Sequential segmental analysis of the crocodilian heart

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ABSTRACT

Differences between hearts of crocodilians and those of mammals and birds are only partly understood because there is no standardized approach and terminology in describing cardiac structure. Whereas most reptiles have an undivided ventricle, crocodilians have a fully septated ventricle. Their hearts, therefore, are more readily comparable with the hearts of mammals and birds. Here, we describe the heart of a crocodile (*Crocodylus noliticus*). We use the versatile sequential segmental approach to analysis, juxtaposing several key views of the crocodilian heart to the comparable views of human hearts. In crocodiles, the atrial and ventricular septums are complete, but unlike in placental mammals, the atrial septum is without an oval fossa. The myocardial component of the crocodilian ventricular septum dominates, but the membranous septum likely takes up a greater proportion than in any mammal. In the crocodile, the aortic trunk taking its origin from the left ventricle is not wedged between the atrioventricular junctions. Consequently, there is a common atrioventricular junction, albeit with separate right and left atrioventricular valvar orifices. As in mammals, nonetheless, the crocodilian left atrioventricular valvar orifice is cranial to the right atrioventricular valvar orifice. By applying a method of analysis and terminology usually restricted to the human heart, we build from the considerable existing literature to show neglected and overlooked shared features, such as the offset between the left and right atrioventricular valvar orifices. Such commonalities are surprising given the substantial evolutionary divergence of the archosaur and synapsid lineages, and likely reflect evolutionarily shared morphogenetic programs.

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INTRODUCTION

George Cuvier (Cuvier, 1836) may have been the first to observe that the cardiac ventricle is divided into left and right ventricles by a full septum, not only in mammals and birds, but also in crocodilians. Nowadays, molecular biology is making an ever stronger case that the hearts of the amniotic vertebrates, specifically mammals, birds and reptiles, share a common building plan during their development whereby evolutionarily conserved transcription factors orchestrate the formation of chambers, junctions, and septal structures (Olson, 2006; Koshiba-Takeuchi et al 2009; Jensen et al. 2012). Anatomical traits shared across vertebrate groups are likely to be fundamental in ensuring their normal function. Comparative studies, therefore, can offer a different line of evidence with which to evaluate the importance of the architecture of the human heart. The human heart is the best described of all hearts, while the crocodilian heart may be the best described reptilian heart (Anderson et al, 2003b; Jensen et al, 2014b). It follows that comparisons between the two hearts, may facilitate our understanding of cardiac evolution, but such comparisons remain difficult. This is due, in part, to a relative scarcity of images of crocodilian hearts. It is also due to the differences used in approach and terminology when examining the hearts. Here, using extensive photographic images, we compare the gross anatomy of the Nile crocodile heart with that of the human heart. Because the human heart is familiar to most, and has been described the best, we describe the crocodilian heart by the process of sequential segmental analysis, this being the approach now accepted as optimal for pathologic analysis of the normal and congenitally malformed human heart. When describing the topographic location of structures in the crocodile, we have chosen to use the terms cranial and caudal, instead of anterior and posterior,

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since the latter terms, when used in human anatomy, describe the features usually called ventral and dorsal in the crocodile.

The crocodilian heart, in terms of its gross anatomy, is distinct from the hearts of other reptiles in possessing fully separated ventricles, and having a large myocardial mural leaflet as part of the right atrioventricular valve, an arrangement that is very similar to the situation found in both birds and monotreme mammals (King, 1837; Van Mierop & Kutsche, 1985; Jensen et al., 2013a, 2014b). In comparison, in the non-crocodilian reptile hearts of the turtles, lizards and snakes, the ventricular mass is sub-divided into three compartments, which connect and permit mixing of oxygen rich and oxygen poor blood (Farrell et al 1998; Hicks, 1998; Jensen et al, 2014b). The situation is of greater interest in that, although the outflow tract of the crocodilian heart is septated into pulmonary and aortic components, the aortic component is split, with one aorta arising from the left ventricle, and a second aorta taking origin, together with the pulmonary trunk, from the right ventricle. The intrapericardial components of the outflow tracts of mammals and birds, of course, are made up of a solitary aorta and the pulmonary trunk (Benninghoff, 1933; Goodrich, 1958; Rowlatt, 1990; Anderson et al 2003b; Jensen et al., 2013b). To clarify further, and identify differences and commonalities, between the hearts of crocodiles and humans, which in many instances can be considered typical of mammals, we have examined in detail the gross anatomy of the crocodilian heart, and compared it to the well-described anatomy of the human heart (Anderson et al, 2003b). We have reinforced our gross anatomic studies with computerised tomographic imaging and serial histologic sections. The terminology of sequential segmental analysis, however, is inconsistent with that generally used for description of the crocodilian aortas. For the purposes of our comparisons, therefore, we emphasise that we have described the

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aorta originating from the left ventricle as the left ventricular aorta. In similar fashion, we have nominated the aorta originating from the right ventricle as the right ventricular aorta. For those concentrating specifically on crocodilian heart, these vessels are usually named the right and left aortas, respectively, due to the location of their arch relative to the bronchial tree (Farrell et al 1998; Hicks, 1998; Jensen et al, 2014b). We describe the side of the aortic arch separately from the aortic ventricular origin. Since connections, rather than relations, are now paramount in the setting of sequential segmental analysis (Anderson et al, 2004), it is the ventricular origins that we consider to represent the most significant feature, whilst still recognising the importance of describing the side of the aortic arch.

MATERIALS & METHODS

We examined 21 hearts of the Nile crocodile (*Crocodylus niloticus*) from the UCL Cardiac Archive, held at the UCL Great Ormond Street Institute of Child Health. The largest heart weighed 150g, while the smallest 5 examples weighed just less than 1g, and are from fetal specimens. Of the hearts, 3 weighing 20.5g were known to be from 2-year old crocodiles. We examined the atrial, ventricular, and arterial components, basing our approach on the system of sequential segmental analysis used to clarify the anatomy of the human heart (Anderson et al, 2004). Prior to, and during dissection, colour photographs were taken using a Nikon D100 digital camera equipped with macro lens and a polarising filter. We subsequently imaged 2 of the largest hearts using computerised tomographic techniques. An additional 2 hearts were sectioned completely in a four-chamber plane, staining every 10th section with either Masson's trichrome or the elastic van Gieson stains. We then compared the images of the

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crocodilian hearts with those prepared from the normal human hearts contained in our archive.

Multi-detector computed tomography was performed using a Sensation 16 scanner (Siemens Medical Systems, Erlangen, Germany). Images were initially acquired using a 0.75 mm beam collimation, and a table feed of 15 millimeters rotation, at 24mAs and 100kV. We then syringed 20 milliliters of 10% diluted non-ionic contrast medium (Omnipaque 300mg iodine/ml, Nycomed, Oslo, Norway) into the great vessels, such that all air was purged from the cardiac cavities and proximal great vessels. A second set of images was then acquired using the same imaging parameters as for the initial acquisition. The time required to acquire each of the two data sets was two seconds. The images were acquired with an axial spiral volume, and multiplanar reformatting performed. Three-dimensional volume-rendered images were created from the post-contrast images on a separate workstation (Leonardo Workstation, Siemens Medical Systems, Iselin, NJ).

For the purposes of comparison with the human heart, we selected images taken over the past decade during our examination of large numbers of specimens housed in the archives of Lurie Children's Hospital, Chicago; University of Florida, Gainesville, and Johns Hopkins All Children's Hospital, St Petersburg, Florida. All hearts had been obtained and stored with appropriate permission, and were photographed in accordance to the regulations of the various Universities.

RESULTS

In all the specimens examined, the long axis of the heart, and the cardiac apex, are directed more towards the right when placing the heart in an orientation approximating its location during life. As a result, the left ventricle is ventral, and the

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right ventricle is dorsally positioned (Figure 1). Arising from the apex of each of the hearts is a broad fibrous strand, previously termed the 'gubernaculum cordis', which anchors the heart to the pericardium.

Chambers of the Right Heart

Unlike the human heart, the most proximal chamber of the right side of the crocodile heart is a discrete systemic venous sinus, the so-called sinus venosus. Bounded by the sinuatrial valves, it receives the right and left superior caval veins, along with the inferior caval veins (Figure 2). Within the small and thin-walled sac enclosed by the sinuatrial valves (Figure 3), the mouth of the left superior caval vein is additionally guarded by its own discrete valve. Unlike the situation in the human heart, the opening of the left caval vein is unrelated to the atrial septum (Figure 3). The crocodilian left superior caval vein is a thin-walled vessel. As in humans with persistence of the left superior caval vein, it runs along the left atrial wall, below the left side of the pulmonary veins, extending between the left atrial appendage and the left pulmonary veins (Figure 2). Unlike in the human heart, however, it runs at some distance from the atrioventricular junction. Also unlike the situation in the human heart, the coronary veins drain to a separate connecting channel, which enters the inferior caval vein caudal to the mouth of the left superior caval vein. In the human heart, the majority of the coronary veins drain to the right atrium via the orifice of the coronary sinus, which in most hearts is the remnant of the left sinus horn (Figure 3). The right atrial chamber, proximal to the venous valves, is separated from the left atrium by a complete atrial septum. It possesses an appendage, a venous component, and a vestibule (Figures 3). Internally, the chamber is highly trabeculated, but contains a network of thin muscular bands, rather than the discrete pectinate muscles seen in the human heart. As in the human heart, nonetheless, the muscular bands

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extend from the tip of the appendage around the right atrioventricular junction (Figure 3). There is an obvious “septum spurium”, or suspensory ligament, to be seen, which is short, and branches caudally into two prominent sinuatrial valves (Figure 3). These then run ventrally to join a central fibrous body, which itself is positioned much more caudally within the heart than in the human.

The smooth non-trabeculated vestibule of the right atrium surrounds the right atrioventricular junction, supporting the leaflets of the right atrioventricular valve (Figure 3). This valve, unlike the arrangement in man, possesses only two leaflets. The septal and ventral of these two leaflets is thin and membranous, while the dorso-caudal, or mural, leaflet is muscular (Figure 4). The ventro-septal leaflet runs caudally from the central fibrous body, with attachments to both the muscular ventricular septum and the ventricular wall. The ends of the zone of apposition between the two leaflets are supported by two papillary muscles. The more dorsal papillary muscle is broad and flat, whereas the more ventral muscle is very short and indistinct. There are no intercordal spaces in the tissues supporting the leaflets, such that the valve largely has the appearances of a funnel.

The apical component of the right ventricle is much trabeculated. A large muscular band is found superficially on the right side of the ventricular septum. It resembles the septomarginal trabeculation as seen in the human heart (Figure 4). This was a solitary band in one heart, but in others it divided into three or more parallel strands. At the ventricular base, it gives rise to a dorsal limb, which runs inferiorly towards the central fibrous body, and a ventral limb which heads towards the pulmonary valve. Arising from the dorsal limb is either a cord, or a papillary muscle, which runs to the underside of the mid-point of the large antero-septal fibrous leaflet of the right

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atrioventricular valve, being reminiscent of the medial papillary muscle in the human heart.

The septal structures

The atrial septum has thick dorsal and thinner ventral components (Figure 3). The thicker dorsal end of the septum is in the same plane as the ventricular septum, whereas the thinner cranial margin is deviated leftward, overlying the orifices of the pulmonary veins (Figure 5). In the fetal hearts, there is no evidence of a well-formed opening within the ventral margin of the septum comparable to the oval foramen as seen in man. Instead, in some of the fetal specimens, there are multiple fenestrations present within the body of the septum (Figure 6). In all specimens, the margins of the septum, as seen from the right atrium, are solid and muscular (Figures 3, 6). Dorsally, a small fold of tissue is interposed between the pulmonary veins and the right atrium. The caudal rim of the septum is a broad solid muscular band, which on the right is continuous mainly with the atrioventricular component of the membranous septum. It is also continuous on the left side with the vestibule of the atrium (Figures 5-6). As we will show, the atrioventricular junction is common in the crocodile. The cavity of the right atrium, nonetheless, is separated from that of the left ventricle by an atrioventricular component of the membranous septum. The feature of the atrioventricular junction of the crocodile heart, therefore, is its commonality, albeit that the junction is divided by the septal structures into separate valvar orifices for the right and left ventricles (Figure 7). When seen in four chamber section, there is a degree of offsetting between the hinges of the right and left atrioventricular valves, as is seen in the human heart. The off-set leaflets in the human heart, however, are hinged from the atrial vestibules rather than the membranous septum (Figure 8). Thus, the septal leaflet of the left atrioventricular valve is hinged from the underside of the

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broad muscular band that anchors the atrial septum dorsally. The membranous leaflet of the right atrioventricular valve, in contrast, is hinged from the large membranous septum, which extends caudally from the central fibrous body (Figure 8). Trans-illumination of the septal surface of the right ventricle reveals the interventricular component of the large membranous septum, which is located on the ventricular aspect of the hinge of the ventro-septal leaflet of the right atrioventricular valve (Figure 9). As in the human heart, therefore, the crocodilian membranous septum possesses both atrioventricular and interventricular components (Figures 8-9). The interventricular component of the membranous septum extends from the caudal margin of the ventricles, at the central fibrous body, between the two ventricles, before curving ventrally to join the underside of the aortic valvar leaflets, where it forms the base of the foramen of Panizza (described further below). The membranous septum, therefore, is positioned so as to wall one of the crocodilian aortas into the left ventricle.

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Outflows from the right heart

The outflow tract of the right ventricle leads not only to the pulmonary trunk, but also to a second aorta. This right ventricular aorta is separated from the right atrioventricular valve by a fold of myocardium. Part of the inner heart curvature, it is known as the Bulboauricularsporn, and is comparable to the human ventriculo-infundibular fold. It contains within it a prominent cartilaginous bar (see below). Within the outflow tract itself, the pulmonary trunk and the right ventricular aorta are separated by a small muscular outlet septum, which has previously been described as the aorticopulmonary septum (Webb, 1979). It is, however, exclusively a right ventricular structure. Still within the right ventricle, the leaflets of the pulmonary valve are supported by a completely muscular infundibulum positioned cranially and ventrally relative to the muscular outlet septum. This infundibulum is only partially separated from the walls of the two aortas, and is not fully free-standing, as it is in the human heart (Figures 7,10).

The pulmonary valvar leaflets are attached within the distal end of the overall myocardial outlet component of the right ventricle. The leaflets guarding the orifice of the aorta arising from the right ventricle are hinged within its proximal end. As a result, there is an offset within the right ventricular outflow tract between the hinges of the valves guarding the orifices of the right ventricular aorta and the pulmonary trunk. The valve of the right ventricular aorta, however, is at the same level within the ventricular base as the valve guarding the origin of the aorta supported by the left ventricle, but is angled away from the septum between the two aortas. The walls of the two aortas are themselves in continuity with each other when traced distally from the level of their valves, as are the arterial walls of the pulmonary trunk and the two

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aortas when traced distally through the common intrapericardial outflow tract (Figures 11-13).

In the largest hearts, a ring of fibrous nodules was found within the right ventricle below the level of the pulmonary valve, which extended distally to the basal attachment of the leaflets (Figures 4, 10, 14). These nodules are not evident in the smallest hearts, including the heart from the fetal specimens, while they are small and indistinct in the hearts of the 2 year crocodiles (Figure 14). At the entrance of the sub-pulmonary infundibulum, a deep and distinct groove is found below the level of the nodules in the largest hearts. It carries the infundibular coronary artery in its epicardial surface. This is the narrowest region of the sub-pulmonary outflow tract. The pulmonary trunk itself divides intrapericardially into right and left pulmonary arteries. The extensive interventricular component of the membranous septum interposes between the origins of the two aortas. It inserts at the base of two of the four leaflets of the aortic valves, with these leaflets being hinged at the same level.

The foramen of Panizza & cartilaginous component of the heart

Immediately distal to the hingepoint of the aortic leaflets, and hence positioned between the two aortic roots, but within the ventricular mass, there is a communication known as the foramen of Panizza (Figures 10-12). This extends distally to the level of the sinutubular junctions. It is surrounded by a hook-shaped band of cartilage, which itself is lined by a band of smooth muscle. The cartilage itself is continuous with two more extensive cartilaginous components, which form the base of each of the sinuses of the two aortas (Figure 15). The first component is formed of cartilage located within the aortic sinuses facing the pulmonary trunk. This is then anchored to the crest of the muscular ventricular septum by a prong which extends caudally, turning to meet the cranial extent of the membranous septum. A crescent-

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shaped arm extends from this first component, running cranially relative to the foramen of Panizza (Figure 15). The second component is found within the two non-adjacent sinuses of the aortic roots. This component meets the first component within the cranial aspect of the foramen of Panizza. The cartilage within the non-adjacent sinus of the right-sided aorta, which arises from the left ventricle and crosses the right bronchus, also forms the outer margin of the Bulboauricularsporn, being located within the inner heart curvature between the aorta arising from the right ventricle and the vestibule of the right atrioventricular valve (Figure 15). Thus, the two components of the cartilage themselves are separated only by a small strip of the interventricular membranous septum, which lies immediately below the hingepoints of the aortic valvar leaflets.

Chambers of the Left Heart

The left atrium, which like the right atrium possesses an appendage and a vestibule, is separated from its partner by the intact atrial septum. It also possesses a large component, less smooth than the vestibule, which intervenes between the pulmonary veins and the appendage (Figure 5). This component is equivalent to the body of the left atrium found in the human heart. The left atrial appendage is rhomboid, with a slightly narrower junction between the atrium and appendage than is found on the right side. Internally, trabeculations are more extensive than in the human heart, where they are confined within the tubular appendage. The crocodilian left atrial body is also less smooth than the vestibule (Figures 5, 8). Two pulmonary veins enter the venous component of the atrium, one on each side. The left atrium then leads via its smooth-walled vestibule into the left atrioventricular valve, which has two fibrous leaflets (Figure 7). In the human heart, it is the mural leaflet of the left atrioventricular valve which is largest. In contrast, the more septally positioned of the crocodilian

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leaflets guards the larger part of the left atrioventricular junction. The leaflets themselves in the crocodile are supported within the left ventricle by two indistinct papillary muscle groups (Figure 5). As in the right atrioventricular valve, the tissues between the papillary muscles and the leaflets are devoid of intercordal spaces, so that the valve has a funnel-like configuration (Figure 5). The trabecular portion of the left ventricle is thick and sponge-like, extending almost half of the way from base to apex. A prominent muscle bar is consistently present, crossing the mid-portion of the left ventricular cavity (Figure 5).

Outflow from the left heart

The outlet from the left ventricle is the left ventricular aorta, with the ventriculo-arterial junction guarded by a bileaflet aortic valve. In contrast to the valve of the aorta arising from the right ventricle, one of the leaflets guarding the orifice of the left ventricular aorta is in close proximity to the foramen of Panizza. There is fibrous continuity between the septally positioned leaflet of the left atrioventricular valve and the leaflets of the left ventricular aorta. As already emphasised, the large interventricular component of the membranous septum forms the base of the fibrous triangle that separates the two leaflets of the aorta arising from the left ventricle.

The Coronary Arteries

Only a solitary coronary artery could be found in all crocodilian hearts examined (Figure 16). It arises within the sinus of the left ventricular aorta that faces the aorta arising from the right ventricle. It divides into two branches. The first, the right coronary artery, runs within the right atrioventricular groove, and then extends caudally to supply the caudal surface of the right and left ventricles. It is this branch that gives rise to the large infundibular vessel, which runs within the deep groove at the base of the sub-pulmonary infundibulum (Figure 1). The second major branch is

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the left coronary artery, which runs behind the aorta arising from the left ventricle and the pulmonary trunk to supply the ventral surface of the heart. This second branch then continues as a circumflex coronary artery, which supplies part of the caudal surface of the left ventricle. Thus, the branches of the solitary coronary artery encircle the pedicle of the three arterial trunks (Figure 16).

DISCUSSION

Building unto the substantial existing descriptions of the crocodilian heart (Cuvier, 1836; Brücke, 1852; Greil, 1903; Davies et al, 1952; White, 1956; White, 1976; Webb, 1979; Axelsson and Franklin, 1997; Farrell et al., 1998; Axelsson, 2001), we have identified several fundamental differences between the hearts of crocodilians and humans. In many instances, these differences can readily be viewed as discrepancies between reptiles and mammals (Table 1). Crocodiles and reptiles, for example, generally have extensive atrial appendages and no oval foramen. Other detailed features, nonetheless, are not found in reptiles generally, but are shared between the hearts of crocodilians and humans, such as the cranial position of the left compared to the right atrioventricular orifices (Figure 8). This suggests, at least in this regard, that the crocodilian heart and hearts of humans, and probably mammals in general, are governed by similar developmental programmes. In our study, we have sought to examine the detailed anatomy of the crocodilian heart using the same logical principles that are now used worldwide for the analysis of human hearts (Van Praagh 1972; Shinebourne et al 1976, Anderson et al 1984, Anderson et al 2004). This permits us to discuss differences and commonalities between the hearts of crocodilians and humans, and to draw in particular on developmental differences so as to explain them.

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The systemic veno-atrial connections

In the developing human heart, it is possible to identify valves at the border of the systemic venous sinus and the right atrium (Steding et al., 1990; Cook et al 2004; Sizarov et al., 2011). These valves come together in the spurious septum of the right atrial roof. We find a very similar configuration in the adult crocodilian heart. This, in turn, is very similar to the configuration found in other ectothermic vertebrates (Jensen et al 2014a; de Bakker at al., 2015). This configuration in the human heart, however, is only temporal, as the sinuatrial junction remodels with ongoing gestation (Steding et al., 1990). The remodelling is commonly referred to as atrialization of the systemic venous sinus, and is absent in reptiles (Jensen et al 2014a - Table 1). During atrialization, the sinuatrial junction widens such that the right and left venous valves can no longer maintain competence. In the postnatal human heart, remnants of the left venous valve are not easily recognized, but may occasionally persist inferiorly adjacent to the atrial septum. In contrast, the right leaflet typically persists to a greater extent, remaining as the Eustachian and Thebesian valves which guard the orifices of the inferior caval vein and the coronary sinus in the normal heart and may persist abnormally as a 'Chiari network' or as membrane dividing within the right atrium (Steding et al., 1990). A further effect of the remodelling is the formation of the terminal crest, at the end of which, and commonly at right angles to it, remains the spurious septum, or sagittal bundle (de Bakker et al., 2015). In the absence of a terminal crest, it is hardly justifiable to equate the right atrial appendage of the crocodilian heart to that of the human heart. The trabeculations of the crocodilian atriums, furthermore, have the appearance of a thick network, in contrast to the

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parallel and large columns of pectinate muscles which generally characterize the human and mammalian hearts. The basic building blocks of the crocodilian atriums, in terms of atrial appendage, vestibule, septum and body, nonetheless, are as found in humans, and indeed in other mammals.

The crocodilian configuration of the systemic veins, with presence of left and right anterior caval veins, together with a posterior caval vein, is much like most other terrestrial vertebrates (Jensen et al 2014a - Table 1). This configuration differs from the human condition, where the left superior, or anterior, caval vein regresses in fetal development, with its proximal component persisting as the coronary sinus. The coronary sinus in the human heart is then nestled in the left atrioventricular groove, rather than being a free-standing vessel as seen in the crocodile. It also receives multiple coronary venous tributaries (Anderson et al 2003a). Although there is but a single coronary vein, coronary venous return in crocodilians is not that different from the mammalian condition, in that the coronary vein drains into the left anterior caval vein near the sinuatrial junction. In mammals, however, multiple coronary veins may drain into the left anterior caval vein, as for instance in humans.

The pulmonary veno-atrial connections

Reptiles and birds do not form any substantial amount of pulmonary venous myocardium and have a very trabeculated left atrium (Jensen et al 2014a). In contrast, during human development the incorporation of substantial amounts of pulmonary venous myocardium produces a large smooth-walled atrial component, which along with retention of the atrial body renders the trabeculated left atrial wall as a small tubular appendage. Mice, like humans, develop extensive amounts of myocardium around the pulmonary veins, but this myocardium is not incorporated to

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the left atrium. There is but a single pulmonary vein connecting to the murine left atrium, and the trabeculated component of the atrium is extensive (Mommersteeg et al, 2007). Across mammals there is much variation in the number of pulmonary veins (Rowlatt, 1990) and, presumably, the extent to which pulmonary venous myocardium is incorporated to the left atrium. As in the mammalian heart, nonetheless, there is a smooth-walled vestibule supporting the leaflets of the left atrioventricular valve.

There are several anecdotal observations, but no firm data, suggesting that the reptile heart may possess a valve-like structure at the entry of the pulmonary veins to the left atrium (Jensen et al., 2014b). In this respect, the bird heart often has a well-developed flap of myocardium in the left atrial roof which partly separates the pulmonary venous orifices from the body of the left atrium (Benninghoff, 1933). In our interpretation, Webb (1979) described the same structure in the crocodilian heart as a "solid rod", and we have illustrated it in Figure 6. Mammalian hearts lack any equivalent structure (Rowlatt, 1990).

The atrial septum

The structure of the intact atrial septum in the crocodile is different from that found in humans, but typical of reptiles (Table 1). Even in the youngest fetal specimens examined, there was no evidence of an oval foramen. Indeed, Rowlatt (1990), extending the work of Röse (1890), observed that the oval fossa, the right atrial feature of the oval foramen, is only found in placental mammals. Formation of the oval foramen, and its closure by an infolding of the atrial roof, commonly referred to by the misnamed "second atrial septum", is therefore unique to placental mammals (Jensen et al, 2017 - Table 1).

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Atrioventricular septation

The atrioventricular septal structures are markedly different in the crocodilian heart from those found in humans, and from mammals in general. Because of the commonality of the atrioventricular junction in the crocodile, there is no postero-inferior atrioventricular muscular 'sandwich', as is seen in fully formed mammalian hearts (Rowlatt, 1990). In mammalian development, the atrioventricular junction is initially a common structure, configured like the Greek letter theta, Θ. Crocodilians, and reptiles generally, have the same configuration. Later mammalian development sees an inferior, or posterior, expansion of the atrioventricular junctions, which renders the atrioventricular configuration as a B (Jensen and Moorman, 2016). As the aorta then wedges itself between the left and right atrioventricular junctions from the anterior side, the atrioventricular junction achieves the configuration of a figure 8. Subsequent to this development, the left and right atrioventricular junctions are separated such that the atrioventricular conduction axis, on its posterior or inferior aspect, is effectively 'sandwiched' between the right and left atrioventricular junctions. In crocodiles, the atrioventricular junction is relatively wide as compared to other reptiles (Jensen et al., 2013) but the Θ-configuration is maintained. It is the "sandwich", in humans, which makes up the larger part of the structures that separate the right and left atrioventricular junctions, and also produces the off-setting of the hinges of the leaflets of the atrioventricular valves. The only true atrioventricular septum found in man is the atrioventricular component of the membranous septum (Anderson et al, 2003a). This component is also to be found in the crocodile,

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producing a degree of off-setting of the leaflets of the two atrioventricular valves (Table 1).

Atrioventricular junctions

Both the atrioventricular valves in the crocodile possess only two leaflets, and hence close along a solitary zone of apposition. They are funnel-shaped, with the leaflets attaching directly to the papillary muscles, or ventricular myocardium, such that the crocodilian atrioventricular valves are without tendinous cords (Table 1). The mural leaflet of the right atrioventricular valve is muscular, as is also seen in the avian heart (Van Mierop & Kutsche, 1985; Jensen et al, 2013; Jensen et al., 2014b). The septal leaflet, in contrast, is a single fibrous sheet, supported at its midpoint by a papillary muscle group reminiscent of a medial papillary muscle in the human heart. The muscularity of the mural leaflet of the right atrioventricular valve appears related to the developmental rightward expansion of the atrioventricular canal that occurs in animals with full ventricular septation (Jensen et al, 2013; Jensen & Moorman, 2016). We found, as has previously been noted (Webb, 1979; Van Mierop & Kutsche, 1985), that the septal leaflet of the left atrioventricular valve occupies the greater proportion of the circumference of the left atrioventricular junction. This is in contrast to the arrangement in the human heart, where it is the mural leaflet of the mitral valve which occupies two-thirds of the valvar circumference. In the crocodile heart, despite the integrity of the atrial and ventricular septums, the two atrioventricular valvar orifices are contained within a common atrioventricular junction. This probably reflects the limited extent of wedging of the left ventricular aorta within the left ventricle.

Ventricular architecture

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The structure of the right ventricle is very similar to that seen in humans, with presence of both an extensive apical trabecular component, and a septomarginal trabeculation with dorsal and ventral limbs (Table 1). Already Gasch (1888) noted the shared feature of the septomarginal trabeculation. Developmental studies have shown it develops in association with the incorporation of the myocardial outflow tract to the right ventricle (Greil 1903; Jensen et al 2013; Poelmann et al 2014). In all examples that we examined, however, the trabecular portion of the left ventricular myocardium was thick in comparison to the compact myocardium, as is the case for reptiles and ectothermic vertebrates generally (Jensen et al., 2014b, Jensen et al 2016a - Table 1). The ventricular septum in both crocodiles and mammals contains both muscular and membranous components, although the proportion occupied by the membranous interventricular septum is far greater in the crocodile than in the normal mammalian heart (Table 1) (Rowlatt, 1990).

In the crocodile heart, the aortic pathway is divided, from valvar level to the level of the arches, into right and left cylinders. In the normal mammalian heart, the aorta is walled from the right ventricle by the interventricular component of the membranous septum. This septal component is in the same plane as the remainder of the extensive muscular ventricular septum. In the normal mammalian heart, therefore, it is not then possible to distinguish a muscular outlet septal component from the remainder of the inner heart curvature. This is why the structures separating the tricuspid from the pulmonary valves in the human heart are grouped together under the generic term ‘supraventricular crest’ (Vricella et al 2004).

In the crocodile heart, the relationship between the muscular outlet septum and the membranous and muscular components of the ventricular septum differs markedly. The muscular outlet septum is a small but discrete structure, and is not in the same

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plane as the muscular ventricular septum, nor the membranous septum. In the normal crocodile, it is an exclusively right ventricular structure, allowing for the unique connection of the right ventricle with an aorta. This situation is seen in human malformations such as double outlet right ventricle or tetralogy of Fallot (Anderson et al 2001), but then with no left ventricular aorta, and with deficient ventricular septation. The membranous septum in the crocodile joins not the base of the muscular outlet septum, but rather the base of the septum which divides the two aortic trunks. In Shaner's analysis of developmental septation in alligator, chicken, and pig, this is one of the cardiac features that set the archosaurs apart from mammals (Shaner 1962). The pulmonary valvar leaflets, of which there are only two, are supported by a short infundibulum (Table 1). This structure is not free-standing, as in the human heart, but is in direct continuity with the arterial walls of the two aortas. In all our specimens, the wall between the two aortas was a true septal structure. The foramen of Panizza is then a window within this wall, situated at sinusal level and surrounded by a cartilaginous skeleton. Our reconstruction of the cartilaginous components is similar to that previously described by White (White 1956). Although there may be species, allometric, and age differences, the cartilage we found was associated within the basal portion of each aortic sinus, with prongs leading into the ventricular septum and over the roof of the foramen of Panizza. Thus, our interpretation of the shape, and the relationship of the two cartilaginous components to the cardiac valves, differs compared to those of White (White 1956). We do not know to what extent the formation of cartilage is an outcome of a developmental program, or is an acquired feature due to, for instance, high shear stress. In the developing Spanish terrapin, a turtle, it has been shown that chondrogenic mesenchyme can be found in the aortic

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base. This region exhibits substantial variation between post-hatched individuals (López et al 2003)

The structure of the outflow tract in the crocodile is that of a common tube divided by discrete septal partitions into two aortic pathways, and a pulmonary pathway (Table 1). This is of potential developmental significance, since even in mammals and birds, which have only one aortic and one pulmonary pathway within the pericardial cavity, these tubes are initially developed from a common muscular tube, albeit that each tube eventually acquires its own arterial wall. In mammals, the division of the initially common tube depends on the fusions of facing and paired endocardial cushions, which extend throughout the outflow tract. In chicken, in contrast, although only two cushions are found proximally, there are three cushions formed distally (Qayyum et al, 2001). In Shaner's developmental analysis, the division of the crocodilian outflow tract resembles that of other reptiles and birds, whereas in the mammalian heart an amphibian-like set of cushions divide the outflow tract (Shaner, 1962).

The coronary arterial supply in all hearts that we examined was through a solitary coronary artery that divides into three branches, which encircle the three arterial trunks. This configuration of the coronary arterial tree on the ventricular surface then resembles that of the American alligator, although in the alligator more than one artery may take origin from the left ventricular aorta (Jensen et al., 2016a). The final feature which is of note in the crocodile heart is the ring of fibrous nodules between the pulmonary valve and the entrance of the sub-pulmonary infundibulum. Previous authors have shown that these structures undergo an ontogenetic change, from

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cushions in young animals, approximately one year, to prominent nodules in large animals (Webb, 1979; Seymour et al., 2004).

Conclusions

We have described the structure of the crocodile heart by the process of sequential segmental analysis as used for humans, and compared the anatomy to the human heart. Although there are similarities in the basic four chamber plan of the heart, and in some components of the atriums and ventricles, our studies further endorse the unique nature of the crocodilian heart in terms of the atrioventricular and ventriculo-arterial junctions and the structure of the arterial trunks. In particular, we have shown that there is a common atrioventricular junction despite the presence of intact atrial and ventricular septal structures, and that the intrapericardial aortic and pulmonary trunks are part of a common arterial structure, separated by true septums. While these differences are not surprising, given the divergence in lineages leading to mammals and crocodiles, this anatomic knowledge may be useful when starting to compare the developmental mechanisms involved in producing these components of the heart.

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FIGURE LEGENDS

Figure 1. External view of a 2 year old crocodile heart showing the relative position of the chambers as they would be seen during life. There is a common arterial trunk arising from the ventricular mass, which is divided internally into two aortic and one pulmonary channel. The proximal extent of the muscular sub-pulmonary infundibulum, which forms part of the pulmonary outflow tract, is marked by a deep recess carrying within it coronary arteries, (blue arrow). RA= Right Atrium, LA= Left Atrium, RV = Right Ventricle; LV = Left Ventricle.

Figure 2. View of the dorso-caudal aspect of the atriums, showing the relationship between the venous channels and the pulmonary veins. The left superior caval vein runs along the left atrial wall between the left atrial appendage and the two pulmonary veins. It is some distance from the left atrioventricular groove, and the coronary veins within it. The three caval veins join together into the venous sinus and enter the right atrium via the venous valves. SCV = Superior Caval Vein; ICV = Inferior Caval Vein.

Figure 3. The opened morphologically right atrium and right atrioventricular valve of the crocodile (A) and human (B) heart. A. The right atrium of the adult crocodile contains similar components as the human heart, specifically an extensive, trabeculated appendage, an atrial septum (AS), a smooth-walled vestibule (blue asterisks), and a venous sinus, the entrance to which is marked by two prominent venous valves. The right atrioventricular valve consists of a large fibrous septal (S) and smaller muscular mural leaflet (M) and is supported by two papillary muscle groups (red triangles). In the crocodilian heart, coronary veins become confluent with the caval veins in the venous sinus dorsal to the venous valves, whereas in the human

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heart the coronary sinus opens into the venous component of the right atrium. The crocodilian heart, furthermore, is without an oval fossa.

Figure 4. The opened morphologically right ventricle and its outflow of the crocodile (A) and human (B) heart. A. The outflow from the morphologically right ventricle of crocodiles leads both to the right ventricular aorta and to the pulmonary trunk. The right ventricular aorta is walled from the morphologically left ventricle by the interventricular component of the membranous septum. The two right ventricular outflows are separated by a small muscular outlet septum (blue asterisk). Below the base of the pulmonary valve is the 'cog-tooth' valve. Also shown are the coarse apical trabeculations, the septomarginal trabeculation (SMT), the septal (S) and mural (M) leaflets of the right atrioventricular valve, and the supporting papillary muscles (red triangles). The human right ventricle has more defined papillary muscles, with the margins of the leaflets being anchored to these by more numerous tendinous cords than in the crocodilian heart. The valve in the human heart also displays three leaflets, all of which are fibrous. AS = anterior-superior leaflet of the tricuspid valve; RV= Right Ventricular.

Figure 5. Corresponding view of the opened morphologically left atrium and left atrioventricular valve of the crocodile (A) and human (B) heart. Again, the crocodilian left atrium can be divided into components that are similar to the human heart. There is a septal component, a pulmonary venous component, a smooth-walled vestibule (blue asterisks), and an appendage, which is much more extensive in the crocodile than in human. In the left atrium of both species, there is also an extensive smooth-walled body. The crocodilian atrial septum is without a secondary foramen.

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The left atrioventricular valve in both species again possesses two leaflets, with both being fibrous. The aortic leaflet (A) in the crocodilian heart occupies a greater proportion of the left atrioventricular junction than does the mural leaflet (M), but these proportions are reversed in the human heart. The extensively trabeculated morphologically left ventricle of the crocodilian heart can also be seen in the image, together with a prominent muscle which extends across the mid part of the cavity (yellow arrow).

Figure 6. Views from the left atrium in a fetal crocodile specimen showing that the structure of the flap valve is similar to that reported in the chick. In A the flap valve is distended to show its pocket-like shape. The cranial margin (green arrow) is intact and is attached towards the left atrial appendage (deviated leftwards). The asterisk marks the site of a prominent muscular strand for orientation purposes. In B, the caudal margin of the flap valve has been distended to show multiple small fenestrations present within the body of the flap valve below the level of the muscular strand (*). LAA= left atrial appendage

Figure 7. Short axis section across the ventricular base of the crocodile (A) and human (B) heart. A. Arterial roots at the level of the sub-pulmonary infundibulum and two aortic valves, and below the level of the pulmonary valve. The cog-teeth can be seen partially closing the sub-pulmonary outflow tract. They are supported by a myocardial sleeve, which is in continuity with the walls of the two aortas. The relationship between the 5 cardiac valves can also be seen, with the left ventricular aortic valve sitting on top of the two atrioventricular valves (LAVV & RAVV) and below the two arterial valves leaving the morphologically right ventricle. The green

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asterisk marks the site of the foramen of Panizza. The aortic roots of the crocodilian heart are positioned less leftwards than in the human heart, and there is no wedging of the left ventricular aorta between the atrioventricular junctions. In consequence, the atrioventricular valvar orifices are less separated in the crocodilian heart, being supported by a common atrioventricular junction. RV= Right Ventricular, LV=Left Ventricular, LAVV = Left Atrioventricular Valve; MV = mitral valve; RAVV = Right Atrioventricular Valve; TV, tricuspid valve.

Figure 8. Four chamber sections of crocodile (A-B) and human heart (C-D) in attitudinal correct positions. The heart of a 2 year old crocodile showing the inferior aspect in A and the superior half in B. The section cuts across the inferior aspect of the fibrous leaflet of the right atrioventricular valve. In the inferior part of the heart (A), the two septally positioned leaflets of the atrioventricular valves are seen to insert at similar levels (circled). There is no evidence of an atrioventricular septal 'sandwich' as seen in the human heart. The fold of tissue between the right pulmonary vein and the right atrium is also evident (red arrow), as are the right (R) and left (L) venous valves. In the superior half of the heart (B), there is a greater degree of offsetting of the atrioventricular valves. This is due to the right atrioventricular valve hinging from the mid-point of the extensive membranous septum (green arrow). LA = Left Atrium; LV = Left Ventricle; PV, pulmonary veins; RA= Right Atrium; RV = Right Ventricle; SS = spurious septum; TC = terminal crest.

Figure 9. Trans-illumination of the membranous septum of the crocodile (A-B) and human heart (C-D). A-B. In this heart from an adult crocodile, the septum has been trans-illuminated to the show the extensive nature of the membranous septum as seen

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from the morphologically right (A) and left ventricle (B). The atrioventricular component, above the hinge line of the septal leaflet of the right atrioventricular valve is outlined in red. On the morphologically right side of the human heart (C), the tendon of Todaro (T) and hinge point (h) of the tricuspid valve converge on the atrioventricular node (the position is approximated by the black circle). AV = Atrioventricular; h = hinge point of the tricuspid valve; LA = Left Atrium; LV = Left Ventricle/Left Ventricular; R = right venous valve (crocodile) or Eustachian valve (human); RA= Right Atrium; RV = Right Ventricle; SMT = septomarginal trabeculation; T = tendon of Todaro.

Figure 10. The section reveals the detailed anatomy of the right ventricular outflows. A common party wall separates the right ventricular aorta from the pulmonary trunk. Within the heart, a small myocardial outlet septum (red asterisk) creates a degree of offsetting between the leaflets of the pulmonary and right ventricular aortic valves. The foramen of Panizza (yellow asterisk) can be seen just behind one of the leaflets of the right ventricular aorta. The foramen itself is surrounded by an arc of cartilage (C) which carries on its leading edge a crescent of smooth muscle. Also shown is the septal leaflet of the right atrioventricular valve (S). RV = Right Ventricular; Pulm. = Pulmonary.

Figure 11. Computerised tomographic images of the great vessels viewed from the left: A cross-sectional slice, no contrast, B thin maximum intensity projection, post-contrast, and c) anatomic correlate. 1) = right ventricle, 2) = left ventricle, 3) = LV aorta, 4) = pulmonary trunk, * = RV aorta, white arrowhead = position of cog-wheel apparatus, and red arrow = foramen of Panizza.

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Figure 12. Volume-rendered, post-contrast computerised tomographical images of the great vessels and ventricles viewed from: A left, B ventrally, and C right. 1) = right ventricle, 2) = left ventricle, 3) = LV aorta, 4) = pulmonary trunk, * = RV aorta, white arrowhead = position of cog-wheel apparatus, and yellow arrow = foramen of Panizza.

Figure 13. Anatomic cross-section A and comparable cross-sectional image obtained with computerised tomography B through the ascending arterial trunks, showing that the three arterial pathways are encased in a common arterial wall. RV = Right Ventricular; LV = Left Ventricular; Pulm. = Pulmonary.

Figure 14. The pulmonary outflow tract has been opened in this specimen from a young crocodile to show the rudimentary nature of the cog-teeth compared to the adult (see figure 10). The location of the myocardial outlet septum is marked by the red asterisk and the septal leaflet of the right atrioventricular valve is seen in the background (S). RV = Right Ventricular; Pulm.= Pulmonary.

Figure 15. These schematic diagrams show the location of the cartilaginous component of the crocodile heart as seen in our examinations, and compared to our interpretation of the initial description by White in 1956 (inset). Our studies show that the cartilage consists of two components, one lateral and one medial. The medial component has prongs running in the base of the aortic sinuses facing the pulmonary trunk and is anchored to the crest of the ventricular septum via a caudal prong. It also encircles the foramen of Panizza cranially (yellow asterisk). This cranial crescent is

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lined by an arc of smooth muscle (blue arc). The lateral component occupies the base of the non-facing sinuses, and runs into the fold of tissue between the right atrioventricular valve and the left ventricular aorta. The extensive membranous septum is shown in green. LAVV = Left Atrioventricular Valve; RAVV = Right Atrioventricular Valve.

Figure 16. These images show the left ventricular aorta as viewed from the right A and left B sides in an adult specimen. There is a single coronary arising from this aorta which gives rise to a right coronary artery (RCA), an infundibular branch (Inf CA), and also passes behind the arterial trunks as a Left Coronary Artery branch (LCA) which then divides into circumflex (Cx) and ventral branches. The coronary arteries thus encircle the arterial pedicle, as can also be seen in figure 1. RA = Right Atrium; RV = Right Ventricle; LV = Left Ventricular.

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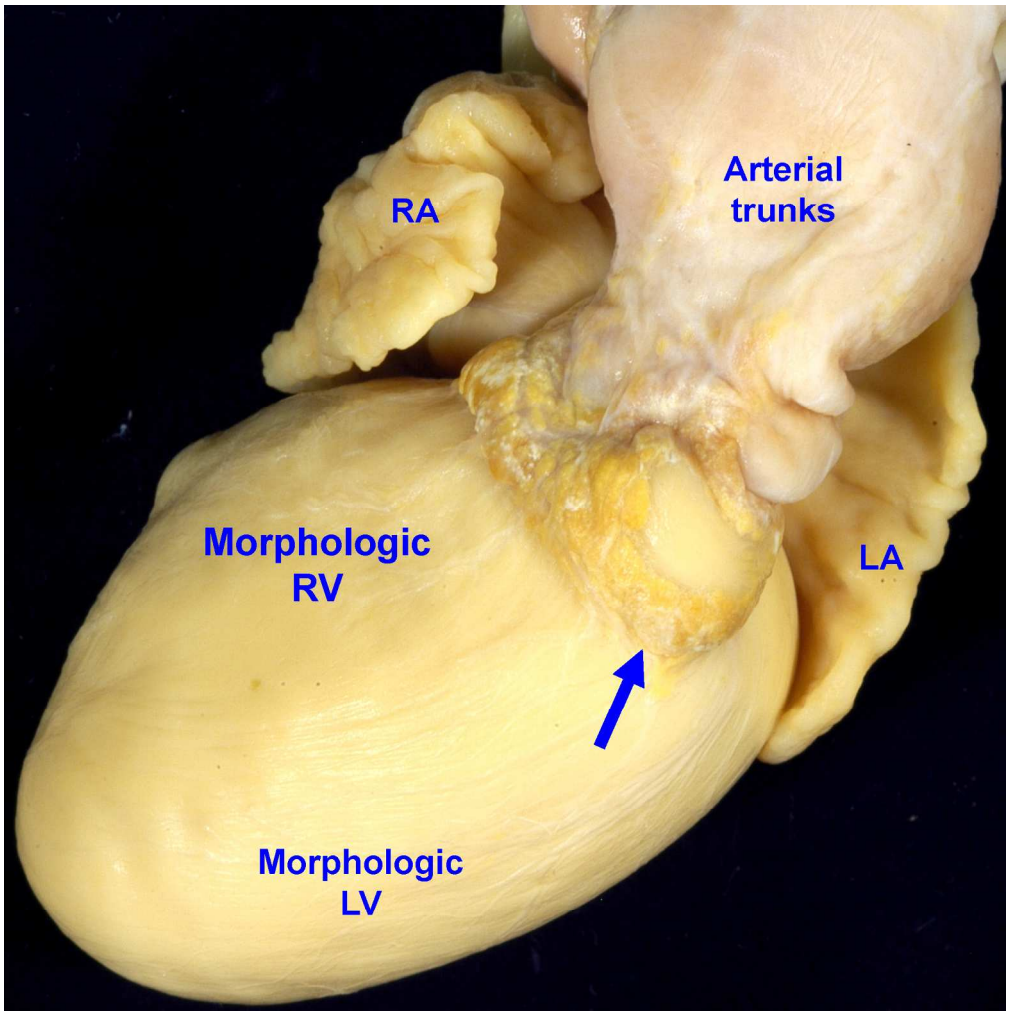

Figure 1. External view of a 2 year old crocodile heart showing the relative position of the chambers as they would be seen during life. There is a common arterial trunk arising from the ventricular mass, which is divided internally into two aortic and one pulmonary channel. The proximal extent of the muscular sub-pulmonary infundibulum, which forms part of the pulmonary outflow tract, is marked by a deep recess carrying within it coronary arteries, (blue arrow). RA= Right Atrium, LA= Left Atrium, RV = Right Ventricle; LV = Left Ventricle.

184x185mm (300 x 300 DPI)

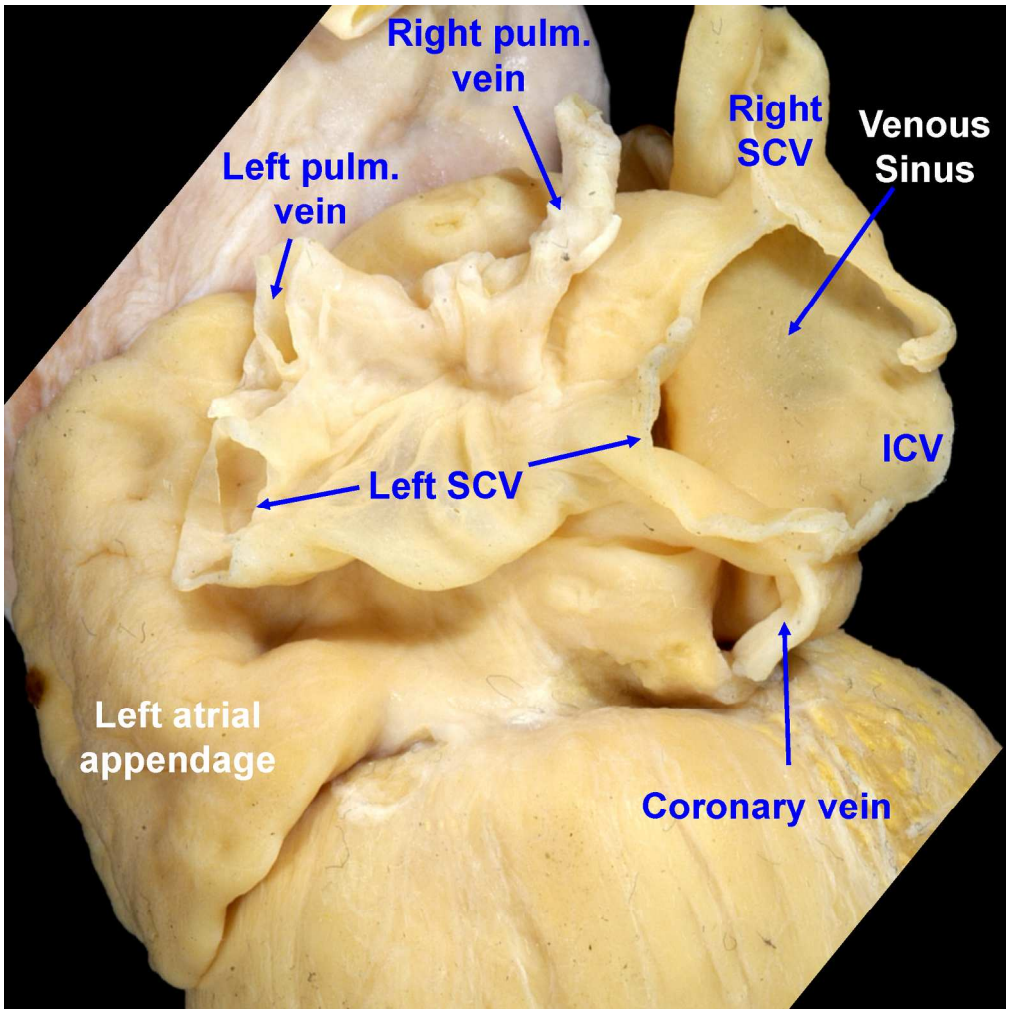

Figure 2. View of the dorso-caudal aspect of the atriums, showing the relationship between the venous channels and the pulmonary veins. The left superior caval vein runs along the left atrial wall between the left atrial appendage and the two pulmonary veins. It is some distance from the left atrioventricular groove, and the coronary veins within it. The three caval veins join together into the venous sinus and enter the right atrium via the venous valves. SCV = Superior Caval Vein; ICV = Inferior Caval Vein.

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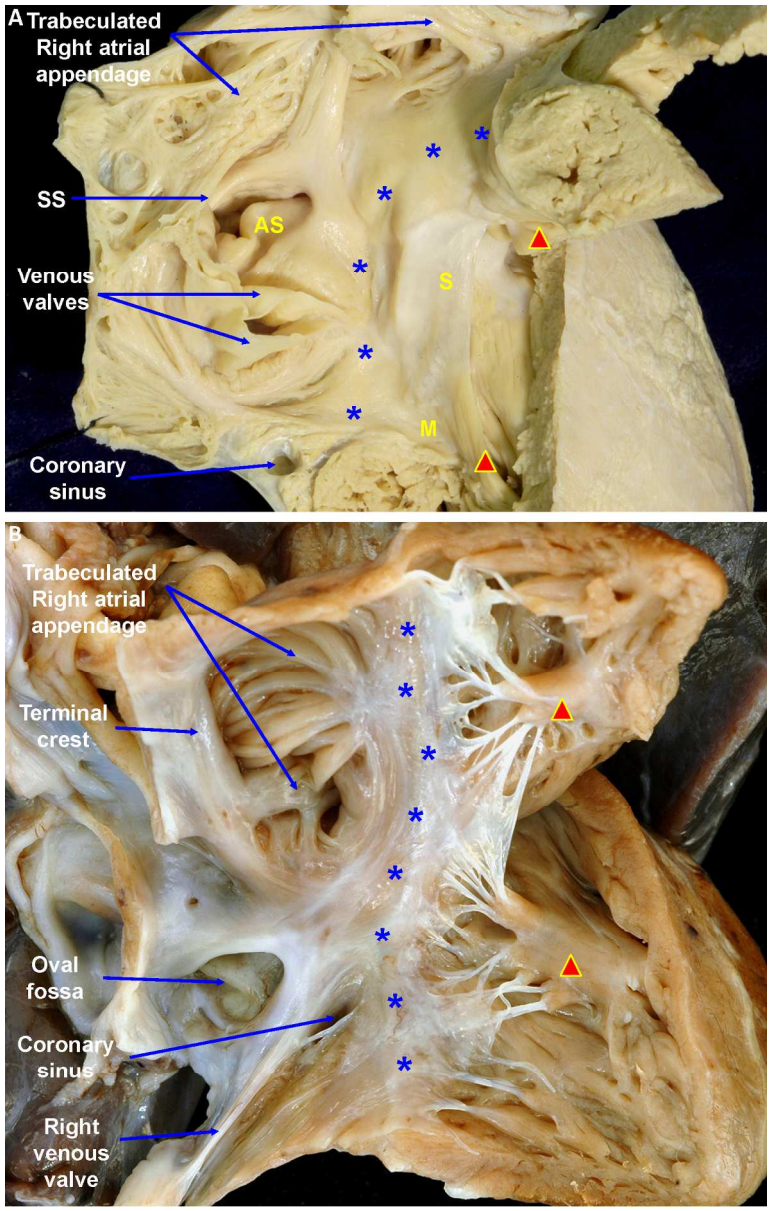

Figure 3. The opened morphologically right atrium and right atrioventricular valve of the crocodile (A) and human (B) heart. A. The right atrium of the adult crocodile contains similar components as the human heart, specifically an extensive, trabeculated appendage, an atrial septum (AS), a smooth-walled vestibule (blue asterisks), and a venous sinus, the entrance to which is marked by two prominent venous valves. The right atrioventricular valve consists of a large fibrous septal (S) and smaller muscular mural leaflet (M) and is supported by two papillary muscle groups (red triangles). In the crocodilian heart, coronary veins become confluent with the caval veins in the venous sinus dorsal to the venous valves, whereas in the human heart the coronary sinus opens into the venous component of the right atrium. The crocodilian heart, furthermore, is without an oval fossa.

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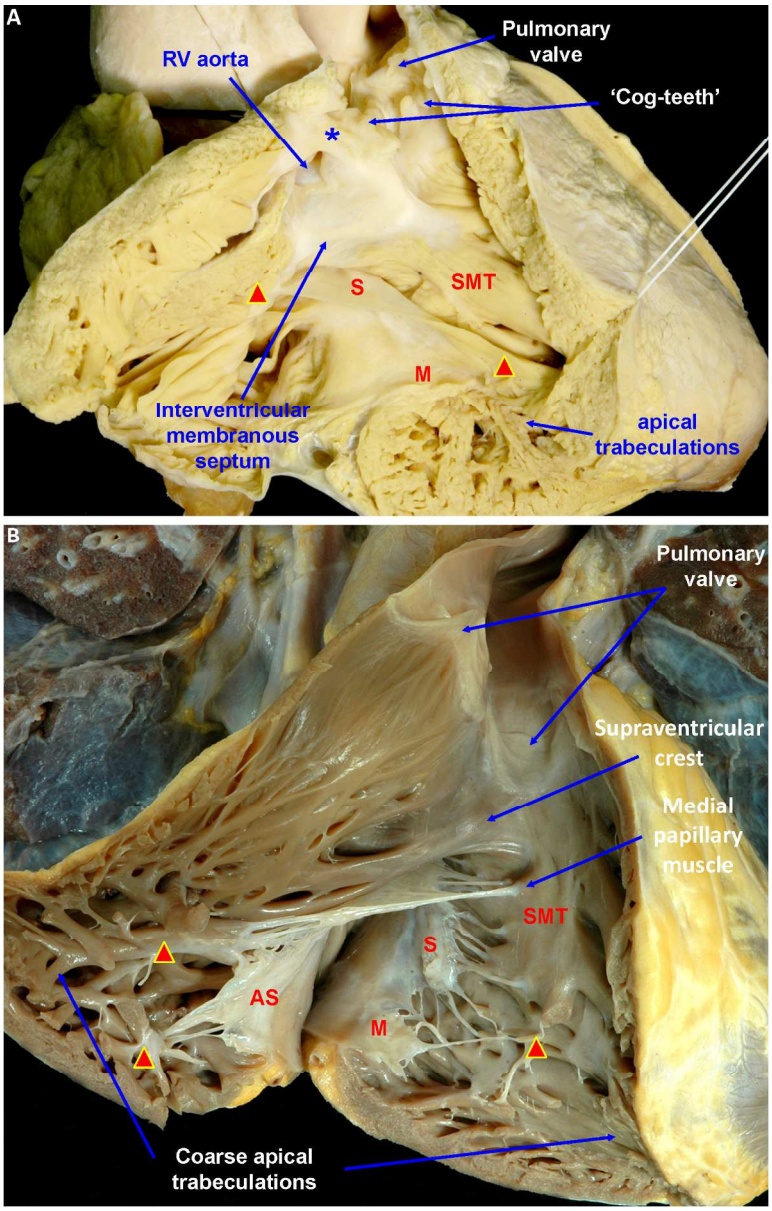

Figure 4. The opened morphologically right ventricle and its outflow of the crocodile (A) and human (B) heart. A. The outflow from the morphologically right ventricle of crocodiles leads both to the right ventricular aorta and to the pulmonary trunk. The right ventricular aorta is walled from the morphologically left ventricle by the interventricular component of the membranous septum. The two right ventricular outflows are separated by a small muscular outlet septum (blue asterisk). Below the base of the pulmonary valve is the 'cog-tooth' valve. Also shown are the coarse apical trabeculations, the septomarginal trabeculation (SMT), the septal (S) and mural (M) leaflets of the right atrioventricular valve, and the supporting papillary muscles (red triangles). The human right ventricle has more defined papillary muscles, with the margins of the leaflets being anchored to these by more numerous tendinous cords than in the crocodilian heart. The valve in the human heart also displays three leaflets, all of which are fibrous. AS = anterior-superior leaflet of the tricuspid valve; RV= Right Ventricular.

152x239mm (300 x 300 DPI)

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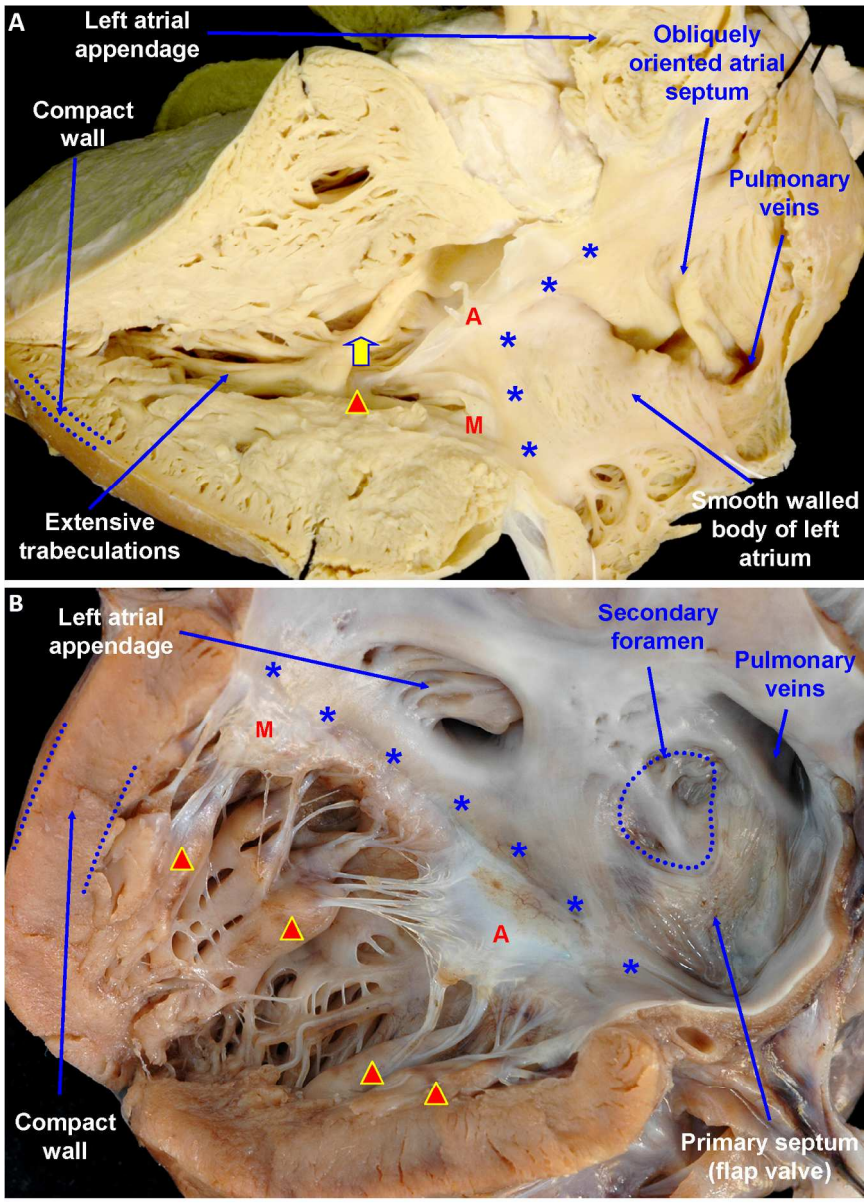

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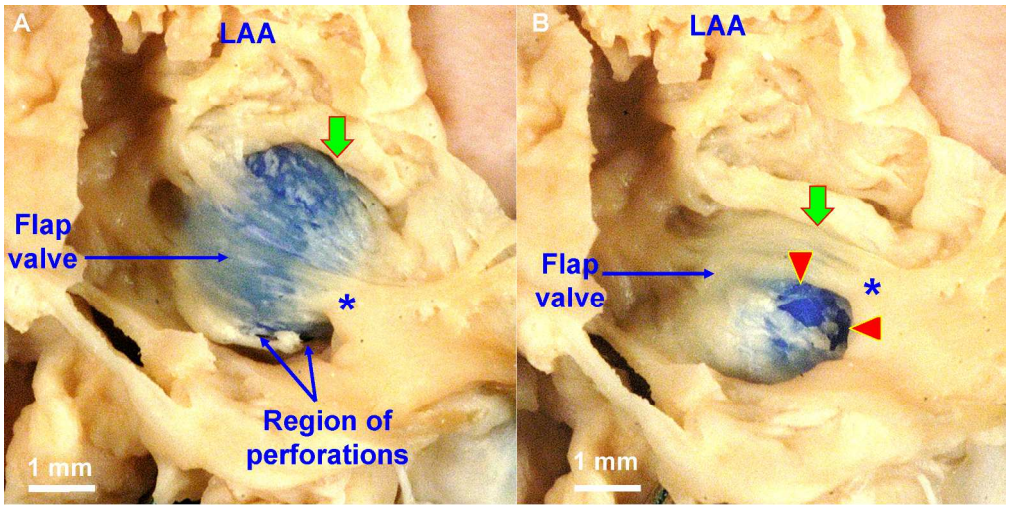

Figure 6. Views from the left atrium in a fetal crocodile specimen showing that the structure of the flap valve is similar to that reported in the chick. In A, the flap valve is distended to show its pocket-like shape. The cranial margin (green arrow) is intact and is attached towards the left atrial appendage (deviated leftwards). The asterisk marks the site of a prominent muscular strand for orientation purposes. In B, the caudal margin of the flap valve has been distended to show multiple small fenestrations present within the body of the flap valve below the level of the muscular strand (*). LAA= left atrial appendage

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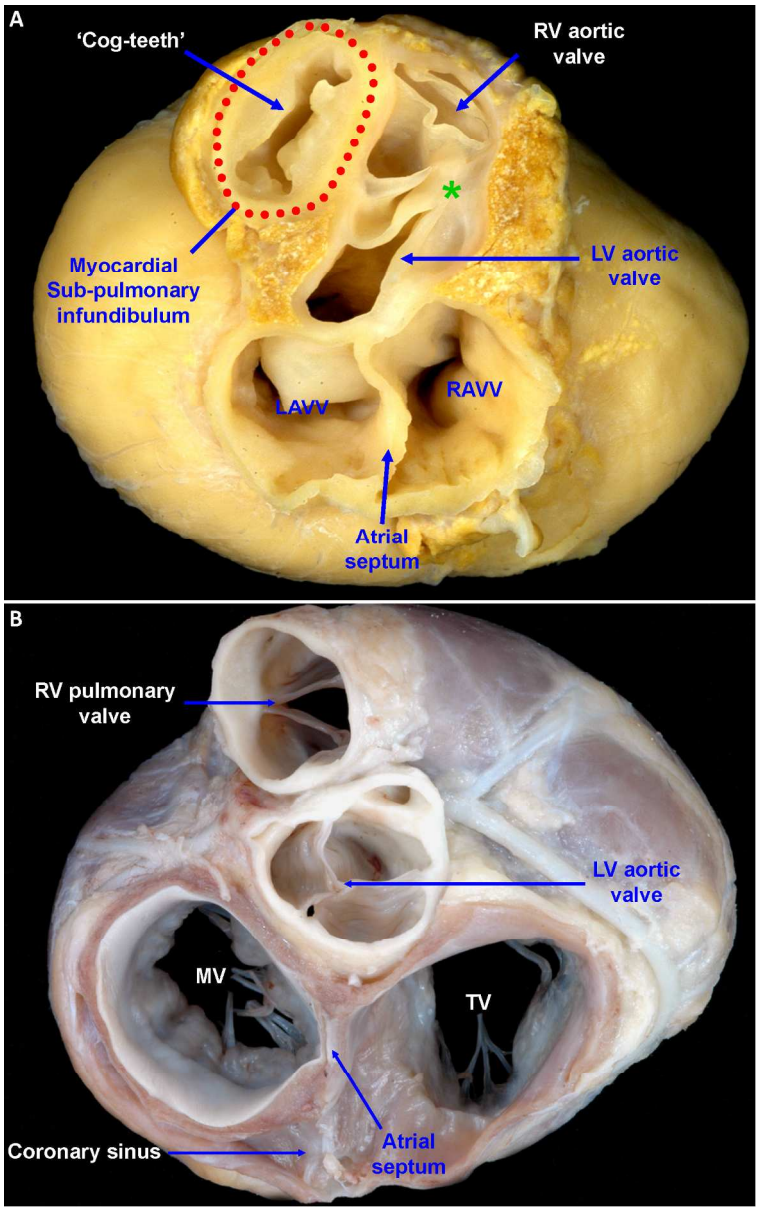

Figure 7. Short axis section across the ventricular base of the crocodile (A) and human (B) heart. A. Arterial roots at the level of the sub-pulmonary infundibulum and two aortic valves, and below the level of the pulmonary valve. The cog-teeth can be seen partially closing the sub-pulmonary outflow tract. They are supported by a myocardial sleeve, which is in continuity with the walls of the two aortas. The relationship between the 5 cardiac valves can also be seen, with the left ventricular aortic valve sitting on top of the two atrioventricular valves (LAVV & RAVV) and below the two arterial valves leaving the morphologically right ventricle. The green asterisk marks the site of the foramen of Panizza. The aortic roots of the crocodilian heart are positioned less leftwards than in the human heart, and there is no wedging of the left ventricular aorta between the atrioventricular junctions. In consequence, the atrioventricular valvar orifices are less separated in the crocodilian heart, being supported by a common atrioventricular junction. RV= Right Ventricular, LV=Left Ventricular, LAVV = Left Atrioventricular Valve; MV = mitral valve; RAVV = Right Atrioventricular Valve; TV, tricuspid valve.

151x242mm (300 x 300 DPI)

For Peer Review Only

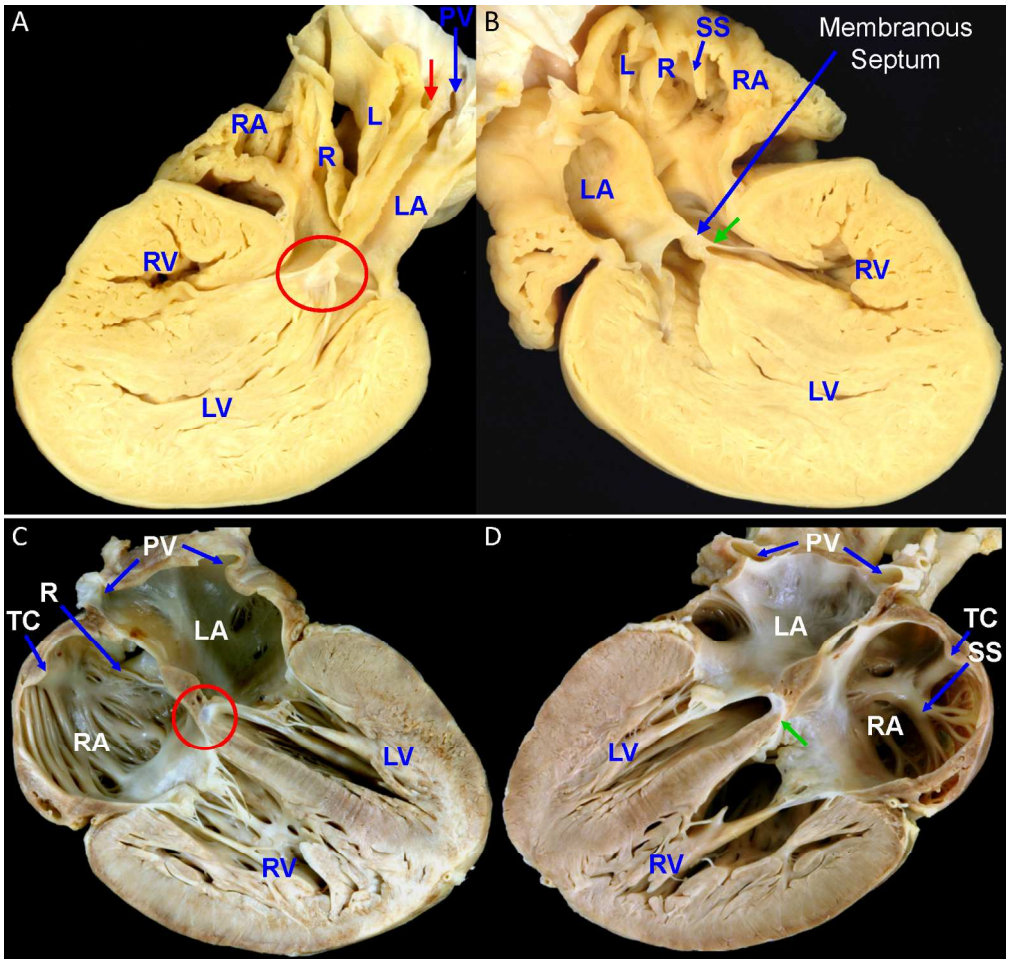

Figure 8. Four chamber sections of crocodile (A-B) and human heart (C-D) in attitudinal correct positions. The heart of a 2 year old crocodile showing the inferior aspect in A and the superior half in B. The section cuts across the inferior aspect of the fibrous leaflet of the right atrioventricular valve. In the inferior part of the heart (A), the two septally positioned leaflets of the atrioventricular valves are seen to insert at similar levels (circled). There is no evidence of an atrioventricular septal 'sandwich' as seen in the human heart. The fold of tissue between the right pulmonary vein and the right atrium is also evident (red arrow), as are the right (R) and left (L) venous valves. In the superior half of the heart (B), there is a greater degree of offsetting of the atrioventricular valves. This is due to the right atrioventricular valve hinging from the mid-point of the extensive membranous septum (green arrow). LA = Left Atrium; LV = Left Ventricle; PV, pulmonary veins; RA= Right Atrium; RV = Right Ventricle; SS = spurious septum; TC = terminal crest.

160x152mm (300 x 300 DPI)

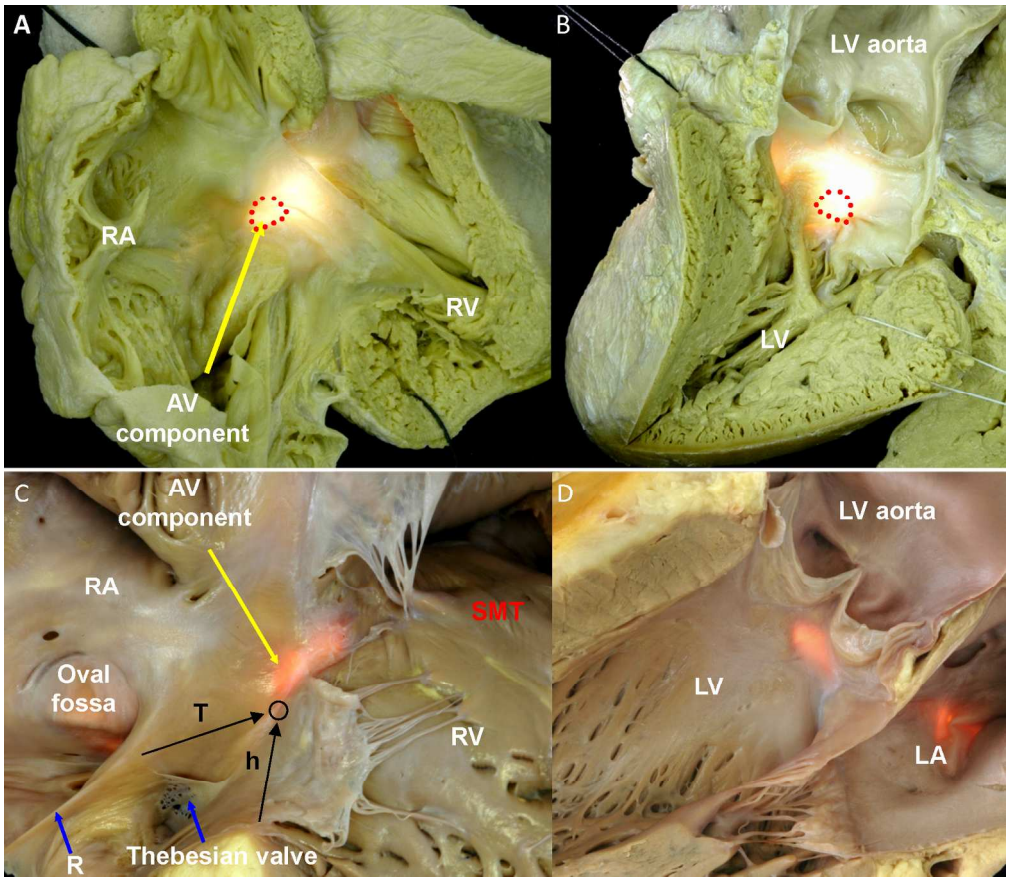

Figure 9. Trans-illumination of the membranous septum of the crocodile (A-B) and human heart (C-D). A-B. In this heart from an adult crocodile, the septum has been trans-illuminated to the show the extensive nature of the membranous septum as seen from the morphologically right (A) and left ventricle (B). The atrioventricular component, above the hinge line of the septal leaflet of the right atrioventricular valve is outlined in red. On the morphologically right side of the human heart (C), the tendon of Todaro (T) and hinge point (h) of the tricuspid valve converge on the atrioventricular node (the position is approximated by the black circle). AV = Atrioventricular; h = hinge point of the tricuspid valve; LA = Left Atrium; LV = Left Ventricle/Left Ventricular; R = right venous valve (crocodile) or Eustachian valve (human); RA= Right Atrium; RV = Right Ventricle; SMT = septomarginal trabeculation; T = tendon of Todaro.

170x148mm (300 x 300 DPI)

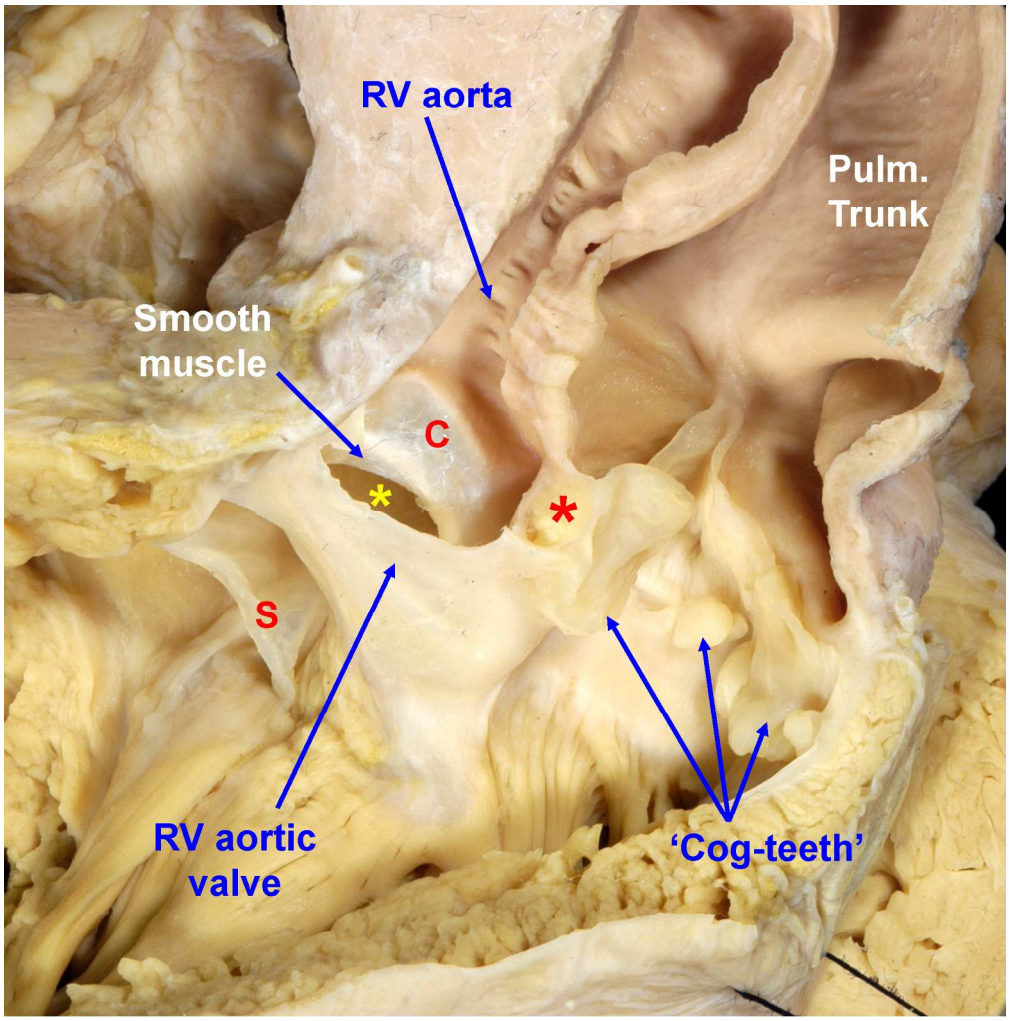

Figure 10. The section reveals the detailed anatomy of the right ventricular outflows. A common party wall separates the right ventricular aorta from the pulmonary trunk. Within the heart, a small myocardial outlet septum (red asterisk) creates a degree of offsetting between the leaflets of the pulmonary and right ventricular aortic valves. The foramen of Panizza (yellow asterisk) can be seen just behind one of the leaflets of the right ventricular aorta. The foramen itself is surrounded by an arc of cartilage (C) which carries on its leading edge a crescent of smooth muscle. Also shown is the septal leaflet of the right atrioventricular valve (S). RV = Right Ventricular; Pulm. = Pulmonary.

188x190mm (300 x 300 DPI)

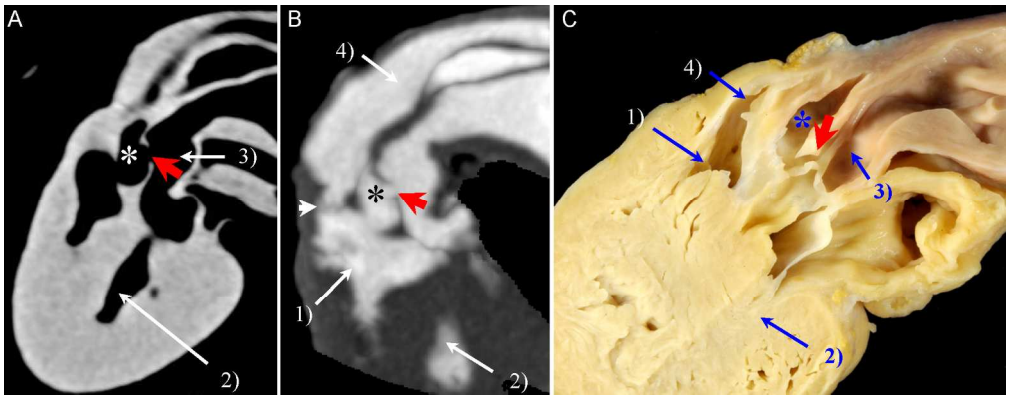

Figure 11. Computerised tomographic images of the great vessels viewed from the left: A cross-sectional slice, no contrast, B thin maximum intensity projection, post-contrast, and C anatomic correlate. 1) = right ventricle, 2) = left ventricle, 3) = LV aorta, 4) = pulmonary trunk, * = RV aorta, white arrowhead = position of cog-wheel apparatus, and red arrow = foramen of Panizza.

187x73mm (300 x 300 DPI)

For Review Only

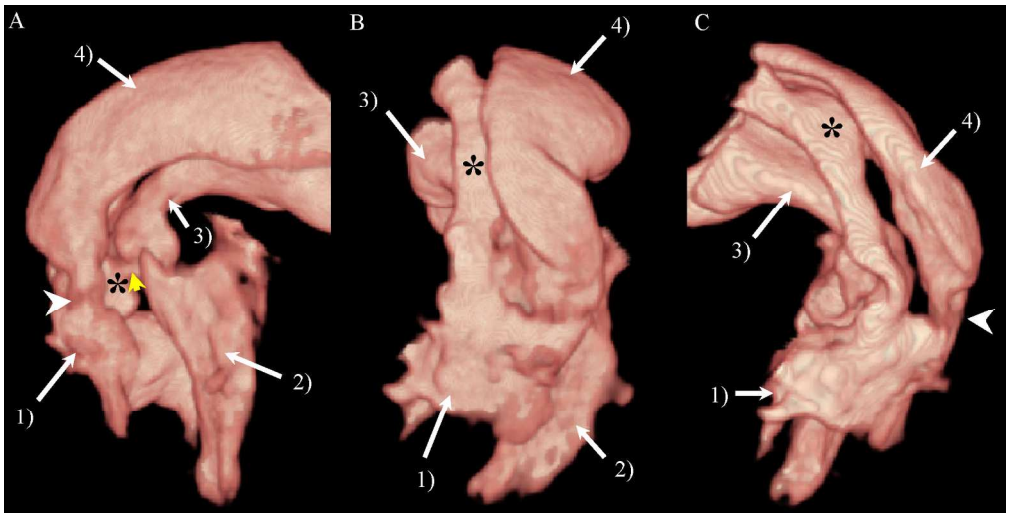

Figure 12. Volume-rendered, post-contrast computerised tomographical images of the great vessels and ventricles viewed from: A left, B ventrally, and C right. 1) = right ventricle, 2) = left ventricle, 3) = LV aorta, 4) = pulmonary trunk, * = RV aorta, white arrowhead = position of cog-wheel apparatus, and yellow arrow = foramen of Panizza.

182x92mm (300 x 300 DPI)

For Review Only

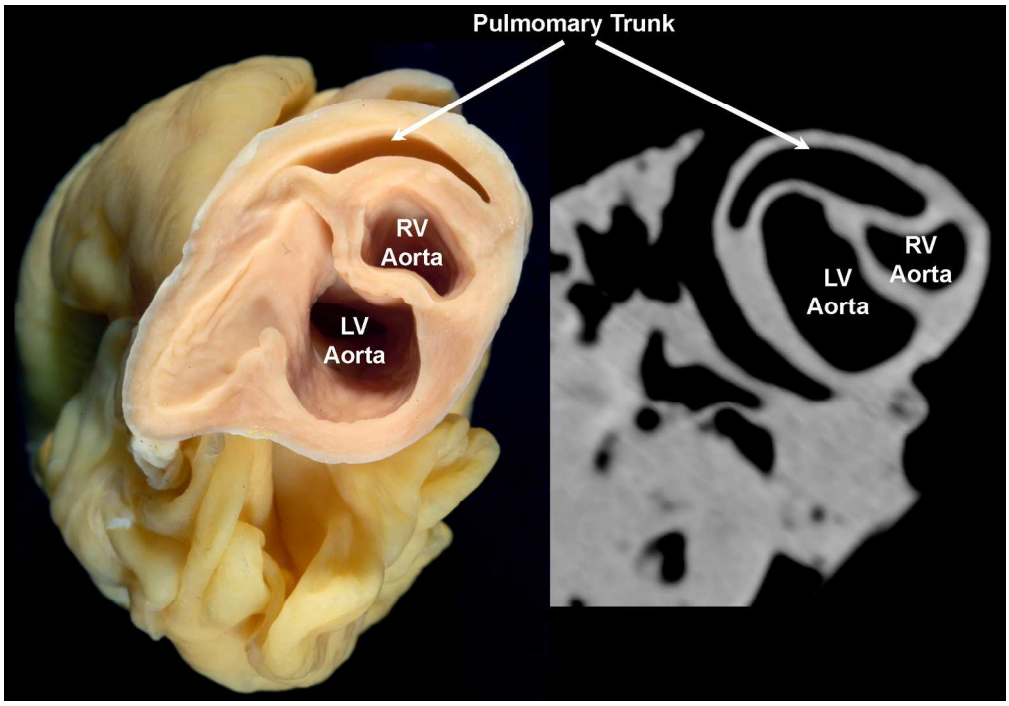

Figure 13. Anatomic cross-section A and comparable cross-sectional image obtained with computerised tomography B through the ascending arterial trunks, showing that the three arterial pathways are encased in a common arterial wall. RV = Right Ventricular; LV = Left Ventricular; Pulm. = Pulmonary.

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For Peer Review Only

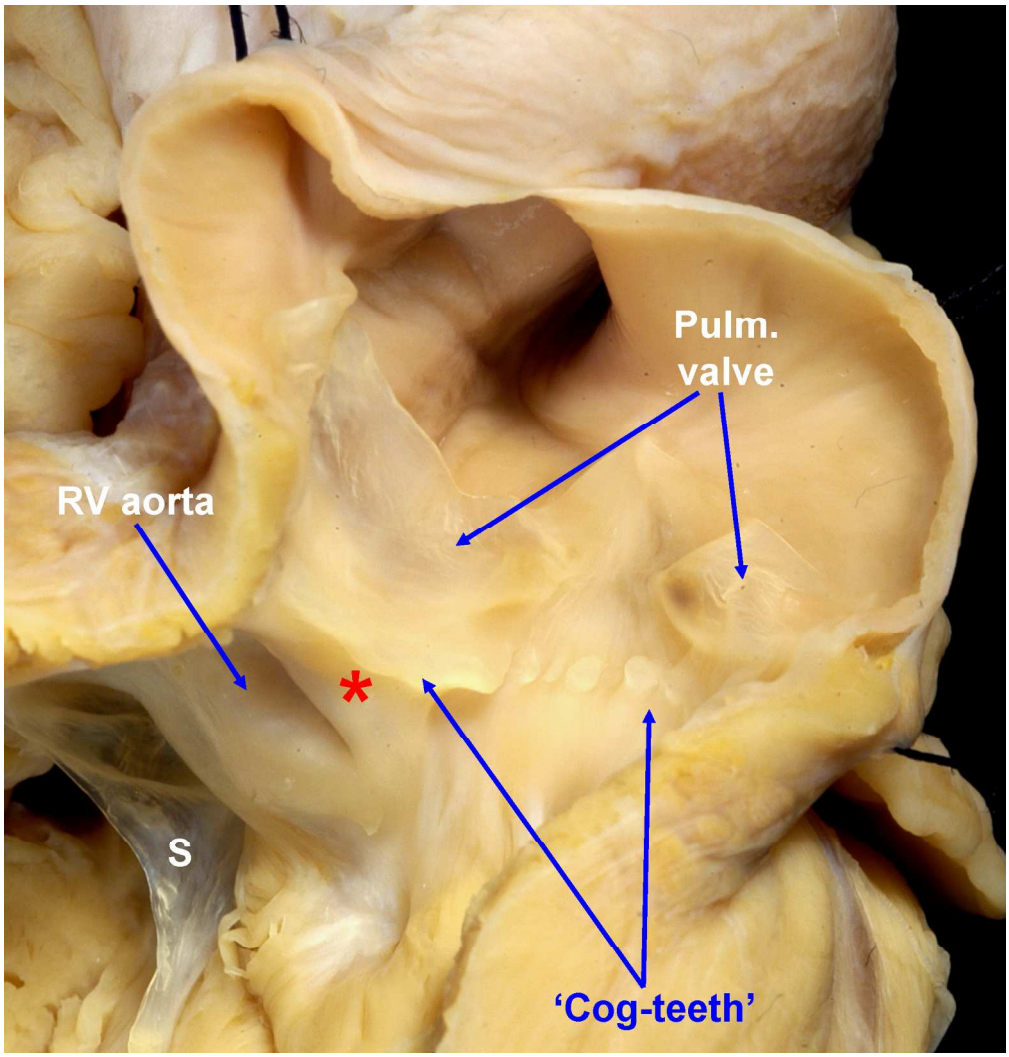

Figure 14. The pulmonary outflow tract has been opened in this specimen from a young crocodile to show the rudimentary nature of the cog-teeth compared to the adult (see figure 10). The location of the myocardial outlet septum is marked by the red asterisk and the septal leaflet of the right atrioventricular valve is seen in the background (S). RV = Right Ventricular; Pulm.= Pulmonary.

181x189mm (300 x 300 DPI)

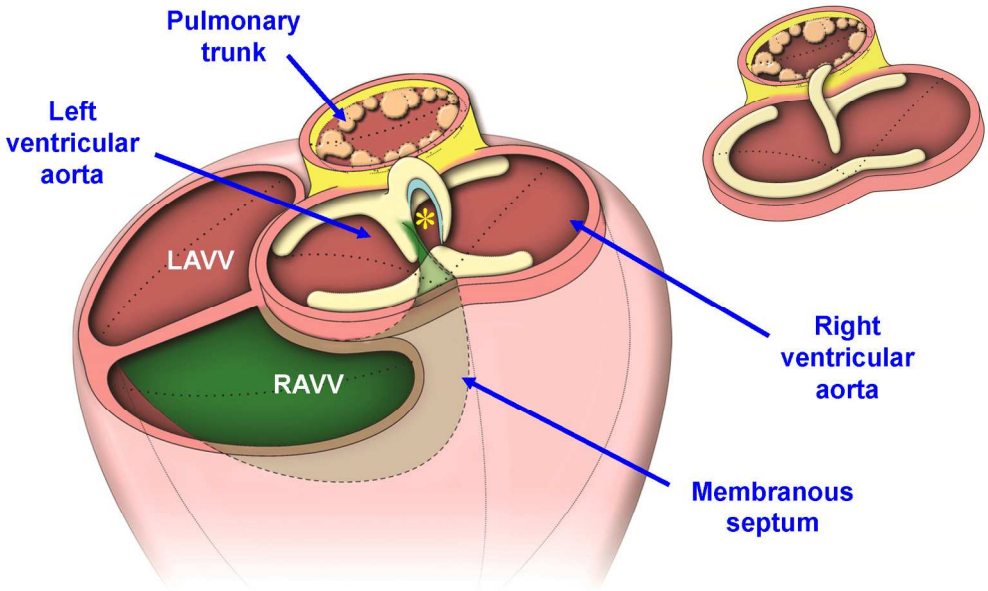

Figure 15. These schematic diagrams show the location of the cartilaginous component of the crocodile heart as seen in our examinations, and compared to our interpretation of the initial description by White in 1956 (inset). Our studies show that the cartilage consists of two components, one lateral and one medial. The medial component has prongs running in the base of the aortic sinuses facing the pulmonary trunk and is anchored to the crest of the ventricular septum via a caudal prong. It also encircles the foramen of Panizza cranially (yellow asterisk). This cranial crescent is lined by an arc of smooth muscle (blue arc). The lateral component occupies the base of the non-facing sinuses, and runs into the fold of tissue between the right atrioventricular valve and the left ventricular aorta. The extensive membranous septum is shown in green. LAVV = Left Atrioventricular Valve; RAVV = Right Atrioventricular Valve.

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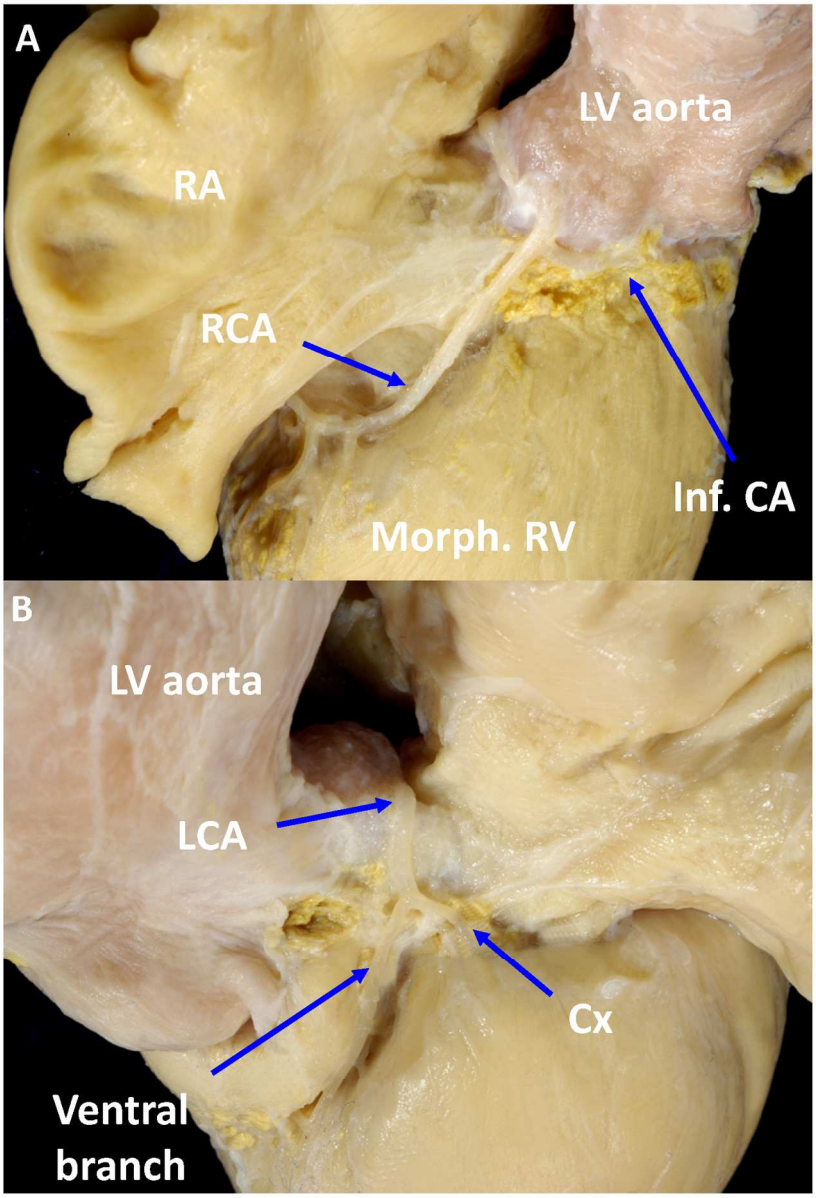

Figure 16. These images show the left ventricular aorta as viewed from the right A and left B sides in an adult specimen. There is a single coronary arising from this aorta which gives rise to a right coronary artery (RCA), an infundibular branch (Inf CA), and also passes behind the arterial trunks as a Left Coronary Artery branch (LCA) which then divides into circumflex (Cx) and ventral branches. The coronary arteries thus encircle the arterial pedicle, as can also be seen in figure 1. RA = Right Atrium; RV = Right Ventricle; LV = Left Ventricular.

125x184mm (300 x 300 DPI)

Table 1. Gross morphological characteristics of the hearts of major groups of amniote vertebrates.

Amniote phylogeny	Noncroc reptiles	Crocodilians	Birds	Monotremes	Marsupials	Placentals
Intracardiac caval veins	3	3	3	3	3	2 or 3
Sinuatrial node	no	no	vague	yes	yes	yes
Sinuatrial valve	yes	yes	varies	yes	reduced	reduced
Atrial septum	1	1	1	1	1	1 + 2*
Atrioventricular node	no	no	vague	yes	yes	yes
Valvar leaflets right avo	1	2	1	1	varies	varies
Left-right av offset	no	yes	yes	yes	yes	yes
RV papillary muscle	no	yes	no	no	on septum	yes
RV moderator band	yes	yes	reduced	yes	yes	yes
Ventricular mus septum	partial	yes	yes	yes	yes	yes
Ventricular mem septum	no	yes	no (myo)	yes	yes	yes
Pulmonary artery	1	1	1	1	1	1
PA valvar leaflets	2	2	3	3	3	3
Pulmonary veins to LA	1	1	2	1	1-4	1-7
Mitral valve	no	yes	yes	yes	yes	yes
LV papillary muscle	no	yes	yes	yes	yes	yes
Number of aortas	2	2	1	1	1	1
Aortic valvar leaflets	2	2	3	3	3	3
Foramen of Panizza	no	yes	no	no	no	n~~N~~o
Compact or spongy ven	spongy	spongy	compact	compact	compact	compact
PA and aorta joined	yes	yes	no	no	no	no
Coronary circulation	yes	yes	yes	yes	yes	yes
Gubernaculum cordis	varies	yes	no	no	no	no
Insulating plane	no	partial	yes	yes	yes	yes
Heart mass/Body mass	0.25%	0.25%	1%	0.5%	0.5%	0.5%

2*, only placental mammals form a secondary foramen and a superior fold, the so-called secondary septum, to close it; av, atrioventricular; avo, atrioventricular orifice; LA, left atrium; LV, left ventricle; mem, membranous; mus, muscular; myo, secondarily myocardialized; PA, pulmonary artery; RV, right ventricle; ven, ventricle. Based on (Röse, 1890; Greil, 1903; Keith & Flack, 1907; Benninghoff, 1933; Davies, 1942; Davies & Francis, 1946; Goodrich, 1958; Nathan & Gloobe, 1970; Webb, 1979; Van Mierop and Kutsche, 1985; Rowlatt, 1990; Steding et al. 1990; Lamers et al., 1991; Runciman et al., 1995; Axelsson & Franklin, 1997; MacDonald et al., 2007; Jensen et al., 2012; Tessadori et al., 2012; Jensen et al., 2013a, 2013b; Burggren et al., 2014; Jensen et al., 2014a, 2014b; Poelmann et al., 2014; de Bakker et al., 2015; ~~Poelmann et al., 2015;~~ Jensen & Moorman, 2016; Jensen et al., ~~in press~~2017).

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