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Gross anatomy and histology of the heart and great vessels of a leopard seal (*Hydrurga leptonyx*)

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Leopard seals (*Hydrurga leptonyx*) are an essential apex predator widely distributed in the Antarctic and sub-Antarctic waters of the Southern Hemisphere (Erickson et al., 1971; Kooyman, 1981; King, 1983a; Hückstädt, 2015; Ferrer, 2018; Staniland et al., 2018). The presence of leopard seals in northern Argentina is directly related to the northward dispersal of juveniles from the Antarctic pack ice (Rodríguez et al., 2003; Bastida et al., 2007). Descriptions of the macroscopic anatomy of the *leopard seal heart* are rare. To our knowledge, only one study has detailed the cardiac anatomy based on a single specimen of this species (Drabek, 1975), and other studies have presented histological descriptions with anatomic aspects of the heart in a few specimens (Gray et al., 2006). This work aims to contribute to the knowledge of the cardiovascular system of the leopard seal through the anatomical and histological description of the heart and great vessels in one specimen studied a few hours postmortem.

Keywords:
pinniped physiology, cardiovascular system, diving adaptations, histology

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One heart of leopard seal was obtained from a young female (2.12 m long), less than three years old, according to Hamilton (1939) and Brown (1957). The animal was found alive at a local beach in Mar del Plata city (Argentina) and died after two days. Although the cause of death was not precisely established, veterinary examination prior to death assumed a poor body condition. It also did not indicate any possible cardiac disorder, so it was assumed that the cardiovascular system was normal. A complete routine necropsy of the specimen was performed, with the corresponding macroscopic examination and extraction, weighing and measurement of all its organs, taking different samples for analysis of contaminants in fat and muscle, genetic analysis, analysis of stomach and intestinal contents, and analysis of stable isotopes. The heart was removed along with the lungs to maintain the pulmonary vessels and the pericardium intact. These organs were then immediately weighed (0.66 kg.), placed in a 10 % formalin solution and studied through dissection, observing first the external conformation and the internal configuration to describe the histomorphology.

Photographs and video were taken with a Canon Powershot SZ50 HS digital camera and a Sony HDR-AS20 video camera, respectively. The terms used were those of the fifth edition of the ICVGAN (2005). Samples from different areas of the heart were taken (n = 9) for histological approach. They were histologically processed, and 5-μm sections were obtained and stained with hematoxylin-eosin. The histoarchitecture was analyzed under a Leica DM500 microscope at 10× and 40×. Photomicrographs were captured with a Leica ICC50W camera and the software Leica LAS EZ 3.4 (Leica Microsystems GmbH, 2016) or Leica Acquire 3.4.6 (Leica Microsystems GmbH, 2021). Up to 15 cardiomyocytes per sample were measured at 100× using open-source software FIJI (Schindelin et al., 2012): cell width, and nuclear length and width. Also, nuclear volume was calculated using the formula:

$$v = \frac{4}{3} \times \pi \times a \times b^2$$

where v is the nuclear volume, a is the semi-major axis, and b is the semi-minor axis (O'Brien et al., 1987). Results are expressed as mean ± (SD) μm for length and width and μm^3 for volume.

Also, samples were stained with Picrosirius Red to analyze myocardial collagen (shown in red over a yellowish background), and with the Taenzer-Unna acid orcein technique to analyze the elastin fibers (shown in purple-brownish over a yellowish background). Fiber distribution was analyzed under the mentioned optical microscope, and their concentration was measured on 4× photomicrographs using FIJI macros (Hadi et al., 2011).

The heart of the leopard seal is flattened dorsoventrally and composed by the four typical chambers (two atria and two ventricles). The base is essentially formed by the right atrium (RA), the left atrium (LA), and the great vessels entering or leaving the heart (aorta and pulmonary trunk). The heart is enclosed within a pericardial sac, composed by the pericardium *fibrosum* and *serosum*. It is attached ventrally to the sternum via the sternopericardial ligament (*ligamentum sternopericardiacum*) and caudally to the diaphragm via the phrenopericardial ligament (*ligamentum phrenicopericardiacum*). The apex of the heart is composed of most of the caudal portions of the right and left ventricles (RV and LV, respectively). The RV protrudes a few millimeters more caudally than the LV.

The aorta (Ao) originates from the LV and courses slightly to the right in a cranial direction. It is bounded ventrally by the pulmonary trunk (Fig. 1A), the *conus arteriosus*, and the atrial appendages, and dorsally by the cranial vena cava. Just cranial to the aortic cusp, the Ao gives off left and right coronary arteries (Fig. 1B). The Ao has an outer diameter of 37.8 mm at the level of the aortic root. Just 3 cm from the aortic root, the ascending Ao expands into a prominently dilated segment (61.6 mm in diameter). This anatomical segment of 130 mm in length in pinnipeds is called the aortic bulb (AoB) or *bulbus aortae* (Fig. 1A). Immediately distal to the AoB, the Ao narrows and curves to the left as the aortic arch. At this level of the Ao arch, three main arterial trunks originate (Fig. 1C): 1) the brachiocephalic trunk arises from the cranial face of the arch. This trunk rises cranially to the right subclavian and common carotid arteries. After the origin of the brachiocephalic trunk, the arch inclines dorsally and to the left to give rise to 2) the left common carotid artery; and 3) the left subclavian artery. Subsequently, the Ao inclines dorsally as the descending Ao gives rise to the intercostal arteries.

The LV is conical in shape, broad at the base, and pointed at the apex. In the cross-section, the cavity of the LV is circular. It has an internal anteroposterior dimension of 70 mm at the midventricular level and a craniocaudal distance of 125 mm (from the apex to the plane of the mitral valve). The thickness of the interventricular septum (IVS) and the lateral wall of the LV is greater at the midventricular level (23.0 mm and 21.6 mm, respectively, Fig. 2A), being less in the apical segments (17.1 mm). The left atrioventricular (AV) orifice is oval, and the left atrioventricular valve is comprised of two cusps (septal or ventral and parietal or dorsal cusp) attached via *chordae tendineae* to papillary muscles of the septal and parietal walls respectively (Fig. 2B). The cusps of the left AV valve are thicker than those of the right AV valve. The aortic valve (*valva aortae*) is also comprised of three semilunar cusps: left, right cusps (ventral) and septal or dorsal (*valvula semilunaris sinistra, dextra et septalis*). The right and left cusps are ventral to their respective sides, whereas the septal cusp is dorsal.

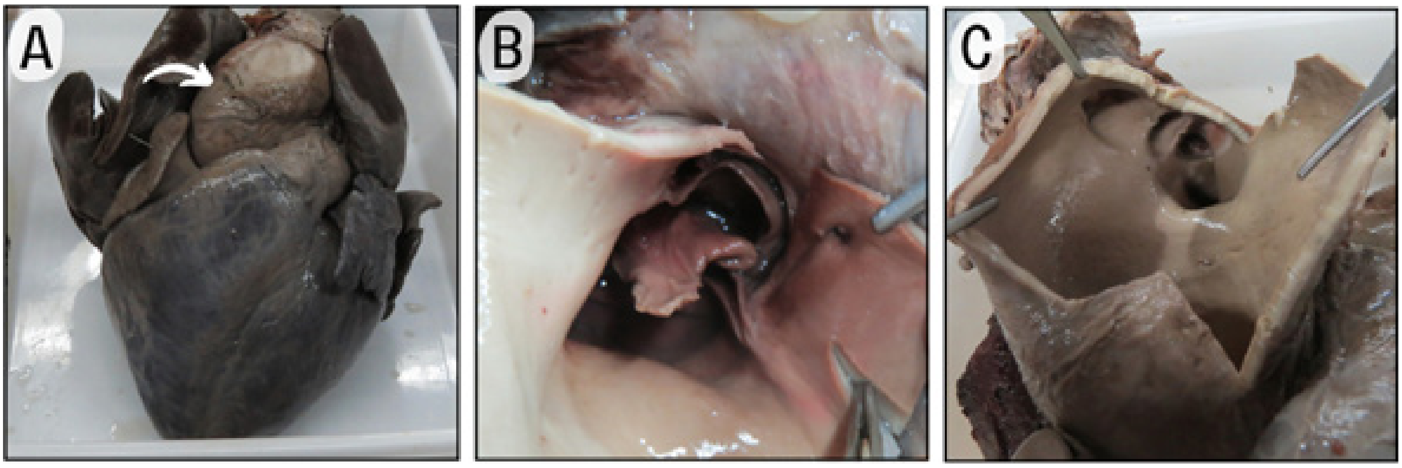

Figure 1. A) Anterior view of a leopard seal's (*Hydrurga leptonyx*) heart (external configuration) and aortic bulb (white arrow); B) Left coronary ostium arises form left aortic valve; C) Main arterial trunks from aorta.

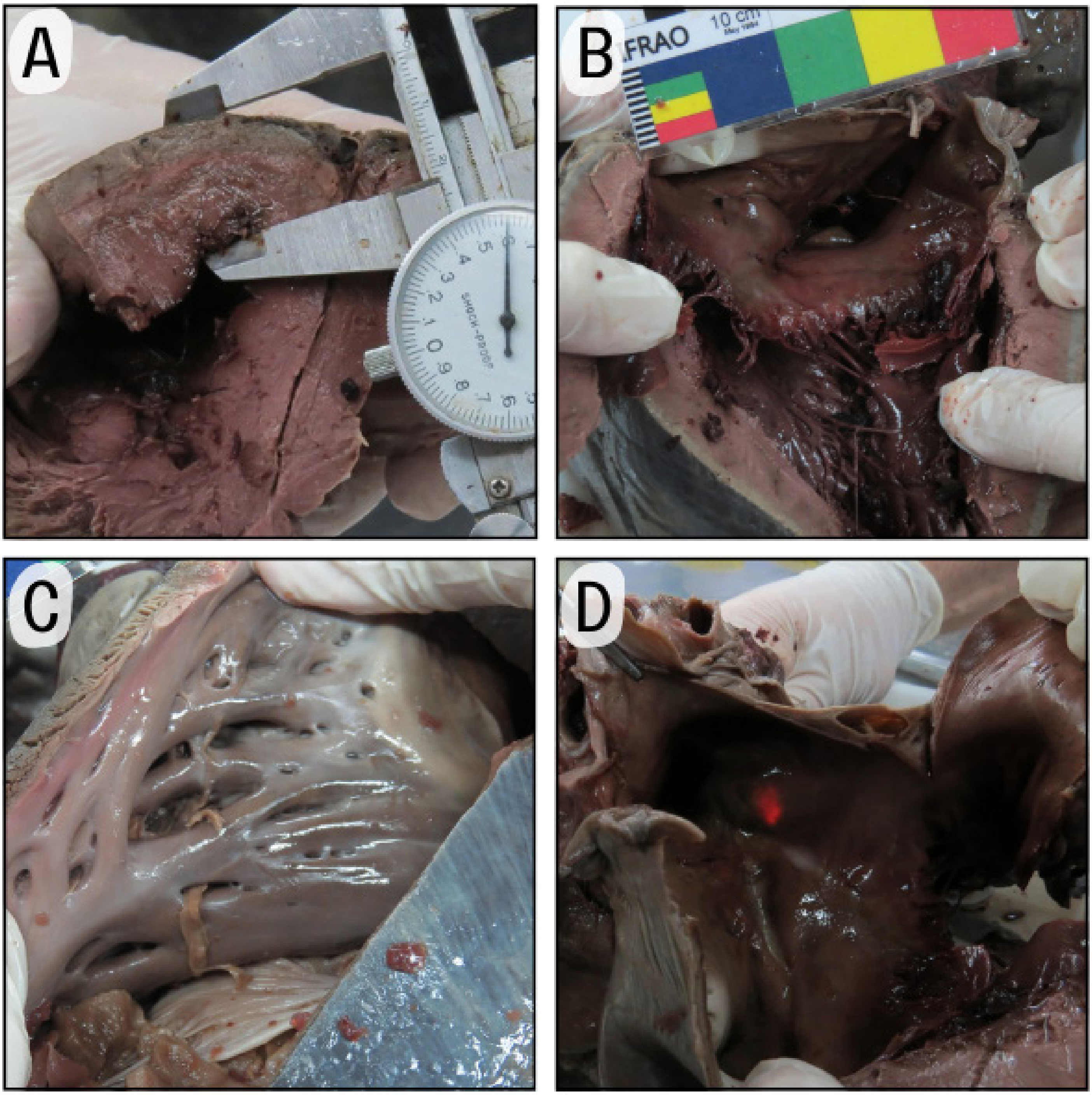

Figure 2. A) The heart of a leopard seal (*Hydrurga leptonyx*). Left ventricular free wall and interventricular septum; B) Internal configuration of the left ventricle, mitral valve and left atrial; C) internal configuration of the right ventricle; D): *Fosa ovalis* shown by transillumination.

The RV is a long, spacious, thin-walled chamber extending caudally to the heart's apex (Fig. 1A). In cross-section, the cavity of the RV is semicircular. The apex is composed of both the right and left ventricles. The craniocaudal dimension of the RV measured from the junction of the IVS with the free wall of the RV to the base of the pulmonary cusps was 120 mm. The thickness of the basal, medial, and apical RV-free walls was 4 mm, 7 mm, and 9 mm, respectively. Inside the RV, a significant development of the *trabeculae carneae* can be seen (their appearance was very similar to that of the *pectineus* muscles) occupying part of the *conus arteriosus* (Fig. 2C). The septal and lateral walls are coarsely trabeculated, except around the *conus arteriosus*. Likewise, two septomarginal bands (*septomarginalis trabeculae*) connecting the septal and lateral walls of the RV are evident. Attached to the septal and lateral wall, three papillary muscles attached to the *chordae tendineae* were identified.

The right AV valve (*valva atrioventriculare dextrum*) is composed of three cusps: 1) one cusp oriented along the ventral wall of the RV; 2) one cusp along the dorsal wall of the RV; and 3) another septal cusp attached to the IVS. The pulmonary valve (*valva trunci* *pulmonalis*) is composed of three semilunar cusps (valves): 1) the left cusp (*valvula semilunaris sinistra*) lies along the IVS; 2) the intermediate cusp (*valvula semilunaris intermedia*) is in the free wall of the pulmonary trunk; and 3) the right cusp (*valvula semilunaris dextra*) is located cranial to the supraventricular crest. The pulmonary artery's trunk originates from the RV's *conus arteriosus* in the ventral midline. It runs between the left and right atria, giving rise to the right and left pulmonary arteries.

The RA presents thin walls, and the interior of the cavity is covered by *pectineus* muscles. The RA is more extensive than LA. In the middle third of the septal wall of the RA, a remnant of the *fossa ovalis* is evident by transillumination (a consequence of the closure of the *foramen ovale*), surrounded by a prominent rim (*limbus fossae ovalis*) (Fig. 2D). A broad-based triangular saccular and blind extension ("ear-shaped") extends from the anterosuperior portion of the lateral wall of the RA (appendage). The RA receives the two *venae cavae* (cranial and caudal) and the coronary sinus. The main body of LA comprises the pulmonary venous portion, the septal portion, and the atrial vestibule (which surrounds the mitral orifice). The LA has a blind appendage (finger-shaped sac) that extends from the anterior and superior segments of the main body of the atrial. The LA appendage is smaller than its counterpart on the right side. The main LA characteristics are the evidence of an atrial portion rich in pectinate muscles with four thin-walled pulmonary veins entering LA separately.

The arterial cardiac irrigation corresponds to the right and left coronary arteries, which emerge from the right and left aortic sinuses, respectively. The left coronary artery arises from the left aortic sinus, 8 mm above the insertion of the left aortic valve. From its origin, it courses to the left and divides into circumflex and interventricular branches. The interventricular branch follows a course along the anterior interventricular sulcus that supplies both ventricles and the IVS until it reaches the cardiac apex. The circumflex branch courses dorsally to the left around the lateral margin of the LA, reaching the atrial surface of the heart to join the right coronary artery in the subsinusal groove. The right coronary artery arises from the right ventral aortic sinus (8 mm above the insertion of the aortic valve) and courses to the right within the coronary sulcus, bending dorsally around the RA, then descending caudally into the subserosal sulcus as the subserosal branch of the right coronary artery. The great cardiac vein originates from the cardiac apex in the interventricular groove. It continues cranially into the coronary sulcus, turns to the left, together with the circumflex branch of the left coronary artery, and finally drains into the coronary sinus.

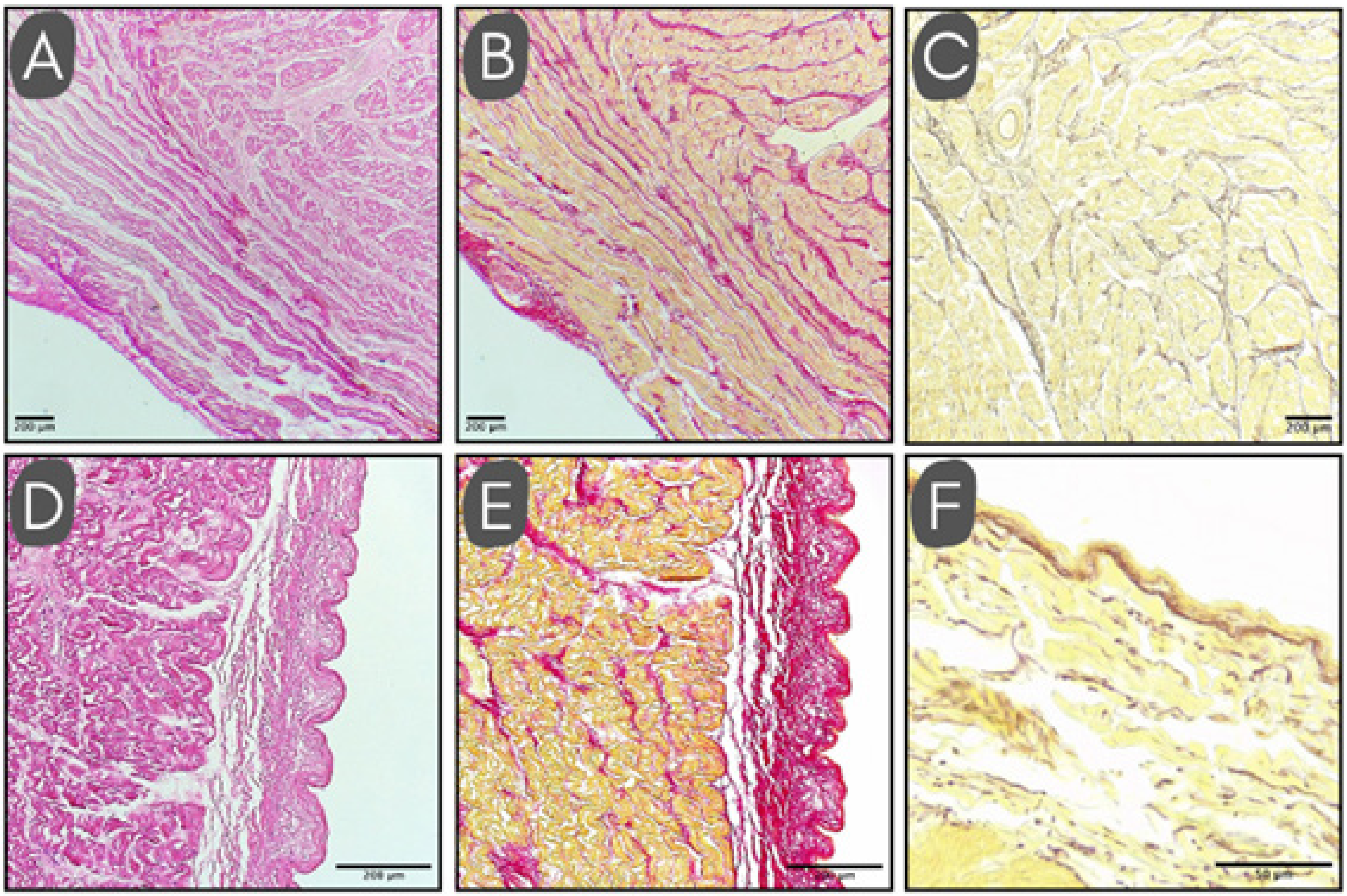

Figure 3: Representative sections of the leopard seal (*Hydrurga leptonyx*) heart with different staining: Hematoxylin-eosin (A, D), picrosirius red (B, E) and Taenzer-Unna acid orcein (C, F). Photomicrographs A–C show visceral epicardium (top-right) and myocardium (bottom), and elastin are noticeable. Magnification: A–C 4×; D–E 10×; F 40×.

Leopard seal left and right ventricles walls are formed by three tunicae: the inner endocardium, the myocardium, and the outer epicardium. The endocardium consists of an endothelium of simple squamous epithelium and a subendothelial loose connective tissue, where large Purkinje cells are visible. The myocardium is the thickest tunica, and consists mainly of striated cardiac muscle fibers with connective tissue interspersed (Fig. 3D). Lastly, the epicardium presents a loose connective tissue with greater blood vessels and nerves, and an external mesothelium of simple squamous epithelium (Fig. 3A). The cardiomyocytes showed a width of 7.11 ± 2.01 μm, and their nuclei showed 12.01 ± 2.33 μm in the major axis, and 3.66 ± 0.83 μm in the minor axis. The cardiomyocyte nuclear volume was 87.48 ± 41.37 μm^3. Collagen in the myocardium forms trabeculae around cardiomyocyte fibers with small blood vessels running through them (Figs 3B, 3E). The mean collagen concentration was 23.8% ±17.3, but a great variability among the different locations was observed, ranging from 0.74 in the apical area of the LV to 52.34% in the area of the AV node. Elastic fibers are present in the three layers of the cardiac wall with some particularities. A thin lamina of elastin immediately under the endothelium was observed, more evident in ventricular walls. Furthermore, the outermost endocardial connective tissue presented a mesh of elastic fibers in different directions (Fig. 3F). The myocardial connective tissue showed variability in the presence of elastin, with a higher concentration among the cardiomyocyte bundles but scarce around each cardiomyocyte (Fig. 3C). The epicardium presented an abundant concentration of elastic fibers with an arrangement in different directions intertwined forming a mesh. The blood vessels observed in the epicardium presented the typical arrangement of elastic laminae. Arteries presented an internal elastic lamina in the tunica intima and an external elastic lamina in the tunica media. An internal elastic lamina was present in the tunica intima of arterioles, whereas elastic fibers were seen in the tunica media of veins.

In general, macroscopic anatomical observations of the leopard seal heart closely correspond with other marine mammals (Drabek, 1977; Slijper, 1979; Berta et al., 2015). Although variations in details of anatomy exist, the general characteristics of the RV and LV of the heart of the leopard seal are comparable to those of other marine mammals. The cardiac apex of the leopard seal is comprised of both left and right ventricles as opposed to the typical terrestrial mammal heart in which it is comprised of just the LV (Berta et al., 2015). Drabek (1975) noted in other pinnipeds that the apex of the heart was comprised of both ventricles. A remarkable feature of the leopard seal heart is a prominent, spacious, thin-walled RV of equal length to the LV. The RV-free wall in the leopard seal is about three times thinner than that of the LV, and can be considered a value comparable to that of most marine mammals (Bisaillon, 1987). In this sense, it is considered that an RV of thinner walls, as in seals, is an advantage for ventricular dilatation during dives and would allow a better response to lung augmentation tissue resistance during deeper dives (Rushmer & Blackmon, 1970; King, 1983b). Moreover, the differences in the proportions of the RV in pinnipeds may represent functional adaptations to deep diving (Drabek, 1975, 1977). A long, narrow RV is considered an advantageous characteristic for mammals that dive to great depths for long periods.

In contrast to the cardiac human anatomy, the most remarkable feature of the leopard seal Ao is an enlarged portion called AoB. This slightly dilated segment in the ascending Ao, is present in many species of aquatic mammals, and its primary function is thought to be the damping of abrupt changes in blood pressure during the bradycardia associated with diving (Chetboul et al., 2012; Kirkwood & Goldsworthy, 2013; Guimaraes et al., 2014; Castro et al., 2018). Using pressure-volume curves, Rhode et al. (1986) demonstrated that the AoB of marine mammals is a very compliant segment and can accommodate more than three times the normal stroke volume of the heart. In addition, Hochachka (2000) found, during a simulated dive of juvenile elephant seals studied using magnetic resonance imaging and magnetic resonance spectroscopy, that the AoB allowed continuous blood flow throughout both systole and diastole, and so removed the large changes in blood flow across the cardiac cycle that are typical of terrestrial mammals. The prominence of an aortic expansion is much more evident when it is expressed as the percentage increase of the bulb over the base (Drabek, 1975). This expression removes any scaling effect due to the size variation of the animals. In this examined specimen, the difference in the base of the bulb was 62.9%, much higher than the 33% reported by Drabek (1975) in the same species.

The study of cardiac anatomy in diving mammals extensively contributes to the understanding of morpho-functional aspects related to diving adaptations. Moreover, it is possible to study the relationship between the anatomy of different cardiac structures and diving abilities, as in the case of the AoB, whose different degree of development among different species has been associated with their feeding habits, in terms of depths and durations reached during diving (Berta et al., 2015). The expansion of the AoB of all Antarctic seals was more impressive when compared to terrestrial mammals (Drabek, 1975). The diameter of the AoB of a leopard seal measured an increase of 62.9% over the base, while the increments of the internal diameters of the bulbs of a dog or a cat are 16.5% and 32% respectively (Drabek, 1975). It has been postulated that the percentage increase in the diameter of the AoB over that of the base in seals corresponds to the ice habitat and the type of diving required by the feeding habits of each pinniped (Drabek, 1975; Drabek & Burns, 2002). The leopard seal, for example, has a smaller AoB than the deep-diving Weddell seal (*Leptonychotes weddellii*; Berta et al., 2015). Furthermore, Van Nie (1985) suggested that the seal's AoB is an innate feature associated with its habitat. These morphological examinations demonstrate a gradual development of the AoB, beginning immediately after birth.

The histological structure of the leopard seal heart is consistent with the limited descriptions of this species (Gray et al., 2006) and similar to that previously described for other marine mammals (Simpson & Gardner, 1972; Pfeiffer & Viers, 1995; Stewardson et al., 1999). It is noticeable that the endocardium is somehow thick, similar to other marine mammals' reports (Black-Schaffer et al., 1965; Pfeiffer & Viers, 1995), yet the physiological significance is unclear. Also, large Purkinje fibers are observed in the endocardium. Gray et al. (2006) proposed that this would provide an advantage for the conduction of the impulses through the heart walls. Unlike previously reported for this species (Gray et al., 2006), no adipocytes were observed in the heart walls. The cardiomyocytes' width is similar to that of sea lion (species not stated) and blue whale (*Balaenoptera musculus*) reported by Black-Schaffer et al. (1965) (considering the compensation for shrinkage done by the authors). It is proposed that this dimension may be related to diffusion rates (Black-Schaffer et al., 1965; Fuson et al., 2003). Furthermore, thickness of the heart wall and elastic fibers presence would be related to the resistance to stretch (Black-Schaffer et al., 1965).

In conclusion, the histomorphology of the leopard seal heart is similar to that of other marine mammals. However, there are some anatomical differences, such as the thinness of the free wall of the prominent right ventricle or the width of the aortic bulb, that are potentially correlated to diving performance.

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Conflict of Interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Author Contributions

D.A., DL.M.C., C.E., and D.M. were responsible for the study planning, data acquisition, and writing the main manuscript. E.V., H.J.M., and F.A. were responsible for histological and histomorphological data acquisition and processing. P.D. and G.J.C.M. participated in data acquisition and analysis, photography processing, and figure creation. All authors participated in the critical reading and final approval of the manuscript.

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