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Structural and ultrastructural analysis of embryonic development of *Prochilodus lineatus* (Valenciennes, 1836) (Characiforme; Prochilodontidae)

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Summary

This survey was performed to characterize the embryogenesis of *Prochilodus lineatus*. Seven stages of embryo development were identified – zygote, cleavage, blastula, gastrula, segmentation, larval and hatching – after a period of incubation of 22 h (24 °C) or 14 h (28 °C). The following cleavage pattern was identified: the first plane was vertical (2 blastomeres); the second was vertical and perpendicular to the first (4 blastomeres); the third was vertical and parallel to the first (4 × 2); the fourth cleavage was vertical and parallel to the second (4 × 4); the fifth was vertical and parallel to the first (4 × 8); and the sixth cleavage was horizontal (64 blastomeres). At the blastula stage (3.0–4.0 h (24 °C); 1.66–2.0 h (28 °C)) irregular spaces were detected and periblast structuring was initiated. At the gastrula stage (4.0–8.0 h (24 °C); 3.0–6.0 h (28 °C)) the epiboly, convergence and cell movements, as well as the formation of embryonic layers, had begun. The segmentation stage (10.0–15.0 h (24 °C); 7.0–10.0 h (28 °C)) was characterized by a rudimentary formation of organs and systems (somites, optic vesicle and intestinal delimitation). The embryo at the larval stage (16.0–21.0 h (24 °C); 11.0–13.0 h (28 °C)) showed a free tail, more than 25 somites, an optic vesicle and a ready-to-hatch larval shape. The blastomeres at cleavage stage had disorganized nuclei indicating high mitotic activity. At gastrula, the blastomeres and the periblast had euchromatic nuclei and a large number of mitochondria and vesicles. The yolk was organized into globose sacs, which were dispersed into small pieces prior to absorption.

Keywords: Characiforme, Embryo, Embryogenesis, *Prochilodus lineatus*, Teleost

Introduction

The 'curimbata', *Prochilodus lineatus*, a member of the family Prochilodontidae, is an iliophagous fish species with wide distribution over southeastern Brazil that undergoes reproductive migration and total spawning (Fowler, 1951). According to Corrêa & Castro (1990), this species is recorded along the entire Paraná–Paraguay and Paraiba river basins. It represents an economically and ecologically important native species of medium to large size.

Knowledge of the embryonic development of different fish species is useful to the management of fishery resources, as well as to surveys related to fish culture, since it provides additional information about species' life cycles. In addition, embryological analysis is helpful in studies about evolutionary relationships, heredity, developmental mechanisms and environmental influences over structural features of distinct organisms (Lagler, 1959).

The embryonic development of fishes is a complex phenomenon, useful for ontogeny studies, experimental modelling, and evaluation of environmental quality and effects of toxic substances on aquatic fauna (Flores *et al.*, 2002), as well as for experiments on *ex situ* species preservation.

Information about the embryology of the curimbata is scarce and restricted to reports such as those by

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Table 1 Embryonic development of *Prochilodus lineatus* at 24 °C

Time (h)	Stage	Description
0–0.75	Zygote	Cytoplasm streams towards the animal pole to form the blastodisc
1.0	Cleavage	50% with 2 cells; 36% with 4 cells; 12% with 8 cells; 2% with 16 cells
1.25	Cleavage	42% with 4 cells; 52% with 5–7 cells; 6% with 8 cells
1.5	Cleavage	2% with 4 cells; 6% with 6 cells; 46% with 8 cells; 10% with 12–14 cells; 15% with 10 cells; 24% with 16 cells
1.75	Cleavage	75% with 16 cells; 25% with 16+ cells
2.0	Cleavage	5% with 16 cells; 95% with 32 cells
2.25	Cleavage	5% with 32 cells; 75% with 64 cells
3.0	Blastula	100% morula
4.0	Blastula; Gastrula	75% morula; 25% gastrula
5.0	Gastrula	100% gastrula (25% epiboly)
6.0	Gastrula	75% gastrula (25% epiboly); 25% gastrula (50% epiboly)
7.0	Gastrula	10% gastrula (25% epiboly); 50% gastrula (50% epiboly); 40% gastrula (75% epiboly)
8.0	Gastrula	20% gastrula (50% epiboly); 80% gastrula (75% epiboly)
9.0	Gastrula	100% epiboly, neural tube
10.0	Segmentation	100% neurula
11.0	Segmentation	4 somites
12.0	Segmentation	100%, otic vesicle, 9 somites and tail attached
13.0	Segmentation	100%, Kupffer and optic vesicles, 13 somites and tail attached
14.0	Segmentation	100%, Kupffer and optic vesicles, 19 somites and tail attached
15.0	Segmentation	100%, optic and otic vesicles, 22 somites and tail attached
16.0	Larval	100%, optic and otic vesicles, 25+ somites and free tail
17.0	Larval	100%, optic and otic vesicles, 30+ somites and free tail
18.0–21.0	Larval	100%, embryo growth
22.0	Hatching	100% hatching

Table 2 Embryonic development of *Prochilodus lineatus* at 28 °C

Time (h)	Stage	Description
0–0.34	Zygote	Cytoplasm streams towards the animal pole to form the blastodisc
0.50	Cleavage	100% with 2 cells
0.66	Cleavage	10% with 2 cells; 90% with 4 cells
0.84	Cleavage	100% with 8 cells
1.00	Cleavage	15% with 8 cells; 85% with 16 cells
1.16	Cleavage	10% with 16 cells; 90% with 32 cells
1.34	Cleavage	100% with 64 cells
1.50	Blastula	64+ cells (morula)
1.66	Blastula	100% morula
1.84	Blastula	100% morula
2.00	Blastula	100% morula
3.00	Gastrula	100% gastrula (25% epiboly)
4.00	Gastrula	100% gastrula (50% epiboly)
5.00	Gastrula	100% gastrula (75% epiboly); early neural tube
6.00	Gastrula	10% gastrula (100% epiboly); 90% gastrula (90% epiboly)
7.00	Segmentation	35% with 4 somites; 65% neurula
8.00	Segmentation	13 somites; optic and Kupffer vesicles and tail attached
9.00	Segmentation	19 somites, optic and Kupffer vesicles and tail attached
10.00	Segmentation	24 somites, optic and otic vesicles, Kupffer vesicle missing and free tail
11.00	Larval	28 somites, optic and otic vesicles and free tail
12.00	Larval	30+ somites; embryo growth
13.00	Larval	100%, embryo growth
14.00	Hatching	100% hatching

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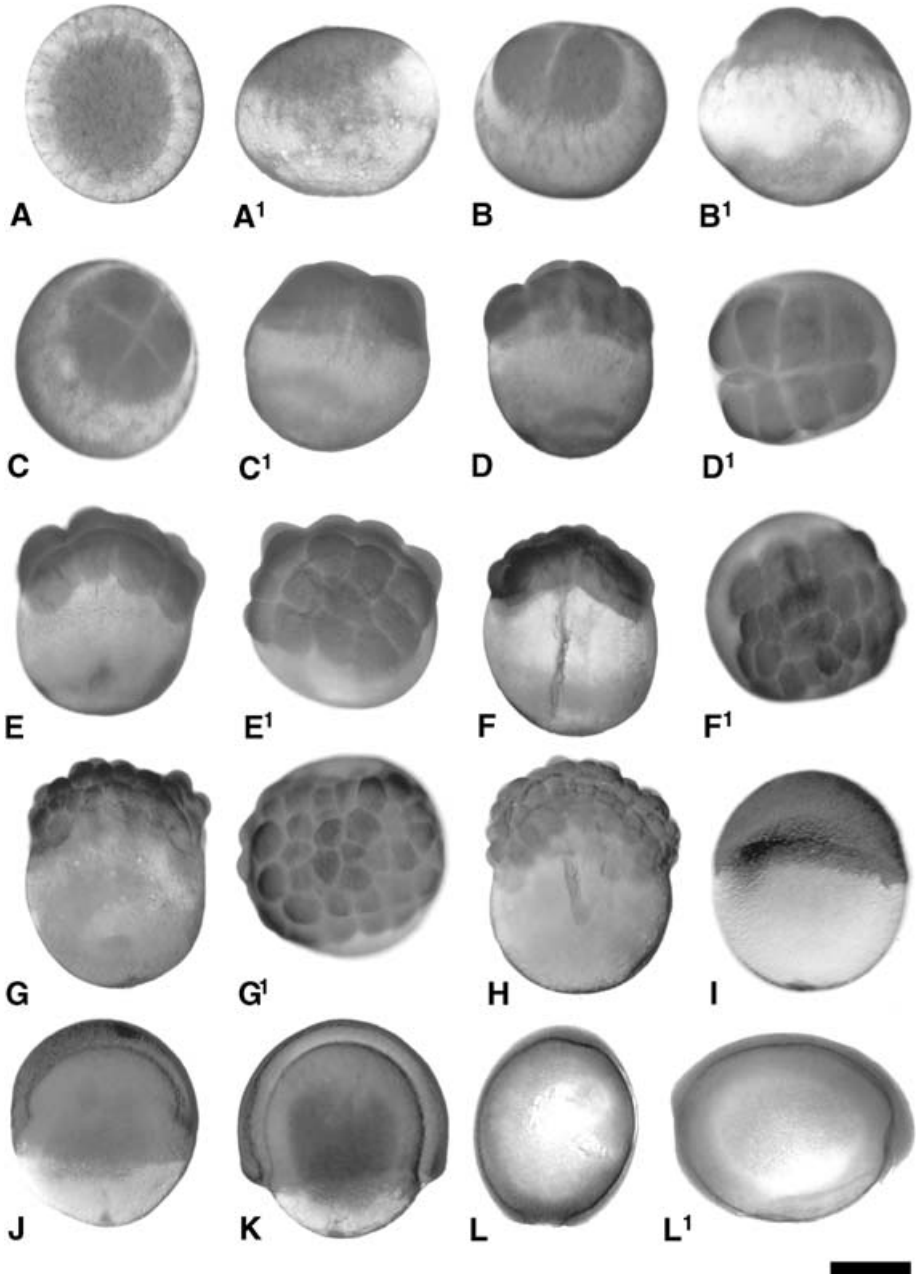

Figure 1 Phases of the embryonic development of *Prochilodus lineatus*. (*A*), (*A′*) Post-fertilization without chorion; (*B*), (*B′*) 2-cell embryo; (*C*), (*C′*) 4-cell embryo; (*D*), (*D′*) 8-cell embryo; (*E*), (*E′*) 16-cell embryo; (*F*), (*F′*) 32-cell embryo; (*G*), (*G′*) 64-cell embryo; (*H*) morula; (*I*) gastrula (25% epiboly); (*J*) gastrula (50% epiboly); (*K*) gastrula (75% epiboly); (*L*), (*L′*) gastrula (90%). Scale bars represent 113.6 μm.

Brazil *et al.* (2002), which studied egg morphology modifications just after fertilization, and that of Castellani *et al.* (1994), who carried out observations of the embryonic development of *P. lineatus* under the light microscope.

Therefore, given on the ecological and economic value of *Prochilodus lineatus*, the present work was performed to analyse the morphological events during the embryonic development of this species at the structural and ultrastructural levels.

Materials and methods

Adult individuals of *Prochilodus lineatus* from the broodstock at the Aquaculture Division of the Faculdade de Medicina Veterinária e Zootecnia, UNESP, Botucatu, São Paulo, Brazil were used to obtain embryos.

Prochilodus lineatus, like many other Brazilian freshwater species, requires an upstream migration to spawn (Godoy, 1975). In captivity, this species is not

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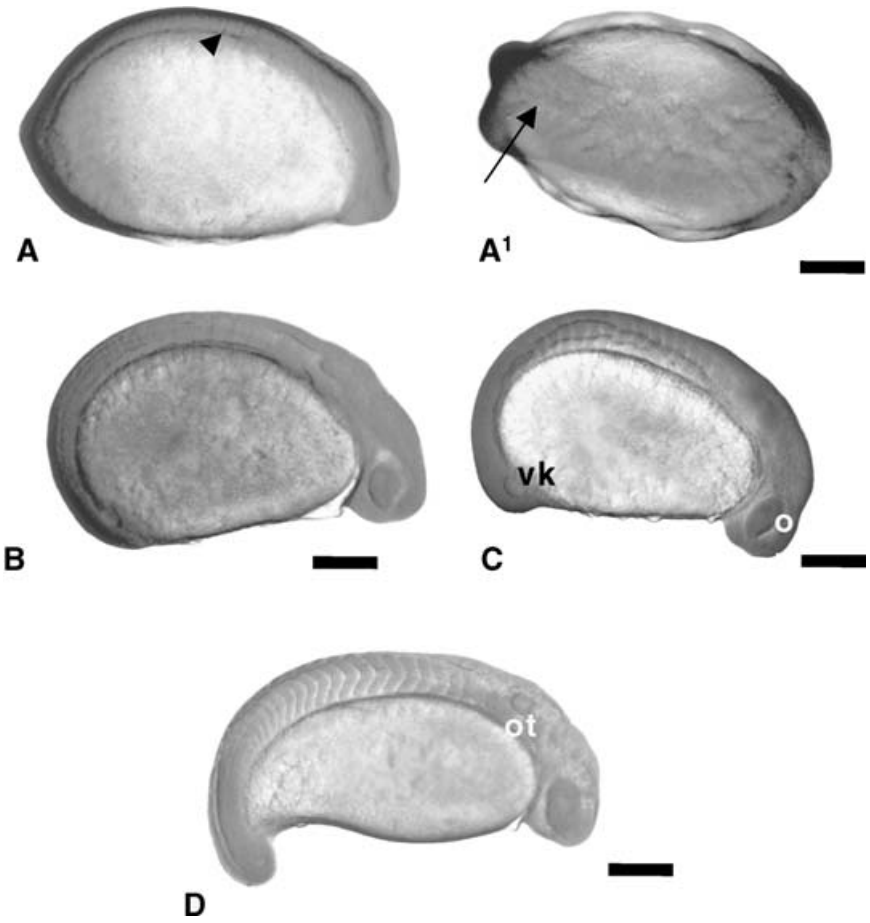

Figure 2 (*A*), (*A′*) Neurula; (*B*) in the presence of about 13 somites, optic vesicle, attached tail; (*C*) embryo bearing nearly 19 somites, optic vesicle, Kupffer's vesicle and attached tail; (*D*) 24 somites, presence of optic and otic vesicles, absence of Kupffer's vesicle and free tail. s, somite; arrow, neural keel; vk, Kupffer's vesicle; op, optic vesicle. Scale bars represent: (*A*), (*A′*) 55 μm; (*B*) 57.1 μm; (*C*) 70.4 μm; (*D*) 76.3 μm.

able to reproduce naturally and hormonal induction is necessary. Breeders were stimulated with carp pituitary extract by inoculating 3 mature females (3 years old) with 0.5 mg and 5 mg/kg body weight, respectively, at an interval of 10 h, and 6 mature males (2 years old) with 1 mg/kg body weight at the time of the second female inoculation. The extrusion of eggs and sperm was performed about 6 h after the last induction.

The dry method was employed for the fertilization process, in which the eggs are mixed with the sperm avoiding contact with water. After that, water was added in order to activate spermatozoa and allow egg hydration. The excess semen was rinsed off and the eggs were incubated in 200 l vertical incubators. To verify a putative effect of water temperature on embryo development, the eggs were divided into two groups incubated at different temperatures (24 °C and 28 °C). The incubators were connected to a closed heated water system coupled with a thermostat.

Embryonic development

To evaluate the possible temporal morphological variation of *P. lineatus* embryos, about 200 embryos were collected at different development stages, defining the moment of fertilization as time zero. The first samples were collected within intervals of 15 and 10 min for eggs incubated at 24 and 28 °C, respectively, until 2 h of embryonic development, while subsequent samples were taken at intervals of 1 h until the point of hatching. The embryo samples were fixed in a solution of 2% glutaraldehyde, 2% paraformaldehyde diluted in sodium phosphate buffer 0.1 M, pH 7.3, for 24 h prior to analyses.

Fifty prefixed embryos were selected for *in toto* analysis. The chorion was extracted using watchmaker's forceps and a needle, and then the embryos were stained with Harries haematoxylin–eosin (H&E) prior to being analysed and photographed using a stereomicroscope.

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Stuctural and ultrastructural analysis

Twenty representative individuals from each development stage were carefully selected and embedded in glycol methacrylate. These samples were submitted to microtomy to obtain serial transverse and longitudinal cuts of from 3 to 5 μm. After that, they were stained with Harries haematoxylin–eosin or toluidine blue and analysed and photographed using a Zeiss Axiophot photomicroscope.

The embryos were postfixed in 1% osmium tetroxide for 2 h, counterstained with an aqueous solution of 0.5% uranyl acetate, dehydrated with acetone and embedded in epoxy resin for analysis by transmission electron microscopy (TEM). The ultrafine sections were caught on a copper net, counterstained with uranyl acetate (Watson, 1958), washed in 50% alcohol and re-counterstained in lead citrate (Reynolds, 1963). The material was analysed and electromicrographed using a Philips CM100 transmission electron microscope.

For analyses by scanning electron microscopy (SEM), the embryos, prefixed in 2.5% glutaraldehyde, were transferred to a 13 mm coverslip, embedded with 1% poly-L-lysine, postfixed in 0.5% osmium tetroxide, dehydrated with ethanol and dried in a critical-point dryer (Balzers CPD-20). The samples were covered with a 10 mm gold pellicle in a Balzers Metalizer MED-010 and observed and electromicrographed using a Philips 515 scanning electron microscope.

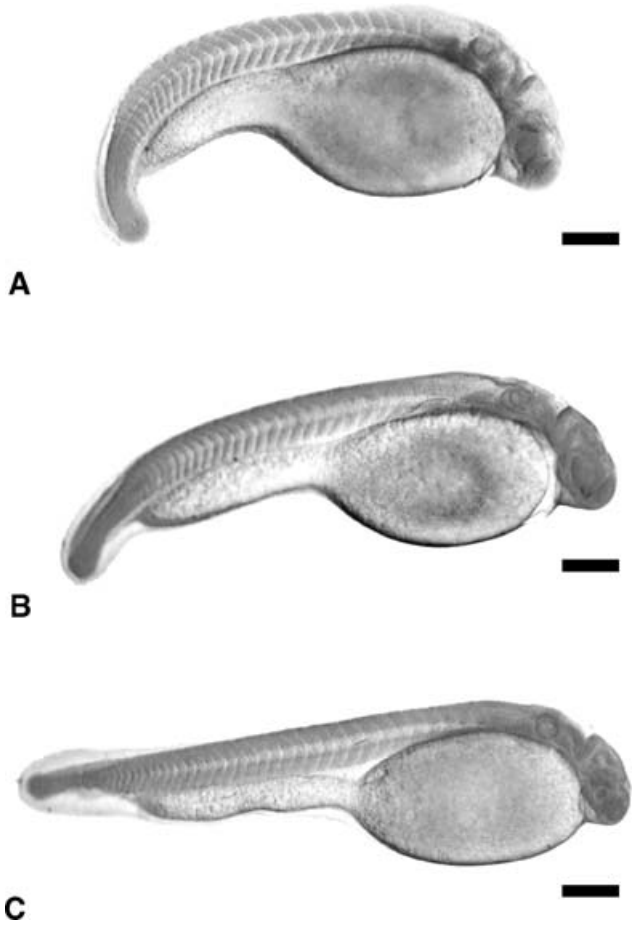

Figure 3 (*A*) 30+ somites, growing larva; (*B*) pre-hatchery embryo; (*C*) hatched embryo. Scale bars represent: (*A*) 63.3 μm; (*B*) 75.6 μm; (*C*) 69.4 μm.

Results

Embryogenesis

The duration of embryonic development in *Prochilodus lineatus*, from fertilization until hatching, has been shown to be dependent on the water temperature. At 24 °C, the incubation period was 22 h, and at 28 °C, it was 14 h. The following stages were identified in the embryonic development of *P. lineatus* after fertilization: zygote, cleavage, blastula, gastrula, segmentation, larval and hatching (Tables 1, 2; Figs. 1, 2, 3).

A higher heterogeneity in embryo development was observed at 24 °C, i.e. embryos at different stages of embryogenesis were detected at the same time, especially at the beginning of cleavage (1–1.5 h) (Fig. 4*A*). Furthermore, embryos displaying 5, 7, 10, 12 or 15 blastomeres were also found (Table 1; Fig. 4*A*, *B*). At 28 °C, embryo development was homogeneous, despite slight variations during the first cleavage phases (0.66–1.66 h) (Table 2; Fig. 4*C*, *D*).

1: Zygote stage (0–0.75 h (24 °C); 0–0.34 h (28 °C))
After fertilization, hydration of the eggs could be observed by the increase in the perivitelline space, pronuclear fusion, and cytoplasm reorganization with the establishment of vegetal and animal poles (Fig. 5*A*–*C*). The animal pole was composed of active cytoplasm and a nucleus, allowing *in vivo* and light microscopic identification, since it is slightly transparent. On the other hand, the vegetal pole was denser at *in vivo* observation and weakly stained in total preparations (Fig. 5*C*), being composed of yolk vesicles (Fig. 6*A*–*C*). Moreover, a thin layer of cytoplasm involving the whole yolk was observed, comprising several central alveoli reminiscent of the cortical reaction during fertilization (Fig. 6*A*).

2: Cleavage stage (1.0–2.25 h (24 °C); 0.50–1.34 h (28 °C))
The cleavage stage was characterized by the beginning of mitotic division, and it continued until the formation of irregular spaces among internal cells, which might be considered a kind of blastocoele. In *P. lineatus*, the cleavages are meroblastic and can be described as follows: the first cleavage plane was vertical, giving rise to 2 blastomeres; the second plane was vertical and

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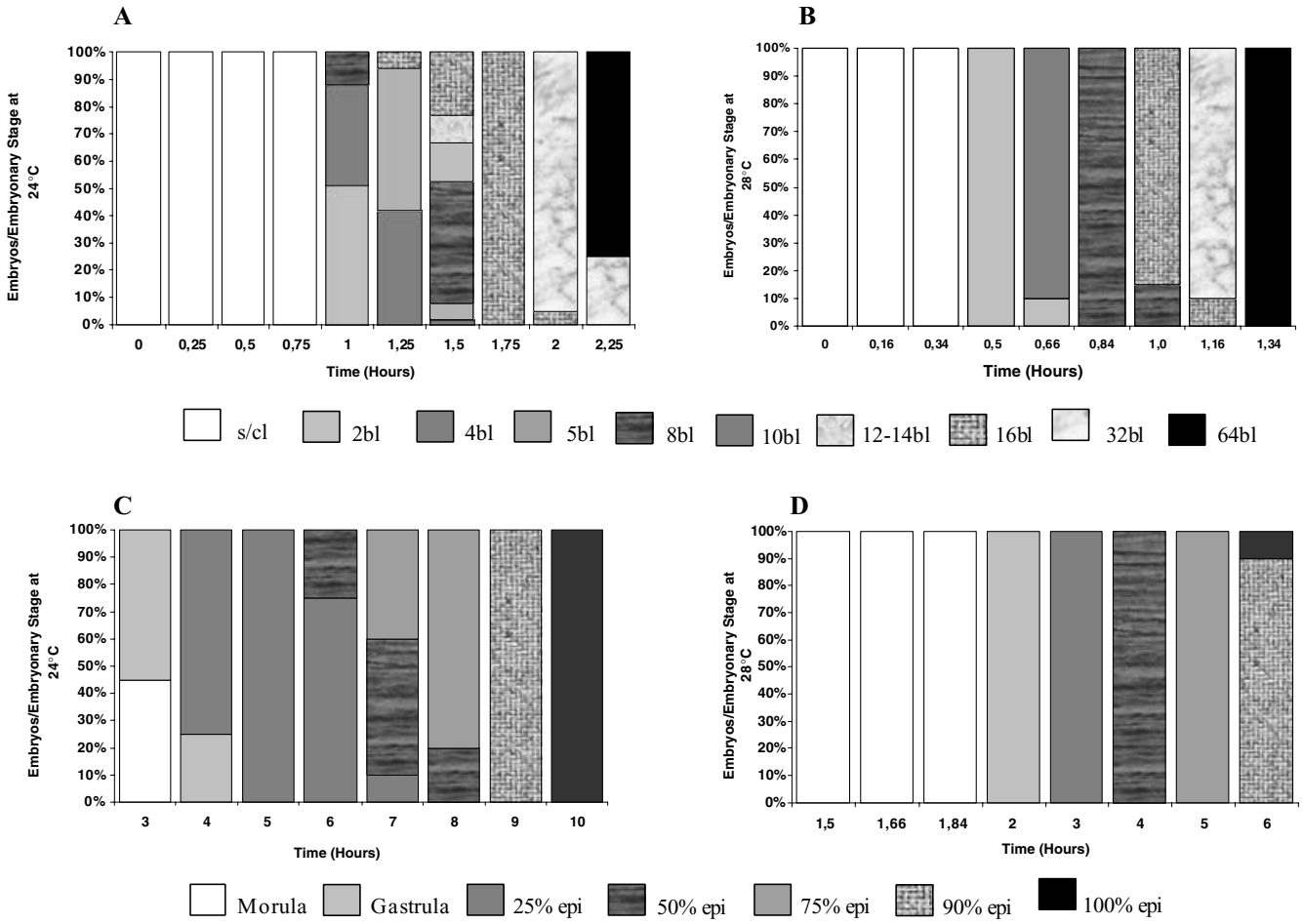

Figure 4 Analysis of the embryonic development of *Prochilodus lineatus* under two temperature conditions (24 °C and 28 °C). (*A*), (*B*) Segmentation period. (*C*), (*D*) Morphogenesis period. s/s, not segmented; bl, blastomeres; epi, epiboly.

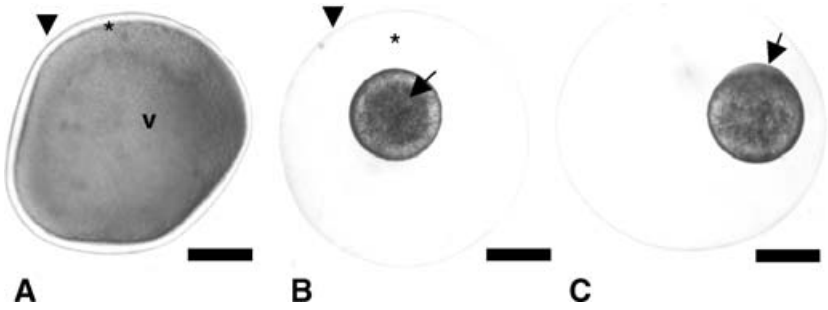

Figure 5 (*A*) Fertilized and non-hydrated egg (×129); (*B*), (*C*) hydrated egg, showing well-defined animal and vegetal poles (×55). arrowhead, chorion; ∗, perivitelline space; arrow, animal pole; v, yolk. Scale bars represent: (*A*) 77.5 μm; (*B*), (*C*) 181.8 μm.

perpendicular to the first, giving rise to 4 blastomeres; the third was vertical and parallel to the first, giving rise to 8 blastomeres displaying a 4 × 2 arrangement; the fourth was vertical and parallel to the second, originating 16 blastomeres in a 4 × 4 formation; the fifth plane was vertical and parallel to the first cleavage, originating 32 blastomeres in a 4 × 8 formation; and the sixth cleavage plane was horizontal, giving rise to two cell layers comprising 64 blastomeres (Fig. 8*A*–*H*).

For as long as the cleavages occur, the number of blastomeres increases and their size decreases. Until the third cleavage the cells keep their homogeneity and,

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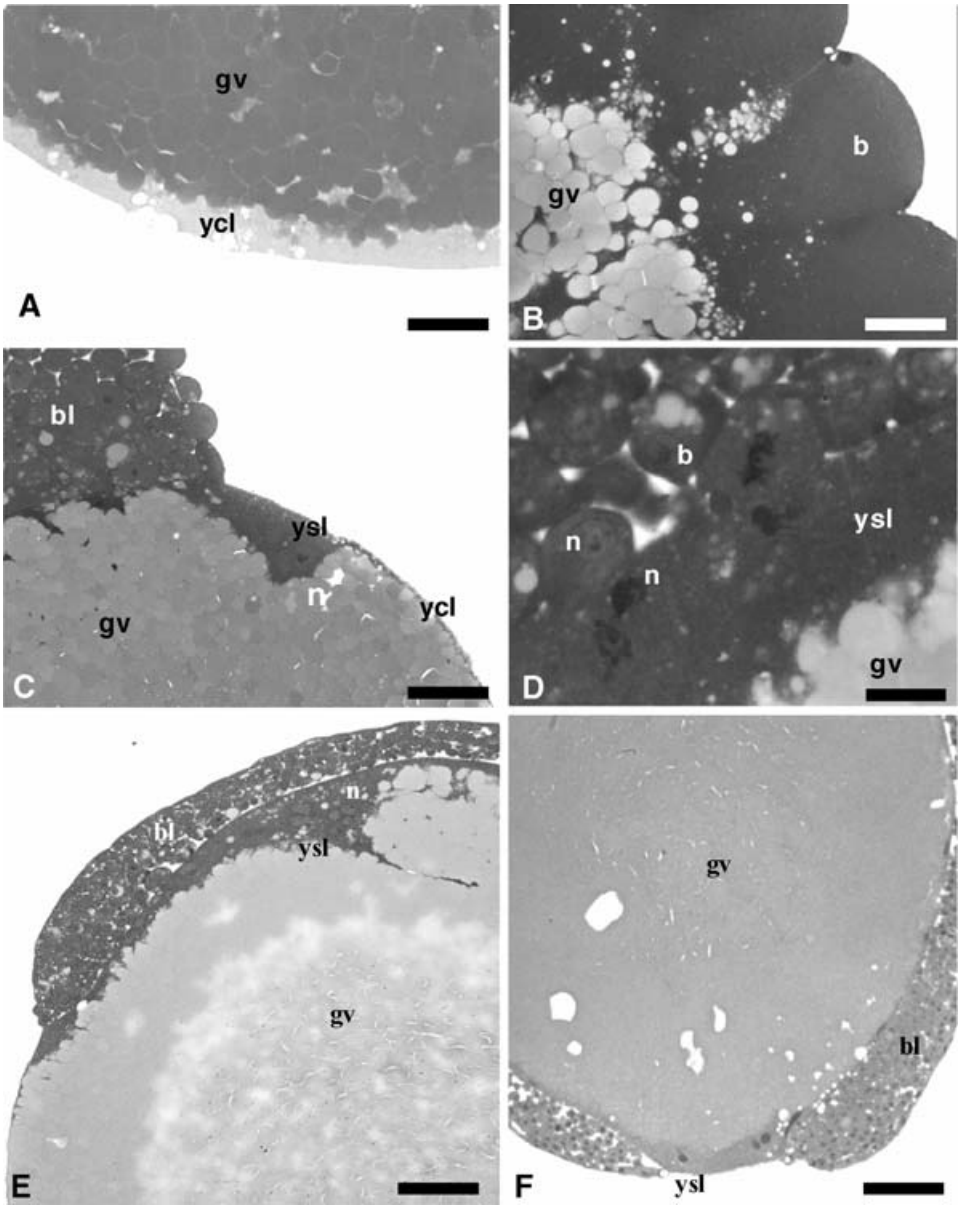

Figure 6 Analysis under a light microscope of embryos of *Prochilodus lineatus*, stained with basic toluidine blue. (*A*) 0.25 h after fertilization, showing the yolk cytoplasmic layer; (*B*) cleavage phase (1.5 h), revealing the penetration of yolk globules into blastomeres; (*C*) detail of the formation of the yolk syncytial layer in an embryo at the blastula stage; (*D*) 4.25 h of development (gastrula stage), characterized by the presence of blastomeres with euchromatic nuclei, yolk syncytial layer and high mitotic activity (MO); (*E*) embryo section at 50% of epiboly, stained with basic toluidine blue; (*F*) embryo section at 90% of epiboly, stained with H&E. gv, yolk globules; ycl, yolk cytoplasmic layer; b, blastomere; bl, blastoderm; ysl, yolk syncytial layer; n, nucleus; gv, yolk globules. Scale bars represent: (*A*) 11.5 µm; (*B*) 17.5 µm; (*C*) 16.7 µm; (*D*) 3.6 µm; (*E*) 80 µm; (*F*) 5.7 µm.

after the fourth cleavage, blastomeres of distinct sizes can be observed (Fig. 8*A*–*H*).

No distinctive layer was observed between the blastoderm and yolk. It was verified that yolk globules penetrate into blastomeres in a fragmented way, probably to facilitate their absorption by cells (Fig. 7*C*). Analyses under the light microscope showed that, during the cleavage stage, individualized nuclei were absent (Fig. 6*C*) while TEM showed blastomeres with a large number of mitochondria, euchromatic nuclei and free ribosomes (Fig. 7*B*, *C*), indicating a high cell metabolism, typical of high mitotic activity.

3: Blastula stage (3.0–4.0 h (24 °C); 1.66–2.0 h (28 °C))

At the beginning of the blastula stage, a dome-shaped blastoderm was present. The cells continuously

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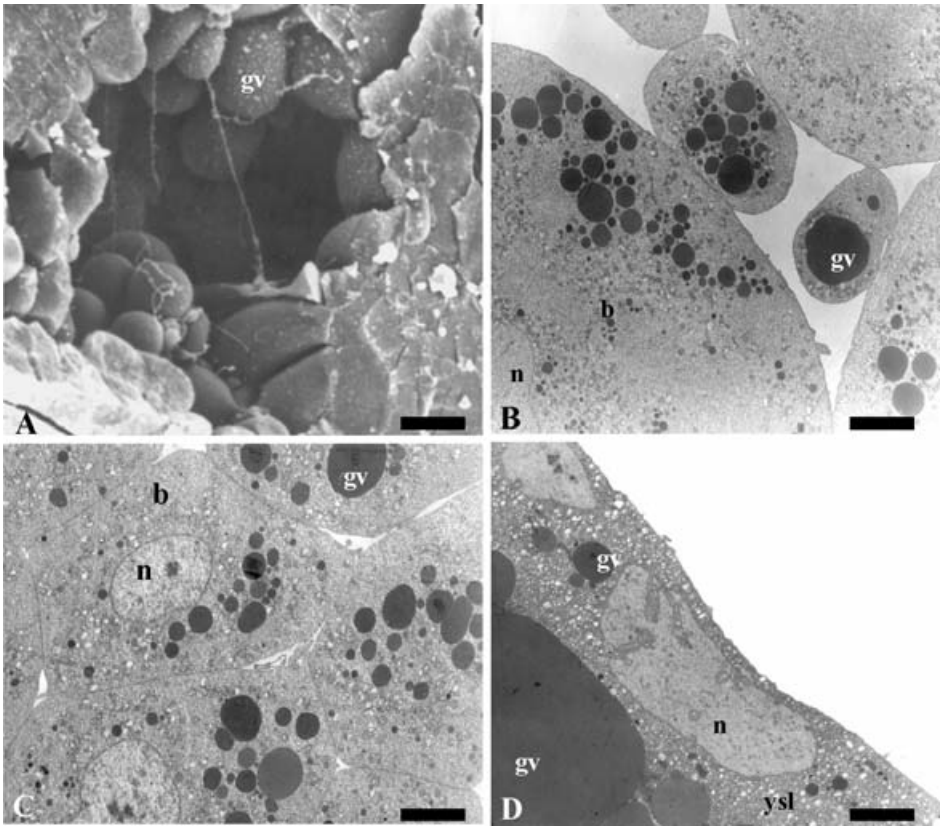

Figure 7 Analysis under an electron microscope of embryos of *Prochilodus lineatus*. (*A*) Embryo blastomeres at cleavage stage, showing euchromatic nucleus and a large number of yolk vesicles in the cytoplasm (TEM); (*B*) Detail of yolk globules (SEM); (*C*) Embryos of *P. lineatus* at the gastrula stage showing the periblast with euchromatic nucleus, cytoplasm with several vesicles, mitochondria and some yolk granules (TEM); (*D*) ultrastructure (TEM) showing irregular nuclei of the yolk syncytial layer and subjacent yolk globules. n, euchromatic nucleus; gv, yolk globules; b, blastomeres; ysl, yolk syncytial layer. Scale bars represent: (*A*) 6.1 μm; (*B*) 20.4 μm; (*C*), 3.8 μm; (*D*) 5.7 μm.

underwent divisions, but the cleavage planes were undetermined. As the number of cell increased, the blastoderm changed into a half-moon shape.

The main characteristics of this stage are the irregular spaces among blastomeres (blastocoele) and the beginning of the formation of a periblast or yolk syncytial layer (Fig. 7*D*, *E*). At the end of this stage, the first epiboly movements could be identified.

4: Gastrula stage (4.0–8.0 h (24 °C); 3.0–6.0 h (28 °C))

The gastrula stage was characterized by epiboly movement and the occurrence of morphogenetic movements of convergence and cell migration that give rise to the first layers and to the head–tail and latero-lateral embryonic axes.

The epiboly movement started after 4 h at 24 °C and after 3 h at 28 °C. It was observed as a fringe, formed by the yolk syncytial layer, across the blastoderm border, from its formation to the closure of the blastopore (Fig. 6*C*, *E*, *F*). The morphogenetic movements of convergence and cell migration began at the border of blastoderm, at about 50% of epiboly (Fig. 6*E*), originating the germ ring and the embryonic shield and culminating with the formation of the two embryonic layers, the epiblast and hypoblast.

The epiboly movement goes on alongside the closure of the yolk plug by the yolk syncytial layer, which is delimited by the blastopore (Fig. 6*F*), following the total recovery of the blastoderm plug.

Ultrastructural analyses demonstrated that the blastomere cytoplasm contains a large number of mitochondria and vesicles, several filled with yolk material. These yolk granules, prior to absorption by blastoderm cells, are fragmented at the periblast region. The blastomeres and periblast nuclei were euchromatic (uncondensed), indicating high metabolic activity (Fig. 7*C*, *D*).

5: Segmentation and organogenesis stage (10.0–15.0 h (24 °C); 7.0–10.0 h (28 °C))

The stage of segmentation and organogenesis was characterized by the formation of rudimentary organs and systems from the embryonic layers. Thus, the somites, the notochord and the neural tube, as well as

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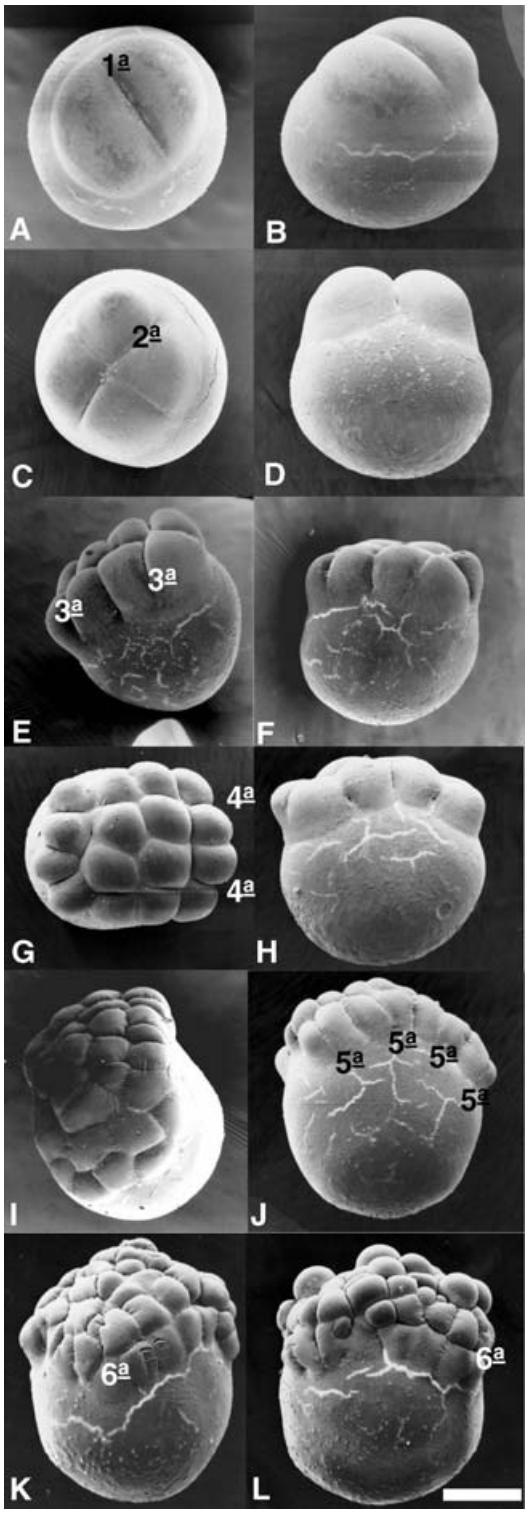

Figure 8 Observation of the first six cleavage planes in embryos of *Prochilodus lineatus* under a scanning electronic microscope. Scale bars represent 263.1 µm.

the initial delimitation of the intestines, were observed, leading to the subsequent growth and elongation of the embryo, particularly along the head–tail axis.

After 10 h of development at 24 °C and 7 h at 28 °C, it was possible to identify the neural keel, the neural plate, the mesendoderm notochord (Fig. 9*A*) and Kupffer's vesicle at the tail region (Fig. 9*B*), as well as segmented somites in some embryos (28 °C) (Fig. 2*A*).

In this phase, the formation of neural tube is initiated (Fig. 10*E*) and as long as its components show differential growth, it is possible to identify the prosencephalon, mesencephalon and rhombencephalon regions (Figs. 2*B*, 9*A*).

After 12 h of development at 24 °C and 8 h at 28 °C, the embryos already contained an optic vesicle and several somites (Fig. 9*D*, *F*). After 15 h at 28 °C and 10 h at 24 °C, the otic vesicle was present, as well as a complete neural tube (Fig. 9*C*, *D*), a rudimentary digestive system and free tail; in addition Kupffer's vesicle was absent (Fig. 2*D*).

6: Larval stage (16.0–21.0 h (24 °C); 11.0–13.0 h (28 °C))
A free tail, the presence of more than 25 pairs of somites and a ready-to-hatch larval shape, characterized the embryos at the larval stage. The embryos showed a well-developed optic calyx, crystalline lens and optic vesicle (Fig. 10*A*, *D*). The notochord extended from the cephalic region to the tail (Fig. 10*E*). The somites showed the beginning of the myogenesis process for the formation of muscles (Fig. 10*B*, *C*) and the posterior primitive intestine was well defined (Fig. 10*E*). Another feature of this stage is the occurrence of spasmodic movements, which increased as embryonic development proceeded.

7: Hatching (22.0–23.0 h (24 °C); 13.0–14.0 h (28 °C))
In the hatching phase the larvae demonstrated vigorous swimming movements, important for chorion rupture. Full larval hatching was observed after nearly 23 and 14 h of development at 24 and 28 °C, respectively (Tables 1, 2).

Discussion

The eggs produced by *Prochilodus lineatus* are pelagic (Nakatani *et al.*, 2001), non-adhesive, greenish, slightly transparent, and show a large perivitelline space after hydration. According to their quantity of yolk and its location, they can be classified as macrolecithal (containing a large amount of yolk) or telolecithal (yolk concentrated at the vegetal pole), while the cytoplasm and its organelles are concentrated at the animal pole. These features are similar to those described for species of the genus *Brycon* (Ganeco, 2003; Andrade-Talmelli *et al.*, 2001; Romagosa *et al.*, 2001; Eckmann, 1984).

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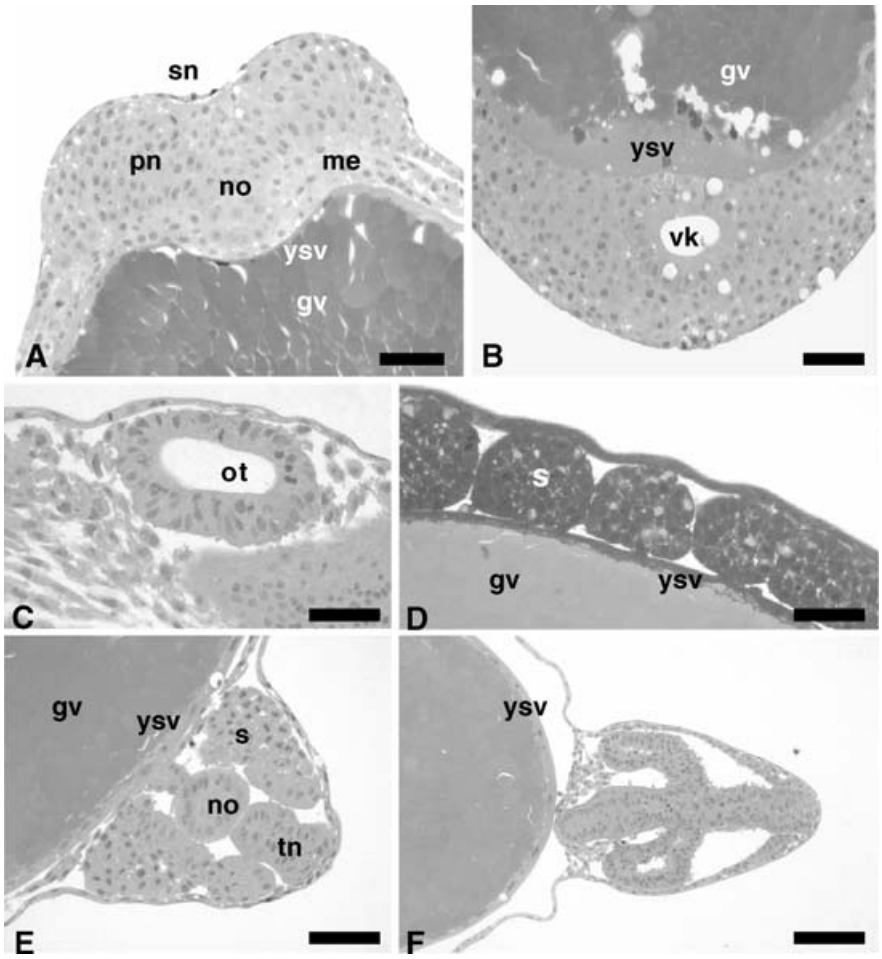

Figure 9 Details under a light microscope of embryos of *Prochilodus lineatus* at the segmentation stage. (*A*) Section showing the notochord, the neural keel, the mesendoderm and the neural plate (H&E); (*B*) detail of the structure of Kupffer's vesicle; (*C*) longitudinal section of the optic vesicle (H&E); (*D*) longitudinal section of somites (toluidine blue); (*E*) transverse section, detailing the notochord, somites and the neural tube (H&E); (*F*) longitudinal section detailing the presence of the optic vesicle (H&E). gv, yolk globules; ysl, yolk syncytial layer; no, notochord; ot, optic vesicle; tn, neural tube; s, somite; me, mesendoderm; pn, neural plate; sn, neural keel; vk, Kupffer's vesicle. Scale bars represent: (*A*) 7.5 μm; (*B*) 7.7 μm; (*C*) 9.1 μm; (*D*) 14.6 μm; (*E*) 22.5 μm; (*F*) 23.4 μm.

Fat droplets inside the yolk sac were absent in *P. lineatus*, similar to what has been observed in other Characiformes, such as *Brycon orbignyanus* (Ganeco, 2003) and *Brycon insignis* (Andrade-Talmelli *et al.*, 2001).

The morphological events identified during the embryogenesis of *P. lineatus*, as well as the short duration of embryonic development, were similar to those reported in other teleosts (Ganeco, 2003; Flores *et al.*, 2002; Andrade-Talmelli *et al.*, 2001; Cardoso *et al.*, 1995; Kimmel *et al.*, 1995; Ribeiro *et al.*, 1995).

The pattern of egg segmentation in vertebrates depends on the amount and distribution of yolk and its proportion in relation to the cytoplasm that composes the blastodisc (Gilbert, 1991). The cleavage of *P. lineatus* follows a meroblastic or partial pattern, as commonly observed in most teleosts (Lagler *et al.*, 1977, Leme dos Santos & Azoubel, 1996). The arrangement of blastomeres (4 × 2; 4 × 4; 4 × 8) at the early cleavage stages is similar to that reported in *Catostomus commersoni* (Long & Ballard, 1976), *Danio rerio* (Kimmel *et al.*, 1995) and *Oreochromis niloticus* (Morrison *et al.*, 2001), but it differs from those observed in *Rhamdia sapo* (Matkovik *et al.*, 1985), *Alosa sapidissima* (Shardo, 1995) and *Brycon orbignyanus* (Ganeco, 2003).

During the cleavage of fish embryos the number of cells increases while their size decreases, a feature previously reported by Castellane *et al.* (1994) in *P. lineatus* and also observed in the present study.

Morrison *et al.* (2001) suggest that the variations in the rate of embryogenesis and embryo development (asynchrony and malformations) are related to the breeders' age and the temperature of incubation. Kimmel *et al.* (1995) and Morrison *et al.* (2001) stated that, even within spawns fertilized and incubated

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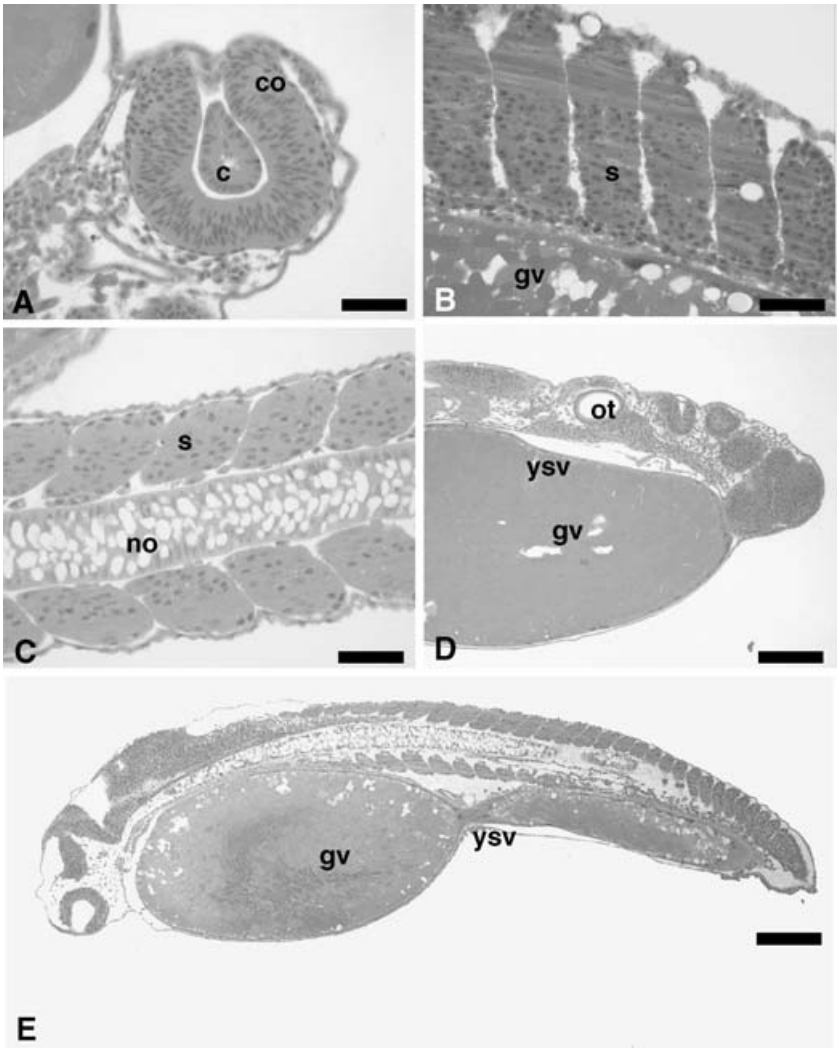

Figure 10 Longitudinal sections of embryos of *P. lineatus* at the larval stage, stained with H&E. (*A*) Detail of the optic calyx and the crystalline lens; (*B*) somites at myogenesis; (*C*) details of somites and notochord; (*D*) details of the optic vesicle and the regions comprising the prosencephalon, mesencephalon and rhombencephalon; (*E*) general details of the embryo, revealing a primitive gut. gv, yolk globules; ysl, yolk syncytial layer; no, notochord; co, optic calyx; c, crystalline lens; s, somite; ot, optic vesicle; mes, mesencephalon; rom, rhombencephalon; pro, prosencephalon; ip, rudimentary intestine. Scale bars represent: (*A*) 18.2 μm; (*B*) 39.1 μm; (*C*) 18.1 μm; (*D*) 51.5 μm; (*E*) 130.2 μm.

under optimal conditions, there is asynchrony of embryonic development. Hisaoka & Firlit (1960) reported that, in zebrafish, the mitotic divisions are synchronous until 32 cells but become asynchronous at 64 cells. Arezon *et al*. (2002) reported that embryos of *Cynolebias melanotaenia* develop faster at 25 °C, but severe abnormalities occur, in contrast to what is detected at lower temperatures (20 and 16 °C).

In the present experiment with *P. lineatus*, despite the utilization of young breeders (2 years old) and a constant water temperature, incubation at 24 °C led to a higher asynchrony of embryonic development and a variation in blastomere division, in some cases, following a non-geometric progression after the third and fourth planes. At 28 °C this variability was reduced and nearly restricted to the cleavage stage. In addition, cleavage progression was geometric, at least until the first 64 cells. These data corroborate the observations of previous reports and stress the important role of incubation temperature in the embryonic development of fish species.

Hisaoka & Firlit (1960) suggested that the protoplasmic movement between the yolk and blastomeres ceases after the 64-cell stage. This fact could be related to the beginning of periblast formation, which occurs at the beginning of the blastula stage and morula stage in *P. lineatus*.

In this species the formation of a blastocoele has also been observed, visible as irregular spaces among some blastoderm cells, as described by several authors

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(Kimmel & Law, 1985; Trinkaus, 1984; Kimmel *et al.*, 1995; Ganeco, 2003) in other teleost species. However, in some species, such as *Oreochromis niloticus* (Morrison, 2001), no characteristic cavity was identified, whereas others, such as *Hippocampus reidi* (Silveira, 2001) and trout (Lagler, 1977), a typical blastocoele cavity was seen between the blastoderm and the periblast.

The gastrula stage begins with the first epiboly movements (Leme dos Santos & Azoubel, 1996) and is completed by closure of the blastopore by the blastoderm and the formation of a tail button (Kimmel *et al.*, 1995). These observations are in accordance with what was detected in *P. lineatus* and also reported by Ganeco (2003) in *Brycon orbignyanus*. On the other hand, in *Oreochromis niloticus*, Morrison *et al.* (2001) reported that, due to the size of yolk, the embryo is not able to extend over the entire vegetal pole and, thus, rudimentary organogenesis (somite segmentation) starts before the epiboly movement is finished.

In *Prochilodus lineatus*, the formation of the embryonic shield is visible when the blastoderm involves 50% of the yolk sphere, similar to previous observations in other species (Kimmel, 1995; Firlit & Hisaoka, 1960; Ganeco, 2003).

According to Hisaoka & Firlit (1960), Kupffer's vesicle represents a remnant structure of the archenteron that is located over the periblast and below the notochord. In *Oncorhynchus keta* (Mahon & Hoar, 1956 cited in Hisaoka & Firlit, 1960) Kupffer's vesicle is described as an oblique and elongated cavity, with walls of columnar epithelium, which is separated from the periblast by a layer of endoderm cells. The histology of this structure in *P. lineatus* was similar to that reported in *O. keta*.

Following Kimmel (1995), the neurula phase was included in the segmentation stage. Brummett & Dumont (1978) and Morrison (2001) identified Kupffer's vesicle in the early phases of the segmentation stage. In the present work, this vesicle was observed at the phase of 13 somites and disappeared after the 24-somite stage; its function remains unknown. However, Brummet & Dumont (1978) hypothesized that it could have a digestive function, favorable to yolk resorption, since several ciliated cells were observed inside Kupffer's vesicle and the intestines of *Fundulus heteroclitus*.

According to Silveira (2001), the ectoderm on the notochord is transformed into a neural plate, which becomes centrally depressed giving rise to the neural keel, which following its posterior enclosure by the fusion of neural filaments, originates the neural tube. As observed in *P. lineatus,* the prosencephalon, mesencephalon and rhombencephalon regions developed from the posterior region of the neural tube, corroborating the description of the embryonic development of *Brycon orbignyanus* carried out by Ganeco (2003).

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