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1 **Quantitative study of developmental biology confirms *Dickinsonia***
2 **as a metazoan**

3
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18

19 **Keywords.** Metazoan evolution, bilaterian, Ediacaran, development, ontogeny

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

35

36 **Introduction.**

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

44 despite fossil assemblages of such organisms having the potential to yield abundant
45 developmental data.

46 The iconic Ediacaran macrofossil *Dickinsonia* (figure 1) offers a prime example of
47 these problems. Initially interpreted as a possible medusoid cnidarian [7–8], *Dickinsonia* has
48 since been variously considered to represent an annelid worm close to the extant *Spinther* [9–
49 11], a platyhelminth [12], a placozoan [4], a ctenophore [13], a polypoid organism [14], a
50 stem-group bilaterian [5,15], an early-branching diploblastic metazoan [3], a lichen [16], a
51 rhizopodan protist [17], or a member of an extinct clade [18]. Lichen and rhizopodan
52 interpretations are refuted by observations of considerable flexibility in *Dickinsonia*
53 specimens [19], as evidenced by twisted, folded [20], and contracted specimens [21], but
54 other suggestions are yet to be categorically confirmed or disproven. Recent studies into
55 growth in *Dickinsonia costata* [22], and arguments for a bilaterian affinity based on ancestral
56 state reconstruction [5], rely on assumptions regarding growth in this taxon that we here
57 argue are incorrect.

58 The fossil record offers numerous assemblages of *Dickinsonia* specimens, most
59 notably from the White Sea and Ural Mountains of Russia [23], and the Ediacara Member of
60 South Australia [10]. Such assemblages include individuals exhibiting significant intra-
61 specific variation in size and number of constructional units, and these are interpreted as
62 recording a wide range of ontogenetic stages in the growth program of this organism.
63 Consideration of morphogenetic relationships between specimens in such assemblages can be
64 used to infer developmental pattern in *Dickinsonia*, and ultimately inform phylogeny [5,24].
65 Here we characterise the morphogenesis of *Dickinsonia*, and show that its growth involved
66 both pre-terminal serial addition, and inflation, of body units. This growth program differs
67 markedly from previous interpretations of growth in this taxon, which view the generative
68 zone as being located in a truly terminal position [5, fig 2], at the opposite end of the

69 organism to that considered herein [5, 19–20;22]. Our new model reconciles *Dickinsonia*
70 with a sub-set of metazoan ontogenetic growth programs, and facilitates its incorporation into
71 discussion of early animal evolution.

72

73 *Growth in Dickinsonia*

74 *Dickinsonia* is inferred to have been a flattened, sheet-like organism [though see 11] with a
75 broadly ovate outline and a bilaterally symmetrical body constructed of multiple elongate
76 units serially arranged along a central growth axis running down the longitudinal midline
77 [5,20,22,25] (figure 1). There is a strong linear relationship between overall length and width
78 of *D. costata* specimens, and a positive correlation between the overall length of the organism
79 and the number of units within local populations [22;26]. Previous assessments of
80 *Dickinsonia* have disagreed about whether the organism grew isometrically [22,25] or
81 allometrically [21], and whether individual units initially expanded in volume before halting
82 their growth after certain dimensions were reached [27], or inflated continuously throughout
83 life [4]. All previous studies have assumed that new units are added terminally, at the end of
84 the organism where the smallest units are located, but this assumption is untested. Early
85 claims for a terminal ‘pygidium’ at this end of the organism, prior to which units may have
86 been added in a sub-terminal position [10], have never been confirmed [28].

87 At one end of the midline there is often a broadly triangular area (here termed the
88 deltoidal region, [cf. 20]), which has previously been interpreted as a ‘head’ [cf. 29]. This
89 triangular region appears to be the most morphologically variable structure within the
90 organism (comprising between 1% and 19% of the areal extent of the organism in our studied
91 Australian material; figure S2). We consider this variability to be inconsistent with the
92 functioning of this region as a ‘head’, which we would expect would comprise a conserved
93 anatomical component. At the opposite end of the midline, the units become progressively

94 smaller in size. We use the neutral terms “deltoidal” (D) and “anti-deltoidal” (AD) to
95 delineate the body axes in *Dickinsonia* (figure 1), and “units” to describe the serially-repeated
96 structures that comprise the organism (see the electronic supplementary information for
97 discussion of historical terminology for *Dickinsonia* morphology). Rarely, deltoidal regions
98 are observed to exhibit grooves, oriented parallel to adjacent units, which extend in from the
99 outer margins of the specimen but do not connect to the central axis to form complete units
100 (figure 1; figure S1, specimens D14 and D17). Such grooves were recognised by Wade [10,
101 p. 174], and although they are relatively rare, can be observed in several well-preserved
102 *Dickinsonia* specimens figured in the literature [e.g. 20, fig. 6; 22, fig. 2A–E]. These features
103 raise the possibility that the deltoidal region could be partially differentiated, and may imply
104 D-end addition at a truly pre-terminal growth zone located at the margins of the deltoidal
105 region, consistent with the pre-terminal growth of many extant bilaterian segmented taxa
106 [28]. In assessing our data, we consider the possibility of both anti-deltoidal and deltoidal
107 addition of new units.

108 *Dickinsonia* specimens may also exhibit faint, radially-arranged, low-relief
109 impressions around the outer margin of the organism, seemingly recording extensions of the
110 positions of individual units (figure 1). These ‘rims’ have been interpreted as evidence for
111 contraction resulting from either active muscular activity [9–10,25] or taphonomic
112 contraction/deflation upon death and burial [19,21]. Contracted specimens are typically
113 smaller than uncontracted specimens with a similar number of units [10], and the extent of
114 contraction undergone by individual specimens was an important consideration in our
115 interpretation of measurements taken from individual specimens. Contraction has not been
116 accounted for in previous studies of growth in *Dickinsonia* [e.g.22].

117

118 **Methods.**

119 Twenty *Dickinsonia* specimens (16 *D. costata* and 4 *D. rex*) from the Ediacara Member in the
120 Flinders Ranges of South Australia were selected for study, each exhibiting a high quality of
121 overall preservation. Specimens span a range of sizes, and are interpreted as snapshots of
122 different ontogenetic stages within the life history of the two taxa. *D. costata* specimens
123 range from 7–134 mm in length, and possess 11–58 units. Contraction is recognised to vary
124 in its extent within the studied population (figure S1). Studied *D. rex* specimens range from
125 14–117 mm in length and possess 23–111 units. Uncertainties related to measurement
126 protocols, taphonomic deformation, and biological variation are discussed in the electronic
127 supplementary information. Although we consider individual units to be connected to one
128 another, we see no evidence for the presence of a membrane in any of our studied specimens
129 [*contra* 22].

130 All specimens were studied from either high resolution photographs, or casts (table
131 S1). Specimen and unit outlines were traced over images of the specimens in the vector-based
132 graphics program Adobe Illustrator CS5. Measurements of unit length (measured from the
133 axial midline to the margin of the specimen for every unit), unit count (total number of units),
134 and unit number (progressive number of appearance of each unit, considering the possibility
135 of generative zones at either the D-end or AD-end of the organism; figure 1; table S2) were
136 obtained for each specimen. Plotting these parameters against one another permits ready
137 visualisation of the data (figure 2; figure S4), with individual specimens plotting as arcs of
138 points at a specific unit count. In each specimen, individual unit lengths were measured from
139 the best preserved side of the specimen, and document the distance from the central axis to
140 the outer margin, following the natural curvature of the unit (figure 1). The length of the
141 resulting curved lines was then calculated in Adobe Illustrator and calibrated to scales in the
142 photographs to translate the measurements into millimetres. The lengths of individual units
143 were indexed by unit number (counted continuously from both the deltoidal and anti-deltoidal

144 terminal units). Our interpretation of growth in *Dickinsonia* assumes that: 1) units can
145 increase or maintain their size, but cannot decrease in size (other than via contraction); 2)
146 units cannot be lost once they have been generated.

147 Wolfram Mathematica, version 9.0 was used for data analysis and programming of
148 the growth model. To construct our model, we assume that in *Dickinsonia*: 1) units are added
149 during ontogeny; 2) units grow during ontogeny; 3) all members of a species follow a similar
150 growth plan, with similar unit lengths at a similar growth stage; 4) units are added either at a
151 terminal AD-end generative zone, or at a pre-terminal D-end generative zone.

152

153 **Results.**

154 Our measurements of unit length, unit number and unit count (table S1; figure 2) confirm that
155 both *D. costata* and *D. rex* exhibit their shortest units at the anti-deltoidal tip of the organism
156 (figure 3iii), while the longest units are near to the centre, being closer to the D-end in unit
157 number (located at $33\pm 7\%$ of the total number of units in *D. costata*, counted from the D-end,
158 and at $35\pm 13\%$ in *D. rex*; figure 3iii; table S1). Larger specimens typically possess more
159 units, which are longer at all positions within the organism, than smaller specimens (figures
160 2–3), though as expected [10], specimens showing signs of significant contraction have
161 smaller unit lengths than uncontracted/less contracted specimens of a similar unit count (see
162 figure S1 for details of the extent of contraction we interpret each specimen to have
163 undergone). Plotting guiding surfaces to connect measurements from similar specimens
164 demonstrates that *Dickinsonia* gradually increased its unit length with increasing unit count,
165 but to varying degrees depending on the position of the unit within the organism (figure 2).
166 We term these guiding surfaces ‘growth surfaces’, since they permit visualisation of the
167 pattern of morphogenesis in individual taxa. *D. rex* specimens (figure 2, blue surface) plot a
168 surface that lies beneath all *D. costata* specimens and extends to a higher unit count, since *D.*

169 *rex* individuals possess a larger number of units relative to *D. costata* specimens of a
170 comparable size. The red (least contracted specimens) and green (most contracted specimens)
171 surfaces reveal variation within the *D. costata* population, with all specimens of that taxon
172 lying on or between these surfaces.

173

174 **Discussion.**

175 *D. costata* from South Australia is revealed to exhibit a consistent growth plan involving unit
176 addition accompanied by concurrent extension of the body axis, and an increase in individual
177 unit length, over the lifetime of the organism. The total number of units (unit count) broadly
178 correlates with overall specimen size [though see 22], with any variation consistent with that
179 observed in natural populations of extant segmented organisms [cf. 30, fig. 4]. Our data are
180 consistent with the suggestion that the number of units can be considered a proxy for relative
181 age [25], but we note that other studies have considered the amount of variation in unit
182 number to be more variable [22].

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

190 We find evidence for a shift in the relative rate of unit addition/inflation, reflected in
191 changes in the gradient of the growth surfaces at ~35 and ~50 units (figures 2–3) (apparent
192 separation of these shifts is likely an artefact of irregular sampling intervals). *D. costata* is
193 rarely found with more than 50–60 units, suggesting that a reduction in the rate of unit

194 addition is the most likely explanation for the observed shift, with unit insertion slowing and
195 inflation becoming the dominant growth mechanism later in the growth program [cf. 4]. This
196 change in gradient is observed in other *Dickinsonia* studies [e.g.22, fig. 3B; 26, fig. 2], but is
197 here interpreted as an ontogenetic shift that may reflect a change in life history, for example
198 as part of a switch to a reproductive phase. However, without knowing the rate of unit
199 insertion, it is not possible to derive an absolute sense of time from these data.

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

208

209 *Where was Dickinsonia's generative zone?*

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

215

216 An anti-deltoidal generative zone

217 If we assume an anti-deltoidal generative zone for *Dickinsonia*, our data can be plotted as in
218 figure 3A, with the D-end units interpreted as the oldest. Growth curves for individual units,

219 produced by connecting measurements from units perceived to be homologous across
220 specimens (figure 3Aii), exhibit variation in their slope. There is little consistency amongst
221 the relative growth trajectories of the specimens (figure 3Aiii), leading to significant overlap
222 in the growth arcs plotted by individuals. The oldest unit generally increases in size with
223 increasing unit count, but the youngest unit, which would be expected to be of a similar size
224 in all specimens if it represents the generative zone, appears to be variable in its length
225 (figure 3Aiii). When the relative position of the longest unit is investigated (figure 3Aiii), the
226 trend in our studied specimens is not unidirectional (as would be expected in an organism
227 with a well-regulated growth programme), and must reverse if AD-end insertion is assumed.
228 We do note that the grain size of the casting medium may limit our ability to observe the very
229 smallest AD units [22].

230

231 A deltoidal generative zone

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

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

256 Abstraction of our measurement data from *Dickinsonia costata* enables construction
257 of a simplified growth model that replicates its growth program (figure 4; electronic
258 supplementary information; see also our interactive downloadable applet:
259 <http://people.maths.ox.ac.uk/hoekzema/Applet/>). The model illustrates that although different
260 *Dickinsonia* species have disparate morphologies, they can be rationalised by a common
261 morphogenetic model, substantiating their coherence as a natural group. Different
262 reconstructed *Dickinsonia* species may look similar at an early stage of growth, but diverge in
263 morphology during ontogeny. It is worth noting that ostensibly similar theoretical
264 morphologies can be created by two quite different growth models (readers can compare AD-
265 end and D-end growth in our applet), emphasising that caution must be exercised when
266 attempting to decipher biological growth programs via modelling techniques [e.g. 32].

267

268 *The phylogenetic affinity of Dickinsonia*

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

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

292 Interpretation of *Epibaion* traces as indicative of external digestion via the ventral
293 surface of *Dickinsonia* [20,33] has been considered irreconcilable with poriferan or
294 eumetazoan lineages [4], and consistent with a placozoan affinity. Impressions interpreted as
295 trace fossils, such as *Epibaion* [29,33], imply that *Dickinsonia* lay static on the underlying
296 microbial mat for long enough to remove the mat beneath it, leading to an interpretation as
297 resting or feeding traces [e.g. 4]. However, in the absence of direct morphological evidence
298 for feeding mechanisms, it is not yet possible to conclude with certainty whether such traces
299 represent feeding by ventral sole digestion as in placozoans [4], cilia-driven grazing [e.g. 29],
300 or even passive reclining on the surface [35]. Modern placozoans have a poorly constrained,
301 non-metameric body plan, but the derived nature of the placozoan crown-group leaves open
302 the possibility that our developmental data may be compatible with a stem-group placozoan
303 position for *Dickinsonia*.

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

315 Our novel description of *Dickinsonia* possessing a deltoidal, pre-terminal growth zone
316 would provide positive support for the potential assignment of *Dickinsonia* within the

317 Bilateria. Indeed, our new model may actually facilitate polarization of *Dickinsonia*'s growth
318 axis, since growth via unit addition in serially-repeated bilaterian taxa typically occurs at the
319 posterior of the organism.

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

328

329 *Implications for contemporaneous Ediacaran Dickinsonia-like organisms:*

330 There have been several attempts to resolve the phylogenetic relationships between
331 *Dickinsonia* and its contemporary Ediacaran organisms, including consideration of the
332 Kingdom *Vendozoa* [21], the Phylum *Vendobionta* [27], and the *Proarticulata* [38], the latter
333 being a phylum characterised by a metameric bodyplan and glide symmetry (a pattern
334 ostensibly similar to bilateral symmetry, but with a distinct offset along the midline) lying
335 outside the Bilateria. Perhaps the most widely discussed grouping in recent years is the
336 morphogroup Dickinsoniomorpha, a grouping of organisms considered to share a
337 morphology constructed of featureless tubes and differentiation across a main body axis [39–
338 40]. The precise taxonomic composition of this group is not yet agreed [23,39].

339 Taxa commonly considered to share close relationships to *Dickinsonia* include *Andiva*
340 [41], and *Yorgia* [42], both of which differ in possessing a large and crescentic
341 undifferentiated region of broadly consistent size at all ontogenetic stages relative to total

342 body size, and distinct unit morphologies. We do not consider the observed morphological
343 differences in unit form to be irreconcilable with our new model, nor do we consider the
344 different symmetries across the dickinsoniomorphs (e.g. the bilateral symmetry of
345 *Dickinsonia* versus the glide symmetry of *Yorgia*) to necessarily preclude a close
346 phylogenetic relationship. Indeed, glide symmetry is known within several extant and extinct
347 bilaterian taxa, including certain machaeridian worms (annelids), where external scales are
348 organised in a glide-symmetrical fashion as a space filling response [28;43]. Different
349 patterns of symmetry are only problematic for the coherence of the proposed
350 dickinsoniomorph group if the units in the bilaterally symmetrical *Dickinsonia* reflect true
351 segments that continue through the entire body, something that is yet to be determined [31].
352 If the units seen on the exterior of *Dickinsonia* are true segments, they cannot be homologous
353 to the externally visible units in *Yorgia*, and so their growth programs would not be amenable
354 to comparison. In such a scenario we would regard it as unlikely that these organisms were
355 closely related. If the units in *Dickinsonia* and *Yorgia* represent annulations, with internal
356 anatomy not governed by the external patterning of the organism, then it is possible that such
357 differences in symmetry could be compatible within a single clade.

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

363

364 *The utility of Dickinsonia in metazoan developmental studies*

365 Resolution of *Dickinsonia* as a placozoan could imply an ancestral diversity of bodyplans,
366 consistent with a rapidly growing body of genetic data that indicate considerable complexity
367 in early metazoans [44]. The Placozoa, once considered sister group to the Bilateria [45],
368 have more recently been interpreted as sister group to the Eumetazoa [46]. The presence of
369 the homeotic gene *Trox2* in the extant Placozoa [47] may suggest secondary simplification
370 and a morphologically complex placozoan stem lineage [48, though see 47], implying that
371 early total-group metazoans could potentially have included organisms with a *Dickinsonia*-
372 like morphology. If *Dickinsonia* is alternatively resolved as lying within the Cnidaria, it
373 would imply secondary loss of (or extinction of organisms showing) concomitant growth of
374 the main body axis and serially-repeated units (regardless of whether those units are regarded
375 as metameres or segments).

376 If *Dickinsonia* is, as our ontogenetic data appear to suggest most strongly, resolvable
377 within the total-group Bilateria, its implications for the evolution of the segmented body plan
378 depend upon its precise position within the Bilateria. The serial anatomical organisation of
379 *Dickinsonia* is compatible with hypotheses of a complex metameric ancestral bilaterian, from
380 which the segmentation mechanisms of chordates, annelids and arthropods were inherited
381 [49]. However, while some authors consider segmentation to be a plesiomorphic bilaterian
382 character [5], others consider simple external annulations to be a precursor to true
383 metamerism [36]. There is increasing evidence that the urbilaterian may not have been a truly
384 metameric organism: independent co-options of pre-existing gene regulatory networks
385 (GRNs, involved in axial elongation) to form a segmentation cascade in the arthropods,
386 annelids and chordates seems more parsimonious than invoking multiple independent losses
387 of the segmented bauplan [36] in all non-metameric bilaterian groups. Metamerism in the
388 chordates proceeds primarily from the mesoderm, rather than (typically) from the ectoderm in
389 the annelids and arthropods [36], suggesting deep differences in the segmentation process

390 [but see 49]. Recent studies propose Xenacoelomorpha (the group including the acoel
391 flatworms and the xenoturbellids) as sister group to the Nephrozoa (protostomes plus
392 deuterostomes) [46]. The xenacoelomorphs are considered to lack the metamerism apparent
393 in some nephrozoan groups, but possess the true bilateral symmetry characteristic of the
394 Bilateria as well as a suite of traits intermediate between the Cnidaria and the Nephrozoa
395 (appearing to justify their position as sister to the Nephrozoa, though see [50]). Since the
396 urbilaterian likely had the GRNs prerequisite to a metameric body plan, it is possible that
397 stem-group xenacoelomorphs could have independently acquired, and subsequently lost, a
398 metameric bauplan (in relation to other bilaterian groups). Future advances in
399 xenacoelomorph ontogeny may allow for better discrimination here.

400 We do not attempt to resolve between true segmentation, annulation, or superficial
401 metamerism in *Dickinsonia*, and nor do we attempt to resolve between placement within the
402 Xenacoelomorpha and the Nephrozoa. However, there are currently no confidently identified
403 apomorphies to tie *Dickinsonia* to any segmented Nephrozoan crown group, and we suggest
404 that if *Dickinsonia* is resolved as belonging to the annelids, arthropods or, indeed, chordates,
405 it would be in a stem-group capacity. If any of these scenarios are true, the apparent variation
406 in unit count observed within the largest *Dickinsonia* specimens would support recent
407 theoretical predictions suggesting that determinate addition of units evolved after both
408 sequential segmentation and the evolution of posterior growth [51] (i.e. the level of flexibility
409 in maximal unit count seen today only in annelids is plesiomorphic to the segmented state).
410 Conversely, if *Dickinsonia* lies outside the segmented Nephrozoa [36], then it may represent
411 an annulated ancestor from which disparate members of the Bilateria diverged to utilise
412 metameric body organisation in different ways [39].

413

414 **Conclusions.**

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

429

430

431

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435

436 **Data accessibility.** The datasets supporting this article are available in an accompanying
437 Electronic Supplementary Information document. Our interactive applet is available for
438 download at: <http://people.maths.ox.ac.uk/hoekzema/Applet/>

439

440 **Competing interests.** We have no competing interests.

441

442 **Author Contributions.** RSH and MDB designed the project approach. RSH carried out the
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444

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450

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571

572 **Figure Legends.**

573

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

579

580 **Figure 2.** Measurements of *Dickinsonia* unit length plotted against unit number and unit
581 count (see text for definitions), assuming traditionally-envisaged AD-end insertion. The
582 measurements from individual specimens plot as arcs of points at a fixed unit count, with the

583 unit number counted consecutively from the D-end. Inset: frontal view of the same plot.

584 Specimens within different groups plot on discrete growth surfaces, with *D. rex* and *D.*

585 *costata* clearly displaying different growth trajectories.

586

587 **Figure 3.** Growth data for *Dickinsonia* specimens assuming (A) an anti-deltoidal (AD-end)

588 and (B) a deltoidal (D-end) generative zone, plotted as (i) complete growth surfaces with

589 units counted from the end interpreted as the oldest, (ii) growth lines of the lengths of

590 individual units as a function of unit count for selected specimens, formed by connecting the

591 measurements of units perceived to be homologous, and (iii) as plots of unit number against

592 unit length, with each continuous line illustrating the measurements of a single specimen, and

593 dotted lines connecting longest units of least and most contracted specimens. Legend for

594 colour coding as in figure 2. The AD-end units of some specimens (particularly D15) could

595 not be measured, so the number of missing units was estimated.

596

597 **Figure 4.** Translation of the growth surface for *D. costata* specimen data (from uncontracted

598 specimens) to a modelled growth surface, which renders unit lengths as a function of unit

599 number and model time. Insets illustrate the measured and modeled growth of an individual

600 unit, analogous to the growth lines in figure 3B. Each unit slows its relative growth after it

601 has become the longest unit. With the additional input of unit angles and widths, this

602 information can be used to render a model morphology at each point in time (see the

603 interactive applet).

604