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Morphological characteristics of the neonate of the white-eared opossum (*Didelphis albiventris* Lund, 1840) (Metatheria: Didelphidae)

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Abstract. Due its post-birth distinction from other mammals, and the absence of detailed work on the morphology of the marsupial neonates, and their respective organogenic development, the objective of this study was to provide macroscopic and microscopic detailed descriptions of the white-eared opossum (*Didelphis albiventris* Lund, 1840) neonates and a potential comparison to embryos/fetuses from other mammalian species described in the literature. For this study, we used 5 *D. albiventris* neonates of four days old. For macroscopic analysis, samples were fixed and photographed, and for microscopic analysis, specimens were processed by routine histology techniques and stained with hematoxylin and eosin. Neonates presented some embryological characteristics, such as pronounced facial immaturity, presence of optic vesicle, gap on the fourth brain, brain vesicles, and underdeveloped lungs and mesonephros. However, they also presented well-developed structures, such as apparent nasal and oral cavities and well-developed pelvic and thoracic limbs, especially the hands, where it was possible to find claws at the ends of the digits. Due to the ease of access to *D. albiventris* neonates, the early development state after birth and not sacrifice of the mother for neonate collected, this species can be considered an excellent model for comparative developmental studies. These results can be extrapolated and compared to morphogenesis of rats, guinea pigs, rabbits, horses, cattle and humans at different stages of development, regardless of the length of the gestation period, considering the particularities of each species.

Keywords: White-eared opossum, Didelphimorphia, Embryology, Histology, Marsupial.

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Introduction

The Genus *Didephis* (Linneaus, 1758) belongs to the Phylum Chordata, the Order Didelphimorphia and the Family Didelphidae (Zeller, 1999; Reis et al., 2006), being popularly named opossums. The group Metatheria (Didelphimorphia) consists of seven orders, 19 families and 81 genera, based on anatomical and physiological similarities, particularly related to reproduction (Hildebrand, 1995; Gonçalves et al., 2009; Bertassoli et al., 2013). Currently marsupials are redistributed into distinct orders, three of these present in the Americas: Didelphimorphia, Microbiotheria and Paucetuberculata (Holz, 2003; Malta and Luppi, 2007). In Brazil, Didelphimorphia is represented by 55 species from 16 different genera. Among them four species of the Genus *Didelphis* (*D. albiventris*, *D. aurita*, *D. marsupialis* and *D. imperfecta*) are common in different biomes (Reis et al., 2006; Paglia et al., 2012; Barros et al., 2013).

American opossums have a breeding season that begins in July, and a sexual rest period from February to June (Nogueira, 1989). These animals prepare their nest with dried leaves in areas of forest, growing vegetation, crops or urban environments. The gestational intra-uterine period has approximately 13 days, and the offspring can happen twice yearly. The number of pups per litter is about eight in the field, and 10 in captivity. As puppies are born very small and embryologically immature, they remain in the marsupium until its morphogenesis is complete (Nogueira, 1989).

Like other marsupial species, *D. albiventris* is in a relatively immature state of development at birth. However, certain organs are functional and/or modified at this stage, in order to allow its survival in the new environment. The young are born with disproportionately well-developed forelimbs and oral apparatus, which allow them to climb and attach to their mother's teat (Goswami et al., 2009). The movement of the newborn from the birth canal to the marsupium is performed by well-developed forelimbs and digits with sharp claws, which are already capable of palmar seizure (Kraus and Fadem, 1987; Krause, 1992).

The young opossum are extremely immature at birth; many organs are at an embryonic stage of development. The skin of the newborn is much tougher and less permeable than that of eutherian fetuses at a similar stage of development. This means that the marsupial newborn withstand handling much better than eutherian fetuses at an equivalent stage of development.

In this study, opossums were chosen as model because of its relevance among mammals, as they represent a link in the transition between Prototheria and Eutheria; Opossums can also be used as models in comparative studies between these two groups. Given its post-birth distinct features, the absence of detailed work on the morphology of the marsupial neonates, and their organogenic development, the objective of this study was to provide macroscopic and microscopic detailed descriptions of the opossum *D. albiventris* neonates and a potential comparison to embryos/fetuses from other mammalian species described in the literature.

Material and methods

D. albiventris neonates collect

It was used 5 *D. albiventris* (Didelphidae, Marsupialia) neonates from the pouch of pregnant females captured during the breeding season (from June to January), at the Ecologic Station of the Universidade Federal de Minas Gerais (19° 52' S, 43° 58' W, Belo Horizonte, MG, Brazil). Subsequent collection of newborns, females were returned to natural habitat. All animal procedures were conducted following instructions of the ethical committee (CEUA/UFMG, ethical approval No. 220/2008), the Brazilian Institute of Natural Environment and Renewable Resources (IBAMA, SISBio No. 27354-2), and the Brazilian laws for the use of animals in scientific experiments.

Samples processing and photodocumentation

For macroscopic analysis, neonates were photographed with a digital camera (MVC-CD500, Nikon, Tokyo, Japan) and a magnifying glass. For microscopic analysis, the same neonates were processed by routine histological techniques. After dehydration using a series of ethanol in increasing concentrations (70-100%), samples were diaphanized in xylene, and embedded in Histosec paraffin as described before (Tolosa et al., 2003). After processing, blocks were subjected to 5 µm cuts on a microtome (RM2165, Leica, Nussloch, Germany), and sections were stained using hematoxylin and eosin (HE). Photomicrographs were made under a microscope (DM 2000, Leica, Nussloch, Germany).

The nomenclature used was according to the International Committee on Veterinary Gross Anatomical Nomenclature, the International Histological Committee on Veterinary Gross and the International Nomenclature Committee on Veterinary Embryological Nomenclature.

Results

Macroscopic analysis

The four day old neonates presented as visible morphological characteristics: pronounced facial immaturity, including the absence of earlobes, and apparent nasal and oral cavities (Figure 1A), showing a small opening; the presence of the optic vesicle (Figure 1B). These neonates have also presented temporal differences between forelimbs and hindlimbs development (Figure 1C and D). It was possible to observe that this opossum species show well formed digits on the forelimbs, with claws on the end of the digits (Figure 1C); which were in more advanced stage of development than the ones found at the hindlimbs (Figure 1C).

Microscopic analysis

Histological sections revealed that this opossum species have some structures/organs showing characteristics found only during embryonic stages; while others are found as well-developed ones. The gap on the fourth brain ventricle, brain vesicles (forebrain, midbrain and hindbrain), underdeveloped lungs and mesonephros (Figure 2), all represent characteristics of structures found during embryonic developmental stages. The presence of the tongue, larynx, trachea, spine, stomach, atrial and ventricular division of the heart, liver and diaphragm, becoming possible the division in the thoracic and abdominal cavity (Figure 2), on the other hand, all show structures are in advanced stages of development.

Nervous system analysis

The analysis of the nervous system allowed the identification of the three primary brain vesicles: forebrain, midbrain and hindbrain (Figure 3A). It was also possible to note the presence of the fourth brain ventricle, which was opened (Figure 3A).

The spinal cord was composed of a common thick neuroepithelium. Flanking the marrow, a thin layer of connective tissue was observed; in opposition to this, it was possible to observe the formation of the spine vertebrae, consisting of both differentiating cartilage and also the fibrocartilaginous tissues, which was found around the vertebral discs (Figure 3B).

Respiratory system analysis

Regarding the respiratory system, it was noticed that the formation of the nasal cavity, larynx cartilage, trachea and lungs

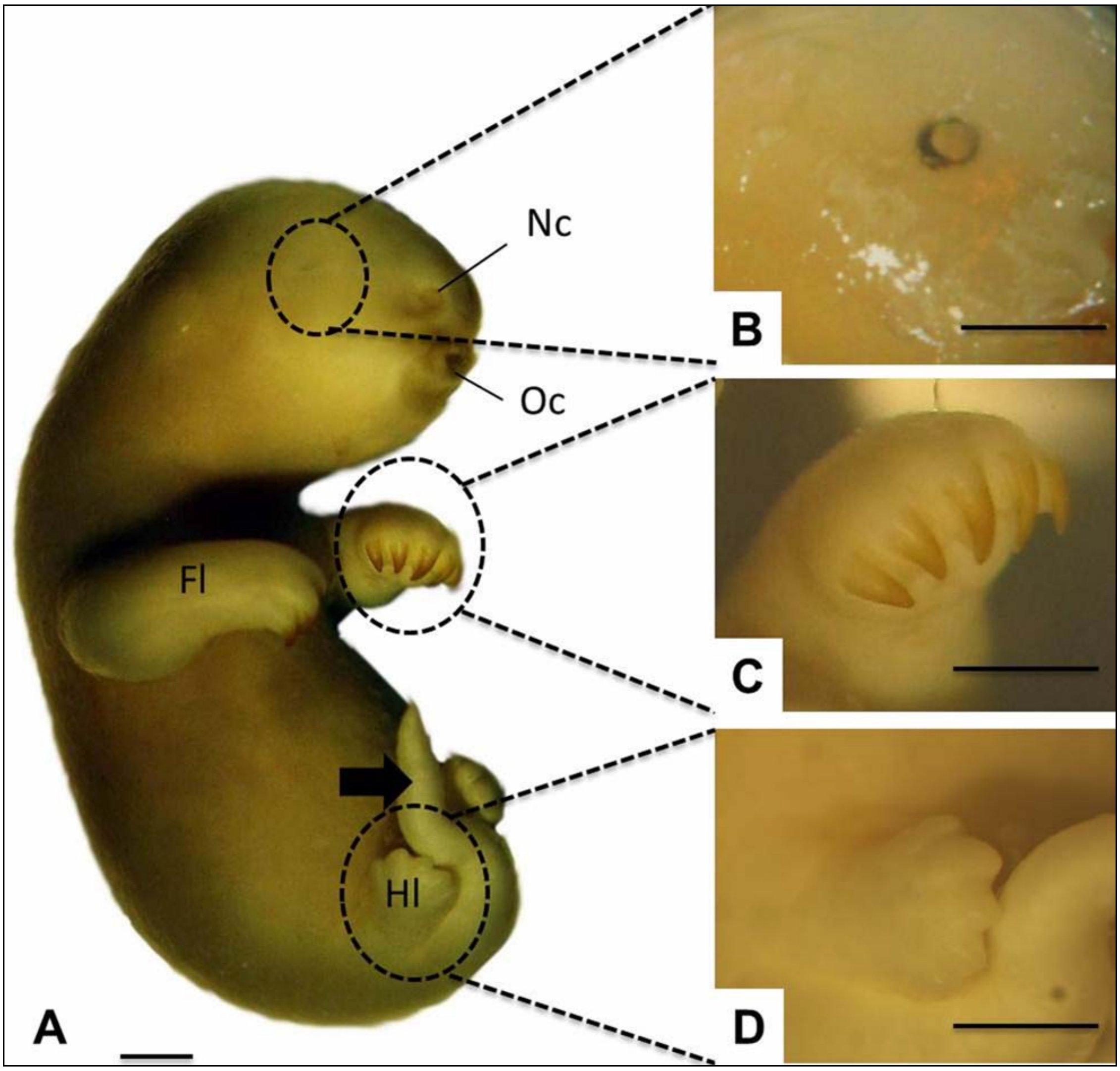

Figure 1. Photomacrography of the *D. albiventris* newborn. A) Four days old neonate: forelimb (Fl), hindlimb (Hl), tail (arrow), nasal cavity (Nc), oral cavity (Oc). B) Diafanization of the head, showing optic vesicle. (C) Zoom in hand. Note the claws. (D) Zoom in foot. Note the digits. Bars: 200 µm.

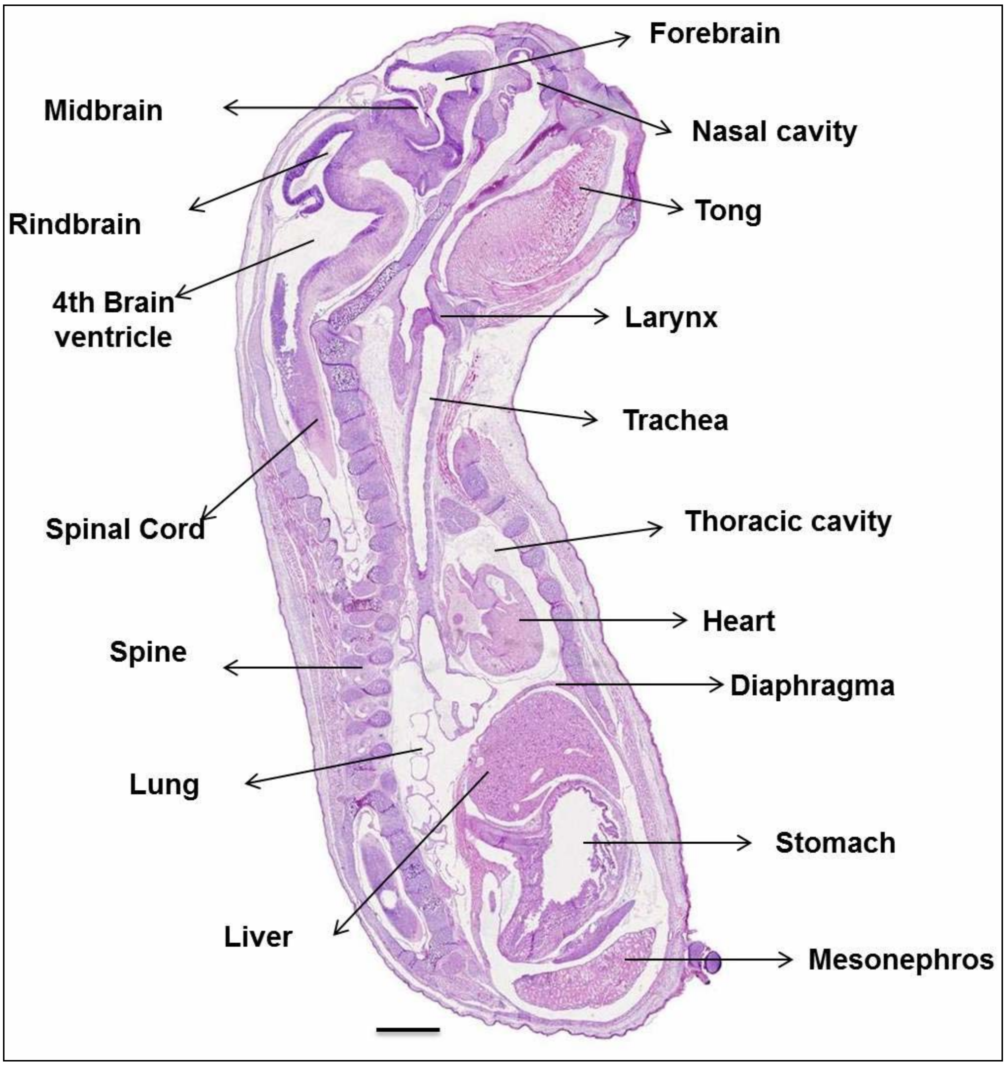

Figure 2. Photomicrography of a four days old *D. albiventris* neonate. Hematoxylin and eosin. Bars, 200 µm.

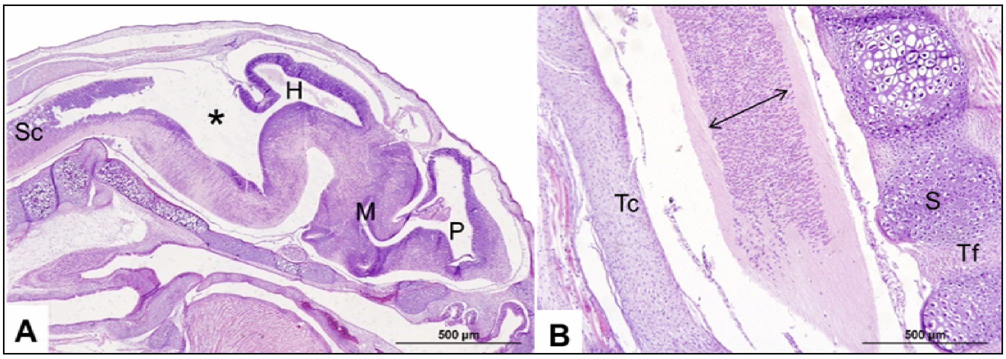

Figure 3. **P**hotomicrography of the nervous system of the *D. albiventris* four days old neonate. A) Three brain vesicles: forebrain (P), midbrain (M) and hindbrain (H), spinal cord (Sc) and fourth cerebral ventricle (*). (B) Spinal cord neuroepithelium (double arrow), connective tissue (Tc), vertebrae (S) and fibrocartilaginous tissue (Tf). Hematoxylin and eosin.

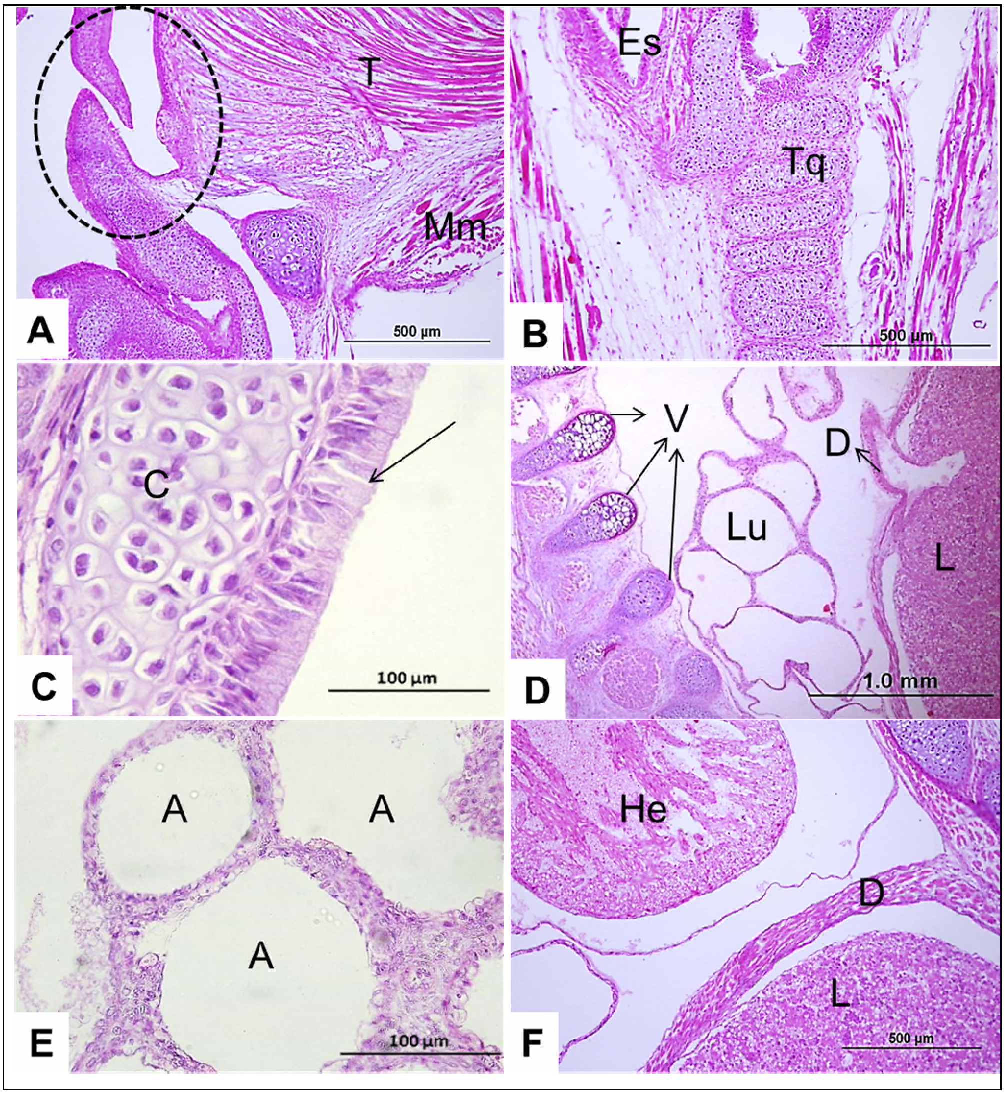

Figure 4. Photomicrography of the respiratory system of the *D. albiventris* four days old neonate. A) Larynx (dotted circle), tong (T), milohiodeum muscle (Mm). B) Esophagus (Es), trachea (Tq). C) Tracheal epithelium (arrow), cartilage (C). D) Vertebrae (V), lung (Lu), diaphragm (D) and liver (L). E) Alveoli (A). F) Heart (He), diaphragm (D) and liver (L). Hematoxylin and eosin.

were still in formation. Larynx was well-developed, being possible to observe the division of its cartilages, such as the epiglottis (Figure 4A). Trachea appeared as a long tube penetrating the lungs and presented areas of cartilage, connective tissue (Figure 4B), and a lining epithelium (Figure 4C). Lungs were found within the thoracic cavity as underdeveloped, being impossible to discriminate its common structures, such as bronchi and bronchioles; therefore, only the alveoli were found (Figure 4D and E). Diaphragm appeared as a little, slender and long muscle, extending from the chest wall to the near end of the spinal column (Figure 4F).

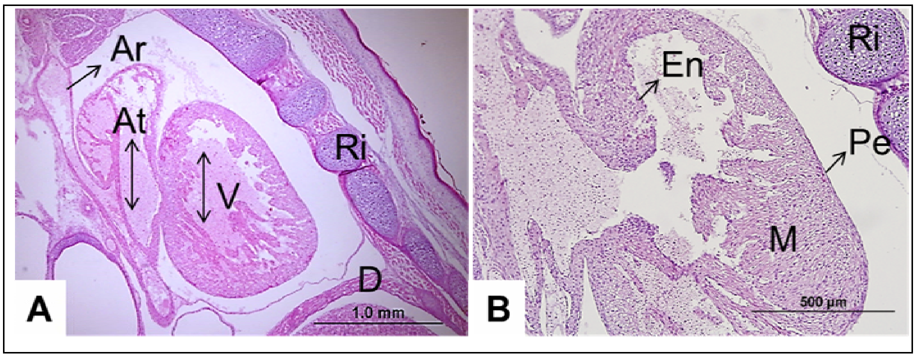

Figure 5. Photomicrography of the cardiovascular system of *D. albiventris* four days old neonate. A) Division of the chambers of the heart: ventricle (V) and atrium (At), hearth communications (arrows), aorta (Ar), diaphragm (D), and ribs (Ri). B) Layers of the heart: pericardium (Pe), myocardium (M), endocardial (En) and Ribs (Ri). Hematoxylin and eosin.

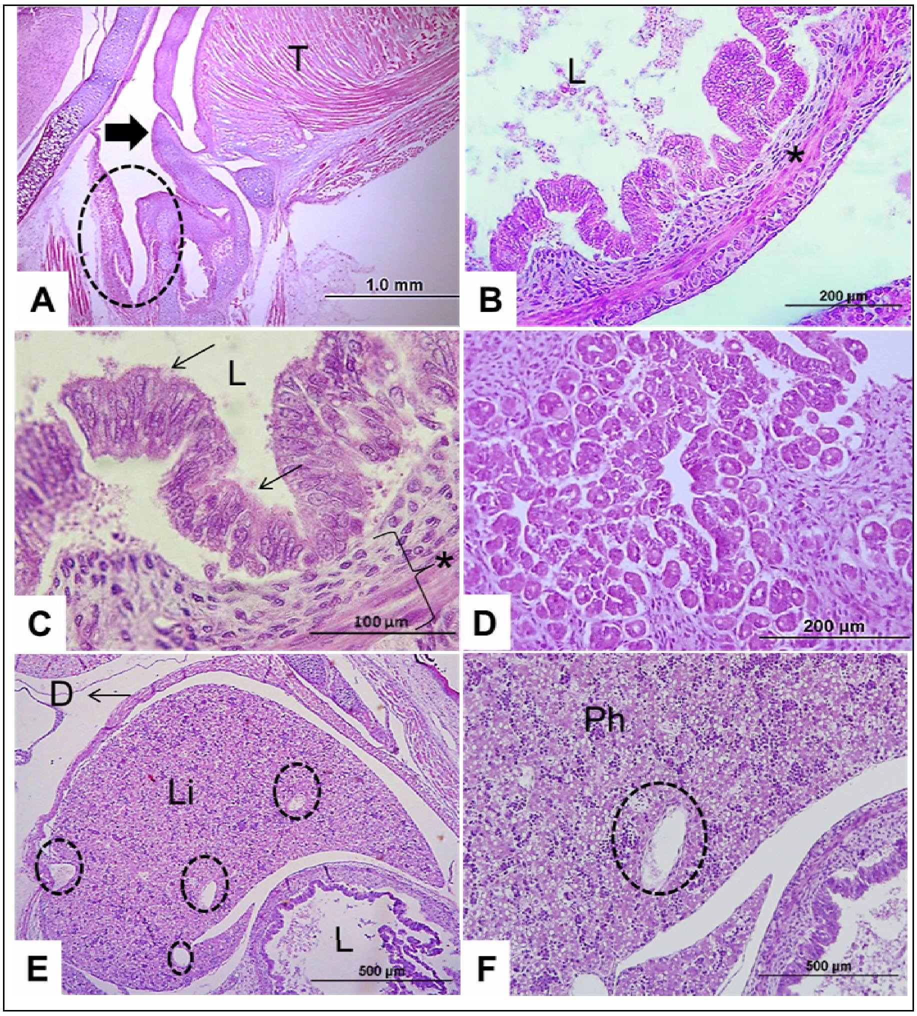

Figure 6. Photomicrography of digestive system of *D. albiventris* four days old neonate. A) Esophagus (dotted circle), tong (T), epiglottis (full arrow). B) Stomach lumen (L), Stomach muscular layer (*). C) Muscular layer (*), stomach lumen (L), stomach epithelium (arrow). D) Layer of the glandular stomach. E) and F) Liver (Li), diaphragm (D), stomach lumen (L), central lobular vein (dotted circles), and liver parenchyma (Ph) with located hepatocytes. Hematoxylin and eosin.

Vascular system analysis

D. albiventris neonates heart was found with its chambers, atrium and ventricle, as well with the dorsal aorta (Figure 5A). At this stage of cardiac development, the left and right atrium, as the right and left ventricles, still have communications (Figure 5A), this feature disappears during the development, where there is subsequent onset of cardiac septum. It was also possible to observe the pericardium, myocardium and endocardium histological layers (Figure 5B).

Digestive system analysis

Histological analysis of the digestive system allowed the identification of the outset of the esophagus as a long tube, supported by the dorsal mesentery formed by mesenchyme (Figure 6A). The stomach and portion of intestine was found as a swelling, with its characteristic epithelium and connective tissue around (Figure 6B and C). The stomach was found to occupy a large portion of the abdominal cavity, and it was possible to visualize the muscular and glandular layers (Figure 6C and D). The liver appeared large, occupying most of the abdominal cavity (Figure 6E). It was possible to visualize the center lobular veins, scattered throughout the liver parenchyma (Figure 6E and F).

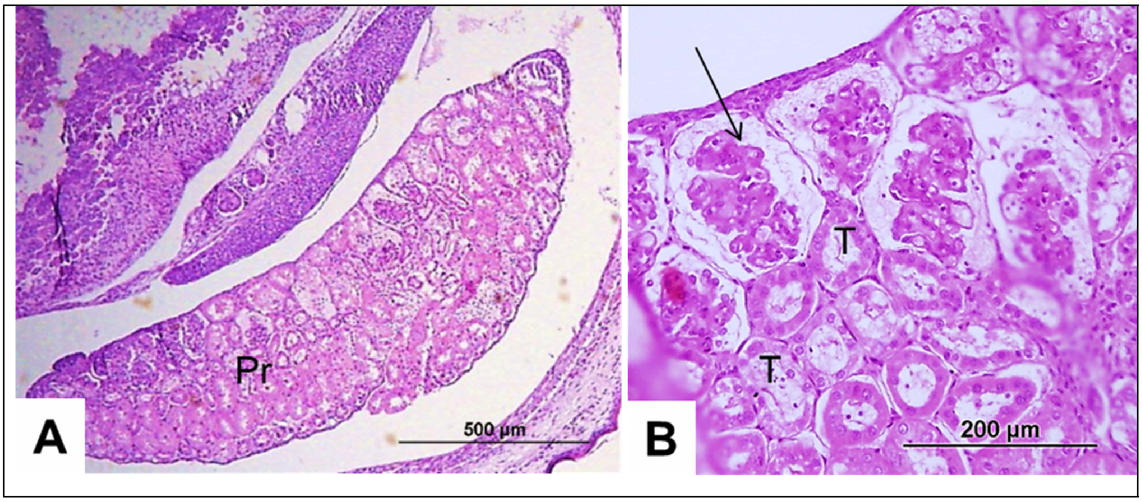

Figure 7. Photomicrography of the urinary system of the four days old *D. albiventris* neonate. A) and B) Renal parenchyma (Pr), renal tubules (T) and glomerulus (arrow). Hematoxylin and eosin.

Urinary system analysis

Neonates of the opossum *D. albiventris* showed mesonephros as a large, well developed organ, consisting of several nephrons separated by delicate connective tissue (Figure 7A). Two components of the mesonephric nephron can be recognized: renal tubules and glomeruli (Figure 7B).

Discussion

Based on external morphological characteristics of rodents fetuses *Myocastor* at 60 days of gestation (Felipe et al., 2006), the four days old *D. albiventris* neonates have similar characteristics, such as the development of limbs (presence of digits in the forelimbs and hindlimbs).

Theiler (1972), on studies with rodent *Rattus rattus* (Linneaus, 1758) embryos at stages 20 and 21, establish as characteristics the presence of the optic vesicle with retinal pigmentation, limbs appear well characterized, with evident digital grooves. These features were observed in rabbit embryos between 12 and 17 days of gestation (Beaudoin et al., 2003); and it resembles the ones reported for the *D. albiventris* four days old neonates in this present study.

D. albiventris, even after birth, show evident embryological features for some structures, such as, three brain vesicles, presence of a gap on the fourth ventricle, ongoing development of the spinal cord, and formation of the spine. These same features were also observed in developing embryos which present a longer intra-uterine gestational period. For guinea pigs *Cavia porcellus* (Linneaus, 1758), for example, these embryological features found after birth in opossums can be observed during the gestational age of 16 up to 22 days (Evans and Sack, 1973); in horses, from 20 to 40 days of gestation (Francioli et al., 2011); in dogs, 17 days (Evans and Sack, 1973); and in cats, from 16 days of gestation (Illanes et al., 2007).

According to New et al. (1977), opossum neonates (*Didelphis* spp.) are born in a developmental stage corresponding to rat fetuses of the same age, with the forelimbs and some organs early developed. Thus, this result corroborate with the ones observed in this study: some organs were found almost entirely developed (such as the stomach, liver and kidney). In guinea pigs, fetuses with 31 days of gestation presented a clear nasal plan, nasal cavity, individualized digits, and limbs with equilateral degree of development (Evans and Sack, 1973), which corroborates with the same features found in *D. albiventris* four days old neonates in this study.

At the fourth week of the human gestation, the fetal lung is in a pseudoglandular stage, which means that it formed the terminal bronchi and bronchioles, and the next phase, an increase in vascularization is observed (Moore and Persaud, 2004; Sadler, 2004). For *D. albiventris* neonates, lungs are in a previous stage of development than the one described above: bronchi or bronchioles were not found in lungs, and only the large alveoli were visible. Opossum lungs characteristics seem to be similar to what was observed for horse embryos at the 36th day of gestation, and cat embryos at 19th day of gestation, as both revealed lungs in developing stage, with the lack of bronchi and bronchioles, being possible to visualize only the alveoli (Francioli et al., 2011; Abreu et al., 2011).

D. albiventris four days old neonates showed well developed larynx and trachea, as it was possible to distinguish their cartilages. This aspect is similar to the observed in domestic cats (*Felis catus*) at 24 days of gestation (Illanes et al., 2007).

The newborn heart showed clear and well developed, already presenting the divisions of the heart chambers, the atrium and ventricle, and containing all muscle layers (endocardium, myocardium and pericardium). These aspects are similar to embryos of cats at 52 days of gestation (Abreu et al., 2011), embryos of horses at 38 days of gestation (Franciolli et al., 2011), mouse embryos at 14 days of gestation, and humans at 48 days of gestation (Vidal, 1997).

D. albiventris four days old neonates have also presented intracardiac connections: connection between the right and left atriums, and connection between the left and right ventricles. These results were also described by Frappell and MacFarlane (2006) in other marsupials such as kangaroos, wallabys and rat kangaroo, being these connections persistent for 2 to 3 days after birth.

Liver is found in the middle of the third week in humans as an invagination from endodermal epithelium, on the distal end of the foregut (Wolpert et al., 2000). The hepatic cords begin to differentiate from the parenchyma and to form the coating of the biliary ducts (Sadler, 2004). These data corroborate with the described for the *D. albiventris* neonate, in which the liver presents its parenchyma in differentiation to form the hepatic cords, as well as the sinusoids.

The first set of kidneys (pronephros) is rudimentary, being similar to the kidneys of primitive fish, the second set (mesonephros) is well developed and operates briefly, being analogous to the amphibians kidneys, the third set (metanephros) becomes the permanent kidney (Moore and Persaud, 2004; Sadler, 2004). Mesonephros are found in all mammals at some stage of their development, but the degree of maturation and duration varies among species. Rats and humans, for example, mesonephros appear early and then rapidly degenerates (Krause et al., 1979), in domestic pigs the mesonephros are visible in 20 days of gestation (Bertassoli et al., 2015), *D. albiventris* showed mesonefrons at 4 days old stage. These results corroborated with Krause et al. (1979), which reported similar results in *D. virginiana*, and have mentioned opossum mesonefros similarity with fetuses from other animals, such as pigs, sheep and cats.

Conclusion

Due to the ease of access to *D. albiventris* neonates, the early development state after birth and not sacrifice of the mother for neonate collected, this species can be considered an excellent model for comparative developmental studies. The embryological development of the four days old neonatal opossum can be compared to rats, guinea pigs, rabbits, horses, cattle and humans in different stages of development, regardless of the length of the gestation period, considering the particularities of each species.

Conflict of interests

Authors declare that they have no conflict of interests.

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