

Early development of coelomic structures in an echinoderm larva and a similarity with coelomic structures in a chordate embryo

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Received: 3 May 2012 / Accepted: 16 August 2012 / Published online: 22 September 2012
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Abstract Early coelomic development in the abbreviated development of the sea urchin *Holopneustes purpureescens* is described and then used in a comparison with coelomic development in chordate embryos to support homology between a single arm of the five-armed radial body plan of an echinoderm and the single bilateral axis of a chordate. The homology depends on a positional similarity between the origin of the hydrocoele in echinoderm development and the origin of the notochord in chordate development, and a positional similarity between the respective origins of the coelomic mesoderm and chordate mesoderm in echinoderm and chordate development. The hydrocoele is homologous with the notochord and the secondary podia are homologous with the somites. The homology between a single echinoderm arm and the chordate axis becomes clear when the aboral to oral growth from the archenteron in the echinoderm larva is turned anteriorly, more in line with the anterior–posterior axis of the early zygote. A dorsoventral axis inversion in chordates is not required in the proposed homology.

Keywords Deuterostome · Evolution · Mesoderm · Morphogenesis · Metamerism

Introduction

Echinoderms and chordates, together with hemichordates, are the main phyla of the deuterostome group whose

phylogenetic affinity is well supported by molecular data (Cameron et al. 2000; Putnam et al. 2008). Echinoderms are more closely related to hemichordates than to chordates, but they differ morphologically from both phyla through their five-armed radial body plan that is very different from the bilateral body plan of a chordate. The morphological connexion between the body plans of echinoderms and chordates has remained unclear. In this report, a similarity between the coelomic structures in an echinoderm larva and those well known for chordate embryos (Gilbert 2010) is described and a homology between echinoderm and chordate body plans is proposed.

The similarity is evident at early stages of development. In an echinoderm, coeloms form at the head of the archenteron, the more prominent of which is the hydrocoele that forms the pentamerous water vascular system of the adult (Hyman 1955). In a chordate, the notochord forms similarly from the archenteron and it is homology between the hydrocoele and the notochord that is proposed here. The coelomic mesoderm that forms more posteriorly from the archenteron in an echinoderm larva shares a similar positional origin to that of the mesoderm in a chordate embryo and homology between these tissues is also proposed.

The early development of echinoderm coeloms is described here in the sea urchin *Holopneustes purpureescens*. Development is direct in this species (Morris 1995), there is no feeding larva, and the coeloms reach the adult form between 1 and 2 days after fertilization. Development of the five primary podia, which form from the hydrocoele and which are the earliest pentamerous structures, was described previously (Morris 2007). Here, laser scanning confocal microscopy and three-dimensional image analysis was used in describing the early coelomic development.

The present description of hydrocoele development challenges the conventional descriptions in Ubaghs (1967), as

Communicated by Hiroki Nishida

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did the description for the echinoid *Heliocidaris erythrogramma* (Morris 2011), which also has an abbreviated development. The emphasis here on coeloms in body plan architecture has parallels with the axial–extraxial theory of Mooi et al. (1994) who divide the echinoderm body into an axial region and an extra-axial region and associate the hydrocoele with the axial region. The differing extents of the axial regions in echinoderms are the basis of a scheme that unites the different forms of the five extant echinoderm classes (David and Mooi 1996).

The homology between echinoderm and chordate body plans depends also on the description (Morris 2009) of the serial development in the juvenile of *H. purpurescens* of secondary podia along an echinoderm arm that was interpreted as a metameric series. In this respect, the conceptual body plan of an echinoderm here resembles that of Turner (1998) who described the bilateral serial repetition of elements along the ambulacra as metamerism, making a link between echinoderms and chordates. The earliest echinoderms were bilateral (Zamora et al. 2012), so it is reasonable to look for bilateral characters in echinoderms in order to relate them morphologically to chordates. A metameric series is a possible bilateral character that would connect echinoderms and chordates since metamerism is a central character of the chordate body plan. Pentamerism would be a consequence of the duplication of arms, as others have suggested (Raff and Popodi 1996; Hotchkiss 1998). Similarity between a single echinoderm arm and the axial structures of a vertebrate embryo was proposed previously by Heinzeller and Welsch (1999).

The data presented and their interpretation sit within the wider contexts of deuterostome evolution (see Swalla and Smith 2008), the ancestry of echinoderms (Smith 2005) and the ancestry of chordates and echinoderms (Jefferies 1990) and is commented on briefly in the “Discussion”. Now the idea of an ancestral role for a dipleurula-like larva has been put aside for being too specialized, as Lacalli (2005) has argued, attention can be placed on adult structures, as here, rather than on adaptive larval characters.

Materials and methods

Cultures of *H. purpurescens* embryos, prepared as described (Morris 2007), were reared at 20 °C in filtered sea water (FSW). Larvae were fixed in 4 % (w/v) paraformaldehyde (Sigma-Aldrich) in FSW for 1–2 h, washed in FSW, then dehydrated in graded methanols to 100 % methanol and stored at –20 °C. For viewing, larvae were cleared and mounted in 2:1 (v/v) methyl benzoate/methyl alcohol (Sigma-Aldrich), in coverslip-sealed chambers.

Larvae, with tissues autofluorescent from the paraformaldehyde fixation, were viewed in the Leica TCS SP5 MP

multiphoton laser scanning confocal system (Leica Microsystems) with a tunable Mai Tai Deep See laser (Spectra-Physics) attached to a Leica DMI6000B-CS inverted microscope. Each specimen was imaged using multiphoton microscopy (Cox 2007) at $\lambda_{ex}=870$ nm with pulses in the 100–200 fs range and detected in a reflected nondescanned detector at λ_{em} 545–605 nm. A Z stack was collected, with default X flipped, averaged over two frames in a 1,024×1,024 pixel array, 12 bits/pixel, at a slice thickness of 1.85 μ m using a Leica HC PL APO 20x/0.70 IMM CORR CS objective lens or at a slice thickness of 0.5 μ m using a Leica HCX PL APO 63x/1.30 GLYC CORR CS 21 °C objective lens.

The Z stacks were viewed in ImageJ (v. 1.43r). XY sections of the Z stack were supplemented by sections through any plane of the Z stack created by the 3-D plugin Volume Viewer. Since the resolution in XZ and YZ sections of the Z stack is lower than in XY sections, by about four times, a property of confocal imaging (Cox 2007), some reduction in resolution can occur in sections created in Volume Viewer.

Larvae were observed at hourly intervals from 27 to 40 h after fertilization. The observations of larvae at 27, 29, 33, 39 and 40 h, illustrated here, represent findings from the developmental range observed. Eight or more larvae were viewed at each of the illustrated times.

Results

The hydrocoele and coelomic mesoderm in *H. purpurescens* form from cells at the head and sides of the archenteron wall. The cells form a structure, which in a feeding larva is the left coelom but which is here the adult, oral coelom that forms on the adult, oral side of the larva. The hydrocoele develops from the anterior region of the oral coelom while the coelomic mesoderm develops from the posterior region of the oral coelom. The oral coelom is bent towards the adult, oral side of the larva. The hydrocoele develops five primary podia and the coelomic mesoderm grows around the primary podia. These findings are illustrated in the figures.

The early development of the hydrocoele and coelomic mesoderm is shown in Figs. 1 and 2 and the later development to the echinus rudiment stage is shown in Fig. 3. The homology between early coelomic structures in an echinoderm and a chordate is illustrated diagrammatically in Fig. 4.

Early coelomic development

Figure 1a–d are low-magnification sections of a 27-h larva in oral view, selected from a Z stack imaged with the 20× objective lens, the sections progress from oral to aboral. The

axes of the larva are shown in Fig. 1a. Anterior (an) is at the animal pole and posterior (po) is at the vegetal pole where the blastopore forms. Left (le) and right (ri) are set by equating the oral view of the larva with a frontal or ventral view.

Figure 1a is a superficial section through the oral face of the oral coelom showing the outline of the coelom (oc) and its prominent left lobe (lo). Figure 1b is a deeper section showing the origin of the hydrocoele (hy) from cells at the anterior tip of the archenteron (ar); the archenteron cavity is open to the cavity of the left lobe of the oral coelom. In Fig. 1c, a ridge of cells (arrow) associated with the hydrocoele is separated from the left lobe by a shallow indentation (arrowheads) of the archenteron wall; the section is through the blastopore (bl). Figure 1d is a section near the aboral wall of the archenteron.

Figure 1e–h are high-magnification sections through the same larva imaged with the 63× objective lens, giving higher resolution images of the tissues. Figure 1e–g are at Z stack levels similar to those in Fig. 1a–c and show the features of Fig. 1a–c. Figure 1h is a section through the Z stack after the XZ face has been tilted towards the observer, using Volume Viewer (Materials and methods), so to obtain an approximate transverse section through the oral coelom at the level of the left lobe. It shows the shape of the left lobe, which curves orally.

Figure 1i–l are sagittal sections of the oral coelom in another 27-h larva that is more advanced in development than the larva in Fig. 1a–h. The view is of the left side of the oral coelom with oral (or) on the left in each panel and aboral (ab) on the right. The sections progress from the left side of the oral coelom to the right side. Figure 1i is a superficial section through the left lobe. Figure 1j is a midsagittal section illustrating the early development: the hydrocoele forms anteriorly, marked out by some epitheliation; the coelomic mesoderm (cm) is a loose aggregation of cells lying posteriorly; the coelomic mesoderm is sourced from the oral wall of the archenteron that here has broken down, undergoing de-epitheliation; the archenteron stem is tilted aborally while the oral coelom comprising the hydrocoele and coelomic mesoderm is bent orally. In Fig. 1k, the XZ face of the Z stack has been tilted away from the observer to show principally the exit of cells from the oral wall of the archenteron, ahead of the arrow. Figure 1l is a section near the right side of the oral coelom where coelomic mesoderm is sourced from the right side of the archenteron wall. Figure 1m is an oral view of the larva in Fig. 1i–l created in Volume Viewer; it shows the shape of the oral coelom, tilted posteriorly on the side of the left lobe; the approximate planes of section in Fig. 1i, j, l are shown in Fig. 1m (arrows).

Figure 1n–p are sections of a 29-h larva that show a more advanced development of the oral coelom. Figure 1n, o are oral views. In Fig. 1n, the hydrocoele, bordered by an

epithelium, is connected with the coelomic mesoderm of the left lobe (lo); coelomic mesoderm (cm) has formed from the right archenteron wall. Figure 1o is a deeper section that is through the opening (ao) into the archenteron cavity created by the breakdown of the oral wall of the archenteron; coelomic mesoderm forms from both side walls of the archenteron (arrowheads). Figure 1p is a sagittal section through the same larva, created in Volume Viewer, to show the relationship between the hydrocoele, the coelomic mesoderm and the archenteron in this larva, and the archenteron opening (ao) open to the archenteron (ar).

Figure 1q–t are sagittal sections of 29-h larvae and a 33-h larva. They summarize features of oral coelom development. Figure 1q shows the anterior position of the hydrocoele with respect to the posterior position of the coelomic mesoderm; Fig. 1r shows the clear origin of cells from the oral wall of the archenteron; Fig. 1s is a section through the right side of the larva showing coelomic mesoderm sourced from the right archenteron wall; Fig. 1t is a section of a 33-h larva showing the later increased mass of coelomic mesoderm, here extending high on the right side of the oral coelom.

Overall, Fig. 1 shows the development of an oral coelom from the head and sides of the archenteron wall, a coelom that bends orally and is slanted clockwise with respect to the anterior–posterior line of the archenteron in oral view. The slant of the oral coelom is presaged in the sections in Fig. 1a–h. The hydrocoele forms from the oral coelom anteriorly and the coelomic mesoderm forms posteriorly.

Early coelomic development in transverse view

Figure 2 is a series of sections approximately transverse to the archenteron and the oral coelom that progress from posterior to anterior. They are to show, in Fig. 2a–d, the exit of cells from the oral and side walls of the archenteron in a 27-h larva, and in Fig. 2e–h, the later growth of the oral coelom in a 29-h larva. The larva in Fig. 2a–d is the larva in Fig. 1i–m and the larva in Fig. 2e–h is the larva in Fig. 1n–p. The sections were created in Volume Viewer and the planes of sections through the archenteron and oral coelom are indicated for the 27-h larva and the 29-h larva respectively in Fig. 2i, j.

Figure 2a is at a level where the coelomic mesoderm is not linked with the archenteron wall. In Fig. 2b, cells have exited from the left side of the archenteron wall in the region of the left lobe; the section is through the posterior region of the oral coelom. Figure 2c is at the level of the start of the breakdown of the oral archenteron wall (arrowhead). In Fig. 2d, much of the oral archenteron wall has broken down; cells have exited from the right archenteron wall (arrowhead); the hydrocoele extends from the aboral wall of the archenteron to the oral face of the oral coelom on the left side; the hydrocoele here is anterior to the coelomic mesoderm lying posteriorly.

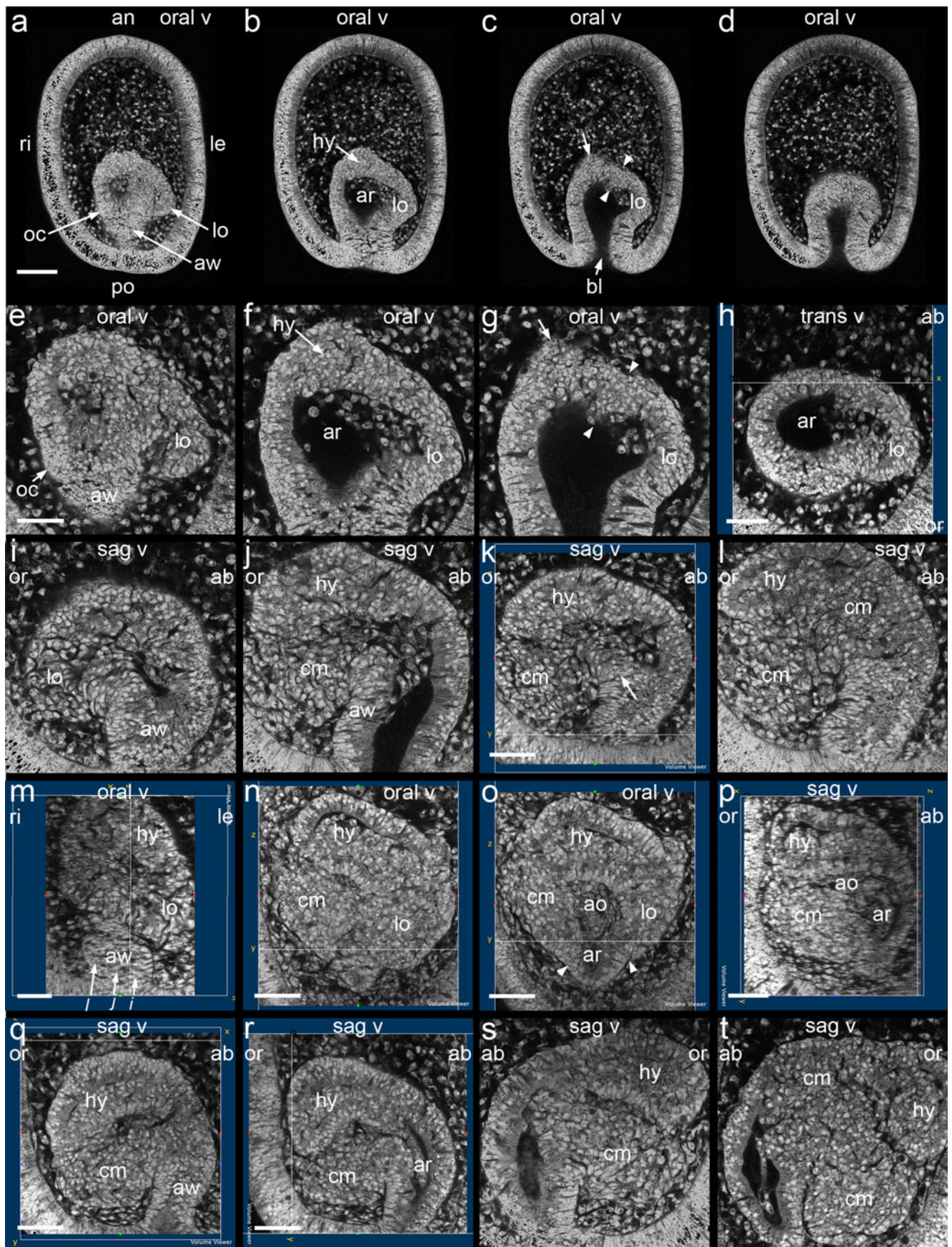


Fig. 1 Early development of the oral coelom in oral and sagittal views (described in text). **a–h** Sections of a 27-h larva imaged with a 20× objective lens (**a–d**) and a 63× objective lens (**e–h**) in oral view, excepting **h** which is a transverse view. **i–m** Sagittal sections of another 27-h larva, in left-side view, excepting **m** which is an oral view. **n, o** Sections of a 29-h larva in oral view; **p** A sagittal view of this larva. **q, r** Left-side sagittal views of another 29-h larva. **s** Right-side sagittal view of another 29-h larva. **t** Sagittal section in right-side view of a 33-h larva. Sections created in Volume Viewer are bordered in blue. Other sections are parallel to the XY face of the Z stack. *ab*, aboral; *an*, anterior; *ao*, archenteron opening; *ar*, archenteron; *arrow* in **c**, ridge of cells; *arrow* in **k**, exit of cells from archenteron wall; *arrows* in **m**, approximate planes of section in **i, j, l**; *arrowheads* in **c** and **g**, indentation of archenteron wall; *arrowheads* in **o**, side walls of archenteron connect with coelomic mesoderm; *aw*, archenteron wall; *bl*, blastopore; *cm*, coelomic mesoderm; *hy*, hydrocoele; *le*, left; *lo*, left lobe; *oc*, oral coelom; *or*, oral; *oral v*, oral view; *po*, posterior; *ri*, right; *sag v*, sagittal view; *trans v*, transverse view. Scale bar 100 μm in **a**, applies also in **b–d**; scale bar 50 μm in **e**, applies also in **f, g, i, j, l, s, t**; scale bars 50 μm elsewhere

In Fig. 2e, a mass of coelomic mesoderm extends from the oral and side walls of the archenteron to the oral ectoderm (oe); the section is at the level of the posterior region of the oral coelom. In Fig. 2f, the coelomic mesoderm is separated into two regions, one on the right and the other, on the left, that is the part of the left lobe where it is connected to the base of the hydrocoele (hb). In Fig. 2g, the separation between the coelomic mesoderm on the right side of the larva and the hydrocoele extends into the archenteron cavity; the hydrocoele also separates from the left lobe (not shown). Figure 2h is a section through the hydrocoele that at this level extends across the oral face of the oral coelom, making contact with oral ectoderm; the epithelium on the oral face of the hydrocoele (arrowheads) is where the primary podia form their shapes.

Overall, Fig. 2 shows the origin of cells from the archenteron walls to form the coelomic mesoderm, the further development of the oral coelom and the contact of the hydrocoele with the oral ectoderm.

Later coelomic development

Figure 3 is a later stage of development where the hydrocoele has formed the five arms that are the hydrocoelic components of the five primary podia. The coelomic mesoderm has grown anteriorly around the sides of the hydrocoele.

Figure 3a–d are low-magnification sections through a 40-h larva in aboral view, progressing from aboral to oral. In aboral view, left is on the left in each panel and right is on the right. Figure 3a is a superficial section through the archenteron wall, the hydrocoele stem anterior to it and a surrounding arc of coelomic mesoderm. Figure 3b, c are progressively deeper sections showing the five hydrocoele arms and the coelomic mesoderm around them. Figure 3d is

at an oral level through the vestibule and the five primary podia (A, B, C, D and E), each composed of a hydrocoele arm encased by vestibular ectoderm.

Figure 3e–g are high-magnification sections through 40-h larvae progressing from aboral to oral. Figure 3e is through the archenteron wall, the hydrocoele stem and the surrounding coelomic mesoderm. Figure 3f is where the hydrocoele has broadened and developed a five-armed shape. Figure 3g is through the five hydrocoele arms; the epithelium of the arms is continuous with the epithelium of the oral face of the hydrocoele that lies centrally between the arms, a region here called the oral plate; the oral plate (op) is slightly aboral to the hydrocoele arms, lying in a depression between the arms which grow outwards and orally.

Figure 3h is a low-magnification sagittal section through a 39-h larva, in right-side view, showing the coelomic mesoderm aboral to hydrocoele which is aboral to the thick vestibular ectoderm (ve) that lines the aboral side of the vestibule (vs).

Figure 3i–k are high-magnification sagittal sections through a 40-h larva showing the relationship between the archenteron, the coelomic mesoderm and the parts of the hydrocoele in the right-side view. In Fig. 3i, the archenteron shares an epithelium with the hydrocoele, which has branched into arms; coelomic mesoderm lies aborally. Figure 3j is a deeper section where the coelomic mesoderm is aboral to the aboral hydrocoele wall. In Fig. 3k, which is a still deeper section, the coelomic mesoderm extends orally towards the separation between two hydrocoele arms; the hydrocoele arms lie close to the vestibular ectoderm.

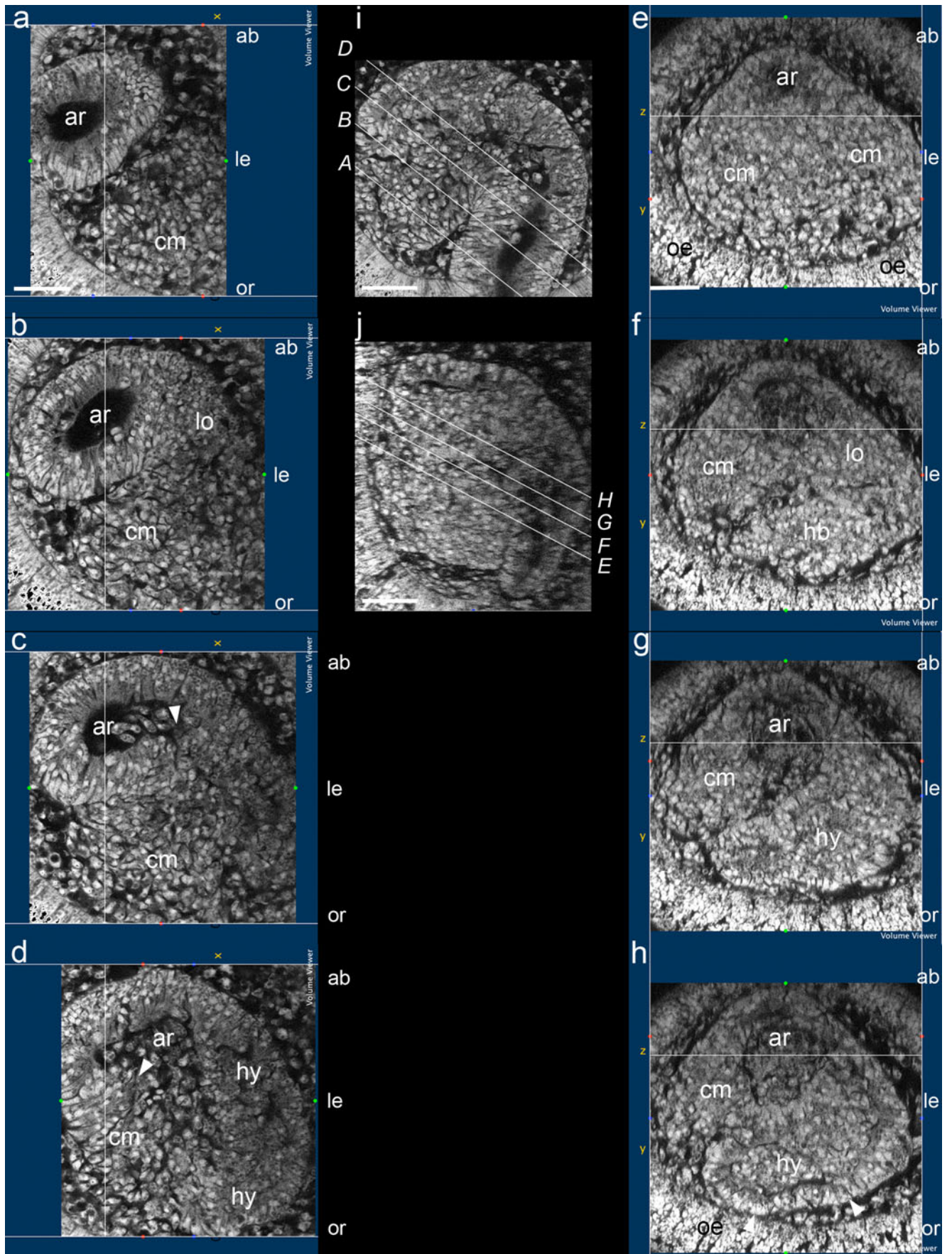
Overall, Fig. 3 shows a pentamerous echinoderm body plan developed from the archenteron products of hydrocoele and coelomic mesoderm.

The direction of growth from the stage of development in Fig. 3 is predominantly oral, extending the tissues outwards from the primary podia, with the source of growth at the primary podia.

Discussion

The early development of coelomic structures in the abbreviated development of the sea urchin *H. purpurascens* exposes a similarity between echinoderm and chordate coelomic development.

The similarity is in the relative positions in which the early hydrocoele and coelomic mesoderm form from cells of the archenteron wall and in the relative positions in which notochord tissue and mesoderm form at the start of gastrulation in chordate embryos. The hydrocoele forms from cells that are anterior to those that form the coelomic mesoderm. There is a similar anterior–posterior relationship in chordates between notochord precursors and mesoderm



◀ **Fig. 2** Early development of the oral coelom in transverse view (described in text). **a–d** The 27-h larva in Fig. 1a–h; sections progress from posterior to anterior at levels (A, B, C, D) shown in **i**. **e–h** The 29-h larva in Fig. 1n–p; sections progress from posterior to anterior at levels (E, F, G, H) shown in **j**. **a–h** Created in Volume Viewer and are bordered in blue. *ab*, aboral; *ar*, archenteron; *arrowhead* in **c**, breakdown of oral archenteron wall; *arrowhead* in **d**, exit of cells from right archenteron wall; *arrowheads* in **h**, oral face of hydrocoele; *cm*, coelomic mesoderm; *hb*, base of hydrocoele; *hy*, hydrocoele; *le*, left; *lo*, left lobe; *oe*, oral ectoderm; *or*, oral. Scale bars 50 μ m; scale bar in **a** applies also in **b–d**; scale bar in **e** applies also in **f–h**

precursors that is seen in the blastomeres of ascidians (Nishida 1987) and in the fate maps of the primitive-streak region in chick (Selleck and Stern 1991) and mouse (Lawson et al. 1991) embryos. This observed similarity leads to the proposal that the hydrocoele is homologous with the notochord and that the coelomic mesoderm is homologous with the chordate mesoderm.

This homology between echinoderms and chordates can be extended by considering the later development of the proposed homologous structures. In echinoderms, the hydrocoele and coelomic mesoderm contribute to the ambulacra. In chordates, structures of the chordate axis are formed: these are the notochord and somites and the more posterior presomitic mesoderm and tail bud mesoderm. Both the ambulacra and the chordate axis are a metameric series, and both require a growth zone.

Whereas in chordates the growth zone that forms the chordate axis is associated with the progeny of cells that involute or ingress at the blastoporal opening, the source of a growth zone for the ambulacra of echinoderms is less clear. However, it has been known for some time that the order in which the secondary podia, or the definitive feet, form is from aboral to oral with the older tube feet nearer the mouth and the younger nearer the terminal, or ocular, plate (Hyman 1955). Mooi et al. (2005) specify a growth zone as the source of the ocular plate rule in which ambulacral plates are laid down in sequence, starting adoral to the ocular plate that forms around the primary podium, with the youngest plates adjacent to the apical system and the oldest plates next to the mouth, a rule they say applies to all echinoderms. Morris (2009), in a description of the development of the secondary podia in a juvenile of *H. purpureus*, showed that the earliest secondary podia develop at the bases of the primary podia where the podia join the emerging radial canals. The present report shows that the primary podia form at the edges of the hydrocoele (Fig. 3f, g) with coelomic mesoderm entering the region between the primary podia (Fig. 3k). It is proposed, therefore, that there is a growth zone associated with the bases of the primary podia and the nearby coelomic mesoderm. At an earlier stage of development, the precursors of cells of this growth zone would have been cells that de-epithelialized at the junction

between the archenteron wall and the developing left coelom. It is thus reasonable to postulate homology between the growth zone for ambulacra in an echinoderm embryo and that for the chordate axis in a chordate embryo.

The extended homology between the echinoderm body plan and the chordate body plan resulting from the growth-zone homology is summarized in Fig. 4, starting with a summary diagram of the echinus rudiment at the latest stage described in the present report (Fig. 4a).

In Fig. 4a, the coelom that formed from the archenteron has developed into the hydrocoele and the coelomic mesoderm. The hydrocoele has turned towards the side of the larva that is the oral side. The primary podia have formed at the ends of the branched hydrocoele: coelomic mesoderm has grown between the branches. The further growth of the rudiment, beyond that described in the present report and represented in grey, will be outwards on the oral side, creating tissues that extend from the bases of the primary podia at the edges of the oral plate to the oral ectoderm. The secondary podia will develop at the bases of the primary podia and protrude orally from the radial canals that form as each hydrocoele branch grows (Morris 2009). These secondary podia are the definitive podia of echinoderms that form successively with new podia formed at an aboral site, leaving the older podia nearer the mouth (Hyman 1955), as noted above.

To appreciate the homology between the body plans, the aboral to oral growth from the bases of the primary podia (Fig. 4a) should be rotated through about 90° so it is more in line with animal–vegetal axis of the early zygote. The view of one radial canal with secondary podia and coelomic mesoderm after such rotation is diagrammed in Fig. 4b.

During the growth of the echinoderm rudiment, the hydrocoele and podia separate from the archenteron and after the mouth forms, the ring canal is around the oesophagus (von Ubisch 1913). Three pairs of secondary podia are shown in Fig. 4b, with the youngest next to the primary podium and the oldest nearest the ring canal around the mouth. The coelomic mesoderm, which will form the muscular and connective tissues of the secondary podia (von Ubisch 1913; Hyman 1955), is shown enveloping the secondary podia. To illustrate the homology with the chordate body plan, the coelomic structures in a generalized amniote vertebrate embryo are diagrammed in Fig. 4c. The homology is between the hydrocoele and the notochord and between the pairs of secondary podia and the pairs of somites. In the amniote vertebrate embryo, notochord has formed anteriorly from the growth zone associated with the primitive streak (Bellairs and Osmond 2005). Mesoderm, formed from the lateral walls of the primitive streak, has formed tail bud mesoderm and presomitic mesoderm, which develops anteriorly into somites (Aulehla and Pourquié 2010) with the older somites lying anteriorly. One difference between

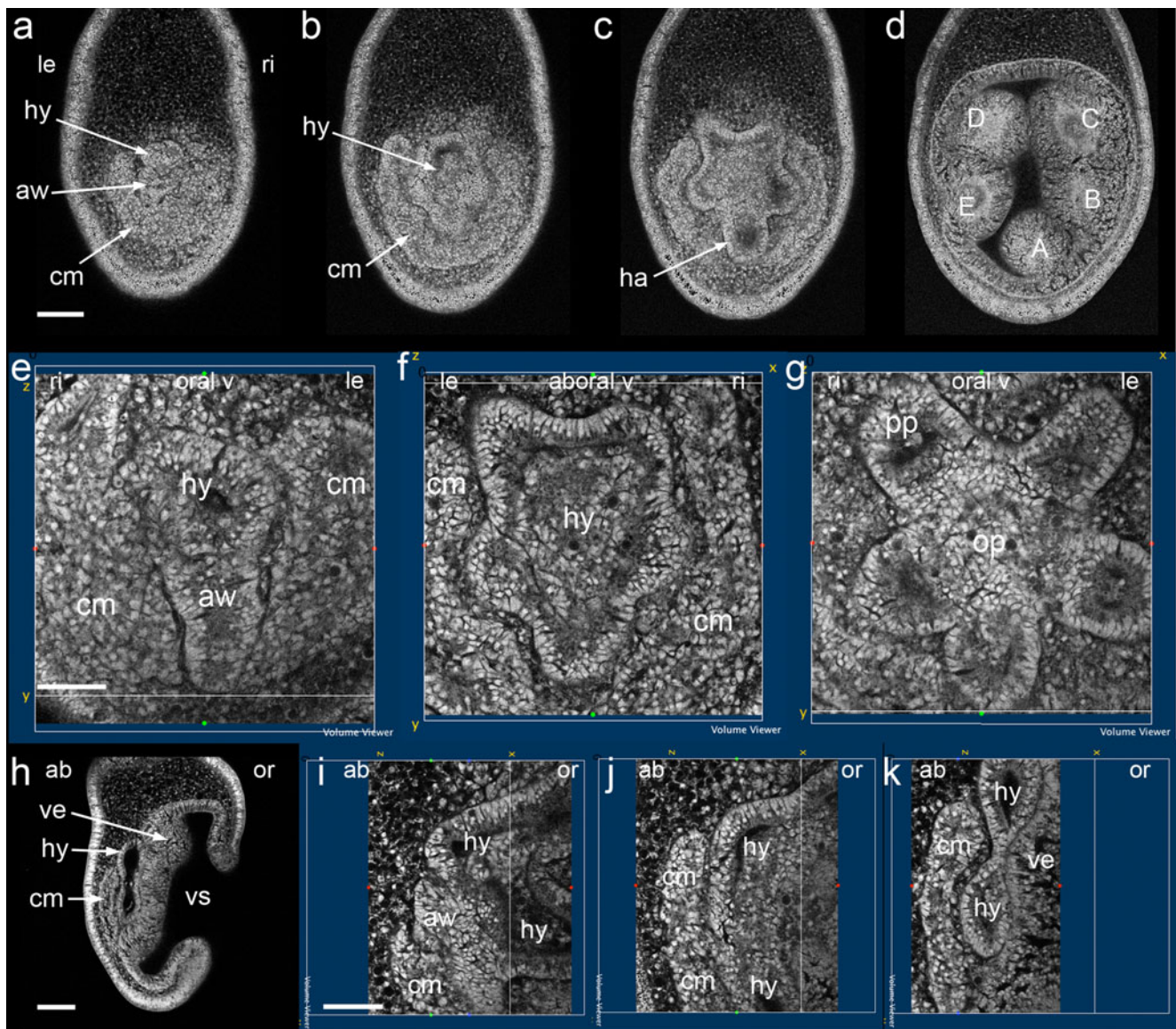


Fig. 3 Later development of the oral coelom to the echinus rudiment stage (described in text). **a–d** Low-magnification sections of a 40-h larva imaged with a 20× objective lens in aboral view; sections progress from aboral to oral. **e–g** High-magnification sections of 40-h larvae imaged with a 63× objective lens, progressing from aboral to oral. **h** Low-magnification sagittal section of a 39-h larva in right-side view. **i–k** High-magnification sagittal sections of a 40-h larva in right-side view; sections progress from right to left. **e–g** and **i–k** created in

Volume Viewer and are bordered in blue. **A, B, C, D** and **E** in **d** are the five primary podia. *aboral v*, aboral view; *aw*, archenteron wall; *cm*, coelomic mesoderm; *ha*, hydrocoele arm; *hy*, hydrocoele; *le*, left; *op*, oral plate; *or*, oral; *oral v*, oral view; *pp*, primary podium; *ri*, right; *ve*, vestibular epithelium; *vs*, vestibule. Scale bar 100 μm in **a** applies also in **b–d**; scale bar 50 μm in **e** applies also in **f, g**; scale bar 100 μm in **h**; scale bar 50 μm in **i** applies also in **j, k**

the echinoderm larva and the vertebrate embryo is in the hydrocoelic component of the secondary podia that contrasts with somites, which lack a notochordal component. Both secondary podia and somites, however, are a coelomic metameric series sourced from a growth zone at the archenteron–coelomic boundary.

To summarize, the homology in Fig. 4 depends on accepting that the cellular origins of the hydrocoele and the notochord have a similar positional identity and that the coelomic mesoderm and chordate mesoderm origins also

have a similar positional identity that is posterior to that of the hydrocoele and notochord. The positional identities claimed for *H. purpurescens* are evident at the earliest appearance of the hydrocoele and coelomic mesoderm cells (Fig. 1a–h).

The homology has support from gene expression studies. In a mouse embryo (Aulehla and Pourquié 2010), signalling gradients involving Wnt/β-catenin, fibroblast growth factor (FGF) and retinoic acid pathways regulate somitogenesis and axial development. In the sea urchin *H. erythrogramma*,

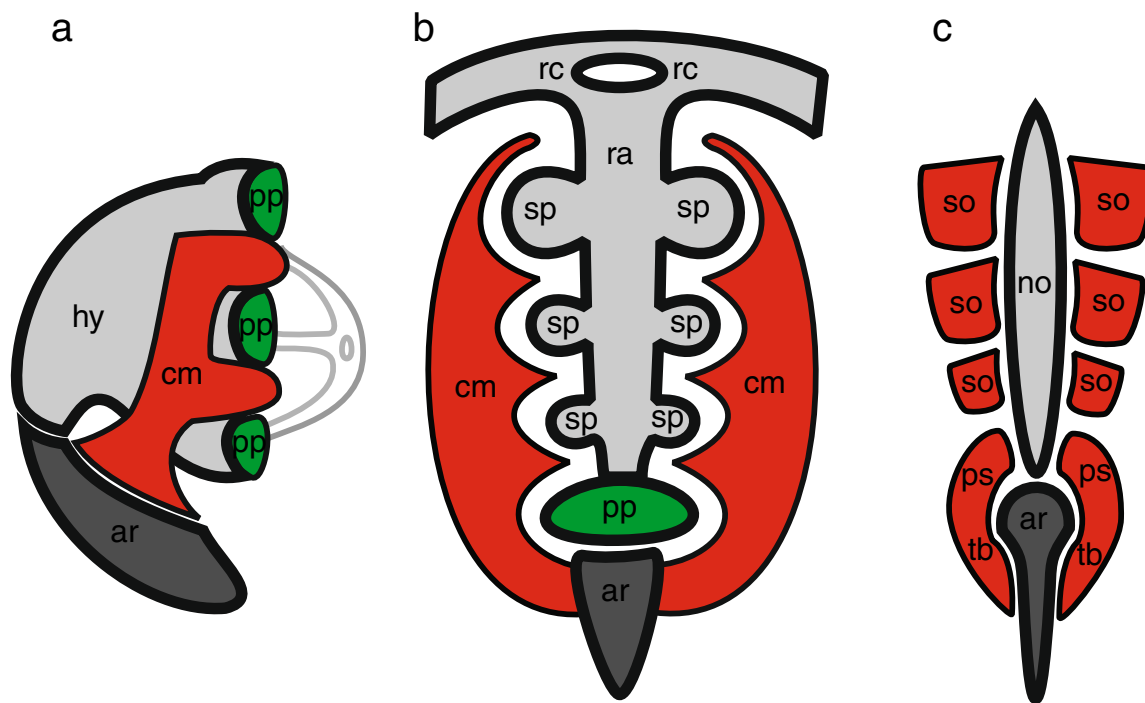


Fig. 4 Echinoderm–chordate homology (described in text). **a** Echinus rudiment in right-side sagittal view; future oral growth (pale grey) to the right of the primary podia (*pp*) is represented by three (out of five) radial canals joined to the ring canal; *ar*, archenteron; *cm*, coelomic mesoderm; *hy*, hydrocoele. **b** A single echinoderm arm with secondary

podia (*sp*) connected to the radial canal (*ra*), surrounded by coelomic mesoderm originating at the archenteron; *rc*, ring canal. **c** Generalized amniote vertebrate embryo of archenteron, notochord (*no*), tail bud mesoderm (*tb*) presomitic mesoderm (*ps*) and somites (*so*)

Wnt genes are expressed in the left coelom (Ferkowicz and Raff 2001); the left coelom is here the oral coelom. An FGF receptor, *FGFR1*, is expressed in the secondary mesenchyme that forms the left coelom in the sea urchin *Strongylocentrotus purpuratus* (Poustka et al. 2007). In mouse, the signalling pathways interact with Hox genes that specify axial identity along the anterior–posterior axis (Aulehla and Pourquié 2010). A vector of Hox genes from *Hox7* to *Hox11/13* is expressed in the somatocoel of *S. purpuratus* (Arenas-Mena et al. 2000); the somatocoel is the coelomic mesoderm of the present report. *Hox5* and *Hox11/13* are expressed in *H. purpurescens* in growth zones next to the primary podia (Morris and Byrne 2005). *Hox11/13* is also expressed in the coelomic mesoderm exiting from the archenteron in *H. purpurescens* larvae (Morris unpublished). A colinear expression of Hox genes has been described in the somatocoel of a crinoid (Hara et al. 2006). *Hox4* is expressed in the left coelom of the sea star *Parvulastra exigua* (Cisternas and Byrne 2009). *Hox3* is expressed in the dental sacs of *S. purpuratus* (Arenas-Mena et al. 1998). The dental sacs form at the oral tips of the coelomic mesoderm next to the ring canal (Morris 2009) and so are nearest to the mouth. Downstream targets of Wnt signalling in mouse include *Brachyury* (Aulehla and Pourquié 2010). *Brachyury* is expressed in the secondary

mesenchyme in echinoids (Hibino et al. 2004); secondary mesenchyme forms the left coelom in echinoderms. Significantly, a later spatial expression of *Brachyury* in the left coelom has been reported for *S. purpuratus* (Peterson et al. 1999a). In amphioxus, *engrailed* is expressed as metameric stripes along the anterior–posterior axis (Holland et al. 1997). In juvenile brittle stars, *engrailed* is expressed at the arm tips overlying the boundaries between newly forming skeletal ossicles (Lowe and Wray 1997).

In mouse, Hox gene expression is also controlled by Notch signalling in the presomitic mesoderm (Aulehla and Pourquié 2010). Poustka et al. (2007) report crosstalk between Wnt, Notch and FGF signalling pathways in secondary mesoderm cell specification and differentiation in *S. purpuratus*, similar to, quote, “signaling cascades that function during development of presomitic mesoderm in mouse embryogenesis”. The secondary mesoderm is the secondary mesenchyme that forms the left coelom in echinoderms (Hyman 1955).

The homology (Fig. 4b, c) between one ambulacrum and the chordate axis implies that the other four ambulacra are an evolutionary duplication from an ancestor with a single ambulacrum. A duplication for the origin of pentamery has been suggested by Raff and Popodi (1996) and Hotchkiss (1998), as has homology between a single echinoderm arm

and the axial structures of a vertebrate embryo (Heinzeller and Welsch 1999). A duplication would fit with the palaeontological evidence that the ancestral echinoderm had a single ambulacrum (Smith 2005). New data on the earliest echinoderms (Zamora et al. 2012) show that the adult body plan was bilateral. These earliest echinoderms have marginal plates that, although of different sizes, are a serial repetition, as are the plates in the tails of cintans. If these plates represent a metameric series, they provide an early link with chordates, supporting a common ancestry for echinoderms and chordates from diverse carpoids, in agreement with Jefferies (1990). A link with the hemichordates is less clear, although based on a similar anterior–posterior identity, the proboscis coelom would be homologous with the hydrocoele and the notochord. The stomochord of hemichordates is not considered homologous with the notochord (Peterson et al. 1999b; Ruppert 2005).

The homology between echinoderms and chordates described here does not require an inversion of the dorsoventral axis of chordates, as has been proposed for the homology between chordates and nonchordate Bilateria (De Robertis and Sasai 1996). In an echinoderm, growth from the archenteron is towards the oral side of the larva where the adult mouth will form. The archenteron, however, remains aboral. In a chordate, the archenteron is dorsal, as is the notochord and as are the somites, and the mouth is on the ventral side. It is the oral growth of the echinus rudiment from its source at the archenteron that leads to the mouth opening on the oral side. When this oral growth is turned anteriorly (Fig. 4b), so the mouth is more anterior in position, the echinoderm and chordate body axes are the same, with the aboral–oral echinoderm axis becoming respectively a dorsoventral axis. A dorsoventral inversion in chordates is then not necessary for the homology between echinoderms and chordates.

Abbreviated development in echinoderms is generally regarded as derived, being a form of development in which the feeding larval stages fail to develop fully. In abbreviated development, however, the early stages of adult development are more easily observed, as here, where an anterior–posterior relationship between the hydrocoele and coelomic mesoderm has been demonstrated. Even so, this same relationship is present in feeding larvae, in the development of the adult structures, where the hydrocoele forms from the anterior part of the left coelom with the somatocoele, here the coelomic mesoderm, more posterior (von Ubisch 1913). Thus, although the earliest hydrocoele development is not clear in feeding larvae, the anterior–posterior relationship is evident as the hydrocoele separates from the somatocoele.

The abbreviated development of *H. purpureescens* is extreme in comparison with the abbreviated development of the sea urchin *H. erythrogramma*. In *H. erythrogramma* (Morris 2011), the left coelom (here, the oral coelom) is a

coelomic cavity encased by an epithelium. The hydrocoele forms from the anterior region of the left coelom, becoming separated from the posterior region by a constriction that starts in the epithelium on the oral face of the left coelom. In *H. purpureescens*, the hydrocoele is separated from the more posterior coelomic mesoderm almost as it forms, with a breakdown of the archenteron wall and the formation of coelomic mesoderm directly from the archenteron wall in a loose aggregation more similar to that in a chordate embryo. The hydrocoele forms as an unbranched structure, anteriorly, at the head of the archenteron. Thus, it is in the extreme abbreviated development of *H. purpureescens* that the similarity between echinoderm and chordate coelomic development is more easily seen.

The findings here implicate echinoderms as a model for investigating how orthologous genes produce radically different phenotypic outcomes at the level of phyla.

Addendum: revisions to Morris (2007)

The region described as the mouth (Morris 2007) is the archenteron opening made by the breakdown of the oral archenteron wall. The formation of the mouth was not observed, but cells in the hydrocoele probably form a channel from the archenteron to the oral plate that is later perforated. Primary podia A and B form from the oral face of an epithelium derived from the posterior face of the hydrocoele, rather than directly from the archenteron wall. The somatocoele (Morris 2007) is the coelomic mesoderm.

Acknowledgments I thank Eleanor Kable and acknowledge the facilities and the scientific and technical assistance of staff of the Australian Microscopy and Microanalysis Facility at the Electron Microscope Unit, The University of Sydney.

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