



## Research

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# Quantitative study of developmental biology confirms *Dickinsonia* as a metazoan

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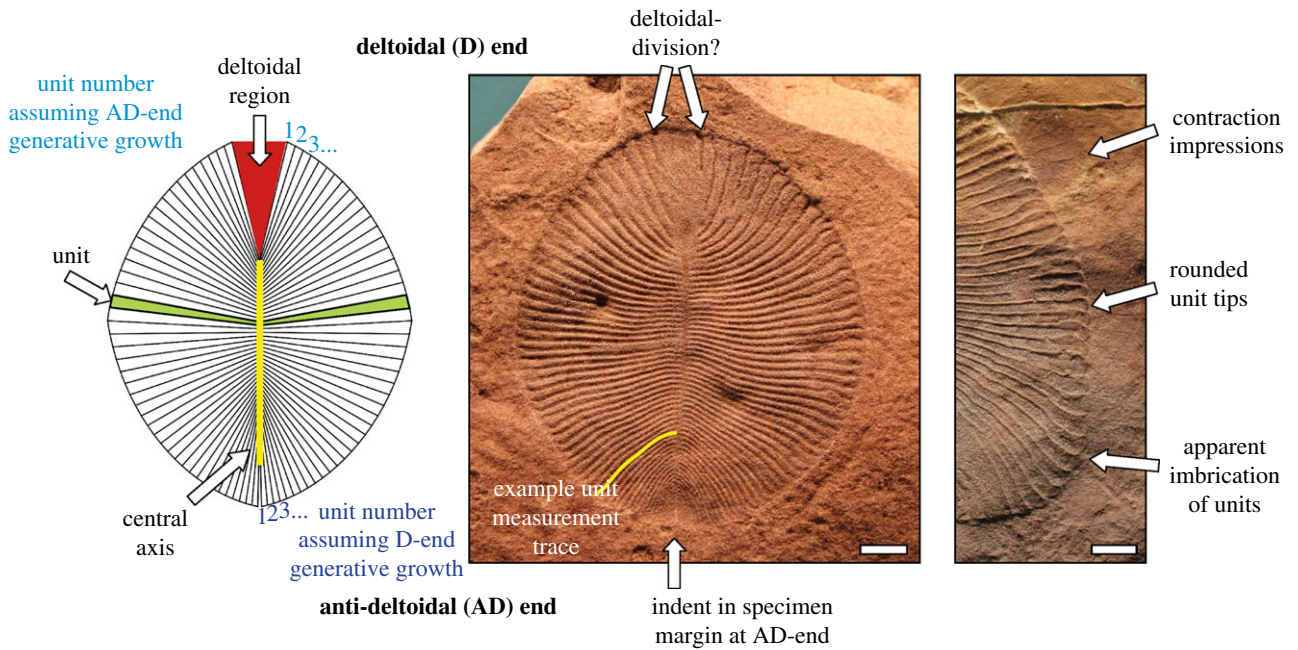
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The late Ediacaran soft-bodied macroorganism *Dickinsonia* (age range approx. 560–550 Ma) has often been interpreted as an early animal, and is increasingly invoked in debate on the evolutionary assembly of eumetazoan body plans. However, conclusive positive evidence in support of such a phylogenetic affinity has not been forthcoming. Here we subject a collection of *Dickinsonia* specimens interpreted to represent multiple ontogenetic stages to a novel, quantitative method for studying growth and development in organisms with an iterative body plan. Our study demonstrates that *Dickinsonia* grew via pre-terminal ‘deltoidal’ insertion and inflation of constructional units, followed by a later inflation-dominated phase of growth. This growth model is contrary to the widely held assumption that *Dickinsonia* grew via terminal addition of units at the end of the organism bearing the smallest units. When considered alongside morphological and behavioural attributes, our developmental data phylogenetically constrain *Dickinsonia* to the Metazoa, specifically the Eumetazoa plus Placozoa total group. Our findings have implications for the use of *Dickinsonia* in developmental debates surrounding the metazoan acquisition of axis specification and metamerism.

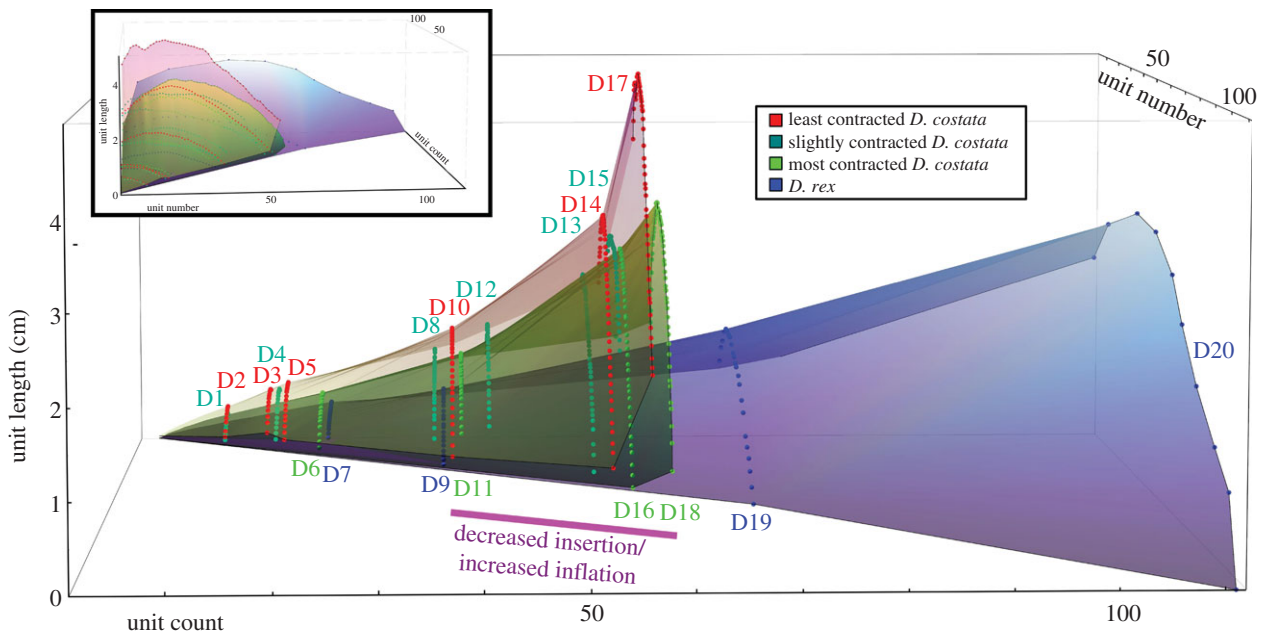
## 1. Introduction

Ediacaran macrofossil assemblages document a variety of large, soft-bodied taxa that have been suggested to include both metazoan and non-metazoan organisms [1]. However, precise determination of the phylogenetic placement of many Ediacaran taxa can be problematic, owing to difficulties in identifying diagnostic morphological characters in available fossil material, and the likelihood that many taxa lie within stem groups to extant clades [2,3]. The resultant phylogenetic uncertainty surrounding Ediacaran macrofossils frustrates efforts to incorporate specific taxa into discussions of metazoan evolution and development (e.g. [4–6]), despite fossil assemblages of such organisms having the potential to yield abundant developmental data.

The iconic Ediacaran macrofossil *Dickinsonia* (figure 1) offers a prime example of these problems. Initially interpreted as a possible medusoid cnidarian [7,8], *Dickinsonia* has since been variously considered to represent an annelid worm close to the extant *Spinther* [9–11], a platyhelminth [12], a placozoan [4], a ctenophore [13], a polypoid organism [14], a stem-group bilaterian [5,15], an early-branching diploblastic metazoan [3], a lichen [16], a rhizopodan protist [17], or a member of an extinct clade [18]. Lichen and rhizopodan interpretations are refuted by observations of considerable flexibility in *Dickinsonia* specimens [19], as evidenced by twisted, folded [20] and contracted specimens [21], but



**Figure 1.** The terminology used herein to describe *Dickinsonia costata*, and various morphological features discussed in the text. Images are of specimens SAM P40135 (D14, centre) and SAM P49355 (D17, right). Unit count is the total number of units counted within a specimen. Unit number denotes the order in which units were added in an individual specimen, assuming growth from a specific generative zone. Scale bars, 10 mm. (Online version in colour.)



**Figure 2.** Measurements of *Dickinsonia* unit length plotted against unit number and unit count (see text for definitions), assuming traditionally envisaged AD-end insertion. The measurements from individual specimens plot as arcs of points at a fixed unit count, with the unit number counted consecutively from the D-end. Inset: frontal view of the same plot. Specimens within different groups plot on distinct growth surfaces, with *D. rex* and *D. costata* clearly displaying different growth trajectories.

other suggestions are yet to be categorically confirmed or disproven. Recent studies into growth in *Dickinsonia costata* [22], and arguments for a bilaterian affinity based on ancestral state reconstruction [5], rely on assumptions regarding growth in this taxon that we here argue are incorrect.

The fossil record offers numerous assemblages of *Dickinsonia* specimens, most notably from the White Sea and Ural Mountains of Russia [23], and the Ediacara Member of South Australia [10]. Such assemblages include individuals exhibiting significant intra-specific variation in size and number of constructional units, and these are interpreted as recording a wide range of ontogenetic stages in the growth

programme of this organism. Consideration of morphogenetic relationships between specimens in such assemblages can be used to infer developmental pattern in *Dickinsonia*, and ultimately inform phylogeny [5,24]. Here we characterize the morphogenesis of *Dickinsonia*, and show that its growth involved both pre-terminal serial addition, and inflation, of body units. This growth programme differs markedly from previous interpretations of growth in this taxon, which view the generative zone as being located in a truly terminal position ([5], figure 2), at the opposite end of the organism to that considered herein ([5,19,20,22]). Our new model reconciles *Dickinsonia* with a sub-set of metazoan ontogenetic

growth programmes, and facilitates its incorporation into discussion of early animal evolution.

### (a) Growth in *Dickinsonia*

*Dickinsonia* is inferred to have been a flattened, sheet-like organism (though see [11]) with a broadly ovate outline and a bilaterally symmetrical body constructed of multiple elongate units serially arranged along a central growth axis running down the longitudinal midline [5,20,22,25] (figure 1). There is a strong linear relationship between overall length and width of *D. costata* specimens, and a positive correlation between the overall length of the organism and the number of units within local populations [22,26]. Previous assessments of *Dickinsonia* have disagreed about whether the organism grew isometrically [22,25] or allometrically [21], and whether individual units initially expanded in volume before halting their growth after certain dimensions were reached [27], or inflated continuously throughout life [4]. All previous studies have assumed that new units are added terminally, at the end of the organism where the smallest units are located, but this assumption is untested. Early claims for a terminal ‘pygidium’ at this end of the organism, prior to which units may have been added in a sub-terminal position [10], have never been confirmed [28].

At one end of the midline there is often a broadly triangular area (here termed the deltoidal region, (cf. [20]), which has previously been interpreted as a ‘head’ (cf. [29]). This triangular region appears to be the most morphologically variable structure within the organism (comprising between 1% and 19% of the areal extent of the organism in our studied Australian material; electronic supplementary material, figure S2). We consider this variability to be inconsistent with the functioning of this region as a ‘head’, which we would expect would comprise a conserved anatomical component. At the opposite end of the midline, the units become progressively smaller in size. We use the neutral terms ‘deltoidal’ (D) and ‘anti-deltoidal’ (AD) to delineate the body axes in *Dickinsonia* (figure 1), and ‘units’ to describe the serially repeated structures that comprise the organism (see the electronic supplementary material for discussion of historical terminology for *Dickinsonia* morphology). Rarely, deltoidal regions are observed to exhibit grooves, oriented parallel to adjacent units, which extend in from the outer margins of the specimen but do not connect to the central axis to form complete units (figure 1; electronic supplementary material, figure S1, specimens D14 and D17). Such grooves were recognised by Wade [10, p. 174], and although they are relatively rare, can be observed in several well-preserved *Dickinsonia* specimens figured in the literature [e.g. [20], fig. 6; [22], fig. 2a–e]. These features raise the possibility that the deltoidal region could be partially differentiated, and may imply D-end addition at a truly pre-terminal growth zone located at the margins of the deltoidal region, consistent with the pre-terminal growth of many extant bilaterian segmented taxa [28]. In assessing our data, we consider the possibility of both anti-deltoidal and deltoidal addition of new units.

*Dickinsonia* specimens may also exhibit faint, radially arranged, low-relief impressions around the outer margin of the organism, seemingly recording extensions of the positions of individual units (figure 1). These ‘rims’ have been interpreted as evidence for contraction resulting from either active muscular activity [9,10,25] or taphonomic contraction/

deflation upon death and burial [19,21]. Contracted specimens are typically smaller than uncontracted specimens with a similar number of units [10], and the extent of contraction undergone by individual specimens was an important consideration in our interpretation of measurements taken from individual specimens. Contraction has not been accounted for in previous studies of growth in *Dickinsonia* (e.g. [22]).

## 2. Material and methods

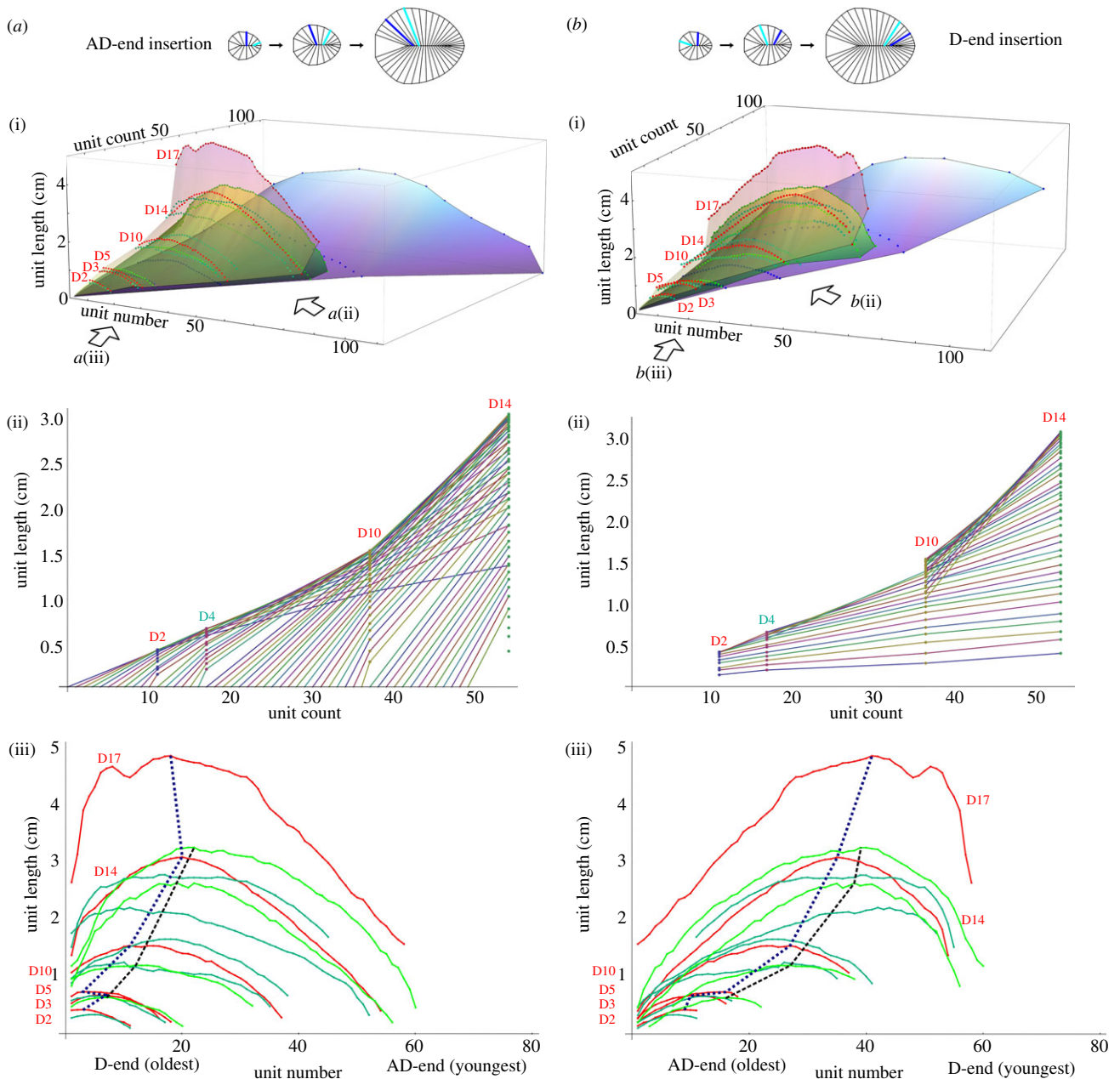
Twenty *Dickinsonia* specimens (16 *D. costata* and 4 *D. rex*) from the Ediacara Member in the Flinders Ranges of South Australia were selected for study, each exhibiting a high quality of overall preservation. Specimens span a range of sizes, and are interpreted as snapshots of different ontogenetic stages within the life history of the two taxa. *Dickinsonia costata* specimens range from 7–134 mm in length, and possess 11–58 units. Contraction is recognized to vary in its extent within the studied population (electronic supplementary material, figure S1). Studied *D. rex* specimens range from 14–117 mm in length and possess 23–111 units. Uncertainties related to measurement protocols, taphonomic deformation and biological variation are discussed in the electronic supplementary material. Although we consider individual units to be connected to one another, we see no evidence for the presence of a membrane in any of our studied specimens (*contra* [22]).

All specimens were studied from either high resolution photographs, or casts (electronic supplementary material, table S1). Specimen and unit outlines were traced over images of the specimens in the vector-based graphics program ADOBE ILLUSTRATOR CS5. Measurements of unit length (measured from the axial midline to the margin of the specimen for every unit), unit count (total number of units), and unit number (progressive number of appearance of each unit, considering the possibility of generative zones at either the D-end or AD-end of the organism; figure 1; electronic supplementary material, table S2) were obtained for each specimen. Plotting these parameters against one another permits ready visualization of the data (figure 2; electronic supplementary material, figure S4) with individual specimens plotting as arcs of points at a specific unit count. In each specimen, individual unit lengths were measured from the best preserved side of the specimen, and document the distance from the central axis to the outer margin, following the natural curvature of the unit (figure 1). The length of the resulting curved lines was then calculated in ADOBE ILLUSTRATOR and calibrated to scales in the photographs to translate the measurements into millimetres. The lengths of individual units were indexed by unit number (counted continuously from both the deltoidal and anti-deltoidal terminal units). Our interpretation of growth in *Dickinsonia* assumes that: (i) units can increase or maintain their size, but cannot decrease in size (other than via contraction); (ii) units cannot be lost once they have been generated.

WOLFRAM MATHEMATICA, version 9.0 was used for data analysis and programming of the growth model. To construct our model, we assume that in *Dickinsonia*: (i) units are added during ontogeny; (ii) units grow during ontogeny; (iii) all members of a species follow a similar growth plan, with similar unit lengths at a similar growth stage; (iv) units are added either at a terminal AD-end generative zone, or at a pre-terminal D-end generative zone.

## 3. Results

Our measurements of unit length, unit number and unit count (electronic supplementary material, table S1; figure 2) confirm that both *D. costata* and *D. rex* exhibit their shortest units at the anti-deltoidal tip of the organism (figure 3iii), while the longest



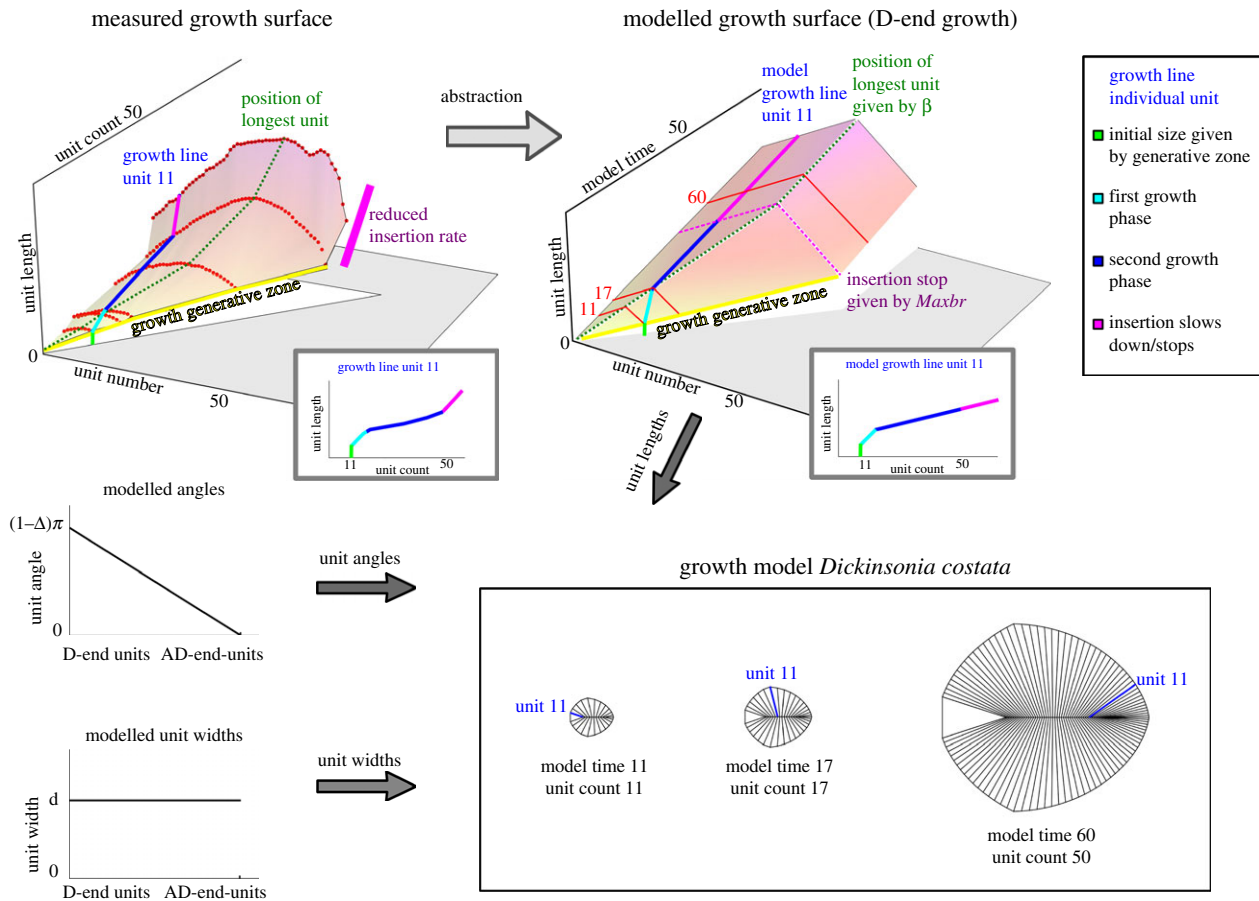
**Figure 3.** Growth data for *Dickinsonia* specimens assuming (a) an anti-deltoidal (AD-end) and (b) a deltoidal (D-end) generative zone, plotted as (i) complete growth surfaces with units counted from the end interpreted as the oldest, (ii) growth lines of the lengths of individual units as a function of unit count for selected specimens, formed by connecting the measurements of units perceived to be homologous, and (iii) as plots of unit number against unit length, with each continuous line illustrating the measurements of a single specimen, and dotted lines connecting longest units of least and most contracted specimens. Legend for colour coding as in figure 2. The AD-end units of some specimens (particularly D15) could not be measured, so the number of missing units was estimated.

units are near to the centre, being closer to the D-end in unit number (located at  $33 \pm 7\%$  of the total number of units in *D. costata*, counted from the D-end, and at  $35 \pm 13\%$  in *D. rex*; figure 3iii; electronic supplementary material, table S1). Larger specimens typically possess more units, which are longer at all positions within the organism, than smaller specimens (figures 2 and 3), though as expected [10], specimens showing signs of significant contraction have smaller unit lengths than uncontracted/less contracted specimens of a similar unit count (see the electronic supplementary material, figure S1 for details of the extent of contraction we interpret each specimen to have undergone). Plotting guiding surfaces to connect measurements from similar specimens demonstrates that *Dickinsonia* gradually increased its unit length with increasing unit count, but to varying degrees depending on the position of the unit within the organism

(figure 2). We term these guiding surfaces ‘growth surfaces’, because they permit visualization of the pattern of morphogenesis in individual taxa. *Dickinsonia rex* specimens (figure 2, blue surface) plot a surface that lies beneath all *D. costata* specimens and extends to a higher unit count, because *D. rex* individuals possess a larger number of units relative to *D. costata* specimens of a comparable size. The red (least contracted specimens) and green (most contracted specimens) surfaces reveal variation within the *D. costata* population, with all specimens of that taxon lying on or between these surfaces.

## 4. Discussion

*Dickinsonia costata* from South Australia is revealed to exhibit a consistent growth plan involving unit addition



**Figure 4.** Translation of the growth surface for *D. costata* specimen data (from uncontracted specimens) to a modelled growth surface, which renders unit lengths as a function of unit number and model time. Insets illustrate the measured and modelled growth of an individual unit, analogous to the growth lines in figure 3b. Each unit slows its relative growth after it has become the longest unit. With the additional input of unit angles and widths, this information can be used to render a model morphology at each point in time (see the interactive applet). (Online version in colour.)

accompanied by concurrent extension of the body axis, and an increase in individual unit length, over the lifetime of the organism. The total number of units (unit count) broadly correlates with overall specimen size (though see [22]), with any variation consistent with that observed in natural populations of extant segmented organisms (cf. [30], figure 4). Our data are consistent with the suggestion that the number of units can be considered a proxy for relative age [25], but we note that other studies have considered the amount of variation in unit number to be more variable [22].

The longest unit appears to maintain its position (as a proportion of unit count) along the axis throughout growth (figure 3iii). In order for this to happen, upon becoming the longest unit a unit must slow its growth rate relative to unit insertion to allow the next unit to overtake it in size. This organized growth programme implies that units did not grow independently, but rather adapted their growth based on their position in the body and the ontogenetic stage of the organism, resulting in a maintained gross morphology that is obtained via an allometric growth programme.

We find evidence for a shift in the relative rate of unit addition/inflation, reflected in changes in the gradient of the growth surfaces at approximately 35 and 50 units (figures 2 and 3) (apparent separation of these shifts is probably an artefact of irregular sampling intervals). *Dickinsonia costata* is rarely found with more than 50–60 units, suggesting that a reduction in the rate of unit addition is the most likely explanation for the observed shift, with unit insertion slowing and inflation becoming the dominant growth mechanism later in

the growth programme (cf. [4]). This change in gradient is observed in other *Dickinsonia* studies (e.g. [22], figure 3b; [26], figure 2), but is here interpreted as an ontogenetic shift that may reflect a change in life history, for example, as part of a switch to a reproductive phase. However, without knowing the rate of unit insertion, it is not possible to derive an absolute sense of time from these data.

The *D. rex* growth surface exhibits a gentle gradient throughout, with little indication of an ontogenetic shift (figure 2), though we note that we do not possess data from sub-centimetre specimens as in *D. costata*. This seemingly stable growth rate with respect to unit insertion is consistent with the apparently indeterminate addition of units in *D. rex*. However, the small sample size for this taxon, and the possibility that ecophenotypic or intra-specific variation may exist within these populations (something that has not previously been investigated in *Dickinsonia*), precludes us from drawing conclusions regarding this species at present.

### (a) Where was *Dickinsonia's* generative zone?

Growth in *Dickinsonia* has universally been assumed to have taken place at the anti-deltoidal end of the organism, because this is where the smallest, perceived to be the youngest, units are located [4,5,19,22,25]. However, our observations of potential deltoidal differentiation in some specimens raise the possibility of a deltoidal generative zone. We here interpret our growth data within both anti-deltoidal and deltoidal frameworks.

### (i) An anti-deltoidal generative zone

If we assume an anti-deltoidal generative zone for *Dickinsonia*, our data can be plotted as in figure 3a, with the D-end units interpreted as the oldest. Growth curves for individual units, produced by connecting measurements from units perceived to be homologous across specimens (figure 3a<sub>ii</sub>), exhibit variation in their slope. There is little consistency among the growth trajectories of the specimens (figure 3a<sub>iii</sub>), leading to significant overlap in the unit measurement arcs plotted by individuals. The oldest unit generally increases in size with increasing unit count, but the youngest unit, which would be expected to be of a similar size in all specimens if it represents the generative zone, appears to be variable in its length (figure 3a<sub>iii</sub>). When the relative position of the longest unit is investigated (figure 3a<sub>iii</sub>), the trend in our studied specimens is not unidirectional (as would be expected in an organism with a well-regulated growth programme), and must reverse if AD-end insertion is assumed. We do note that the grain size of the casting medium may limit our ability to observe the very smallest AD units [22].

### (ii) A deltoidal generative zone

When a deltoidal generative zone is assumed for *Dickinsonia*, with new units being added by differentiation of the deltoidal unit, we see that new units neatly and consistently exhibit increasingly greater lengths as they are added (figure 3b<sub>iii</sub>). Individual units grow at a relatively slower rate throughout life than when AD growth is assumed (compare the gentle and constant slopes in figure 3b<sub>ii</sub> with those in figure 3a<sub>ii</sub>). The AD-end regions of different specimens in figure 3b<sub>iii</sub> (which would reflect the oldest units in this model) only slightly increase their size during growth. Unit length in general increases first relatively quickly and then gradually and consistently among the sampled specimens, as one would expect if the AD-end units were homologous (figure 3b<sub>ii</sub>). D-end insertion further results in a consistent, unidirectional positive trend in the relative position of the longest unit (figure 3b<sub>iii</sub>), in contrast to the reversed trend observed if AD-end insertion is assumed (figure 3a<sub>iii</sub>).

When combined with the aforementioned anatomical evidence for apparent differentiation within the deltoidal region (e.g. figure 1), and what appears to be a consistent decrease in the size of the deltoidal area relative to the total organism with increasing unit count (electronic supplementary material, figure S2), a deltoidal generative zone more parsimoniously explains the patterns observed in our data. We therefore conclude that, contrary to all previous interpretations of growth in this organism, *D. costata* added units at a D-end generative zone, with morphological evidence suggesting that this unit addition may have occurred in a pre-terminal position. These units inflated during life as part of an organized, intricate growth programme. Our study suggests the position of the smallest units alone may not be a robust indicator of the generative zone in this taxon; a finding with potential implications for developmental and phylogenetic studies into other Ediacaran taxa (e.g. *Charnia* [31]). Alternative suggestions that *Dickinsonia* might have been bipolar [21] are considered unlikely given the clear asymmetry of its termini.

Abstraction of our measurement data from *D. costata* enables construction of a simplified growth model that replicates its growth programme (figure 4; electronic

supplementary material; see also our interactive downloadable applet: <http://people.maths.ox.ac.uk/hoekzema/Applet/>). The model illustrates that although different *Dickinsonia* species have disparate morphologies, they can be rationalized by a common morphogenetic model, substantiating their coherence as a natural group. Different reconstructed *Dickinsonia* species may look similar at an early stage of growth, but diverge in morphology during ontogeny. It is worth noting that ostensibly similar theoretical morphologies can be created by two quite different growth models (readers can compare AD-end and D-end growth in our applet), emphasizing that caution must be exercised when attempting to decipher biological growth programmes via modelling techniques (e.g. [32]).

### (b) The phylogenetic affinity of *Dickinsonia*

The seemingly tightly constrained growth programme of *Dickinsonia*, whereby individual units change their growth rate in order to maintain the overall shape of the organism, reveals a growth programme with a greater level of organization than that observed in extant slime moulds. The combination of both additional and inflationary growth in *Dickinsonia* [4] is confirmed by our data, and is incompatible with the insertion-only growth seen in extant foraminifera and xenophyophores [4]. The close spatial relationship and resemblance of *Dickinsonia* to the ichnotaxon *Epibaion* [20,29,33] implies that it was benthic and motile [4]. Such motility would refute fungal, algal and lichen biological affinities [4].

Evidence for putative biradial symmetry and internal structures was purported to demonstrate that *Dickinsonia* was a ctenophore [13], but relies heavily on a single, potentially unrepresentative, specimen. We note that no anatomical evidence has been presented to suggest that features inferred as meridional canals [13] connect to the 'gut'—a characteristic of true meridional canals. Putative internal anatomy in *Dickinsonia* [34] shows more than eight 'canals' in total, and no evidence for any transverse canals. We, therefore, do not find the anatomical evidence in support of a ctenophoran affinity for *Dickinsonia* compelling. An alternative suggestion that the longitudinal axis of *Dickinsonia* is homologous to the oral–aboral axis of ctenophores is intriguing [3], but requires acceptance of a range of equivocal morphological similarities between *Dickinsonia* and radial taxa. *Dickinsonia*'s axial growth contrasts starkly with the concentric isometric addition of units in corals such as *Fungia*, refuting some polypoid affinities [5]. However, given the developmental and morphological diversity exhibited by extant cnidarians, and the presence of a pre-terminal growth zone in some cnidarians [5], we consider it possible that *Dickinsonia* could potentially be allied with this group.

Interpretation of *Epibaion* traces as indicative of external digestion via the ventral surface of *Dickinsonia* [20,33] has been considered irreconcilable with poriferan or eumetazoan lineages [4], and consistent with a placozoan affinity. Impressions interpreted as trace fossils, such as *Epibaion* [29,33], imply that *Dickinsonia* lay static on the underlying microbial mat for long enough to remove the mat beneath it, leading to an interpretation as resting or feeding traces (e.g. [4]). However, in the absence of direct morphological evidence for feeding mechanisms, it is not yet possible to conclude with certainty whether such traces represent feeding by ventral sole digestion as in placozoans [4], cilia-driven

grazing (e.g. [29]) or even passive reclining on the surface [35]. Modern placozoans have a poorly constrained, non-metameric body plan, but the derived nature of the placozoan crown-group leaves open the possibility that our developmental data may be compatible with a stem-group placozoan position for *Dickinsonia*.

Possible merging or branching of units in *Dickinsonia* specimens has been claimed to be incompatible with a bilaterian body plan [19], but we consider such observations to result from superposition of flexible, poorly (spatially) constrained individual units (figure 1). Rare morphological evidence for musculature [10] or internal organs [11,13,34] has largely been treated with caution, but would be consistent with a bilaterian affinity. Gold *et al.* [5] infer an anti-deltoidal, 'terminal' (i.e. pre-terminal *sensu* [28]) generative zone for *Dickinsonia*, which would support a bilaterian phylogenetic placement, because many bilaterian groups—and the anticipated bilaterian ancestor—are considered to grow in this way [36] (although certain derived bilaterian groups such as the Onychophora do possess truly terminal growth zones). However, the generative zone figured by Gold *et al.* appears truly terminal ([5], figure 2), a scenario that would inadvertently set *Dickinsonia* apart from most members of the Bilateria.

Our novel description of *Dickinsonia* possessing a deltoidal, pre-terminal growth zone would provide positive support for the potential assignment of *Dickinsonia* within the Bilateria. Indeed, our new model may actually facilitate polarization of *Dickinsonia*'s growth axis, because growth via unit addition in serially repeated bilaterian taxa typically occurs at the posterior of the organism.

In summary, when combined with other evidence, our developmental data indicate that *Dickinsonia* was a metazoan, to the exclusion of all previously proposed alternative extant clades (electronic supplementary material, figure S7). More specifically, *Dickinsonia* is considered in light of developmental, behavioural and morphological information to have lain within the Eumetazoa plus Placozoa total-group. Although comparisons to the Bilateria are attractive in the absence of direct developmental evidence to ally *Dickinsonia* to the Placozoa or Cnidaria, on the basis of current data it would be premature to constrain its phylogenetic position more tightly.

### (c) Implications for contemporaneous Ediacaran

#### *Dickinsonia*-like organisms

There have been several attempts to resolve the phylogenetic relationships between *Dickinsonia* and its contemporary Ediacaran organisms, including consideration of the Kingdom Vendozoa [21], the Phylum Vendobionta [27] and the Proarticulata [37], the latter being a phylum characterized by a metameric body plan and glide symmetry (a pattern ostensibly similar to bilateral symmetry, but with a distinct offset along the midline) lying outside the Bilateria. Perhaps the most widely discussed grouping in recent years is the morphogroup Dickinsoniomorpha, a grouping of organisms considered to share a morphology constructed of featureless tubes and differentiation across a main body axis [38,39]. The precise taxonomic composition of this group is not yet agreed [23,38].

Taxa commonly considered to share close relationships to *Dickinsonia* include *Andiva* [40] and *Yorgia* [41], both of which differ in possessing a large and crescentic undifferentiated region of broadly consistent size at all ontogenetic stages

relative to total body size, and distinct unit morphologies. We do not consider the observed morphological differences in unit form to be irreconcilable with our new model, nor do we consider the different symmetries across the dickinsoniormorphs (e.g. the bilateral symmetry of *Dickinsonia* versus the glide symmetry of *Yorgia*) to necessarily preclude a close phylogenetic relationship. Indeed, glide symmetry is known within several extant and extinct bilaterian taxa, including certain machaeridian worms (annelids), where external scales are organized in a glide-symmetrical fashion as a space filling response [28,42]. Different patterns of symmetry are only problematic for the coherence of the proposed dickinsoniormorph group if the units in the bilaterally symmetrical *Dickinsonia* reflect true segments that continue through the entire body, something that is yet to be determined [31]. If the units seen on the exterior of *Dickinsonia* are true segments, they cannot be homologous to the externally visible units in *Yorgia*, and so their growth programmes would not be amenable to comparison. In such a scenario we would regard it as unlikely that these organisms were closely related. If the units in *Dickinsonia* and *Yorgia* represent annulations, with internal anatomy not governed by the external patterning of the organism, then it is possible that such differences in symmetry could be compatible within a single clade.

The quantitative methodology presented in this study can be applied via our abstracted model and applet to investigate the growth plans of morphologically similar Ediacaran and non-Ediacaran taxa including other Dickinsoniormorphs (extended electronic supplementary material). This technique could open up new avenues through which to explore ontogenesis and development in taxa with iterative growth.

### (d) The use of *Dickinsonia* in metazoan developmental studies

Resolution of *Dickinsonia* as a placozoan could imply an ancestral diversity of body plans, consistent with a rapidly growing body of genetic data that indicate considerable complexity in early metazoans [43]. The Placozoa, once considered sister group to the Bilateria [44], have more recently been interpreted as sister group to the Eumetazoa [45]. The presence of the homeotic gene *Trox2* in the extant Placozoa [46] may suggest secondary simplification and a morphologically complex placozoan stem lineage ([47], though see [46]), implying that early total-group metazoans could potentially have included organisms with a *Dickinsonia*-like morphology. If *Dickinsonia* is alternatively resolved as lying within the Cnidaria, it would imply secondary loss of (or extinction of organisms showing) concomitant growth of the main body axis and serially repeated units (regardless of whether those units are regarded as metameres or segments).

If *Dickinsonia* is, as our ontogenetic data appear to suggest most strongly, resolvable within the total-group Bilateria, its implications for the evolution of the segmented body plan depend upon its precise position within the Bilateria. The serial anatomical organization of *Dickinsonia* is compatible with hypotheses of a complex metameric ancestral bilaterian, from which the segmentation mechanisms of chordates, annelids and arthropods were inherited [48]. However, while some authors consider segmentation to be a plesiomorphic bilaterian character [5], others consider simple external annulations to be a precursor to true metamerism

[36]. There is increasing evidence that the urbilaterian may not have been a truly metameric organism: independent co-options of pre-existing gene regulatory networks (GRNs, involved in axial elongation) to form a segmentation cascade in the arthropods, annelids and chordates seems more parsimonious than invoking multiple independent losses of the segmented bauplan [36] in all non-metameric bilaterian groups. Metamerism in the chordates proceeds primarily from the mesoderm, rather than (typically) from the ectoderm in the annelids and arthropods [36], suggesting deep differences in the segmentation process (but see [48]). Recent studies propose Xenacoelomorpha (the group including the acoel flatworms and the xenoturbellids) as sister group to the Nephrozoa (protostomes plus deuterostomes) [45]. The xenacoelomorphs are considered to lack the metamerism apparent in some nephrozoan groups, but possess the true bilateral symmetry characteristic of the Bilateria as well as a suite of traits intermediate between the Cnidaria and the Nephrozoa (appearing to justify their position as sister to the Nephrozoa, though see [49]). Since the urbilaterian probably had the GRNs prerequisite to a metameric body plan, it is possible that stem-group xenacoelomorphs could have independently acquired, and subsequently lost, a metameric bauplan (in relation to other bilaterian groups). Future advances in xenacoelomorph ontogeny may allow for better discrimination here.

We do not attempt to resolve between true segmentation, annulation, or superficial metamerism in *Dickinsonia*, and nor do we attempt to resolve between placement within the Xenacoelomorpha and the Nephrozoa. However, there are currently no confidently identified apomorphies to tie *Dickinsonia* to any segmented Nephrozoan crown group, and we suggest that if *Dickinsonia* is resolved as belonging to the annelids, arthropods or, indeed, chordates, it would be in a stem-group capacity. If any of these scenarios are true, the apparent variation in unit count observed within the largest *Dickinsonia* specimens would support recent theoretical predictions suggesting that determinate addition of units evolved after both sequential segmentation and the evolution of posterior growth [50] (i.e. the level of flexibility in maximal unit count seen today only in annelids is plesiomorphic to the segmented state). Conversely, if *Dickinsonia* lies outside the segmented Nephrozoa [36], then it may represent an annulated ancestor from which disparate members of the

Bilateria diverged to use metameric body organization in different ways [38].

## 5. Conclusion

Our data demonstrate that *Dickinsonia* grew by addition of serial units via differentiation at a probable pre-terminal (del-toidal) generative zone, concurrent with elongation of the main body axis as well as lateral and axial growth of those units. This study emphasizes that growth and development offer powerful tools with which to constrain the phylogenetic position of problematic fossil taxa. Assignment of *Dickinsonia*, a particularly enigmatic taxon, to the Placozoa plus Eumetazoa total group enables us to draw a line under previous suggestions of non-metazoan biological affinities, and move forward with more focused studies that can distinguish between remaining hypotheses; something that is imperative if we are to unlock this taxon's considerable potential in unravelling the origins of metamerism. Investigation of *Dickinsonia*'s serially repeated body plan to determine whether it reflects annulation, metamerism or segmentation, represents the next key challenge in understanding this organism. We are confident that expansion of a developmental approach to the study of Ediacaran macro-organisms will enable palaeontological data to contribute substantial insights to developmental studies into early metazoan evolution.

**Data accessibility.** The datasets supporting this article are available in an accompanying electronic supplementary material. Our interactive applet is available for download at: <http://people.maths.ox.ac.uk/hoekzema/Applet/>.

**Authors' contributions.** R.S.H. and M.D.B. designed the project approach. R.S.H. carried out the research. All authors interpreted the data and R.S.H., F.S.D. and A.G.L. wrote the paper.

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