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

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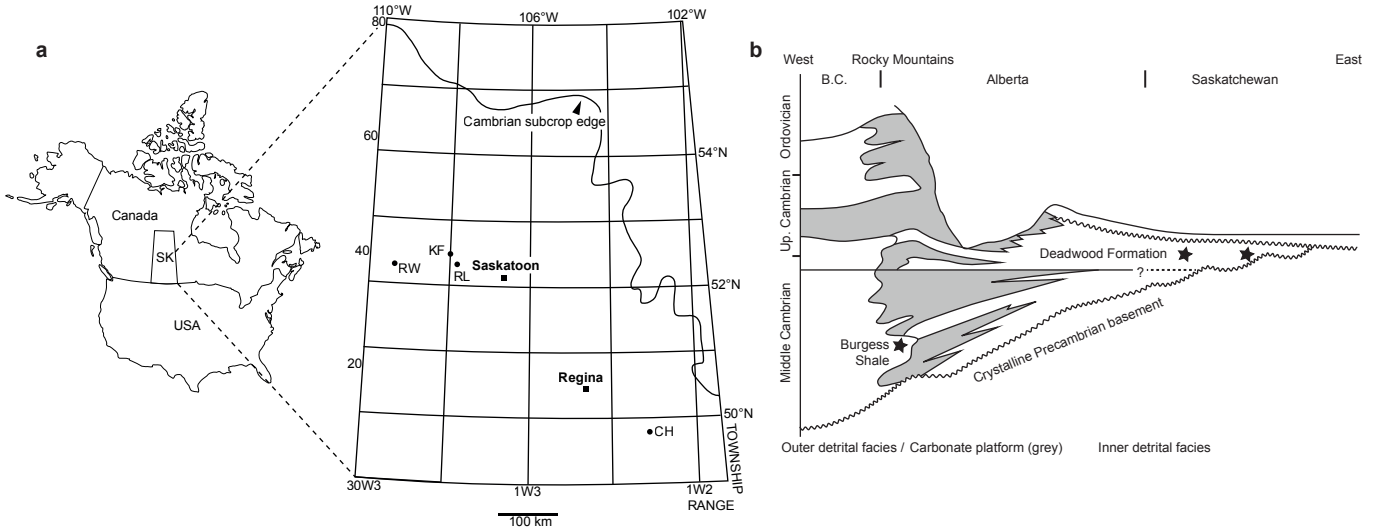

Supplementary Fig. 1. Localities and geological setting. **a**, Localities of sampled drillcores that have yielded specimens of *Eolorica deadwoodensis* in southern Sasketchewan (SK), Canada. CH = CPEC *et al*. Hartaven (also known as FH, Founders *et al*. Hartaven) 142/12-01-101-09W2, sample FH3-4-5 (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[50,51], 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 Alberta[52]. 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 Reward[53] and lithostratigraphic correspondence in the other drillcores (drawing on data in ref. 50). The loriciferan fossils co-occur in samples containing a diverse range of Cambrian SCFs[54,55]. (Figure adapted from ref. 55; Cambrian subcrop edge from ref. 50.) **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'[56]. (Figure adapted from ref. 56.)

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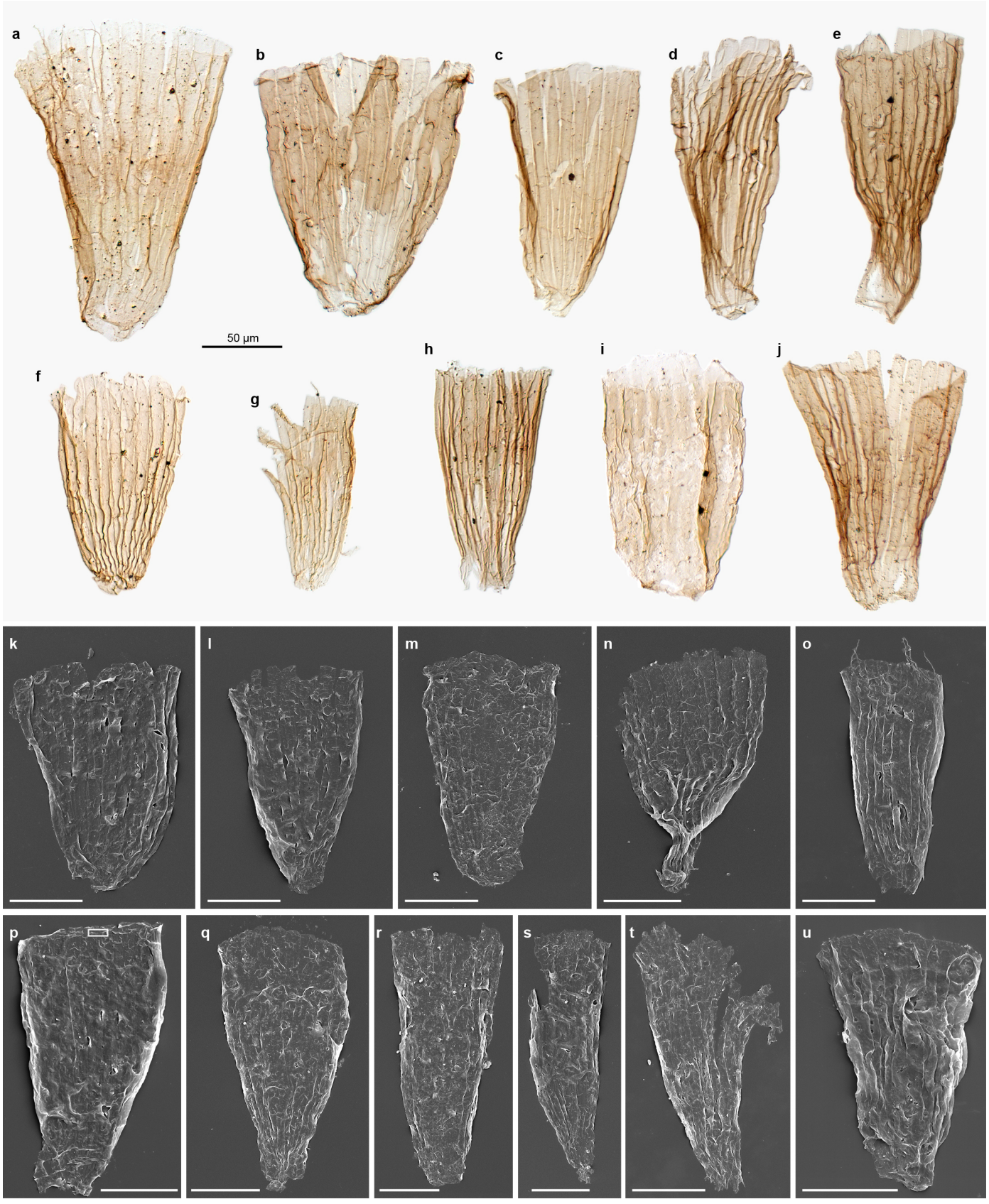
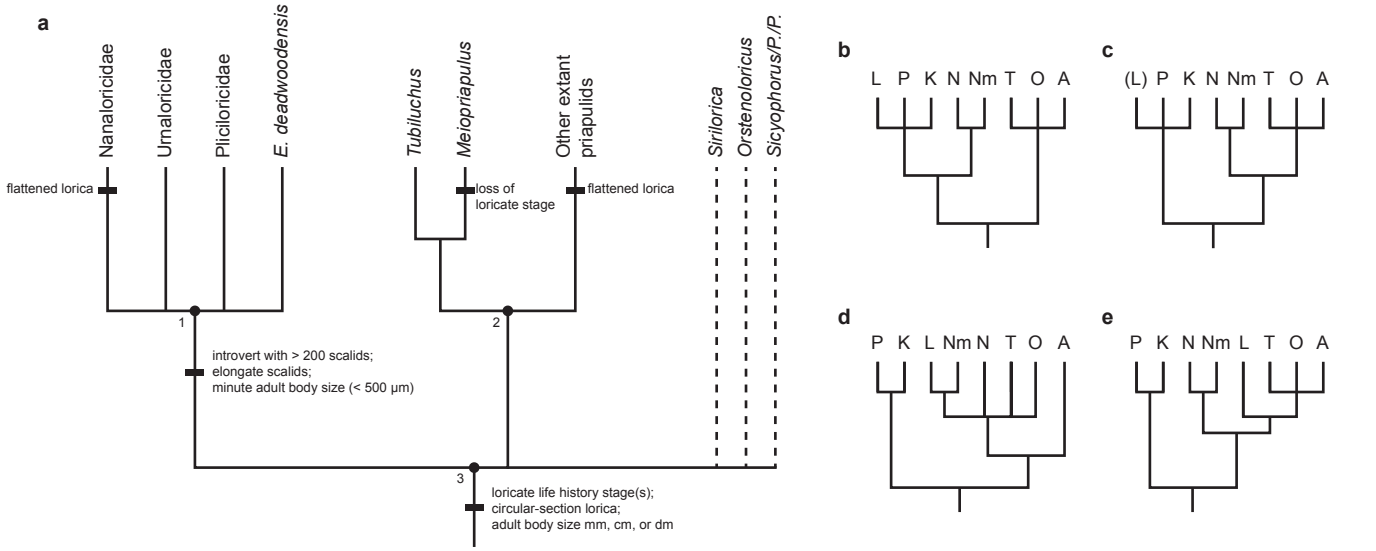


Supplementary Fig. 2. Anatomical and preservational variation in *Eolorica deadwoodensis*. **a**–**u**, Selected fossil specimens, including three with remnant protruding scalids (**a**, **g**, **o**), to show variation in size, proportions, thickness of preserved cuticle, degree of plica separation/disarticulation, 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.



Supplementary Fig. 3. Phylogenetic contexts. **a**, Phylogenetic tree summarizing a proposed parsimonious scenario of loricate 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[11,57]; note that a more derived position for *Tubiluchus*[58] would imply a less parsimonious pattern of lorica evolution. Node 3 unites Loricifera and Priapulida (=Vinctiplicata)[9,57] 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[32,59] 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[11,57] 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 data[12] suggests that 'Cycloneuralia'/'Introverta' is paraphyletic[5] 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)[15]; these topologies are suspected to be influenced by long branch attraction and limited sequence availability[12,16].

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

Character	*Eolorica deadwoodensis*	Adult Pliciloricidae[9]	Adult Nanaloricidae[9]	larval *Tubiluchus*[13,21]	Other loricate-larval priapulids[13]	*Sirilorica*[17]	*Orstenoloricus*[20]	*Sicyophorus*/*Palaeo.*/*Proto.*[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		