



The relative length of the cardiac bulbus arteriosus reflects phylogenetic relationships among elasmobranchs

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ABSTRACT

Previous work on the anatomy of embryonic and adult hearts of the shark *Galeus atlanticus* showed that the cardiac outflow tract consists not of a single component but of two, the myocardial conus arteriosus and the nonmyocardial bulbus arteriosus. However, it was still uncertain whether a bulbus occurs regularly in elasmobranchs. The present study was designed to gain new insight into this issue. Moreover, we investigated the interspecific variation in the relative length of the bulbus in order to decide whether such variation is related to the lifestyle or to the phylogenetic position of sharks and rays. The material examined consisted of hearts of 42 species belonging to seven families of squalomorphs, six families of galeomorphs and six families of batoids. Our findings confirmed that the bulbus arteriosus is indeed a cardiac outflow tract component commonly present in all elasmobranchs examined. In addition, we propose that the relative length of the bulbus arteriosus is rather related to the phylogenetic position of the species than to its lifestyle. Overall, the bulbus arteriosus is notably longer in galeomorphs than in squalomorphs and batoids, with the exception of the two representatives of *Torpedo*, which possess a bulbus arteriosus longer than other batoids. Likewise, the anatomical origin of the outflow tract from the ventricle is closely related to the relative length of the bulbus. We suggest that both anatomical traits are associated with the phylogenetic position of the sharks and rays investigated.

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1. Introduction

In a previous paper (Durán et al., 2008) we showed that the cardiac outflow tract (OFT) of *Galeus atlanticus* (Vaillant, 1888) consists of two components, proximal and distal with regard to the ventricle. The proximal component is the conus arteriosus (CA); it is a contractile segment, characterized by the presence of myocardial muscle in its walls and valves at its luminal side. The distal component is the bulbus arteriosus (BA); it lies within the pericardial cavity, connecting the conus with the ventral aorta, and lacks both myocardium and valves. Histologically, the BA displays an arterial-like structure. However, it differs from the aorta because it is covered by the epicardium, shows a different arrangement of the histological elements, and its walls are crossed longitudinally by the main coronary artery trunks, which enter the cardiac OFT at the distal boundary of the pericardial cavity. It should be noted that we used the term bulbus arteriosus to designate the distal component

of the cardiac OFT because we provided morphological evidence of its homology with the actinopterygian BA (Durán et al., 2008). Further support to this notion came from our study on the formation of the cardiac OFT in *G. atlanticus* embryos (Rodríguez et al., 2013). Therefore, we concluded that the BA can no longer be regarded as an exclusive trait of teleosts (reviewed in Durán et al., 2008 and Rodríguez et al., 2013), as was classically assumed (Santer, 1985).

The presence of a BA has been briefly reported in a limited number of shark and batoid species (Durán et al., 2008; Grimes et al., 2010; Borucinska et al., 2012). However, it remains uncertain whether this OFT component occurs regularly in elasmobranchs. We have increased our collection of elasmobranch hearts with specimens from animals captured in various campaigns for the evaluation of fishery resources performed by the Spanish Institute of Oceanography. In the present study, we focused our attention on the gross anatomy of the OFT. Our first goal was to test if all species examined have actually a BA at the arterial pole of the heart, in order to verify whether the BA is an evolutionary conserved trait in elasmobranchs. Our second objective was to assess whether the potential variations in the length of the BA are related to the lifestyles and the phylogenetic position of the species, and

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additionally whether there is any relation between the length of the BA and the anatomical origin of the entire OFT from the ventricle.

2. Materials and methods

2.1. Animals

We examined the hearts from 125 sharks and batoids of the 42 species listed in [Table 1](#), which belonged to 19 families and 7 orders.

Overall, 95 specimens were collected by scientific vessels of the Spanish Institute of Oceanography in the Western Mediterranean and in the areas of Mauritania and Guinea Bissau of the Eastern Atlantic. The remaining hearts ($n=30$) belonged to the collection of the Department of Animal Biology of the University of Málaga. They were removed from sharks and rays collected in the Western Mediterranean, Eastern Atlantic and Eastern Pacific. The total length (size) of the animals was measured from the snout to a point on the horizontal axis intersecting a perpendicular line extending downward from the tip of the upper caudal lobe to form a right angle ([Kohler et al., 1996](#)).

The hearts collected in campaigns of the Spanish Institute of Oceanography were removed, and rinsed in elasmobranch buffer (16.38 g/l NaCl, 0.89 g/l KCl, 1.11 g/l CaCl₂, 0.38 g/l NaHCO₃, 0.06 g/l NaHPO₄, 21.6 g/l urea, pH 7.2). Then, they were fixed in the fixatives routinely used in our laboratory: in 4% paraformaldehyde in elasmobranch buffer, in MAW (methanol/acetone/water 2:2:1), or in Bouin fixative, for subsequent histological studies. The hearts stored in our Department were preserved in 4% formaldehyde.

2.2. Anatomical data

We studied the gross anatomy of the hearts to determine the origin of the cardiac OFT from the anterior aspect of the ventricle. We defined that the OFT origin was located in the middle of the anterior aspect of the ventricle when the CA arose from within 25% of the midline of the ventricle with respect to both the right-left and dorsoventral axes. When CAs arose outside of these boundaries the OFT origin was designed right or left, or shifted dorsally or ventrally, respectively.

The cardiac OFT of all species included in our study consisted of two components, the anatomical characteristics of which enabled their identification as CA and BA, as in previously studied elasmobranchs ([Durán et al., 2008](#); [Grimes et al., 2010](#)). According to these authors, we considered the ventriculo-conal sulcus as the proximal external limit of the CA. The anterior boundary of the conal myocardium defined the distal limit of the CA and the proximal limit of the BA. The distal limit of the BA and, consequently, that of the cardiac OFT was the anterior boundary of the pericardial cavity ([Fig. 1A](#) and [B](#)). It should be noted that, at that point, vessels coming ultimately from the hypobranquial arteries traversed the pericardium and entered the subepicardial space of the OFT, becoming the coronary arteries that irrigate the wall of both BA and CA.

The hearts were photographed with a Nikon D300S camera provided with a SIGMA DG macro lens. We took the ventral midpoint of the ventriculo-conal junction as the proximal measuring point in both the hearts where the junction ran parallel to the left-right cardiac axis ([Fig. 1A](#)) and hearts in which the CA arose from the right corner, obliquely to the left-right axis ([Fig. 1B](#)). We measured the lineal length of the CA, BA and the whole OFT. All measurements were taken in ventral view with the Image J software (NIH).

We took the relative length of the BA (RLBA) with respect to the total length of the cardiac OFT for comparative purposes. The use of relative and not absolute values counteracted errors due to

the effect of the fixatives. We calculated the RLBA by dividing the absolute length of the BA by the total length of the cardiac OFT.

2.3. Histochemical techniques for light microscopy

The hearts were dehydrated in graded ethanol and embedded in paraffin (Histosec, Merck KGaA, Darmstadt, Germany). Serial sections, sagittally cut at 8–10 μ m and fixed in either fixative, were stained with Masson-Goldner's trichrome stain for a general assessment of the tissue structure, in especial for connective tissue and musculature. Observations were made with a Leica DMSL microscope. Images were acquired by an Olympus VS120 virtual microscopy slide scanning system (Tokyo, Japan) equipped with the VS-ASW software (Olympus) and viewed using the free of charge software OlyVIA (Informer Technologies, Inc., Walnut, CA, USA).

2.4. Information about the lifestyle and phylogenetic relationships of species

We examined literature sources providing information on the habitats, biological aspects and activity of the species included in the study ([Compagno et al., 2005](#); [FishBase, 2016](#); [Fowler et al., 2005](#); [Guisande-González et al., 2011](#); [Carrier et al., 2012](#)).

To evaluate our observations from the phylogenetic viewpoint, we relied on the following assumptions about elasmobranch phylogenetic relationships. We assumed the reciprocal monophyly of sharks and batoids ([Douady et al., 2003](#); [Pavan-Kumar et al., 2014](#)). As for sharks, we took into account the notion that they are divided into two major groups, namely, galeomorphs and squalomorphs ([Vélez-Zuazo and Agnarsson, 2011](#); [Pavan-Kumar et al., 2014](#)). Finally, we assumed that the torpediniforms are the sister group to all other batoids ([Rocco et al., 2007](#)). In the material examined, the squalomorphs were represented by species belonging to the orders Hexanchiformes and Squaliformes, the galeomorphs by species of the orders Lamniformes and Carcharhiniformes, and the batoids by species of the orders Rajiformes, Myliobatiformes and Torpediniformes ([Table 1](#)).

2.5. Quantitative assessment

In some species, the sample was composed of specimens of different size, i.e., age. To find out whether the RLBA varied according to the total length of the animals, we compared the RLBA of specimens of two species, namely, *Scyliorhinus canicula* (Linnaeus, 1758) and *Dalatias licha* (Bonnaterre, 1788). We then calculated the Pearson Correlation Coefficient (Pearson's r) for each of the two species, under the null hypothesis that there is no relationship between the RLBA and size of the animals. RLBA and size were previously log-transformed to meet the parametric conditions.

We performed simple ANOVAs to seek any relationship between the RLBA and (1) the lifestyle of the species, (2) their phylogenetic position and (3) the anatomical origin of the OFT from the ventricle. Each species was considered as a sample. In the case of more than one specimen available for a given species, we calculated the median RLBA value, which was then log-transformed. Thereafter, we conducted post-hoc multiple comparisons among lifestyles, superorders or OFT origins by means of the Tukey's HSD analysis.

When statistically significant differences related to RLBA values were found, we used multiple-factor ANOVA to determine the partial contribution of these factors to explain the RLBA variance.

All statistical analyses were implemented using the software IBM SPSS Statistics 23.

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