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Morphological Study in Heart of Wild Chinese Alligator (*Alligator sinensis*)

Estudio Morfológico del Corazón del Caimán Chino Salvaje (*Alligator sinensis*)

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XIE, Z.; SHI, P.; YANG, X. & LU, M. Morphological study in heart of wild Chinese alligator (*Alligator sinensis*). *Int. J. Morphol., 41(1)*:324-330, 2023.

SUMMARY: The Chinese alligator (*Alligator sinensis*) belongs to the genus Alligator, which is a unique crocodile in China. In order to study the macroscopic structure of the heart of Chinese alligator, we performed detailed cardiac anatomy on five specimens. The heart is in the cranial mediastinum. It is caudally involved by the liver cranial margins, and ventrally by the ribs, intercostal muscles, and sternum and dorsally by the lungs. The wild Chinese alligator heart is a typical four-chamber heart, with two (right and left) atria and ventricles, left and right aorta, pulmonary artery and subclavian artery branch from the aorta. Morphology measures the circumference (129.36 mm), weight (44.14 g), and length of the heart from apex to bottom (52.50 mm). Studies have shown that the shape of the wild Chinese alligator's heart is consistent with the anatomy of other crocodiles.

KEY WORDS: Chinese alligator; Heart; Morphology; Reptile.

INTRODUCTION

Alligator sinensis, is endemic to China, has been listed as China's level of endangered species, and listed as critically endangered on the IUCN red list (Jiang & Wu, 2018).Wild Chinese alligators are distributed in subtropical and warm temperate climates, living in fresh water and live mainly in lakes, marsh beaches or hills covered with grass poling wet areas (Jiang & Wu, 2018). By 2015, the wild Chinese alligator remaining Xuancheng, Nanling, Jingxian, Wuhu, Longxi, and Guangde (Jiang & Wu, 2018). According to the survey, only 68 to 86 mature wild Chinese alligators remained in the wild as of 2018 (Jiang & Wu, 2018). The Chinese alligator has an evolutionary history of nearly 150 million years, which is of great significance in evolutionary biology research.

According to statistics, 793 Chinese alligators were low level of genetic diversity (Pan *et al*., 2019). In addition, niche simulations showed that the habitat area of the critically endangered Chinese alligator was reduced by 8 to 15 times compared to previous climatic conditions, suggesting that the decrease in population size and habitat loss may be one of the factors affecting the current situation of the Chinese alligator, and may also be the direct cause of the decline in genetic diversity of the Chinese alligator (Pan *et al*., 2019). Currently, the progress of habitat restoration of Chinese alligator is not comprehensive. Habitat connectivity, quality and habitat area all need to be improved to prevent future time-delayed extinctions (Jochen *et al*., 2010; Margules & Pressey, 2000). Research on the heart of the Chinese alligator can help people better understand its living habits and physiological mechanism, so as to establish high-quality, high-utilization habitat or conservation area, that is, to protect it more efficiently.

Crocodiles have four-chambered hearts, different from the three-chambered hearts of other reptiles, and slightly different from the four-chambered hearts of mammal (Webb, 1979; Franklin, 2000; Axelsson, 2001; Dominique *et al*., 2014; Grigg & Kirshner, 2015; Cook *et al*., 2017). The crocodile heart has a special structure called the Panizza hole, where blood is mixed to meet the crocodile's diving needs (Kirshner, 2015). In addition, the blood circulation of crocodiles is typically double-circulatory, which is the same as that of most reptiles and amphibians (Dominique *et al*., 2014). Crocodile's heart has special structures such as the 'cog-teeth valve' and semilunar valve, which can help the

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heart to divert blood and achieve double circulation (Axelsson, 2001; Dominique *et al*., 2014). In this study, the heart structure and functional mechanism of the Chinese alligator were compared with those of other species, so as to supplement the anatomical database of the alligator heart.

There have been few reported anatomical studies of the hearts of the Chinese alligators (Webb, 1979; Axelsson *et al*., 1997; Altimiras *et al*., 1998; Eme *et al*., 2009; Alves *et al*., 2016; Jensen *et al*., 2016; De Oliveira Lima *et al*., 2020). This study focuses on the anatomy of the heart of the Chinese alligator, providing reference for the protection of the species of the Chinese alligator, and at the same time verifying the mechanism of the heart circulation of the alligator, supplementing the database of the heart anatomy of the alligator, and providing basic information for biological evolution, ancient reptiles, paleogeology.

MATERIAL AND METHOD

Five adult wild Chinese Alligator were donated by the Wildlife Rescue and Rehabilitation Center of Henan province, China. Adult males and three females wild Chinese Alligator that died eventually due to the lack of rescue experience and guidance were used in our study. The anatomy method used in this study is the general zoological anatomy method, the alligator front up, opened along the cloaca to the mandibular symphysis, opened the ribs make heart naked, before removing the heart to look at the position of the heart to describe and photograph, after the separation of the heart. After that, the heart was separated carefully to preserve the great vessels and then fixed in 10 % formalin for 48 h to dissection.

Measuring instruments are digital calipers and a string (for circumference). Measurement is the total length of the body; total length of heart (apex to bottom); length and width of the head; length of the lips; length of the tail; length, width and thickness of the right atrium; length, width and thickness of the left atrium; length, width and thickness of the right ventricle; length, width and thickness of the left ventricle and total circumference of the heart. After that, the hearts were placed in an analytical balance for weight measurement, and then the heart were cut from the apex to the longitudinally sectioned along the apex-to-base axis, exposing the atrial and ventricular cavities for description. At the same time, the inter ventricular septum and wall thickness of left and right ventricle respectively were measured. In the left ventricle, measurements were taken both at the base and apex, whereas in the right ventricle they were measured only at the base.

RESULTS

Topography. It was observed that the heart of *Alligator sinensis* is located in the cranial mediastinum, surrounded by two pericardial sac and surrounded by a large amount of adipocytes. The tail of the heart is near the top of the liver, and the back of the heart is in contact with the lungs. It is located in the middle and upper part of the abdominal cavity (Fig. 1).

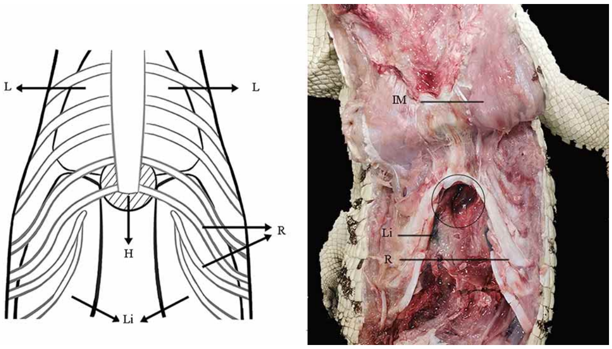

Fig. 1 Ventral view of the coelomic cavity of an adult female specimen (Sample-3) of Chinese alligator (*Alligator sinensis*). The heart (H) is located in the pericardial sac. The tail of the heart is near the top of the liver(Li), and the back of the heart is in contact with the lungs (L). The outer heart is protected by the ribs (R), intercostal muscles (IM) and the sternum.

Morphology. The heart is surrounded by two pericardial sac, which are dissected to reveal a gubernaculum cordis connecting the pericardium to the heart apex. This morphology can be observed from the left and right sides in all specimens. Heart has a developed truncus arteriosus and four chamber: two atria and ventricles. The atrium is near the head of the crocodile, the ventricle is near the tail of the crocodile, and the arterial trunk of the heart is located on the cranial ventral surface of the heart (Figs. 2 and 3). Along the longitudinal incision ventricular apex to cardiac axis, to display in the middle of the ventricle separated intact ventricular wall between the right and left ventricles (Fig. 4). The interventricular septum is a thick inner wall with an irregular spongy appearance. The pulmonary artery and the left and right aortic arches start from the ventricle and leave the heart at this region, branching into six vessels. The pulmonary artery is divided into the left pulmonary artery and the right pulmonary artery, most of which are located on the dorsal side of the truncus arteriosus. The right aortic arch is divided into the right aorta, the right subclavian artery, and the left subclavian artery, located ventrally in the truncus arteriosus. Here there is a hole at the base of the left subclavian artery and left aorta called the foramen of Panizza. At the bottom of the pulmonary artery has a cogteeth valve (Fig. 4).

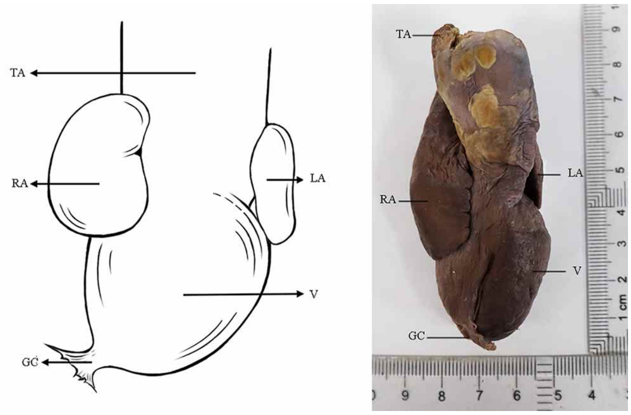

Fig. 2. The heart of a male, adult specimen Sample-3 (*Alligator sinensis*) in ventral views.Truncus arteriosus (TA), right atrium (RA), left atrium (LA), ventricle (V), gubernaculum cordis (GC).

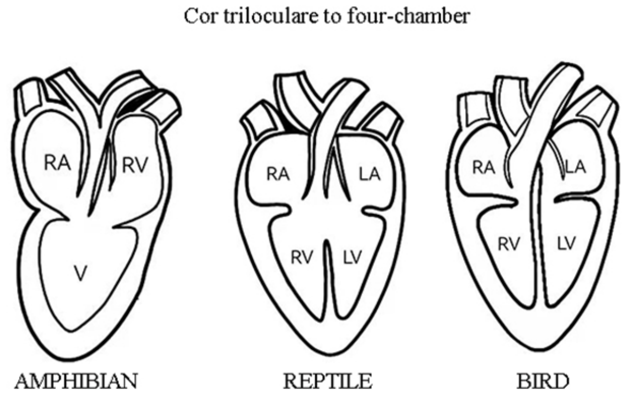

Fig. 5. The transition from cor triloculare heart to four-chamber heart. Truncus Arteriosus (TA), right atrium (RA), left atrium (LA), ventricle (V), right ventricle (RV), left ventricle (LV), gubernaculum cordis (GC).

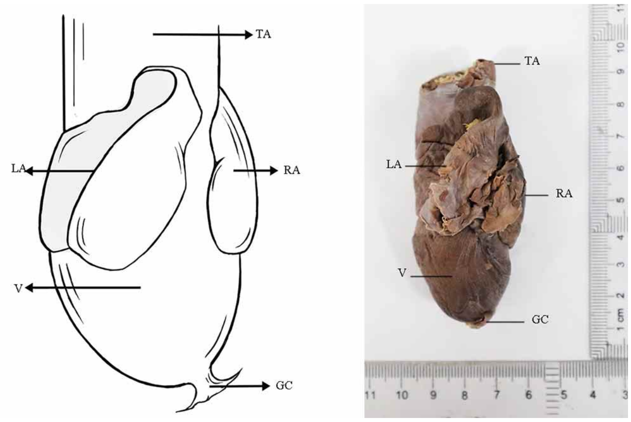

Fig. 3. The heart of a male,adult specimen Sample-3 (*Alligator sinensis*) in dorsal views. Truncus arteriosus (TA), right atrium (RA), left atrium (LA), ventricle (V), gubernaculum cordis (GC).

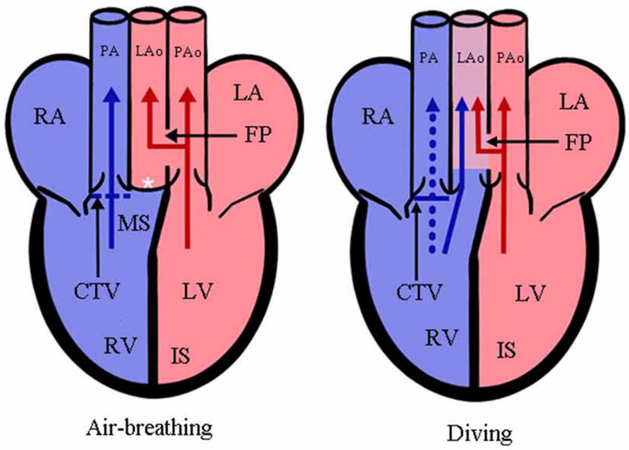

Fig. 6. The blood flow of crocodiles as they breathe and dive. Right atrium (RA), left atrium (LA), right ventricle (RV), left ventricle (LV), interventricular septum (IS), membranous septum (MS), pulmonary artery (PA), left aortic arch (LAo), right aortic arch(RAo), foramen of Panizza (FP), 'cog-teeth valve' (CTV), semilunar valve (*).

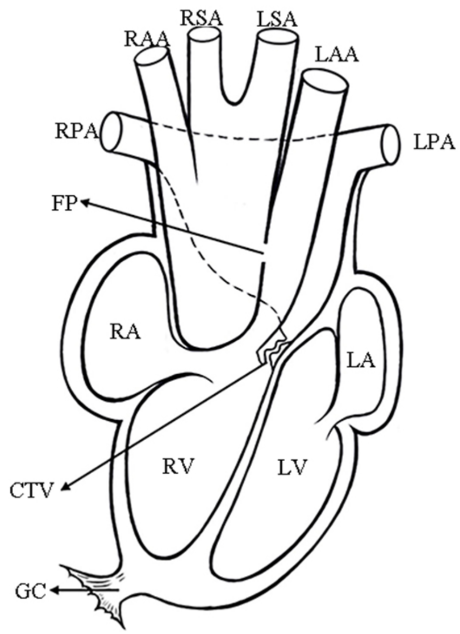

Fig. 4. The heart of a male, adult specimen Sample-3 (*Alligator sinensis*) in ventral section views.Truncus arteriosus (TA), right atrium (RA), left atrium (LA), right ventricle (RV), left ventricle (LV), right subclavian artery (RSA), left subclavian artery (LSA), right aorta (RAA), left aortic arche (LAA), right pulmonary artery (RPA), left pulmonary artery (LPA), foramen of Panizza (FP), 'cog-teeth valve' (CTV), gubernaculum cordis(GC).

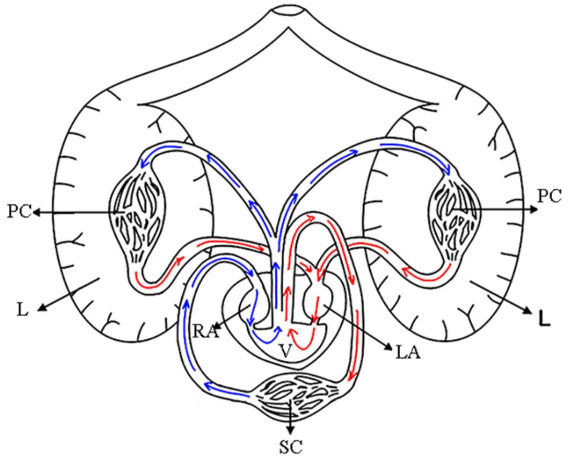

Fig. 7. Description about double the circulation of the blood, Right atrium (RA), left atrium (LA), ventricle (V), lung (L), pulmonary circulation (PC), systemic circulation (SC).Blue arrows indicate anaerobic blood, red arrows indicate aerobic blood.

Morphometry. The morphometry of *Alligator sinensis* heart was recorded in Table I, and the main data are presented as follows. In all specimens, the length, width, and thickness of the right atrium were larger than that of the left atrium.

In the specimen Sample-1, the left ventricular was larger in length, width than the left atrium, but not in thickness. The thickness of right ventricle wall was 2.54 mm, the thickness of left ventricle wall was 2.02 mm at the apex and 3.98 mm at the base, with an average of 3.00 mm. The thickness of interventricular septum was 9.54 mm. The heart circumference was 140.77 mm.

In the specimen Sample-2, the left ventricular was larger in length, thickness than the left atrium, but not in width. The thickness of right ventricle wall was 2.78 mm, the thickness of left ventricle wall was 2.06 mm at the apex and 4.58 mm at the base, with an average of 3.32 mm. The thickness of interventricular septum was 11.54 mm. The heart circumference was 135.78 mm.

In the specimen Sample-3, the left ventricular was larger in length than the left atrium, but not in width and thickness. The thickness of right ventricle wall was 2.12 mm, the thickness of left ventricle wall was 1.22 mm at the apex and 2.86 mm at the base, with an average of 2.04 mm. The thickness of interventricular septum was 10.69 mm. The heart circumference was 126.90 mm.

Table I. Heart morphometry of the specimens of *Alligator sinensis*

Name	Sample-1	Sample-2	Sample-3	Sample-4	Sample-5	SD
Sex	Male	Male	Female	Female	Female	
Age class	Adult	Adult	Adult	Adult	Adult	
Total Le (cm)	195	202	172	174	178	12.03993
Snout-vent Le (cm)	102	108	90	90	94	7.11056
Tail Le (cm)	100	105	81	84	85	9.61249
B-A (mm)	59.86	62.12	47.24	44.58	48.69	7.094
Heart weight (g)	54.88	58.45	36.45	33.64	37.28	10.35924
IS (mm)	9.54	11.54	10.69	8.24	7.66	1.45236
RA Le (mm)	59.53	62.38	54.22	50.78	53.46	4.24279
RA Wi (mm)	34.45	35.78	29.15	27.44	26.29	3.80307
RA Th (mm)	11.78	12.04	9.33	8.96	8.42	1.50338
LA Le (mm)	38.95	40.24	35.66	34.81	32.44	2.82855
LA Wi (mm)	27.48	25.94	23.14	23.54	25.89	1.62612
LA Th (mm)	10.53	11.29	8.25	7.92	9.96	1.30346
RV Le (mm)	38.23	37.76	33.25	30.57	29.58	3.57267
RV Wi (mm)	22.48	24.86	21.26	20.93	24.35	1.58834
RV Th (mm)	10. 78	9.56	7.55	7.43	8.03	1.29968
RV wall (mm)	2.54	2.78	2.12	2.38	1.68	0.37715
LV Le (mm)	44.29	47.16	37.95	34.82	30.43	6.10844
LV Wi (mm)	25.44	23.78	19.80	16.73	17.88	3.36077
LV Th (mm)	8.74	10.59	7.33	7.48	6.79	1.36133
LV Apex wall (mm)	2.02	2.06	1.22	1.48	1.35	0.34811
LV Base wall (mm)	3.98	4.58	2.86	2.46	3.07	0.7762
TA Le (mm)	50.12	53.11	44.25	40.45	44.78	4.50358
TA Wi (mm)	38.78	34.58	31.22	29.78	34.45	3.119
TA Th (mm)	20.78	18.44	16.62	15.48	14.33	2.27426
Circumference (mm)	140.77	135.78	126.90	124.78	118.56	7.93817

Abbreviations: SD, Standard Deviation; B-A, Base to Apex; IS, Interventricular Septum; RA, Right Atrium; LA, Left Atrium; RV, Right Ventricle; LV, Left Ventricle; TA, Truncus Arteriosus; Le, length; Wi, width; Th, thickness.

In the specimen Sample-4, the left ventricular was larger in length than the left atrium, but not in width, with almost the same thickness. The thickness of right ventricle wall was 2.38 mm, the thickness of left ventricle wall was 1.48 mm at the apex and 2.46 mm at the base, with an average of 1.97 mm. The thickness of interventricular septum was 8.24 mm. The heart circumference was 124.78 mm.

In the specimen Sample-5, the left ventricular was larger in length than the left atrium, but not in width and thickness. The thickness of right ventricle wall was 1.68 mm, the thickness of left ventricle wall was 1.35 mm at the apex and 3.07 mm at the base, with an average of 2.21 mm. The thickness of interventricular septum was 7.66 mm. The heart circumference was 118.56 mm.

In all female specimens, the average length, width and thickness of the right atrium are 52.82 mm, 27.63 mm and 8.90 mm, the average length, width and thickness of the left atrium are 34.30 mm, 24.19 mm and 8.71 mm, the average length, width and thickness of the right ventricle were 31.13 mm, 22.18 mm and 7.67 mm, the average length, width and thickness of the left ventricle were 34.40 mm, 18.14 mm and 7.20 mm. The average thickness of the right ventricular wall was 2.06 mm. The average thickness of the left ventricular wall was 3.35 mm at the apex and 2.80 mm at the base.

In all male specimens, the average length, width and thickness of the right atrium are 60.96 mm, 35.12 mm and 11.91 mm, the average length, width and thickness of the left atrium are 39.60 mm, 26.71 mm and 10.91 mm, the average length, width and thickness of the right ventricle were 38.00mm, 23.67 mm and 10.17 mm, the average length, width and thickness of the left ventricle were 45.73 mm, 24.61 mm and 9.67 mm. The average thickness of the right ventricular wall was 2.66mm. The average thickness of the left ventricular wall was 2.04 mm at the apex and 4.28 mm at the base.

Comparative morphometry of the right atrium and left atrium of all specimens of Chinese Alligator (*Alligator sinensis*) is seen in Fig. 8, and comparative morphometry of the right ventricle and left ventricle of all specimens of Chinese Alligator (*Alligator sinensis*) is seen in Fig. 9.

The truncus arteriosus of the male specimen Sample-1 and Sample-2 show mean was 51.62 mm length, 36.68 mm wide and 19.61 mm thick. In comparison, the female specimens Sample-3, Sample-4 and Sample-5 show mean length of 43.16 mm, mean width of 31.82 mm and mean thickness of 15.48 mm (Fig. 10).

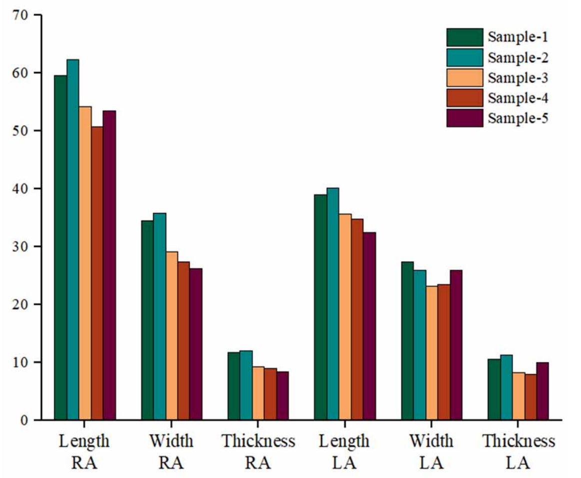

Fig. 8 Comparative morphometry of the right atrium (RA) and left atrium (LA) of all specimens of Chinese Alligator (*Alligator sinensis*). The measurements are relative to length, width and thickness in mm

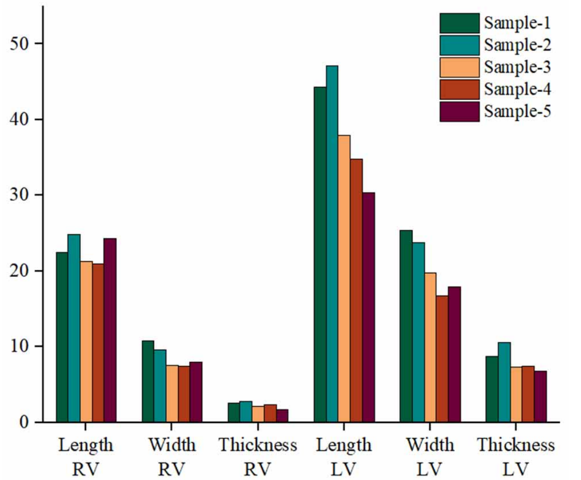

Fig. 9 Comparative morphometry of the right ventricle (RV) and left ventricle (LV) of all specimens of Chinese Alligator (*Alligator sinensis*). The measurements are relative to length, width and thickness in mm.

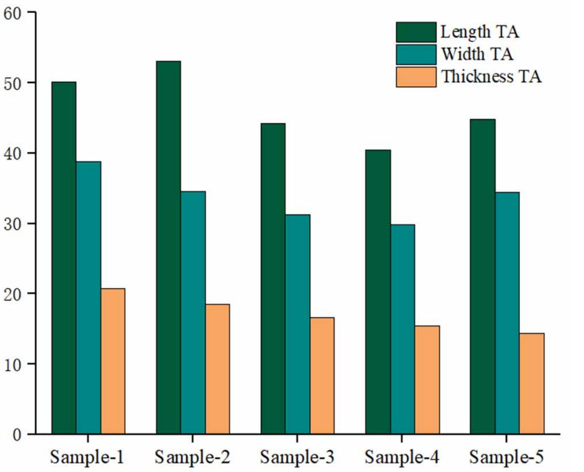

Fig. 10 Comparative morphometry of the truncus arteriosus (TA) of all specimens of Chinese Alligator (*Alligator sinensis*). The measurements are relative to length, width and thickness in mm.

DISCUSSION

The results show that the heart of *Alligator sinensis* is located in the cranial mediastinum, adjacent to liver, lungs and other organs. It is protected by ribs and wrapped by two pericardial sac (Fig. 1). Heart has a developed truncus arteriosus and four chamber: two atria and two ventricles. The left and right ventricles are separated by an interventricular septum with a thick inner wall with an irregular spongy appearance (Fig. 4).The study found that the Chinese alligator heart belongs to the typical crocodile heart, and other types of crocodiles heart in addition to the size has no obvious difference (Webb, 1979; Axelsson *et al*., 1997; Altimiras *et al*., 1998; Eme *et al*., 2009; Alves *et al*., 2016; Jensen *et al*., 2016; De Oliveira Lima *et al*., 2020). Unlike other reptiles, such as snakes and lizards, the crocodile heart has a complete interventricular septum. Unlike mammals, crocodiles have a foramen of Panizza, where blood fuses to meet their diving needs (Webb, 1979; Franklin, 2000; Axelsson, 2001; Dominique *et al*., 2014; Grigg & Kirshner, 2015; Cook *et al*., 2017).

It was observed that all specimens have 6 arteries, are surrounded by a thick sheath of connective tissue, formation of truncus arteriosus. After deep dissection, the pulmonary artery and the left right aortic arch start from the right ventricle, the right aortic arch start from the left ventricle. The pulmonary artery is divided into the left pulmonary artery and the right pulmonary artery, most of which are located on the dorsal side of the truncus arteriosus. The right aortic arch is divided into the right aorta, the right subclavian artery,and the left subclavian artery, located ventrally in the truncus arteriosus (Fig. 4). Crocodile of right ventricle and pulmonary artery and aorta is linked together, the right ventricle to pulmonary circulation, not only is used in the systemic circulation, the blood to other parts of the body (Fig. 7). These vessels are designed for efficient blood circulation and are similar in shape to the heart vessels of other crocodiles (Webb, 1979; Axelsson *et al*., 1997; Altimiras *et al*., 1998; Eme *et al*., 2009; Alves *et al*., 2016; Jensen *et al*., 2016; De Oliveira Lima *et al*., 2020).

It was found that the heart of Chinese alligator has four chambers and the foramen of Panizza, which is a typical type of crocodile heart (Axelsson, 2001). Heart of the structure is caused by many reasons, the main reason is evolution. According to data, the heart of early fish was an atrium and a ventricle. The atria store blood and the ventricles pump it. Atrioventricular valves exists between the atrium and ventricle to prevent blood reflux (Marcos, 2005; Dominique *et al*., 2014; Stephenson *et al*., 2017). The amphibian heart evolved from fish into two atria and one ventricle. This evolution reduces the mixing of anaerobic blood and aerobic blood in the atrium, improves the utilization rate of oxygen, and provides the species with stronger exercise capacity and metabolic capacity (Marcos, 2005; Dominique *et al*., 2014; Stephenson *et al*., 2017). Reptiles evolved from amphibians; at this time, due to changes in their living environment, animals needed more efficient locomotion and metabolism, so non-crocodile reptiles developed an incomplete interventricular septum in the ventricle to separate the entire ventricle (Fig. 5). The interventricular septum and various folds in the ventricles allow non-crocodile reptiles to almost completely separate anaerobic and aerobic blood in the ventricles, resulting in more efficient oxygen utilization (Webb, 1979; Marcos, 2005; Dominique *et al*., 2014; Stephenson *et al*., 2017). To make more efficient use of anaerobic and aerobic blood, mammals and crocodiles have developed a complete interventricular septum, forming a complete four-chamber heart (Fig. 5). However, the difference is that the heart of the crocodile has a special structure in the panizza hole, anaerobic and aerobic blood converge here (Webb, 1979; Axelsson, 2001; Marcos, 2005; Dominique *et al*., 2014; Stephenson *et al*., 2017; Cook *et al*., 2017).

The Foramen of Panizza is an important anatomical feature of the crocodile, where blood is mixed to meet the crocodile's diving needs. It is located in the left between the aorta and right aortic arch, cartilaginous skeleton around it (Axelsson *et al*., 1997; Axelsson, 2001; Cook *et al*., 2017; De Oliveira Lima *et al*., 2020). When the crocodile breathes, the 'cog-teeth valve' opens, the semilunar valve closes, and the blood from the right ventricle flows only into the pulmonary artery (Fig. 6). The blood from the left ventricle flows first into the right aortic arch, and then through the Panizza hole to the left aortic arch. At this time, both sides of the aortic arch are filled with oxygen blood. When the crocodile is diving, the semilunar valve opens, the 'cog-teeth valve' closes, and the blood from the right ventricle flows only into the left aortic arch (Fig. 6). The blood from the left ventricle flows first into the right aortic arch, and because the aerobic blood high blood pressure, then the aerobic blood through the Panizza hole to the left aortic arch. At this point, the blood flows from the right aortic arch for aerobic blood, the blood from the left aortic arch mostly anaerobic blood, and a few for aerobic blood (Axelsson, 2001; Dominique *et al*., 2014). At this point, the crocodile does not breathe or engage in pulmonary circulation. The crocodile's oxygen demand can only be satisfied from aerobic blood in the right aortic arch and a small amount of aerobic blood in the left aortic arch. The existence of the Foramen of Panizza provides the basis for blood circulation while the crocodile dives. This is also one of the reasons why crocodiles cannot dive for a long time (Dominique *et al*., 2014).

The 'cog-teeth valve' is another important anatomical feature of the crocodile. It is located in the lower part of the pulmonary artery and right ventricle, is mainly composed of connective tissue nodules, main function is to control the blood flowing to the pulmonary artery (Axelsson, 2001): when the right ventricle blood pressure is higher than the base of the pulmonary artery blood pressure, the 'cog-teeth valve' is opened, the blood flowing from the right ventricle to pulmonary artery into the pulmonary circulation again; When the base of the pulmonary artery blood pressure is higher than the right ventricle blood pressure, the 'cog-teeth valve' closes and blood flow to the lungs is reduced (Fig. 6) (Axelsson, 2001; Dominique *et al*., 2014).

There is a valve called semilunar valve at the junction between the left aorta and the right ventricle, which mainly controls the blood flow to the left aortic arch (Axelsson, 2001): when the right ventricle blood pressure is higher than the base of the pulmonary artery blood pressure and the 'cog-teeth valve' closes, the semilunar valve is opened, the blood flowing from the right ventricle to left aortic arch; When the pulmonary artery blood pressure is higher than the base of the right ventricle blood pressure and the 'cog-teeth valv-e' closes, the semilunar valve closes and blood flow to the left aortic arch is reduced (Axelsson, 2001; Dominique *et al*., 2014).

While observing the heart of the Chinese alligator structure, it can be concluded that the blood circulation is a typical double circulation (Fig. 7). Reptiles and amphibians all use a double circulation in which the heart pumps anaerobic blood to the lungs through the left subclavian artery and the right subclavian artery with a weak beat, and then back to the heart. The heart then beats a little harder, pumping oxygen through the left aortic arch and the right aortic arch to the body for systemic circulation, and back to the heart to form a circulation.

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RESUMEN: El caimán chino (*Alligator sinensis*) pertenece al género Alligator, que es un cocodrilo único en China. Para estudiar la estructura macroscópica del corazón del caimán chino, revisamos detalladamente la anatomía cardíaca de cinco especímenes. El corazón está en el mediastino craneal. Está limitado caudalmente por los márgenes craneales del hígado, y ventralmente por las costillas, los músculos intercostales y el esternón, y dorsalmente por los pulmones. El corazón de cocodrilo chino salvaje es un corazón típico de cuatro cámaras, con dos atrios y dos ventrículos (derecho e izquierdo), aortas izquierda y derecha, arteria pulmonar y rama de la arteria subclavia de la aorta. La morfología mide la circunferencia (129,36 mm), el peso (44,14 g) y la longitud del corazón desde el ápice hasta la base (52,50 mm). Los estudios han demostrado que la forma del corazón del caimán chino salvaje es consistente con la anatomía de otros cocodrilos.

PALABRAS CLAVE: Caimán chino; Corazón; Morfología; Reptil.

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