Zygote

cambridge.org/zyg

Embryonic development of teleost *Brycon orbignyanus*

Luciana Nakaghi Ganeco-Kirschnik[1], Irene Bastos Franceschini-Vicentini[2,3], Maria do Carmo Faria Paes[4] and Laura Satiko Okada Nakaghi[2,4]

[1]Embrapa, Palmas; Aquaculture Center of São Paulo State University, Jaboticabal; Department of Biological Sciences – São Paulo State University, Bauru; and Department of Animal Morphology and Physiology – São Paulo State University, Jaboticabal, Brazil, [2]Aquaculture Center of São Paulo State University, Jaboticabal – SP, Brazil, [3]Department of Biological Sciences – São Paulo State University, Bauru – SP, Brazil and [4]Department of Animal Morphology and Physiology – São Paulo State University, Jaboticabal – SP, Brazil

Article

Cite this article:
Ganeco-Kirschnik LN *et al* (2018) Embryonic development of teleost *Brycon orbignyanus*. *Zygote* **26**: 294–300. doi:10.1017/S0967199418000229

Received: 24 February 2018
Revised: 3 May 2018
Accepted: 20 June 2018
First published online: 18 September 2018

Keywords:
Embryology; Fish; Histology; Reproduction; Ultrastructure

Author for correspondence:
Luciana Nakaghi Ganeco-Kirschnik. Embrapa, Caixa Postal No. 90 CEP:77001-970. Palmas – TO, Brazil. E-mail: luciana.ganeco@embrapa.br

Summary

Brycon orbignyanus is an important large teleost that is currently on the list of endangered species, therefore studies on its reproductive biology and embryology are fundamental to help species conservation and recovery. The objective of this research was to characterize the events that occur during extrusion, fertilization and embryonic development of the species. The samples were collected at predetermined times, fixed and processed for light microscopy and scanning electron microscopy. The greenish oocytes were spherical, had translucent chorion and a mean diameter of 1.3 ± 0.11 mm. The eggs had well defined animal and vegetative poles approximately 18 min post-fertilization. Stages from 2 to 128 blastomeres occurred between 20 min and 3 h post-fertilization (hPF), when the morula was characterized. The blastula stage was observed between 2 and 3 hPF, and the gastrula between 3 and 7 hPF, when the embryonic shield emerged and the cellular migration with the consequent formation of epiblast and hypoblast. At 8 hPF, the formation of the neural tube, above the notochord and the encephalic region, was observed, delimiting the forebrain, mesencephalon and rhombencephalon regions. From 11 hPF onward, the optic vesicle was formed close to the forebrain and the embryo tail was well developed. The optic vesicle was observed from 12 hPF onward, and the tail showed an intense movement that culminated with the rupture of the chorion and consequent hatching of the larva at 13 hPF and 27°C.

Introduction

Brycon orbignyanus is a large tropical species, indigenous to the Paraná and Uruguay River Basins (Carolsfeld *et al*., 2004) that is highly dependent on riparian vegetation (Panarari-Antunes *et al*., 2011). In addition to excessive fishing and environmental degradation, the construction of hydroelectric dams in the area has led the species to be classified as close to extinction as the dams impede their reproductive migration (Oliveira *et al*., 2017).

Although induced artificial reproduction and incubation are well understood in different teleost species (Faustino *et al*., 2007), there are few research groups dedicated to studying the basic aspects of the fish embryonic period, which extends from fertilization to hatching. Studies on the early stages of the life cycle are fundamental for knowledge of both taxonomy and species ecology (Sanches *et al*., 1999). Reynalte-Tataje *et al*. (2004) found that embryos and larvae of a species are greatly important and a useful tool to locate spawning areas and study the species growth in the natural environment. Knowledge of the embryonic development stages allows species ontogeny to be studied while also being used in biotechnological processes, as bioindicators of environmental quality to evaluate the effect of toxic substances on aquatic fauna, in repopulation programmes and conservation of rare species (Flores *et al*., 2002; Botero *et al*., 2004; Ninhaus-Silveira *et al*., 2006; Paes *et al*., 2014; Santos *et al*., 2016).

Fish embryonic development has been studied and divided into stages in several species; among these are *Brycon cephalus* (Romagosa *et al*., 2001), *Brycon insignis* (Andrade-Talmelli *et al*., 2001), *Brycon gouldingi* (Faustino *et al*., 2015), *Brycon amazonicus* (Neumann *et al*., 2013; Silva *et al*., 2017); *Brycon nattereri* (Maria *et al*., 2017) and *Brycon orthotaenia* (Sampaio *et al*., 2015). Thus, this research aims at characterizing in depth the external and internal morphological events that occur during oocyte extrusion, fertilization and embryonic development of *B. orbignyanus* over known time periods. All knowledge regarding its biology is extremely important as it can contribute to studies on repopulation, cryopreservation and reproductive management, aimed at species conservation and survival.

© Cambridge University Press 2018.

CAMBRIDGE UNIVERSITY PRESS

https://doi.org/10.1017/S0967199418000229 Published online by Cambridge University Press

Materials and methods

Collections were carried out during the species reproductive period from October to January. To perform induced reproduction, breeding animals from captivity were selected and were made dry fertilization. Females with an average weight of 4.8 kg and standard length of 46 cm received three doses of common carp pituitary (*Cyprinus carpio*). The first dose of 0.25 mg/kg body weight (BW) was followed by the second and third doses of 0.5 mg/kg BW each, injected 4 and 16 h after the first dose. Spawning occurred 4 h after the third application. Males with an average weight of 2.1 kg and standard length of 33 cm received a dose of 5.0 mg/kg BW at the same time as the females received the last dose.

After extrusion, the oocytes were placed in a beaker and gently mixed with the added semen. Hydration occurred 1 min later and the eggs were placed in a 1600 litre incubator with water circulation and aeration. The water temperature was kept at 27°C throughout development through heaters with thermostats.

Sampling occurred at the following times: oocyte extrusion, oocyte and semen mixing (time zero), every minute until 10 min were completed, and at 12, 14, 16, 18, 20, 24, 26, 28, 30, 45 and 60 min and after that, every hour until the larvae hatched.

Light microscopy

Samples were fixed in Karnovsky solution (2.5% glutaraldehyde + 2.5% paraformaldehyde) for 24 h, washed in 0.1 M sodium cacodylate buffer, pH 7.4, dehydrated in increasing ethanol series solutions, diaphanized in xylol followed by infiltration and inclusion in paraplast. Subsequently, 4-µm thick histological sections were stained with haematoxylin-eosin and masson trichrome. The analysis and photograph documentation were performed using an Olympus BX 50 photomicroscope.

Scanning electron microscopy

Samples were also fixed in Karnovsky solution for 24 h and then washed in 0.1 M sodium cacodylate buffer, pH 7.2; post-fixed in 1% osmium tetroxide solution for 2 h and washed in the same buffer. Subsequently, the samples were dehydrated in an increasing ethanol series (30, 50, 70, 80, 90, 95, 100%), for 15 min each. Then, they were processed in a liquid crystal (BAL-TEC) Crushed Dryer, mounted on a copper support, metallized with palladium-gold and observed under a scanning electron microscope (JEOL-JSM 5410).

Morphometric analysis

The diameters of the oocytes/eggs were measured under a stereomicroscope using the micrometric ocular lens. Twenty oocytes/eggs were measured from each of the sampling times, and three measurements of diameter were taken for each one. The values were statistically evaluated by Tukey test at 5% ($P < 0.05$) using ESTAT software.

Results

B. orbignyanus embryonic development lasted 13 h from fertilization to hatching at 27°C. The greenish eggs remained spherical throughout development, and non-adhesive, with a translucent chorion limiting a wide perivitelline space.

The size of the perivitelline space increased up to 30 min after fertilization and after that, diameter is practically maintained. At the time of extrusion oocyte average diameter was 1.31 ± 0.11 mm while, 30 min after fertilization, the egg size, considering the perivitelline space, was 3.32 ± 0.12 mm. At hatching, the larvae measured 3.47 ± 0.15 mm.

Stage 1 (0–20 min)

This stage lasted from fertilization to the first cleavage. The female and male pro-nuclei approached for further fusion in the cytoplasm in the animal pole or blastodisc. This region comprising the cortical cytoplasm appeared thick and convex over the yolk sphere, which, at this stage, represented the vegetative pole (Fig. 1*A*). It is noteworthy that, at this developmental stage, the cortical cytoplasm was observed to form a thin layer covering the whole yolk.

Stage 2 (20 to 30 min)

The beginning of the first cleavage occurred in the centre of the blastodisc (Fig. 1*B*) and extended to the periphery while simultaneously deepening to the perimeter of the yolk sac, presenting meroblastic or partial type segmentation.

Stage 3 (26–45 min)

The first cleavage furrow, marking the second cleavage developed in two blastomeres, and was vertically oriented (Fig. 1*C*). This furrow deepened to divide each blastomere into two of the same size, totalling four blastomeres (Fig. 1*D, E*).

Stage 4 (45 min to 1 h)

The third cleavage, which consisted of dividing the four existing blastomeres into eight blastomeres, occurred in a plane parallel to the first cleavage (Fig. 1*F*). At this development stage, the resulting eight blastomeres were arranged in two tiers of four blastomeres each, oriented according to the plane of the second cleavage.

Stage 5 (1 h)

Similarly, the fourth cleavage that divided the two rows of four blastomeres into four rows of four blastomeres each, occurred parallel to the second cleavage. The resulting 16 blastomeres were arranged in 12 marginal and four central blastomeres (Fig. 1*G*). At this stage, the central projection of the yolk was observed under the blastodisc, raising the four central blastomeres above the cleavage plane.

Stage 6 (1–2 h)

The fifth cleavage was characterized by the formation of 32 cells. The fifth cleavage plane meridionally divided the 12 marginal blastomeres into 24, and horizontally the four central blastomeres into eight, forming one outer and one inner layer, each with four cells in the central region (Fig. 1*H*).

Stage 7 (2–3 h)

From the sixth cleavage, the embryo presented cell division plans with diverse orientation, constituting initially 64 to 128 cells, characterizing the morula stage (Fig. 1*I*). These cells were arranged in two or more layers forming the dome-shaped blastoderm. The successive cleavages increased the number of cells but not their size.

https://doi.org/10.1017/S0967199418000229 Published online by Cambridge University Press

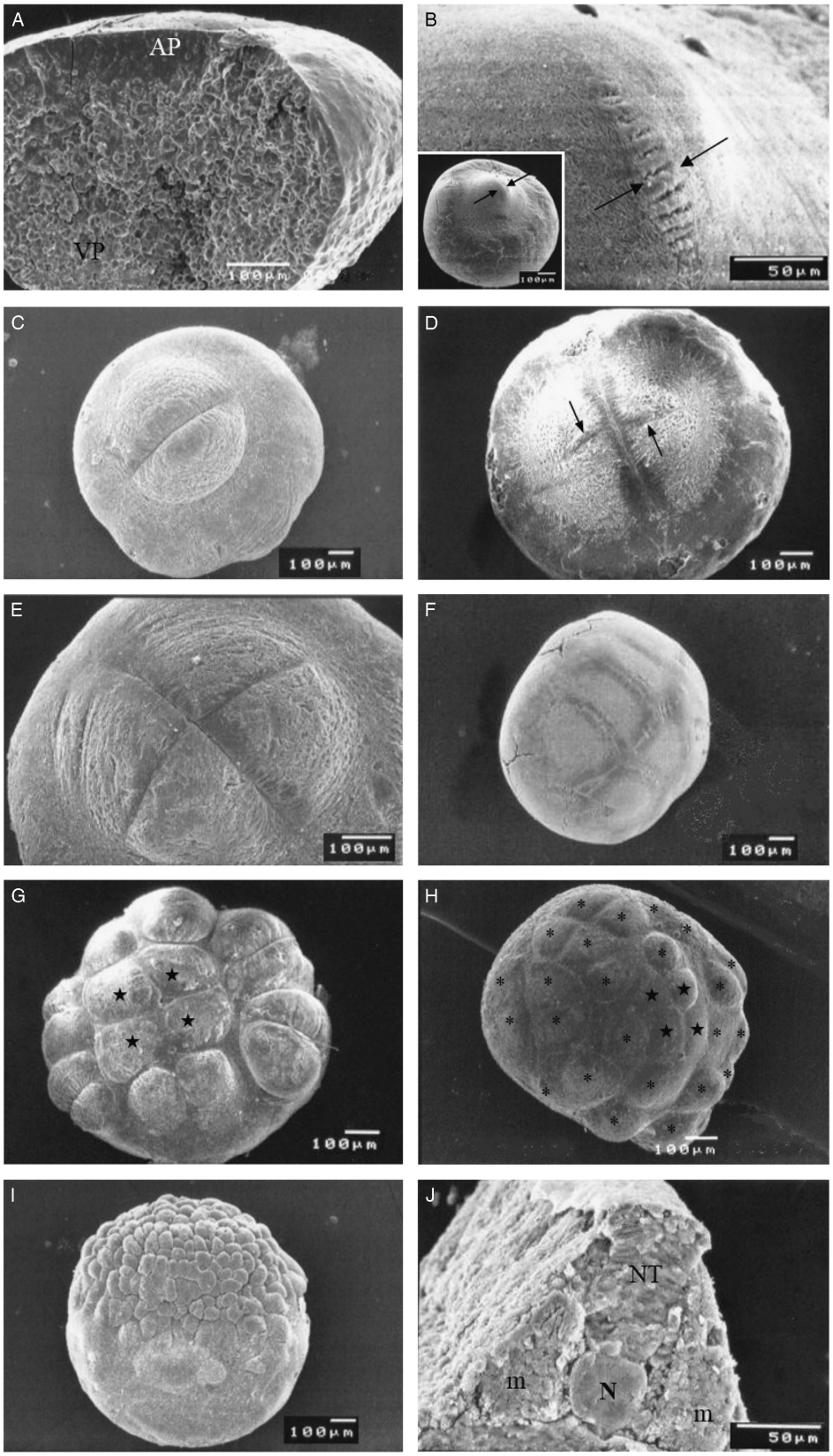


Figure 1. Scanning electron micrographs (SEM) of *B. orbignyanus* eggs. (*A*) Stage 1, time '9 min', showing the formation of the animal and vegetative poles. (*B*) Stage 2, time '20 min', evidencing the beginning of the first cleavage (arrows). (*C*) Stage 2, time '20 min', showing egg with 2 blastomeres. (*D*) Stage 2–3, time '26 min', highlighting the beginning of second cleavage (arrows). (*E*) Stage 3, time '30 min', egg with four blastomeres. (*F*) Stage 4, time '45 min', egg with eight blastomeres. (*G*) Stage 5, time '1 h', egg with 16 blastomeres showing arrangement with four central blastomeres (★) and 12 marginal blastomeres. (*H*) Stage 6, time '1 h', egg in the 32-cell phase, showing that only 28 blastomeres are observed on the surface, being four central (★) and 24 marginal (*). (*I*) Stage 7, time '2 h', egg in the morula stage. (*J*) Stage 10, time '8 h', evidencing the presence of the neural tube (TN), notochord (N) and para-axial mesoderm (m).

https://doi.org/10.1017/S0967199418000229 Published online by Cambridge University Press

Stage 8 (2–3 h)

The blastoderm was equally thick in the blastula and at the end of the morula stages. The marginal blastomeres of the blastoderm migrated and positioned themselves in an arranged manner, limiting the vitellus. These cells arranged in a row constitute the periblast, observed around the blastoderm (Fig. 2*A*). At this developmental stage, the periblast cells collapse and release their cytoplasm along with their nucleus into the cytoplasm that houses the embryo yolk giving rise to the yolk syncytial layer (YSL) (Fig. 2*B*). It was also noted that this stage was characterized by irregular spaces between the cells that constitute the blastoderm (Fig. 2*C*). At the end of this stage, epiboly began, that is the blastoderm starts to cover the yolk.

Stage 9 (3–7 h)

The gastrula stage was characterized by the epiboly movement, emergence of the embryonic shield, cell migration with consequent formation of two germ layers. Epiboly was characterized by the continued blastoderm movement to cover the yolk. When 50% of the yolk was covered, the marginal border of the blastoderm thickened forming the germ ring (Fig. 2*C*). In this stage, epiboly stops temporarily while cells accumulate perpendicular to the germ ring, forming the embryonic shield. Once the shield is formed, epiboly restarts and the margin of the blastoderm advances along the yolk boundary, completely covering it (Fig. 2*D*), forming the yolk sac. At the time epiboly restarted, cells had migrated in the germ ring region forming a fold that projected below and opposite to the movement of blastoderm epiboly (Fig. 2*D*, *E*). At this stage, the embryo presented two germ layers, the external or epiblast and the internal or hypoblast (Fig. 2*E*).

When the gastrula stage presented more than 50% epiboly, it was noticed that several cell layers started to form between the hypoblast and epiblast. At this stage, this cellular accumulation was considered as the appearance of a possible third embryonic tissue called the mesendoderm (Fig. 2*F*). The cells of this new tissue, located below and centrally to the embryonic shield, were considered as axial mesoderm. When epiboly is close to the end, cells start to accumulate in this region, probably forming the second axis of the embryo, known as notochord (Fig. 2*F*).

At the end of the gastrula, i.e., when the yolk plug was formed, the onset of the dorsal epiblast thickening was observed immediately above the formed notochord (Fig. 2*F*). This region was called the neural plaque, the primordia of the nervous system.

Stage 10 (8 h)

At this stage, thickening of the dorsal epiblast causes a sulcus to form, which later fused its edges into the neural tube (Figs 1*J* and 2*G*).

Laterally to the notochord was the para-axial mesoderm (Figs 1*J* and 2*G*), which in the anterior portion gave rise to the musculature associated with the organs of this region. Subsequently, the para-axial mesoderm represented the segmentation plate that gave rise to the somites (Fig. 2*H*).

Previously, a region representing the pre-chordal plaque was found in the hypoblast. The region of the neural tube above this plate thickened originating the encephalic region, delimiting the forebrain, mesencephalon, and rhombencephalon regions (Fig. 2*I*). At this stage, the subcephalic and subcaudal folds appeared caused by the decreasing yolk. The appearance of the folds defined the body shape of the embryo.

Stage 11 (11–13 h)

After 11 h, the optic vesicle started to form by the evagination of the lateral wall of the posterior portion of the forebrain (Fig. 2*I*). At this stage, the tail of the embryo was well developed.

The optic vesicle was observed after 12 h (Fig. 2*J*) while the tail showed a more intense growth, greatly increasing the length of the embryo. Subsequently, the tail started to move, culminating with the rupture of the chorion and consequent hatching of the larva from 13 h.

At the time of hatching, the digestive system was rudimentarily developed as a massive cord of cells in the mid-posterior region of the embryo, and was characterized by the formation of the posterior intestine.

Discussion

It is known that incubation temperature directly influences the developmental speed of teleosts while possibly leading to anomalies during the embryo and larva formation stages (Santos *et al*., 2016). The embryonic development of *B. orbignyanus* lasted 13 h at 27°C, considered an ideal temperature for the species according to Nakatani *et al.* (1999). Another parameter that should also be considered in the speed of the embryonic development is the size of oocytes and eggs as species with smaller eggs have faster development (Paes *et al*., 2014). The *Brycon orbignyanus* oocytes had an average diameter of 1.31 ± 0.11 mm at the time of extrusion. It is also known that oocyte and egg diameters vary among species and are associated with both reproductive behaviour and fecundity, presenting inter- and intraspecific alterations (Marques *et al*., 2017). Generally, migratory species that exhibit total spawning, external fertilization and no parental care have a high fertility rate and smaller oocytes/eggs (Vazzoler, 1996).

These fish eggs are classified as megalecithal due to the large yolk, and telolecithal because the yolk appears unevenly distributed, concentrated at the vegetative pole and the cytoplasm with the organelles is concentrated in the animal pole (Ribeiro *et al*., 1995). The eggs of *Brycon orbignyanus*, similar to those of other *Brycon* species, can also be classified as such (Romagosa *et al*., 2001; Andrade-Talmelli *et al*., 2001; Faustino *et al*., 2015; Sampaio *et al*., 2015).

The *B. orbignyanus* eggs had a wide perivitelline space, which was also observed in other species of teleost fish (Sampaio *et al*., 2015; Marques *et al*., 2017). However, Valentin *et al*. (2015) and Santos *et al*. (2016) reported that *Betta splendens* and *Astyanax altiparanae* eggs hydrate very little, while presenting a small perivitelline space. The size of perivitelline space depends on the reproductive characteristics of each species (Paes *et al*., 2014), the environment in which the eggs develop and the functions attributed to it such as mechanical protection, osmotic regulation, nutrition and prevention to polyspermia (Laale, 1980).

Embryogenesis extends from fertilization to hatching and, for teaching purposes, is usually divided into stages such as cleavage, blastula, gastrula and segmentation (Valentin *et al*., 2015; Santos *et al*., 2016; Marques *et al*., 2017). In *B. orbignyanus*, the first cleavage divided the egg cell into two same size blastomeres that appeared indistinguishable, similar to *Danio rerio* (Kimmel *et al*., 1995) and *Zungaru jahu* (Marques *et al*., 2017). According to Castellani *et al.* (1994), the number of blastomeres increased as their size decreased during cleavage, a fact also observed in *B. orbignyanus*. The first cleavage in *B. orbignyanus* started at the centre of the

https://doi.org/10.1017/S0967199418000229 Published online by Cambridge University Press

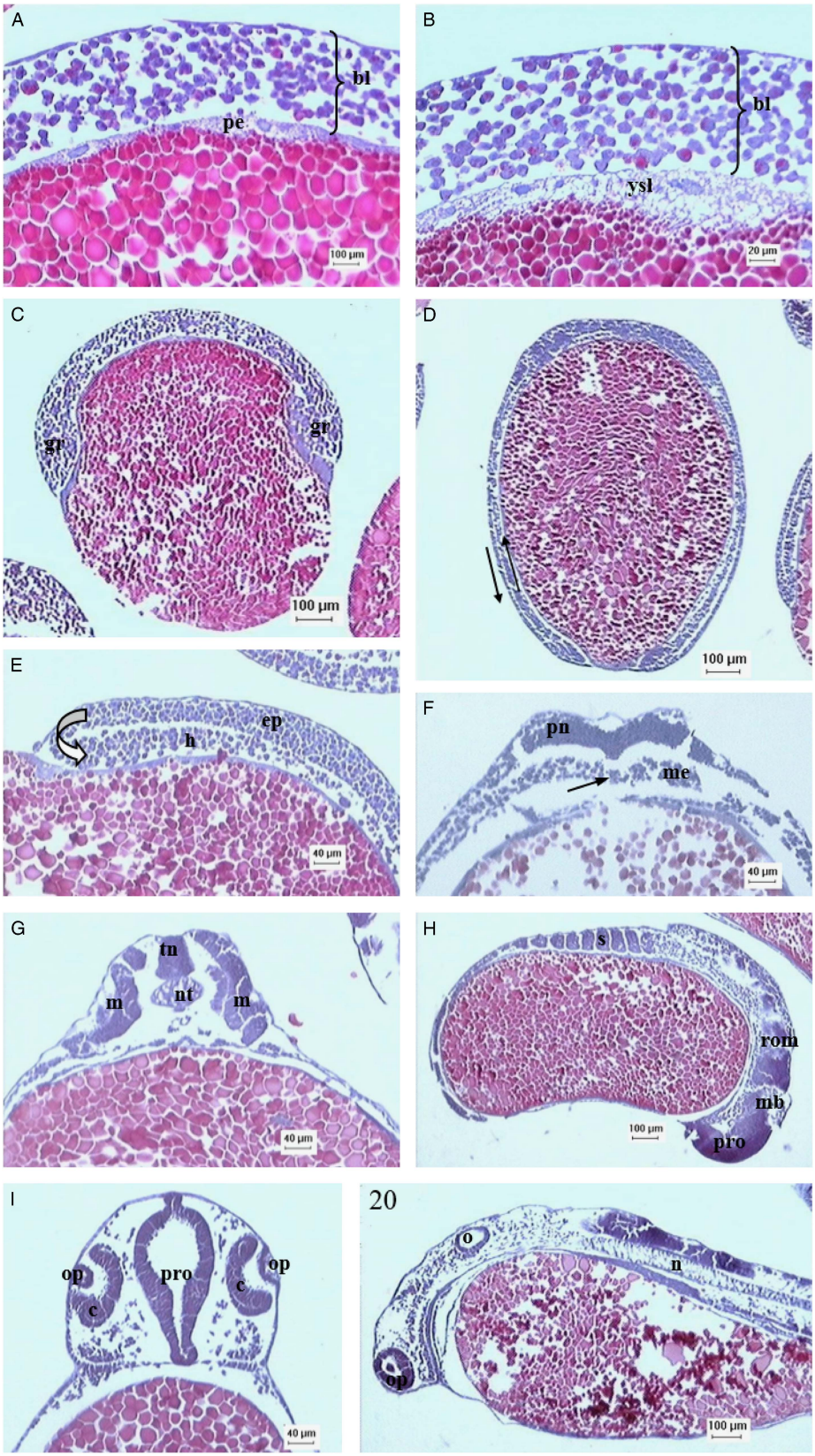


Figure 2. Photomicrographs of eggs/embryo of *B. orbignyanus*. (*A*) Stage 9, time '4 h', egg in the blastula phase, evidencing periblast (pe) and blastoderm (bl). (*B*) Stage 9, time '4 h', showing the formation of the yolk syncytial layer (ysl) and the blastoderm (bl). (*C*) Stage 9, time '4 h', showing the blastula with irregular spaces between blastoderm cells and formation of the germ ring (gr). (*D*) Stage 9, time '6 h', showing gastrula at the end of epiboly, highlighting the direction of epiboly (l) (up arrow) and migration (down arrow) (┌) movements. (*E*) Stage 9, time '5 h', evidencing the migration movement (arrow) performed by the blastoderm cells to form the epiblast (ep) and hypoblast (h). (*F*) Stage 9, time '7 h', highlighting the formation of a furrow in the neural plate (pn), formed above the notochord (arrow). The mesendoderm (me) is present. (*G*) Stage 10, time '10 h', showing neural tube (tn), notochord (n) and para-axial mesoderm (m). (*H*) Stage 10, time '8 h', highlighting the presence of somites (s) and formation of forebrain (pro), midbrain (mes) and rhombencephalon (rom). (*I*) Stage 11, time '11 h', showing optic or crystalline vesicle (op), optic cup (c) and forebrain (pro). (*J*) Stage 11, time '12 h', highlighting the optic vesicle (op), optic (o) and notochord (n).

https://doi.org/10.1017/S0967199418000229 Published online by Cambridge University Press

blastodisc, extending laterally and deepened to divide the cell completely, similar to *Alosa sapidissima* (Shardo, 1995).

In the blastoderm stage, irregular spaces formed between the blastoderm cells, a fact also observed in zebrafish by Kimmel *et al.* (1995). These authors suggest that this stage is better defined as 'stereoblastula' as it does not present a typical blastocoele.

In the blastula stage, formation of the periblast and the YSL was observed. The YSL is a cytoplasmic continuous layer, located between the blastoderm disc and the yolk, resulting from the incomplete division of the blastomeres (Ninhaus-Silveira *et al.*, 2007). It is noteworthy that the appearance of periblast in teleosts can occur at the morula stage, as reported for *Z. jahu* by Marques *et al.* (2017) or at the blastula stage (Kimmel *et al.*, 1995).

At the end of blastula and beginning of gastrula stages, epiboly movement was evidenced in *B. orbignyanus* eggs. This movement was accompanied by the appearance of a germ ring at the marginal border of the blastoderm, and consequent formation of the embryonic shield. Similar events have been observed in *Oryzias latipes* (Iwamatsu, 1994), *Danio rerio* (Kimmel *et al.*, 1995) and *Alosa sapidissima* (Shardo, 1995). As previously mentioned, in *D. rerio*, *O. latipes* and *A. sapidissima*, formation of the embryonic shield has a beginning delimited by the dorsal lip of the blastoderm, whereas in *B. orbignyanus* it was not possible to observe beginning of formation of the germ shield through a region delimited such as the dorsal lip.

In *B. orbignyanus*, when epiboly reached 50% coverage, cells migrated forming a fold that projected below and opposite the epiboly movement of the blastoderm, therefore forming the epiblast and the hypoblast. The epiblast corresponds to the definitive ectoderm, which gives rise to the epidermis, central nervous system, neural crest and sensory placodes (Kimmel *et al.*, 1995; Shardo, 1995). The hypoblast gives rise to a joint layer described as mesoderm and endoderm (Kimmel *et al.*, 1995; Shardo, 1995). Thisse *et al.* (1993) named this tissue the mesendoderm, emphasizing that only two embryonic layers are present in the gastrula; how this leaflet is subdivided into mesoderm and endoderm remains unknown (Kimmel *et al.*, 1995).

The central nervous system in *B. orbignyanus* rising from the ectoderm coincides with the formation of this organ system in the other vertebrates (Iwamatsu, 1994; Kimmel *et al.*, 1995; Shardo, 1995). The mesodermal tissues that appeared on both sides of the notochord during the gastrula were organized into segments called somites, similar to those described in *Danio rerio* (Kimmel *et al.*, 1995); *Oreochromis niloticus* (Morrison *et al.*, 2001) and *Oryzias latipes* (Iwamatsu, 1994).

In *B. orbignyanus*, the anterior region of the neural tube expanded to form the forebrain, mesencephalon, and rhombencephalon regions, similar to that described for *Z. jahu* (Marques *et al.*, 2017) and *P. lineatus* (Ninhaus-Silveira *et al.*, 2006), emphasizing that in these species the optic primordia developed at the beginning of the prosencephalon formation.

Larvae began to hatching after 13 h, when intense tail movement was observed, culminating with rupture of the chorion. According to Zhang *et al.* (2009), hatching of many bonefish occurs when glands start to secrete proteolytic enzymes that are associated with tail and body movements.

Chardon *et al.* (2003) stated that rapid embryonic development is typical of migratory species with seasonal reproductive strategies, high fertility and no parental care. Embryonic development in *B. orbignyanus* consisted of events that have already been described for other tropical teleosts, but differed regarding duration and some species-specific characteristics.

Vandewalle *et al.* (2005) stated that rapid embryonic development is typical of migratory species with seasonal reproductive strategies, high fertility and no parental care. Embryonic development of *B. orbignyanus* was divided into 11 stages that consisted of events already described for other tropical teleosts, but differ regarding duration and some species-specific characteristics.

Acknowledgements. The authors thank Buriti Fisheries (Nova Mutum, MT, Brazil) for providing the eggs and fish and to Orandi Mateus, histo-technician from the Histology and Embryology Laboratory of FCAV/UNESP, Jaboticabal-SP, Brazil, for his help in material processing.

Financial support. The authors would like to thank the National Council for Scientific and Technological Development – CNPq for their financial support.

Conflicts of interest. There are no conflicts of interest.

References

Andrade-Talmelli EF, Kavamoto ET, Romagosa E and Fenerich-Verani N (2001) Embryonic and larval development of the 'piabanha', *Brycon insignis*, Steindachner, 1876 (Pisces, Characidae). *Bol Inst Pesca* **27**, 21–8.

Botero M, Fresneda A, Montoya AF and Olivera MA (2004) Descripción del desarrollo embrionário de zigotos híbridos obtenidos por el cruce de machos de Cachama Blanca (*Piaractus brachypomus*) y hembras de Cachama Negra (*Colossoma macropomum*). *Rev Colomb Ciências Pec* **17**, 38–45.

Carolsfeld J, Harvey B, Ross C and Baer A (2004) *Migratory Fishes of South America – Biology Fisheries and Conservation Status*. World Fisheries Trust, Victoria, Canada, 372 pp.

Castellani LR, Tse HG, Leme dos Santos HS, Faria RHS and Santos MLS (1994) Desenvolvimento embrionário do curimbatá *Prochilodus lineatus* (VALENCIENNES, 1836) (Cypriniformes, Prochidontidae). *Rev Bras Ciências Morfol* **11**, 99–105.

Chardon M, Parmentier E and Vandewalle P (2003) *Morphology, development and evolution of the Weberian apparatus in catfish*. In *Catfishes* (eds G Arratia, BG Kappor, M Chardon and R Diogo), pp. 72–120. Enfield, CT: Scientific Publisher.

Faustino F, Nakaghi LSO, Marques C, Makino LC and Senhorini JA (2007) Fertilização e desenvolvimento embrionário: morfometria e análise estereomicroscópica dos ovos dos híbridos de surubins (pintado, *Pseudoplatystoma corruscans* × cachara, *Pseudoplatystoma fasciatum*). *Acta Sci* **29**, 49–55.

Faustino F, Makino LC, Neumann E and Nakaghi LSO (2015) Morphological and morphometric aspects of early life stages of piabanha *Brycon gouldingi* (Characidae). *J Fish Biol* **86**, 1491–506.

Flores J.C.B, Araiza M.A.F and Valle M.R.G (2002) Desarrollo embrionário de *Ctenopharyngodon indellus* (Carpa herbívora). CIVA 2002 (http://www.civa2002.org), 792–7.

Iwamatsu T (1994) Stages of normal development in the medaka *Oryzias latipes*. *Zool Sci* **11**, 825–39.

Kimmel CB, Ballard WW, Kimmel SR and Ullmann B (1995) Stages of embryonic development of the zebrafish. *Dev Dyn* **203**, 253–310.

Laale W.H (1980) The perivitelline space and egg envelopes of bony fishes: a review. *Copeia* **2**, 210–26.

Maria AN, Ninhaus-Silveira A, Órfão LH and Viveiros ATM (2017) Embryonic development and larval growth of *Brycon nattereri* Günther, 1864 (Characidae) and its implications for captive rearing. *Zygote* **25**, 711–8.

Marques C, Faustino F, Bertolucci B, Paes MCF, Silva R.C and Nakaghi L.S.O (2017) Embryonic development in *Zungaro jahu*. *Zygote* **25**, 17–31.

Morrison CM, Miyake T and Wright JR (2001) Histological study of development of the embryo and early larva of *Oreochromis niloticus* (Pisces, Cichlidae). *J Morphol* **247**, 172–95.

Nakatani K, Agostinho AA, Baumgartner G, Bialetzki A, Sanches PV and Cavicchioli M (1999) *Ovos e larvas de peixes de água doce, desenvolvimento e manual de identificação*. Maringá: UEM, Nupelia, 359 pp.

https://doi.org/10.1017/S0967199418000229 Published online by Cambridge University Press

Neumann E, Nakaghi LSO, Braga FMS, Faustino F and Mendes JMRM (2013) Moments of induced spawning and embryonic development of *Brycon amazonicus* (Teleostei, Characidae). *Zygote* **22**, 549–57.

Ninhaus-Silveira A, Foresti F and Azevedo A (2006) Structural and ultrastructural analysis of embryonic development of *Prochilodus lineatus* (Valenciennes, 1836) (Characiformes; Prochilodontidae). *Zygote* **14**, 217–29.

Ninhaus-Silveira A, Foresti F, Azevedo A, Agostinho CA and Veríssimo-Silveira R (2007) Structural and ultrastructural characteristics of the yolk syncytial layer in *Prochilodus lineatus* (Valenciennes, 1836) (Teleostei; Prochilodontidae). *Zygote* **15**, 267–71.

Oliveira DJ, Ashikaga FY, Foresti F and Senhorini JA (2017) Conservation status of the 'Piracanjuba' *Brycon orbignyanus* (Valenciennes, 1850) (Characiformes, Bryconidae): Basis for Management Programs. *Biod Bras* **1**, 18–33.

Paes MCF, Silva RC, Nascimaneto NF, Valentin FN, Senhorini JA and Nakaghi LSO (2014) Hatching, survival and deformities of piracanjuba (*Brycon orbignyanus*) embryos subjected to different cooling protocols. *Cryobiology* **69**, 451–6.

Panarari-Antunes RS, Prioli AJ, Prioli SMAP, Galdino AS, Julio HF Jr and Prioli LM (2011) Genetic variability of *Brycon orbignyanus* (Valenciennes, 1850) (characiformes: Characidae) in cultivated and natural populations of the upper paraná river, and implications for the conservation of the species. *Braz Arch Biol Technol* **54**, 1–10.

Ribeiro CR, Leme dos Santos HS and Bolzan AA (1995) Estudo comparativo da embriogênese de peixes ósseos (Pacu, *Piaractus mesopotamicus*; Tambaqui, *Colossoma macropomum* e híbrido tambacu). *Rev Brasil Biol* **55**, 65–78.

Reynalte-Tataje D, Zaniboni-Filho E and Esquivel JR (2004) Embryonic and larvae development of piracanjuba, *Brycon orbignyanus* Valenciennes, 1849 (Pisces, Characidae). *Acta Sci* **26**, 67–71.

Romagosa E, Narahara MY and Fenerich-Verani N (2001) Stages of embryonic development of the 'matrinxã', *Brycon cephalus* (Pisces, Characidae). *Bol Inst Pesca* **27**, 29–32.

Sampaio KH, Prado PS, Sato Y, Bazzoli N and Rizzo E (2015) Comparative morphology of the oocyte surface and early development in four characiformes from the São Francisco River, Brazil. *J Morphol* **276**, 1258–72.

Sanches PV, Nakatani K and Bialetzki A (1999) Morphological description of the developmental stages of *Parauchenipterus galeatus* (Linnaeus, 1766) (Siluriformes, Auchenipteridae) on the floodplain of the upper Paraná river. *Rev Brasil Biol* **59**, 429–38.

Santos MP, Yasui GS, Xavier PL, Macedo ANS, Nascimento NF, Fujimoto T, Senhorini JA and Nakaghi LSO (2016) Morphology of gametes, post-fertilization events and the effect of temperature on the embryonic development of *Astyanax altiparanae* (Teleostei, Characidae). *Zygote* **24**, 795–807.

Shardo JD (1995) Comparative embryology of teleostean fishes. I. Development and staging of the American Shad, *Alosa sapidissima* (Wilson, 1811). *J Morphol* **225**, 125–67.

Silva RC, Santos MP, Senhorini JA, Paes MCF, Valentin FN, Fujimoto T, Nascimento NF, Yasui GS and Nakaghi LSO (2017) The effect of temperature on the initial development of *Brycon amazonicus* Spix & Agassiz, 1829 as tools for micromanipulation of embryos. *Zygote* **5**, 637–51.

Thisse C, Thisse B, Schiling TF and Postlethwait JH (1993) Structure of the zebrafish snail1 gene and its expression in wild-type, spade tail and no tail mutant embryos. *Development* **119**, 1203–15.

Valentin FN, Nascimento NF, Silva RC, Fernandes JBK, Giannecchini LG and Nakaghi L.S.O (2015) Early development of *Betta splendens* under stereomicroscopy and scanning electron microscopy. *Zygote* **23**, 247–56.

Vandewalle P, Germeau G, Besancenet P, Parmentier E and Baras E (2005) Early development of the head skeleton in *Brycon moorei* (Pisces, Ostariophysi, Characidae). *J Fish Biol* **66**, 996–1024.

Vazzoler AEAM (1996) *Reproductive Biology of Teleosteos Fish: Theory and Practice*. Maringá: Eduem, 169 pp.

Zhang S, Bhattacharya H and Li H (2009) Embryogenesis and development. In *Reproductive Biology and Phylogeny of Fishes (Agnathans and Bony Fishes)* (ed. BGM Jamieson), pp. 485–511. Enfield, NH, USA: CRC Press.

https://doi.org/10.1017/S0967199418000229 Published online by Cambridge University Press