Received: 19 March 2020 | Accepted: 7 June 2020

DOI: 10.1111/jfb.14427

REGULAR PAPER

JOURNAL OF FISH BIOLOGY fsbi

Myosin heavy chain isoforms in the myocardium of the atrioventricular junction of *Scyliorhinus canicula* (Chondrichthyes, Carcharhiniformes)

Miguel A. López-Unzu[1,2] | María Teresa Soto-Navarrete[1,2] | Valentín Sans-Coma[1,2] | Borja Fernández[1,2,3] | Ana Carmen Durán[1,2,3]

[1]Departamento de Biología Animal, Facultad de Ciencias, Universidad de Málaga, Málaga, Spain

[2]Instituto de Biomedicina de Málaga (IBIMA), Málaga, Spain

[3]Instituto de Biotecnología y Desarrollo Azul (IBYDA), Málaga, Spain

Correspondence
Ana Carmen Durán, Department of Animal Biology, Faculty of Science, University of Málaga, 29071 Málaga, Spain.
Email: acduran@uma.es

Funding information
FEDER Funds; Ministerio de Ciencia e Innovación, Grant/Award Numbers: CGL2017-85090-P, FPU15/03209, PRE2018-083176

Abstract

The atrioventricular junction of the fish heart, namely the segment interposed between the single atrium and the single ventricle, has been studied anatomically and histologically in several chondrichthyan and teleost species. Nonetheless, knowledge about myosin heavy chain (MyHC) in the atrioventricular myocardium remains scarce. The present report is the first one to provide data on the MyHC isoform distribution in the myocardium of the atrioventricular junction in chondrichthyans, specifically in the lesser spotted dogfish, *Scyliorhinus canicula*, a shark species whose heart reflects the primitive cardiac anatomical design in gnathostomes. Hearts from five dogfish were examined using histochemical and immunohistochemical techniques. The anti-MyHC A4.1025 antibody was used to detect differences in the occurrence of MyHC isoforms in the dogfish, as the fast-twitch isoforms MYH2 and MYH6 have a higher affinity for this antibody than the slow-twitch isoforms MYH7 and MYH7B. The histochemical findings show that myocardium of the atrioventricular junction connects the trabeculated myocardium of the atrium with the trabeculated layer of the ventricular myocardium. The immunohistochemical results indicate that the distribution of MyHC isoforms in the atrioventricular junction is not homogeneous. The atrial portion of the atrioventricular myocardium shows a positive reactivity against the A4.1025 antibody similar to that of the atrial myocardium. In contrast, the ventricular portion of the atrioventricular junction is not labelled, as is the case with the ventricular myocardium. This dual condition suggests that the myocardium of the atrioventricular junction has two contraction patterns: the myocardium of the atrial portion contracts in line with the atrial myocardium, whereas that of the ventricular portion follows the contraction pattern of the ventricular myocardium. Thus, the transition of the contraction wave from the atrium to the ventricle may be established in the atrioventricular segment because of its heterogeneous MyHC isoform distribution. The findings support the hypothesis that a distinct MyHC isoform distribution in the atrioventricular myocardium enables a synchronous contraction of inflow and outflow cardiac segments in vertebrates lacking a specialized cardiac conduction system.

KEYWORDS
atrioventricular junction, Chondrichthyes, heart, myocardium, myosin heavy chain

1 | INTRODUCTION

According to multidisciplinary studies carried out during the past two decades (Christoffels *et al*., 2000; Moorman *et al*., 2004; Moorman & Christoffels, 2003; Pérez-Pomares *et al*., 2009), the heart of the jawed vertebrates (gnathostomes) is currently considered to be composed of a set of segments devoted to two haemodynamic functions, namely "to receive blood upstream (inflow) and to pump it downstream (outflow)" (Simoes-Costa *et al*., 2005). The junction between the inflow and outflow segments, or more precisely, between the atria and the ventricles, is generally known as the atrioventricular junction, region or canal. It supports the atrioventricular valves that prevent blood flow from the ventricle to the atria.

The atrioventricular junction of the fish heart has been studied anatomically and histologically in several chondrichthyan (Gegenbaur, 1901; Hamlett *et al*., 1996; Victor *et al*., 1995) and teleost species (Farrell & Jones, 1992; Icardo & Colvee, 2011; Santer & Cobb, 1972; Satchell, 1991). In this context, the only work that has provided data on the myosin heavy chain (MyHC) total content in the myocardium is that of Franco *et al*. (2002). The authors carried out a comparative study between different vertebrate species: dogfish, chicken, rat and mouse. As to the dogfish, used as a chondrichthyan representative, Franco *et al*. (2002) concluded that there is a distinct "atrioventricular region" interposed between the atrium and the ventricle. They suggested that this region could be responsible for a delay in the propagation of the cardiac impulse throughout the heart, allowing the integrated performance of the atrial and ventricular myocardia.

The reason why researchers are interested on studying the dogfish heart is the fact that its gross anatomical composition, like that of other chondrichthyans, almost certainly reflects the primitive cardiac design of the gnathostomes. It consists of a sinus venosus, an atrium, an atrioventricular region (junction), a ventricle and an outflow tract composed of a myocardial conus arteriosus and a non-myocardial bulbus arteriosus (Durán *et al*., 2008; Lorenzale *et al*., 2018).

A previous work using proteomic techniques described the MyHC isoform distribution in adult dogfish cardiac segments and assessed the anti-MyHC A4.1025 antibody reactivity against the myocardium of representative species from different vertebrate groups (López-Unzu *et al*., 2019). It was found that in the dogfish and other chondrichthyans, MYH2 and MYH6 predominate in the inflow segments, whereas MYH7 and MYH7B predominate in the outflow segments. In addition, it was shown that the fast-twitch isoforms MYH2 and MYH6 have a higher affinity for the A4.1025 antibody than the slow-twitch isoforms MYH7 and MYH7B. This study was mainly devoted to the atrium and the ventricle. The main aim of the present study was to elucidate the distribution of fast- and slow-twitch MyHC isoforms in the myocardium of the atrioventricular junction of the dogfish, which is crucial for the proper propagation of the cardiac impulse throughout the heart. The results raise the hypothesis that an asymmetric distribution of fast- and slow MyHC in this cardiac segment could contribute to the synchronization of the cardiac cycle. In addition, the findings advance several issues on the structural characteristics of the atrioventricular junction of the gnathostome primitive heart that should be considered in further physiological and evolutionary research on this cardiac region.

2 | MATERIALS AND METHODS

2.1 | Animals

The sample examined consisted of hearts from five adult (two males, three females) lesser spotted dogfish (*Scyliorhinus canicula*). The specimens were caught in the Western Mediterranean by fishing vessels of Caleta de Vélez, Málaga, Spain. The animals were already dead when they were collected from the vessels. All the methodological procedures were approved by the Spanish funding agency and performed at the University of Málaga, which is a licensed establishment under the European and Spanish guidelines on the protection of animals used for scientific purposes (directive 86/609/EEC).

The ventral region of the pericardial cavity was exposed by means of a longitudinal incision along the anterior midventral line of the animal, and the heart was removed and rinsed in elasmobranch buffer (16.38 g l^{-1} NaCl, 0.89 g l^{-1} KCl, 1.11 g l^{-1} $CaCl_2$, 0.38 g l^{-1} $NaHCO_3$, 0.06 g l^{-1} $NaHPO_4$, 21.6 g l^{-1} urea, pH 7.2), as described elsewhere (Macías *et al*., 1998; Muñoz-Chápuli *et al*., 1996).

2.2 | Histochemical techniques for light microscopy

Two hearts were fixed in methanol/acetone/water (2:2:1), and three in 4% paraformaldehyde diluted in elasmobranch buffer. Then, the samples were dehydrated in a graded series of ethanol and embedded in Histosec (Merck KGaA, Darmstadt, Germany). Serial sections sagittally cut at 8 or 10 μm using a Leitz 1512 microtome (Leitz, Wetzlar, Germany) were stained with Masson–Goldner's trichrome stain for a general assessment of the tissue structure and picrosirius staining and polarization microscopy (Junqueira *et al*., 1979) for the detection of collagen.

2.3 | Immunohistochemical techniques

Paraffin sections were dewaxed, hydrated and washed in Tris-phosphate-buffered saline (TPBS, pH 7.8). For immunoperoxidase, endogenous peroxidase activity was quenched by incubation for 30 min with 3% hydrogen peroxide in TPBS. After washing with TPBS, non-specific binding sites were saturated for 30 min with 10% sheep serum, 1% bovine serum albumin and 0.1% Triton X-100 in TPBS (SBT). The slides were then incubated overnight at 4°C in the primary antibody diluted in SBT. The primary antibody was A4.1025 (DSHB category number A4.1025, RRID: AB_528356) anti-MyHC monoclonal antibody (Developmental Studies Hybridoma Bank, University of Iowa) or anti-alpha-smooth muscle (SM) actin antibody (Clone 1A4, Sigma-

Aldrich Chemical Co., Poole, UK). It was used at dilutions of 1:200 and 1:300 of the respective supernatants. Negative control sections were incubated in SBT only. After incubation, the slides were washed in TPBS, incubated for 1.5 h at room temperature in biotin-conjugated anti-mouse IgG (Sigma-Aldrich) diluted 1:1000 in SBT, washed again and incubated for 1 h in ExtraAvidin-peroxidase complex (Sigma-Aldrich) diluted 1:250 in TPBS. Peroxidase activity was detected using Sigma Fast 3,3′-diaminobenzidine tablets (Sigma-Aldrich) following the indications of the supplier. Sections were counterstained with haematoxylin. All the sections were observed using a Leica DMSL light microscope or an Olympus VS120 virtual microscopy slide scanning system (Olympus, Tokyo, Japan) equipped with the *VS*-ASW software (Olympus) and viewed using the free-of-charge software OlyVIA (Informer Technologies, Inc., Walnut, CA, USA).

3 | RESULTS

Macroscopically, the heart of the adult lesser spotted dogfish is composed of sinus venosus, atrium, ventricle, conus arteriosus and bulbus arteriosus. Except for the bulbus arteriosus, all these components have myocardium in their walls (Figure 1a). The myoarchitecture differs between the different cardiac chambers. The sinus venosus has a thin wall in which myocardial cells form small bundles. The atrium shows a thicker wall, with a slender trabeculated myocardium, which projects the so-called pectinate muscles towards the lumen. The ventricle possesses mixed-type myocardium consisting of a thick, inner spongy (trabeculated) layer covered by an outer layer of compact cardiac muscle (Figure 1a). The conus arteriosus has compact myocardium. Histology also reveals the presence of a segment intercalated between the atrium and the ventricle, the atrioventricular junction, which is imperceptible macroscopically.

Immunostaining with the anti-MyHC antibody A4.1025 shows a heterogeneous mark in the dogfish heart. The myocardial walls of the sinus venosus and the atrium are positive to A4.1025. The ventricular mixed myocardium is negative, except for the apex region where the trabecular layer is slightly positive (Figure 1b). The conus arteriosus is also negative.

The atrioventricular junction is composed of compact myocardium that is continuous with both the trabeculated myocardium of the atrium and the trabeculated layer of the ventricle. The atrioventricular myocardium consists of bundles, mainly arranged circumferentially, which are surrounded by connective tissue that is continuous with the fibrous tissue of the atrioventricular leaflet (Figure 2a). Picrosirius staining and polarization microscopy enhance the natural birefringence of collagen bundles. This technique was used to detect the arrangement of collagen at the atrioventricular junction. In the subendocardial space, collagen fibres, mainly type I, are densely arranged. In the mid-myocardial layer of the atrioventricular region, collagen fibres types I and III are oriented parallel and perpendicular to the endocardium, respectively, forming a network (Figure 2b). The atrioventricular myocardium is well supplied by small coronary branches arising from the left ventricular coronary artery that runs next to the atrioventricular venous ring (Figure 2c). A4.1025 labelling differs throughout the atrioventricular segment. The myocardium next to the atrium, which mainly supports the atrioventricular leaflet, is positive. Nonetheless, the atrioventricular myocardium in contact with the ventricle shows no mark (Figure 2d).

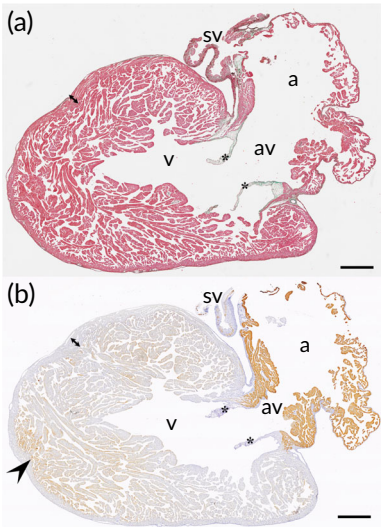

FIGURE 1 Sagittal sections of the heart of an adult *Scyliorhinus canicula*. (a) Masson–Goldner's trichrome stain. The atrium (a) possesses trabeculated myocardium. The ventricle has mixed myocardium composed of a compact (double-headed arrow) and a trabeculated layer. The atrioventricular junction (av), with compact myocardium, supports the atrioventricular valves (asterisks). (b) Immunolabelling with A4.1025 anti-myosin heavy chain antibody counterstained with haematoxylin. The myocardia of the sinus venosus (sv) and atrium (a) are intensely labelled with the antibody. The ventricular (v) myocardium shows no reactivity against the antibody, except for the trabecular layer of the apex region (arrowhead). Scale bars: 1 mm

4 | DISCUSSION

This is the first study to report on the distribution of MyHC isoforms in the myocardium of the atrioventricular junction in an adult vertebrate species. It was carried out in the lesser spotted dogfish, *S. canicula*, considering that the heart of this species reflects, almost certainly, the primitive anatomical design in gnathostomes.

A previous molecular study conducted in the adult dogfish observed that the myocardium of the different cardiac segments displays a distinct composition in terms of MyHC isoforms (López-Unzu *et al*., 2019). The venous pole (sinus venosus and atrium) has a higher concentration of fast-twitch MyHC isoforms, specifically MYH2 and MYH6, compared to the myocardium of the arterial pole (ventricle and conus arteriosus), which is rich in slow-twitch and tonic isoforms (MYH7 and MYH7B). This distribution of fast-twitch *vs*. slow-twitch MyHC isoforms coincides with that in other vertebrate groups. It has been described that orthologs of the human fast-twitch cardiac myosin MYH6, as the atrial myosin of *Danio rerio* or *Gallus gallus* (aMyHC)

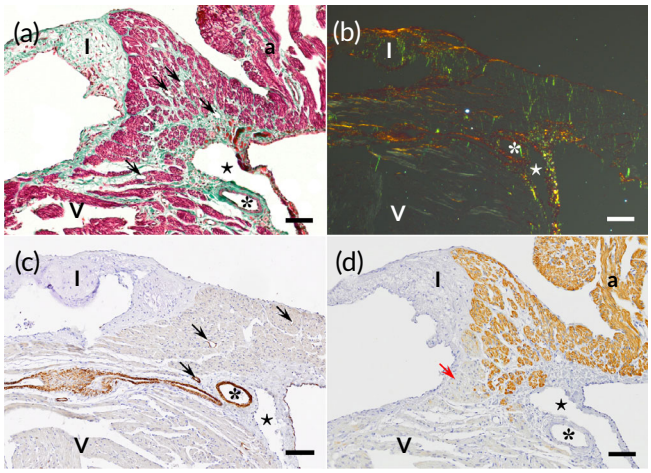

FIGURE 2 Sagittal sections of the atrioventricular junction of an adult *Scyliorhinus canicula*. (a) Masson–Goldner's trichrome stain. Detailed view of the atrioventricular junction. The compact myocardium is well irrigated by several coronary arteries (arrows) arising from the left ventricular coronary artery. (b) Picrosirius staining and polarization microscopy. The luminal side of the leaflet and the atrioventricular subendocardium are enriched with collagen fibres type I. In the mid-myocardial layer of the atrioventricular junction, type I fibres (reddish) are parallel and type III fibres (greenish) are oriented perpendicular with respect to the endocardium. (c) Immunolabelling with anti-alpha-smooth muscle actin counterstained with haematoxylin. The coronary arteries are positive. The atrioventricular myocardium is fully irrigated (arrows). (d) Immunolabelling with A4.1025 anti-myosin heavy chain antibody counterstained with haematoxylin. The myocardium next to the atrium is positive. The atrioventricular myocardium contacting the ventricle shows no signal (red arrow). a: atrium; l: atrioventricular leaflet; v: ventricle; asterisk: left ventricular coronary artery; star: atrioventricular venous ring. Scale bars: 100 μm

and alpha-myosin of *Mus musculus* (α-MyHC), are more abundant in the atrial myocardium of each species. Furthermore, orthologs of the slow-twitch myosin of the human myosin MYH7, as the ventricular myosin of *D. rerio* or *G. gallus* (vMyHC) and beta myosin of *M. musculus* (β-MyHC), are more abundant in the ventricular myocardium (Berdougo *et al*., 2003; Bisaha & Bader, 1991; England & Loughna, 2013; Mascarello *et al*., 2009; Moore *et al*., 1992; Morkin, 2000; Reiser & Kliner, 1998; Somi *et al*., 2005; Yelon *et al*., 1992; Yuztey *et al*., 1994). Thus, it has been concluded that the atrial myocardium of the vertebrates is enriched with fast myosin isoforms, whereas the ventricular myocardium displays a higher concentration of slow isoforms (England & Loughna, 2013). A previous study (López-Unzu *et al*., 2019) also demonstrated that in the dogfish, the anti-MyHC antibody A4.1025 does not bind all the MyHC isoforms with the same affinity. The fast-twitch isoforms MYH2 and MYH6 have a higher affinity for the antibody than the slow-twitch isoforms MYH7 and MYH7B (López-Unzu *et al*., 2019). The myocardium of the venous pole, with a high concentration of fast-twitch MyHC, can be morphologically recognized because of the intense positive staining with the A4.1025 antibody (López-Unzu *et al*., 2019).

The results obtained in the present work using A4.1025 show that in the atrioventricular myocardium of the dogfish, two portions can be distinguished based on the isomyosin distribution, namely an atrial portion and a ventricular portion. The atrial portion is in continuity with the atrial trabeculated myocardium and is strongly positive for A4.1025. This intense mark with A4.1025 indicates that the myocardiocytes of the atrial portion express fast-twitch MYH2 and MYH6, revealed by the distinct affinity of this antibody for different MyHC isoforms (López-Unzu *et al*., 2019). The ventricular portion includes the wedge-form compact myocardium which is in continuity with the trabeculated layer of the ventricular myocardium. The lack of A4.1025 mark indicates that MYH7 and MYH7B, both slow-twitch MyHC, are abundant in this portion (see López-Unzu *et al*., 2019). These results suggest that the myocardium of the dogfish atrioventricular junction has two contraction patterns: the myocardium of the atrial portion follows the contraction pattern of the atrial myocardium, whereas that of the ventricular portion contracts in line with the ventricular myocardium.

In addition to the distribution of MyHC isoforms, the histochemical and immunohistochemical studies of the present work are consistent with the notion that the atrioventricular junction is composed of compact myocardia, which is in continuity with the trabeculated myocardium of the atrium and the ventricle. The immunohistochemical results with anti-alpha-SM actin antibodies show that the left ventricular coronary artery runs through the atrioventricular junction, giving off branches that irrigate the whole atrioventricular myocardium, a characteristic of the compact myocardium. In addition, the picrosirius staining reveals the existence of a slight collagen meshwork between muscle fibres of the atrioventricular myocardium, similar to the interstitium of the atrial and ventricular myocardia. No real fascia enveloping the atrioventricular myocardium was found. This pool of data indicates that there is a continuity between the myocardia of the atrium, the atrioventricular junction and the ventricle in terms of both the tissue constitution and the MyHC isoform distribution.

This myocardial continuity has been previously observed in several teleost species and in tetrapods like *Xenopus* using histochemical techniques and semithin sections (Icardo & Colvee, 2011; Jensen *et al*., 2012). Both teleosts and amphibians lack the components of the mammalian conduction system responsible for transferring the cardiac impulse from the atrium to the ventricle, that is, the atrioventricular node and the His bundle. In birds and mammals, the myocardial atrioventricular junction found in fishes had been substituted during evolution by a connective tissue ring between the atria and ventricles which isolates the conduction in the ventricle from that in the atrium (Jensen *et al*., 2012). In birds and mammals, the impulse from the inflow to the outflow segments is transferred only through the specialized myocardium of the atrioventricular node and bundle of His, thus causing a delay in the impulse necessary for the correct contraction of the heart (reviewed by Jensen *et al*., 2012). The present results raise the hypothesis that in vertebrates lacking a specialized conduction myocardium, the atrioventricular junction may perform a coordination role in the cardiac cycle by transferring the cardiac impulse from the atrium to the

ventricle by virtue of its histological continuity with the adjacent myocardia and its dual MyHC isoform constitution.

A similar hypothesis was put forward by Franco *et al.* (2002). They described a lower mRNA and protein expression of total MyHC in the atrioventricular junction of the embryonic and adult dogfish heart in comparison with the atrium and ventricle. The authors suggested that the reduced MyHC concentration in the atrioventricular junction would cause a delay in the cardiac impulse from the atrium to the ventricle, thus providing a coordination role of this segment in the cardiac cycle. In addition, they proposed that in birds and mammals, this pattern of cardiac impulse conduction was achieved during late embryonic stages. In early embryos, after the tubular heart stage, a myocardial atrioventricular junction develops, which has a different composition in isomyosins as compared to the adjacent cardiac segments, namely the atrium and ventricle. In particular, there is a similar concentration of α-MyHC (MYH6) and β-MyHC (MYH7). Because of this differential molecular profile, the atrioventricular junction has been considered as a distinct functional segment during the development but not in the adult heart, in which the myocardial atrioventricular portion is substituted by a connective ring (De Groot *et al.*, 1989; De Jong *et al.*, 1990; Franco *et al.*, 2002). De Groot *et al.* (1989) indicate that the zonation of each isomyosin in the developing heart coincides with the change in the pattern of contraction, becoming synchronous between the venous and arterial poles. Thus, the present study's results, together with those in the literature, suggest that the distinct distribution of fast-twitch and slow-twitch MyHC isoforms in the atrioventricular junction of vertebrates without a complete specialized conduction system, including avian and mammalian embryos, may serve to assure the proper cardiac contraction.

In conclusion, the atrioventricular myocardium of the dogfish is composed of two portions which are distinguished according to the isomyosin distribution, namely an atrial portion and a ventricular portion. This study proposes that in vertebrates without a well-developed atrioventricular conduction system, this asymmetric isomyosin zonation be used to coordinate the cardiac cycle. Further studies on the MyHC isoforms' distribution in the atrioventricular myocardium of other vertebrate groups with a myocardial atrioventricular junction (*e.g.*, teleost or *Xenopus*) are needed to assess whether this trait is a synapomorphy for chondrichthyans or a symplesiomorphy for gnathostomes lacking a well-developed conduction system.

ACKNOWLEDGEMENTS

This work was supported by Ministerio de Ciencia e Innovación, Madrid, Spain (grants numbers: CGL2017-85090-P and FPU15/03209 to M.A.L.-U. and PRE2018-083176 to M.T.S.-N.) and FEDER funds. We thank Dr. David Navas from the Servicios Centrales de Apoyo a la Investigación (University of Málaga, Spain) for his assistance in microscopy techniques. We also thank Mr. Sebastián Martín, Mr. Marcos Fernández (Caleta de Vélez, Málaga, Spain) and Cofradía de Pescadores Caleta de Vélez (Málaga, Spain) for their kind contribution in obtaining the material examined.

AUTHOR CONTRIBUTIONS

M.A.L.-U. and M.T.S.-N. initiated the study and carried out the morphological and histochemical studies of the heart of *S. canicula*, assisted by B.F., V.S.C. and A.C.D. M.T.S.-N. and M.A.L.-U. performed the immunohistochemical study. All authors contributed to the interpretation of the results obtained. M.A.L.-U. and V.S.C. drafted the manuscript with critical revising by all authors and major improvements by A.C.D. and B.F. All authors read and approved the final manuscript.

ORCID

Miguel A. López-Unzu https://orcid.org/0000-0002-8538-0713

REFERENCES

Berdougo, E., Coleman, H., Lee, D. H., Stainier, D. Y. R., & Yelon, D. (2003). Mutation of weak atrium/atrial myosin heavy chain disrupts atrial function and influences ventricular morphogenesis in zebrafish. *Development*, *130*(24), 6121–6129.

Bisaha, J. G., & Bader, D. (1991). Identification and characterization of a ventricular specific avian myosin heavy chain, VMYHC1: expression in differentiating cardiac and skeletal muscle. *Developmental Biology*, *148*, 355–364.

Christoffels, V. M., Habets, P. E., Franco, D., Campione, M., de Jong, F., Lamers, W. H., … Moorman, A. F. M. (2000). Chamber formation and morphogenesis in the developing mammalian heart. *Developmental Biology*, *223*, 266–278.

De Groot, I. J. M., Lamers, W. H., & Moorman, A. F. M. (1989). Isomyosin expression pattern during rat heart morphogenesis: an immunohistochemical study. *The Anatomical Record*, *224*, 365–373.

De Jong, F., Geerts, W. J. C., Lamers, W. H., Los, J. A., & Moorman, A. F. M. (1990). Isomyosin expression pattern during the formation of the tubular chicken heart: a 3D immunohistochemical analysis. *The Anatomical Record*, *226*, 213–227.

Durán, A. C., Fernández, B., Grimes, A. C., Rodríguez, C., Arqué, J. M., & Sans-Coma, V. (2008). Chondrichthyans have a bulbus arteriosus at the arterial pole of the heart: morphological and evolutionary implications. *Journal of Anatomy*, *213*(5), 597–606.

England, J., & Loughna, S. (2013). Heavy and light roles: myosin in the morphogenesis of the heart. *Cellular and Molecular Life Sciences*, *70*(7), 1221–1239.

Farrell, A. P., & Jones, D. R. (1992). The heart. In W. S. Hoar, D. Randall, & A. P. Farrell (Eds.), *Fish physiology. The cardiovascular system, Part A* (pp. 1–87). San Diego, CA: Academic Press.

Franco, D., Gallego, A., Habets, P. E. M. H., Sans-Coma, V., & Moorman, A. F. M. (2002). Species-specific differences of myosin content in the developing cardiac chambers of fish, birds, and mammals. *The Anatomical Record*, *268*, 27–37.

Gegenbaur, C. (1901). *Vergleichende Anatomie der Wirbelthiere mit Berücksichtigung der Wirbellosen*. Leipzig: Wilhelm Engelman.

Hamlett, W. C., Schwartz, F. J., Schmeinda, R., & Cuevas, E. (1996). Anatomy, histology and development of the cardiac valvular system in elasmobranchs. *Journal of Experimental Zoology*, *275*, 83–94.

Icardo, J. M., & Colvee, E. (2011). The atrioventricular region of the teleost heart. A distinct heart segment. *The Anatomical Record*, *294*, 236–242.

Jensen, B., Boukens, B. J. D., Postma, A. V., Gunst, Q. D., Van Den Hoff, M. J. B., Moorman, A. F. M., … Christoffels, V. M. (2012). Identifying the evolutionary building blocks of the cardiac conduction system. *PLoS One*, *7*(9), e44231.

Junqueira, L. C. V., Bignolas, G., & Brentani, R. R. (1979). Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *The Histochemical Journal*, *11*, 447–435.

López-Unzu, M. A., Durán, A. C., Soto-Navarrete, M. T., Sans-Coma, V., & Fernández, B. (2019). Differential expression of myosin heavy chain isoforms in cardiac segments of gnathostome vertebrates and its evolutionary implications. *Frontiers in Zoology*, *16*(1), 18.

Lorenzale, M., López-Unzu, M. A., Rodríguez, C., Fernández, B., Durán, A. C., & Sans-Coma, V. (2018). The anatomical components of the cardiac outflow tract of chondrichthyans and actinopterygians. *Biological Reviews*, *93*, 1604–1619.

Macías, D., Pérez-Pomares, J. M., García-Garrido, L., & Muñoz-Chápuli, R. (1998). Immunohistochemical study of the origin of the subepicardial mesenchyme in the dogfish (*Scyliorhinus canicula*). *Acta Zoologica*, *79* (4), 335–342.

Mascarello, F., Toniolo, L., Cancellara, P., Reggiani, C., & Maccatrozzo, L. (2009). Expression and identification of 10 sarcomeric MyHC isoforms in human skeletal muscles of different embryological origin. Diversity and similarity in mammalian species. *Annals of Anatomy*, *207*, 9–20.

Moore, L. A., Tidyman, W. E., Arrizubieta, M. J., & Bandman, E. (1992). Gene conversions within the skeletal myosin multigene family. *Journal of Molecular Biology*, *223*, 368–387.

Moorman, A. F. M., & Christoffels, V. M. (2003). Cardiac chamber formation: development, genes, and evolution. *Physiological Reviews*, *83*, 1223–1267.

Moorman, A. F. M., Soufan, A. T., Hagoort, J., De Boer, P. A. J., & Christoffels, V. M. (2004). Development of the building plan of the heart. *Annals of the New York Academy of Sciences*, *1015*, 171–181.

Morkin, E. (2000). Control of cardiac myosin heavy chain gene expression. *Microscopy Research and Techniques*, *50*, 522–531.

Muñoz-Chápuli, R., Macías, D., Ramos, C., Gallego, A., & De Andrés, V. (1996). Development of the subepicardial mesenchyme and the early cardiac vessels in the dogfish (*Scyliorhinus canincula*). *The Journal of Experimental Zoology*, *275*, 95–111.

Pérez-Pomares, J. M., González-Rosa, J. M., & Muñoz-Chápuli, R. (2009). Building the vertebrate heart – an evolutionary approach to cardiac development. *The International Journal of Developmental Biology*, *53*, 1427–1443.

Reiser, P. J., & Kliner, W. O. (1998). Electrophoretic separation and quantitation of cardiac myosin heavy chain isoforms in eight mammalian species. *American Journal of Physiology*, *273*(3), H1048–H1053.

Santer, R. M., & Cobb, J. L. S. (1972). The fine structure of the heart of the teleost *Pleuronectes platessa L. Zeitschrift fur Zellforschung und mikroskopische Anatomie*, *131*, 1–14.

Satchell, G. H. (1991). *Physiology and form of fish circulation*. Cambridge: Cambridge University Press.

Simoes-Costa, M. S., Vasconcelos, M., Sampaio, A. C., Cravo, R. M., Linhares, V. L., Hochgreb, T., … Xavier-Neto, J. (2005). The evolutionary origin of cardiac chambers. *Developmental Biology*, *277*, 1–15.

Somi, S., Klein, A. T. J., Houweling, A. C., Ruijter, J. M., Buffing, A. A. M., Moorman, A. F. M., & Van den Hoff, M. J. B. (2005). Atrial and ventricular myosin heavy-chain expression in the developing chicken heart: strengths and limitations of non-radioactive *in situ* hybridization. *Journal of Histochemistry & Cytochemistry*, *54*(6), 649–664.

Victor, S., Nayak, V. M., Raveen, R., & Gladstone, M. (1995). Bicuspid evolution of the arterial and venous atrioventricular valves. *Journal of Heart Valve Disease*, *4*, 78–87.

Yelon, D., Horne, S. A., & Stainier, Y. R. (1992). Restricted expression of cardiac myosin genes reveals regulated aspects of heart tube assembly in zebrafish. *Developmental Biology*, *214*, 23–37.

Yuztey, K. E., Rhee, J. T., & Bader, D. (1994). Expression of the atrial-specific myosin heavy chain AMHC1 and the establishment of anteroposterior polarity in the developing chicken heart. *Development*, *120*, 871–883.

How to cite this article: López-Unzu MA, Soto-Navarrete MT, Sans-Coma V, Fernández B, Durán AC. Myosin heavy chain isoforms in the myocardium of the atrioventricular junction of *Scyliorhinus canicula* (Chondrichthyes, Carcharhiniformes). *J Fish Biol*. 2020;1–6. https://doi.org/10.1111/jfb.14427