1 Exceptionally preserved Cambrian loriciferans and the early animal invasion of the
2 meiobenthos

3
4 Harvey, Thomas H.P.^{1*} and Butterfield, Nicholas J.²

5
6 ¹Department of Geology, University of Leicester, University Road, Leicester LE1 7RH, UK.

7 ²Department of Earth Sciences, University of Cambridge, Downing Street, Cambridge CB2
8 3EQ, UK.

9 *Corresponding author, email: thph2@le.ac.uk

10
11 Introductory paragraph:

12 Microscopic animals that live among and between sediment grains (meiobenthic metazoans)
13 are key constituents of modern aquatic ecosystems, but are effectively absent from the fossil
14 record. Here we describe an assemblage of microscopic fossil loriciferans (Ecdysozoa,
15 Loricifera) from the late Cambrian Deadwood Formation of western Canada. The fossils
16 share a characteristic head structure and minute adult body size (c. 300 µm) with modern
17 loriciferans, indicating the early evolution and subsequent conservation of an obligate,
18 permanently meiobenthic lifestyle. The unsuspected fossilization potential of such small
19 animals in marine mudstones offers a new search image for the earliest ecdysozoans and
20 other animals, even while the anatomical complexity of loriciferans points to their
21 evolutionary miniaturization from a larger-bodied ancestor. The invasion of animals into
22 ecospace previously monopolized by protists will have contributed importantly to the
23 revolutionary geobiological feedbacks of the Proterozoic–Phanerozoic transition.

24
25 [Main text:]

26 Animals with minute body size are among the most abundant and diverse organisms in the
27 marine benthos¹, but most meiobenthic (= meiofaunal) animal lineages are palaeontologically
28 invisible. Instead, inferences on the origins of microscopic body size rely on molecular
29 phylogenetic analyses^{2,3}, though many groups remain poorly sampled or difficult to place in
30 the tree of life. As such, there are conflicting predictions of ancestral body size for the major
31 metazoan clades, with important implications for addressing the marked discordance between
32 their first unequivocal fossil appearances in the terminal Ediacaran and Cambrian, and
33 predictions from molecular clocks of early- (or pre-)Ediacaran divergence dates^{4,5}. Certainly,
34 biases in the ‘conventional’ fossil record leave open the possibility that the Cambrian
35 ‘explosion’ might artefactually conflate ecological and phylogenetic innovations by recording
36 mostly macroscopic animals and their activities⁶. Here, we describe exceptionally preserved
37 fossils demonstrating that microscopic loriciferans – a key lineage of meiobenthic
38 ecdysozoans – had arisen by at least the late Cambrian. In addition to establishing a unique
39 stratigraphic calibration point for this recently recognized phylum, these fossils provide
40 important constraints on functional and phylogenetic models of early animal evolution.

41

42 Results

43 Systematic Palaeontology

44 Ecdysozoa; Scalidophora; Phylum Loricifera (total-group)

45 *Eolorica deadwoodensis* gen. et sp. nov.

46 Etymology: From Greek, *eos* (dawn) + *loricus* (corset), alluding to the antiquity of the fossil
47 loriciferan, and its occurrence in the Deadwood Formation.

48 Holotype: GSC138579 (Fig. 1d–g), catalogued along with other figured specimens in the
49 Type Collection of the Geological Survey of Canada (GSC), 601 Booth St., Ottawa, Ontario,
50 Canada.

51 Referred material: 104 additional loricae (Figs 1, 2 and Supplementary Fig. 2), including 17
52 with remnant scalids preserved (Figs 1b and 2b,d–f , and Supplementary Fig. 2a,g,o).
53

54 Locality, stratigraphy and preservation: Five sample horizons in the Deadwood Formation
55 from across four geographically widespread drillcores in southern Saskatchewan, Canada;
56 dated biostratigraphically to the late Cambrian Furongian epoch (c. 485–497 million years
57 ago). All specimens occur as ‘small carbonaceous fossils’ (SCFs) recovered from unoxidized
58 mudstone horizons using a low-manipulation HF extraction process⁷. Fossilization occurred
59 in a shallow marine, muddy/sandy shelf environment (shoreface to offshore) in a notably
60 more oxygenated, epicratonic setting than classical ‘Burgess Shale-type’ environments (see
61 Supplementary Fig. 1 for further details).
62

63 Diagnosis of genus and species: Body with lorica and introvert; lorica length 120–215 μm ;
64 total length including introvert c. 180–300 μm . Introvert almost as wide as the anterior lorica,
65 and around half the lorica’s length when everted. Introvert with > 200 elongate scalids,
66 including robust, jointed and reflexed anterior spinoscalids, and more gracile spinoscalids
67 and/or trichoscalids posteriorly, some with fringed tips. Mouth region delimited by c. 6 short
68 clavoscalids or mouth cone elements. Lorica constructed from 20 longitudinal strip-like
69 plicae, each 5–10 μm -wide with squared or bluntly rounded anterior margins ornamented
70 with a fine external fringe of 1.5 μm long fimbriae; plicae undivided and undifferentiated,
71 becoming poorly defined posteriorly. Lorica with a circular aperture and a slight curvature
72 towards the (presumed) ventral side.
73

74 Discussion

75 The large number of scalids (> 200) on the introvert (eversible spiny ‘head’) of *E.*
76 *deadwoodensis*, together with the highly elongate scalid morphologies (Fig. 1d–g) and
77 loricate body, are uniquely shared with modern loriciferans, a recently discovered phylum of
78 microscopic marine metazoans⁸. Just as in modern species⁹, the introvert scalids of *E.*
79 *deadwoodensis* exhibit anterior-posterior differentiation, with notably robust, jointed and
80 reflexed frontal scalids (spinoscalids) (Fig. 1f) followed by more gracile posterior
81 spinoscalids and/or trichoscalids (Fig. 1g). The lorica (posterior ‘corset-like’ region) of *E.*
82 *deadwoodensis* (Figs 1a–d and 2a–c, and Supplementary Fig. 2) is conspicuously similar to
83 the multi-plicate loricae of modern Pliciloricidae (one of the two principal loriciferan sub-
84 groups), with features reminiscent of both *Pliciloricus* (which is characterized by 22 lorica
85 plicae, compared with 20 in the fossils; cf. Fig. 2j) and *Rugiloricus* (which has a higher plica-
86 count of 30–60, but shares with the fossils a lack of differentiation among the plicae)⁹. All
87 modern pliciloricids have an approximately circular lorica cross-section, in common with the
88 fossil specimens (which exhibit an oval aperture regardless of the angle of flattening; Fig.
89 2d–f). In contrast, modern loriciferans belonging to Nanaloricidae are characterized by
90 relatively flattened loricae composed of 6–10 strongly differentiated plates⁹. Together with
91 the loriciferan synapomorphies exhibited by the introvert, the preserved lorica characters are
92 consistent with a phylogenetic position for *E. deadwoodensis* among pliciloricid loriciferans
93 (Supplementary Table 1), and therefore with a crown-group loriciferan position overall.
94 Alternatively, if pliciloricid-type loricae are plesiomorphic for loriciferans, *E. deadwoodensis*
95 could conceivably lie in the loriciferan stem (see Supplementary Fig. 3). A stem-group
96 position could explain the absence of differentiated larval stages (such as a ‘Higgins-larva’
97 with its specialized lorica structures), if not simply a result of differential decay, or a species-
98 specific trait. Another possible plesiomorphic character is the unusual perioral armature in *E.*
99 *deadwoodensis*: its ring of c. six robust structures (possibly clavoscalids or mouth cone

elements) compare in number (but not shape) to the six oral styles of adult nanaloricids and the six oral teeth of larval pliciloricids⁹. Ultimately, these characters will help to place *E. deadwoodensis* once the interrelationships of modern loriciferans are better resolved.

Besides the striking anatomical correspondence to modern loriciferans, *E. deadwoodensis* is remarkable for its microscopically small body size. Based on the scaling relationship between the most intact specimen (lorica length 135 μm ; overall length including introvert < 200 μm), and the longest lorica (c. 215 μm), we estimate a maximum overall body length of less than 300 μm . A taphonomically imposed upper limit to body size in the fossil populations can be ruled out by the fact that larger (up to millimetre-size) SCFs co-occur in each of the five loriciferan-bearing samples. The presence of elongate scalid morphologies in the fossils, along with the absence of posterior lorica projections (tubuli, setae, or locomotory ‘appendages’), indicates that the fossils are adults, despite their small size. Their size is typical of modern adult loriciferans, which range between 115 and 425 μm in maximum dimension⁹, and even the largest-known ‘giant’ larvae of *Titaniloricus* are only 800 μm long¹⁰ (see Table 1).

Phylogenetic significance

Establishing the evolutionary significance of minute body size in loriciferans requires consideration of their wider phylogenetic relationships. A suite of morphological characters support a grouping of loriciferans, kinorhynchs and priapulids in a clade (Scalidophora) within the Ecdysozoa, to the exclusion of panarthropods and nematoids^{5,11–13}. A lorica is present during the larval (but never the adult) stages of all but one extant priapulid species, suggesting a close relationship between the two phyla. Some characters, though, support a closer relationship with kinorhynchs or even nematomorphs⁸, which would imply additional

losses or convergent evolution of a lorica. Loriciferans have seldom been included in molecular phylogenetic analyses, and preliminary datasets incorporating loriciferan 18S +/- 28S rRNA sequences^{14,15,16} have thus far failed to recover a monophyletic Scalidophora (Supplementary Fig. 3), although the results are likely to be distorted by long branch attraction¹². The picture is further complicated by a range of problematic Cambrian fossils that possess a lorica-like structure but are quite unlike modern loriciferans. *Sirilorica* from the early Cambrian (Series 2) Sirius Passet biota of North Greenland, for example, has a bizarrely interdigitated ‘lorica’ some 70 mm long – orders of magnitude larger than found in any living loriciferan¹⁷. More conventional lorica-like structures are present in comparably macroscopic (up to 25 mm) *Palaeopriapulites*, *Protopriapulites* and *Sicyophorus* from the early Cambrian (Series 2) Chengjiang biota of south China^{18,19} (Table 1), though their priapulid-type introverts with comparatively few, short scalids¹³ gives them the appearance of giant priapulid larvae; indeed, they commonly group with priapulids in cladistic analyses¹¹. Middle Cambrian (Series 3) *Orstenoloricus*, known from incomplete specimens lacking an introvert, has also been compared to loriciferans²⁰, though the concertina-like folding of its lorica, extensive neck region and millimetric body size (Table 1) ally it more closely with larvae of the extant priapulid *Tubiluchus*.

In contrast, *E. deadwoodensis* provides the first unambiguous evidence for microscopic loriciferans in the fossil record, and establishes their long evolutionary history independent of priapulids and other ecdysozoans. At the same time, it exhibits lorica characters that can be interpreted as plesiomorphic for both priapulids and loriciferans: a lorica with a circular cross-section also occurs in the earliest loricated larval stages of *Priapulites*¹³, in the candidate ‘basal’ priapulid *Tubiluchus*^{13,21}, and in the ‘giant’ Cambrian loricated fossils^{17,18,19}. To this extent, *E. deadwoodensis* supports the homology of loricae between the two phyla. It has

been suggested that loriciferans derive from a larger-bodied, priapulid-like ancestor via progenesis from a form with a loricate larva (i.e., precocious sexual maturity leading to retention of a larval morphology)²². The presence in the Cambrian fossil record of macroscopic loricate forms offers an alternative mechanism of secondary miniaturization^{3,23} from a large loricate adult. Either way, a larger-bodied ancestor for microscopic loriciferans is predicted by their anatomical complexity, most strikingly expressed by their intricate myoanatomy²⁴, hundreds of innervated scalids, and thousands of cells¹. Indeed, a parsimonious reconstruction of lorica evolution suggests that *E. deadwoodensis* and modern loriciferans are derived from a larger-bodied (millimetric, centimetric or even decimetric) ancestor, given the stem-loriciferan, stem-priapulid and/or deeper positions for other Cambrian loricate worms, and the macroscopic size of non-loricate outgroups (see Supplementary Fig. 3).

A hypothesis that minute animals existed for tens or hundreds of millions of years before the Cambrian ‘explosion’^{6,25} is attractive insofar as it reconciles deep molecular clock predications of divergences^{4,5} with the well-established empirical pattern from the fossil record. A long pre-Cambrian history of microscopic loriciferans seems unlikely, considering their derived phylogenetic position and a lack of plausible (i.e., macroscopic) ancestors prior to the Cambrian (or identifiable animal traces prior to the latest Ediacaran)²⁶. At a deeper level, however, ambiguities in the metazoan tree allow for either a large-bodied²⁷ or small-bodied³ ecdysozoan ancestor. The discovery of *E. deadwoodensis* demonstrates the potential for even the tiniest cuticularized metazoans to be preserved as ‘small carbonaceous fossils’, augmenting the existing SCF record of comparatively tough cuticular sclerites of larger-bodied relatives^{7,28}. The absence of any such cuticular remains prior to the Cambrian

therefore points to a pronounced late-stage radiation of ecdysozoans, challenging molecular-clock predictions of $> 580 \text{ Ma}^5$ or $> 630 \text{ Ma}^4$ intra-ecdysozoan divergences.

Early evolution of the metazoan meiobenthos

The exceptionally small body size of modern loriciferans – which are among the smallest known metazoans – is attributed to their specialized ecologies as members of the obligate, permanent metazoan meiobenthos; i.e., those animals that spend their entire life cycle living interstitially between sand grains or among finer-grained sediments on the sea floor⁹.

Following their first formal description⁸ in 1983, loriciferans have been discovered in a wide range of habitats, from the intertidal zone to $> 8 \text{ km}$ -deep ocean trenches, and from sandy sediments to anoxic muds⁹. Even so, they consistently exhibit key ecological traits linked to microscopic body size²⁹, including the absence of a planktonic larval stage, asymptotic growth, (weak) motility, and a particle-discriminating (apparently bacterivorous or micro-algal) diet⁹. A comparable ecology for *E. deadwoodensis* is supported by the equivalence of scale, the restricted size range in the fossil populations, and the various detailed scalid adaptations linked to feeding and locomotion.

Modern meiobenthic organisms are distinguished operationally from larger macrobenthos and smaller microbenthos if they pass through a mesh of 1 mm (or sometimes $500 \mu\text{m}$) but not one of 63 (or $45 \mu\text{m}$)¹. By this measure, the larvae of various Cambrian animals would have been temporary members of the meiobenthos. Identifying fossils as members of the permanent meiobenthos, however, requires additional evidence of both adult anatomy and benthic ecology. With its adult-type lorica and introvert armature, and necessarily bottom-dwelling, non-swimming ecology, *E. deadwoodensis* readily passes these tests – but the case is less clear for other Cambrian candidates. Fossils from phosphatized ‘Orsten-type’

assemblages often exhibit meiobenthic dimensions, including a variety of panarthropods^{30,31}, scalidophorans^{32,33}, and cnidarians³⁴, but most of these forms are known to have grown to at least millimetric adult body sizes (Table 1). One possible exception is a single 350 µm-long larval tardigrade³⁰ that may have retained its small size as an adult. A few of the Orsten pancrustaceans might also fall into this category, in particular *Bredocaris*, which has a maximum known (?adult) body length of 850 µm, and *Skara*, which is somewhat longer (1.2 mm) but has an elongate body similar to some modern meiobenthic crustaceans³¹. In any event, *E. deadwoodensis* is the smallest animal yet identified in the Cambrian fossil record, and the clearest evidence for early animals living permanently among and between sedimentary grains.

The discovery of *E. deadwoodensis* establishes the presence of a specialized metazoan component to the meiobenthos by at least the late Cambrian, adding a fundamental new dimension to the geobiological feedbacks attending the Cambrian ‘explosion’. In the modern world, meiobenthic animals can be enormously abundant in marine sediments (10^6 – 10^7 individuals/m²), constituting a significant fraction of sedimentary biomass and profoundly influencing local sedimentological and biogeochemical expression, including sediment cohesion, solute transport, and organic-matter cycling^{1,35–39}. In turn, the ‘engineering’ effects of meiobenthic animals inevitably shape the surrounding microbenthic (smaller protistan) and macrobenthic communities – just as the activities of macroscopic animals locally either promote or suppress meiobenthic abundance and diversity^{1,36,38}.

Not all small interstitial organisms are animals, of course, and there is significant degree of overlap in size and ecosystem function with larger benthic protists, most notably ciliates and foraminifera^{1,25}. Moreover, protistan-grade forms are likely to have been established well

224 before the rise of animals, as suggested by accumulations of testate amoebae in mid-
 225 Neoproterozoic (ca. 750 Ma) strata⁴⁰. Importantly, however, the habits and geobiological
 226 impact of these two components of the benthic meiofauna can be profoundly different.
 227 Organ-grade metazoans express a fundamentally greater repertoire of habits and interactions
 228 than their unicellular counterparts, not least the dramatically enhanced levels of
 229 motility/dexterity conferred by a bilaterian musculoskeletal system, and the revolutionary
 230 impact of processing food via a through-gut. The Cambrian colonization of meiobenthic
 231 habitats by metazoans clearly attests to this adaptive potential. At one level it presumably
 232 entailed a competitive displacement of incumbent protists, though the accompanying
 233 engineering effects may also have facilitated their subsequent Phanerozoic diversification.
 234
 235 Among the most obvious and characteristic features of the Cambrian ‘explosion’ is a
 236 dramatic increase in maximum body size, culminating in metre-long anomalocaridids⁴¹. No
 237 less impressive, considering the competitive demands on early meiobenthic pioneers, is the
 238 extremely small size of Cambrian loriciferans. Size increase through this interval is often
 239 viewed as an exploration of vacant ecospace coincident with a permissive rise in ambient
 240 oxygen levels⁴ – but extremes in body size can also indicate strong selective displacement
 241 away from more optimal ranges⁴². Miniaturization in a Cambrian loriciferan to near the lower
 242 limit for free-living animals implies that the prevailing benthic ecology was sufficiently
 243 ‘escalated’ to drive novel defensive strategies (via a small-size ‘refuge’ or life-history
 244 modification) and/or or the exploitation of a rich but inaccessible food source²³. Certainly, the
 245 advent of burrowing by larger metazoans in the Cambrian resulted in a systematic
 246 restructuring of the benthic environment by converting matgrounds to mixgrounds⁴³,
 247 potentially opening new ecospace for smaller metazoans via increased sediment oxygenation,
 248 in particular. A wider analysis, however, suggests that there is no straightforward relationship

between oxygen, macrofaunal burrowing, and meiobenthic opportunities: seemingly inhospitable modern-day microbial mat environments can host abundant and diverse interstitial animals as well as protists⁴⁴, and some extant loriciferans apparently occupy permanently anoxic habitats⁴⁵, albeit as a derived ecology.

Eolorica deadwoodensis documents the evolution of extreme miniaturization in one lineage of Cambrian animals, but there is a conspicuous absence of others, notably nematodes, harpacticoid copepods, turbellarians and micro-annelids – by far the most geobiologically important modern groups¹. Neither the taxonomic composition or ecological contribution of the Cambrian meiobenthos is likely to have been fully modern, but the challenge is to distinguish genuine absence from taphonomic loss. Meiobenthic animals have long been of interest to palaeobiologists precisely because of their perceived invisibility as fossils – but not all are impossible to preserve. While molecular phylogenetic analyses^{2,3}, preserved benthic biomarkers⁴⁶ and sedimentary (bio)fabrics^{39,47} provide important constraints on meiobenthic evolution, ‘small carbonaceous fossils’ offer a direct window onto cryptic animal ecosystems through deep time.

Data availability

The specimens on which this study is based are accessioned in the collections of the Geological Survey of Canada (GSC), 601 Booth St., Ottawa, Ontario, Canada (accession numbers GSC138576–138606, plus associated unfigured material). Additional images of specimens are available in figshare with the identifier doi: 10.6084/m9.figshare.4036104.

References

- 273 1. Giere, O. *Meiobenthology - The Microscopic Motile Fauna of Aquatic Sediments*
274 (Springer-Verlag, 2009).
275
- 276 2. Laumer, C. E. *et al.* Spiralian phylogeny informs the evolution of microscopic lineages.
277 *Curr. Biol.* **25**, 2000–2006 (2015).
278
- 279 3. Struck, T. H. *et al.* The evolution of annelids reveals two adaptive routes to the interstitial
280 realm. *Curr. Biol.* **25**, 1993–1999 (2015).
281
- 282 4. Erwin, D. H. *et al.* The Cambrian conundrum: early divergence and later ecological
283 success in the early history of animals. *Science* **334**, 1091–1097 (2011).
284
- 285 5. Rota-Stabelli, O., Daley, A. C. & Pisani, D. Molecular timetrees reveal a Cambrian
286 colonization of land and a new scenario for ecdysozoan evolution. *Curr. Biol.* **23**, 392–398
287 (2013).
288
- 289 6. Fortey, R. A., Briggs, D. E. G. & Wills, M. A. The Cambrian evolutionary ‘explosion’:
290 decoupling cladogenesis from morphological disparity. *Biol. J. Linn. Soc.* **57**, 13–33 (1996).
291
- 292 7. Butterfield, N. J. & Harvey, T. H. P. Small carbonaceous fossils (SCFs): A new measure of
293 early Paleozoic paleobiology. *Geology* **40**, 71–74 (2012).
294
- 295 8. Kristensen, R. M. Loricifera, a new phylum with Aschelminthes characters from the
296 meiobenthos. *Z. Zool. Syst. Evol.* **21**, 163–180 (1983).
297

- 298 9. Neves, R. C., Reichert, H., Sørensen, M. V. & Kristensen, R. M. Systematics of phylum
299 Loricifera: Identification keys of families, genera and species. *Zool. Anz.* (in press 2016)
300 doi:10.1016/j.jcz.2016.06.002
301
- 302 10. Gad, G. Giant Higgins-larvae with paedogenetic reproduction from the deep sea of the
303 Angola Basin - evidence for a new life cycle and for abyssal gigantism in Loricifera? *Org.*
304 *Divers. Evol.* **5**, 59–75 (2005).
305
- 306 11. Wills, M. A., Gerber, S., Ruta, M. & Hughes, M. The disparity of priapulid,
307 archaeopriapulid and palaeoscolecid worms in the light of new data. *J. Evol. Biol.* **25**, 2056–
308 2076 (2012).
309
- 310 12. Hejnol, A. & Lowe, C. J. Embracing the comparative approach: how robust phylogenies
311 and broader developmental sampling impacts the understanding of nervous system evolution.
312 *Phil. Trans. R. Soc. B* **370**, 20150045 (2015).
313
- 314 13. Schmidt-Rhaesa, A. in *Handbook of Zoology Vol. 1 Nematomorpha, Priapulida,*
315 *Kinorhyncha, Loricifera* (ed. Schmidt-Rhaesa, A.) 147–180 (De Gruyter, 2013).
316
- 317 14. Sørensen, M. V. *et al.* New data from an enigmatic phylum: evidence from molecular
318 sequence data supports a sister-group relationship between Loricifera and Nematomorpha. *J.*
319 *Zool. Syst. Evol. Res.* **46**, 231–239 (2008).
320
- 321 15. Yamasaki, H., Fujimoto, S. & Miyazaki, K. Phylogenetic position of Loricifera inferred
322 from nearly complete 18S and 28S rRNA gene sequences. *Zoological Lett* **1**, 1–9 (2015).

323

324 16. Park, J. K., Rho, H. S., Kristensen, R. M., Kim, W. & Giribet, G. First molecular data on
325 the phylum Loricifera: an investigation into the phylogeny of Ecdysozoa with emphasis on
326 the positions of Loricifera and Priapulida. *Zool. Sci.* **23**, 943–954 (2006).

327

328 17. Peel, J. S., Stein, M. & Kristensen, R. M. Life cycle and morphology of a Cambrian stem-
329 lineage loriciferan. *PLoS ONE* **8**, e73583 (2013).

330

331 18. Huang, D., Chen, J. & Vannier, J. Discussion on the systematic position of the Early
332 Cambrian priapulomorph worms. *Chinese Sci. Bull.* **51**, 243–249 (2006).

333

334 19. Maas, A., Huang, D., Chen, J., Waloszek, D. & Braun, A. Maotianshan-Shale
335 nemathelminthes - morphology, biology, and the phylogeny of Nemathelminthes.
336 *Palaeogeogr. Palaeocl.* **254**, 288–306 (2007).

337

338 20. Maas, A., Waloszek, D., Haug, J. T. & Müller, K. J. Loricata larvae (Scalidophora) from
339 the Middle Cambrian of Australia. *Memoirs of the Association of Australasian*
340 *Palaeontologists* **37**, 281–302 (2009).

341

342 21. Kirsteuer, E. Notes on adult morphology and larval development of *Tubiluchus*
343 *corallicola* (Priapulida), based on in vivo and scanning electron microscope examinations of
344 specimens from Bermuda. *Zool. Scr.* **5**, 239–255 (1976).

345

346 22. Warwick, R. M. Are loriciferans paedomorphic (progenetic) priapulids? *Vie et Milieu* **50**,
347 191–193 (2000)

348

349 23. Hanken, J. & Wake, D. B. Miniaturization of Body Size: Organismal Consequences and
350 Evolutionary Significance. *Ann. Rev. Ecol. Syst.* **24**, 501–519 (1993).

351

352 24. Neves, R. C. *et al.* A complete three-dimensional reconstruction of the myoanatomy of
353 Loricifera: comparative morphology of an adult and a Higgins larva stage. *Front. Zool* **10**, 19
354 (2013).

355

356 25. Rundell, R. J. & Leander, B. S. Masters of miniaturization: convergent evolution among
357 interstitial eukaryotes. *BioEssays* **32**, 430–437 (2010).

358

359 26. Budd, G. E. & Jensen, S. A critical reappraisal of the fossil record of the bilaterian phyla.
360 *Biol. Rev.* **75**, 253–295 (2000).

361

362 27. Webster, B. L. *et al.* Mitogenomics and phylogenomics reveal priapulid worms as extant
363 models of the ancestral Ecdysozoan. *Evol. Dev.* **8**, 502–510 (2006).

364

365 28. Smith, M. R., Harvey, T. H. P. & Butterfield, N. J. The macro- and microfossil record of
366 the Cambrian priapulid *Ottoia*. *Palaeontology* **58**, 705–721 (2015).

367

368 29. Warwick, R. M. Meiobenthos and macrobenthos are discrete entities and not artefacts of
369 sampling a size continuum: Comment on Bett (2013). *Mar. Ecol.-Prog. Ser.* **505**, 295–298
370 (2014).

371

- 372 30. Müller, K. J., Walossek, D. & Zakharov, A. 'Orsten' type phosphatized soft-integument
373 preservation and a new record from the Middle Cambrian Kuonamka Formation in Siberia.
374 *Neue Jahrb. Geol. P.-A.* **197**, 101–118 (1995).
- 375
- 376 31. Walossek, D. & Müller, K. J. in *Arthropod Fossils and Phylogeny* (ed. Edgecombe, G.
377 D.) 185–231 (Columbia University Press, 1998).
- 378
- 379 32. Dong, X.-P., Donoghue, P. C. J., Cheng, H. & Liu, J.-B. Fossil embryos from the Middle
380 and Late Cambrian period of Hunan, south China. *Nature* **427**, 237–240 (2004).
- 381
- 382 33. Zhang, H. *et al.* Armored kinorhynch-like scalidophoran animals from the early
383 Cambrian. *Scientific Reports* **5**, 16521 (2015).
- 384
- 385 34. Dong, X.-P. *et al.* Developmental biology of the early Cambrian cnidarian *Olivoooides*.
386 *Palaeontology* **59**, 387–407.
- 387
- 388 35. Cullen, D. J. Bioturbation of superficial marine sediments by interstitial meiobenthos.
389 *Nature* **242**, 323–324 (1973).
- 390
- 391 36. Gerlach, S. A. Food-chain relationships in subtidal silty sand marine sediments and the
392 role of meiofauna in stimulating bacterial productivity. *Oecologia* **33**, 55–69 (1978).
- 393
- 394 37. Aller, R. C. & Aller, J. Y. Meiofauna and solute transport in marine muds. *Limnol.*
395 *Oceanogr.* **37**, 1018–1033 (1992).
- 396

397 38. Coull, B. C. Role of meiofauna in estuarine soft-bottom habitats. *Aust. J. Ecol.* **24**, 327–
398 343 (1999).
399
400 39. Pike, J., Bernhard, J. M., Moreton, S. G. & Butler, I. B. Microbioirrigation of marine
401 sediments in dysoxic environments: Implications for early sediment fabric formation and
402 diagenetic processes. *Geology* **29**, 923–926 (2001).
403
404 40. Porter, S. M. Tiny vampires in ancient seas: evidence for predation via perforation in
405 fossils from the 780–740 million-year-old Chuar Group, Grand Canyon, USA. *Proc. R. Soc.*
406 *B* **283**, 20160221 (2016).
407
408 41. Smith, F. A. *et al.* Body size evolution across the Geozoic. *Ann. Rev. Earth Pl. Sc.* **44**,
409 523–553 (2016).
410
411 42. Brown, J. H., Marquet, P. A. & Taper, M. L. Evolution of body size: consequences of an
412 energetic definition of fitness. *Am. Nat.* **142**, 573–584 (1993).
413
414 43. Mángano, M. G. & Buatois, L. A. Decoupling of body-plan diversification and ecological
415 structuring during the Ediacaran–Cambrian transition: evolutionary and geobiological
416 feedbacks. *Proc. R. Soc. B* **281**, 20140038 (2014).
417
418 44. Tarhan, L. G., Planavsky, N. J., Laumer, C. E., Stolz, J. F. & Reid, R. P. Microbial mat
419 controls on infaunal abundance and diversity in modern marine microbialites. *Geobiology* **11**,
420 485–497 (2013).
421

- 422 45. Danovaro, R. *et al.* The challenge of proving the existence of metazoan life in
423 permanently anoxic deep-sea sediments. *BMC Biology* **14**, 43 (2016).
- 424
- 425 46. Pawlowska, M. M., Butterfield, N. J. & Brocks, J. J. Lipid taphonomy in the Proterozoic
426 and the effect of microbial mats on biomarker preservation. *Geology* **41**, 103-106 (2012).
- 427
- 428 47. Löhr, S. C. & Kennedy, M. J. Micro-trace fossils reveal pervasive reworking of Pliocene
429 sapropels by low-oxygen-adapted benthic meiofauna. *Nat. Commun.* **6**, 6589 (2015).
- 430
- 431 48. Heiner, I. & Kristensen, R. M. Two new species of the genus *Pliciloricus* (Loricifera,
432 Pliciloricidae) from the Faroe Bank, North Atlantic. *Zool. Anz.* **243**, 121–138 (2005).

433 Acknowledgements

434 We thank staff at the Geological Subsurface Laboratory, Regina, Saskatchewan and Maria
435 Vélez, University of Regina, for help with core sampling, and geoLOGIC for generous access
436 to subsurface data. This work was supported by Natural Environment Research Council
437 Grant NE/H009914/1.

439 Author contributions

440 T.H.P.H. and N.J.B. designed and performed the research and wrote the paper.

442 Competing financial interests

443 The authors declare no competing financial interests.

445 Table 1. Body length of the new fossil loriciferan, *Eolorica deadwoodensis*, compared to
 446 other fossil and extant loricated scalidophorans, and other small-bodied Cambrian animals. BL
 447 = body length.

	Taxon and growth stage	Max. length (µm)
Deadwood fossil loriciferan	<i>Eolorica deadwoodensis</i> gen. et sp. nov. (adult)	215 (lorica only)
		300 (reconstructed BL)
Extant loriciferans	adults ⁹	115-425
	larval stages ^{9,10}	48-800
Other Cambrian loricated taxa	<i>Orstenoloricus shergoldii</i> (larval) ²⁰	1,100 (reconstructed BL)
	<i>Sicyophorus</i> / <i>Palaeopriapulites</i> / <i>Protopriapulites</i> (adult) ¹⁸	25,000
	<i>Sirilorica</i> (adult) ¹⁷	70,000 (lorica only)
Extant priapulids with loricated larvae	meiobenthic priapulids (larval <i>Tubiluchus</i>) ²¹	465 (lorica only)
	macropriapulids (incl. larval <i>Priapulid</i> , <i>Halicryptus</i>) ^{13,18}	c. 2,000
Selected small-bodied benthic Cambrian animals	unnamed Orsten stem-tardigrade (larval) ³⁰	350
	<i>Bredocaris admirabilis</i> (Pancrustacea) (?adult) ³¹	850
	other Orsten arthropods (reconstructed adult stages) ³¹	> 1,000 (reconstructed BL)
	<i>Eokinorhynchus rarus</i> (?stem-kinorhynch) (?adult) ³³	c. 2,500 (reconstructed BL)
	<i>Markuelia</i> (Scalidophora) (late-stage embryo) ³²	> 3,000 (reconstructed BL)
	<i>Olivoooides</i> (Cnidaria) ³⁴	> 3,200

448

449 Figure 1. *Eolorica deadwoodensis*, a fossil loriciferan from the upper Cambrian Deadwood
450 Formation of Canada. **a–c**, Isolated loricae including one with remnant protruding scalids (**b**)
451 and one with partially disarticulated plicae (**c**). **d–g**, Specimen with articulated lorica,
452 introvert and scalids; holotype. **e**, Detail of the peri-oral structures (arrowed). **f**, Detail of the
453 robust anterior spinoscalids, with joints and long setules (arrowed). **g**, Detail of the more
454 posterior scalids, including forms with fringing setules (arrowed). Photographs are extended
455 depth of focus images using differential interference contrast in transmitted light. Scale bar in
456 **a** applies to **a–c**; scale bar in **g** applies to **e–g**. Specimen identifiers: **a**, RW12-4-10-13-N15,
457 GSC138576; **b**, RW12-4-10-24-X20, GSC138577; **c**, RW12-4-10-27-P16, GSC138578,
458 image flipped horizontally; **d–g**, FH3-4-5-01-P22, GSC138579.

459 Figure 2. *E. deadwoodensis* from the upper Cambrian Deadwood Formation (**a–i**) and a
 460 modern pliciloricid loriciferan (**j**). **a–c**, Overviews of isolated loricae, including one with
 461 remnant scalids (**b**). **d–f**, Lorica apertures, now flattened, with protruding remnant scalids.
 462 **g,h**, Details of scalids from panel **d** (**g**) and panel **f** (**h**) showing ornamentation of short
 463 setules. **i**, Fringed external anterior edge of a single plica (see Supplementary Fig. 2p for
 464 specimen overview). **j**, Modern pliciloricid loriciferan, *Pliciloricus leocaudatus*; image
 465 adapted from ref. 48 (Zoologischer Anzeiger vol. 243, Heiner, I. and Kristensen, R. M., “Two
 466 new species of the genus *Pliciloricus* (Loricifera, Pliciloricidae) from the Faroe Bank, North
 467 Atlantic”, page 128, Copyright (2005), with permission from Elsevier). Images are scanning
 468 electron micrographs. Scale bar in **a** applies to **a–c**. Specimen identifiers: **a**, RW12-4-10-02-
 469 04, GSC138580; **b**, RW12-4-10-06-09, GSC138581; **c**, RW12-4-10-03-08, GSC138582; **d,g**,
 470 RW12-4-10-03-02, GSC138583; **e**, RW12-4-10-04-13, GSC138584; **f,h**, RW12-4-10-04-07,
 471 GSC138585; **i**, RW12-4-10-01-07; GSC138586.

	Taxon and growth stage	Max. length (µm)
Deadwood fossil loriciferan	<i>Eolorica deadwoodensis</i> gen. et sp. nov. (adult)	215 (lorica only)
		300 (reconstructed BL)
Extant loriciferans	adults ⁹	115-425
	larval stages ^{9,10}	48-800
Other Cambrian loricata taxa	<i>Orstenoloricus shergoldii</i> (larval) ²⁰	1,100 (reconstructed BL)
	<i>Sicyophorus</i> / <i>Palaeopriapulites</i> / <i>Protopriapulites</i> (adult) ¹⁸	25,000
	<i>Siriloric</i> (adult) ¹⁷	70,000 (lorica only)
Extant priapulids with loricata larvae	meiobenthic priapulids (larval <i>Tubiluchus</i>) ²¹	465 (lorica only)
	macropriapulids (incl. larval <i>Priapul</i> , <i>Halicryptus</i>) ^{13,18}	c. 2,000
Selected small-bodied benthic Cambrian animals	unnamed Orsten stem-tardigrade (larval) ³⁰	350
	<i>Bredocaris admirabilis</i> (Pancrustacea) (?adult) ³¹	850
	other Orsten arthropods (reconstructed adult stages) ³¹	> 1,000 (reconstructed BL)
	<i>Eokinorhynchus rarus</i> (?stem-kinorhynch) (?adult) ³³	c. 2,500 (reconstructed BL)
	<i>Markuelia</i> (Scalidophora) (late-stage embryo) ³²	> 3,000 (reconstructed BL)

Exceptionally preserved Cambrian loriciferans and the early animal invasion of the
meiobenthos

Harvey, Thomas H.P.^{1*} and Butterfield, Nicholas J.²

¹Department of Geology, University of Leicester, University Road, Leicester LE1 7RH, UK.

²Department of Earth Sciences, University of Cambridge, Downing Street, Cambridge CB2
3EQ, UK.

*Corresponding author, email: thph2@le.ac.uk

a **b**

Supplementary Fig. 1. Localities and geological setting. **a**, Localities of sampled drillcores that have yielded specimens of *Eolorica deadwoodensis* in southern Saskatchewan (SK), Canada. CH = CPEC *et al.* Hartaven (also known as FH, Founders *et al.* Hartaven) 142/12-01-101-09W2, sample FH3-4-5 ($n = 1$; holotype) and sample FH3-4-65 ($n = 2$), depths c. 2447 m; RW = Ceepee Reward 101/04-28-038-24W3, sample RW12-4-10, depth c. 1592 m ($n = 100$, from rock sample weighing 38.3 g); KF = Ceepee Keppel Forest 101/08-03-040-14W3, sample KF12-6-B, depth c. 1529 m ($n = 1$; palynological recovery); and RL = Ceepee Riley Lake 101/03-04-039-13W3, sample RL13-4-110, depth c. 1641 m ($n = 1$). All specimens come from the Deadwood Formation^{49,50}, which regionally is dated to Cambrian Series 3 to Furongian (middle to late Cambrian) based on lithostratigraphic relationships with trilobite-bearing units in the northern US, and paraconodonts, trilobites and graptolites in Alberta⁵¹. Samples bearing *E. deadwoodensis* are further constrained to the Furongian (c. 485–497 million years ago) based on stratigraphic proximity to linguliformean brachiopods in Ceepee Reward⁵² and lithostratigraphic correspondence in the other drillcores (drawing on data in ref. 31). The loriciferan fossils co-occur in samples containing a diverse range of Cambrian SCFs^{53,54}. (Figure adapted from ref. 55; Cambrian subcrop edge from ref. 49.) **b**, Schematic W–E cross section through Cambrian and Ordovician rocks in the Western Canada Sedimentary Basin. Not to scale; approximate horizontal extent c. 1,000 km. In southern Saskatchewan, the Deadwood Formation encompasses the upper part of a sandstone-rich siliciclastic succession with minimal carbonates, representing deposition onto an eroded continental margin (overlying the ‘Great Unconformity’) in an extensive epicratonic sea during a major transgressive phase. Note that the Burgess Shale was deposited in a contrasting setting, in a more distal, mud-dominated environment now represented within a succession of shale-carbonate ‘grand cycles’⁵⁵. (Figure adapted from ref. 55.)

49. Dixon, J. Stratigraphy and facies of Cambrian to Lower Ordovician strata in Saskatchewan. *B. Can. Petrol. Geol.* **56**, 93–117 (2008).
50. Buatois, L. & Mángano, M. G. Paleoenvironmental variability of the Lower Palaeozoic Earlie and Deadwood formations in subsurface Saskatchewan: a preliminary assessment. *Summary of Investigations 2013, Volume 1, Saskatchewan Geological Survey, Sask. Ministry of the Economy, Misc. Rep.* **2013 4.1**, Paper A-3, 8 p. (2013).
51. Hein, F. J. & Nowlan, G. S. Regional sedimentology, conodont biostratigraphy and correlation of Middle Cambrian - Lower Ordovician strata of the 'Finnegan' and Deadwood formations, Alberta subsurface, Western Canada Sedimentary Basin. *B. Can. Petrol. Geol.* **46**, 166–188 (1998).
52. Robson, S. P., Nowlan, G. S. & Pratt, B. R. Middle to Upper Cambrian linguliformean brachiopods from the Deadwood Formation of subsurface Alberta and Saskatchewan, Canada. *J. Paleontol.* **77**, 201–211 (2003).
53. Harvey, T. H. P., Vélez, M. I. & Butterfield, N. J. Exceptionally preserved crustaceans from western Canada reveal a cryptic Cambrian radiation. *PNAS* **109**, 1589–1594 (2012).
54. Harvey, T. H. P., Vélez, M. I. & Butterfield, N. J. Small carbonaceous fossils from the Earlie and Deadwood formations (middle Cambrian to lower Ordovician) of southern Saskatchewan. *Summary of Investigations 2012, Volume 1, Saskatchewan Geological Survey, Sask. Ministry of the Economy, Misc. Rep.* **2012-4.1**, Paper A-1, 8 p. (2012).

55. Slind, O. L. *et al.* in *Geological atlas of the Western Canada Sedimentary Basin* (eds. Mossop, G. D. & Shetsen, I.) 87–108 (Canadian Society of Petroleum Geologists and Alberta Research Council, 1994).

Supplementary Fig. 2. Anatomical and preservational variation in *Eolorica deadwoodensis*.

a–u, Selected fossil specimens, including three with remnant protruding scalids (**a**, **g**, **o**), to show variation in size, proportions, thickness of preserved cuticle, degree of plica separation/disarticulation, degree of constriction, and angle of flattening (from bilaterally symmetrical, dorso-ventrally compressed to posteriorly curved, laterally compressed). All scale bars equal 50 μm . **a–j**, extended depth of focus images using differential interference contrast in transmitted light, figured to a common scale bar; **k–u**, scanning electron micrographs, with individual scale bars. Specimen identifiers: **a**, RW12-4-10-19-W38, GSC138587; **b**, RW12-4-10-13-W11, GSC138588; **c**, RW12-4-10-26-Y45, GSC138589; **d**, RW12-4-10-27-H21, GSC138590; **e**, RW12-4-10-09-Q11, GSC138591; **f**, RW12-4-10-24-K22, GSC138592; **g**, RW12-4-10-17-N37, GSC138593; **h**, RW12-4-10-25-M21, GSC138594; **i**, RL13-4-110-06-V4, GSC138595; **j**, RW12-4-10-36-O22, GSC138596; **k**, RW12-4-10-04-08, GSC138597; **l**, RW12-4-10-04-20, GSC138598; **m**, RW12-4-10-04-11, GSC138599; **n**, RW12-4-10-04-18, GSC138600; **o**, RW12-4-10-02-02, GSC138601; **p**, RW12-4-10-01-07, GSC138586 (white box indicates position of plica-fringe detail in Fig. 2i); **q**, RW12-4-10-03-04, GSC138602; **r**, RW12-4-10-05-05, GSC138603; **s**, RW12-4-10-04-01, GSC138604; **t**, RW12-4-10-02-20, GSC138605; **u**, RW12-4-10-06-08, GSC138606.

a **b** **c** **d** **e**

Supplementary Fig. 3. Phylogenetic contexts. **a**, Phylogenetic tree summarizing a proposed parsimonious scenario of loricated ecdysozoan evolution and the implied derived status of extreme small body size in meiobenthic loriciferans. Node 1 unites *Eolorica deadwoodensis* with the three families of modern loriciferans (including the monospecific Urnaloricidae, adult stage unknown)⁹; the stem- or crown-loriciferan status of *E. deadwoodensis* is contingent upon the interrelationships of the modern groups, which remain to be tested. Node 2 unites crown-group priapulids in a topology that is supported by morphological cladistic analyses^{11,56}; note that a more derived position for *Tubiluchus*⁵⁷ would imply a less parsimonious pattern of lorica evolution. Node 3 unites Loricifera and Priapulida (=Vinctiplicata)^{9,56} among scalidophorans to the exclusion of kinorhynchs, which lack a lorica; alternative pair-wise groupings would imply additional convergence or loss of lorica characters. Other Cambrian lorica-bearing fossils (*Sirilorica*¹⁷, *Orstenoloricus*²⁰ and *Sicyophorus* /*Palaeopriapulites* /*Protopriapulites*^{18,19}), whatever their precise positions in the tree, support a larger-bodied (mm, cm or dm) vinctiplicatan ancestor, consistent with the mm-plus body sizes of proposed stem-scalidophorans^{32,58} and a stem-kinorhynch^{12,33}, two possible outgroups. Distributions of key characters are summarized in Supplementary Table 1. **b–e**, Hypotheses of relationships within Ecdysozoa, encompassing Loricifera (L), Priapulida (P), Kinorhyncha (K), Nematoda (N), Nematomorpha (Nm), Tardigrada (T), Onychophora (O), and Arthropoda/Euarthropoda (A). Uncertainties in the tree limit confident inferences about branching order and, therefore, the ancestral body size for Ecdysozoa, because various microscopic lineages (K, T) occupy pivotal positions. **b**, A topology based on analyses of morphological data^{11,56} includes the clades Scalidophora (L+P+K), Nematoida (N+Nm), Cycloneuralia/Introverta (Scalidophora+Nematoida) and Panarthropoda (T+O+A). **c**, An emerging topology from analyses of molecular data¹² suggests that ‘Cycloneuralia’/‘Introverta’ is paraphyletic⁵ but so far lacks support for an assumed

scalidophoran position for Loricifera. **d,e**, Unexpected topologies resulting from inclusion of loriciferan sequence data in molecular phylogenetic analyses, including **(d)** a Loricifera+Nematomorpha clade (18S rRNA + Histone 3 loriciferan data)¹⁴ and **(e)** a Loricifera+Panarthropoda clade (18S + 28S rRNA)⁴⁵; these topologies are suspected to be influenced by long branch attraction and limited sequence availability^{12,16}.

56. Harvey, T. H. P., Dong, X. & Donoghue, P. C. J. Are palaeoscolecsids ancestral ecdysozoans? *Evol. Dev.* **12**, 177–200 (2010).

57. Adrianov, A. V. & Malakhov, V. V. The phylogeny, classification and zoogeography of the class Priapulida. I. Phylogeny and classification. *Zoosystematica Rossica* **4**, 219–238 (1996).

58. Liu, Y., Xiao, S., Shao, T., Broce, J. & Zhang, H. The oldest known priapulid-like scalidophoran animal and its implications for the early evolution of cycloneuralians and ecdysozoans. *Evol. Dev.* **16**, 155–165 (2014).

Supplementary Table 1: Distributions of key characters in *Eolorica deadwoodensis* and other loricata ecdysozoans.

Character	<i>Eolorica deadwoodensis</i>	Adult Plicioricidae ⁹	Adult Nanaloricidae ⁹	larval <i>Tubiluchus</i> ^{13,21}	Other loricata-larval priapulids ¹³	<i>Siriloric</i> ¹⁷	<i>Orstenoloricus</i> ²⁰	<i>Sicyophorus/Palaeo./Proto.</i> ^{18,19}	Interpretation
Lorica	x	x	x	x	x	x	x	x	Vinctiplicatan plesiomorphy
Lorica with circular cross-section	x	x		x		x	x	x	Vinctiplicatan plesiomorphy
Lorica with flattened cross-section			x		x				Convergent in nanaloricids and priapulids
Introvert with hundreds of elongate scalids	x	x	x				?		Derived loriciferan character
Robust jointed anterior scalids	x	x	x				?		Derived loriciferan character
Sub-millimetric adult body size	x	x	x						Derived loriciferan character
Concertina-like lorica folding pattern				x			x		