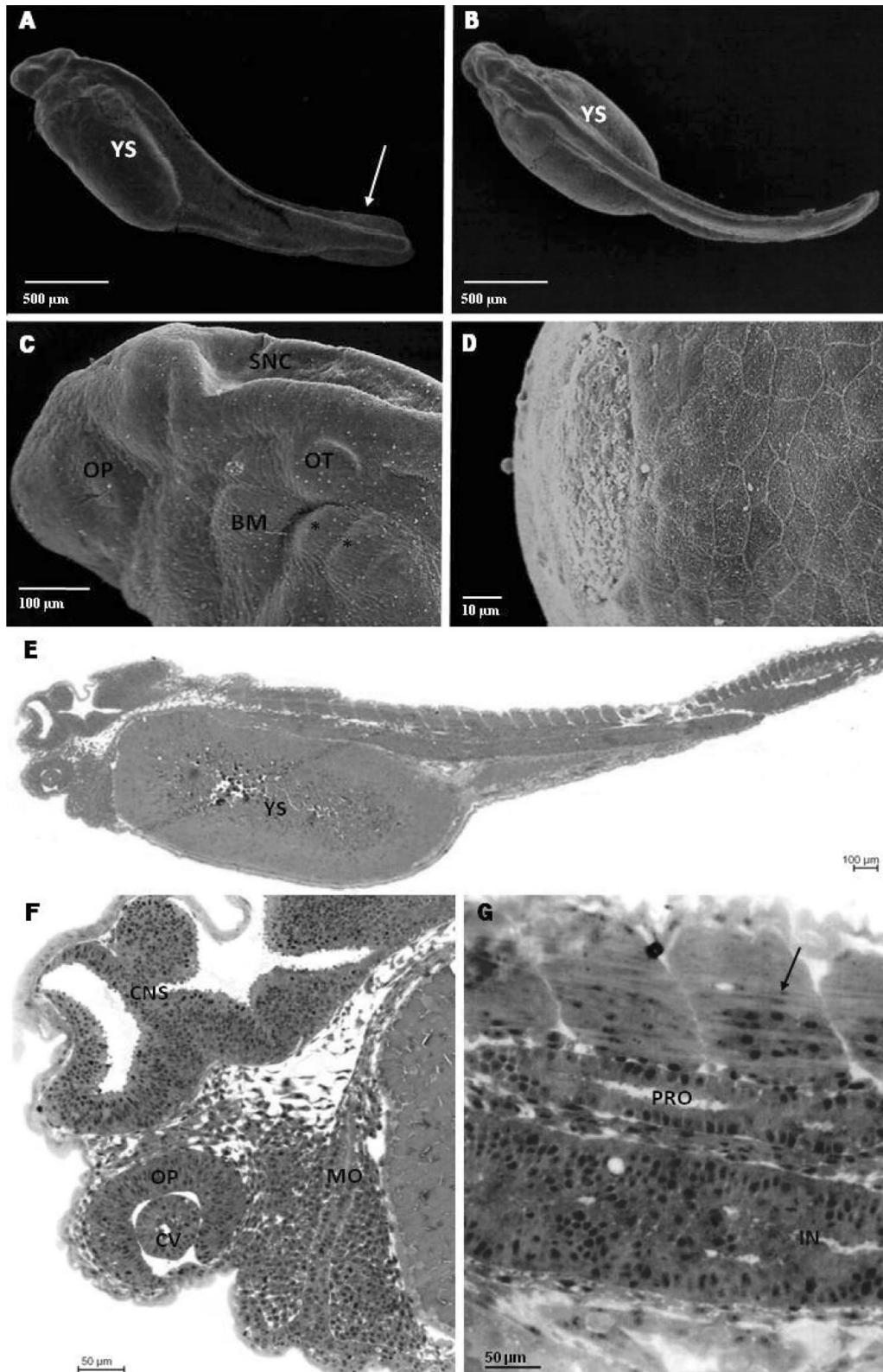
At this moment, the larvae presented primordial gill arches while the primordium of the branchiostegal





**Figure 5** Scanning electron micrographs (A,B) and photomicrographs (C–H) of embryos of *B. gouldingi*. (A) 12 hpf: Embryo elongation by the cephalic–caudal axis and development of olfactory placodes (arrow). (B) 12 hpf: Detail of olfactory placodes. (C) 12 hpf: Observation of optic cup and optic vesicle (circle), central nervous system (CNS), otic vesicle (OT). (D) 12 hpf: Detail of notochord (no). (E) 12 hpf: Detail of otic vesicle. (F) 12 hpf: Detail of optic cup (OC) and crystalline primordium (CP). (G) 13 hpf: Larval cephalic region: otic vesicle (OT), rudimentary heart (circle); development of central nervous system with division of prosencephalon (PRO), mesencephalon (MES) and rhombencephalon (ROM), presence of epiphysis (E) and cerebellum primordium (\*). (H) 13 hpf: Larval cephalic region: optic cup (OC), crystalline vesicle (CV), mouth primordium (MO), olfactory placode (OP).





**Figure 6** Scanning electron micrographs (A–D) and photomicrographs (E–G) of larvae of *B. gouldingi*. 14 hpf: Larval hatching. (A) Distended posture, yolk sac (YS) and presence of embryonic fin (→). (B) Dorsal view of recently hatched larva showing the yolk sac (YS). (C) Detail of cephalic region of hatched larva showing rudimentary gill arches (\*), optic vesicle (OP), otic vesicle (OT) and development of branchiostegal membrane (BM) and central nervous system (SNC). (D) Detail of olfactory placodes filled with ciliated cells. (E) Distended posture and presence of final portion of the digestive tract (highlighted), yolk sac (YS). (F) Detail of cephalic region of hatched larva: mouth primordium (MO), optic cup (OP), crystalline vesicle (CV) CNS, central nervous system. (G) Detail of hatched larva: differentiation of miomeres (→), visualization of pronephros (PRO) and intestine (IN).

membrane, that eventually will cover such arches, was under formation (Fig. 6C). The olfactory placodes were seen as shallow rounded depressions filled with tiny rudimentary cilia (Fig. 6D). The digestive tract was undifferentiated, with only an epithelium indicating the region of the further development of the buccopharyngeal cavity (Fig. 6F), represented by a single cell layer throughout the extension of the yolk sac, that changed onto several undifferentiated cell layers where the intestine will develop (Fig. 6E). At this moment, the pronephros (rudimentary kidney) was being formed, right above the intestine (Fig. 6G). It was also possible to observe the differentiation of miomeres (muscular segment) (Fig. 6G).

## Discussion

The presence of a single micropyle with a funnel-like vestibule and several rectilinear folds in the oocytes of *B. gouldingi*, is a common reproductive feature within species of the genus *Brycon*, as also reported by Ganeco & Nakaghi (2003) and Ganeco *et al.* (2008) in *B. orbignyanus*, Sampaio (2006) in *B. orthotaenia* and Neumann (2008) in *B. amazonius*. The micropyle represents a small opening in the animal pole of the cell (Laale, 1980), through which the spermatozoon penetrates the oocyte (Riehl, 1993).

According to Rizzo & Bazzoli (1993), funnel-like micropyles are observed in most of teleosts and allow the entrance of a single spermatozoon through the intern aperture.

Riehl (1993) and Li *et al.* (2000) stated that the micropyle microstructure is an important criterion to characterize and identify fish eggs. Chen *et al.* (1999) also suggested that the micropyle can be an important tool in the identification of eggs and for phylogenetic studies in fishes, once it might present differences among species of the same genus or family, as a way to prevent inter-specific hybridization. However, some studies reported similarities between the micropyle apparatus in different species, indicating putative relationships among systematic groups (Rizzo *et al.*, 2002).

The sequence of events observed in the embryogenesis of the species studied in the present work is similar to that described for other *Brycon* species, like *B. insignis* (Andrade-Talmelli *et al.*, 2001), *B. cephalus* (Lopes *et al.*, 1995; Romagosa *et al.*, 2001), *B. orbignyanus* (Landinez *et al.*, 2004; Reynalte-Tataje *et al.*, 2004) and other teleosts as well, such as the hybrid between *P. corruscans* and *P. fasciatum* (Faustino *et al.*, 2007), *P. lineatus* (Ninhaus-Silveira *et al.*, 2006) and *P. corruscans* (Marques *et al.*, 2008), being differentiated only by the chronology of the events.

The presence of several spermatozoa in the micropyle vestibule from the moment the semen and

the oocytes were mixed to 1 mpf suggested that fertilization had taken place within this interval. As some fertilization cones could be seen after 30 s, it is possible to infer that several eggs were rapidly fertilized. Kudo (1980) reported that the time between gamete fusion and subsequent formation of the fertilization cone is usually very short. He also stated that this cone would be composed of a granular material with few cell organelles. Iwamatsu (2000) observed that the fertilization cone is composed of perivitelline fluid released through the micropyle canal, forming a bubble. The beginning of formation of such cone occurs simultaneously to the exocytose of cortical alveoli (Iwamatsu & Ohta, 1981).

Right after the fertilization, the eggs of *B. gouldingi* became flattened. This behaviour was also observed by Neumann (2008) in *B. amazonicus*. The author also relates such 'compression' to the cell movements to form the animal pole. The egg cleavages in *B. gouldingi* occurred only in the animal pole, while the vegetative pole was composed of yolk. Such division pattern is typical of fish eggs and it is known as meroblastic or partial, once it involves exclusively the animal pole (Balinsky, 1970; Leme dos Santos & Azoubel, 1996).

The mitotic divisions during the cleavage take place in order to promote a new balance in the relationship between the nucleus and the cytoplasm, i.e., the high volume of the zygote cytoplasm is divided into increasingly smaller cells, while the cytoplasm volume does not increase (Gilbert, 2003). This feature was visualized in *B. gouldingi*: as the number of blastomeres increased, their size decreased corroborating the reports by Wourms & Evans (1974) and Castellani *et al.* (1994), being possible to observe several events of mitotic divisions as previously reported by Marques (2008).

In the blastula stage, the differentiation of blastoderm (embryonic cells) and periblast could be detected. The periblast (also called yolk syncytial layer) plays an important role once, before taking exogenous food sources, the embryo/larvae feeds on the yolk which is constantly reabsorbed via yolk syncytial layer (Balinsky, 1970). According to Kimmel *et al.* (1995), this layer is an organ and it is found only in teleosts, as an extra-embryonic structure, thereby not contributing to the embryo formation.

The appearance of periblast was also reported at blastula stage by other authors, such as Cardoso *et al.* (1995) in *P. corruscans*, Ninhaus-Silveira *et al.* (2006) in *P. lineatus* and González-Doncel *et al.* (2005) in *O. latipes*. However, it should be pointed out that the appearance of this layer was observed at morula stage by Long and Ballard (1976) in *C. commersoni*, by Matkovic *et al.* (1985) in *R. sapo* and by Marques (2008) in *Z. jahu*.

The initialization of cell movement characterizes the epiboly (Warga & Kimmel, 1990, Leme dos Santos,

1996). During gastrulation, the blastodisc cells are moved, differentiating the germ follicles, with the separation of epiblast and hypoblast and the beginning of notochord formation (Stickney *et al.*, 2000). During epiboly, the epiblast and the yolk syncytial layer (periblast) expand over the yolk until the blastopore is closed (Kimmel *et al.*, 1995), as observed in *B. gouldingi* and also in *B. insignis* (Andrade-Talmelli *et al.*, 2001), *L. piau* (Borçato *et al.*, 2004), and *P. lineatus* (Ninhaus-Silveira *et al.*, 2006).

Following the development, comes the organogenesis/histogenesis stage. The formation of somites (somitogenesis stage) is necessary in the organization of the segmental pattern of vertebrate embryos. These structures are transitory muscular precursors that develop into cell blocks (Gilbert, 2003).

The visualization of notochord in *B. gouldingi* occurred at this stage and, according to Gilbert (2003), it is a transitory organ whose main function is to induce the formation of the neural tube (further central nervous system) and establish the cephalic-caudal corporal axis. Most part of notochord degenerates, but the portion between the vertebrae originates the tissue of inter-vertebral disks. According to Falk-Petersen (2005), the notochord is composed of vacuolated cells separated by thin cell membranes.

In *B. gouldingi*, the anterior region of the neural tube was expanded, giving rise to anterior brain (prosencephalon), medium brain (mesencephalon) and posterior brain (rhombencephalon) as described in *D. rerio* by Kimmel *et al.* (1995), *G. morhua* by Hall *et al.* (2004), *P. lineatus* by Ninhaus-Silveira *et al.* (2006) and by Marques *et al.* (2008) in *P. corruscans*.

In the present study, we observed the epiphysis, derived from diencephalon. This structure is the pineal primordium (Kimmel *et al.*, 1995) and it appears on the upper limit of diencephalon (Hall *et al.*, 2004). The cerebellum primordium was also identified in *B. gouldingi* and, according to Kimmel *et al.* (1995) and Hall *et al.* (2004); it is derived from the metencephalon, the most posterior portion of the rhombencephalon.

With the heart formation during the somitogenesis of the embryo, the development of the circulatory system is initiated, and it continues throughout the whole larval development (Langeland & Kimmel, 1997; Yelon & Stainier, 1999; Hu *et al.*, 2000).

The heart primordium visualized in the anterior region of the yolk in *B. gouldingi*, has also been observed by several authors who described that the heart initially appears as a simple tube with a wall of several layers (Morrison *et al.*, 2001; Hall *et al.*, 2004).

After the organogenesis/histogenesis stage follows the larval hatching. Most freshwater fish larvae hatch with non-pigmented eyes, large yolk sac and underdeveloped jaw and mouth (Nakatani *et al.*, 2001), just like the larvae of *B. gouldingi*. Furthermore,

the *B. gouldingi* larvae lacked fin primordia (only an embryonic fin surrounded the whole tail) and the rudimentary digestive tract without oral and anal apertures at hatching. According to Önal *et al.* (2009), larvae that hatch with a large yolk sac and undifferentiated digestive tract, such as *B. gouldingi*, can be classified as altricial larvae.

The information obtained in the present work is described for the first time as being important because several biological traits were characterized about this new *Brycon* species, endemic to the Brazilian Tocantins-Araguaia basin, with great interest to fish farmers for captivity rearing as a food source to many riverine communities, but threatened by several projects of dam constructions for hydroelectric power along the basin. The present data are useful to elucidate questions on the rearing of *Brycon gouldingi* under laboratory conditions during the embryonic stage, besides being a tool for taxonomic, ecological and conservationist studies in this species.

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