

Ontogeny of the Postcranial Axial Skeleton of *Melanosuchus niger* (Crocodylia, Alligatoridae)

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ABSTRACT

This study proposes the description of the development of the postcranial axial skeleton, including vertebrae, gastralia, ribs, sternum, and interclavicle, in *Melanosuchus niger*. Six nests were marked and two eggs removed from each nest at 24-hr intervals until hatching. For posthatching evaluation, 30 hatchlings were kept in captivity and one exemplar was euthanized at three-day intervals. Samples were diaphanized using potassium hydroxide (KOH), alizarin red S, and Alcian blue. A routinely generally used method was applied for histological evaluation. It was difficult to define in which vertebrae the development of cartilaginous centers began, but it was possible to observe that this condensation advanced in the craniocaudal direction. The condensation started in the vertebral arches and was visibly stronger in the cervical and dorsal regions, advancing to the lumbar, sacral and, last, to the caudal region. The atlas showed a highly different morphology compared with the other cervical vertebrae, with a short intercenter, two neural arches, and a proatlas. The ossification process began in the body of cervical vertebrae III to VIII and alizarin retention decreased in the last vertebrae, indicating a craniocaudal direction in bone development, similar to cartilage formation. In the histological sections of gastralia and interclavicles of *M. niger* at several development stages, it was possible to observe that these elements showed intramembranous development. Anat Rec, 00:000–000, 2017. © 2017 Wiley Periodicals, Inc.

Key words: black caiman; chondrogenesis; embryology; gastralia; vertebral column

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The skeleton of vertebrates is one of the most important systems analyzed in morphological studies. As the mineralized structure is preserved in fossils, it enables comparisons with living groups, determination of homology, and the drawing of relationships between structures with the same origin in the different groups. The wide variety in skeletal elements observed throughout the evolutionary history of the great groups reflects the plasticity of this system in the response to the different habitats, besides providing important information related to lifestyle, such as posture, size, locomotion pattern, and feeding habits (Kardong, 2010). On the other hand, bone and cartilage development patterns show the mechanisms responsible for the origin of these evolutionary variations, and are important indications of kinship (Maisano, 2002).

Each group of animals has ontogenetic particularities in the formation of their axial skeleton, leading to an adult form that responds to specific demands (Burke, 1989). In Testudines, for example, the shell is a highly specialized synapomorphic evolutionary characteristic that makes them a restricted clad (Lee, 1996; Gilbert et al., 2001). In other reptiles, these particularities are less remarkable and, consequently, less explored. The axial skeleton of the specimens in the Crocodylia order, among others, presents particular and little known characteristics. These modifications may be understood by means of ontogenic studies related to evolutionary patterns of the taxon (Hildebrand and Goslow, 2006).

The sequence in which ossification takes place is a source of data that has been widely investigated. Recently, Maisano (2002), Sánchez-Villagra (2002), and Schoch (2006), emphasized the potential of this type of information when used in phylogenetic analyses. Although these sequences are normally preserved inside a species, the factors that affect their evolution are speculative, such as the effect of heterochronic ossification and morphological evolution (Sánchez-Villagra, 2002; Prochel, 2006; Sánchez-Villagra et al., 2008; Maxwell, 2009).

In the development of the vertebral elements, the notochord, which supports the axis of the body of the embryo in its initial stages, is functionally replaced by a series of vertebrae formed around it and in the neural tube. The vertebral series may be divided in presacral, sacral, and caudal regions. Each vertebra is made up by a center, a neural arch that grows to form, dorsally, the spinous process and, in many cases, an intercenter that is ventrally located between each vertebral center. In the caudal region, the hemal arches may extend from the ventral part of the intercenter throughout the tail (Vickaryous and Hall, 2008).

The ribs are articulated to some vertebrae in Crocodylia. They are connected to the sternum by means of the ventral ribs. Originally, the ribs extended throughout the length of the vertebral column to increase direct contact between the axial muscles and the skeleton. As the ribs increased in length, they started to protect the viscera, but the flexibility and minimum weight were maintained. They are specialized to contribute to respiratory movements only among amniotes (Farmer 2010, 2015).

Although there are no studies about the ontogeny pattern of post-cranial axial skeleton of *Melanosuchus niger*, reports describe skeletal development in others Crocodylia species. One example is the contribution of Bellairs

and Kamal (1981), who analyzed the development of the cranium in crocodiles. Some data on the ossification sequence were also provided by Müller (1967), whereas Deraniyagala (1939) emphasized the study of ossification in the species *Crocodylus palustris* and *Crocodylus porosus*, without much detail. Klembara (1991) evaluated the relationship between dermal bones and the chondrocranium of *A. mississippiensis*. Other organogenesis aspects, including the postcranial axial skeleton, were reviewed by Ferguson (1985). Limb development in crocodylians was studied by Steiner (1934), Müller and Alberch (1990), and Vieira et al. (2016b). Rieppel (1993) also analyzed the ossification pattern in the species *A. mississippiensis*. More recently, Lima (2011a, 2011b, 2011c) described the sequence of appearance of ossification centers in *C. yacare*, but this report was restricted to bone formation. Some aspects of postembryonic development and postcranial skeleton growth were analyzed by Mook (1921).

Given the living species of Crocodylia, studies on the formation of the skeleton remain little explored. Based on the perception that development may crucially contribute to the understanding of the evolutionary processes, this study proposes the description of the development of the postcranial axial skeleton in *Melanosuchus niger* based on a sequence of temporally organized stages in relation to cartilage and ossification center development, no matter if the process is endochondral or intramembranous. This study represents the most complete description of postcranial skeletal development in *M. niger*, and focuses on the discussion of the regionalization of the vertebral column, rib homology, and origin of the gastralium, ventral ribs, and sternum.

MATERIALS AND METHODS

Six nests of *Melanosuchus niger* (Spix, 1825) were marked and protected with a metal screen after oviposition between October and December 2008, in the Extractive reserve of Lago Cuniã, state of Rondônia, Brazil, under the license no. 12243-1/2007 RAN/IBAMA. Each nest was kept under natural incubation conditions, and two eggs were removed every 24 hr, starting the tenth day of development until the moment of hatching. An ontogenetic sequence with 186 samples was obtained (Table 1). All procedures of this research were approved by the Animal Ethics Committee from the Institute of Biological Sciences at University of Biology (UnBDOC no. 100271/2009).

After surgically remotion of the embryos from eggs, they were euthanized with an overdose of thiopental (50mg/kg), via the intracoelomic route, and preserved in formaldehyde 3.7%. For post-hatching evaluation, 30 hatchlings were kept in a water tank warmed with electric heater in environment temperature of 27°C and *ad libitum* feeding. One exemplar was euthanized every three days with the same protocol described above and was also preserved in formaldehyde 3.7%.

At the end of the collection, the specimens were sent to the Laboratory of Comparative Anatomy of Vertebrates (LACV), at University of Brasilia - UnB, for analysis.

TABLE 1. Number of embryos of *Melanosuchus niger* used per embryonic stage

Number of embryos	Days of incubation	Embryonic stages
2	10	4
4	11 and 12	5
4	13 and 14	6
4	15 and 16	7
4	17 and 18	8
4	19 and 20	9
4	21 and 22	10
6	23 and 25	11
2	26	12
6	27 and 29	13
4	30 and 31	14
2	32	15
12	33 and 38	16
2	39	17
12	40 and 45	18
4	46 and 47	19
6	48 and 50	20
4	51 and 52	21
6	53 and 55	22
2	56	23
20	57 and 66	24
10	67 and 71	25
8	72 and 75	26
4	76 and 77	27
8	78 and 91	28

Diaphanization and Staining of Bones and Cartilages

Samples were diaphanized using potassium hydroxide (KOH), Alizarin red S for bone stained and Alcian blue for cartilages following the methods proposed by Davis and Gore (1936), Dingerkus and Uhler (1977), with some modifications and Vieira et al. (2016a).

The specimens were analyzed with the aid of a stereomicroscope (Leica, DM 1000) quipped with a camera (Leica, Camera Software DFU Twain 6.7.0). In this study, we used the term “ossification center” for a small area of ossification observed in each bone, recognized by means of alizarin staining, which shows the accumulation of calcium; this is not necessarily the first event in bone development.

Histology Processing

Histology processing was carried out in the Laboratory of Histopathology at Federal University of Uberlândia (UFU), following a routinely generally used method of hematoxylin and eosin staining (Behmer et al., 1976; Vieira et al., 2016b). The microtomography was developed at Department of Genetics and Morphology of Institute of Biological Sciences (UNB), using the methodology described by Vieira et al. (2016a).

The images were captured using 5× and 10× magnification objective lenses.

Terminology

For identification of cartilage condensations of postcranial skeleton and the ossification pattern, the terminology proposed by Burke and Alberch (1985), Shubin and Alberch (1986), Müller and Alberch (1990), and

Bellairs and Kamal (1981) was used. Embryonic stages were determined using external morphological characteristics, following the methods proposed by Vieira et al. (2011) and structures of skeleton description were based in studies of Mook (1921), Iordansky (1973), Romer (1956), and Vieira et al. (2016a).

RESULTS

For characteristics of the components and structures that form the postcranial axial skeleton in embryos and hatchlings of *Melanosuchus niger* we used the description presented in the study by Vieira et al. (2016a), based in an adult specimen. Adult specimen of *M. niger* presents 63 vertebrae in vertebral column. They are divided in 8 cervical, 13 dorsal, 3 lumbar, 2 sacral, and 37 caudal vertebrae.

Chondrogenesis Pattern

The information about the stages of chondrogenesis pattern is summarized in Table 2.

During Stage 7, cartilaginous centers appeared in some vertebrae. It was difficult to define, in the beginning of the process, in which vertebrae the development began, but it was possible to observe that this condensation advanced in the craniocaudal direction. This condensation started in the vertebral arches and was visibly stronger in the cervical and dorsal regions, advancing to the lumbar, sacral and, last, to the caudal region. These elements of the vertebral column appeared as paired structures that grew towards the median dorsal line (Fig. 1). In the following stages, cartilage formation increased gradually. However, until the beginning of Stage 11, vertebral arches still remained unfused at their dorsal end.

In Stage 12, the paired elements of the vertebral arches of the cervical, dorsal, and lumbar regions of the vertebral column were found fused above the spinal cord to form the vertebral arch. Soon after that, the spinal and transverse processes developed from extensions of the vertebral arch (Figs. 2A–C and 3). Chondrogenesis continued until the cartilaginous vertebral column was formed and these processes became differentiated. Sacral and caudal ribs were developed by means of an independent cartilaginous condensation centers and, in later stages, they touched the vertebra near the neurocentral suture (Figs. 2D–G and 4). The greater the proximity with the caudal region, the lesser the condensation, indicating a craniocaudal progression in the development of these structures (Fig. 1).

However, the spinous processes showed greater retention of Alcian blue in the last cervical vertebrae and the first dorsal ones. The vertebral arch, and its processes, developed in a similar manner throughout the vertebral column.

The atlas showed a highly different morphology compared with the other cervical vertebrae, with a short intercenter, two neural arches, and a proatlas. From these, only one pair of neural arches and the intercenter showed retention of Alcian blue with small cartilaginous condensation in stage 12. The proatlas developed from two different foci of chondrogenesis, one in each antimer, at the end of Stage 13 (Fig. 5A). These became fused beginning in Stage 22.

TABLE 2. Chondrogenesis sequence of cartilaginous development process of the postcranial axial skeleton of *Melanosuchus niger* - INICIO E + FIM

Segment	Stage bone	7	8	-12	+12	13	14	+16	18
Ribs	Cervical								
	Dorsal								
	Ventral								
	Sacral								
	Caudal								
Vertebrae	Vertebral arch	Cervical							
		Dorsal							
		Lumbar							
		Sacral							
		Caudal							
	Vertebral body	Cervical							
		Dorsal							
		Lumbar							
		Sacral							
		Caudal							
Other elements	Gastralium								
	Interclavicle								
	Hemal process								
	Sternum								

–, beginning of stage; +, end of stage.

In the body of the vertebrae, cartilaginous condensation began in the centrum and moved towards the neural arch, fusing to it, in more advanced stages, to form the cartilaginous vertebra. Similar to the neural arch, the progression occurred in the craniocaudal direction (Fig. 1).

Ventrally to the body of the first caudal vertebrae, small cartilaginous condensations shaped like a “V” appeared at the end of Stage 12 and continued caudally, representing the hemal processes. These processes were located between each vertebral body without, however, following its pattern, as the vertebral body develops earlier (Figs. 1D–G and 4C–H).

Still in the end of Stage 12, both cervical and dorsal ribs presented chondrogenic formations, with the dorsal ones in a more advanced stage (Figs. 1 and 5). It was also observed that the ventral ribs showed cartilage development, but it still was free in the abdominal region (Fig. 1D–G). At Stage 14, the development of the sternum was observed, and its connection with the ventral ribs. The fusion of these structures occurred at stage 18 (Fig. 6A,C).

At the beginning of Stage 13, the development of other five hemal processes was observed, but with very light cartilaginous condensation. At the end of this stage, the vertebral body was more defined. The vertebral body of the first four caudal vertebrae was also visible.

At the end of Stage 14, the embryo showed four other caudal ribs, beginning in the 15th caudal vertebra. From this one, the ribs were reduced and not visible any more. The caudal vertebrae developed gradually in the craniocaudal direction. Condensation started in the vertebral arch, was followed by the body of the vertebra, and soon by the spinous processes and the rib. The latter one was not always present (Fig. 1).

Ossification Pattern

The information about the stages of ossification pattern is summarized in Table 3.

The ossification process began in the body of cervical vertebrae III to VIII, in Stage 19 (Figs. 1G and 3B,C). Alizarin retention decreased in the last vertebrae, indicating a craniocaudal direction in bone development, similar to cartilage formation. Still in Stage 19, a small ossification center was observed in the cranial extremity of the interclavicle (Fig. 6A,C). At the beginning of Stage 20, the odontoid process showed an ossification center and, at the end of this stage, the pair of neural arches of the atlas started to develop by means of two ossification centers, one in each antimere (Fig. 5D,E). The other structures remained temporarily cartilaginous.

Soon at the beginning of Stage 21, all dorsal, lumbar, sacral vertebrae and the ten first caudal vertebrae showed ossification centers in the vertebral body. Besides, the seven first dorsal ribs began their bone development, whereas the cervical ribs remained cartilaginous (Fig. 7B).

At Stage 22, the proatlas showed two ossification centers, one in each antimere, which were fused in Stage 26 forming the adult proatlas. The ossification process of the vertebral arches was observed, for the first time, in cervical vertebrae II and III, also in Stage 22, beginning in two ossification centers, one in each antimere (Fig. 7C).

Starting in Stage 23, bone development in the vertebral arches continued in the craniocaudal direction in the cervical vertebrae, followed by the dorsal and lumbar vertebrae in Stages 25 and 28 (Figs. 7D–F and 3F,H). From the components of the atlas, the intercenter was the last one to present ossification centers, which occurred in Stage 24 (Fig. 5F).

At the beginning of Stage 26, the vertebral arches of most caudal vertebrae showed ossification centers. Still in this stage, in the cervical region, these elements started to fuse, with the formation of the first spinous processes, which developed following the vertebral arch, and progressed in the craniocaudal direction.

In the histological sections of gastralium and interclavicles of *M. niger* at several development stages, it was

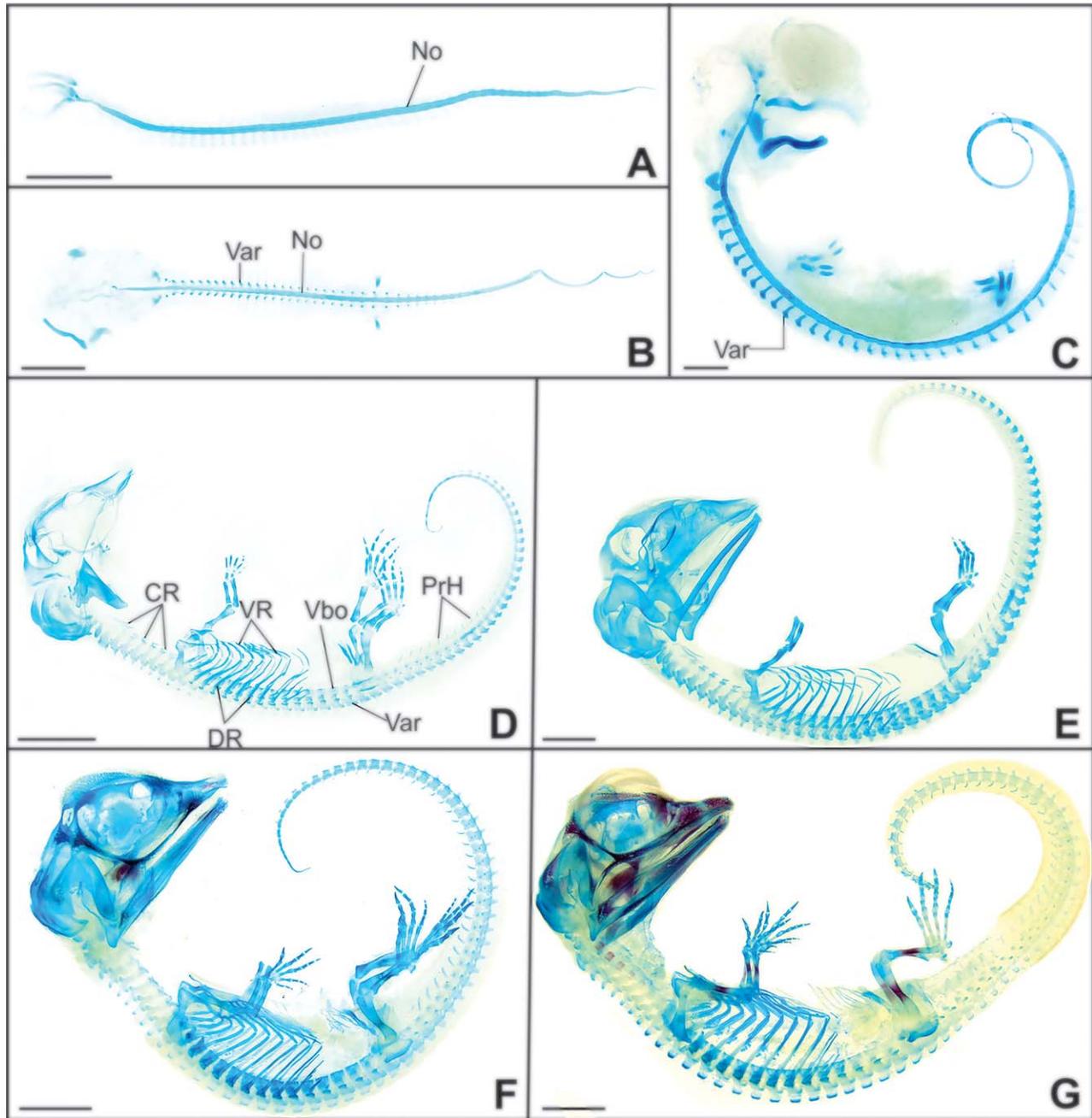


Fig. 1. Chondrogenesis of the postaxial skeleton in *Melanosuchus niger* in dorsal (A and B) and lateral (C–G) view. (A) stage 7, (B) stage 8, (C) stage 10, (D) beginning of stage 13, (E) end of stage 13, (F) beginning of stage 16, (G) stage 19. Abbreviations: Var, vertebral arch; CR, cervical ribs; DR, dorsal ribs; Vbo, vertebral body; No, notochord; VR, ventral ribs; PrH, hemal processes. Diaphanized with KOH and stained with Alizarin red S and Alcian Blue. Scale: A–C, 3 mm; D–G, 5 mm.

possible to observe that these elements showed intra-membranous development (Fig. 8). The number of bone elements that forms the gastralium is variable, and these elements were elongated and very thin, beginning their ossification in Stage 22 (Fig. 6D). Each gastralium element was formed from a single ossification center. Until the end of the embryonic period, these elements were organized in rows united in the median line forming the shape “V” with its apex turned to the caudal region.

At the end of Stage 23, all cervical ribs showed ossification centers, and vertebral bodies in most of the caudal vertebrae. The cervical ribs may be seen in greater detail in Figures 5F,H–J and 3A–C. These images also show the pre- and post-zygapophyses that may be seen in the cervical vertebrae. The first hemal processes showed ossification centers at the end of Stage 25 (Fig. 7E).

In Stage 24, the transverse processes of the dorsal vertebrae also presented very discrete ossification

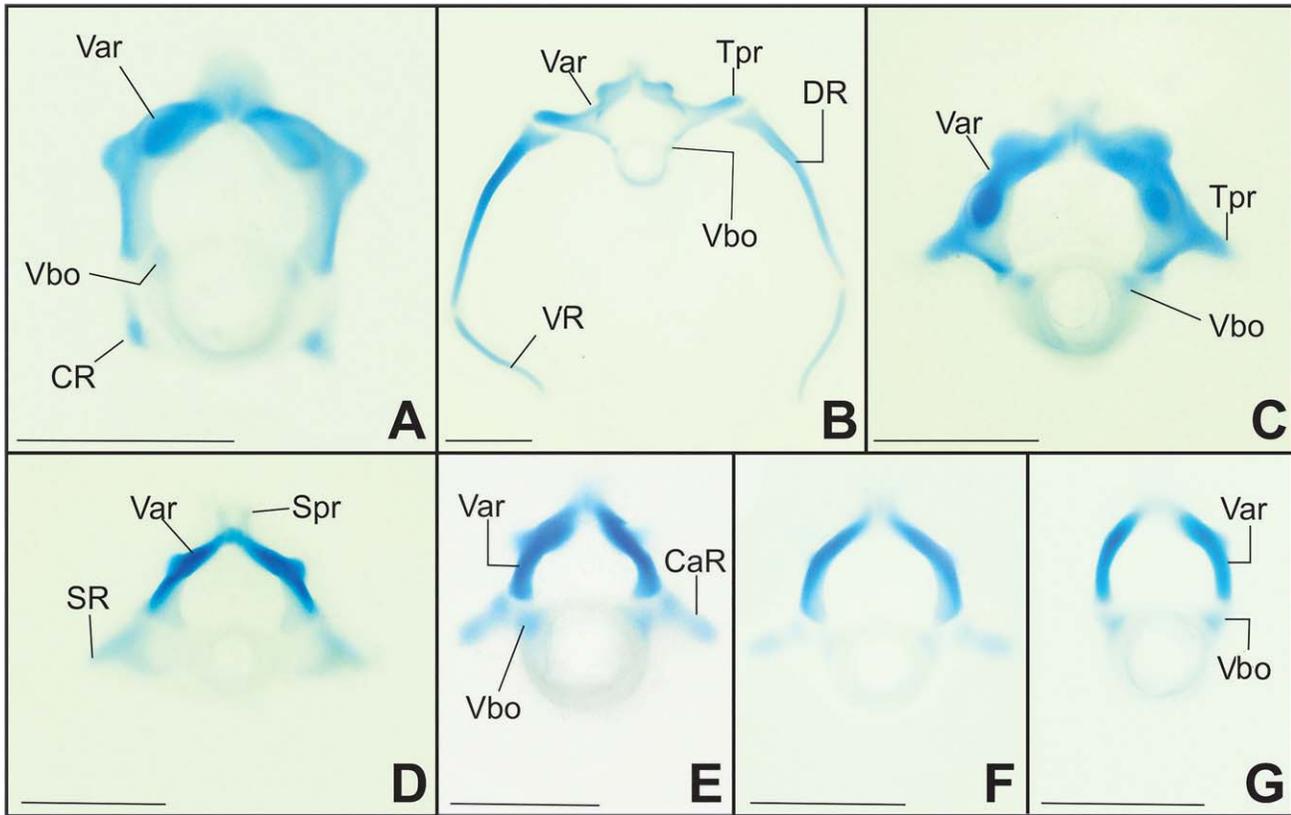


Fig. 2. Chondrogenesis of the vertebrae in *Melanosuchus niger* in the end of stage 12, cranial view. (A) cervical vertebra, (B) dorsal vertebra, (C) lumbar vertebra, (D) sacral vertebra, (E) caudal cranial vertebra, (F) caudal media vertebra, (G) distal caudal vertebra. Abbreviations: Var, vertebral arch; CR, cervical ribs; CaR, caudal ribs; DR, dorsal ribs; Vbo, vertebral body; SR, sacral ribs; Spr, spinous process; Tpr, transverse processes; VR, ventral ribs. Diaphanized with KOH and stained with Alizarin red S and Alcian Blue. Scale: 1 mm.

centers, following the vertebral arch, as well (Figs. 6E,F and 3F). In the lumbar vertebrae, the transverse processes appeared beginning in Stage 25, also following the arch and in the craniocaudal direction (Fig. 3H). Still in Stage 25, there were ossification centers in the caudal and sacral ribs (Figs. 7E and 4B). In Figures 6H,I and 9, it is possible to observe a small space between the rib and the vertebra in the sacral and caudal regions, indicating that synostosis did not occur in the prenatal period.

At the end of the prenatal period, in Stage 27, the vertebral arches began to fuse in all dorsal vertebrae, with the formation of the spinous process. At the beginning of Stage 28, it was possible to observe the development of all hemal processes (Fig. 4D,F,H).

DISCUSSION

Vertebrae and Ribs

In the initial development stages of *M. niger*, it is difficult to differentiate in which vertebrae the cartilage development started, but it was possible to note that the progression of the chondrogenesis foci took place predominantly in the craniocaudal direction. This condensation began in each antimer of the vertebral arches, and was more visible in the cervical and dorsal regions, followed by the lumbar, sacral and, last, the caudal region.

Rieppel (1993), in the analysis of *A. mississippiensis*, observed the same pattern. According to Starck (1996), the development of the vertebral column in birds and other amniotes occurs in the same direction observed in *M. niger* and *A. mississippiensis*.

In the cervical segment, the elements of the atlas in *M. niger* started their cartilage development by the pair of neural arches and the intercenter, followed by the proatlas. The two first cervical vertebrae, the atlas and the axis, are highly modified, and are considerably different from the following vertebrae. These distinctions were very apparent in the species studied here. It is a characteristic that is found in the other terrestrial vertebrates due to the importance of independent movement of the head (Souza et al., 2000; Hildebrand and Goslow, 2006; Vieira, 2008). Thus, modifications of the most cranial vertebrae and their articulation with the cranium enable considerable freedom of movement (Romer, 1956). The same characteristic is found in *C. yacare* (Lima et al., 2011c).

Still in relation to the atlas, it is important to emphasize that two morphological types of intercenter may be found in Crocodylia. The first type, found in *M. niger*, shows an intercenter that is both long and wide, and has a bifurcated caudal end where it articulates with the first pair of cervical ribs. This form is also seen in *Caiman latirostris*, *C. crocodylus* (Mook, 1921), and *C.*

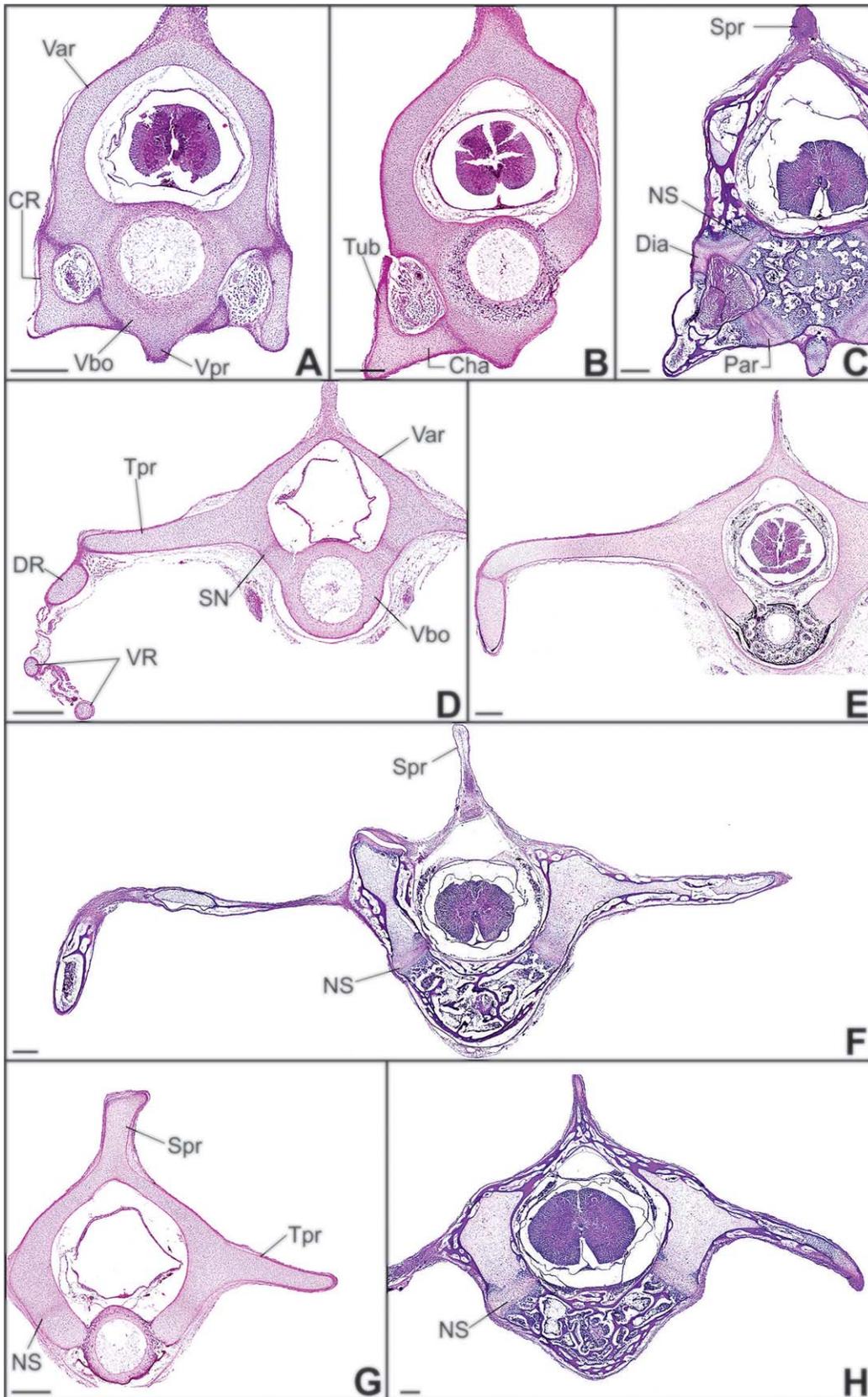


Fig. 3. Histological sections of the cervical (A–C), dorsal (D–F) and lumbar (G, H) vertebral column in *Melanosuchus niger*. (A and D) stage 14, (B and G) stage 19, (C and F) beginning of stage 25, (E) end of stage 24, (H) beginning of stage 27. Abbreviations: Var, vertebral arch; Cha, chapter; CR, cervical ribs; DR, dorsal ribs; Vbo, vertebral body; Dia, diapophysis; Par, parapophysis; Spr, spinous process; Tpr, transverse processes; VR, ventral ribs; Vpr, ventral process; NS, neurocentral suture; Tub, tubercle. Staining: hematoxylin and eosin. Scale: 400 μm .

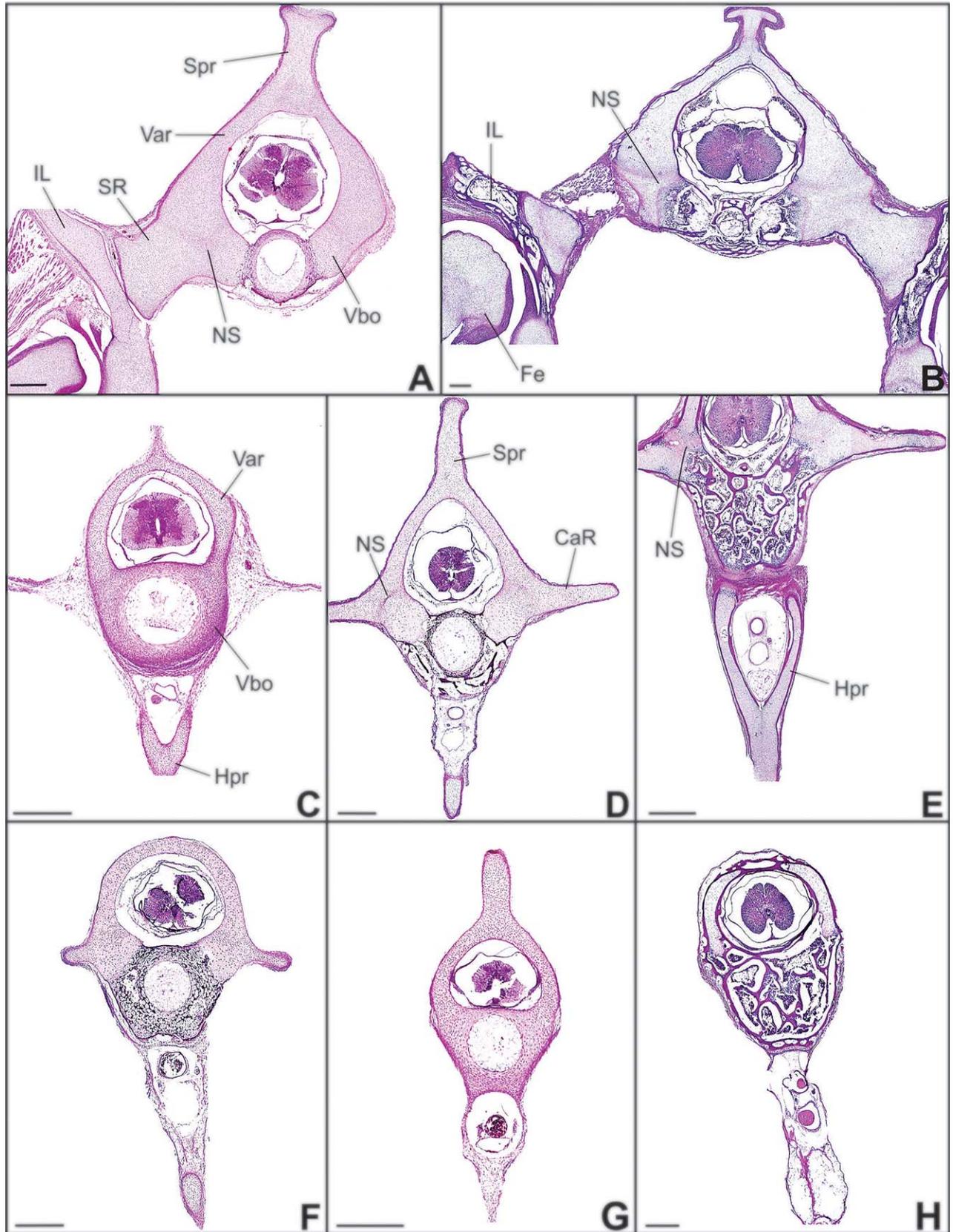


Fig. 4. Histological sections of the sacral (A, B), caudal (C–H) vertebral column in *Melanosuchus niger*. (A and G) stage 19, (B and E) beginning of stage 25, (C) stage 14, (D and F) end of stage 24, (H) beginning of stage 27. Abbreviations: Var, vertebral arch; CaR, caudal ribs; SR, sacral ribs; IL: ilium; Vbo, vertebral body; Fe, femur; Hpr hemal processes; Spr, spinous process; NS, neurocentral suture. Staining: hematoxylin and eosin. Scale: 400 μ m.

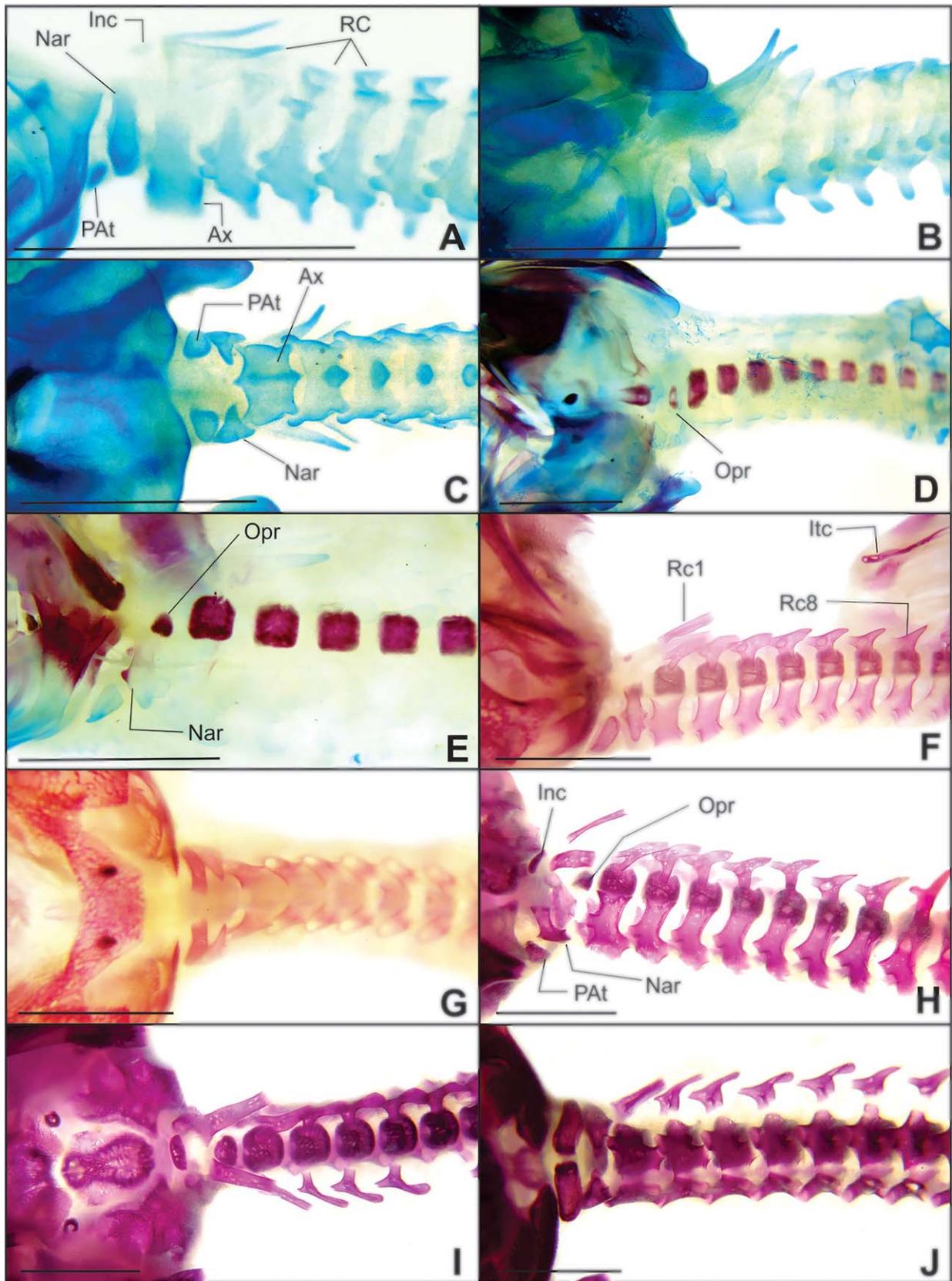


Fig. 5. Chondrogenesis and ossification of embryos of *Melanosuchus niger* in the lateral view. (A) end of stage 13, (B) beginning of stage 16, (C) beginning of stage 16, (D) beginning of stage 20, (E) end of stage 20, (F) beginning of stage 24, (G) beginning of stage 24, (H–J) end of stage 25. Abbreviations: Nar, neural arch; Ax, axis; RC, cervical ribs; Rc1, cervical ribs 1; Rc8, cervical ribs 8; ltc, intercenter; ltc, interclavicle; PAAt; proatlas; Opr, odontoid process. Diaphanized with KOH and stained with Alizarin red S and Alcian Blue. Scale: 5 mm.

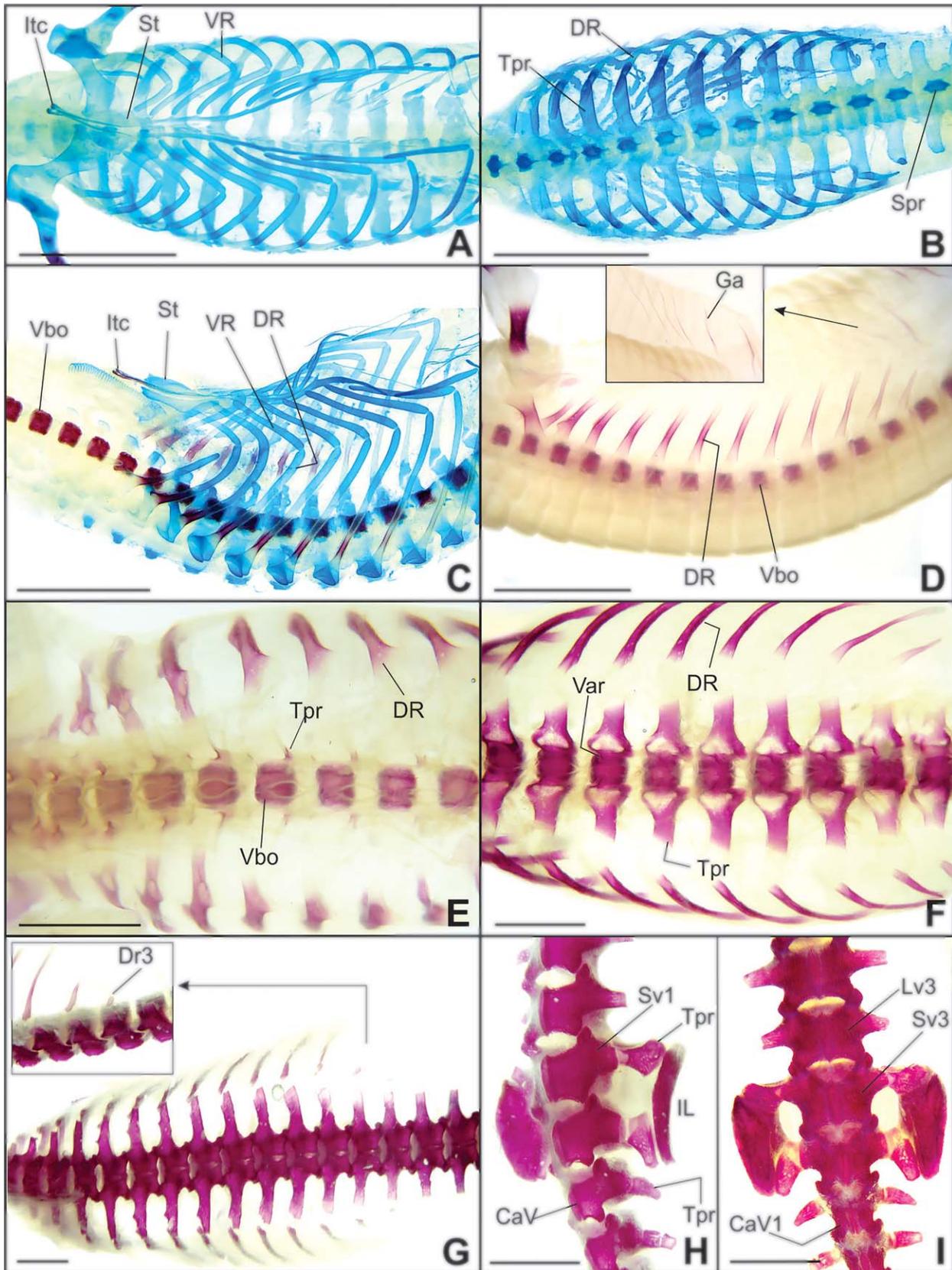


Fig. 6. Chondrogenesis and ossification of embryos of *Melanosuchus niger* in the lateral view. (A and B) stage 19, (C) beginning of stage 22, (D) end of stage 22, (E) beginning of stage 24, (F) end of stage 25, (G-I) stage 28. Abbreviations: Var, vertebral arch; DR, dorsal ribs; Spr, spinous process; Vbo, vertebral body; St, sternum; Ga, gastralia; IL, ilium; Itc, interclavicle; Tpr, transverse process; VR, ventral ribs; CaV, caudal vertebra; CaV1, caudal vertebra 1; Dr3: dorsal rib 3; Lv3, lumbar vertebra 3; Sv1, sacral vertebra 1; Sv3, sacral vertebra 3. Diaphanized with KOH and stained with Alizarin red S and Alcian Blue. Scale: 5 mm.

TABLE 3. Ossification sequence of the postcranial axial skeleton of *Melanosuchus niger*

Segment	Stage bone	19	20	21	22	23	25	26	27
Ribs	Cervical								
	Dorsal			-					
	Ventral								
	Sacral								
	Caudal								
Vertebrae	Vertebral arch	Cervical							
		Dorsal							
		Lumbar							
		Sacral							
		Caudal							
	Vertebral body	Cervical							
		Dorsal							
		Lumbar							
		Sacral							
		Caudal							
Other elements	Gastralium								
	Interclavicle								
	Hemal process								
	Sternum								

-, beginning of stage; +, end of stage.

yacare (Lima et al., 2011c). In the second type, the atlas is wider, more than longer. This type of intercenter is found in *Crocodylus americanus* and *Gavialis gangeticus* (Mook, 1921). This characteristic may contribute to the elucidation of some phylogenetic relationships with other living species in the Crocodylia order, and other extinct species.

It is worthy emphasizing that in *M. niger*, and in *C. yacare* (Lima et al., 2011c), the odontoid process is found cranially to the vertebral body showing a very clear ossification center separated from the body of the axis. In the *M. niger* skeletons that were prepared and analyzed in the current study, it was possible to clearly see the suture between these elements of the axis (Vieira et al., 2016a), which is a result of the individual development of the odontoid process, both in cartilage and bone development (Fig. 5). The odontoid process, together with the atlas, makes up the joint with the single occipital bone found in crocodylians. According to Romer (1956), the odontoid process is a complex element that results from the fusion of the vertebral body of the atlas with intercenter of the axis, and it connects with the body of the axis by a suture that is visible in the adult. Mook (1921) reinforces the presence of this suture in living Crocodylia.

In spite of the similarity found with the other cervical vertebrae, some peculiar differences between these vertebrae were observed and are important to be pointed out. For example, the width and height of the spinous process: in C3 to C5, this process is larger than in the other vertebrae, but in C8, it is taller. As for their development, cartilage process begins in the vertebral arches followed by the vertebral bodies, whereas in the ossification process the sequence is inverted. However, development always takes place in the craniocaudal direction, both in cartilage and bone development (Figs. 1 and 7). This cartilage and bone development pattern was observed in the other segments of the vertebral column. Similar observations were made by Rieppel (1993) and Lima et al. (2011c) for *A. mississippiensis* and *C. yacare*, respectively.

The first dorsal vertebra in *M. niger*, and in *Crocodylus americanus* (Mook, 1921), *A. Mississippiensis* (Rieppel, 1993), and *C. yacare* (Lima et al., 2011c) is very similar to the last cervical one. However, some particular changes, like the development of facet joints for the ribs and the presence of transverse processes, are criteria used to determine the division of the cervical and dorsal regions of the vertebral column.

Cartilage development began in the vertebral arches, transverse and spinous processes, followed by the body of the vertebra. Cartilaginous condensation of the most cranial dorsal vertebra was simultaneous to the most caudal cervical vertebrae. As observed in *M. niger* and in the studies carried out by Lima et al. (2011c) and Rieppel (1993) for *C. yacare* and *A. mississippiensis*, respectively, the ossification process of all dorsal vertebrae begins early, simultaneously to the cervical vertebrae. The level of alizarin retention throughout the vertebrae of this dorsal region enables us to state that there was a craniocaudal development gradient, as occurred in cartilage development.

In *M. niger*, the transverse processes of the dorsal and lumbar vertebrae, and the spinous process of all vertebrae are developed from extensions of the vertebral arch (Figs. 2–4). From this cartilage model, the ossification centers appear in the arch, expand, and delimit these processes, without new ossification centers appearing. According to Lima et al. (2011c), the transverse processes in *C. yacare* are formed from different ossification centers in the vertebral body. However, the author did not describe in details the relationship between the development of the transverse process and the vertebral arch.

Three lumbar vertebrae were identified in *M. niger* interposed between the dorsal and sacral vertebrae. This segment of the vertebral column does not have ribs, and the transverse processes became gradually narrower and shorter. The development of these vertebrae followed the same pattern described above. Lima et al. (2011c) and Rieppel (1993) did not report information about lumbar vertebrae in *C. yacare* and *A. mississippiensis*,

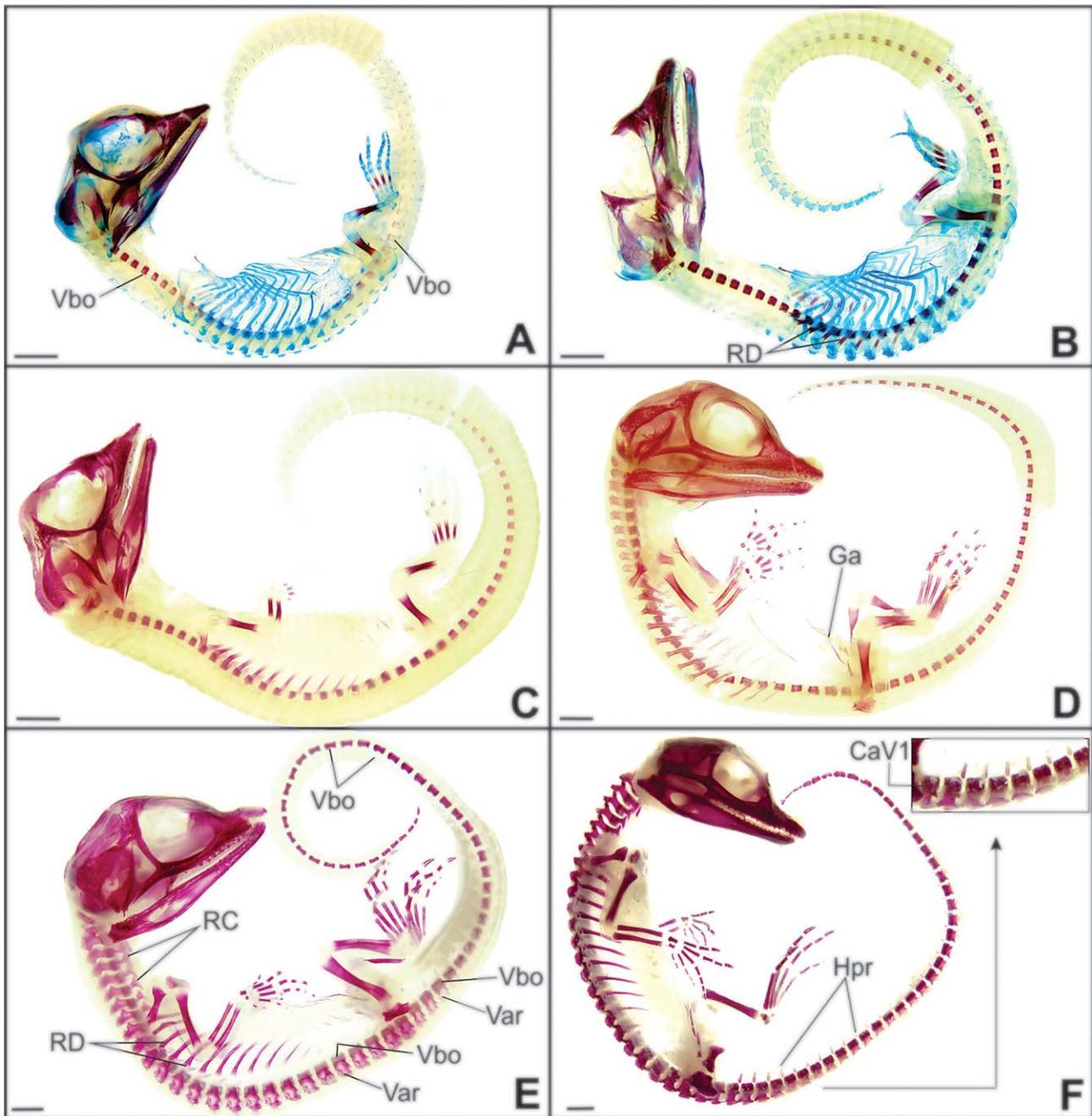


Fig. 7. Process of ossification of the of the post-axial skeleton in *Melanosuchus niger* in lateral view. **(A)** beginning of stage 20, **(B)** end of stage 21, **(C)** stage 22, **(D)** beginning of stage 24, **(E)** end of stage 25, **(F)** stage 28. Abbreviations: Var, vertebral arch; CaV1, caudal vertebra 1; RC, cervical ribs; Ga, gastralia; RD, dorsal ribs; Vbo, vertebral body; Hpr, hemal processes. Diaphanized with KOH and stained with Alizarin red S and Alcian Blue. Scale: 5 mm.

respectively. According to Romer (1956), the absence of floating ribs may characterize the lumbar region, and this criterion was also used in the analysis of *M. niger* in the present study. Also according to this reasoning, Mook (1921) also observed three lumbar vertebrae in a study with several species of the Crocodylia order.

In *M. niger*, there were two sacral vertebrae, each of them articulating with the corresponding sacral rib. It

may be stated, however, that in living Crocodylia, there are only two sacral vertebrae. According to Romer (1956) and Hutchinson (2001), this is the primitive number in Archosauromorpha. In *C. yacare* and *A. mississippiensis*, this number was confirmed (Lima et al., 2011c; Rieppel, 1993).

As for the development of this vertebral column segment, the cartilaginous condensation of the ribs occurred

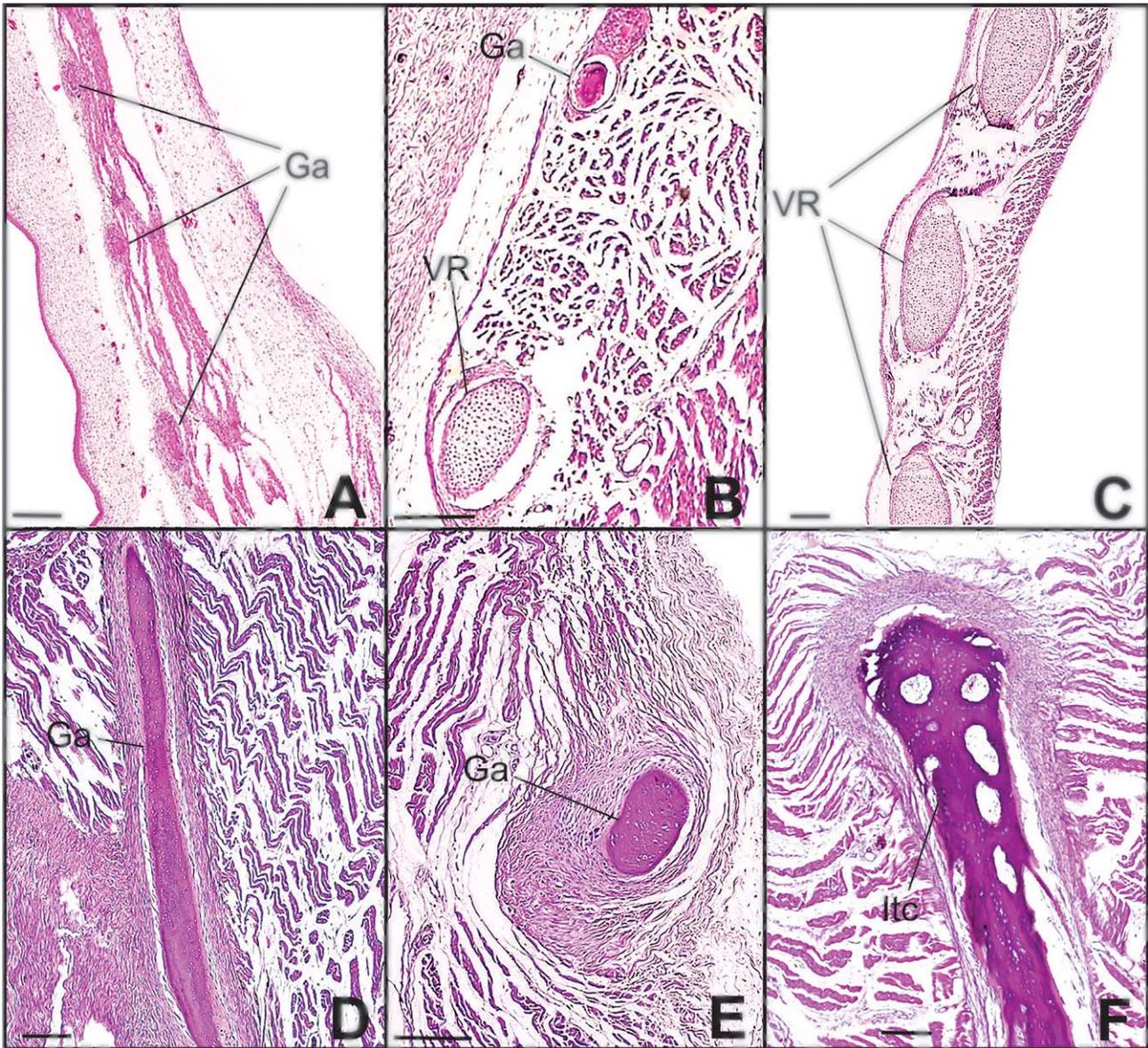


Fig. 8. Histological sections of the gastralia and ventral rib in *Melanosuchus niger*. (A) end of stage 16, (B and C) end of stage 24, (D and E) beginning of stage 27, (F) beginning of stage 25. Abbreviations: Ga, gastralia; Itc, interclavicle; VR, ventral ribs. Staining: hematoxylin and eosin. Scale: 200 μ m.

individually and, later on, took the vertebra near the future neurocentral joint with a complete fusion without spaces. This pattern was different from the cervical and dorsal ribs, in which the connection points are the head and tubercle of the rib directly on the transverse process of the vertebra, with some space for passage of the vessels and nerves.

The development of the caudal vertebrae was similar to the sacral ones. The caudal rib was in place of the transverse process, and it was formed by an independent cartilaginous condensation; its connection with the corresponding vertebrae occurred near the neurocentral suture (Fig. 2). Higgins (1923) and Emelianov (1937), in embryological studies with crocodiles, confirmed these observations.

Independent ossification centers were observed in *M. niger* in both the sacral and caudal ribs (Figs. 6H,I and 9). Although Rieppel (1993) reported this finding in *A. mississippiensis*, caudal ribs were called transverse processes. Rieppel (1993) also reported that, in the transverse processes of the dorsal vertebrae, ossification begin with independent centers, as well, and dorsal transverse processes fuse with the neural arch early in embryonic development, whereas caudal and sacral ribs fused later, during post-hatching growth. This finding was not confirmed in *M. niger*, once dorsal transverse processes developed following the vertebral arch, without the development of individual ossification centers (Figs. 3 and 9F).

Rieppel (1993) recognized that independent ossification centers in the dorsal transverse processes were not

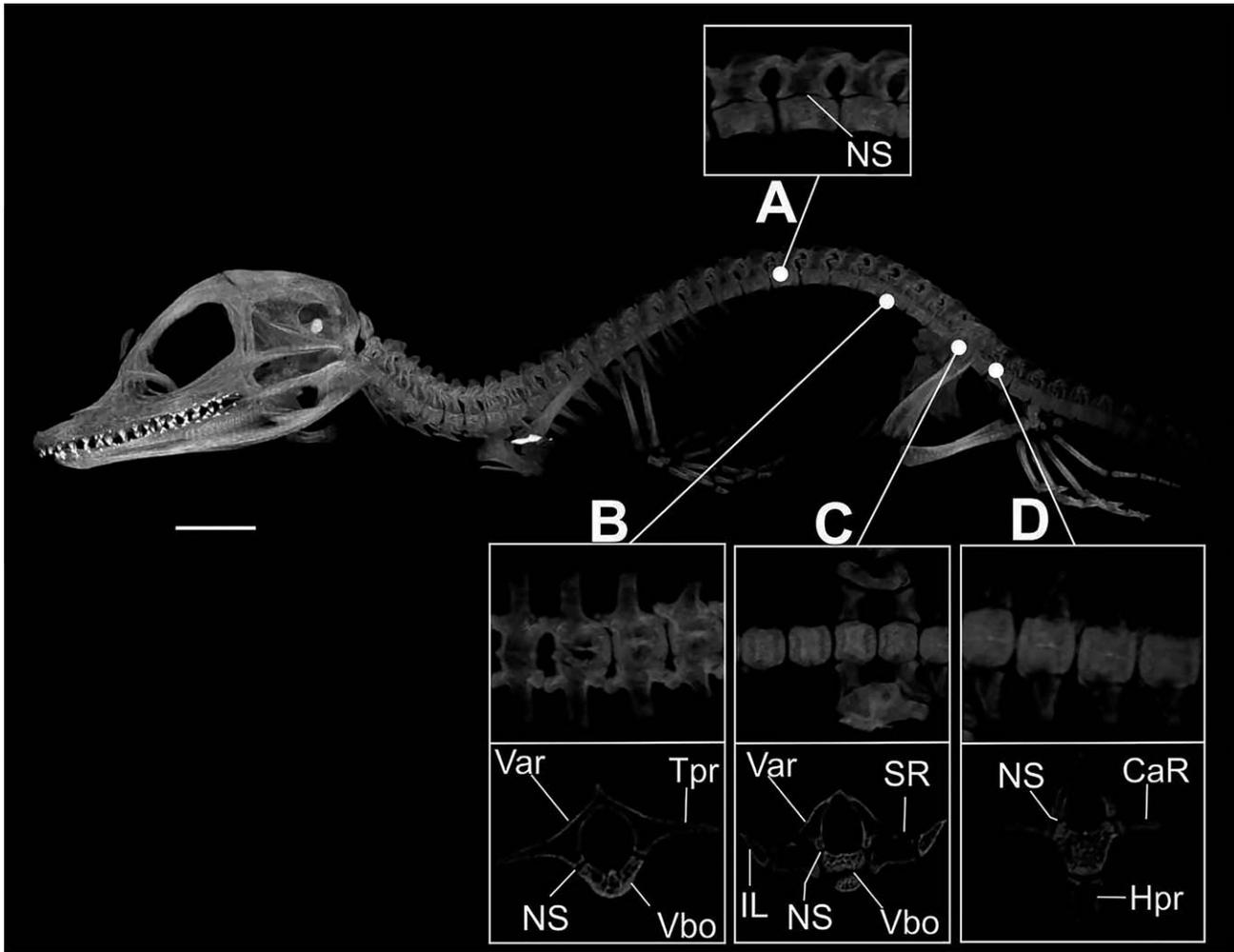


Fig. 9. Embryo of *Melanosuchus niger* in stage 28 in lateral view. (A) segment of the dorsal vertebral column; (B) segment of the lumbar vertebral column; (C) segment of the sacral vertebral column; (D) segment of the caudal vertebral column. Abbreviations: Var, vertebral arch; CaR, caudal ribs; Vbo, vertebral body; SR, sacral ribs; I, ilium; NS, neurocentral suture; Tpr, transverse processes; Hpr, hemal processes. Microtomography and 3D reconstruction. Scale: 10 mm.

mentioned in other reptiles. According to this author, this may be due to the lengthening of the transverse processes, which is a synapomorphy of Archosauromorpha (Benton, 1985). We agree with the synapomorphy of the transverse processes, but independent development of these processes was not observed in any of the specimens analyzed.

In *M. niger*, the dorsal, sacral, and caudal ribs showed similar development patterns, once they were formed by independent chondrogenesis foci and ossification centers; only later they touched the corresponding vertebra. The name of these structures reinforces the idea of serial homology in dorsal, sacral, and caudal ribs, which supports the use of the term “rib” instead of “transverse process” in the sacral and caudal segments of Crocodylia.

In general, we agree with Rieppel (1993) that this is a complex situation, as these changes involve the fusion of chondrogenic and ossified elements during embryogenesis. The homology between dorsal, caudal, and sacral ribs has been debated. Gegenbaur (1871) was the first to

raise the issue. Claus (1876) *apud* Rieppel (1993) found the caudal transverse process only with sutures in juvenile crocodiles, and considered this series homologous to the series of dorsal ribs. Mook (1921) also recognized the homology of sacral ribs and caudal transverse processes with dorsal ribs in crocodiles, a conclusion that was supported by Higgins (1923) based on the embryology findings of this author.

According to Gauthier and De Queiroz (1988), the ossification of the sacral ribs and caudal transverse processes (caudal ribs) originating from different ossification centers represents the plesiomorphic status of Lepidosauromorpha and Archosauromorpha.

Based on that, Mook (1921) and Rieppel (1993) concluded that the transverse processes in caudal vertebrae are nothing more than ribs fused with the vertebral body. According to Hoffstetter and Gasc (1969), offspring of living Crocodylia have these elements still separated in the vertebrae, and they become fused with time. In *M. niger* we noted the presence of these sutures in the sacral and caudal ribs of recently hatched offspring. In

skeletons of adult *M. niger* specimens, Vieira et al. (2016a) described this occurrence only in the most cranial vertebrae, and reported that the most caudal region these sutures are not visible any more. Besides, other changes may be observed in the adult skeleton, such as the transverse process originating more ventrally to the zygapophysis, at the level of the neurocentral suture. In the dorsal segment, the transverse processes originate directly from the neural arch.

In *M. niger*, as well in the other representatives of the order Crocodylia (Hoffstetter and Gasc, 1969), the caudal ribs and the hemal processes gradually decrease in size throughout the tail of the animal, until they are vestigial, and then absent. Therefore, the most distal caudal vertebrae do not present ribs, following the pattern observed in Crocodyliformes (Nascimento, 2008). Rieppel (1993) described bone development of 9–10 hemal processes associated with the first caudal vertebrae, in a craniocaudal gradient. Lima et al. (2011c) also observed ossification centers in the first hemal processes of *C. yacare*, and emphasized that they occur in the same direction.

The hemal processes in *M. niger* are bones in the caudal region located ventrally to the vertebrae. According to Romer (1956), these elements originate in the intercenter, a minuscule piece located between the vertebral centers, which is lost in Archosauria (Benton and Clark, 1988), present only in atlas and in the shape of hemal processes, as observed in *M. niger* (Vieira et al., 2016a), *C. yacare* (Lima et al., 2011c), and *A. mississippiensis* (Rieppel, 1993). Romer (1956) reported that the hemal processes are normally absent in the first caudal vertebrae because of the pelvic passage in this region. In living Crocodylia, the presence or absence of hemal processes in the second caudal vertebra is normally used to characterize sexual dimorphism, as the female loses the second hemal process to better accommodate the reproductive organs (Romer 1956). When the location of the first hemal process was analyzed in embryos that showed this ossified structure, and in *M. niger* offspring as well, it was noted that they were articulated between the second and third caudal vertebra. According to Romer's (1956) reasoning, all the specimens analyzed would be males. This observation makes us disagree with this author, only in relation to this information on sexual dimorphism.

Gastralium

In some vertebrate, caudally to the sternum, there is a set of bones that originates separately from the skeletal elements, the gastralium. In *M. niger*, this set is formed by thin bones, placed on metameric lines in the ventral portion of the abdominal wall, between the pubis and the sternum. The gastralium is made up by seven lines, each composed by four bones (Vieira et al., 2016a). According to Claessens (2004), the number of lines varies considerably: in prosauropods and theropods, the gastralial system ranges from 8 to 21 lines. In *C. yacare*, Lima et al. (2011c) state that the number of lines is variable, and the gastralium originates from a single ossification center, but in tangent and ipsilateral pairs that are joined in the shape of a “V,” with the apex cranially turned.

In *A. mississippiensis*, Rieppel (1993) only described the moment at which the gastralium elements start to ossify. On the other hand, Vickaryous and Hall (2008) described gastralial development in *A. mississippiensis* in greater detail. According to these authors, the elements of the gastralium are sequentially developed. Although these authors reported the presence of eight pairs of lines, they could only show seven lines, similar to the number of lines observed in *M. niger*.

In *M. niger*, gastralium showed strong alizarin retention, which suggests a dermal origin of these elements. Romer (1956) described this origin and emphasized that although the gastralium is, many times, referred to as the abdominal ventral ribs, this name is inadequate as the origin of the gastralium is completely dermal, different from actual ribs. Therefore, they are more comparable to osteoderms than to ribs. Still according to this author, the gastralium derives from the cover of ventral scales, which is an inheritance of basal crossopterygians. Knox (1869) was the first to recognize their dermal origin.

Although Vickaryous and Hall (2008) did not observe any signs of cartilage associated with any of the gastralium elements in *A. mississippiensis*, with weak Alcian blue retention, these authors stated that the gastralium is not adequately understood, probably due to the fact that its occurrence is limited to few living vertebrates, such as crocodylians (Romer, 1956). Besides, as they are floating bones, without any continuous connection with any other skeletal elements, they are rarely preserved in fossils, and there is little information on their ontogenetic development.

Ventral Ribs, Sternum, and Interclavicle

The ventral ribs in *M. niger* are little ossified projections that extend caudoventrally originating in the distal end of the dorsal ribs (Vieira et al., 2016a). These structures are found in most of the living birds (Bellairs and Jenkin, 1960), and are also found in some theropod dinosaurs (Paul, 2002).

In *M. niger*, the ventral ribs showed strong retention of Alcian blue, which may be seen in stage 13. Its ossification process was not observed during the prenatal period, or in recently hatched animals. This finding indicates that ossification of this structure will occur later on, after birth. The ventral ribs have not been discussed in members of the Crocodylia order. However, in spite of little information on it, its presence was reported by Hoffstetter and Gasc (1969), and Romer (1956), as either a cartilaginous or an ossified structure. Studies on the ontogenetic development of the ventral ribs in Crocodylia species were not found in the specialized literature. However, this absence suggests the need continue and improve the descriptive anatomy and ontogenetic study of this taxon.

The ventral ribs are essential for the mechanics of breathing in birds, due to the insertion of muscles that are involved both in inspiration and expiration; they are also important in bird locomotion (Codd et al., 2005, 2008; Codd, 2010). Owing to its location and similarity with birds, these structures in *M. niger* may have the same function in breathing. Once the expansion of the ventral ribs occurs, there is an increase in the

respiratory capacity that enables the animal to remain longer under water.

The presence of a median element in the pectoral waist is a plesiomorphic characteristic. In Crocodylia, including *M. niger*, this element is identified as the interclavicle (Rieppel, 1993; Vickaryous and Hall, 2010; Vieira et al., 2016a); in Testudines, as the endoplastron (Lane, 1910); and in birds, as the furcal bone (Russell and Joffe, 1985). In the histological sections of the interclavicle of *M. niger*, in several development stages, it was possible to observe that this element was developed as an intramembranous structure (Fig. 8F). This structure remains surrounded by the sternal cartilage in *M. niger*, *A. mississippiensis* (Vickaryous and Hall, 2010; Rieppel, 1993), and *C. yacare* (Lima et al., 2011c). Similar to the studies by Rieppel (1993) and Vickaryous and Hall (2010), our data demonstrated that ossification started in the cranial part of the interclavicle and continued caudally in the median plane.

According to the findings on interclavicle development in *M. niger* and literature data (Vickaryous and Hall, 2010; Bryant and Russell, 1993), it may be stated that the furcal bone in birds and the interclavicle are homologous structures. This statement is supported by the following criteria: the two elements share the same anatomical position; they never coexist in the same individual; they share common ancestry: both Crocodylia and birds belong to the superorder Archosauria; and they are developed by intramembranous ossification. Although the “V” shape of the furcal bone is different from the interclavicle shape of living Crocodylia, morphology alone is inadequate to determine evidence of homology, as already discussed by Bock (1989).

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