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BRIEF COMMUNICATION

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Laterality defect of the heart in non-teleost fish

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Abstract

Dextrocardia is a rare congenital malformation in humans in which most of the heart mass is positioned in the right hemithorax rather than on the left. The heart itself may be normal and dextrocardia is sometimes diagnosed during non-related explorations. A few reports have documented atypical positions of the cardiac chambers in farmed teleost fish. Here, we report the casual finding of a left–right mirrored heart in an 85 cm long wild-caught spiny dogfish (*Squalus acanthias*) with several organ malformations. Macroscopic observations showed an outflow tract originating from the left side of the ventricular mass, rather than from the right. Internal inspection revealed the expected structures and a looped cavity. The inner curvature of the loop comprised a large trabeculation, the bulboventricular fold, as expected. The junction between the sinus venosus and the atrium appeared normal, only mirrored. MRI data acquired at 0.7 mm isotropic resolution and subsequent 3D-modeling revealed the atrioventricular canal was to the right of the bulboventricular fold, rather than on the left. Spurred by the finding of dextrocardia in the shark, we revisit our previously published material on farmed Adriatic sturgeon (*Acipenser naccarii*), a non-teleost bony fish. We found several alevins with inverted (left-loop) hearts, amounting to an approximate incidence of 1%–2%. Additionally, an adult sturgeon measuring 90 cm in length showed abnormal topology of the cardiac chambers, but normal position of the abdominal organs. In conclusion, left–right mirrored hearts, a setting that resembles human dextrocardia, can occur in both farmed and wild non-teleost fish.

KEYWORDS
atrioventricular canal, MRI, shark, sturgeon

1 | INTRODUCTION

While dissecting formalin-fixed spiny dogfish (*Squalus acanthias*) as part of an elective course for medical students at our institute, we came across one case of apparent dextrocardia in a male specimen that was outwardly mostly normal. A setting resembling situs inversus has been reported in inbred strains of platyfish (Baker-Cohen, 1961) and farmed salmonids can exhibit various cardiac malformations, including abnormal chamber topology (Frisk et al., 2020; Poppe et al., 2002). Given the high degree of left–right symmetry of the heart in the vast majority of bony fish (Icardo, 2017), even when a substantial conus arteriosus is present (Icardo, 2006), it is challenging to assess whether the cardiac chambers may be affected by a laterality defect such as situs inversus (Baker-Cohen, 1961). Early in development, however, bony fish hearts exhibit much more asymmetry than in adult stages, both

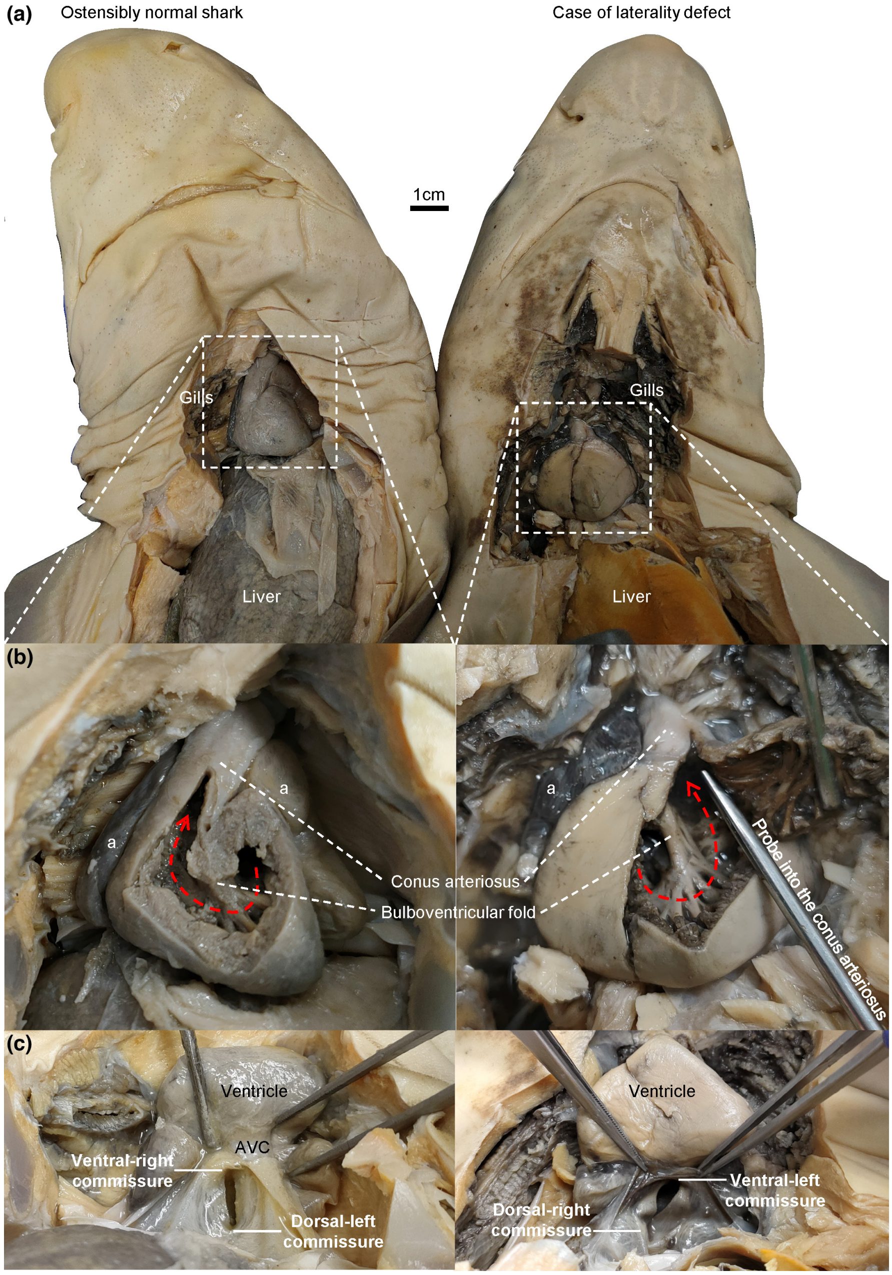
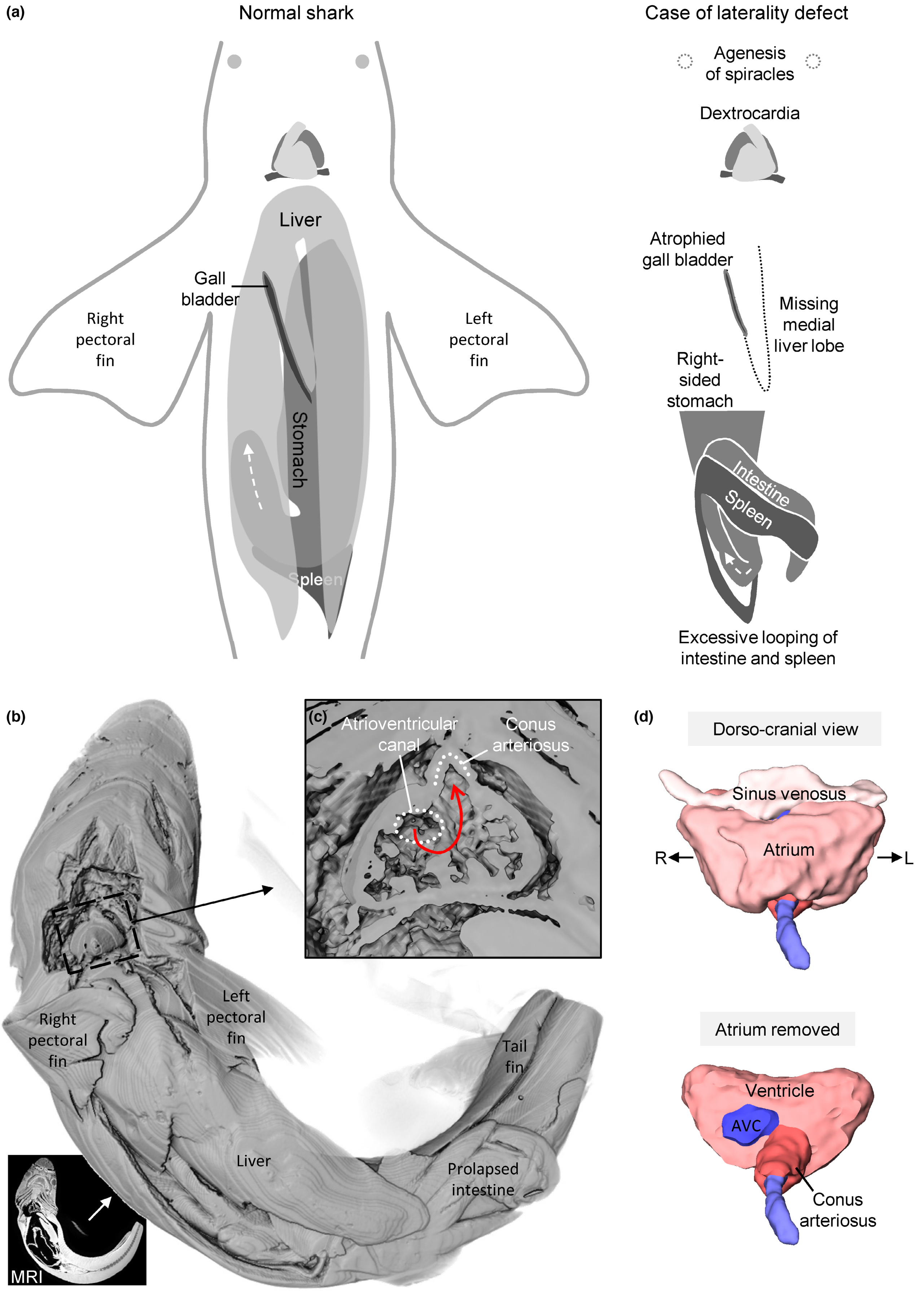


FIGURE 1 Spiny dogfish with laterality defects. (a) The shark that exhibited signs of laterality defects (right panel) looks normal. (b) The heart of the case specimen shows all the expected structural components, but the outflow tract is located to the left. Thus, it mirrors the normal setting (compare the dashed red arrows). (c) Caudal view of the sinuatrial valve, showing abnormal positions of the valve commissures in the case specimen.

anatomically and molecularly (Icardo et al., 2004; Noël et al., 2013; Smith & Uribe, 2021). In humans, dextrocardia together with situs inversus of other organs can be a casual finding and only in about a quarter of the cases present with overt cardiac malformations (Bohun et al., 2007). To our knowledge, this is the first report of a left–right mirrored heart in a cartilaginous fish. In addition, we revisited the material of a series of studies on the heart of the Adriatic sturgeon (Icardo et al., 2002, 2004, 2009) and report left–right mirrored hearts in several alevins and in a specimen of approximately 90 cm total length (TL).

FIGURE 2 Overview of malformations in the Spiny dogfish with laterality defect. (a) On the left is shown in ventral view the approximate normal position and morphology of organs that were overtly malformed in the case shark. On the right are schematic representations of the malformations seen in the shark with laterality defect. (b) Volume rendering of the whole shark in ventral view, based on MRI. (c) The reconstructed ventricle recapitulates the macroscopic inspection shown in Figure 1b. (d) Reconstructed model of the heart in which the removal of the atrium reveals the right-sided position of the atrioventricular canal (AVC). L, left; R, right.


(a)
Normal shark
Case of laterality defect
Agenesis of spiracles
Dextrocardia
Liver
Gall bladder
Right pectoral fin
Left pectoral fin
Stomach
Spleen
Atrophied gall bladder
Missing medial liver lobe
Right-sided stomach
Intestine
Spleen
Excessive looping of intestine and spleen
(b)
(c)
Atrioventricular canal
Conus arteriosus
Right pectoral fin
Left pectoral fin
Tail fin
Liver
Prolapsed intestine
MRI
(d)
Dorso-cranial view
Sinus venosus
Atrium
R
L
Atrium removed
Ventricle
AVC
Conus arteriosus

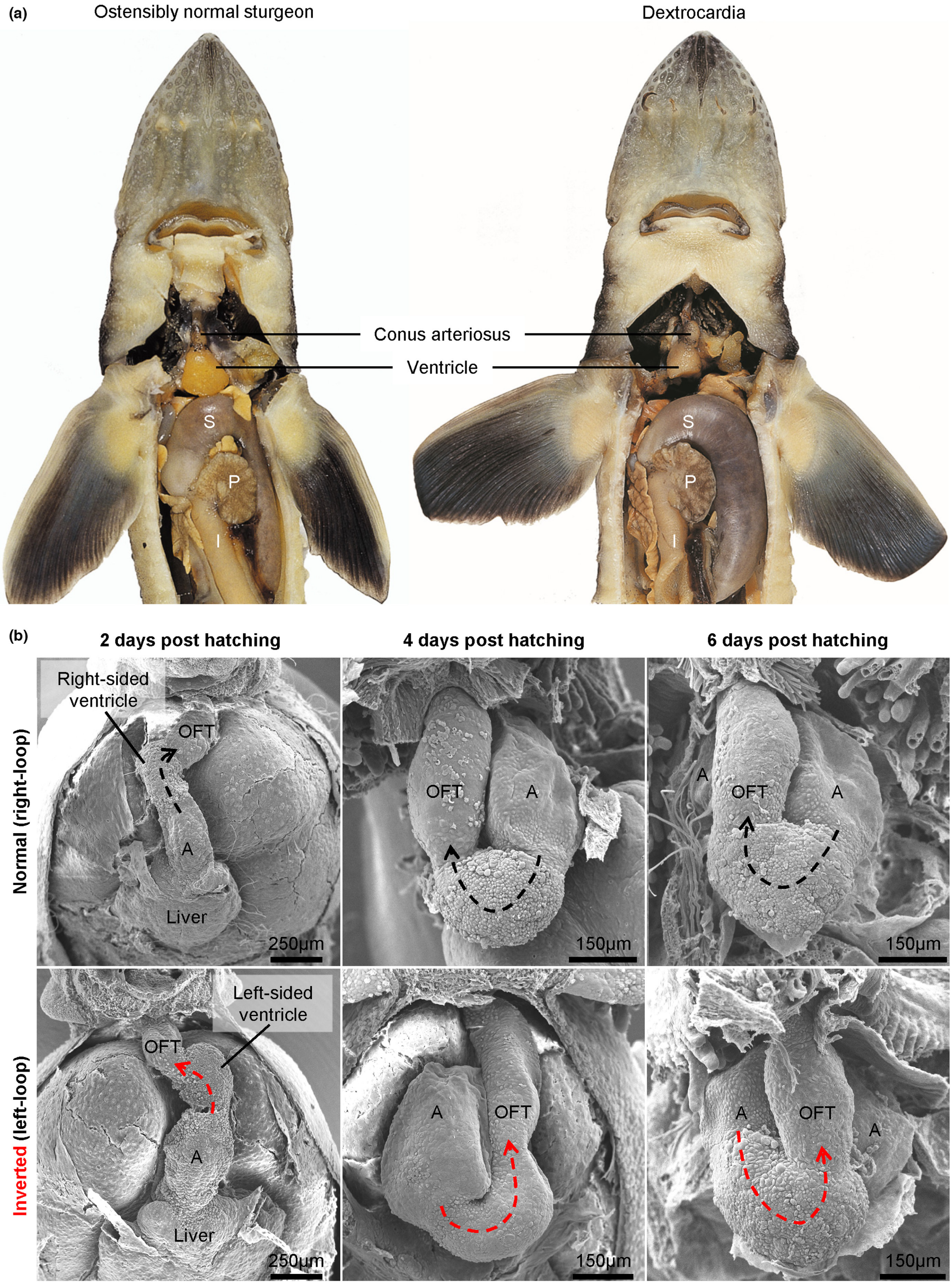

(a)
Ostensibly normal sturgeon
Dextrocardia
Conus arteriosus
Ventricle
S
P
I
S
P
I
(b)
2 days post hatching
4 days post hatching
6 days post hatching
Normal (right-loop)
Right-sided ventricle
OFT
A
Liver
250μm
OFT
A
150μm
A
OFT
A
150μm
Inverted (left-loop)
Left-sided ventricle
OFT
A
Liver
250μm
A
OFT
150μm
A
OFT
A
150μm

FIGURE 3 Adriatic sturgeon (*Acipenser naccarii*) with laterality defects. (a) The adult sturgeon with signs of laterality defects (right panel) looks normal generally. However, the ventricle is displaced to the right and the outflow tract is displaced to the left. Other organs keep the normal spatial relationships, such as the U-shaped stomach (S), the pyloric appendage (P), and the anterior intestine (I). (b) Hearts of sturgeon alevins with normal, right-loop of the ventricle (top row) and cases of mirrored looping, or left-loop (bottom row). A, atrium; OFT, outflow tract.

2 | MATERIALS AND METHODS

2.1 | Spiny dogfish

Six spiny dogfish of approximately 85 cm length and both sexes, previously fixed, were dissected as part of an elective course on evolution at Amsterdam UMC. The case specimen and an ostensibly normal shark were kept afterward in a preservation fluid containing low concentrations of ethanol, glycerol, and phenol. For MRI, to wash out compounds in the preservation fluid that diminishes the tissue-free fluid contrast, the two sharks were placed under running tap water for 3 days. After that, over the weekend, they were stored at 5°C in a sealed plastic bag before being MRI scanned on Whit Monday. They were scanned on a 3T Ingenia clinical MRI scanner (Philips, Best, the Netherlands) using a standard 16 channel anterior coil in combination with 12 posterior coil elements fixed in the MRI table. A high-resolution isotropic 3D T1-weighted gradient-echo sequence was used in order to suppress the fluid for optimal contrast. Specific sequence parameters were: TR/TE = 10/2.1 ms, flip angle = 20°, FOV = 400 × 280 × 150 mm, resolution = 0.7 × 0.7 × 0.7 mm^3, total scan time = 14 min. The MRI image stack of the case specimen is given as Supporting Information Figure S1. We imported the image stack to the 3D software Amira (version 3D 2021.2, FEI SAS, Thermo Fisher Scientific). A Volume Rendering was made of the whole scan and a plane of sectioning was made using the Slice module. Next, labeling of the cardiac chambers and the atrioventricular canal was done in the Segmentation Editor module. From the label file, using the Generate Surface function, a surface file was made that was visualized using the Surface View function. This was done in the same window as the volume rendering whereby the heart model was projected into it. The conversion of the 3D model to an interactive pdf was done as previously described (de Bakker et al., 2016).

2.2 | Adriatic sturgeons

Cardiac malformations in alevins of the autochthonous Adriatic sturgeon *Acipenser naccarii* (*A. naccarii* Bonaparte 1836) were found among specimens from previous studies (Icardo et al., 2004, 2009). Briefly, alevins obtained from the Sierra Nevada Fishery at Riofrío, Granada, Spain, were collected between days 1 and 28 post-hatching (dph). For scanning electron microscopy (SEM), the specimens were fixed in 3% glutaraldehyde in phosphate-buffered saline (PBS) for 3–5 h. Then, the samples were microdissected under a stereomicroscope, dehydrated in graded acetone, dried by the critical-point method with CO_2 as the transitional fluid and coated with gold following routine procedures. Samples were observed with an Inspect S microscope (FEI Company) working at 15–20 KV. In addition, an unpublished case of laterality defect in a sturgeon of approximately 90 cm TL was found among the material of a previous study (Icardo et al., 2002).

3 | RESULTS AND DISCUSSION

The dissected sharks measured 85 cm TL (Figure 1). Due to dissection and preparation, several organs were displaced. The heart of the case specimen, however, was in situ when it was realized that the ventricle was left–right mirrored. The stomach of the case specimen was full with partially digested fish and the testis were enlarged indicating that the shark had entered the reproductive stage. There was bilateral agenesis of the spiracles, whereas the pseudo branches and the gills appeared normal. Most of the spiral intestine was prolapsed out of the cloaca. It was not clear whether the prolapse had already occurred when the shark was still alive. The liver was relatively large, yellowish in color. It comprised two large lobes, as expected, but it lacked the third, smaller, right-sided medial lobe found in the other specimens (Figure 2a). On the left cranial-medial side of the right liver lobe, a small fibrotic sack-like structure was found. Presumably, this is an atrophied gall bladder. The stomach was mostly right-sided instead of being mostly on the left. Possibly, the filling of the stomach by prey combined with the cloacal prolapse, may have contributed to its shifted position. The pylorus curved to the left. From the pylorus, the small intestine curved cranially, then crossed to the left side and, finally, curved caudally (Figure 2a). Both the spleen and pancreas had an abnormal morphology. Instead of the normal triangular shape, the spleen had an elongated shape, was located on the ventro-lateral side of the stomach and continued over the additional looping of the intestines (Figure 2a). The dorsal pancreas was also excessively elongated and followed the looping of the small intestine. The ventral pancreas had a normal position with respect to the intestine. Both pancreatic lobes were situated on the left side of the body. In aggregate, the visceral organs gave multiple indications of pronounced deviations from the normal topology.

The heart of cartilaginous fish has a pronounced left–right asymmetry. The ventricular inlet, or atrioventricular canal, is in the dorsal-left part of the ventricular mass (Hirasaki et al., 2018; Icardo, 2017; Sanchez-Quintana & Hurle, 1987). Blood ejected from the ventricle exits on the right, into the myocardial conus arteriosus (Grimes & Kirby, 2009; Lorenzale et al., 2018). There is then a pronounced loop in the central cavity of the ventricle, around the so-named bulboventricular fold (Goor et al., 1972; Icardo et al., 2004; Jensen et al., 2013; Van Mierop & Kutsche, 1984). This configuration, conserved through evolution, is the retention of the cardiac looping that took place during embryogenesis (Pelster & Bemis, 1991). On the luminal side of the ventricle, the bulboventricular fold appears as a large ridge-like aggregate of trabecular muscle (Figure 1b).

The bulboventricular fold was readily seen both in the normal hearts and in the heart with the laterality defect (Figure 1b). This suggests that the ventricle of the case specimen contained all the normal structural components. However, whereas the conus arteriosus is normally found to the right of the bulboventricular fold, the conus arteriosus of the case specimen was located to the left of the bulboventricular fold (Figure 1b). A large coronary vessel was present on either side of the conus arteriosus in all sharks. In the normal shark, the sinuatrial junction was located in the midline and appeared guarded by a two-leaflet valve (Figure 1c) (Gallego et al., 1997; Hirasaki et al., 2018). The free margin of the leaflets formed a midline slit that was slightly askew to the dorsoventral axis (Figure 1c). This orientation was inverted in the case specimen (Figure 1c).

The high-resolution MRI volume rendering recapitulated the anatomy of the whole shark and the inner anatomy of the ventricle (Figure 2b,c). Based on the MRI, we made a 3D reconstruction of the heart which revealed a right-sided atrioventricular canal and the left-sided position of the conus arteriosus in the ventricular mass (Figure 2d). This gave further support for the notion of dextrocardia in this specimen. The atrium and sinus venosus did not exhibit clear left–right asymmetries on the MRI. This likely reflected the limitations of the spatial resolution and the sinuatrial valve slit, for example, was only seen on two image slices. The 3D model is available as an interactive pdf (Supporting Information Figure S2).

Based on the material of previous studies on the Adriatic sturgeon (Icardo et al., 2002, 2004), we report here a cardiac chamber topology defect in a specimen of 90 cm TL (Figure 3a). In this specimen, other organs had the normal spatial relationships. In addition, out of the approximately 300 hundred alevins of sturgeon that were previously analyzed to study heart development (Icardo et al., 2004, 2009), about 10 per cent exhibited cardiac defects including mirror-looping of the heart, or dextrocardia. Several examples are shown in Figure 3b. In early alevins, the single sign of organ asymmetry is the heart looping since the liver occupies a middle position and the intestine has not differentiated. Intestine development begins at 3–4 days post hatching when the yolk sac adopts a tubular shape and the separate anlage of the gastric and intestinal portions can first be recognized (Ostos-Garrido et al., 2009). Also, at 4dph, the liver appears divided into left and right lobes with the gallbladder developing at the cranioventral portion of the right lobe. While laterality defects of the gastrointestinal tract could not be discarded, our records indicate that the gallbladder was always located under the liver right lobe irrespective of heart loop direction. The incidence of dextrocardia in the alevins is estimated to be between one and two per cent. This incidence is approximately similar to that reported for developing *Xenopus* frogs (van Veenendaal et al., 2013), but it is two orders of magnitude more frequent than the approximate 1:10.000 occurrence of dextrocardia reported in humans (Bohun et al., 2007). Of note, dextrocardia was found more frequently in younger than in older alevins. This suggests that either the malformation is detrimental in later stages, and/or, is associated with severe extracardiac malformations which, at least in humans, can occur (Bohun et al., 2007).

In conclusion, left–right mirrored hearts occur in both farmed and wild fish. Our findings indicate that laterality defects can easily be identified in cartilaginous fish due to heart asymmetry. These defects may be more difficult to identify in bony (and especially in teleost) fish due to the more left–right symmetric arrangement of the heart chambers.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

Baker-Cohen, K.F. (1961) Visceral and vascular transposition in fishes, and a comparison with similar anomalies in man. *American Journal of Anatomy*, 109(1), 37–55.

Bohun, C.M., Potts, J.E., Casey, B.M. & Sandor, G.G. (2007) A population-based study of cardiac malformations and outcomes associated with dextrocardia. *The American Journal of Cardiology*, 100(2), 305–309.

de Bakker, B.S., de Jong, K.H., Hagoort, J., de Bree, K., Besselink, C.T., de Kanter, F.E.C. et al. (2016). An interactive three-dimensional digital atlas and quantitative database of human development. *Science*, 354(6315). Available from: https://doi.org/10.1126/science.aag0053

Frisk, M., Høyland, M., Zhang, L., Vindas, M.A., Øverli, Ø. & Johansen, I.B. (2020) Intensive smolt production is associated with deviating cardiac morphology in Atlantic salmon (Salmo salar L.). *Aquaculture*, 529, 735615.

Gallego, A., Durán, A.C., De Andrés, A.V., Navarro, P. & Muñoz-Chapuli, R. (1997) Anatomy and development of the sinoatrial valves in the dogfish (*Scyliorhinus canicula*). *The Anatomical Record*, 248(2), 224–232.

Goor, D.A., Dische, R. & Lillehei, C.W. (1972) The conotruncus: I. its normal inversion and conus absorption. *Circulation*, 46(2), 375–384.

Grimes, A.C. & Kirby, M.L. (2009) The outflow tract of the heart in fishes: anatomy, genes and evolution. *Journal of Fish Biology*, 74(5), 983–1036.

Hirasaki, Y., Tomita, T., Yanagisawa, M., Ueda, K., Sato, K. & Okabe, M. (2018) Heart anatomy of Rhincodon typus: three-dimensional X-ray computed tomography of plastinated specimens. *The Anatomical Record*, 301(11), 1801–1808.

Icardo, J.M. (2006) Conus arteriosus of the teleost heart: dismissed, but not missed. *The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology: An Official Publication of the American Association of Anatomists*, 288(8), 900–908.

Icardo, J.M. (2017) Heart morphology and anatomy. In: *Fish physiology*, Vol. 36. Cambridge, MA: Academic Press, pp. 1–54.

Icardo, J.M., Colvee, E., Cerra, M.C. & Tota, B. (2002) Structure of the conus arteriosus of the sturgeon (*Acipenser naccarii*) heart. I: the conus valves and the subendocardium. *The Anatomical Record: An*

Official Publication of the American Association of Anatomists, 267(1), 17–27.

Icardo, J.M., Guerrero, A., Durán, A.C., Colvee, E., Domezain, A. & Sans-Coma, V. (2009) The development of the epicardium in the sturgeon *Acipenser naccarii*. *Anatomical Record*, 292, 1593–1601.

Icardo, J.M., Guerrero, A., Durán, A.C., Domezain, A., Colvee, E. & Sans-Coma, V. (2004) The development of the sturgeon heart. *Anatomy and Embryology*, 208, 439–449.

Jensen, B., van den Berg, G., van den Doel, R., Oostra, R.J., Wang, T. & Moorman, A.F. (2013) Development of the hearts of lizards and snakes and perspectives to cardiac evolution. *PLoS One*, 8(6), e63651.

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(3), 1604–1619.

Noël, E.S., Verhoeven, M., Lagendijk, A.K., Tessadori, F., Smith, K., Choorapoikayil, S. et al. (2013) A nodal-independent and tissue-intrinsic mechanism controls heart-looping chirality. *Nature Communications*, 4(1), 2754.

Ostos-Garrido, M.V., Llorente, J.I., Camacho, S., Garcia-Gallego, M., Sanz, A., Domezain, A. et al. (2009) Histological, histochemical and ultrastructural changes in the digestive tract of sturgeon Acipenser naccarii during early ontogeny. In: Carmona, R., Domezain, A., Garcia-Gallego, M., Hernando, J.A., Rodriguez, F. & Ruiz-Rejón, M. (Eds.) *Biology, conservation and sustainable development of sturgeons*. Heidelberg: Springer Science Business Media B.V, pp. 121–136.

Pelster, B. & Bemis, W.E. (1991) Ontogeny of heart function in the little skate Raja erinacea. *Journal of Experimental Biology*, 156(1), 387–398.

Poppe, T.T., Johansen, R. & Tørud, B. (2002) Cardiac abnormality with associated hernia in farmed rainbow trout *Oncorhynchus mykiss*. *Diseases of Aquatic Organisms*, 50(2), 153–155.

Sanchez-Quintana, D. & Hurle, J.M. (1987) Ventricular myocardial architecture in marine fishes. *The Anatomical Record*, 217(3), 263–273.

Smith, K.A. & Uribe, V. (2021) Getting to the heart of left–right asymmetry: contributions from the zebrafish model. *Journal of Cardiovascular Development and Disease*, 8(6), 64.

Van Mierop, L.H.S. & Kutsche, L.M. (1984) Comparative anatomy and embryology of the ventricles and arterial pole of the vertebrate heart. In: J.J. Nora & A. Takao (Eds.), *Congenital heart disease: Causes and processes*. Mount Kisco, NY: Futura Publishing, pp. 459–479.

van Veenendaal, N.R., Ulmer, B., Boskovski, M.T., Fang, X., Khokha, M.K., Wendler, C.C. et al. (2013) Embryonic exposure to propylthiouracil disrupts left-right patterning in Xenopus embryos. *The FASEB Journal*, 27(2), 684–691.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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