

## Research



**Cite this article:** Evans SD, Droser ML, Erwin DH. 2021 Developmental processes in Ediacara macrofossils. *Proc. R. Soc. B* **288**: 20203055. <https://doi.org/10.1098/rspb.2020.3055>

Received: 7 December 2020

Accepted: 1 February 2021

**Subject Category:**

Palaeobiology

**Subject Areas:**

developmental biology, evolution, palaeontology

**Keywords:**

development, Ediacaran, Ediacara biota, evolution, regulatory genes, Metazoa

**Author for correspondence:**

Scott D. Evans

e-mail: [scotte23@vt.edu](mailto:scotte23@vt.edu)

<sup>†</sup>Present address: Department of Geosciences, Virginia Tech, Blacksburg, VA 24061, USA.

# Developmental processes in Ediacara macrofossils

Scott D. Evans<sup>1,†</sup>, Mary L. Droser<sup>2</sup> and Douglas H. Erwin<sup>1</sup>

<sup>1</sup>Department of Paleobiology MRC-121, National Museum of Natural History, Washington, DC 20013-7012, USA

<sup>2</sup>Department of Earth and Planetary Sciences, University of California, Riverside, CA 92521, USA

SDE, 0000-0001-5654-8495; MLD, 0000-0001-7112-5669; DHE, 0000-0003-2518-5614

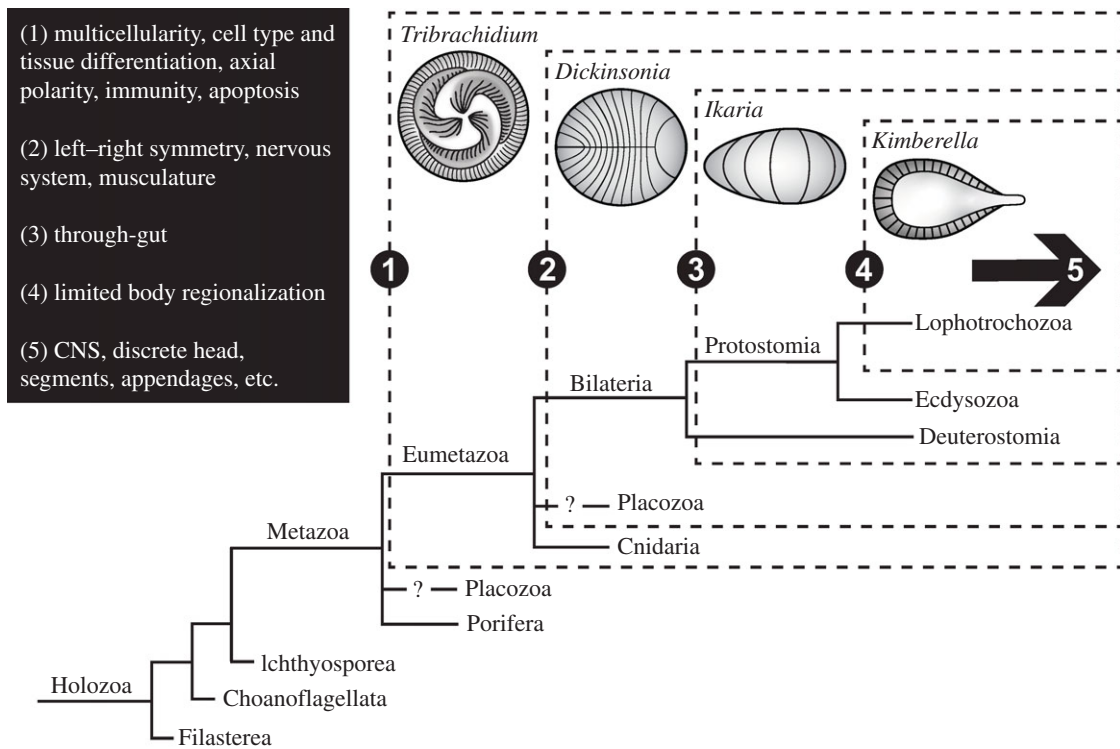
The Ediacara Biota preserves the oldest fossil evidence of abundant, complex metazoans. Despite their significance, assigning individual taxa to specific phylogenetic groups has proved problematic. To better understand these forms, we identify developmentally controlled characters in representative taxa from the Ediacaran White Sea assemblage and compare them with the regulatory tools underlying similar traits in modern organisms. This analysis demonstrates that the genetic pathways for multicellularity, axial polarity, musculature, and a nervous system were likely present in some of these early animals. Equally meaningful is the absence of evidence for major differentiation of macroscopic body units, including distinct organs, localized sensory machinery or appendages. Together these traits help to better constrain the phylogenetic position of several key Ediacara taxa and inform our views of early metazoan evolution. An apparent lack of heads with concentrated sensory machinery or ventral nerve cords in such taxa supports the hypothesis that these evolved independently in disparate bilaterian clades.

## 1. Introduction

The fossil record of complex, macroscopic community-forming organisms, including animals, begins with the Ediacara Biota (570–539 Ma). Molecular clock estimates suggest that major metazoan phyla and their constituent clades evolved prior to this period [1–4]; however, phylogenetic affinities for most of the Ediacara Biota remain enigmatic [5]. Potential explanations for this phylogenetic uncertainty include the simplicity of early animal forms, preservational biases, and lags between character acquisition and ecological success (e.g. [3,6,7]). Many Ediacara taxa may represent stem lineages of animal phyla but their diagnostic characters either were not preserved or had not yet evolved.

Comparative developmental studies of modern organisms reveal a wealth of information regarding the underlying genetic controls responsible for specific characters (e.g. [8]). Many genes highly conserved in bilaterians are present in all animals and can be found among their closest metazoan relatives (see review in [9]). Importantly, there is a growing database of information regarding developmental characters, their phylogenetic distribution and the genetic machinery underlying their expression.

Here, we use the expression of developmentally controlled features, or their absence, to evaluate the position of select Ediacara taxa. We identify characters of these organisms controlled by conserved developmental processes and suggest genetic elements likely responsible for their expression. Based on recent work, we assume that these taxa were animals. Although alternatives have been proposed (e.g. [10,11]) and certain fossils from this biota have been identified as non-metazoans [12,13], multiple independent lines of evidence support the interpretation of key taxa as animals (e.g. [1,5,14–16]).



**Figure 1.** Holozoan phylogeny with inferred placement of representative White Sea taxa (dashed boxes) based on developmentally relevant characters (1–5, black box). Characters represent those that can be identified based on morphological expression in representative Ediacara fossils, and thus are not indicative of their earliest appearance. Arrow represents increased combinatorial complexity of transcription factor interactions in all three groups of bilaterians. Question marks represent uncertainty of placozoan placement. Ctenophores omitted to avoid uncertainty. CNS, central nervous system.

Observations from the fossil record rely on preserved morphological traits for recognizing potential regulatory mechanisms. Regulatory elements controlling features that are not preserved will not be recognized. Consequently, an inferred lack of a given character necessarily represents the absence of evidence.

### (a) Metazoan framework

We are concerned with the main axis of animal phylogeny, from sponges through cnidarians to the three clades of bilaterians (figure 1). Lophotrochozoans and ecdysozoans compose the protostomes, and chordates and echinoderms belong to the deuterostomes [17]. A number of problematic issues remain in metazoan phylogeny, including the position of ctenophores, placozoans and Xenacoelomorpha and the topology of major branches within the Panarthropoda and the Lophotrochozoa. The issues relevant here are the position of ctenophores, discussed below, and placozoans. In most studies, placozoans emerged after sponges and are the sister clade to all other metazoans. Recent work suggests they may be sister to cnidarians, but this result is sensitive to the position of ctenophores (e.g. [18]). We retain placozoans in their traditional position, but note that if they are sister to cnidarians they may be the remnants of a now largely missing clade of diploblastic forms, possibly including members of the Ediacara Biota.

Metazoans are classified within Holozoa, which broadly contain many of the developmental tools exploited by animals. Non-metazoan holozoans are small and have limited cellular differentiation. However, studies of major clades (filastereans, ichthyosporeans and choanoflagellates) reveal that the regulatory capacity for multicellularity, including

spatial and temporal differentiation of multiple cell types, is shared across holozoans [19,20] and thus likely present by 900 Ma [9]. A substantial increase in genome size and regulatory complexity occurred at the base of Metazoa [9,21–24]. Both morphological and more recent single cell RNA sequencing (scRNA-seq) studies have identified a dozen or more cell types in sponges, cnidarians and ctenophores (although fewer in placozoans [25,26]; summarized in [9]).

Despite expansion of the regulatory genome before and during the origin of animals, key regulatory components were not widely used until the origin of Bilateria (e.g. [27]). One example is the use of distal enhancers, regulatory sequences that lie well away from the target gene (in contrast to proximal enhancers, which are immediately upstream of their target gene). Distal enhancers are present at the base of Metazoa [28], but surprisingly, are not common in sponges, cnidarians or placozoans, possibly because more highly structured chromosome architectures were required to efficiently deploy them.

Erwin [9] proposed the following scenario: Many significant genetic processes were initially controlled by relatively flat regulatory networks largely proximal to the coding gene, limiting developmental and morphological complexity. Under this scenario, the protostome–deuterostome ancestor (PDA) was morphologically fairly simple with at most tens of different cell types. Anteroposterior (A/P) patterning was achieved largely via Wnts, while *distalless* helped generate proximo-distal patterning and Pax genes were associated with sensory activities. Near the origin of the PDA, new genes arose, the number of transcription factors (TFs) increased and regulatory potential escalated through use of distal enhancers and more structured chromosome architectures. This allowed independent co-option of conserved

genes and expansion of developmental patterning to generate complex bilaterian characters, including appendages, eyes and gut. Evidence from segmentation is consistent with this model, as it apparently arose multiple times in different bilaterian clades (e.g. [29]), and other clades, such as molluscs, that are metameric but lack true segments [30].

Although there has been considerable interest in the history of nerve cells and the early evolution of the nervous system, achieving consensus on the topic has been hampered by recent debates over the position of ctenophores. Most studies place ctenophores after sponges [31–33]. However, some analyses of molecular data support that they arose before sponges [34,35], with nervous systems evolving independently in ctenophores and eumetazoans [36,37].

Notwithstanding these issues, three primary evolutionary stages are recognized: (i) the origin of discrete neurons, likely from multifunctional sensory cells; (ii) the evolution of a diffuse nerve net; and (iii) the coalescence of a central nervous system (CNS) [38–41]. Analysis of non-metazoan holozoan clades has found evidence of proto-synaptic proteins for cell–cell communication [42] and thus, as is generally the case, many of the elements of the nervous system were present before the origin of metazoans. This facilitated the appearance of specialized neuronal cells followed by the origin of nervous systems in ctenophores, cnidarians and bilaterians (see discussion in [43]). One of the startling results from the scRNA-seq analysis is the diversity of cnidarian neuronal cell types [26].

## (b) Representative taxa

The Ediacara Biota is divided into three temporally distinct assemblages of soft-bodied, macroscopic taxa [44]. The middle White Sea assemblage is well-known from extensive deposits in Russia and South Australia [45] and both is the most diverse and has the highest morphological disparity of the three assemblages [46]. Of more than 40 recognized species, we concentrate on four representative taxa (figure 1). These exhibit features for which developmental processes are well documented among living taxa.

*Kimberella* is an approximately ovoid fossil with a broad, rounded end opposite a narrow, truncated region (figure 2a). The long axis can exceed 10 cm. Preservation of a significant vertical component (depth) suggests that the body was relatively thick and resilient to compaction. Association with repeated sets of scratch marks (figure 2b) demonstrates mobility and feeding by excavation of organic mats that lined the Ediacaran seafloor [47–50]. Morphological evidence for a projection at one end of the organism is reconstructed as a proboscis used in mat excavation [48]. The main body is interpreted with a muscular foot or analogous structure, possibly evidenced by an outer rim or ‘frill’ [48,49,51].

*Ikaria* are millimetre-scale, elliptical fossils (figure 2c) recently described from South Australia and consistent with the generation of associated horizontal burrows (figure 2d), *Helminthoidichnites* [52]. The preservation of negative *Helminthoidichnites* on bed soles with positive levees indicates that *Ikaria* was capable of moving through and displacing sand grains [53,54]. *Helminthoidichnites* are limited to thin (less than 15 mm) sand layers and are observed penetrating organic mats and macroscopic Ediacara taxa, evidence of scavenging [53].

*Dickinsonia* is an ovoid fossil (figure 2e), with one species that could grow to almost a metre in total length. It is divided

down the long axis by a midline, with modular body divisions roughly perpendicular. One end of the long axis is undivided by modules. Associated trace fossils represent feeding via external ventral digestion between periods of directed, active mobility [55–58]. Claims that *Dickinsonia* may have been a giant single-celled organism [10] are contradicted by large maximum dimensions [59], mobility [55–58] and possible tissue differentiation [14,60].

The circular, triradially symmetrical *Tribrachidium* (figure 2f) was likely a sessile, benthic organism (although see [61]) with a maximum diameter of approximately 3 cm [62]. Threefold symmetry is rare in modern animals but is found in several White Sea taxa [63]. Results from computational fluid dynamics are consistent with suspension feeding [64].

## 2. Developmentally controlled characters

Multicellular organisms generate multiple cell types, with tissues representing combinations of cell types, and organs spatially arranged tissues (e.g. [65]). The scale and morphological patterning of many Ediacara taxa is evidence of multiple cell types and some degree of regional differentiation [66]. Suspension feeding activity directing water to specific regions of the body in *Tribrachidium* [64] suggests the concentration of distinct cell types forming an isolated local environment consistent with tissue-grade organization [65].

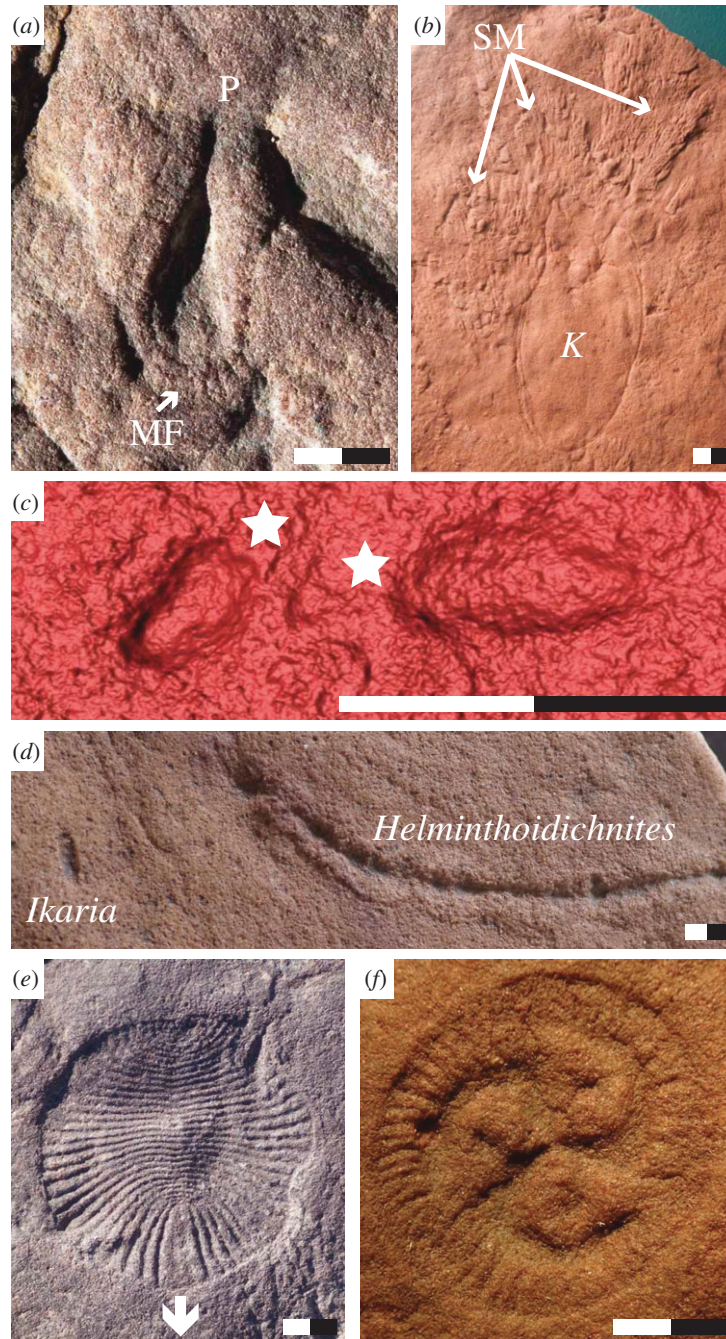
Mobility in *Kimberella*, *Ikaria* and *Dickinsonia* has been attributed to muscular activity [52,55,56,67]. Muscles are composite tissues consisting of multiple cell types [65]. Feeding in *Kimberella* [48,49] and *Ikaria* [52,53] suggests the presence of a mouth and gut, potentially a through-gut, although such structures are not preserved. It is unclear whether these represent true organs, but, if present, a gut likely required multiple tissue layers, including muscles for particle transport.

*Kimberella* presents perhaps the strongest evidence for regional patterning of discrete functional units. One end of the long axis is specialized for excavation of the organic mat but remains ambiguous with respect to organ-grade differentiation. Regardless, morphological distinction, including between the surface facing upward, into the water column, and that in contact with the seafloor, represents functional regionalization.

Axial polarity and related body patterning are observed in all four taxa considered. Flipped *Tribrachidium* exhibit concentric circles on the surface in direct contact with the sediment–water interface distinct from the three-armed morphology facing upward [62]. Although polarization in *Kimberella*, *Ikaria* and *Dickinsonia* may not be homologous to A/P or dorsoventral (D/V) axes in bilaterians [15], these taxa possessed the developmental capacity to produce morphologically distinct perpendicular axes. Expression of such axes results in left–right symmetry in all three, although this may be offset in *Dickinsonia* ([57], but see [56,60]). Precise maintenance of symmetry was likely integral for functions such as mobility [55].

Despite cell-type differentiation, axial polarity and probable gastrulation, no evidence for differentiated appendages, tagmata, or sensory organs has been identified in any White Sea taxon. The absence of observable differentiation related to the long axis within repeated units in *Dickinsonia* precludes assignment as segments [68,69]. This likely extends to all contemporaneous serially divided taxa, although a segmented organism has recently been described from younger





**Figure 2.** Representative taxa of the White Sea assemblage from the Ediacara Member, South Australia, including: (a,b) *Kimberella quadrata* (*K*) with frill or muscular foot (MF), proboscis (P) and associated scratch marks (SM); (c,d) *Icaria variootia* with wider end indicated by white stars and with associated trace fossil *Helminthoidichnites*; (e) *Dickinsonia costata* with white arrow indicating the direction of movement; and (f) *Tribrachidium heraldicum*. Fossils are external moulds preserved in negative relief on the base of fossil beds (hyporelief). (a,b) and (d–f) are photographs of original fossils and (c) is a three-dimensional laser scan. (f) Photo courtesy Christine Hall. (a) LV-FUN 001; (b) P35660; (c) 1T-A 001; (d) P57686; (e) TB-ARB 001; (f) P12898. Scale bars total 1 cm. (Online version in colour.)

Ediacaran strata in South China [70]. Representative taxa show no evidence for serial homology of repeated divisions into distinct functional units, and thus appear to lack true segmentation as observed among multiple Cambrian clades. Despite clear axial polarity, there is no evidence for a discrete head with concentrated sensory organs.

A nervous system is an assembly of neurons [71,72]. What constitutes a neuron is less clear [71,73] and such cells are unlikely to be identified in the fossil record. Nervous systems allow rapid communication over significant distances, often integrating multiple sensory inputs to produce a response (e.g. [74]). Sponges lack a nervous system but move and respond

to external stimuli. Movement is slow and responses are limited [72,73,75,76]. Placozoans move via epithelia and respond to food availability [77,78] relying on concentrated neurosecretory cells at the body periphery [79].

*Kimberella*, *Icaria* and *Dickinsonia* fed directly on organic matter covering the Ediacaran seafloor [48,52,55,58] and likely moved to access new resources. Trace fossils associated with *Kimberella* and *Dickinsonia* suggest that they were able to determine when they had consumed sufficient nutrients in a particular area such that movement was more productive than continued feeding. Comparisons with growth rates in modern mats require that, in order to leave traces of such

**Table 1.** Developmental characters of four representative Ediacara taxa and the genetic controls that regulate their formation in modern organisms. ‘Basal clade’ refers to the earliest appearance of identified regulatory control, in most cases predating the earliest appearance of the character. References cited are for the identification of regulatory control in the basal clade indicated. See also references in [86].

character	representative White Sea taxa with character	regulatory control	basal clade with regulatory element	references
multicellularity	all	actomyosin, cadherins, C-type lectins, LIM Homeobox, Type IV collagens, tyrosine kinases	Holozoa	[22,23,87–89]
cell-type differentiation	all	autocrine signalling, microRNAs, Myc, p53, PCR2, SOX/TCF	Holozoa	[23,90]
mesoderm	<i>Kimberella</i> , <i>Ikaria</i>	$\beta$ -catenin, Nodal, Notch/Delta	Holozoa	[28,91,92]
musculature	<i>Kimberella</i> , <i>Ikaria</i> , <i>Dickinsonia</i>	actin, Mef2, MyHC, myocardin	Eukarya	[93,94]
axial polarity	all	BMP, Hox, ParaHox, Wnt	Metazoa	[95–99]
left–right symmetry	<i>Kimberella</i> , <i>Ikaria</i> , <i>Dickinsonia</i>	Nodal	Bilateria	[92]
body regionalization (organs, appendages, segmentation, etc.)	<i>Kimberella</i> ?	CTFCs, distal enhancers, Hox, Notch/Delta, TADs	Metazoa	[90,100–102]
nervous system	<i>Kimberella</i> , <i>Ikaria</i> , <i>Dickinsonia</i>	bHLH, Notch, SoxB2	Metazoa	[40,103]
CNS	absent	HOX, NK cluster, Nodal, Numb, PAX, Prospero	Bilateria	[40,103,104]
immunity	<i>Dickinsonia</i>	Toll-like receptors	Holozoa	[24,105]
apoptosis	all	Hippo, Myc	Holozoa	[106]

behaviour, mobility—in some cases extending over several metres of the seafloor—must have occurred relatively recently, likely within hours prior to burial [55]. Restriction of *Helminthoichnites* to thin sandstone horizons indicates the chemosensory ability of *Ikaria* to seek out both beneficial oxygenated and toxic sulfidic environments, possibly in response to daily cycles of oxygenic photosynthesis within mats [52,53,80,81].

Ecological similarity between *Dickinsonia* and Placozoa [58] suggests that similar behaviours are possible without a nervous system, although it is unclear if this is scalable to the sizes achieved by *Dickinsonia*. Rapid mobility over large areas and associated with sediment displacement by *Ikaria* is beyond the capacity of Porifera or Placozoa. Burrowing in response to both food availability and environmental suitability suggests a behavioural response integrating distinct sources of information. Systematic excavation observed in scratch marks associated with *Kimberella* [47,48] indicates coordination between a proboscis and ‘frill’ structure adapted for mobility, separated by centimetres. These features strongly suggest the presence of a nervous system.

These three taxa with possible evidence for neural activity exhibit bilateral symmetry but lack signs of neural condensation. Both morphological and behavioural evidence establishes that complex sensory organs and a CNS were present in the early Cambrian (e.g. [82,83]). Thus, the absence of evidence for a CNS as found in many bilaterians is potentially meaningful.

Future palaeontological studies will likely reveal additional developmentally significant characters. The recent discovery of

*Dickinsonia* with morphological defects followed by a return to regular modularity [61,84] indicates repair functions and possibly an immune response. The highly regulated growth and maintenance of constant morphologies in a variety of Ediacara taxa [15,68,85] is difficult to envision without apoptosis.

### (a) Inferred developmental capacity

The developmentally relevant characters described above have conserved regulatory elements that control their expression in modern animals. In table 1 and the discussion below, we use these relationships to identify likely regulatory machinery responsible for their production in representative Ediacara taxa.

Given the assumption of animal affinities, regulatory elements essential for multicellularity and found in holozoans were likely operating in Ediacara taxa. These likely included multiple extracellular matrix domains and TF families, such as cadherins, C-type lectins, tyrosine kinases, LIM Homeobox and canonical Type IV collagens, among others [22,23,86–89].

Different animal cell types are produced via changes to their core regulatory complex of TFs [107]. Other controls, such as microRNAs and autocrine signalling, help maintain individualized cellular identities [24,28,65,108]. Tissue formation builds upon tools involved in the establishment of an extracellular matrix, such as  $\beta$ -catenin [28,91]. These coordinate different life stages in single-celled holozoans (e.g. [23,28,91]). White Sea

taxa likely employed these same genetic elements to produce differentiated cell types and tissue-grade organization.

Actomyosin-based contraction, essential in metazoan musculature, is conserved among eukaryotes for functions including cell division and shape change [93,94]. Thus, common contractile proteins, such as actin and myosin heavy chain, were present in Ediacaran animals. Metazoan lineages constructed individualized TF pathways to build and control muscles, including different muscle types within bilaterian groups [93,94], limiting further classification of muscle-specific gene regulatory pathways.

Axial patterning is normally achieved by antagonistic interactions between morphogenic gradients [95–99,109,110]. A/P differentiation is controlled in bilaterians by the canonical Wnt/ $\beta$ -catenin pathway, with later co-option and expansion by Hox and ParaHox genes [95–99]. Polarized expression of Wnt in non-bilaterian metazoans, including poriferan larvae, ctenophores and cnidarians, suggests a conserved role for these proteins [95,98,111–113]. Antagonistic chordin-BMP signalling for D/V patterning is conserved across bilaterians [95–98,114]. Although homology between cnidarian and bilaterian body axes is unresolved (as discussed by [115]), similar regulatory mechanisms—including Wnt, BMP signalling and Hox genes [111,113,116,117]—likely operated in Ediacaran metazoans with axial differentiation. In modern bilaterians, left–right symmetry, as observed in *Kimberella*, *Ikaria* and *Dickinsonia*, requires the Nodal pathway as an extension of the transforming growth factor (TGF- $\beta$ ) pathway [92].

Increased body regionalization characteristic of bilaterians appears to have required enhanced combinatorial complexity of interactions between existing TFs, and thus deployment of distal enhancers, topologically associated domains (TADs) and insulator proteins (e.g. CTCFs), which jointly structure three-dimensional chromatin interactions [9,28]. For example, Hox genes are integral in the formation of specific anatomical structures such as organs and appendages [118] as well as more basal functions involving axial patterning [95–99,117,119]. Evidence for some degree of gross morphological regionalization in *Kimberella* indicates potential, but limited use of similar regulatory elements.

The Notch receptor and Delta ligand promote cell identity in populations of regionally adjacent cells [90], which Davidson & Erwin described as a reusable ‘plug-in’ [120]. Although unique to metazoans, these pathways likely evolved from the reshuffling and co-option of protein domains found in single-celled eukaryotes [100]. This signalling pathway is found in non-bilaterian metazoans, for example, in nematocyte and germ cell differentiation in cnidarians [101] and sensory cells in poriferans [121]. Notch/Delta signalling is associated with a range of differentiated systems in bilaterians, including the brain, heart and limbs (see [102] and references therein), apparently absent in the Ediacara Biota. Although patterns of segmentation are variably regulated, a common theme among vertebrates, arthropods and annelids is co-option of Notch/Delta signalling [122]. The absence of a CNS, segmentation and appendages in the Ediacara Biota suggests that Notch/Delta signalling was likely restricted to germ cell differentiation and/or establishment of the nervous system in Ediacara taxa such as *Kimberella* and *Ikaria*. Apparently, many bilaterian co-options had not yet occurred.

Based on common expression in the neuronal regions of ctenophores, cnidarians and bilaterians, the establishment of a nervous system in Ediacara taxa likely involved SoxB2,

Notch and bHLH signalling [40,103,113]. An absence of evidence for the arrangement of this system leaves open the question of whether such taxa used regulatory elements, such as the Nodal pathway, important in establishing neural organization in bilaterians [92].

Immunity was possibly triggered by Toll-like receptors common to cnidarians and bilaterians [105] and recently identified in choanoflagellates [24]. Apoptosis may have been achieved by conserved signalling pathways, such as Hippo, functional in holozoans and used to coordinate cell proliferation and apoptosis in a variety of animals [106].

## (b) Inferred phylogenetic affinities of representative Ediacara taxa

Insights into the developmental capacity of representative Ediacara taxa can be integrated with comparative developmental studies to constrain potential phylogenetic positions (figure 1). Non-metazoans exhibit similar traits to those highlighted here, albeit with different developmental control. Namely, plants are spatially patterned with repeated units oriented relative to the growth axis and controlled via regulatory mechanisms distinct from those used by animals [123,124]. Thus, the identification of specific genetic programming in representative taxa relies on the assumption of metazoan affinity.

Developmental characters are interpreted exclusively from fossil evidence, independent of phylogenetic classification. We consider it most parsimonious that the number of developmentally relevant characters consistent with those found in metazoans add to the growing body of evidence that the Ediacara Biota records the early evolution of animals, rather than the independent evolution of a variety of metazoan features in a ‘failed evolutionary experiment’ of non-animal taxa [125]. Further, traits identified share many similarities with those of animals not found in other complex organisms. For example, although both use antagonistic gene expression integrating local and global signalling to establish axial polarity, plants maximize morphological flexibility in order to respond to variability in their external environment [124,126]. Animals, as well as the Ediacara Biota, instead use these systems to maintain symmetry and scaling with growth, important for functions such as mobility [124]. Therefore, developmental characters may represent further, independent support for many Ediacara Biota taxa belonging within Metazoa.

Despite unfamiliar body plans, *Tribrachidium* and *Dickinsonia* display cellular and tissue differentiation as well as polarity about at least one body axis. *Tribrachidium* used regulatory programming similar to that present in modern cnidarians. *Dickinsonia* appears to occupy a unique space in metazoan development. It had the capacity to coordinate behaviour across great cellular distances as well as generate polarity, left–right symmetry and patterning relative to the direction of movement, similar to bilaterians. However, there is no evidence for the more complex body regionalization evident in crown bilaterians.

Previous interpretations have indicated that *Ikaria* shares many attributes associated with the PDA (although the PDA probably existed tens of millions of years earlier [1,2]), including the likely presence of a nervous system and a through-gut [52]. *Kimberella* has been allied with Lophotrochozoa [1] and displays axial and regional patterning to produce a proboscis and foot (or analogous structures). These traits are consistent



with the use of bilaterian specific regulatory elements, including  $\beta$ -catenin, distal enhancers, Notch/Delta and Nodal signalling [92].

### 3. Implications for regulatory evolution

There is an absence of evidence for segmentation, appendages, or concentrated sensory organs in representative White Sea taxa. Molecular clocks suggest that numerous bilaterian lineages existed at this time, and thus these characters might be expected [1,2]. Such absence, however, is consistent with phylogenetic and developmental support for morphologically simple basal bilaterians [3,7,9,103,120]. Although speculative, lack of these features may reflect the absence or limited expression of localized boundaries established by systems of cross-repression (for example, Notch/Delta).

Among bilaterians, formation of a discrete head is developmentally decoupled from A/P patterning (e.g. [127]). Axial polarization in Ediacara fossils with no evidence for focused sensory organs is consistent with the independent evolution of a head and ventral nerve cord in several bilaterian clades by co-opting common body patterning sub-modules [104,127]. An apparent unifying feature of bilaterian cephalization, and similar structures such as lophophores, is a lack of Hox expression and deactivation of Wnt signalling via antagonistic gene expression [127,128]. The hypothesized involvement of Hox and Wnt in the development of *Dickinsonia*, *Ikaria* and *Kimberella* is consistent with the use of these genetic controls prior to the anterior concentration of sensory organs.

Organization of the CNS in bilaterians is related to A/P and D/V patterning, with developmental control involving Hox genes, Wnt and BMP pathways [40,103,104,113,129]. However, cnidarians and some bilaterians (e.g. Xenacoelomorpha) exhibit axial patterning and a non-centralized nervous system [103,104,117]. We propose that several Ediacara taxa represent lineages that similarly had not co-opted these pathways for more complex regionalization. While it is possible that a CNS was present in the PDA and that this absence represents subsequent loss, we consider this unlikely

as it would require the earliest bilaterians in the fossil record to be highly derived relative to this common ancestor. Our proposal is consistent with the hypothesis that rudimentary nervous systems were present in early metazoan lineages and persisted for a long period before multiple independent origins of a CNS [7].

### 4. Concluding remarks

We have evaluated the developmental capacity of representative White Sea taxa, identified several metazoan-specific morphogenetic processes and the likely regulatory elements responsible for their expression. This allows us to bound potential phylogenetic positions of these taxa relative to extant metazoans. At least three occupy the significant gap between the ability to produce body polarization and a nervous system and the subsequent evolutionary adaptations required for more complex regionalization and the formation of a CNS. Although diverse bilaterian body plans do not appear until the Cambrian, bilaterians and gene regulatory elements critical for their later success were represented in the Ediacara Biota. Future work focused on resolving additional developmentally important characters in Ediacara taxa (e.g. morphogenesis [15]), incorporating novel discoveries of gene regulatory networks in modern organisms and identifying variations through time will further refine our understanding of early animal evolution and diversification.

**Data accessibility.** This article has no additional data.

**Authors' contributions.** S.D.E., M.L.D. and D.H.E. conceived of this study and edited the manuscript, S.D.E. and D.H.E. composed the manuscript and S.D.E. created the figures and table.

**Competing interests.** We declare we have no competing interests.

**Funding.** This work was supported by a Peter Buck postdoctoral fellowship to S.D.E. and a NASA Exobiology Grant no. (NNX14AJ86G) to M.L.D.

**Acknowledgements.** We thank Jim Gehling, Christine Hall, Ian Hughes and Karma Nanglu for insightful discussion while preparing this manuscript, Michelle Droser for providing line drawings of representative taxa used in figure 1, and the reviewers for useful comments.

### References

- Erwin DH, Laflamme M, Tweedt SM, Sperling EA, Pisani D, Peterson KJ. 2011 The Cambrian conundrum: early divergence and later ecological success in the early history of animals. *Science* **334**, 1091–1097. (doi:10.1126/science.1206375)
- dos Reis M, Thawornwattana Y, Angelis K, Telford MJ, Donoghue PC, Yang Z. 2015 Uncertainty in the timing of origin of animals and the limits of precision in molecular timescales. *Curr. Biol.* **25**, 2939–2950. (doi:10.1016/j.cub.2015.09.066)
- Cunningham JA, Liu AG, Bengtson S, Donoghue PCJ. 2017 The origin of animals: can molecular clocks and the fossil record be reconciled? *Bioessays* **39**, 1–12 e201600120. (doi:10.1002/bies.201600120)
- Sperling EA, Stockey RG. 2018 The temporal and environmental context of early animal evolution: considering all the ingredients of an 'explosion'. *Int. Comp. Biol.* **58**, 605–622. (doi:10.1093/icb/icy088)
- Droser ML, Gehling JG. 2015 The advent of animals: the view from the Ediacaran. *Proc. Natl Acad. Sci. USA* **112**, 4865–4870. (doi:10.1073/pnas.1403669112)
- Erwin DH, Valentine JW. 2013 *The Cambrian explosion: the construction of animal biodiversity*. Greenwood, CO: Roberts & Co.
- Erwin DH. 2015 Novelty and innovation in the history of life. *Curr. Biol.* **25**, R930–R940. (doi:10.1016/j.cub.2015.08.019)
- Peter IS, Davidson EH. 2015 *Genomic control processes. Development and evolution*. London, UK: Academic Press.
- Erwin DH. 2020 Origin of animal bodyplans: a view from the regulatory genome. *Development* **147**, dev182899. (doi:10.1242/dev.182899)
- Seilacher A, Grazhdankin DV, Legouta A. 2003 Ediacaran biota: the dawn of animal life in the shadow of giant protists. *Paleontol. Res.* **7**, 43–54. (doi:10.2517/prpsj.7.43)
- Retallack GJ. 2007 Growth, decay and burial compaction of *Dickinsonia*, an iconic Ediacaran fossil. *Alcheringa* **31**, 215–240. (doi:10.1080/03115510701484705)
- Bobrovskiy I, Hope JM, Krasnova A, Ivantsov AY, Brocks JJ. 2018 Molecular fossils from organically preserved Ediacara biota reveal cyanobacterial origin for *Beltanelliformis*. *Nat. Ecol. Evol.* **2**, 437–440. (doi:10.1038/s41559-017-0438-6)
- Xiao S, Gehling JG, Evans SD, Hughes IV, Droser ML. 2020 Probable benthic macroalgae from the Ediacara Member, South Australia. *Precam. Res.* **350**, 105903. (doi:10.1016/j.precamres.2020.105903)
- Bobrovskiy I, Hope JM, Ivantsov AY, Nettersheim BJ, Hallmann C, Brocks JJ. 2018 Ancient steroids

- establish the Ediacaran fossil *Dickinsonia* as one of the earliest animals. *Science* **361**, 1246–1249. (doi:10.1126/science.aat7228)
15. Dunn FS, Liu AG, Donoghue PCJ. 2018 Ediacaran developmental biology. *Biol. Rev.* **93**, 914–932. (doi:10.1111/brv.12379)
  16. Xiao SH, Laflamme M. 2008 On the eve of animal radiation: phylogeny, ecology and evolution of the Ediacara biota. *Trends Ecol. Evol.* **24**, 31–40. (doi:10.1016/j.tree.2008.07.015)
  17. Dunn CW, Giribet G, Edgecombe GD, Hejnol A. 2014 Animal phylogeny and its evolutionary implications. *Annu. Rev. Ecol. Syst.* **45**, 371–395. (doi:10.1146/annurev-ecolsys-120213-091627)
  18. Laumer CE, Gruber-Vodicka H, Hadfield MG, Pearse VB, Riesgo A, Marioni JC, Giribet G. 2018 Support for a clade of Placozoa and Cnidaria in genes with minimal compositional bias. *eLife* **7**, e36278. (doi:10.7554/eLife.36278)
  19. Brunet T, King N. 2017 The origin of animal multicellularity and cell differentiation. *Dev. Cell* **43**, 124–140. (doi:10.1016/j.devcel.2017.09.016)
  20. de Mendoza A, Suga H, Permanyer J, Irimia M, Ruiz-Trillo I. 2015 Complex transcriptional regulation and independent evolution of fungal-like traits in a relative of animals. *eLife* **4**, e08904. (doi:10.7554/eLife.08904)
  21. Sebe-Pedros A, Degnan BM, Ruiz-Trillo I. 2017 The origin of Metazoa: a unicellular perspective. *Nat. Rev. Genet.* **18**, 498–512. (doi:10.1038/nrg.2017.21)
  22. Grau-Bove X, Torruella G, Donachie S, Suga H, Leonard G, Richards TA, Ruiz-Trillo I. 2017 Dynamics of genomic innovation in the unicellular ancestry of animals. *eLife* **6**, e26036. (doi:10.7554/eLife.26036)
  23. King N *et al.* 2008 The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans. *Nature* **451**, 783–788. (doi:10.1038/nature06617)
  24. Richter DJ, Fozouni P, Eisen MB, King N. 2018 Gene family innovation, conservation and loss on the animal stem lineage. *eLife* **7**, e34226. (doi:10.7554/eLife.34226)
  25. Sebe-Pedros A *et al.* 2018 Early metazoan cell type diversity and the evolution of multicellular gene regulation. *Nat. Ecol. Evol.* **2**, 1176–1188. (doi:10.1038/s41559-018-0575-6)
  26. Sebe-Pedros A *et al.* 2018 Cnidarian cell type diversity and regulation revealed by whole-organism single-cell RNA-Seq. *Cell* **173**, 1520–1534. (doi:10.1016/j.cell.2018.05.019)
  27. Gaiti F, Calcino AD, Tanurdzic M, Degnan BM. 2017 Origin and evolution of the metazoan non-coding regulatory genome. *Dev. Biol.* **427**, 193–202. (doi:10.1016/j.ydbio.2016.11.013)
  28. Sebe-Pedros A, Ballare C, Parra-Acero H, Chiva C, Tena JJ, Sabido E, Gomez-Skarmeta JL, Di Croce L, Ruiz-Trillo I. 2016 The dynamic regulatory genome of *Capsaspora* and the origin of animal multicellularity. *Cell* **165**, 1224–1237. (doi:10.1016/j.cell.2016.03.034)
  29. Chipman AD. 2010 Parallel evolution of segmentation by co-option of ancestral gene regulatory networks. *Bioessays* **32**, 60–70. (doi:10.1002/bies.200900130)
  30. Minelli A, Fusco G. 2004 Evo-devo perspectives on segmentation: model organisms, and beyond. *Trends Ecol. Evol.* **19**, 423–429. (doi:10.1016/j.tree.2004.06.007)
  31. Feuda R, Dohrmann M, Pett W, Philippe H, Rota-Stabelli O, Lartillot N, Worheide G, Pisani D. 2017 Improved modeling of compositional heterogeneity supports sponges as sister to all other animals. *Curr. Biol.* **27**, 3864–3870. (doi:10.1016/j.cub.2017.11.008)
  32. Pisani D, Pett W, Dohrmann M, Feuda R, Rota-Stabelli O, Philippe H, Lartillot N, Worheide G. 2015 Genomic data do not support comb jellies as the sister group to all other animals. *Proc. Natl Acad. Sci. USA* **112**, 15 402–15 407. (doi:10.1073/pnas.1518127112)
  33. Simion P *et al.* 2017 A large and consistent phylogenomic dataset supports sponges as the sister group to all other animals. *Curr. Biol.* **27**, 958–967. (doi:10.1016/j.cub.2017.02.031)
  34. Moroz LL *et al.* 2014 The ctenophore genome and the evolutionary origins of neural systems. *Nature* **510**, 109–114. (doi:10.1038/nature13400)
  35. Ryan JF *et al.* 2013 The genome of the ctenophore *Mnemiopsis leidyi* and its implications for cell type evolution. *Science* **342**, 1336. (doi:10.1126/science.1242592)
  36. Moroz LL. 2009 On the independent origins of complex brains and neurons. *Brain Behav. Evol.* **74**, 177–190. (doi:10.1159/000258665)
  37. Jekely G, Paps J, Nielsen C. 2015 The phylogenetic position of ctenophores and the origin(s) of nervous systems. *Evodevo* **6**, 1. (doi:10.1186/2041-9139-6-1)
  38. Jacobs DK, Gold DA, Nakanishi N, Yuan D, Camara A, Nichols SA, Hartenstein V. 2010 Basal metazoan sensory evolution. In *Key transitions in animal evolution*, vol. 220 (eds R Desalle, B Schierwater), pp. 175–193. Boca Raton, FL: CRC Press.
  39. Arendt D, Bertucci PY, Achim K, Musser JM. 2019 Evolution of neuronal types and families. *Curr. Opin. Neurobiol.* **56**, 144–152. (doi:10.1016/j.conb.2019.01.022)
  40. Arendt D, Tosches MA, Marlow H. 2016 From nerve net to nerve ring, nerve cord and brain – evolution of the nervous system. *Nat. Rev. Neurosci.* **17**, 61–72. (doi:10.1038/nrn.2015.15)
  41. Liebeskind BJ, Hillis DM, Zakon HH, Hofmann HA. 2016 Complex homology and the evolution of nervous systems. *Trends Ecol. Evol.* **31**, 127–135. (doi:10.1016/j.tree.2015.12.005)
  42. Burkhardt P, Sprecher SG. 2017 Evolutionary origin of synapses and neurons – bridging the gap. *Bioessays* **39**, 1700024. (doi:10.1002/bies.201700024)
  43. Erwin DH. 2015 Early metazoan life: divergence, environment and ecology. *Phil. Trans. R. Soc. B* **370**, 20150036. (doi:10.1098/rstb.2015.0036)
  44. Waggoner BM. 2003 The Ediacaran biotas in space and time. *Int. Comp. Biol.* **43**, 104–113. (doi:10.1093/icb/43.1.104)
  45. Narbonne GM. 2005 The Ediacara biota: Neoproterozoic origin of animals and their ecosystems. *Annu. Rev. Earth Planet. Sci.* **33**, 421–442. (doi:10.1146/annurev.earth.33.092203.122519)
  46. Droser ML, Tarhan LG, Gehling JG. 2017 The rise of animals in a changing environment: global ecological innovation in the late Ediacaran. *Annu. Rev. Earth Planet. Sci.* **45**, 593–617. (doi:10.1146/annurev-earth-063016-015645)
  47. Fedonkin MA, Waggoner BM. 1997 The late Precambrian fossil *Kimberella* is a mollusc-like bilaterian organism. *Nature* **388**, 868–871. (doi:10.1038/42242)
  48. Gehling JG, Runnegar BN, Droser ML. 2014 Scratch traces of large Ediacara bilaterian animals. *J. Paleontol.* **88**, 284–298. (doi:10.1666/13-054)
  49. Ivantsov AY. 2013 Trace fossils of Precambrian metazoans 'Vendobionta' and 'Mollusks'. *Stratigr. Geol. Correl.* **21**, 252–264. (doi:10.1134/S0869593813030039)
  50. Seilacher A. 1999 Biomat-related lifestyles in the Precambrian. *Palaios* **14**, 86–93. (doi:10.2307/3515363)
  51. Ivantsov AY, Nagovitsyn A, Zakrevskaya M. 2019 Traces of locomotion in Ediacaran macroorganisms. *Geosciences* **9**, 395. (doi:10.3990/geosciences.9090395)
  52. Evans SD, Hughes IV, Gehling JG, Droser ML. 2020 Discovery of the oldest bilaterian from the Ediacaran of South Australia. *Proc. Natl Acad. Sci. USA* **117**, 7845–7850. (doi:10.1073/pnas.2001045117)
  53. Gehling JG, Droser ML. 2018 Ediacaran scavenging as a prelude to predation. *Emerg. Top. Life Sci.* **2**, 213–222. (doi:10.1042/ETLS20170166)
  54. Jensen S. 2003 The Proterozoic and earliest Cambrian trace fossil record: patterns, problems and perspectives. *Int. Comp. Biol.* **43**, 219–228. (doi:10.1093/icb/43.1.219)
  55. Evans SD, Gehling JG, Droser ML. 2019 Slime travelers: early evidence of animal mobility and feeding in an organic mat world. *Geobiology* **17**, 490–509. (doi:10.1111/gbi.12351)
  56. Gehling JG, Droser ML, Jensen SR, Runnegar BN. 2005 Ediacara organisms: relating form to function. In *Form and function: fossils and development* (ed. DEG Briggs), pp. 43–66. New Haven, CT: Peabody Museum of Natural History, Yale University.
  57. Ivantsov AY, Malakhovskaya YE. 2002 Giant traces of Vendian animals. *Dokl. Earth Sci.* **385**, 618–622.
  58. Sperling EA, Vinther J. 2010 A placozoan affinity for *Dickinsonia* and the evolution of late Proterozoic metazoan feeding modes. *Evol. Dev.* **12**, 201–209. (doi:10.1111/j.1525-142X.2010.00404.x)
  59. Jenkins RJF. 1992 Functional and ecological aspects of Ediacaran assemblages. In *Origin and early evolution of the Metazoa* (eds JH Lipps, PW Signor), pp. 131–176. New York, NY: Plenum.
  60. Evans SD, Huang W, Gehling JG, Kivalus D, Droser ML. 2019 Stretched, mangled and torn: responses of the Ediacaran fossil *Dickinsonia* to variable forces. *Geology* **47**, 1049–1053. (doi:10.1130/G46574.1)
  61. Ivantsov AY, Zakrevskaya M, Nagovitsyn A. 2019 Morphology of integuments of the Precambrian



- animals: Proarticulata. *Invert. Zool.* **16**, 19–26. (doi:10.15298/invertzool.16.1.03)
62. Hall CMS, Droser ML, Gehling JG, Dzaugis ME. 2015 Paleocology of the enigmatic *Tribrachidium*: new data from the Ediacaran of South Australia. *Precam. Res.* **269**, 183–194. (doi:10.1016/j.precamres.2015.08.009)
63. Hall CMS, Droser ML, Clites EC, Gehling JG. 2018 The short-lived but successful tri-radial body plan: a view from the Ediacaran of Australia. *Aust. J. Earth Sci.* **67**, 885–895. (doi:10.1080/08120099.2018.1472666)
64. Rahman IA, Darroch SA, Racicot RA, Laflamme M. 2015 Suspension feeding in the enigmatic Ediacaran organism *Tribrachidium* demonstrates complexity of Neoproterozoic ecosystems. *Sci. Adv.* **1**, e1500800. (doi:10.1126/sciadv.1500800)
65. DeFrisco J, Love AC, Wagner GP. 2020 Character identity mechanisms: a conceptual model for comparative-mechanistic biology. *Biol. Philos.* **35**, 44. (doi:10.1007/s10539-020-09762-2)
66. Butterfield NJ. In press. Constructional and functional anatomy of Ediacaran rangeomorphs. *Geol. Mag.* (doi:10.1017/S0016756820000734)
67. Budd GE, Jensen S. 2016 The origin of animals and a ‘Savannah’ hypothesis for early bilaterian evolution. *Biol. Rev.* **92**, 446–473. (doi:10.1111/brv.12239)
68. Evans SD, Droser ML, Gehling JG. 2017 Highly regulated growth and development of the Ediacara microfossil *Dickinsonia costata*. *PLoS ONE* **12**, e0176874. (doi:10.1371/journal.pone.0176874)
69. Hannibal RL, Patel NH. 2013 What is a segment? *Evodevo* **4**, 35. (doi:10.1186/2041-9139-4-35)
70. Chen Z, Zhou C, Yuan X, Xiao S. 2019 Death march of a segmented and trilobate bilaterian elucidates early animal evolution. *Nature* **573**, 412–415. (doi:10.1038/s41586-019-1522-7)
71. Anderson PAV. 2015 On the origins of that most transformative of biological systems – the nervous system. *J. Exp. Biol.* **218**, 504–505. (doi:10.1242/jeb.119412)
72. Mackie GO. 1990 The elementary nervous system revisited. *Am. Zool.* **30**, 907–920. (doi:10.1093/icb/30.4.907)
73. Ryan JF, Choidin M. 2016 Where is my mind? How sponges and placozoans may have lost neural cell types. *Phil. Trans. R. Soc. B* **370**, 20150059. (doi:10.1098/rstb.2015.0059)
74. Bucher D, Anderson PAV. 2015 Evolution of the first nervous system – what can we surmise? *J. Exp. Biol.* **218**, 501–503. (doi:10.1242/jeb.111799)
75. Jones WC. 1962 Is there a nervous system in sponges? *Biol. Rev.* **37**, 1–50. (doi:10.1111/j.1469-185X.1962.tb01602.x)
76. Nickel M. 2006 Like a ‘rolling stone’: quantitative analysis of the body movement and skeletal dynamics of the sponge *Tethya wilhelma*. *J. Exp. Biol.* **209**, 2839–2846. (doi:10.1242/jeb.02337)
77. Pearse VB. 1989 Growth and behavior of *Trichoplax adhaerens*: first record of the phylum Placozoa in Hawaii. *Pacific Sci.* **43**, 117–121.
78. Ueda T, Koya S, Maruyama YK. 1999 Dynamic patterns in the locomotion and feeding behaviors by the placozoan *Trichoplax adhaerens*. *Biosystems* **54**, 65–70. (doi:10.1016/S0303-2647(99)00066-0)
79. Smith CL, Varoqueaux F, Kittelmann M, Azzam RN, Cooper B, Winters CA, Eitel M, Fasshauer D, Reese TS. 2014 Novel cell types, neurosecretory cells, and body plan of the early-diverging metazoan *Trichoplax adhaerens*. *Curr. Biol.* **24**, 1565–1572. (doi:10.1016/j.cub.2014.05.046)
80. Gingras MK, Hagadorn JW, Seilacher A, Lalonde SV, Pecoits E, Petrush D, Konhouser KO. 2011 Possible evolution of mobile animals in association with microbial mats. *Nat. Geosci.* **4**, 372–375. (doi:10.1038/ngeo1142)
81. Olson S. 2018 Earth’s oxygenation: causes, consequences, and implications for exoplanet life detection, pp. 86–99. DPhil dissertation, University of California Riverside.
82. Edgecombe GD, Ma X, Strausfeld NJ. 2015 Unlocking the early fossil record of the arthropod central nervous system. *Phil. Trans. R. Soc. B* **370**, 20150038. (doi:10.1098/rstb.2015.0038)
83. Plotnick RE, Dornbos SQ, Junyuan C. 2010 Information landscapes and sensory ecology of the Cambrian radiation. *Paleobiology* **36**, 303–317. (doi:10.1666/08062.1)
84. Ivantsov AY, Zakrevskaya M, Nagovitsin K, Krasnova A, Bobrovskiy I, Luzhnaya E. 2020 Intravital damage to the body of *Dickinsonia* (Metazoa of the late Ediacaran). *J. Paleontol.* **94**, 1019–1033. (doi:10.1017/jpa.2020.65)
85. Hoyal Cuthill JF, Conway Morris S. 2014 Fractal branching organizations of Ediacaran rangeomorph fronds reveal a lost Proterozoic body plan. *Proc. Natl Acad. Sci. USA* **111**, 13 122–13 126. (doi:10.1073/pnas.1408542111)
86. Tweedt SM, Erwin DH. 2015 Origin of metazoan developmental toolkits and their expression in the fossil record. In *Evolution of multicellularity* (eds I Ruiz-Trillo, AM Nedelcu), pp. 47–77. New York, NY: Academic Press.
87. Brunet T, Larson BT, Linden TA, Vermeij MJA, McDonald K, King N. 2019 Light-regulated collective contractility in a multicellular choanoflagellate. *Science* **366**, 326–334. (doi:10.1126/science.aay2346)
88. King N, Hittinger CT, Carroll SB. 2003 Evolution of key cell signaling and adhesion protein families predates animal origins. *Science* **301**, 361–363. (doi:10.1126/science.1083853)
89. Dudin O, Ondracka A, Grau-Bové X, Haraldsen AA, Toyoda A, Suga H, Brate J, Ruiz-Trillo I. 2019 A unicellular relative of animals generates a layer of polarized cells by actomyosin-dependent cellularization. *eLife* **8**, e49801. (doi:10.7554/eLife.49801)
90. Babonis LS, Martindale MQ. 2017 Phylogenetic evidence for the modular evolution of metazoan signalling pathways. *Phil. Trans. R. Soc. B* **372**, 20150477. (doi:10.1098/rstb.2015.0477)
91. Martindale MQ. 2005 The evolution of metazoan axial properties. *Nat. Rev. Genet.* **6**, 917–926. (doi:10.1038/nrg1725)
92. Heger P, Zheng W, Rottmann A, Panfilio KA, Wiehe T. 2020 The genetic factors of bilaterian evolution. *eLife* **9**, e45530. (doi:10.7554/eLife.45530)
93. Brunet T, Fischer AHL, Steinmetz PRH, Lauri A, Bertucci P, Arendt D. 2016 The evolutionary origin of bilaterian smooth and striated myocytes. *eLife* **5**, e19607. (doi:10.7554/eLife.19607)
94. Steinmetz PRH *et al.* 2012 Independent evolution of striated muscles in cnidarians and bilaterians. *Nature* **487**, 231–234. (doi:10.1038/nature11180)
95. Holstein TW. 2012 The evolution of the Wnt pathway. *Cold Spring Harb. Pers. Biol.* **4**, a007922. (doi:10.1101/cshperspect.a007922)
96. Loh KM, van Amerongen R, Nusse R. 2016 Generating cellular diversity and spatial form: Wnt signaling and the evolution of multicellular animals. *Dev. Cell* **38**, 643–655. (doi:10.1016/j.devcel.2016.08.011)
97. Niehrs C. 2010 On growth and form: a Cartesian coordinate system of Wnt and BMP signaling specifies bilaterian body axes. *Development* **137**, 845–857. (doi:10.1242/dev.039651)
98. Petersen CP, Reddien PW. 2009 Wnt signaling and the polarity of the primary body axis. *Cell* **139**, 1056–1068. (doi:10.1016/j.cell.2009.11.035)
99. Ryan JF, Baxeavanis AD. 2007 Hox, Wnt, and the evolution of the primary body axis: insights from the early-divergent phyla. *Biol. Direct* **2**, 37. (doi:10.1186/1745-6150-2-37)
100. Gazave E, Lapebie P, Richards GS, Brunet F, Ereskovsky AV, Degnan BM, Borchellini C, Vervoort M, Renard E. 2009 Origin and evolution of the Notch signalling pathway: an overview from eukaryotic genomes. *BMC Evol. Biol.* **9**, 249. (doi:10.1186/1471-2148-9-249)
101. Kasbauer T, Towb P, Alexandrova O, David CN, Dall’armi E, Staudigl A, Stiening B, Bottger A. 2007 The Notch signaling pathway in the cnidarian *Hydra*. *Dev. Biol.* **303**, 376–390. (doi:10.1016/j.ydbio.2006.11.022)
102. Andersson ER, Sandberg R, Lendahl U. 2011 Notch signaling: simplicity in design, versatility in function. *Development* **138**, 3593–3612. (doi:10.1242/dev.063610)
103. Hartenstein V, Stollewerk A. 2015 The evolution of early neurogenesis. *Dev. Cell* **32**, 390–407. (doi:10.1016/j.devcel.2015.02.004)
104. Martin-Duran JM, Pang K, Borve A, Le HS, Furu A, Cannon JT, Jondelius U, Hejnol A. 2018 Convergent evolution of bilaterian nerve cords. *Nature* **553**, 45–50. (doi:10.1038/nature25030)
105. Leulier F, Lemaitre B. 2008 Toll-like receptors – taking an evolutionary approach. *Nat. Rev. Genet.* **9**, 165–178. (doi:10.1038/nrg2303)
106. Sebe-Pedros A, Zheng Y, Ruiz-Trillo I, Pan D. 2012 Premetazoan origin of the Hippo signaling pathway. *Cell Rep.* **1**, 13–20. (doi:10.1016/j.celrep.2011.11.004)
107. Ardent D *et al.* 2016 The origin and evolution of cell types. *Nature* **17**, 744–757. (doi:10.1038/nrg.2016.127)
108. Makayev EV, Maniatis T. 2008 Multilevel regulation of gene expression by microRNAs. *Science* **319**, 1789–1790. (doi:10.1126/science.1152326)

109. Aulehla A, Pourquié O. 2010 Signaling gradients during paraxial mesoderm development. *Cold Spring Harb. Persp. Biol.* **2**, a000869. (doi:10.1101/cshperspect.a000869)
110. Gurdon JB, Bourillot P-Y. 2001 Morphogen gradient interpretation. *Nature* **413**, 797–803. (doi:10.1038/35101500)
111. Finnerty JR, Pang K, Burton P, Paulson D, Martindale MQ. 2004 Origins of bilateral symmetry: *Hox* and *Dpp* expression in a sea anemone. *Science* **304**, 1335–1337. (doi:10.1126/science.1091946)
112. Kusserow A *et al.* 2005 Unexpected complexity of the *Wnt* gene family in a sea anemone. *Nature* **433**, 156–160. (doi:10.1038/nature03158)
113. Rentzsch F, Technau U. 2016 Genomics and development of *Nematostella vectensis* and other anthozoans. *Curr. Opin. Genet. Dev.* **39**, 63–70. (doi:10.1016/j.gde.2016.05.024)
114. Holley SA, Jackson PD, Sasai Y, Lu B, De Robertis EM, Hoffmann FM, Ferguson EL. 1995 A conserved system for dorsal-ventral patterning in insects and vertebrates involving *sog* and *chordin*. *Nature* **376**, 249–253. (doi:10.1038/376249a0)
115. Nielson C, Brunet T, Arendt D. 2018 Evolution of the bilaterian mouth and anus. *Nat. Ecol. Evol.* **2**, 1358–1376. (doi:10.1038/s41559-018-0641-0)
116. Chourrout D *et al.* 2006 Minimal ProtoHox cluster inferred from bilaterian and cnidarian Hox complements. *Nature* **442**, 684–687. (doi:10.1038/nature04863)
117. He S, de Viso F, Chen C-Y, Ikmi A, Kroesen AE, Gibson MC. 2018 An axial Hox code controls tissue segmentation and body patterning in *Nematostella vectensis*. *Science* **361**, 1377–1380. (doi:10.1126/science.aar8384)
118. Pearson JC, Lemons D, McGinnis W. 2005 Modulating Hox gene functions during animal body patterning. *Nat. Rev. Genet.* **6**, 893–904. (doi:10.1038/nrg1726)
119. Slack JMW, Holland PWH, Graham CF. 1993 The zootype and the phylotypic stage. *Nature* **361**, 490–492. (doi:10.1038/361490a0)
120. Davidson EH, Erwin DH. 2006 Gene regulatory networks and the evolution of animal body plans. *Science* **311**, 796–800. (doi:10.1126/science.1113832)
121. Richards GS, Simionato E, Perron M, Adamska M, Vervoort M, Degnan BM. 2008 Sponge genes provide new insight into the evolutionary origin of the neurogenic circuit. *Curr. Biol.* **18**, 1156–1161. (doi:10.1016/j.cub.2008.06.074)
122. Janssen A, Budd GE. 2016 Gene expression analysis reveals that Delta/Notch signalling is not involved in onychophoran segmentation. *Dev. Genes Evol.* **226**, 69–77. (doi:10.1007/s00427-016-0529-4)
123. Meyerwitz EM. 2002 Plants compared to animals: the broadest comparative study of development. *Science* **295**, 1482–1485. (doi:10.1126/science.1066609)
124. Tusscher KT. 2020 Of mice and plants: comparative developmental systems biology. *Dev. Biol.* **460**, 32–39. (doi:10.1016/j.ydbio.2018.10.024)
125. Dunn FS, Liu AG. 2019 Viewing the Ediacaran biota as a failed experiment is unhelpful. *Nat. Ecol. Evol.* **3**, 512–514. (doi:10.1038/s41559-019-0815-4)
126. Mounier E, Pervent M, Ljung K, Gojon A, Nacry P. 2014 Auxin-mediated nitrate signalling by NRT1.1 partides in the adaptive responses of *Arabidopsis* root architecture to the spatial heterogeneity of nitrate availability. *Plant Cell Environ.* **37**, 162–174. (doi:10.1111/pce.12143)
127. Luo YJ, Kanda M, Koyanagi R, Hisata K, Akiyama T, Sakamoto H, Sakamoto T, Satoh N. 2018 Nemertean and phoronid genomes reveal lophotrochozoan evolution and the origin of bilaterian heads. *Nat. Ecol. Evol.* **2**, 141–151. (doi:10.1038/s41559-017-0389-y)
128. Glinka A, Wu W, Onichtchouk D, Blumenstock C, Niehrs C. 1997 Head induction by simultaneous repression of *Bmp* and *Wnt* signalling in *Xenopus*. *Nature* **389**, 517–519. (doi:10.1038/39092)
129. Hejnol A, Pang K. 2016 Xenacoelomorpha's significance for understanding bilaterian evolution. *Curr. Opin. Genet. Dev.* **39**, 48–54. (doi:10.1016/j.gde.2016.05.019)