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Embryonic development in *Zungaro jahu*

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Summary

The aim of this study was to characterize the embryonic development of *Zungaro jahu*, a fresh water teleostei commonly known as 'jaú'. Samples were collected at pre-determined times from oocyte release to larval hatching and analysed under light microscopy, transmission electron microscopy and scanning electron microscopy. At the first collection times, the oocytes and eggs were spherical and yellowish, with an evident micropyle. Embryo development took place at 29.4 ± 1.5°C and was divided into seven stages: zygote, cleavage, morula, blastula, gastrula, organogenesis, and hatching. The differentiation of the animal and vegetative poles occured during the zygote stage, at 10 min post-fertilization (mpf), leading to the development of the egg cell at 15 mpf. From 20 to 75 mpf, successive cleavages resulted in the formation of 2, 4, 8, 16, 32 and 64 blastomeres. The morula stage was observed between 90 and 105 mpf, and the blastula and gastrula stage at 120 and 180 mpf; respectively. The end of the gastrula stage was characterized by the presence of the yolk plug at 360 mpf. Organogenesis followed, with differentiation of the cephalic and caudal regions, elongation of the embryo by the cephalo-caudal axis, and somitogenesis. Hatching occurred at 780 mpf, with mean larval total length of 3.79 ± 0.11 mm.

Keywords: Embryo, Fish, Histology, Structure, Ultrastructure

Introduction

The species *Zungaro jahu* belongs to the order Siluriformes. It is the largest fish in the Paraná-Paraguay and Uruguay River basins, reaching up to 144 cm in length and 150 kg in weight (Agostinho *et al.*, 2004). First maturation occurs when adults are 70 cm long and spawning takes place between December and February, with animals migrating upriver during the reproductive season (Vaz *et al.*, 2000).

Induced reproduction and incubation techniques are well understood in different fish species (Shardo, 1995). However, few studies have analysed the basic aspects of the embryonic period from fertilization to hatching. A good understanding of the initial phases of the life cycle is of extreme importance in taxonomy and species ecology studies (Sanches *et al.*, 1999).

Embryonic development is a complex process that begins when the oocyte is fertilized by the spermatozoon in the micropyle and consists in the reorganization of the egg components (Depêche & Billard, 1994). Embryo studies are of great importance in fish reproductive biology (Coward *et al.*, 2002) and are essential in a great variety of research, including species ontogeny and biotechnology, where they can be used as bioindicators of environmental quality, and to determine the effects of toxic substances on aquatic fauna (Flores *et al.*, 2002; Botero *et al.*, 2004; Ninhaus-Silveira *et al.*, 2006). However, in order to be fully

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understood, embryonic studies must be combined with topological analysis of fertilized eggs (Depêche & Billard, 1994).

Embryonic development has been studied in several fish species and is often classified into stages. Currently, the following native Siluriformes species have been studied: *Rhamdia hilarii* (Godinho *et al.*, 1978), *Rhamdia sapo* (Cussac *et al.*, 1985; Matkovic *et al.*, 1985); *Pseudoplatystoma coruscans* (Cardoso *et al.*, 1995; Landines *et al.*, 2003), and *Pimelodus maculatus* (Luz *et al.*, 2001). However, in the majority of studies, embryonic development was analysed only by stereomicroscopy, with only a few studies using light microscopy.

Data on the biology and ecology of *Z. jahu* are scarce, with most data being related to nutrition or parasitology (Agostinho *et al.*, 2004). Successful rearing of fish depends on the understanding of the species' initial biology, including fertilization and embryonic development characteristics, which directly influence fertilization and hatching rates (Matkovic *et al.*, 1985).

The *Z. jahu* is a vulnerable species with a reduced natural stock and is in danger of extinction due to changes and destruction of its habitat. Thus, it has been included in the *List of Fauna Species in Danger of Extinction in the State of Minas Gerais* under the category 'Critically Endangered' (Minas Gerais, 2008) and in the *List of Vertebrate and Invertebrate Species of the Wild Fauna in Danger of Extinction in the State of São Paulo* (São Paulo, 2014). The *Z. jahu* has not been included in the national list of endangered species in Brazil because it can also be found in the Paraguay River basin where, in spite of overfishing, it cannot be considered endangered due to the relatively intact environmental state of the Pantanal ecosystem (Machado *et al.*, 2008).

The aim of this study was to structurally and ultrastructurally identify the events that take place during the embryonic development of *Z. jahu* in order to better understand its biology and reproduction, and thus improve husbandry practices in this species.

Materials and methods

Collection and fertilization

Broodstock of the species *Z. jahu* Ihering, 1898 (= *Paulicea luetkeni* Steindachner, 1875) from the fishery Usina Hidrelétrica Engenheiro Souza Dias (Companhia Energética do Estado de São Paulo, Castilho, SP, Brazil) were subjected to induced reproduction and dry fertilization during the reproductive period of the species, from December to February, according to the techniques by Woynarovich & Horváth (1983).

The females ($n = 5$) received two injections of common carp (*Cyprinus carpio*) pituitary extract (0.8 and 5.0 mg /kg body weight) in the coelomic cavity at 12-h intervals. The males ($n = 8$) received a single dose (2.5 mg/kg body weight) concomitantly to the females' second dose.

Approximately 12 h after the effect of the hormone, the gametes were obtained by manual craniocaudal massage of the abdomen and subsequently mixed and hydrated.

After extrusion, semen and oocytes were gently homogenized and water was added to the mixture for gamete activation and egg hydration. The eggs were washed under running water to remove excess semen and transferred to 60-litre incubators with a continuous water flow, where they remained for the whole experimental procedure. The following physical–chemical characteristics of the water were recorded: temperature and dissolved oxygen (mg/L), by YSI 550 A equipment, pH, conductivity (μS/cm), by YSI 63 equipment, and alkalinity (mg/L) ammonia (μg/L) by the Goltermann *et al.* (1978) method.

Samples were collected at oocyte extrusion; when oocytes and semen were mixed together (fertilization–time zero); at 10 and 30 seconds post-fertilization; at 1, 2, 5, 7, 10, 15, 20, 30, and 45 min post-fertilization (mpf), then every 15 min up to 2 h post-fertilization, and at every hour until hatching. Fertilization rate (developing eggs/total eggs collected in 1 g) ×100, was determined 5 h after the male and female gametes were mixed (fertilization time).

Twenty-five oocytes/eggs/larvae were collected at each sampling time. These samples were fixed in modified Karnovsky solution (2.5% glutaraldehyde + 2.5% paraformaldehyde) for 24 h, washed in 0.1 M sodium cacodylate buffer solution (pH 7.4) and stored in 70% alcohol for further analysis at the School of Agrarian Sciences and Veterinary Medicine (FCAV), UNESP – Univ Estadual Paulista, Campus Jaboticabal, Department of Morphology and Animal Physiology, Laboratory of Histology and Animal Embryology.

Stereomicroscopy and morphometry

Samples were analysed and photographed using LEICA DFC 280 photomicrography equipment attached to a LEICA MZ8 stereomicroscope. Linear measures (oocyte/egg/embryo diameter and larval total length) were obtained using the programme IM 50-LEICA. Twenty oocytes/eggs/larvae were measured for each collection time.

Light microscopy (LM)

Samples were prepared and embedded in historesin (kit Leica Historesin®) according to the manufacture's recommendations and the histological sections (3 μm) were stained with haematoxylin-phloxine (Tolosa *et al.*, 2003). Analysis of slides and photodocumentation

Table 1 Characteristics of the different stages of embryonic development in *Zungaro jahu* at 29.4°C

Time (mpf)	Stage	Characteristics
0 to 15	Zygote	Cytoplasm migration and formation of the animal pole
20	Cleavage	100% with two blastomeres
30	Cleavage	100% with 4 blastomeres
45	Cleavage	100% with eight blastomeres
60	Cleavage	13% with 16 blastomeres; 87% with 32 blastomeres
75	Cleavage	45% with 32 blastomeres; 55% with 64 blastomeres
90	Morula	70% initial morula; 30% final morula
105	Morula	14% initial morula; 86% final morula
120	Blastula	100% blastula
180	Gastrula	100% gastrula (25% epiboly)
240	Gastrula	20% gastrula (25% epiboly); 80% gastrula (50% epiboly)
300	Gastrula	19% gastrula (50%epiboly); 81% gastrula (75% epiboly)
360	Gastrula	100% yolk plug
420	Organogenesis	Differentiation of the cephalic and caudal regions
480	Organogenesis	Prominent cephalic region
540	Organogenesis	100% optical and Kupffer vesicles, 9 somites
600	Organogenesis	100% caudal elongation, start of body and yolk pigmentation, 17 somites
660	Organogenesis	100% free tail, 24 somites
720	Organogenesis	100% free tail, 30 somites, increase in pigmentation
780	Hatching	100% hatched

mpf: minutes post-fertilization.

were performed using Leica DFC 280 photomicrography equipment attached to a LEICA DM 2500 photomicroscope.

Scanning electron microscopy (SEM)

Samples were post-fixed in 1% osmium tetroxide solution for 2 h, washed in 0.1 M sodium cacodylate buffer, and dehydrated in a graded series of ethanol (30, 50, 70, 80, 90, and 95%) for 5 min each. Subsequently, the slides were dried to critical point in liquid CO_2 (BAL-TEC dryer), mounted onto a copper grid, coated in palladium/gold (Desk II Denton Vacuum equipment), and analysed under a scanning electron microscope (JEOL-JSM 5410).

Transmission electron microscopy (TEM)

Oocytes and eggs collected during the first hour of development were analysed by TEM. These samples were post-fixed in 2% osmium tetroxide for 2 h and dehydrated in a graded series of ethanol (similarly to SEM). Samples were infiltrated with a mixture of araldite and acetone and subsequently embedded in araldite. Semi-fine sections (0.5 µm) were obtained and stained with 1% toluidine blue in saturated boric acid for selection and analysis of the areas of interest. The best sections were selected and ultra-fine sections (60 nm) obtained using a diamond blade. These sections were contrasted, analysed, and electron micrographed with a transmission electron microscope (JEOL – JEM 1010).

Results

The embryonic period of *Z. jahu* comprised the period from fertilization to hatching and lasted for 780 mpf (13 h) at 29.4 ± 1.5°C. Fertilization rate was 95%. The physical–chemical characteristics of the water from the incubators were: ammonia = 11.13 µg/l, pH = 8.14, conductivity = 34 µS/cm, dissolved oxygen = 7.26 mg/l, and alkalinity = 21 mg/l.

The embryonic development of *Z. jahu* was divided into seven stages and classified according to Faustino *et al.* (2011 and 2015) into: zygote, cleavage, morula, blastula, gastrula, organogenesis, and hatching. The different stages and their corresponding characteristics are detailed in Table 1. The main characteristics of each stage are illustrated in Figures 1*b–j* and 2*a–f*.

The term 'oocyte' refered to the female gamete before fertilization while 'egg' comprised the stages from zygote (fertilization time) to the end of the gastrula stage, when the embryonic axis was formed (organogenesis stage) and the term 'embryo' used forthwith. The denomination 'larvae' was used from the time of chorion rupture.

Oocytes and zygotes (extrusion, 0–15 mpf)

At the time of extrusion, oocytes showed a mean diameter of 1.38 ± 0.06 mm, which remained virtually unaltered until organogenesis. The oocytes were spherical, yellowish, with a large perivitelline space (Figures 1*a* and 3*b*), and a gelatinous layer over the chorion.

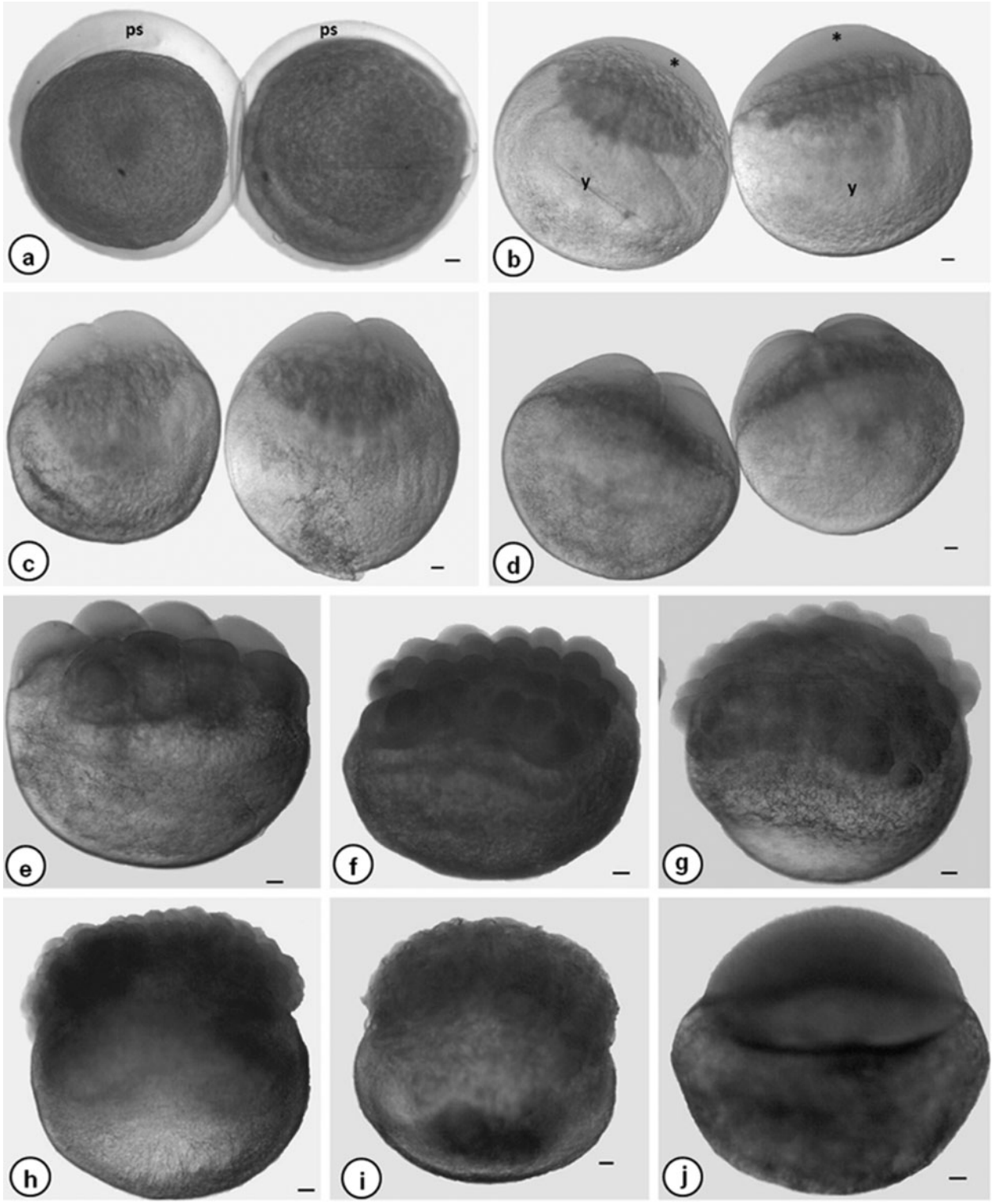

Figure 1 Photomicrography of oocytes (*a*) and eggs (*b–j*) of *Z. jahu*. (*a*) Oocytes (extrusion). (*b*) Zygote phase (0–15 mpf), development of the egg cell or zygote (∗). (*c–h*) Cleavage phase: (*c*) presence of two blastomeres (20 mpf); (*d*) four blastomeres (30 mpf); (*e*) eight blastomeres (45 mpf); (*f*) 16 blastomeres (60 mpf); (*g*) 32 blastomeres (60 mpf); (*h*) 64 blastomeres (75 mpf). (*i*) Morula phase (90 mpf). (*j*) Blastula phase (120 mpf). mpf, minutes post-fertilization; ps, perivitelline space; y, yolk. Bars represent 100 μm.

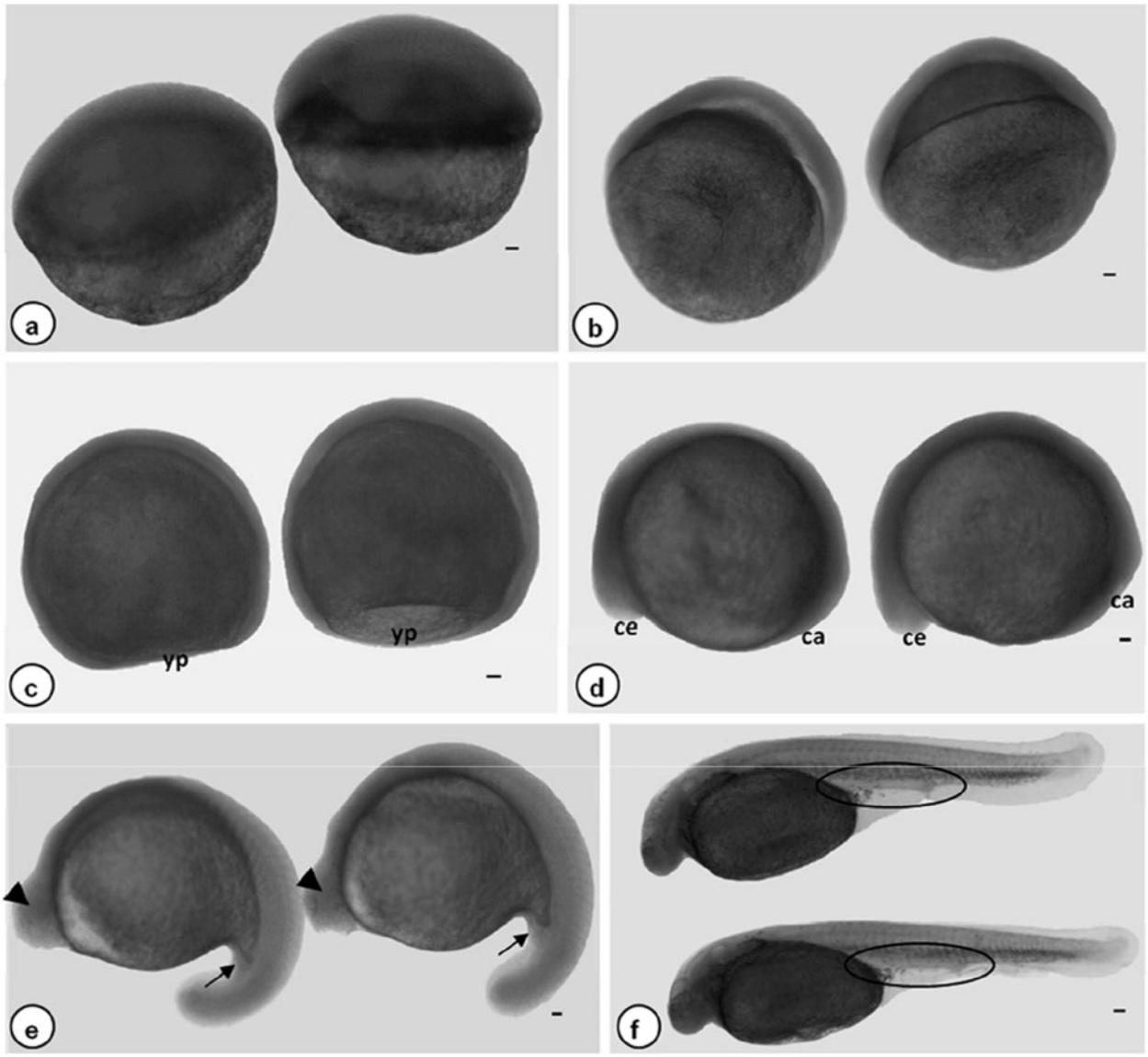

Figure 2 Photomicrography of eggs (*a*–*c*), embryos (*d*, *e*) and hatched larvae (*f*) of *Z. jahu*. (*a*–*c*) Gastrula phase: (*a*) 25% epiboly (180 mpf); (*b*) 50% epiboly (240 mpf); (*c*) formation of yolk plug (360 mpf). (*d*, *e*) Organogenesis phase: (*d*) differentiation of cephalic and caudal regions (420 mpf); (*e*) caudal elongation of the embryo, presence of somites, optic (arrowhead) and Kupffer vesicles (arrow), and start of pigmentation (600 mpf). (*f*) Hatching phase (780 mpf), detail of primitive gut (circle) and embryonic fin (broken arrow). ca, caudal region; ce, cephalic region; yp, yolk plug. Bars represent 100 μm.

Each oocyte showed a single micropyle, a small opening where the spermatozoon penetrated during fertilization (Figure 4*a*). The micropylar apparatus consisted of two vestibules and a short micropylar canal (Figures 3*a* and 4*a*).

Transversal sectioning (TEM) revealed a perforated chorion containing several pores responsible for water intake and exchange of substances necessary for embryonic development (Figure 4*b*), as well as the presence of a thin gelatinous layer (Figure 3*c*).

Under LM and TEM, it was possible to observe several cortical alveoli of various sizes distributed throughout the whole periphery of the oocyte, forming a thin basophilic layer (ML) that constituted the cortical cytoplasm (Figures 3*c* and 4*c*).

The zygote stage comprised the period from fertilization to the development of the animal pole, also known as the egg cell.

Under LM, at the time of fertilization, it was possible to observe a cytoplasmic movement towards the egg's micropyle region, characterizing the animal pole; which was formed by the union between the male and female pronucleus and the dislodged cytoplasm. The animal pole was basophilic and homogeneous (Figure 3*d*), while the vegetative pole consisted of a large amount of yolk.

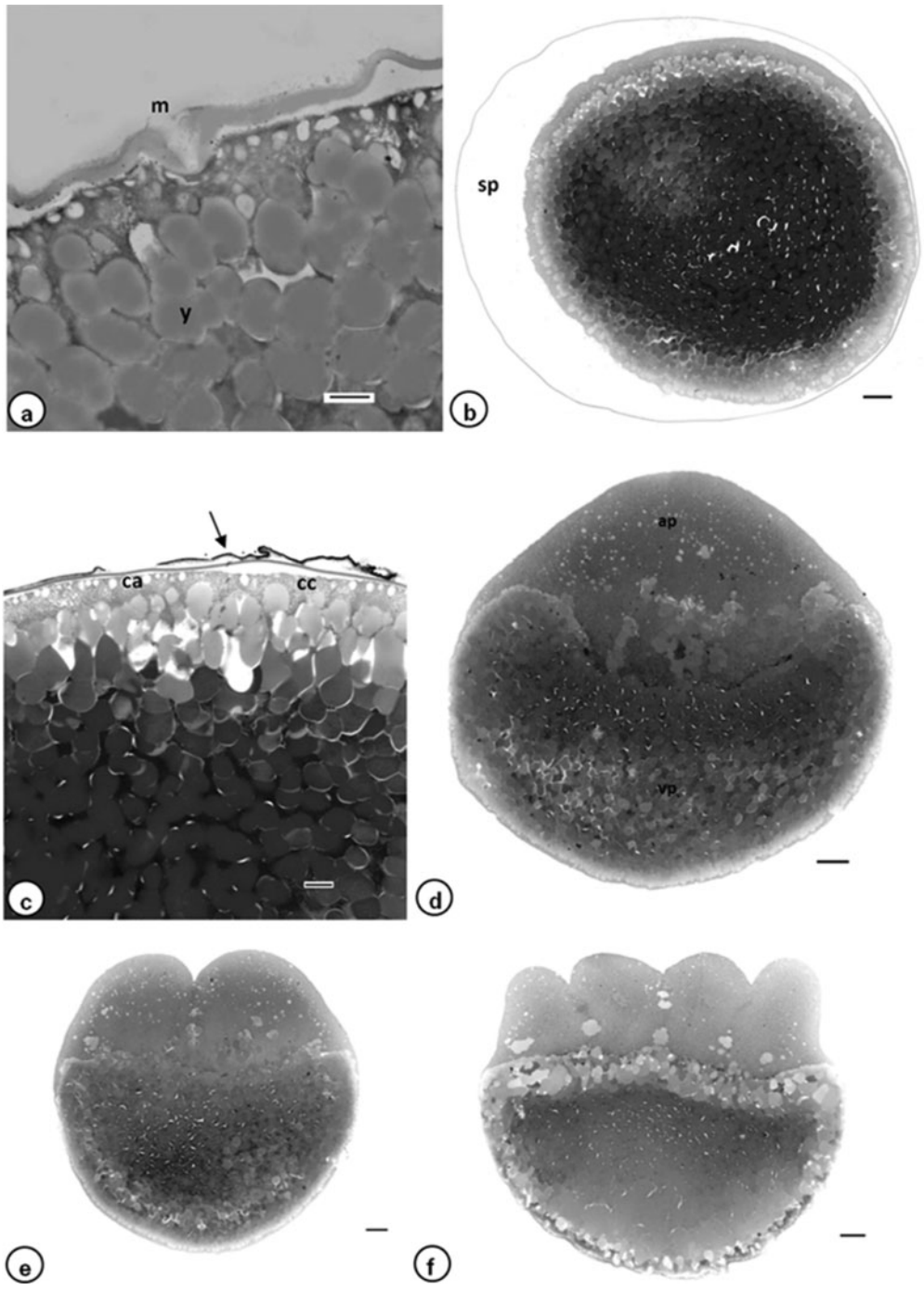

Figure 3 Photomicrography of oocytes (extrusion; *a–c*) and eggs (*d–f*) of *Z. jahu*. (*a*) Detail of the micropyle. (*b*) Oocyte during fertilization. (*c*) Cortical cytoplasm with cortical alveoli distributed in the periphery, chorion with a thin gelatinous layer (arrow). (*d*) Development of the egg cell (0–15 mpf). (*e*) Two blastomeres (20 mpf). (*f*) Eight blastomeres (45 mpf). ap, animal pole; ca, cortical alveolus; cc, cortical cytoplasm; m, micropyle; ps, perivitelline space; vp, vegetative pole; y, yolk. Bars: (*a*) represent 20 μm; (*b*, *d–f*) represent 100 μm; (*c*) represent 30 μm.

The alveoli initially seen in the oocytes ruptured after fertilization, contributing to the elevation of the chorion and consequent increase in the perivitelline space. By 5 mpf, the cortical alveoli were absent. The development of the perivitelline space and its increase resulted in the separation of the chorion from the egg membrane. It was also observed that the cortical cytoplasm contained mitochondria, ribosomes, and secretion vesicles (Figure 4*c*).

Under SEM, several spermatozoa could be seen at the entrance of the micropyle from the time of fertilization up to 1 mpf (Figure 4*d*). By 30 s

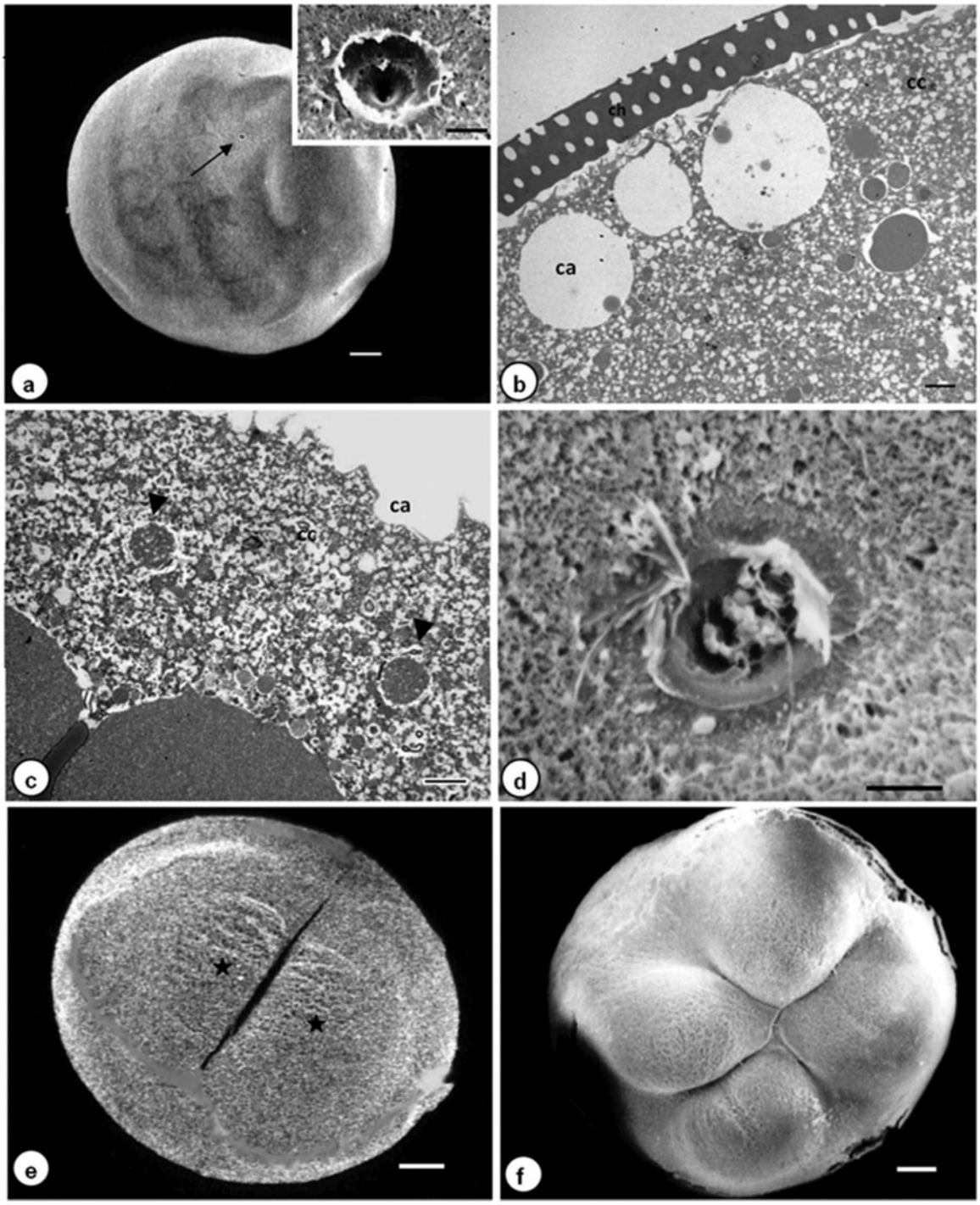

Figure 4 Scanning (*a*, *d–f*) and transmission electron micrography (*b*, *c*) of oocytes (extrusion, *a–d*) and eggs (0–15 mpf; *e*, *f*) of *Z. jahu*. (*a*) Oocyte, presence of a single micropyle (arrow and inset). (*b*) Cortical cytoplasm with cortical alveoli distributed in the periphery, chorion with pores. (*c*) Cortical cytoplasm with mitochondria (arrowhead) and ruptured cortical alveoli. (*d*) Presence of multiple spermatozoa at the entrance of the oocyte micropyle. (*e*) Two blastomeres (∗). (*f*) Four blastomeres. ca, cortical alveolus; cc, cortical cytoplasm; ch, chorion. Bars: (*a*) represent 100 μm; (*b*, *e*, *f*) represent 10 μm; inset, (*d*) represent 2 μm.

post-fertilization, some eggs had been fertilized and were protected from polyspermia by the development of a fertilization cone, a round structure that projected from the micropyle and obstructed its opening.

Cleavage stage (20–75 mpf)

This stage began with the first cleavage, on the vertical plan of the animal pole, which divided the blastodisc into two cells (blastomeres) of equal size (Figures 1*c*, 3*e* and 4*e*). Subsequently, another division took place in the vertical plane, perpendicular to the first, generating four blastomeres (Figures 1*d* and 3*f*). The third division was vertical and parallel to the first, resulting in eight blastomeres arranged 4 × 2 (Figures 1*e*, 4*f* and 5*a*). The fourth division was vertical and parallel to the second, forming 16 blastomeres arranged 4 × 4 (Figures 1*f* and 5*b*). The fifth division was vertical and parallel to the first, originating 32 blastomeres arranged 4 × 8 (Figures 1*g*, 5*c* and 6*a*) and the sixth cleavage was on the horizontal plane, resulting in two layers of cells and 64 blastomeres (Figures 1*h* and 6*b*).

As the egg developed, the number of blastomeres increased while their size decreased. It was also noted that small globules of yolk penetrated the area of the blastomeres in a fragmented way (Figure 6*a*).

Morula stage (90–105 mpf)

This stage was characterized by the presence of more than 64 blastomeres in the eggs. The final phase of the morula stage was determined when the blastomeres formed a cellular mass shaped like a half berry (Figures 1*i*, 5*d* and 6*c*). At this stage it was possible to observe the development of the yolk syncytial layer, known as a periblast (Figure 6*c*).

Blastula stage (120 mpf)

This stage was characterized by intense cellular division, with no distinction between the boundaries of the blastomeres. There was the formation of the blastoderm, a dome-shaped structure over the yolk, and the appearance of the blastocele, characterized as a large space between the yolk and the blastomeres (Figures 1*j* and 6*d*).

Gastrula stage (180–360 mpf)

The gastrula stage was defined by the start of the epiboly movement in the eggs, the migration of the embryonic cells from the animal to the vegetative pole (Figure 2*a*). Once 50% of the yolk was covered, the involution movement began. It projected below and in the opposite direction to the epiboly movement of the blastoderm, generating the epiblast and hypoblast (Figures 2*b* and 6*e*). This stage ended with the end of the epiboly, characterized by the complete coverage of the yolk by the embryonic cells, forming the vitelline plug or blastopore (Figures 2*c* and 6*f*).

Organogenesis stage (420–720 mpf)

This stage began with the differentiation of the cephalic and caudal regions of the embryo (Figures 2*d*, 5*e* and 7*a*), followed by the appearance of somites (somitogenesis), notochord, otic vesicle, and Kupffer vesicle (Figures 2*e* and 7*b*). Subsequently, there was elongation of the embryo by the cephalo-caudal axis, differentiation of large round nuclei myoblasts into flat nuclei myomeres (Figure 7*c*), and pigmentation of the body and yolk. The anterior region of the neural tube expanded, forming the prosencephalon and mesencephalon, and the optic vesicle was evident (Figure 7*d*). The embryo tail became free and began to move, and would later cause the rupture of the chorion.

Hatching stage (780 mpf)

This stage was characterized by the total rupture of the chorion and free swimming of the larvae (Figures 2*f*, 5*f* and 7*e*). Under SEM, contrast revealed the primordium of the first pair of maxillary barbels, oral cavity, developing branchiae, olfactory organ with few cilia, embryonic membrane covering the whole caudal region, and large yolk sack (Figure 5*f*). Under a stereomicroscope, it was possible to observe a rudimentary digestive system in the mid-posterior region of the larvae (Figure 2*f*), and LM revealed the outline of the heart in the region anterior to the yolk sac (Figure 7*e*).

Discussion

The physical–chemical parameters of the water from the incubators, where the embryonic development of *Z. jahu* took place, were considered good for the development of fresh water fish (Sipaúba-Tavares, 1995).

According to Vazzoler (1996), the fecundity and diameter of fish oocytes can vary and show inter- and intra-specific variations between reproductive periods. Furthermore, these characteristics are associated with reproductive behaviour; in which migratory species with complete spawning, external fertilization, and no parental care produce smaller oocytes and have greater fecundity rates. This is the case for *Z. jahu*, whose mean diameter of the oocytes, eggs and embryos are considered small (1.38 ± 0.06 mm) when compared with species of no migratory fish. Oocyte diameter also affects incubation time, with species that produce

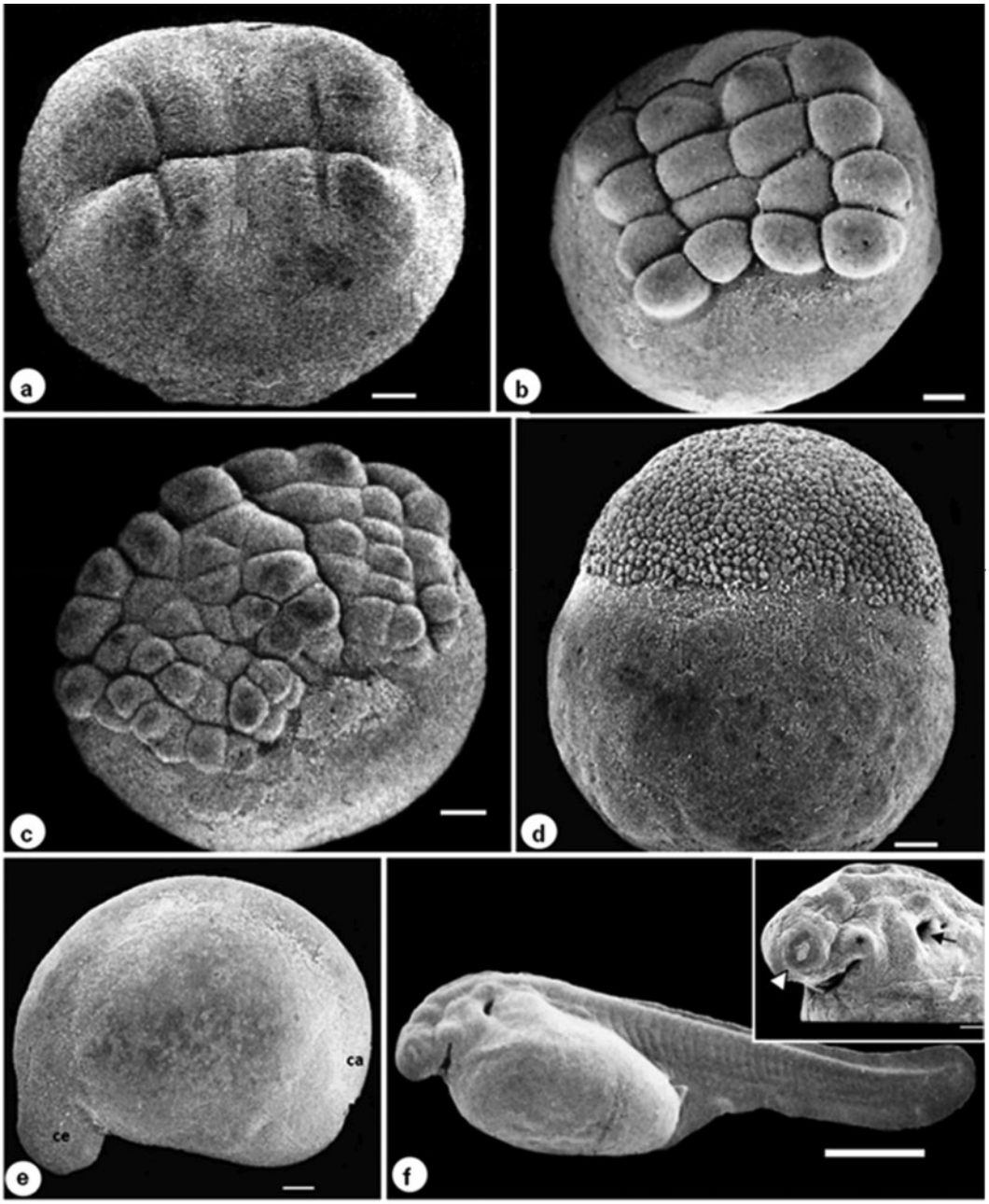

Figure 5 Scanning electron micrography of eggs (*a–d*), embryo (*e*) and larva (*f*) of *Z. jahu*. (*a–c*) Presence of eight, 16, 32 blastomeres (45, 60, 75 mpf), respectively. (*d*) Morula phase (90 mpf). (*e*) Differentiation of the cephalic and caudal regions (420 mpf). (*f*) Hatched larva with embryonic fins (780 mpf) (broken arrow); inset highlighting primordial barbels (∗), oral cavity (white open arrow), respiratory cavity (arrow) and nostril region (white arrowhead). ca, caudal region; ce, cephalic region. Bars: *a–e*, detail represent 100 μm, *f* represent 500 μm.

smaller oocytes having shorter incubation periods than those that have large oocytes, such as the majority of sedentary species (Sargent *et al.*, 1987).

Z. jahu produced free non-adhesive oocytes with a chorion surrounded by a thin gelatinous layer. According to Rizzo *et al.* (2002), this layer is made of an entanglement of delicate fibrils and is often found in Siluriformes. It does not seem to be related to the adhesiveness of the eggs, as it has been reported in both adhesive and non-adhesive fish eggs. Furthermore, in species that do not provide parental care, this gelatinous layer enables oocytes and eggs to adhere to substrates, thus protecting them from water currents (Rizzo *et al.*, 2002). In species with parental care, this facilitates parental behaviour (Melo *et al.*, 2011). This layer has been reported by Faustino *et al.*

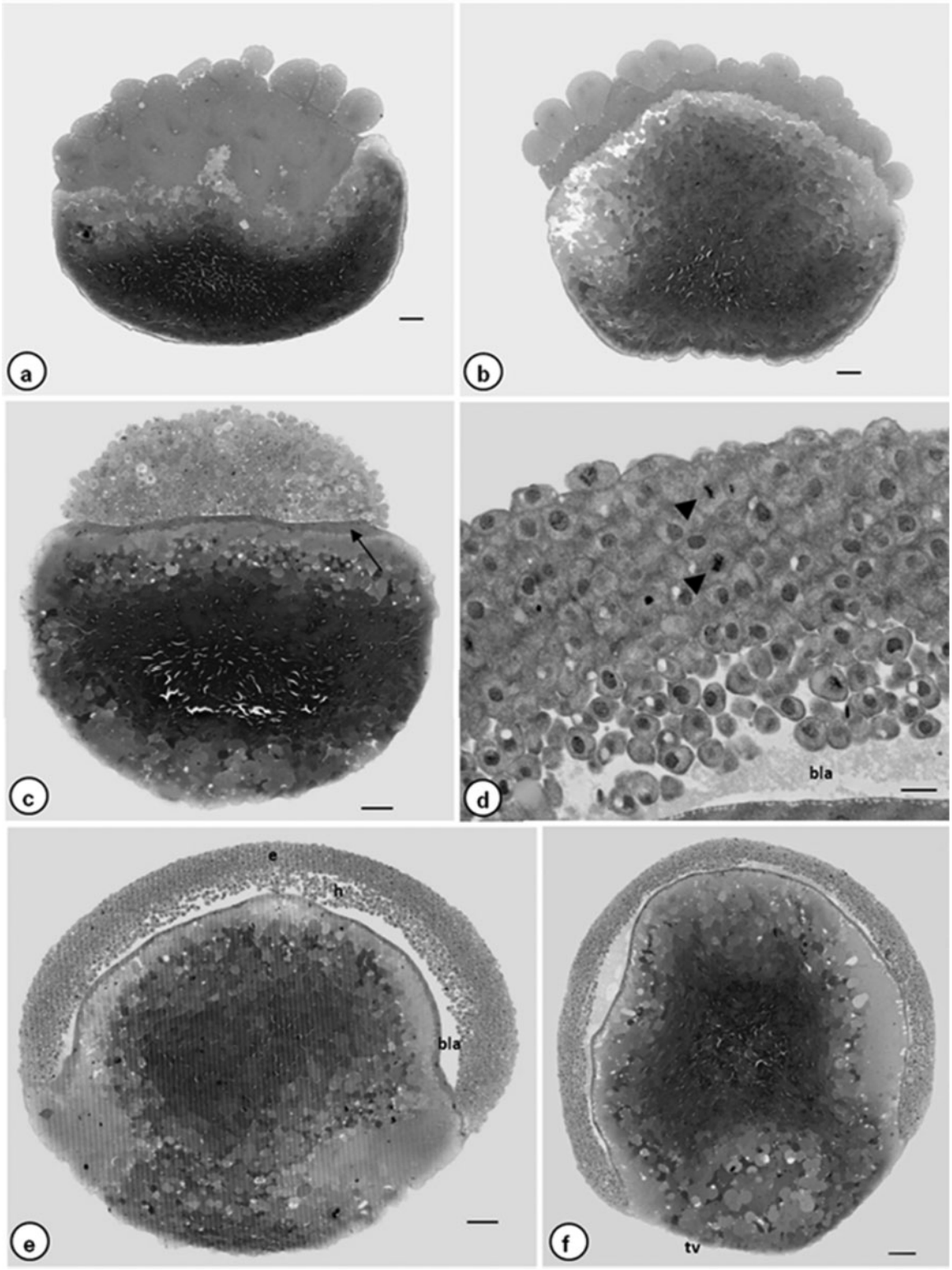

Figure 6 Photomicrography of the eggs of *Z. jahu*. (*a*) Thirty-two blastomeres (60 mpf). (*b*) Sixty-four blastomeres (75 mpf). (*c*) Morula phase (90 mpf), with yolk syncytial layer (arrow). (*d*) Large number of mitosis (90 mpf) (arrowhead). (*e*, *f*) Gastrula phase: (*e*) highlighting the epiboly movement with the development of epiblast and hypoblast (300 mpf); (*f*) presence of yolk plug (360 mpf). bla, blastocele; e, epiblast; h, hypoblast; yp, yolk plug. Bars: (*a–c*, *e*, *f*) represent 100 μm, (*d*) represent 20 μm.

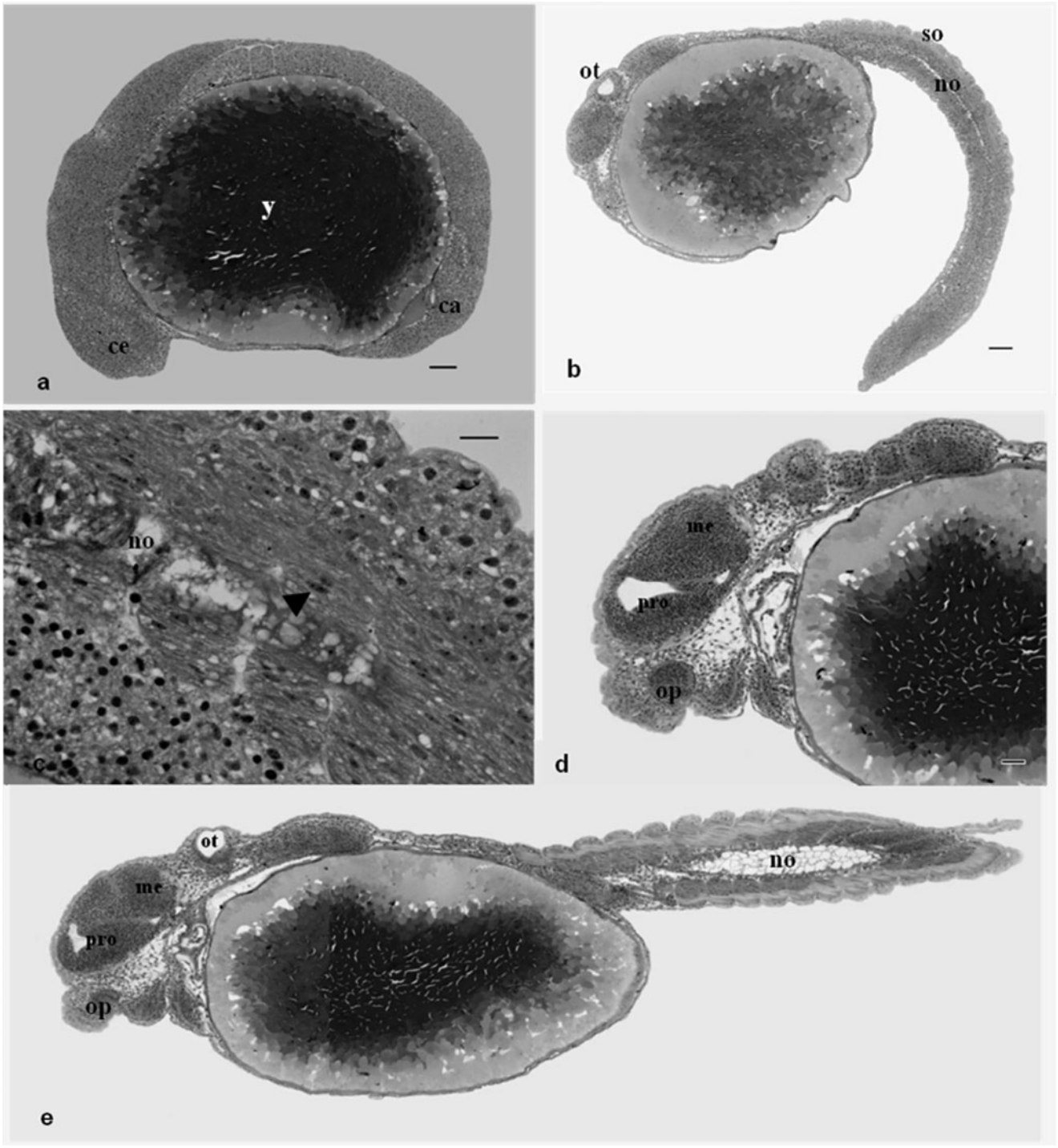

Figure 7 Photomicrography of embryos (*a–d*) and larva (*e*) of *Z. jahu*. (*a*) Differentiation of the cephalic and caudal regions (420 mpf). (*b*) Elongation of the embryo by the cephalo-caudal axis (600 mpf). (*c*) Differentiation of myomeres (arrowhead). (*d*) Development of the nervous system and optical vesicle (720 mpf). (*e*) Hatched larva (780 mpf. ca, caudal region; ce, cephalic region; me, mesencephalus; no, notochord; op, optic vesicle; ot, otic vesicle; pr, prosencephalon; so, somites; y, yolk. Bars: (*a*, *b*, *e*) represent 100 μm; (*c*) represent 20 μm; (*d*) represent 50 μm.

(2007) and Perini *et al.* (2009) in *P. corruscans* × *P. fasciatum* hybrid and in *Rhinelepis aspera*, respectively.

The micropylar apparatus of the oocytes of *Z. jahu* had two vestibules, a smooth surface, and a short and narrow micropylar canal. According to Rizzo *et al.* (2002) and Ganeco & Nakaghi (2003), the micropyle is a concave area located in the chorion, consisting of a continuous vestibule with an internal canal that narrows towards the plasmatic membrane of the egg. Rizzo & Bazzoli (1993) reported that the funnel-shaped micropyle is present in the majority of teleostei and allows the passage of a single spermatozoon. Studies suggest that differences in the micropyle can be found among species of the same genus or family, as a mechanism to prevent interspecific hybridization (Chen *et al.*, 1999). Conversely, some studies have reported similarities between the micropylar apparatus in different species, suggesting a possible relationship between systematic groups (Rizzo *et al.*, 2002).

At the time of fertilization, a cytoplasmic movement towards the micropyle region begun in the eggs of *Z. jahu*, characterizing the animal pole. Similar findings have been reported by Kimmel *et al.* (1995) in *Danio rerio*, by Ninhaus-Silveira *et al.* (2006) in *Prochilodus lineatus*, by Marques *et al.* (2008) in *P. corruscans*, and by Faustino *et al.* (2011) in *Brycon gouldingi*, who have reported stimulation of this cytoplasmic movement after fertilization. However, Ganeco (2003) observed this cytoplasmic movement in *Brycon orbignyanus* oocytes at extrusion, before fertilization.

Oocytes and eggs of *Z. jahu* showed a large perivitelline space throughout the embryonic development, which according to Matsumura (1972) would protect the embryos from environmental damages and contribute to greater survival in running water.

The eggs from *Z. jahu* are classified as telolecithal due to the large amount and distribution of the yolk, showing partial or meroblastic segmentation restricted to the animal pole; which is characteristic of fish eggs (Lagler *et al.*, 1977; Leme dos Santos & Azoubel, 1996).

At the cleavage stage, it was observed that the cell divisions began from the centre towards the edge of the blastodisc, as also reported by Matkovic *et al.* (1985) in *Rhamdia sapo* and by Shardo (1995) in *Alosa sapidissima*. Successive cleavage occurred up to the 64 blastomeres phase, when the number of blastomeres was inversely proportional to their size, as also reported by Wourms & Evans (1974) in *Xiphister atropurpureus* and by Faustino *et al.* (2011) in *B. gouldingi.* According to Gilbert (2003), these cleavages (mitotic divisions) are essential for a new balance to be established between the nucleus and cytoplasm of the animal pole. The yolk globules observed entering the blastomeres in *Z. jahu* probably aid their absorption by the cells, a fact also noted by Ninhaus-Silveira *et al.* (2006).

At the morula stage, the yolk syncytial layer or periblast was formed, a continuous cytoplasmic layer located between the blastoderm disc and the yolk that resulted from the incomplete division of the blastomeres (Ninhaus-Silveira *et al.*, 2007). According to Kimmel *et al.* (1995), this layer constitutes an organ and can only be found in teleostei, positioned extra-embryonically and thus not contributing to the development of the embryo. According to Balinsky (1970), this layer is important in the breakdown of the yolk, converting it into available energy for embryo development. The development of the periblast during the morula stage has also been observed by Long & Ballard (1976) in *Catostomus commersoni* and by Matkovic *et al.* (1985) in *Rhamdia sapo.* However, in *Oryzias latipes* (González-Doncel *et al.*, 2005), *Prochilodus lineatus* (Ninhaus-Silveira *et al.*, 2006), and *B. gouldingi* (Faustino *et al.*, 2011) this layer was observed at the blastula stage.

In *Z. jahu*, the morula stage was followed by the blastula stage, which was characterized by the formation of the blastocele, a single and significant space between the blastoderm and periblast; which was also observed in *Oreochromis niloticus* by Galman & Avtalion (1989). Conversely, Kimmel *et al.* (1995), in a study in *B. rerio*, have suggested that the majority of teleostei only shows irregular extracellular spaces between the blastomeres without a well defined blastocele as observed by Faustino *et al.* (2011) in *B. gouldingi.*

The start of the gastrula stage in *Z. jahu* was characterized by the epiboly movement, when the embryonic cells moved from the animal to the vegetative pole (Leme dos Santos & Azoubel, 1996). The phases of the epiboly movement are often characterized according to the percentage of yolk coverage, as previously reported by Buzzolo *et al.* (2011) in *Pimelodus maculatus* and Faustino *et al.* (2015) in *B. gouldingi.* In *Z. jahu* eggs, when approximately 50% of the yolk was covered, the most superficial cells began to move contrary to the epiboly movement (involution), originating two embryonic layers: epiblast and hypoblast. The end of the gastrula stage was characterized by the formation of the blastopore or vitelline plug, in agreement with the findings by Buzzolo *et al.* (2011) in *Pimelodus maculatus* and Faustino *et al.* (2011) in *B. gouldingi.*

Organogenesis was defined by tissue and organ development. It began with the appearance of the embryonic axis, formed by the differentiation of the cephalic and caudal regions (Faustino *et al.*, 2011, 2015). This was followed by the appearance of somites, which are muscle precursors that develop in blocks of transient cells and are necessary for segmental organisation of embryos (Gilbert, 2003). The notochord was also present, a transitory organ related to the development of the neural tube (future central nervous

system, CNS) and the establishment of the antero-posterior body axis (Gilbert, 2003). The expansion of the neural tube culminated in the development of the three primary vesicles of the CNS, similarly to that observed in *R. sapo* by Cussac *et al.* (1985), *D. rerio* by Kimmel *et al.* (1995), *P. lineatus* by Ninhaus-Silveira *et al.* (2006) and *B. gouldingi* by Faustino *et al.* (2011, 2015). According to Kimmel *et al.* (1995), the optical vesicles form at the start of prosencephalon development and the otic vesicles originate from the posterior region of the rhombencephalon.

The organogenesis stage was followed by larval hatching, the final stage in the embryonic development of *Z. jahu*. Hatching occurs after vigorous muscle contractions in the tail and body of the larvae (Nakatani *et al.*, 2001), leading to the softening of the chorion by enzymatic activity (Blaxter, 1988). At this stage in *Z. jahu*, the primordial heart was observed in the anterior region of the yolk, which according to Hu *et al.* (2000) is the first definite organ to develop and become functional during embryogenesis, signalling the formation of the circulatory system.

Conversely, the presence of a rudimentary digestive system, an embryonic fin covering the whole body, primordial branchiae and barbels, oral cavity with small fissures hinting at the separation of the lips, olfactory organ with few cilia, outline of the optic and otic vesicles, and large yolk sac represent a group of morphological characteristics that demonstrate that *Z. jahu* larvae have low swimming ability and little perception of the environment at hatching. This situation makes the search for food and scape from predators difficult, thus making the larvae dependent on endogenous energy reserves.

These findings corroborate with Nakatani *et al.* (2001), who reported that the majority of fresh water fish larvae are not fully developed at hatching and often follow this pattern until the first stage of development after hatching (vitelline larvae). However, it is important to note that, especially in migratory species, the development of structures that enable the adaptation to adverse environment conditions occurs within a short time (Vandewalle *et al.*, 2005).

The data presented in this study are the first of its kind to be reported in *Z. jahu* and could be used to improve production in captivity as well as to aid conservation studies in this species.

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