Description of embryonic stages in *Melanosuchus niger* (Spix, 1825) (Crocodylia: Alligatoridae)

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Abstract

The determination of embryonic stages is very useful in comparative and evolutionary anatomy, experimental research and ecological studies. Although tables of development stages already exist for some crocodilian species, no table has yet been published for *Melanosuchus niger*. Therefore, the aim of this work is to monitor the development of this species of Alligatoridae and characterize its stages based on the main morphological transformations the embryo undergoes during the incubation process. Under natural conditions, two samples were collected daily in the Lake Cuniã Extractive Reserve in Rondônia, Brazil, making a total of 186 eggs. The incubation period was 90 days at an ambient temperature varying from 25.2 to 31.7 °C. We established 24 embryonic stages between the formation of the pharyngeal arch and hatching. Primordia for the sensory organs and somites were observed in the early stages. The buds of the pelvic and thoracic limbs emerged simultaneously, also in the early stages. An analysis of these structures showed evidence of considerable evolutionary modifications when compared with embryos of different species. In stage 12 we observed subtle pigmentation in the region of the head and, in stage 25, the pigmentation and striped pattern were typical of the newly hatched specimen. The eyetooth was visible in stage 18, disappearing within the first few weeks after hatching. The vitellus was completely absorbed in the penultimate stage. Direct comparisons of the table of embryonic stages were made between *M. niger* and other crocodilian taxa, and the main differences identified were chronological variations.

Keywords: black caiman, development, embryology, incubation, reptiles.

1 Introduction

Reptile embryology has long been investigated by different researchers (PARKER, 1879; KAMAL and HAMMOUDA, 1965; KAMAL, HAMMOUDA and MOKHTAR, 1970). Yntema (1968) was among the first to provide a normal pattern of embryonic stages for *Chelydra serpentina*, and since then the stages of development of several other species of Testudines have been determined. There is still a paucity of studies of this nature on the order Crocodylia. Magnusson and Taylor (1980) were pioneers in the characterization of the embryonic stages based on the external morphological traits of *Crocodylus porosus* (Crocodylidae), and were followed by Ferguson (1987) for *Alligator mississippiensis* (Alligatoridae) and Iungman, Piña and Siroski (2008) for *Caiman latirostris* (Alligatoridae). Although earlier studies such as that of Voeltzkow (1899) on *Crocodylus madagascariensis* and that of Reese (1915) on *Alligator mississippiensis* described some embryonic traits, none of them established a complete series.

Prior to the description and standardization of these stages, many researchers used standard body measurements to estimate the approximate age of the embryo (RISLEY, 1933), which to constant errors. In fact, if a series of morphometric dimensions and morphological traits could

be correlated to real or structural age, it could be possible to estimate the egg-laying time, the period of incubation, predict the hatch and or know how long a given embryo should be incubated to reach a given stage. Moreover, classification based solely on chronological age is very faulty, especially in terms of incubation time, which is normally variable since changes in the development rate may occur among embryos of the same age and even in the same nest (DONAYO, PINÃ and LARRIERA, 2002).

The incubation time of *Melanosuchus niger* eggs comprises the period from egg-laying to hatching, and may vary from two to three months (MEDEM, 1963; HERRON, EMMONS and CADLE,1990). Studies with crocodilians have demonstrated that temperature is an important abiotic factor that influences the duration of embryonic development, although it may also be affected by other variables such as humidity (PACKARD and PACKARD, 1984). The embryonic stages of *M. niger*, as well as for the species of Crocodylia *C. porosus* (MAGNUSSON and TAYLOR, 1980), *A. mississippiensis* (FERGUSON, 1987) and *C. latirostris* (IUNGMAN, PIÑA and SIROSKI, 2008), and for *Apalone spinifera* (GREENBAUM and CARR, 2002), *Python sebae*

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(BOUGHNER, BUCHTOVÁ, FU et al., 2007), *Gallus gallus domesticus* (HAMBURGER and HAMILTON, 1992), *Coturnix coturnix japonica* (AINSWORTH, STANLEY and EVANS, 2010), *Eleutherodactylus coqui* (TOWNSEND and STEWART, 1985) and *Carofilia perspicillata* (CRETEKOS, WEATHERBEE, CHEN et al., 2005), were established according to the continual appearance of distinct external morphological traits at a given moment of development. These traits are described in detail and are considered criteria for the differential recognition of each stage.

The black caiman (*M. niger*) is considered a species at low risk of biological extinction in nature (DA SILVEIRA, MAGNUSSON and CAMPOS, 1997). Horna, Zimmermann, Cintra et al.(2003) reported that this reptile may reach a total length of six meters. In fact, it is considered the largest South American predator distributed throughout the Amazon basin including Bolivia, Brazil, Colombia, Ecuador, Peru, Venezuela and some areas of Guyana and French Guiana.

It is extremely important to determine the stages of embryonic development because, in addition to providing information for comparative and evolutionary anatomy, it can contribute to descriptive or experimental research (BEGGS, YOUNG, GEORGES et al., 2000; HAMBURGER and HAMILTON, 1992; HUA, WANG and CHEN, 2004),

in addition to serving as a useful instrument for ecological studies. Therefore, the purpose of the present study was to monitor the embryonic development of *M.niger* and to characterize its embryonic stages based on the main morphological transformations the embryo undergoes during the process of natural incubation.

2 Material and methods

Melanosuchus niger eggs were collected daily from an area of natural incubation in the Lake Cuniã Extractive Reserve located in the state of Rondônia, Brazil. Collections were made between October and December, under ICMBio/IBAMA Permit No. 12243-1/2007. In a monitored area, the nests found after egg-laying were tagged and covered with a sheet of metal grill (Figure 1).

Under conditions of natural incubation, only two eggs were collected daily from the nests, starting on the tenth day of incubation. The other eggs were left in the nest until the next collection, throughout the period of incubation (Figure 1). A total of 186 eggs were collected from six different nests situated in the following geographic locations: nest 1 – 08° 19' 08.8" S and 63° 28' 54.3" W; nest 2 – 08° 18' 32.7" S and 63° 29' 09.5" W; nest 3 – 08° 19' 13.6" S and 63° 28' 07.3" W;

Figure 1. Marking system and control of the nests of *Melanosuchus niger*. a) Observation and counting of eggs found in the nest after laying; b) marking and protection the nest with metal screen; c) manipulation of nest for collection; d) thermometer to record the air temperature and in the nest.
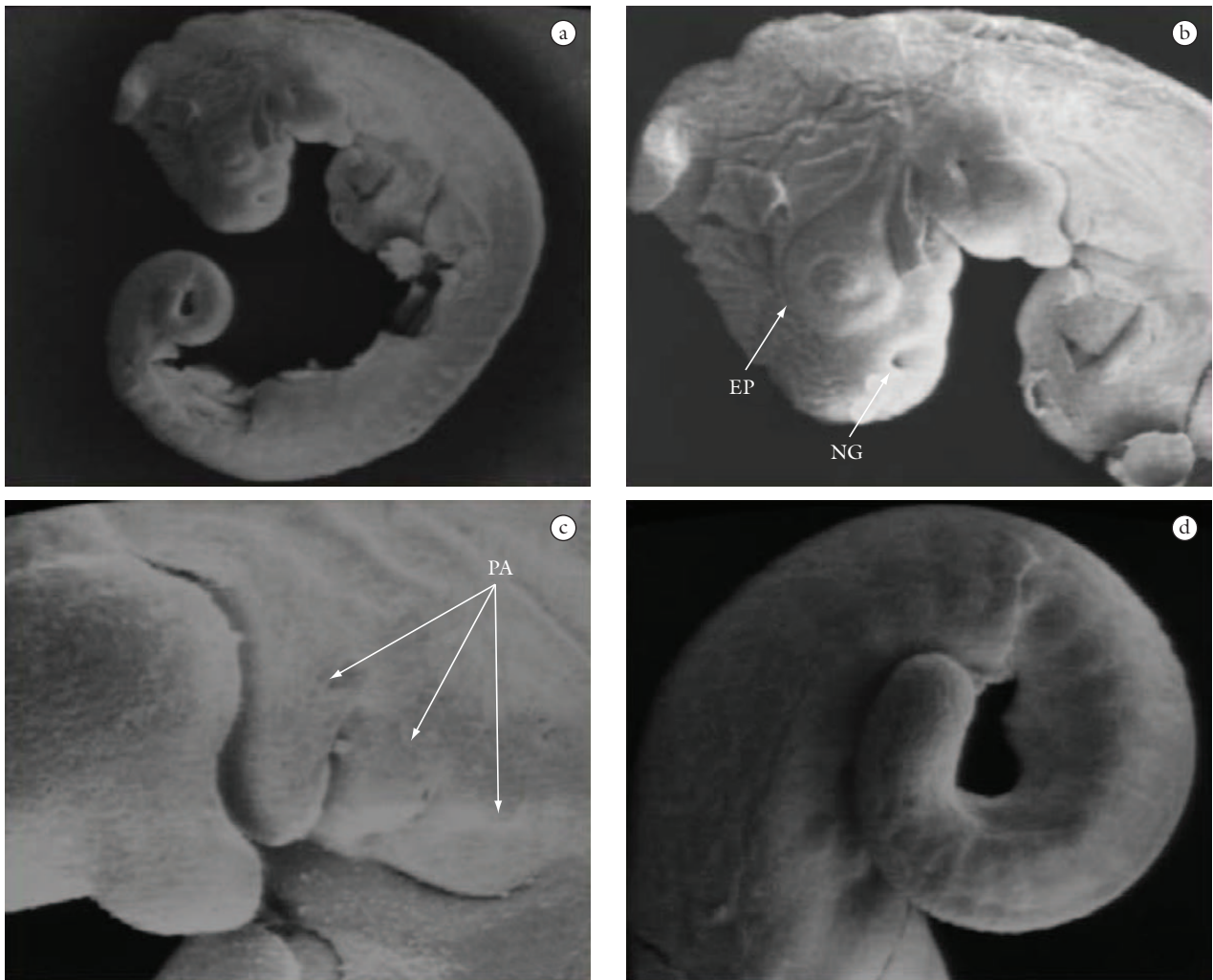
Figure 2. Scanning electron microscopy of stage 4 of *M. niger* in lateral view. a) Panoramic view; b) details of eye primordium and nasal groove; c) pharyngeal arches; d) extremity of the tail. OP, optic placode; NG, nasal groove; EP, eye primordium; PA, pharyngeal arches.

nest 4 – 08° 19' 15.3" S and 63° 28' 02.8" W; nest 5 – 08° 19' 59.5" S and 63° 27' 29.5" W and nest 6 – 08° 19' 12.0" S and 63° 28' 24.1" W. The air temperature varied from 25.2 to 31.7 °C, while the temperature in the nests ranged from 28.2 to 31.5 °C throughout the period of incubation. The embryos were removed from their shells with surgical scissors and the specimens fixed in 10% formalin. At the end of each collection, the specimens were sent to the Laboratory of Comparative Vertebrate Anatomy (LACV) at the University of Brasília – UnB for processing and analysis.

This *M. niger* reproduction area is strictly controlled by the RAN (National Center for Research and Conservation of Reptiles and Amphibians), an agency that conducts scientific research and management actions for the conservation and recovery of endangered species throughout the Brazilian territory. All the procedures involving the animals were described in detail and approved by the Research Ethics Committee of UnB (UnBDOC No. 100271/2009). All specimens were deposited in the LACV at the University of Brasília.

The embryonic stages were determined according to the appearance of different external morphological traits at a given moment of development. The main structures used as parameters were: pharyngeal arches, somites, choroidal fissure, limb buds, fore and hindlimb claws, nasal structures, development of the urogenital papilla, cloaca, nictitating membrane, eyelids, pigmentation, amount of vitellus absorption, and emergence of teeth and eggtooth. The terminology employed here followed that used by Iungman, Piña and Siroski (2008), Ferguson (1985) and Magnusson and Taylor (1980). These structures were examined in detail under a stereoscopic microscope (Leica S6D) and the information was visually recorded with a digital camera (Sony DSC H20 10.1).

The first embryos were also analyzed by scanning electron microscopy (SEM) to reveal some of the principal elements present in the early stages of development. These specimens were fixed in a solution of glutaraldehyde and paraformaldehyde immediately after their removal. After fixation, the material was preserved in sodium cacodylate buffer to await the post-fixation process, when it was

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immersed in an osmium tetroxide solution. The material was then subjected to a process of gradual dehydration in baths of aqueous solutions of 30, 50, 70 and 90% of acetone and three 15-minute baths of pure acetone followed by critical point drying with CO_2 and surface metallization in a sputter coater. The material was then photographed in a JEOL JSM 840A scanning electron microscope.

We established 24 stages of embryonic development (stages 4 to 28) between the formation of the pharyngeal arch and hatching. Our description began in stage 4 due to two factors. First, the nonavailability of material during the initial stages of cleavage to neurulation, and second, our youngest embryo presented very similar traits to those described in stage 4 by Iungman, Piña and Siroski (2008).

3 Results

Below is the description of the stages of embryonic development of Alligatoridae *M. niger*, based on the main external morphological traits starting from the tenth day of oviposition.

Stage 4 (10-day old embryo): Approximately 26 pairs of somites are visible on the dorsum of the embryo. The mandibular arch is the largest of the three pharyngeal arches that are present. Two clefts are open laterally, the second cleft covered by the hyoid arch. Five cephalic vesicles are visible in the rostral portion of the embryo. The optic placodes appear conspicuously, while the auditory placodes are present dorsally to the pharyngeal arches on the lateral aspect of the body. There are signs of a nasal placode in the rostral portion (Figures 2, 7a). The heart primordium appears as a prominence on the ventral side and a protuberance immediately caudal to this organ indicates the liver primordium. The notochord extends to the tip of the tail. The curvature of the body begins with flexion of the trunk at the level of the heart, and J-shaped flexion of the tail is already visible.

Stage 5 (11 to 12-day old embryo): There are 28 pairs of somites. The maxillary process extends ventrally to the mandibular process. Thoracic and pelvic limbs are present as small distinct buds. The axis of the limb is caudoventrally oriented. The flexion of the trunk is greater, giving the embryo a U-shape, and the tip of the tail is much more curled (Figure 3a).

Stage 6 (13 to 14-day old embryo): The mandibular process is more conspicuous. Thoracic and pelvic limb buds are larger and show signs of digital plate primordial (Figures 3b, 5a, 6a). The embryo's flexion increases gradually and the tip of the snout lightly touches the tail. The eye has weak pigmentation of the choroid layer. The nasal placode is deeper and extends caudally (Figure 3b).

Stage 7 (15 to 16-day old embryo): The digital plate of the pelvic limb buds is evident, but still lacks digital grooves, and the thoracic limb buds are less developed. Eye pigmentation is stronger, and invagination of the optic vesicles creates the choroid fissure.

Stage 8 (17 to 18-day old embryo): The digital plates of the thoracic and pelvic limbs are wider and show slight signs of digital grooves. Elbows are recognizable in the thoracic limb buds, while the knees of the pelvic limb buds are still imperceptible. The rostral edge of the mandibular process is situated at the level of the caudal edge of the crystalline lens

of the eye. A small protuberance indicates the primordium of the urogenital papilla located on the ventral surface at the root of the tail between the pelvic limbs. The primordium of the external auditory meatus is clearly visible in the rostrolateral region of the embryo (Figures 3c, 5b, 6b).

Stage 9 (19 to 20-day old embryo): The digital plates of the pelvic and thoracic limbs are completely developed, but without digital serration. The eyeball is prominent, with clearly visible upper eyelid. The mandibular process extends rostrally, encompassing two thirds of the maxilla.

Stage 10 (21 to 22-day old embryo): The upper eyelid is developed, invagination of the optic vesicles is complete, and the choroidal fissure narrows and closes. The mouth is well developed, extending toward the cardiac prominence. The digital grooves of the pelvic limbs are more pronounced. The horizontal projection of the snout is prominent on the lateral sides (Figures 3d, 6c).

Stage 11 (23 to 25-day old embryo): The periphery of the digital plate of the thoracic limbs shows distinct grooves and a weak demarcation of four digits is visible by serration. The upper eyelid reaches the upper edge of the iris. The abdominal wall is developed, but the ventricles, liver and other organs are still visible and the intestine is visible through the transparent abdominal wall (Figures 5c, 6d).

Stage 12 (26-day old embryo): In this stage, the embryo is notably longer and the outlines of the fore- and hind-limb digits are visible in the expanded discs of the limbs. The presence of the lower eyelid and the nictitating and tympanic membranes is evident. The brain is still visible through the transparent tissues at the top of the head and still presents a median groove. The body's ventral wall musculature is still open in the median line. The tail is clearly bent at the distal end distal. Elbows and knees are recognizable in the limbs and are slightly flexed at the joint. Subtle pigmentation appears first on the head, specifically between the eyes and snout (Figures 3e, 5d, 6e).

Stage 13 (27 to 29-day old embryo): The median groove of the brain is more evident. Fore and hindlimb digits are clearly demarcated around the digital plate and are slightly serrated in both limbs. The eyelids are well formed and cover most of the eye (Figure 7b). The urogenital papilla between the pelvic limbs is now less exposed. The body's pigmentation is stronger, and the dorsal surface and tip of the tail are darker (Figure 3f).

Stage 14 (30 to 31-day old embryo): Interdigital grooves are more distinctly formed, demarcating the digits of fore and hindlimbs. Pigmentation areas are now concentrated in the tail, limbs and dorsal side of the neck. Facial pigmentation is more intense and pigment spots are visible on the mandible. Presence of the cloacal primordium with two small lobes. In this stage, claws begin to develop in the first, second and third digits of the foot and in the first and second digits of the hand (Figures 3g, 5e).

Stage 15 (32-day old embryo): The cloacal orifice now involves the urogenital papilla (Figure 7e). Claws are clearly visible in the digits of both hands and feet and the tip of the claw of the second digit is slightly curved. There is large-scale dorsal and tail pigmentation, giving the embryo a slightly brownish appearance. Another marked characteristic is the emergence of the first rows of nuchal scutes.

Stage 16 (33 to 38-day old embryo): The upper and lower eyelids are separated only by a narrow cleft. There

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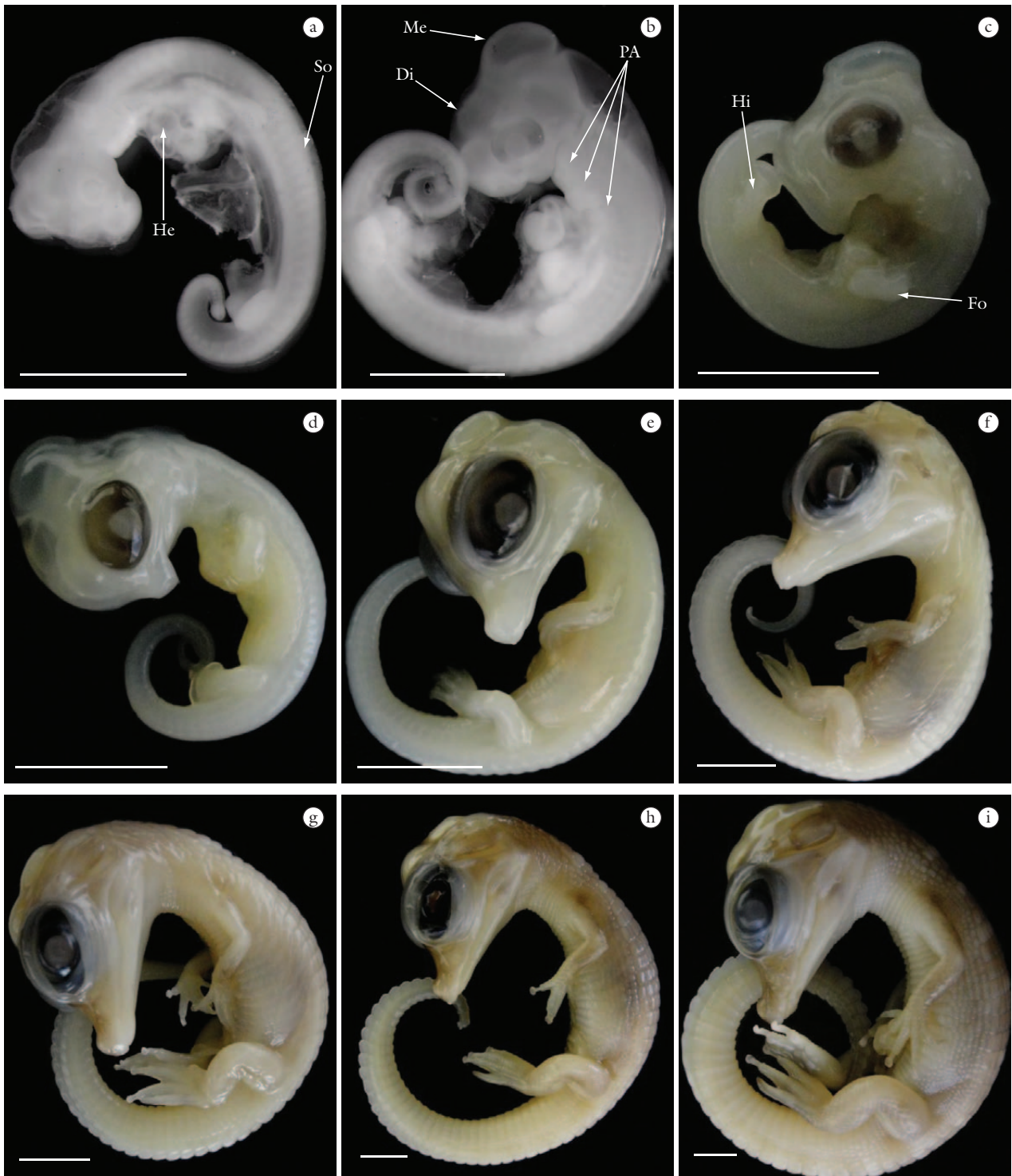J. Morphol. Sci., 2011, vol. 28, no. 1, p. 11-22

Figure 3. Photographs of the embryonic stages of *M. niger* in lateral view. a) Stage 5; b) stage 6; c) stage 8; d) stage 10; e) stage 12; f) stage 13; g) stage 14; h) stage 16; and i) stage 18. Di, diencephalon; Mi, midbrain; He, heart, Fo, forelimb; Hi, hindlimb. PA, pharyngeal arches; So, somites. Scale bar: 1 cm.

are five rows of nuchal scutes, slightly pigmented. A series of protrusions in the digits mark the beginning of the interphalangeal joints. Pigmentation extends toward the thoracic and pelvic limbs. The claw of the third digit is laterally flattened and ventrally curved, while the other claws are still flat (Figures 3h, 5f, 6f).

Stage 17 (39-day old embryo): The abdominal wall musculature is now completely fused along the median ventral line except in the umbilical region. Thus, the previously exposed intestine is now completely retracted. The outlines of the digits are totally individualized and the differences in their sizes clearly visible.

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Stage 18 (40 to 45-day old embryo): Seven rows of nuchal scutes are present on the dorsal side of the embryo. A marked characteristic in this stage is the emergence of a small eggtooth at the tip of the maxilla (Figures 7c, d). The claws of the digits are curved. The entire body of the embryo shows increased pigmentation, changing from pale to dark gray (Figures 3i, 5g, 6g).

Stage 19 (46 to 47-day old embryo): In this stage the pigmentation pattern of the whole body appears as on hatchling, with the typical black dorsal stripes alternating with light brown stripes. The head is brown, but lighter than the hatchling.

Stage 20 (48 to 50-day old embryo): The size of the umbilical opening is now smaller. The surface of the head shows dark brown pigmentation. Cranial ossification is practically complete except for a small oval window, the fontanelle in the center of the dorsal platform of the skull, through which the brain is still visible.

Stage 21 (51 to 52-day old embryo): The cloacal orifice is completely closed and the urogenital papilla no longer visible. The entire body shows more intense pigmentation. Musk glands are barely visible along the caudolateral edges of the intergular floor of the mandible (Figure 4a).

Stage 22 (53 to 55-day old embryo): The tail scutes emerge, showing more intense pigmentation. The pupil presents a vertical cleft. The tympanic membrane covers the entire external auditory meatus (Figure 4b).

Stage 23 (56-day old embryo): The cranially oriented scutes of the tail are bristled, like those of hatchling. The mandible and maxilla begin to show the emergence of protuberances signaling tooth primordia. Pigmentation does not change perceptibly from that found in stage 22 (Figure 4c).

Stage 24 (57 to 66-day old embryo): The eyelid remains open but covers more than half of the eye. Both eyes
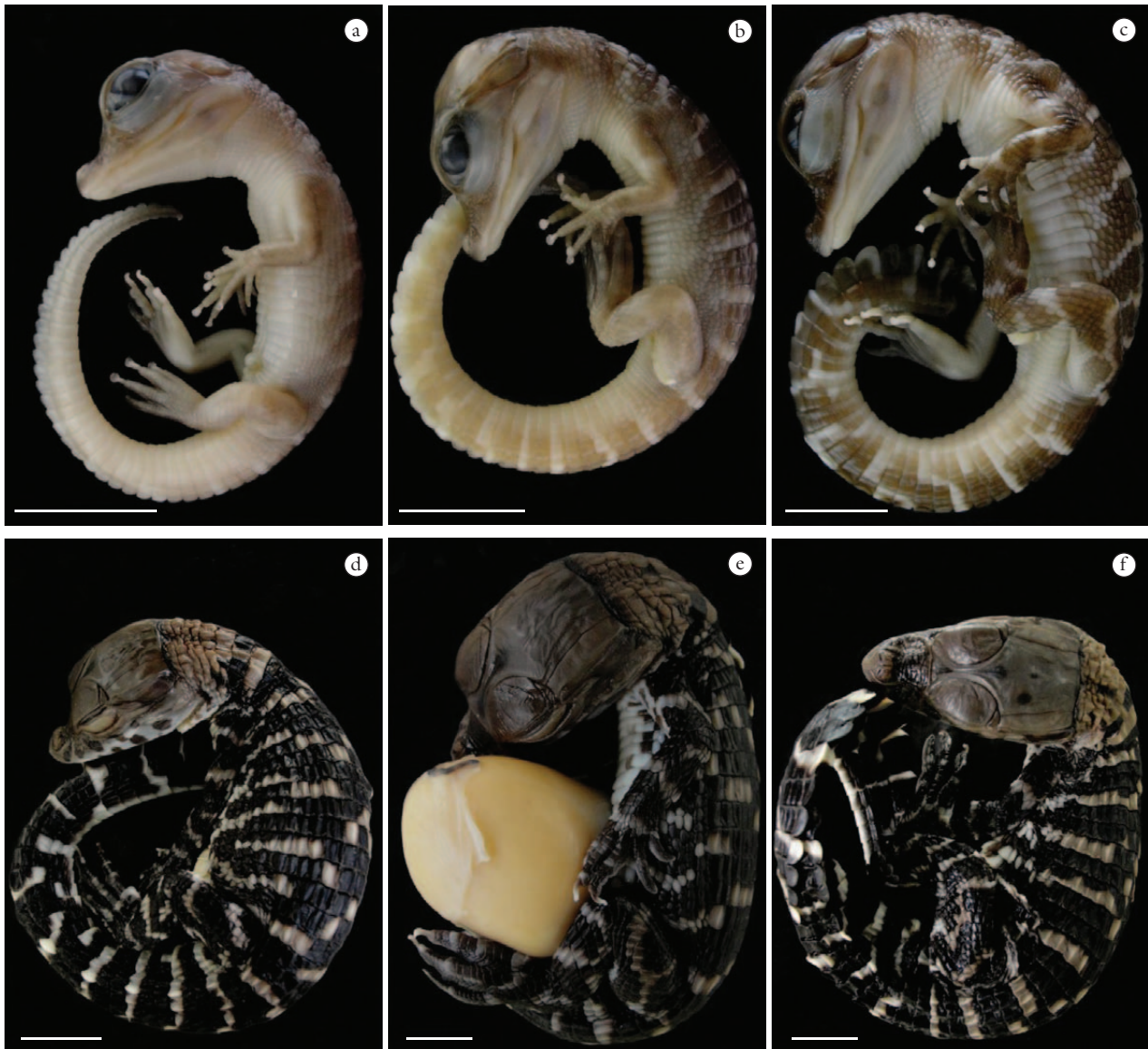
Figure 4. Photographs of the embryonic stages of *M. niger* in lateral view. a) Stage 21; b) stage 22; c) stage 23; d) stage 24; e) stage 27; and f) stage 28. Scale bar: 2 cm.
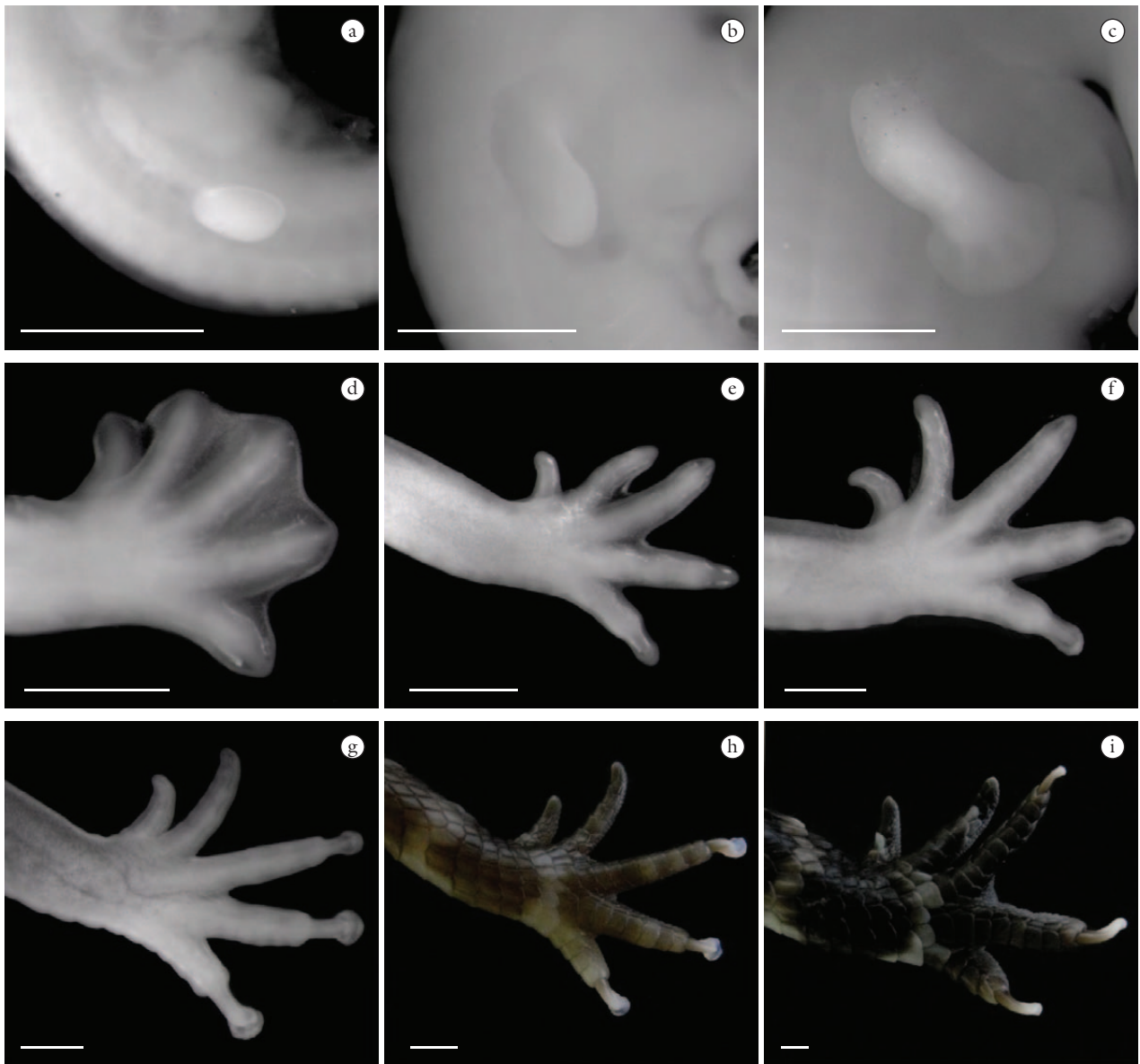
Figure 5. Photographs of the development of the forelimb of *M. niger*. a-c) Lateral view of right forelimb in stages 6, 8 and 11, respectively; d-i) dorsal view of right hand; d) stage 12; e) stage 14; f) stage 16; g) stage 18; h) stage 24; and i) stage 27. Note the progressive developement of the digital plate and digital serration in limb and the appearance of claws. Scale bar: 1 mm.

and body of the embryo show more intense pigmentation (Figure 4d).

Stage 25 (67 to 71-day old embryo): Both eyelids are completely formed and completely cover the surface of the eye. Albeit no longer visible through the cranial tissue, the cranial fontanelle is still present and can be felt through palpation. Now both the pigmentation and striped pattern are typical of the hatchling. Another marked characteristic in this stage is the beginning of vitelline absorption, which begins to be encompassed by the embryo.

Stage 26 (72 to 75-day old embryo): About 50% of the vitelline sac is encompassed by the embryo. The first teeth emerge in the mandible and maxilla. Other traits remain unaltered.

Stage 27 (76 to 77-day old embryo): The vitelline sac is completely encompassed, leaving only an median raphe (Figures 4f, 7f).

Stage 28 (78 to 91-day old embryo): Embryos in this stage are very close to hatching, and their median raphe is long and narrow. The cranial fontanelle is closed and the roof of the skull is well developed (Figures 4f).

4 Discussion

The series of stages of normal embryonic development found in the present study enables us to compare *M. niger* with three other crocodilian species in order to observe the similarities and differences among these strongly related taxa, as well as make comparisons with other reptile, bird and mammal species.

Unlike other groups of vertebrates such as *Ichthyophis kohtaoensis* of the order Gymnophiona (DUNKER, WAKE and OLSON, 2000), the anuran *Hyperolius puncticulatus* (CHIPMAN, HAAS, TCHERNOV et al., 2000) and
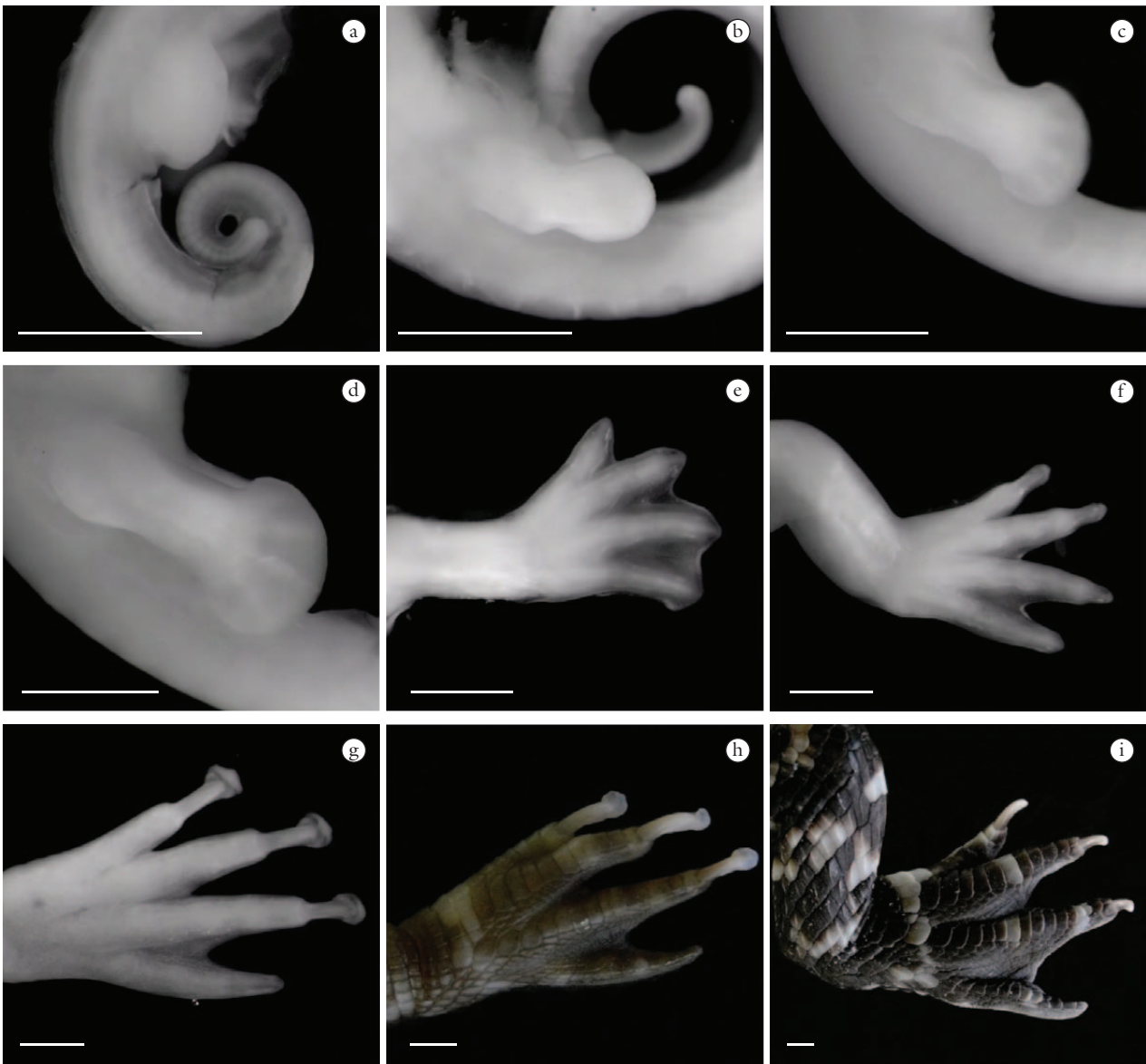
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Figure 6. Photographs of the development of the hindlimb of *M. niger*. a-d) Lateral view of right hindlim b in stages 6, 8, 10 and 11, respectively; e-i) dorsal view the right foot; e) stage 12; f) stage 16; g) stage 18; h) stage 24; and i) stage 27. Note the progressive development of the digital plate and digital serration in limb and the appearance of claws. Scale bar: 1 mm.

Squamata (HUBERT, 1985), embryos of Testudines do not present species-specific traits in early stages (RENOUS, RIMBLOT-BALY, FRETEY et al., 1989), as has been found in the aforementioned crocodilians and in *M. niger*. For a better comparison, we have organized the stages of development in chronological sequence.

In the vertebrates *Paroedura pictus* (NORO, UEJIMA, ABE et al., 2009), lizards of the genus Anolis (SANGER, LOSOS and GIBSON-BROWN, 2008), *Pelodiscus sinensis* (TOKITA and KURATANI, 2001), *A. spinifera* (GREENBAUM and CARR, 2002), *G.g.domesticus* (HAMBURGER and HAMILTON 1992), *A. mississippiensis* (FERGUSON, 1987), *C. latirostris* (IUNGMAN, PIÑA and SIROSKI, 2008) and *C. porosus* (MAGNUSSON and TAYLOR, 1980), the early stages were determined by the emergence of the primary brain vesicles, placodes,

pharyngeal arches, number of somites, and body flexion and rotation. The intermediate stages were marked by the emergence and morphology of the limbs and craniofacial traits, while the later stages were detected by differentiated pigmentation and the scutal patterns were the most marked traits in both crocodilians and Testudines. The embryonic stages of *M. niger* were determined according to these criteria, since these traits have become practically a standard for comparative studies of reptile embryos (GREENBAUM and CARR, 2002).

According to Graham (2001), the pharyngeal arches contribute significantly to the external appearance of the embryo in the early stages. Initially, they consist of segments of mesenchymatous tissue separated by the branchial clefts. These arches begin to emerge before stage 7 in the embryological stages of *A. mississippiensis* (FERGUSON,
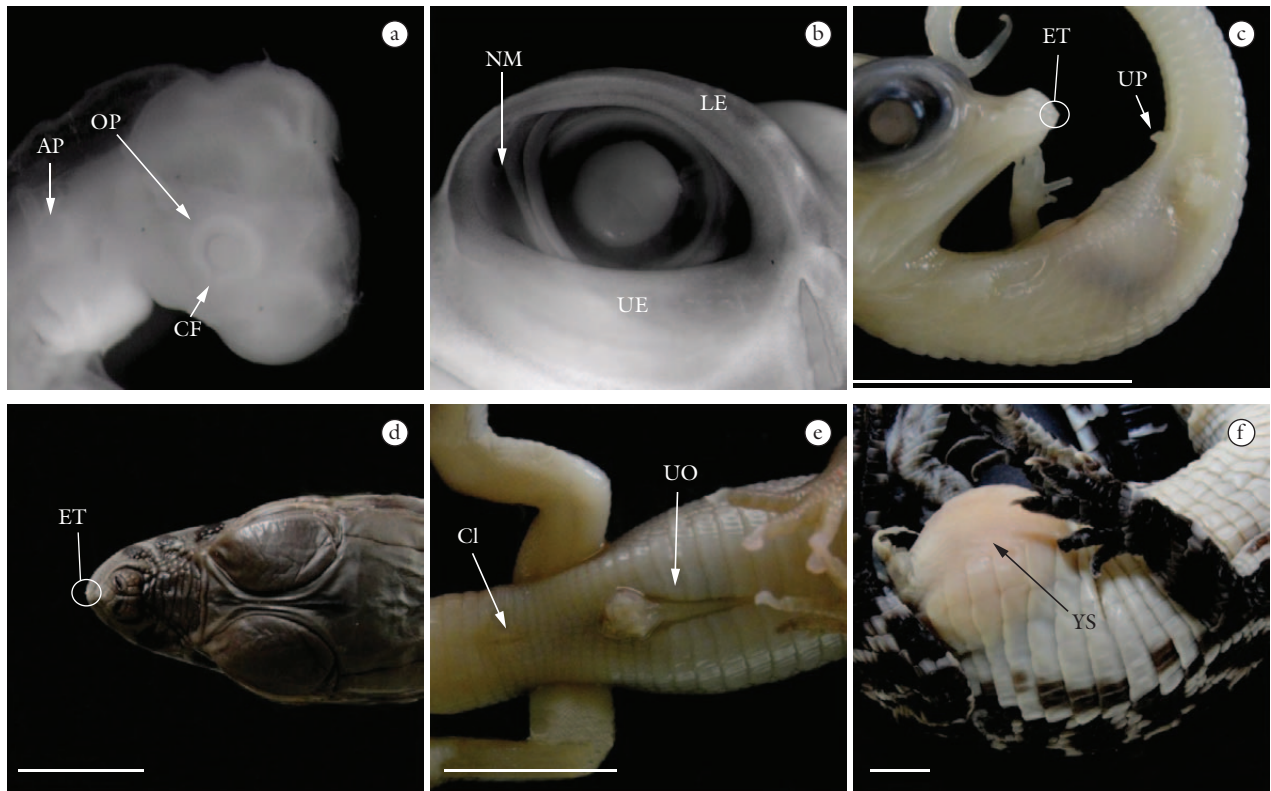
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Figure 7. Photographs of the embryonic stages of *M. niger*. a-c) Lateral view; a) stage 5; b) stage 15; c) stage 18; d) stage 24 in dorsal view; e) stage 15 in ventral view; and f) stage 27. CF, Choroid fissure; OP, optic placode; AP, auditive placode; LE, lower eyelids, UE; upper eyelids; NM, nictitating membrane; UP, urogenital papilla; ET, eggtooth; UO, umbilical opening; Cl, cloaca; YS, yolk sac. Scale bar: 1 cm.

1987), and in *M. niger* specifically in stage 4, while for Iungman, Piña and Siroski (2008), these mesenchymatous structures are visible precisely in stage 7, and Magnusson and Taylor (1980) described the emergence of two pharyngeal arches in *C. porosus* already in stage 1.

The optic placodes in *M. niger* emerge in stage 4, similar to what Ferguson (1987) observed in *A. mississippiensis* and Iungman, Piña and Siroski (2008) in *C. latirostris*, where the development of this structure begins in stage 4, although it is completed only in stage 6, while for *C. porosus* (MAGNUSSON and TAYLOR, 1980) this structure is described in stage 2. The formation of the auditory placode is reported by Ferguson (1987) for *A. mississippiensis*, by Iungman, Piña and Siroski (2008) for *C. latirostris* and by Magnusson and Taylor (1980) for *C. porosus* in stage 2, unlike *M. niger*, where it was only observed in stage 4. In *G.g.domesticus* (HAMBURGER and HAMILTON, 1992), the auditory placode is present in stage 14. A trait shared by the crocodilians *A. mississippiensis*, *C. latirostris*, *C. porosus* and *M. niger* is the emergence of the choroidal fissure in stage 7 and of the urogenital papilla in stage 8, which represent pronounced structures in these stages for the Crocodylia species, except for the choroid fissure in *C. porosus*, which was not described by Magnusson and Taylor (1980).

The emergence of the somites was evidenced principally by a dorsal protuberance in stage 4 of *M. niger*. Magnusson and Taylor (1980) reported their emergence in *C. porosus*

right at the beginning of development in stage 1, unlike the species *C. latirostris* (IUNGMAN, PIÑA and SIROSKI, 2008) and *A. mississippiensis* (FERGUSON, 1987), where they emerge in stage 2. It is important to note that although the somites appear early in these species, their number varies considerably during the primordium of their development, with 20 somites in *A. mississippiensis* and *C. latirostris*, 25 in *C. porosus* and 26 in *M. niger*. In our observations of *M. niger*, consistent with the descriptions of Iungman, Piña and Siroski (2008) for *C. latirostris*, individual observation of the somites became increasingly difficult starting from stage 5, which for *G.g.domesticus* (HAMBURGER and HAMILTON, 1992) occurred starting from stage 14. According to Hamburger and Hamilton (1992), this is in part due to dispersion of the mesoderm of the more cranial somites and, in the final stages, to the curvature of the tail. Therefore, other structures, such as the formation of the limbs, were used as identification criteria from stage 5 onward in crocodilians.

With regard to the development of the limbs, the Testudines exhibit a tendency for differentiation of the forelimbs before the hindlimbs, except for marine turtles, in which the limbs develop synchronously (MILLER, 1985). In birds, bats, *C. latirostris*, *C. porosus* and *M. niger* (IUNGMAN, PIÑA and SIROSKI, 2008; BEGGS, YOUNG, GEORGES et al., 2000; CRETEKOS et al., 2005; HAMBURGER and HAMILTON, 1992; MAGNUSSON and TAYLOR, 1980), the forelimb buds emerge prior or

simultaneously with the hindlimb buds. This information is not consistent with the descriptions of Ferguson (1985, 1987) for *A. mississippiensis*, in which the emergence of the hindlimbs occurs first.

Among the amphibians, even within a single genus, there is considerable variation in the sequence of emergence of the limbs. In *Xenopus*, the hindlimb buds appear first (NIEUWKOOP and FABER, 1994), unlike *Ambystoma* (HARRISON, 1969; SCHRECKENBERG and JACOBSON, 1975), which still present significant differences among several species (SCHRECKENBERG and JACOBSON, 1975), as well as in *Rana*, where the hindlimb buds emerge in different stages (SHUMWAY, 1940). Variations in the chronology limbs development may reflect a functional value. The forelimb buds in the possum (McCRADY, 1938), for instance, develop more rapidly than in other mammal species. According to the author, this heterochrony reveals an adaptation to the characteristics of marsupial reproductive biology, since newborns must use these limbs to climb up into the pouch.

Unfortunately, we were unable to observe whether there is an order in the emergence of these limbs in *M. niger* due to their rapid development, even with specimens collected daily. We believe new studies may reflect the lack of a complete ontogenetic series, making it difficult to observe the development of this trait. We also suggest that the unequivocal observation of these possible events will require more precise analyses of many specimens in the stages in which these episodes occur, possibly at intervals shorter than 24 hours.

In this context, we point out that the time of emergence of the hind and forelimb buds is extremely variable. This diverges notably from the phylotypic concept put forward by Haeckel (1891), in which the morphology of vertebrate embryos in the early stages of development is considered resistant to evolutionary changes. However, upon analyzing these and other prominent traits on the planes of the body of these vertebrates, we see evidence of considerable evolutionary modifications in the comparison of embryos of different species.

In the final stages, unlike other crocodilian species, *C. latirostris* and *M. niger* exhibit apparent pigmentation only after the formation of the jaws and claws. In both these crocodilians, pigmentation begins along the limbs, spreading up the ventral side of the flanks, jaws and back, but showing little or no ventral pigmentation, unlike other crocodiles that present pigmentation first in the ventral portion which then extends dorsally (FERGUSON, 1985, 1987). Iungman, Piña and Siroski (2008) reported that the coloration pattern of *C. latirostris* during its development is a unique trait, since it differs from that reported for the other crocodilians. However, our investigation of *M. niger* revealed a pigmentation pattern with aspects resembling those of *C. latirostris*, indicating a closely associated trait between these two species.

An interesting trait to highlight is the o emergence of the egg tooth which, according to Ferguson (1987), marks the beginning of stage 26, although the author did not describe this stage due to the absence of this structure in his series of embryos of *A. mississippiensis*. Later, Iungman, Piña and Siroski (2008) concurred with this statement, although they reported that the egg tooth of *C. latirostris* begin to emerge in stage 23. On the other hand, in our

observations of embryos of *M. niger*, the egg tooth became visible in stage 18. We also found that this structure was very fleeting, disappearing a week after its emergence, as has been seen in most birds (the egg tooth disappears within the first week after hatching). However, this structure may also remain for longer times, as Wiebe (2010) observed. This author concludes that in some bird species, the size and persistence of the egg tooth suggests an alternative or complementary function, such as orientation of the parents while feeding their young in nests located in sites with little light (WIEBE, 2010). The lack of quantitative data about the persistence of the egg tooth in crocodilians rendered it impossible to associate them with any other function in these reptiles.

The variations in the chronological emergence of the structures can be explained, in part, by the incubation temperature, which may vary under natural conditions within and among nests; hence, the incubation period is often variable (LANG, ANDREWS and WHITAKER, 1989; PIÑA, LARRIERA, MEDINA et al., 2007). In addition to temperature, development may be influenced by other variables such as nest humidity (PACKARD and PACKARD, 1984), gas environment (WARBURTON, HASTINGS and WANG, 1995) and acid-base equilibrium (ETCHBERGER, EWERT, PHILLIPS et al., 1992). This may explain the differences between the species reported by Ferguson (1987), Iungman, Piña and Siroski (2008), Magnusson and Taylor (1980), and *M. niger*, considering that the latter was collected in an area of natural incubation. Thus, the main differences are concentrated in chronological variations. Further studies are needed for a comparative phylogenetic analysis due to the high potential for intra and interspecific variations in species.

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