Article

Cambrian stem-group ambulacrarians and the nature of the ancestral deuterostome

Graphical abstract

New fossil discovery & the origin of deuterostomes
Rotadiscus grandis

Chordata
Vetulicolians†

Ambulacraria
Cambroernids† Echinoderms Hemichordates

Reduction in seriation
Tentaculate feeding strategy
Chordate-like coelomopore covering

Ambulacrarians evolved from a chordate-like ancestor
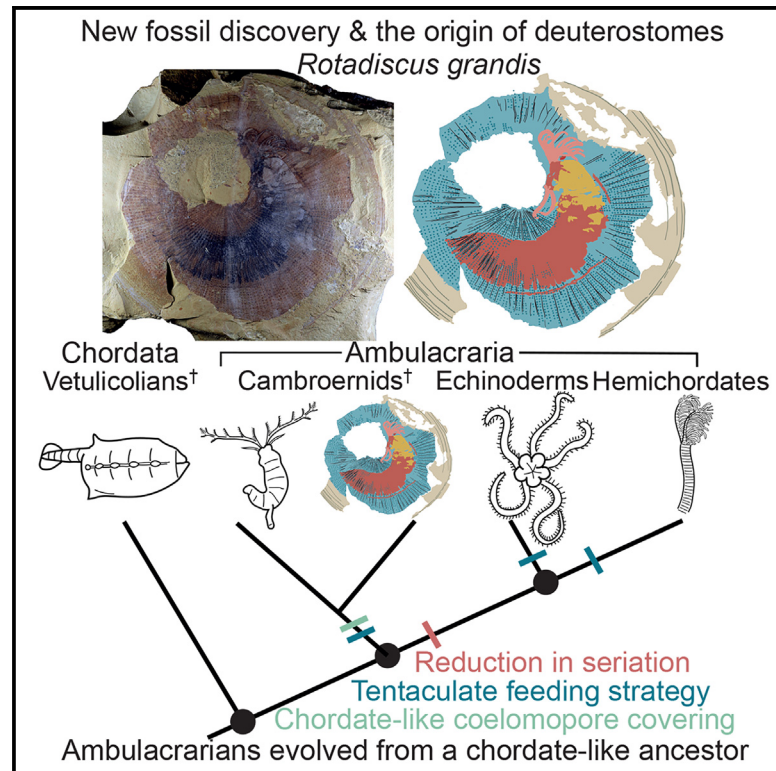
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In brief

Li et al. describe exceptionally preserved fossils of the enigmatic animal *Rotadiscus* from the Chengjiang biota of China. This reveals new anatomical details, including a chordate-like covering to a coelomopore. *Rotadiscus* and closely related species are recovered as stem ambulacrarians, demonstrating that convergence is rife among deuterostomes.

Highlights

- Exceptionally preserved fossils of the discoidal animal *Rotadiscus* are described

- *Rotadiscus* exhibits a combination of ambulacrarian and chordate characters

- Phylogenetic analyses recover *Rotadiscus* as a stem ambulacrarian

- Major deuterostome characters evolved through convergence

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Article

Cambrian stem-group ambulacrarians and the nature of the ancestral deuterostome

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SUMMARY

Deuterostomes are characterized by some of the most widely divergent body plans in the animal kingdom. These striking morphological differences have hindered efforts to predict ancestral characters, with the origin and earliest evolution of the group remaining ambiguous. Several iconic Cambrian fossils have been suggested to be early deuterostomes and hence could help elucidate ancestral character states. However, their phylogenetic relationships are controversial. Here, we describe new, exceptionally preserved specimens of the discoidal metazoan *Rotadiscus grandis* from the early Cambrian Chengjiang biota of China. These reveal a previously unknown double spiral structure, which we interpret as a chordate-like covering to a coelomopore, located adjacent to a horseshoe-shaped tentacle complex. The tentacles differ in key aspects from those seen in lophophorates and are instead more similar to the tentacular systems of extant pterobranchs and echinoderms. Thus, *Rotadiscus* exhibits a chimeric combination of ambulacrarian and chordate characters. Phylogenetic analyses recover *Rotadiscus* and closely related fossil taxa as stem ambulacrarians, filling a significant morphological gap in the deuterostome tree of life. These results allow us to reconstruct the ancestral body plans of major clades of deuterostomes, revealing that key traits of extant forms, such as a post-anal region, gill bars, and a U-shaped gut, evolved through convergence.

INTRODUCTION

Reconstructing the most recent common ancestor of deuterostomes[1] is crucial for uncovering the earliest steps in the evolution of this hyper-diverse clade. Comparative anatomy, developmental biology, and molecular phylogenetics have provided valuable insights into patterns of character evolution,[2–5] but important aspects of the evolution of the group remain unclear due to the widely divergent body plans exhibited by extant deuterostomes. Putative stem deuterostomes,[6–8] stem chordates,[9,10] stem echinoderms,[11–13] and stem hemichordates[14–16] have been reported from the Cambrian, potentially bridging these morphological gaps, but the phylogenetic positions of these taxa are contentious. One such fossil is the enigmatic metazoan *Rotadiscus grandis*.[17] Characterized by an unusual combination of characters, including a discoidal body and paired tentacles, it has been variously interpreted as a cnidarian,[17] a lophophorate,[18] or an ambulacrarian.[19] Distinguishing between these competing hypotheses is critical for establishing the evolutionary significance of this peculiar animal, but this has proved challenging owing to uncertainty over key aspects of its anatomy.

Here, we describe three exceptionally preserved specimens of *Rotadiscus* (two comprising part and counterpart) from the early Cambrian (~518 Ma) Chengjiang biota, Yunnan Province, China, which reveal novel details of the anatomy of this enigmatic taxon. These new data allow us to carry out phylogenetic analyses of extant and extinct bilaterians, the results of which strongly support the placement of *Rotadiscus* and closely related fossil taxa (cambroernids) as stem ambulacrarians. Cambroernids possess several characters that are observed in chordates but not in living ambulacrarians, suggesting that their most recent common ancestor was more chordate-like than previously thought.

RESULTS

The fossils are reddish-brown or pale yellow in color and are preserved flattened sub-parallel to bedding across several lamellae, with some parts showing low relief (Figures 1 and S1). They are relatively large in size (>90 mm in diameter), with a discoidal shape consisting of two distinct surfaces or discs; a thin layer of sediment fills the space between these surfaces (Figure 1A). One surface is rough and covered with fine concentric and radial ornament (Figures 1 and S1); where broken, it appears
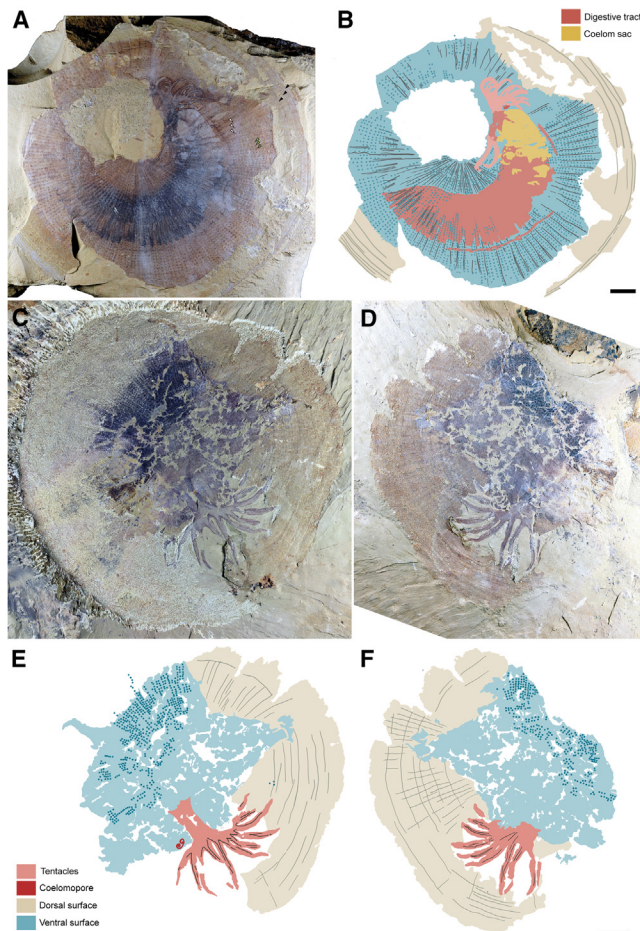
Figure 1. *Rotadiscus grandis* from the early Cambrian Chengjiang biota (Yunnan Province, China)

(A and B) YKLP 13090. Photograph (A) and interpretive drawing (B). Black arrows indicate concentric ornament, green arrows indicate pustules, and white arrows indicate radial ornament.

(C–F) CJHMD 00038. Photographs (C and D) and interpretative drawings (E and F) of the part (D and F) and counterpart (C and E).

Scale bars, 10 mm.

See also Figure S1.

noticeably thicker than the opposing surface. Together with the absence of folding or deformation, this suggests that the surface was relatively rigid. The opposing surface is smooth and more irregular in outline, often with both fine and coarse wrinkles (Figure S1A), indicating that it was softer. It exhibits two sets of radial structures: about 90 pairs of radiating lines, extending from the center to the margin of the disc and bifurcating in the marginal area (Figure 1); and a series of small (about 0.5 mm in diameter) circular pustules arranged in rows between the radiating lines (Figures 1 and S1). The pustules form a single row in the inner part of the disc and a double row in the outer part. Radially arranged lobes, indicated by areas of slight relief between the radiating lines, are evident in CJHMD 00039 (Figure S1A).

A curved structure (about 20 mm wide) is positioned approximately one-third of the distance from the center to the margin (Figures 1 and S1). It can be divided into three parts: a short, indistinct region; a long medial region with corrugated margins

defined by a dark stain; and a long region similar in color to the rest of the fossil. Based on its size, shape, and position, this structure is interpreted as a U-shaped gut.[18,20] The short indistinct region connects to a bilaterally symmetrical pair of tentacles (Figures 1A, 1B, and 2A) and is thus inferred to have led to the mouth, with the anus assumed to have been located at the opposing end. The three regions of the gut probably represent distinct parts of the digestive tract, i.e., esophagus, stomach, and intestine.[18,20] An additional structure partially surrounds the anterior of the gut in YKLP 13090; it is paler in color and shows higher relief than the gut, with weak divisions roughly aligned with the radiating lines of the disc (Figures 1A, 1B, and 2A). The outline coincides well with the anterior part of the gut, showing a sharp margin. We interpret this structure as a partially preserved coelomic sac surrounding the gut.

The tentacles occur on the soft surface characterized by radial structures, showing stronger relief than most of the fossil (Figures 1, 2, and S1); this relative three-dimensionality might explain their high fidelity of preservation. The mode of preservation of the tentacles and the similarly high-relief coelomic sac (Figures 2A–2C) probably results from sediment infilling, given that there is no evidence of mineralization in the SEM-energy dispersive X-ray spectroscopy (EDX) analyses (Figures 3E–3G). This has previously been recognized as an important mechanism for preserving hollow anatomical features (including tentacles) in several eldonioid taxa.[18] The tentacles arise from a horseshoe shaped basal structure (about 6 mm wide), which fans into proximal and distal zones on both the left and right sides. The proximal zone gives rise to four or five tentacles, while the distal zone gives rise to seven or eight (Figures 2D, 2E, and 2I), all of which emerge on the outer side of the tentacle complex (i.e., unilateral branching). The tentacles are up to about 19 mm long, weakly to strongly curved, and taper in width distally before an abrupt blunt termination (Figures 2D, 2E, and 2H). Fine dark lines run along the outer edges of the tentacles (black arrows in Figures 2D and 2E) and are most likely wrinkles resulting from the collapse of originally hollow structures. Additionally, there are dark lines running down the middle of parts of the proximal and distal zones and associated tentacles (white arrows in Figures 2D and 2E), which do not connect to these wrinkles; their size, shape, and arrangement indicate that they are best interpreted as part of a tentacular nervous system.[21–23] The tentacle complex shows similarities to the lophophores of living brachiopods, bryozoans, and phoronids, yet differs from them in that the tentacles of *Rotadiscus* taper distally, a feature that is not seen in any living lophophorates[21] and only weakly expressed in fossil forms[24] but is observed in some ambulacrarians.[21] In addition, the mouth is not directly associated with the tentacles in *Rotadiscus*, unlike all lophophorates,[23] and this arrangement is instead more similar to the situation in extant pterobranchs and crinoids.[21] This separation between the mouth and tentacles of *Rotadiscus* is incompatible with lophophore function, strongly arguing against the homology of these structures. Instead, the morphology and organization of the tentacle complex of *Rotadiscus* are more consistent with the tentacular systems of extant ambulacrarians, as previously suggested.[19]

In YKLP 13090 and CJHMD 00038, a double spiral structure (about 3 mm wide) occurs on the disc surface adjacent to the

anatomies (Figures 3A and 3C), seemingly independently acquired by different tunicate lineages.[25,26]

The presence of a rigid surface showing fine concentric and radial ornament together with a soft surface characterized by about 90 pairs of radiating lines and a horseshoe-shaped tentacle complex with unilateral branching demonstrates that the fossils belong to *Rotadiscus grandis*.[17,18,20,27] In other eldonioids, the number of radiating lines is generally much lower and the tentacles, when preserved, show multiple orders of bilateral branching.[17,18,20,28] The putative trifid mouth described by Sun & Hou,[17] not evident in any of the fossil specimens we studied, is in all likelihood a taphonomic artefact.[27] The double spiral structure adjacent to the base of the tentacle complex in two specimens (Figures 1, 2, and 3) had never previously been documented in *Rotadiscus* or any other eldonioids; however, the consistent size and shape of this structure across multiple specimens demonstrate that it was an original feature of the animal.

Eldonioids have been variously interpreted as holothurians,[29–31] cnidarians,[17,32] lophophorates,[18,33,34] stem echinoderms[35] or early ambulacrarians,[19,36] and, as such, researchers have not been able to leverage the phylogenetic utility of these strange fossils. The presence of a U-shaped through-gut and bilateral symmetry alongside the absence of any cnidarian synapomorphies strongly refutes non-bilaterian comparators, but to establish the phylogenetic position of the group with greater precision, we assembled a comprehensive matrix of extant and extinct bilaterians consisting of 330 characters for 41 living groups and 20 fossil taxa, including the eldonioids *Rotadiscus*, *Pararotadiscus*, *Eldonia ludwigi*, and *Eldonia eumorpha*. Bayesian analyses of this dataset support the placement of eldonioids in a clade with the Cambrian tentaculate taxa *Herpetogaster* and *Phlogites*. The results of our sensitivity tests (Figures S3 and S4) were largely unchanged from the main analysis (Figure 4A). Morphological characters
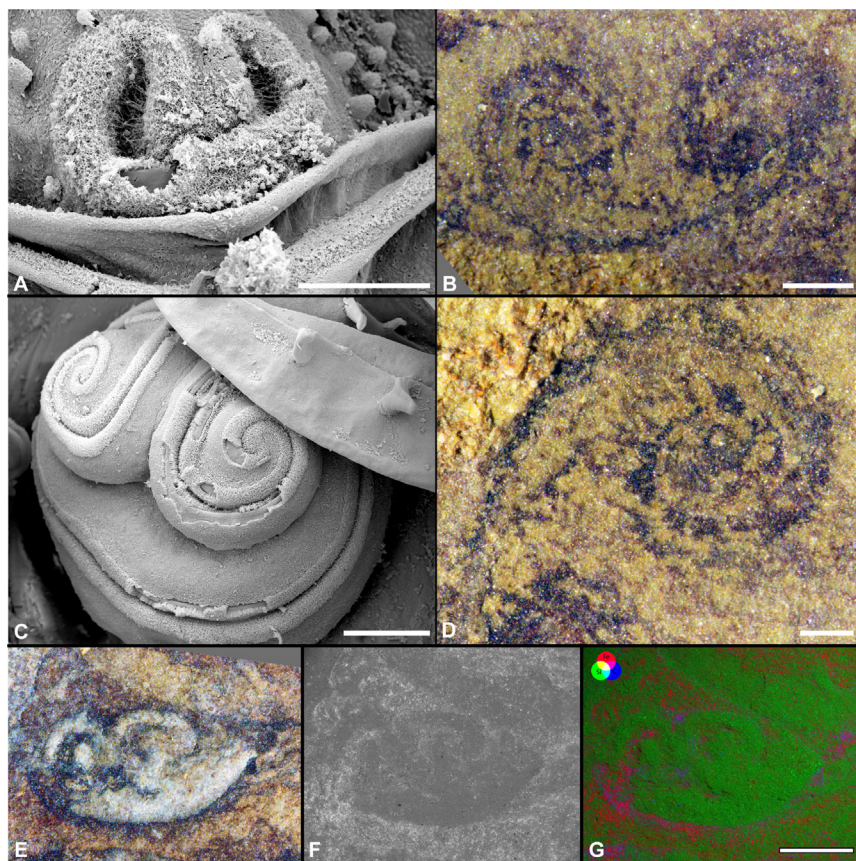
base of the tentacle complex (Figures 1, 2, and 3). It consists of a mirrored pair of tight spirals that are connected at their distal ends to form a "C" shape (Figures 2B, 2C, 2F, 2G, 3B, and 3D–3G). The orientation of the double spiral relative to the tentacles is inconsistent between the specimens (Figures 1 and S1). The close proximity and consistent mode of preservation of the double spiral and the tentacles (Figures 3E–3G) suggest that the former might have served as an opening to the exterior for the latter. We infer that the tentacles were coelomate, based on their originally hollow nature and the close similarity in preservation to the coelomic sac surrounding the gut (Figures 1A, 1B, and 2A–2C), and thus the double spiral is interpreted as a covering to a coelomopore (an opening connecting the tentacular system to the exterior). Extant cephalochordates and tunicates show similar dorsal coverings (opening of Hatschek's pit and the dorsal tubercle, respectively), which are ciliated tracts leading to homologous structures.[25,26] In tunicates, the dorsal tubercle can even show comparably intricate spiraling

is a connection between these external opening(s) and the anteriormost head coelom[21,37,38] (see Stach[39] for an alternative hypothesis), and this is supported by conserved gene expression patterns in Rathke's pouch (the embryonic structure that gives rise to the adenohypophysis in adult vertebrates) and the coelomic ducts of echinoderms and hemichordates.[40] We therefore hypothesize homology of the coelomopore in *Rotadiscus* with the opening to Hatschek's pit in cephalochordates, the dorsal tubercle and neural gland in tunicates, the adenohypophysis in vertebrates, the hydropore of echinoderms, and the proboscis pores of hemichordates (Figure 5). In extant ambulacrarians, the coelomopore plays an important role in the excretory and water vascular systems.[2,4,21] In chordates, the homologous structures are associated with neurological structures and the endocrine system, secreting gonadotropin which plays a role in sexual development and reproductive function.[21,25,26] The close association with a complex tentacular system in *Rotadiscus* (Figure 2) strongly suggests a similar function to that of extant ambulacrarians. Because the tentacular systems of modern echinoderms and pterobranch hemichordates are derived from homologous coelomic cavities[2,4,41] (hydrocoel and mesocoel, respectively; Figure 5), this could indicate that the tentacles of cambroernids were constructed from the same body coelom.

Our results strongly support the placement of cambroernids as stem-group ambulacrarians (Figure 4A), and they are the first group to be recovered in this position through phylogenetic systematic analysis. Perhaps unsurprisingly, we recover eldonioids as derived members of the cambroernid clade, confirming that body coiling increased throughout the evolution of the group[19] (Figure 4B). *Herpetogaster* is recovered as the earliest-diverging cambroernid, indicating that it might be closest in morphology to the most recent common ancestor of the group.

Ancestral state reconstructions are equivocal as to whether a branched tentacular system is plesiomorphic for ambulacrarians (probability of presence = 0.48; Figure 4A), perhaps suggesting that the echinoderm water vascular system and the pterobranch tentacular system share a deep homology but evolved
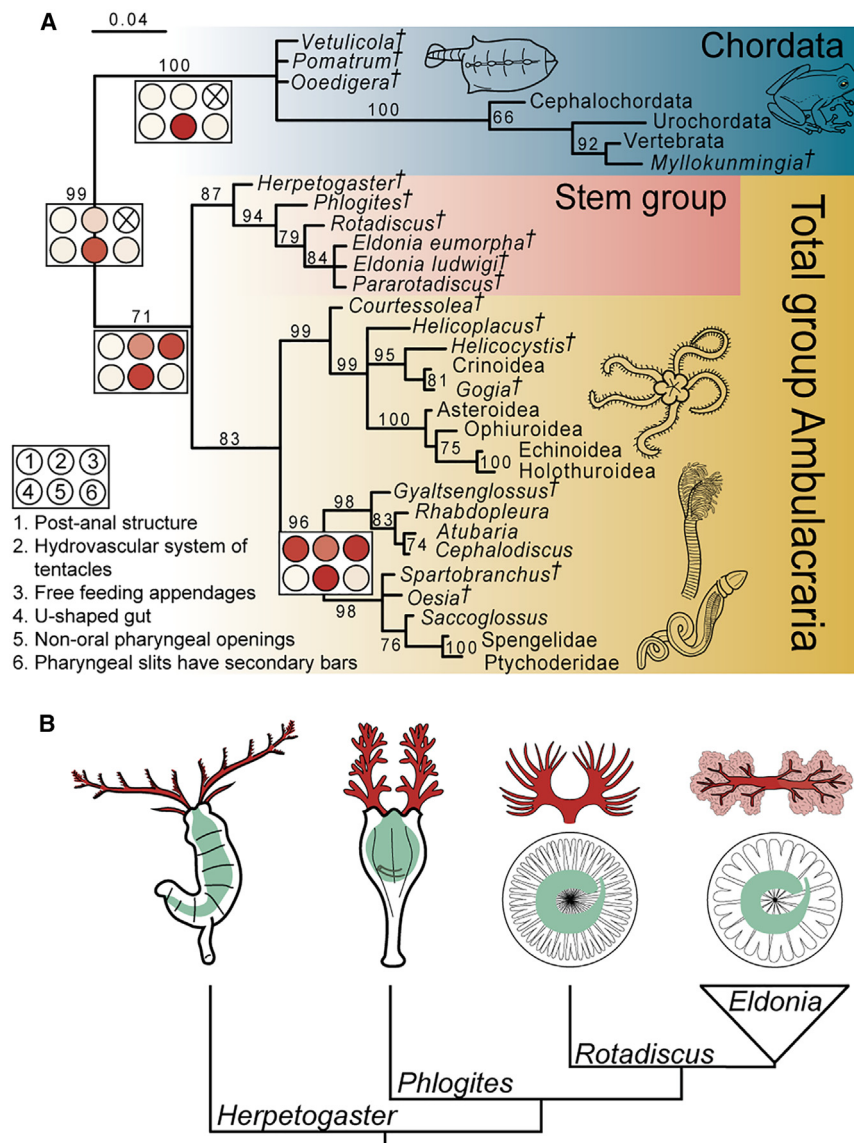
uniting these forms include a tentacle complex that bifurcates and branches and body rotation about the anterior-posterior axis. The tentacle complex shows variation within the group (Figure 4B): *Rotadiscus* and *Herpetogaster* share a similar unilateral branching pattern of the tentacles, while in *Eldonia* and *Pararotadiscus*, the tentacles branch bilaterally.[17,18,20,28] In addition, *Herpetogaster* and *Eldonia* have more plumose tentacles than other members of the clade. This group, named Cambroernida,[19] is recovered with strong support as a clade of stem ambulacrarians, expanding the disparity of this group to include the unique cambroernid body plan.

DISCUSSION

Comparison with extant deuterostomes allows us to propose homologies between cambroernids, ambulacrarians, and chordates (Figure 5). The covering of the coelomopore of *Rotadiscus* is morphologically similar to the opening to Hatschek's pit in cephalochordates and the dorsal tubercle in tunicates (Figure 3), although the intricate shape of the dorsal tubercle in different lineages of tunicates (Figures 3A and 3C) likely evolved independently.[25,26] These structures have been homologized with the vertebrate adenohypophysis—the anterior part of the pituitary gland—based on positional, anatomical, and functional similarities.[25,26] The homology of Hatschek's pit with the coelomopores of extant ambulacrarians (echinoderm hydropore and hemichordate proboscis pores) has long been suggested because in echinoderms, hemichordates, and larval cephalochordates there

Figure 4. Phylogenetic position of *Rotadiscus grandis* and other extinct deuterostomes

(A) Majority-rule consensus tree from Bayesian phylogenetic analyses with ancestral state reconstructions for key characters (colors show probability of presence, with red equal to 1 and white equal to 0). Characters 1–6 relate to characters 232, 156, 157, 36, 196, and 199 in Data S1 and S2. Crosses for character 3 (157 in Data S1 and S2) indicate inapplicability due to high probability of absence of character 2 (156 in Data S1 and S2), on which it is contingent. Posterior probabilities shown at resolved nodes. Fossil taxa indicated with a dagger symbol.

(B) Tentacle morphology in cambroernids.

See also Figures S3 and S4 and Data S1 and S2.

developed further adaptations for tentaculate feeding,[16,43] or enlarged or serialized in forms without a complex tentacular system.[15]

Although non-oral pharyngeal openings are absent in all extant echinoderms, their presence has been inferred in some fossil taxa.[42,45,46] Furthermore, several putative stem-group echinoderms have been interpreted as pharyngeal basket feeders,[12,47,48] implying the presence of typical chordate characteristics in other early ambulacrarians. Among extant deuterostomes, pharyngeal basket feeding is observed in tunicates, while the double spiral structure we document in *Rotadiscus* is most similar to the dorsal covering of the coelomopore derivative in cephalochordates and tunicates.[21,25,26] Moreover, *Herpetogaster* has a serialized body,[19] a feature that is otherwise absent in ambulacrarians but is present in chordates. Together, this confirms that early ambulacrarians had chimeric combinations of characters and indicates that several features that were previously thought to have been restricted to chordates may have been plesiomorphic. This is consistent with our ancestral state reconstruction, which was equivocal about the presence of some traditionally hypothesized ambulacrarian characters (e.g., a tentacular system) in the most recent common ancestor of ambulacrarians (Figure 4A).

Additionally, our analysis sheds light on the body plan of the most recent common ancestor of deuterostomes. Surprisingly, the results indicate that a post-anal region is not plesiomorphic for deuterostomes (probability of presence = 0.01; Figure 4A), as often assumed,[2,49,50] but instead evolved independently in crown chordates and hemichordates. Although these posterior extensions have similar patterns of *hox* gene expression in living species,[51,52] this may be because posterior *hox* genes were already expressed in the posterior ectoderm.[53] Indeed, there are major morphological differences between the post-anal tails of chordates and hemichordates; the chordate tail is

independently. However, if this tentacular system was present in the ancestral ambulacrarian, we find that it was most probably arranged into a series of free feeding appendages (probability of presence = 0.85; Figure 4A), similar to those seen in cambroernids and pterobranchs. Many extant ambulacrarians with developed tentacular systems have either lost non-oral pharyngeal openings entirely (echinoderms[42,43]) or retain only rudimentary structures (the pterobranch *Rhabdopleura*[44]), and this might be due to the dominance of tentaculate feeding in those groups. *Herpetogaster*, the earliest-diverging cambroernid, has a pair of pore-like structures, interpreted as non-oral pharyngeal openings, proximal to a pair of branched tentacles,[19] but there is no evidence for pharyngeal openings in any other cambroernids. This might indicate that non-oral pharyngeal openings were lost in more derived cambroernids as they became better suited to a tentaculate feeding strategy. The presence of both characters in *Herpetogaster* is likely indicative of the plesiomorphic state for ambulacrarians, with pharyngeal openings either becoming redundant as taxa
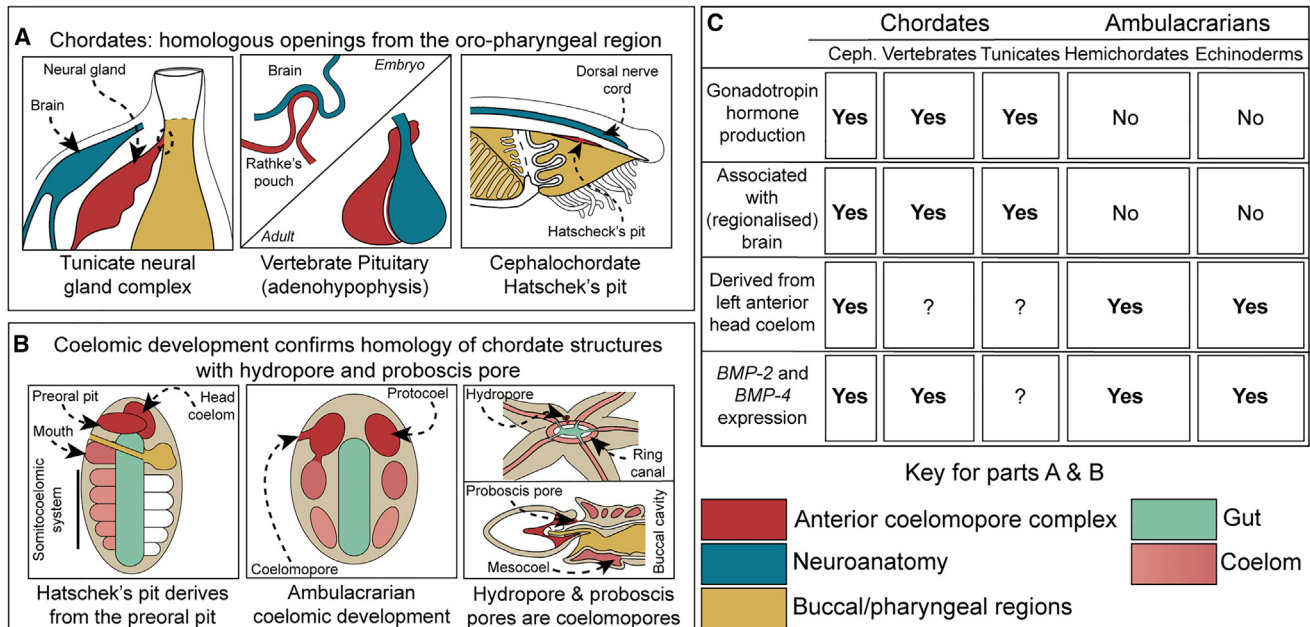
Figure 5. Homology of the coelomopore and related structures in extant deuterostomes
(A) Homologies between tunicates, vertebrates, and cephalochordates. Dashed oval in left-hand panel corresponds to dorsal tubercle.
(B) Homologies between cephalochordates and ambulacrarians.
(C) Homologies across deuterostomes. Abbreviation: Ceph., cephalochordates.
Colors signify homologous structures. See also Data S1.

characterized by a central skeletal element, longitudinal nerve cord, and endodermal tissue—features that are absent in the post-anal tail of the enteropneust *Saccoglossus kowalevskii*.[54] Our analyses confirm that pharyngeal openings were present in the most recent ancestor of crown deuterostomes (probability of presence = 0.84; Figure 4A), but they strongly suggest the gill bars that support these openings in extant cephalochordates and enteropneusts were not present in their most recent common ancestor (probability of presence = 0.06; Figure 4A). The ancestral deuterostome also lacked a U-shaped gut (probability of presence = 0.10; Figure 4A), which our data suggest evolved multiple times across deuterostomes.

Given these data, we reconstruct the ancestral deuterostome as a bilaterally symmetrical chordate-like animal with a terminal anus and pharyngeal openings (consistent with morphological and developmental genetic evidence[2–5,49,50]), which were most likely used for active suspension feeding, as in extant cephalochordates, urochordates, and enteropneusts. This argues against the traditional hypothesis of a sessile tentaculate ancestor[55] and is instead more compatible with the mobile filter-feeding worm proposed by Cameron.[41,56]

Eldonioids have proven controversial since their discovery over a century ago.[29] Until now, the apparent absence of any derived characters shared with extant phyla had led to their exclusion from phylogenetic analyses concerned with deuterostome evolution.[8,13,16] Our novel interpretations of the anatomy of *Rotadiscus*, based on exceptionally preserved fossil specimens, allow us to rigorously constrain the phylogenetic position of eldonioids for the first time, thereby elucidating their evolutionary significance. Historically, uncertainty regarding the

plesiomorphic character states of deuterostomes has hindered efforts to reconstruct ancestral body plans[2–5]; our analyses suggest this is because major characters, including a post-anal region, gill bars, and a U-shaped gut, evolved through convergence rather than shared ancestry. These results confirm that the integration of unique fossil anatomies is critical for uncovering the origin and earliest evolution of deuterostomes.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2023.04.048.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited Data		
Character matrix	This study	Data S1 and S2
Software and Algorithms		
Adobe Photoshop 2021	Adobe	https://www.adobe.com
MrBayes v.3.2.7	Ronquist et al.[57]	https://nbisweden.github.io/MrBayes/index.html
Other		
Fossil specimens	Chengjiang Fossil Site World Heritage Museum (CJHMD)	CJHMD 00038, CJHMD 00039
Fossil specimen	Yunnan Key Laboratory for Palaeobiology, Yunnan University (YKLP)	YKLP 13090

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Imran Rahman (imran.rahman@nhm.ac.uk).

Materials availability
Newly described fossil specimens (CJHMD 00038, CJHMD 00039, and YKLP 13090) are located in the Chengjiang Fossil Site World Heritage Museum (CJHMD) and the Yunnan Key Laboratory for Palaeobiology, Yunnan University (YKLP).

Data and code availability
The character matrix used for phylogenetic analyses is available as supplemental data (Data S1 and S2).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Material
Fossil specimens of *Rotadiscus grandis* were collected from the Chengjiang biota of Yunnan Province, southern China, which is early Cambrian (Cambrian Series 2, Stage 3) in age (~518 Ma).[58] The fossils are housed at the Chengjiang Fossil Site World Heritage Museum (CJHMD) and the Yunnan Key Laboratory for Palaeobiology, Yunnan University (YKLP).

METHOD DETAILS

Fossil preparation and photography
Specimens were prepared with needles under a Nikon SMZ1270 stereo microscope. Photographs were taken using a Canon EOS 5DS digital SLR with a Sigma 70 mm macro lens. Colour levels of images were adjusted using Adobe Photoshop 2021 to improve balance without removing any data.

SEM-EDX
YKLP 13090 was imaged using an FEI Quanta 650 FEG scanning electron microscope (SEM) at Yunnan University. Elemental mapping was undertaken using an EDAX Pegasus energy dispersive X-ray spectroscopy (EDX) system, 112× magnification, 10.3 mm working distance, and an accelerating voltage of 20 kV.

Phylogenetic analyses
Phylogenetic analyses were performed using a character matrix based on published matrices[41,59–62] supplemented with new characters and additional fossil data. The matrix generally samples to phylum level, but includes class-level sampling for ambulacrarians and lophophorates as these are the two main current hypotheses of affinity for eldonioids. The final matrix (Data S1 and S2) consists of 61 taxa (41 extant groups and 20 extinct taxa) and 330 characters.

Bayesian analyses were run in MrBayes v.3.2.7[57] under the Mk model.[63] The tree presented in our main text shows the results of using a gamma distribution to model rate variation, but we additionally carried out analyses using a log normal distribution. Our main

analysis employed symmetry in transition rates, i.e., all characters have an equal rate when moving from state 0 to state 1 and back again, which is the default setting in MrBayes; we also ran analyses specifying character asymmetry with the command symdirihyperpr=fixed(0.1). 0.1 fixes a uniform prior on the proportions of state frequencies. All analyses we run using only parsimony informative characters to account for ascertainment bias. Other parameters not mentioned here were left as the MrBayes default settings (i.e. shapepr=exponential(1.0) and brlenspr=Unconstrained:GammaDir(1.0,0.100,1.0,1.0)). Analyses were run for 10,000,000 generations, sampling every 100 generations, with 25% of samples discarded as burnin. Convergence was assessed based on the effective sample size (>200), potential scale-reduction factor (1.0) and the average derivation of split frequencies (<0.01).

To test if the recovered topology was sensitive to our choice of parameters, we ran analyses under a gamma distribution for modelled rate variation specifying either symmetry (the default state in MrBayes) (Figure S3A) or asymmetry (Figure S3B) of transition rate. We also repeated analyses under a log normal distribution for modelled rate variation, again specifying both symmetry (Figure S3C) and asymmetry (Figure S3D) in transition rate.

Some phylogenomic studies have recovered xenacoelomorphs as sister-group to ambulacrarians[64,65] and not as sister to Nephrozoa (the traditional phylogenetic hypothesis[66]), so we ran an additional analysis in which monophyly of Xenambulacraria was specified, but all fossils were allowed to wander (Figure S4A). Additional recent work has suggested that deuterostomes may not be monophyletic,[67,68] and so we constrained the non-monophyly of deuterostomes by forcing a sister relationship between chordates and protostomes, again with all fossils allowed to wander fully (Figure S4B).

Ancestral state reconstructions

Ancestral state reconstructions of all 330 characters were performed in MrBayes using the 'report ancstates' function, which incorporates topological uncertainty. We placed hard monophyly constraints on four nodes of interest, Deuterostomia, Ambulacraria, Chordata, and Hemichordata, before running these analyses, but allowed taxa to wander within these clades. Convergence was assessed based on the effective sample size (>200).