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## FRONT MATTER

## Title

- **Preservation and early evolution of scalidophoran ventral nerve cord**
- **Short title: Nervous system of early Scalidophora**

## Authors

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## Abstract

Ecdysozoan worms (Nematoida + Scalidophora) are typified by disparate grades of neural organization reflecting a complex evolutionary history. The fossil record offers a unique opportunity to reconstruct the early character evolution of the nervous system via the exceptional preservation of extinct representatives. We focus here on their nervous system as it appears in early and mid-Cambrian fossils. We show that some of the oldest known representatives of the group either preserved in carbonaceous compression (early and mid-Cambrian Burgess-type preservation) or secondarily phosphatized in three-dimensions (3D) (e.g. basal Cambrian Kuanchuanpu Formation, ca. 535 ma) had an unpaired ventral nerve cord (VNC) that ran along the trunk in an eccentric position as in modern priapulids and nematodes. A phylogenetic analysis integrating these fossil data suggests that ancestral scalidophorans had an unpaired VNC and that paired nervous systems probably evolved independently in Kinorhyncha and Loricifera, and, more importantly, in panarthropods in possible relation with the rise of paired appendages and bilaterally coordinated motricity.

## Teaser

Fossils from the early Cambrian indicate that arthropods evolved from worm-like ancestors with a single ventral nerve cord.

## 43 Introduction

44 Throughout evolution, bilaterian animals have developed extraordinarily sophisticated  
45 nervous systems that dramatically increased their capacity to analyze, coordinate, and  
46 transmit signals, thus allowing them to perform vital functions such as motricity and  
47 adequate responses to external stimuli (1). Most groups possess peripheral nerve networks  
48 and a central nervous system (CNS) with a differentiated brain in the cephalic region that  
49 extends posteriorly into an axial structure known as the nerve cord, which runs ventrally in  
50 ecdysozoans (1-3). Fossilized remains of brains and the ventral nerve cord (VNC) are now  
51 known in various early ctenophores (4), annelids (5) and especially panarthropods from  
52 celebrated Cambrian Lagerstätten, such as that of Chengjiang and the Burgess Shale. These  
53 are mainly euarthropods, such as *Fuxianhuia protensa* (6, 7) and *Mollisonia symmetrica*  
54 (8), and have far-reaching evolutionary implications (e.g. coupling of brain elements with  
55 cephalic appendages, (9-11)). However, their interpretation is difficult, especially in fossils  
56 preserved in compression where different anatomical features overlap, and accurate  
57 recognition criteria are required (12). Fossilized nervous systems undoubtedly represent a  
58 critical source of phylogenetic information but have until now been used mainly to explore  
59 the evolutionary relationships within euarthropods or panarthropods (8, 12-14). In contrast,  
60 the nervous systems of other early ecdysozoan worms have drawn less attention (15),  
61 although they are potentially important to clarify the relationships between Nematoida,  
62 Scalidophora, and Panarthropoda (16, 17). Ecdysozoan worms comprise Nematoida  
63 (Nematoda and Nematomorpha, (18)) and Scalidophora (Priapulida, Loricifera, and  
64 Kinorhyncha, (19)) and are characterized by a ring-like circumpharyngeal brain, except for  
65 nematomorphs (17, 20, 21). While sometimes referred to as Cycloneuralia (20), the  
66 monophyly of this group remains controversial, with molecular evidence strongly  
67 supporting nematoids and panarthropods as a clade (e.g. (22-24)). Notably, the VNC of  
68 ecdysozoan worms is either an unpaired (Priapulida, Nematoda and Nematomorpha) or  
69 paired (Loricifera and Kinorhyncha (17, 21)) structure. Longitudinal features interpreted as  
70 possible VNCs have been described in several Cambrian representatives of the group, such  
71 as *Ottoia prolifica* (25), *Yunnanpriapulites halteriformis* (26), *Xiaoheiqingella peculiaris*  
72 (27), *Acosmia maotiania* (28), and the phosphatized palaeoscolecid *Austroscolex*  
73 *spatiolatus* (29, 30). However, these remains are either incomplete and/or extremely  
74 difficult to interpret and make it difficult to draw conclusions about the evolution of the  
75 nervous system of early ecdysozoan worms (e.g. (31) but see (16, 32)).

76 To fill this knowledge gap, we describe here the oldest known VNC in scalidophoran  
77 worms occurring in *Eopriapulites sphinx* (33-35) and *Eokinorhynchus rarus* (36) from the  
78 ca. 535 ma basal Cambrian of China. We also re-describe the VNC of other key Cambrian  
79 species of the group, such as *Xiaoheiqingella peculiaris* and *Ottoia prolifica*, from the  
80 Chengjiang biota and Burgess Shale, respectively, in the light of comparisons with modern  
81 priapulid worms, giving particular importance to their mode of preservation. The fossil  
82 dataset obtained here is used to define the possible VNC ground pattern of Priapulida and  
83 Scalidophora and propose scenarios about the evolution of the VNC among early  
84 ecdysozoan worms.

## 86 Results

### 87 Eccentric longitudinal feature in Cambrian scalidophorans

88 *Eopriapulites sphinx*. Three 3D-preserved, secondarily phosphatized specimens  
89 (ELIXX118-96, ELIXX139-256, and UMCU 14CHD0816-008) are represented by curved  
90 cylindrical bodies bearing a closely spaced pattern of fine annulated folds and wrinkles  
91 (Figs. 1C, G, 2A; fig. S1). Their diameter slightly enlarges at one end and gradually

tappers at the opposite. The thicker end is truncated and incomplete, whereas the thinner one shows a central anal depression (34) with a smooth rounded rim and tiny pits (Fig. 1C, D). Specimens of comparable size and external ornament from the same locality as the present material were described (34) as belonging to *Eopriapulites sphinx*, one of the oldest known scalidophorans (Kuanchuanpu Formation, Xixiang section ca. 535 ma). Complete specimens of *Eopriapulites sphinx* display a swollen introvert lined with 18 rows of stout scalids, a terminal mouth surrounded by monodentate pharyngeal teeth, a finely annulated trunk with no sclerotized ornament and a tapering posterior end with no circum-anal features (Figs. 1A–C, 2A, see (33, 34) for detailed descriptions).

Two compressed specimens are also shown here. UMCU 14CHD0816-007 is strongly flattened and twisted (Fig. 2D, E). ELIXX42-269 is fragmentary and deformed, with a larger diameter than the other studied specimens (Fig. 2A–C, table S1). Its annuli are well-defined and ca. 25–35  $\mu\text{m}$  distant from each other. Importantly, five of our studied specimens show a remarkable longitudinal feature that runs along the trunk in an eccentric position. It is a slightly elevated strip (width ca. 40–60  $\mu\text{m}$ , table S1) that, in places, forms two longitudinal lobes separated by a very shallow furrow. Its relief gradually vanishes posteriorly when reaching the peri-anal rounded rim (bursa). Locally, the longitudinal structure shows a single thick rod (Fig. 1F, H). As the studied specimens are incomplete, it is impossible to determine how far this strip extended anteriorly (e.g. circum-oral or introvert region). A transverse section through *Eopriapulites sphinx* (Fig. 1B) shows that the VNC is not preserved as its own tissue but only materialized by the thin cuticular layer that covers it externally. In flattened specimens, the bilobate structure has a more eroded relief (Fig. 2D, E) or is truncated (deep furrow flanked by ridges), thus revealing a double-layered structure (Fig. 2C).

***Eokinatorhynchus rarus*.** ELIXX140-23, also from the Kuanchuanpu Formation, displays an incomplete (a little of proximal introvert) annulated trunk bearing small and larger sclerites, as well as small plates (without a spine pointing outwards). This specimen resembles *Eokinatorhynchus rarus* (36). One side of the trunk exhibits a longitudinal strip of constant width with a positive relief. Abundant fine cuticular folds cross the boundary between annulations (Fig. 2F, G).

***Ottoia prolifica*.** A comparable longitudinal feature distinct from the digestive tract (black band along the trunk, Fig. 3A) is clearly present in two specimens of *Ottoia prolifica* from the mid-Cambrian Burgess Shale Lagerstätte. In contrast to those from Kuanchuanpu, these fossils are preserved in carbonaceous compression and clearly show a longitudinal ladder-like reflective feature running perpendicular to the trunk annulations. This feature consists of two thin parallel strips (continuous or locally interrupted; Fig. 3B, C) that connect at a right angle to the inter-annular boundaries similarly preserved as carbon films. A faint additional linear feature occurs in places between these two strips (Fig. 3D, E, white arrows). One of these Burgess Shale specimens (USNM 188635) is likely to be an incomplete, partly folded exuvia - i.e., a thin cuticle-only flimsy structure, with well-marked boundaries between annulations but no trace of soft tissues (e.g. muscles).

**Cambrian scalidophorans from China.** Poorly preserved colored longitudinal traces also occur in at least three specimens of the early Cambrian of China. These belong to: *Yunnanpriapululus halteriformis* (26), *Xiaoheiqingella peculiaris* (27), and *Acosmia maotiania* (28) but are extremely difficult to characterize. Similarly questionable are the fine linear features seen along part of the trunk of *Acosmia maotiania* and the mid-Cambrian phosphatized palaeoscolecoid *Austroscolex spatiolatus* (29, 30). However, a new specimen of *Mafangscoclex yunnanensis* from the Chengjiang Lagerstätte (Fig. 3J, K) much more clearly shows a fine longitudinal feature that co-occurs with the digestive tract. In addition, one specimen of *Xiaoheiqingella peculiaris*, also from Chengjiang,

142 shows a curved linear feature running from the introvert to the pre-caudal region that  
143 locally intersects the sinuous gut tract (Fig. 3F–I).

### 144 **Muscle and nervous systems of living priapulids**

145 Extant priapulids have a dense network of circular and longitudinal muscle fibres  
146 within their body wall (i.e., below the epidermis; Fig. 4D, E, K), plus longitudinal, oblique  
147 bundles of retractile muscles (e.g. from trunk to introvert). The VNC of priapulids is  
148 intraepidermal (i.e., within the epidermal cell layer), sandwiched between the thin cuticle  
149 and the double layer of circular and longitudinal muscle fibers (Fig. 4D, E), and  
150 always appears as an unpaired structure in live specimens (*Priapulopsis australis*, Fig.  
151 4A; *Priapululus caudatus*, Fig. 4B, C). However, specimens of *Priapululus caudatus*  
152 preserved in 70% alcohol or desiccated (critical point) for scanning electron  
153 microscopy (Fig. 4D, E) exhibit local positive and negative deformation, particularly  
154 along the nerve cord that thus takes the form of a bilobate structure (Fig. 4F–H). The very  
155 thin cuticle (ca. 10  $\mu\text{m}$ , exuvia) is also affected by micro-folding forming multi-folds (Fig.  
156 4I, J).

### 158 **Phylogeny**

159 Phylogenetic analyses were performed to explore the phylogeny of ecdysozoan worms and  
160 possibly clarify the evolution of their nervous system, exemplified by their VNC. The  
161 monophyly of Cycloneuralia based on anatomical characters is not supported by molecular  
162 evidence (e.g. (23, 24)), which instead supports a clade composed of Nematoida plus  
163 Panarthropoda. Accordingly, the clade Nematoida plus Panarthropoda was constrained in  
164 cladistic analysis. Our results resolve Cambrian scalidophorans such as *Eokinorhynchus*  
165 *rarus* as stem-group Scalidophora and *Xiaoheiqingella peculiaris* as stem-group Priapulida  
166 (Fig. 6, fig. S2). *Mafangscolex yunnanensis*, *Ottoia prolifica*, and *Eopriapulites sphinx*  
167 form a clade as the sister group to *Eokinorhynchus rarus* plus crown-group Scalidophora  
168 (Fig. 6, fig. S2). The position of *Acosmia maotiania*, which is a separate branch within  
169 Ecdysozoa, is not well resolved.

### 171 **Discussion**

#### 172 **Nature of the longitudinal structure**

173 Extant scalidophorans have two major longitudinal anatomical structures, the digestive  
174 tract (gut) that runs centrally from the mouth to the anus throughout the animal's body  
175 cavity and the ventral nerve cord nested within the body wall. The gut is relatively narrow,  
176 cylindrical and, importantly, not attached to the trunk wall (37–39). In contrast, the  
177 longitudinal strip seen in *Eopriapulites sphinx* and *Eokinorhynchus rarus* has an eccentric  
178 location and is clearly embedded within the body wall. It is unlikely to represent the gut  
179 that would have collapsed onto the inner body wall after death or its imprint.

180 Extant priapulids have an orthogonal muscle system (see above), whereas adult  
181 kinorhynchs and loriciferans have only longitudinal muscles along their trunk (40, 41). No  
182 single thick muscle bundle running along the trunk is known to occur in any extant  
183 scalidophoran. Besides, experimental taphonomy shows that muscles are labile tissues that  
184 decay rapidly after death ((42) for priapulids, lost after 4 or 6 days). For these reasons, the  
185 single strip seen in *Eopriapulites sphinx* and *Eokinorhynchus rarus*, is unlikely to  
186 represent any of these muscle types.

187 Rare cases of muscle preservation result from the extremely rapid post-mortem  
188 precipitation of calcium phosphate over undecayed structures (43, 44). Well-preserved  
189 muscle fibres are exceptionally found in some lobopodians (45, 46) and cnidarians (47)  
190 but have no equivalent in any Cambrian worm preserved in carbonaceous compression  
191 (Burgess Shale-type localities). Gonads are arranged in longitudinal pairs in all extant

192 scalidophorans and occupy a large part of the pseudocoel in extant (see (37)) and  
193 Cambrian (48) priapulids. By their clustered shape and location, these organs differ  
194 markedly from the single strip seen in *Eopriapulites sphinx* and *Eokinorhynchus rarus*. In  
195 summary, the most meaningful interpretation is that the three-dimensionally preserved  
196 longitudinal strip seen in *Eopriapulites sphinx* and *Eokinorhynchus rarus* corresponds to  
197 the ventral nerve cord (VNC). No other typical elements of the nervous system known in  
198 extant scalidophorans (e.g. the circumpharyngeal ring and caudal ganglia) could be  
199 observed in our incomplete specimens.

### 200 **VNC preservation modes**

201 Cambrian scalidophorans are preserved either in fine shales as carbonaceous compression  
202 (e.g. Chengjiang, Burgess) or mineralized in three-dimensional (3D) via secondary  
203 phosphatization (e.g. phosphatic limestones of the Kuanchuanpu Formation).  
204 Experimental taphonomy (see (42) for priapulids) shows that muscle fibers (lost after 4 to  
205 6 days) and epidermal cells (lost after 8 days) rapidly decompose after death, contrasting  
206 with the more decay-resistant cuticle. Paradoxically, the VNC retains its consistency and  
207 external configuration over about 4 days, allowing the overlying thin cuticle to imprint its  
208 shape before its final breakdown (42). This information on the timing of decay processes  
209 supports the hypothesis that the mineralized cuticle of the scalidophorans from  
210 Kuanchuanpu faithfully replicated the overall shape of the VNC after the latter had  
211 completely decomposed.

212 In the Burgess Shale-type preservation (e.g. Burgess Shale, Chengjiang localities), the  
213 nervous tissues of numerous Cambrian arthropods (8, 12, 49) and annelids (5) are  
214 typically preserved as reflective carbon films with a greater or lesser proportion of iron  
215 oxide (e.g. brain (6, 8); originally microcrystalline pyrite). Similarly, the VNC of  
216 scalidophorans typically appears as a brownish strip, presumably slightly enriched in iron  
217 oxides and carbon.

218 We suggest a possible taphonomic scenario for these two different modes of preservation  
219 (Fig. 5) in which Steps 1 and 2 (Fig. 5A, B) would correspond to the post-mortem collapse  
220 of most tissues creating cuticular deformation and folding; Step 3a (Fig. 5C, D; in  
221 environments favorable to phosphatic precipitation) to the mineralization of the folded  
222 cuticle (50, 51); Step 3b (Fig. 5E–G; in environments where worms are buried in fine  
223 mud) to the compression of all tissues (possibly including VNC) and cuticular remains  
224 and their fossilization as carbon films (12).

### 225 **Was the VNC of the oldest known scalidophorans paired or unpaired?**

226 The VNC of *Eopriapulites sphinx* is seemingly made of juxtaposed longitudinal lobes in  
227 preservation, at least locally (see Figs. 1H, 2), raising the question of its possible paired  
228 nature. Our decay experiments conducted with priapulids indicate that post-mortem  
229 bilobate and micro-folded structures appear along the VNC, which seem to result from the  
230 shrinkage and collapse of underlying tissues (e.g. muscles) at various stages of post-  
231 mortem evolution or preparation. The very similar configuration seen in the fossil  
232 specimens from Kuanchuanpu (bilobate relief of VNC, see Figs. 1H, 2) is most probably  
233 artefactual and does not represent the remains of a paired structure originally made of two  
234 closely juxtaposed nerve cords.

235 Interestingly, the VNC of *Eopriapulites sphinx* is preserved as its externally cuticular  
236 imprint instead of its own tissue (see above). As with most labile tissues, the VNC  
237 probably collapsed and decayed before the phosphatic mineralization of the cuticle while  
238 leaving its external 3D-imprint. In this context, the hypothesis of cuticular deformation  
239 over the VNC appears most plausible. Additionally, the truncated lobes seen in some  
240  
241

specimens (Fig. 2D, E) seem to result from the shrinkage of the cuticular structure followed by relief abrasion after phosphatic deposition.

It should also be noted that, unlike *Eopriapulites sphinx*, *Eokinorhynchus rarus* clearly displays an unequivocal unpaired VNC (Fig. 2F, G). Thus, the VNC of the oldest known scalidophorans from Kuanchuanpu had most likely an unpaired structure, as seen in other Cambrian scalidophorans preserved in compression and in extant priapulids.

Interestingly, the ladder-like structure seen in the exuvia of *Ottoia prolifica* from the Burgess Shale (Fig. 3C–E) has an exact equivalent in that of *Priapululus caudatus* (Fig. 4I, J), where crisscrossed cuticular folds occur both at the boundary between annulations and longitudinally on both sides of the VNC. The same pattern observed in *Ottoia prolifica* is, therefore, unlikely to represent the fossilized remains of neural tissues, and, a fortiori, a paired nerve cord (16, 49).

To summarize, none of the early and mid-Cambrian scalidophorans currently known shows evidence of a paired VNC, suggesting that ancestors and modern representatives (e.g. priapulids) of the group are characterized by an unpaired structure.

### Early evolution of the nervous system in ecdysozoan worms

Many uncertainties remain concerning the early evolution of ecdysozoans, such as the rise of leg-bearing panarthropods from assumed vermiform ancestors (35, 52) and the chronology of divergence between the main vermiform lineages (Priapulida, Loricifera, Kinorhyncha, Nematomorpha, Nematoda; see (20, 22, 23)). Despite persisting debates concerning the monophyly of Cycloneuralia (molecular versus anatomical data, (23), summarized in (53)), it is largely admitted that ecdysozoan worms except Nematomorpha share important features in the general organization of their nervous systems (e.g. circumpharyngeal ring (17, 21)) and could potentially provide key information on the neural organization of more remote ecdysozoan ancestors.

However, the coexistence of distinct nervous system arrangements within ecdysozoans raises important questions about how this organ system evolved and diversified among ecdysozoan worms over time (9, 17, 20, 54, 55). There are marked differences between the central nervous system of panarthropods and that of ecdysozoan worms (17): ganglionated versus ring-like brain, respectively, and their VNC (with ganglia in panarthropods except Onychophora). In addition, the VNC is unpaired in priapulids and nematoids (37, 56, 57) but paired in kinorhynchs, loriciferans (32, 39, 40) and panarthropods (54, 55, 58, 59).

Our phylogenetic results support an unpaired VNC as the ancestral condition of Scalidophora (e.g. *Eokinorhynchus rarus*) and Priapulida (e.g. *Xiaoheiqingella peculiaris*) and seem to be conserved throughout the total-group Priapulida (16, 60) from the Cambrian to the present day (Fig. 6, fig. S2). This contradicts previous hypotheses that considered the unpaired VNC of priapulids as a derived condition possibly resulting from the fusion of a hypothetical paired structure present in the ancestors of the group (31). Although lacking Cambrian representatives, both extant nematodes and nematomorphs have an unpaired VNC, too (20, 21, 56, 57). Altogether, this evidence suggests that the nervous system of early scalidophorans was characterized by an unpaired VNC (Fig. 6, fig. S2).

Interestingly, two groups of meiobenthic scalidophorans, the Kinorhyncha and Loricifera, complicate this apparently simple evolutionary scenario. Kinorhynchs differ from other scalidophorans (e.g. annulated priapulids) by the presence of (i) 11 cuticularized and articulated segments (either ring-like or divided into articulating tergal (dorsal) and sternal (ventral) plates, and (ii) segmentally arranged muscles (32, 38, 41, 61). These externally well-differentiated segments correlate internally with segmentally arranged elements of

292 the muscular and nervous systems (32), thus recalling arthropods. In kinorhynchs, ten  
293 longitudinal neurite bundles originate from the brain and converge into five units along the  
294 trunk, where the VNC diverges again posteriorly (32, 38, 60). As a result, their VNC  
295 appears as a paired structure made of more or less closely juxtaposed (but never fused)  
296 neurite bundles (32). Like in kinorhynchs, the VNC of loriciferans similarly consists of  
297 two longitudinal neurite bundles (62), thus contrasting with the unpaired strand of  
298 priapulids (37).

299 Another peculiar feature of kinorhynchs is the presence of paired ganglia along the VNC  
300 (21, 38), corresponding (or with a slight offset) to the trunk segments (32, 61, 63). These  
301 paired ganglia might be concomitant to the evolution of body segmentation (e.g. to  
302 innerve segmental muscles). We hypothesize that paired VNCs and paired associated  
303 ganglia may have arisen independently among ecdysozoans as a possible evolutionary  
304 response to body segmentation and the development of lateral appendages (e.g.  
305 panarthropods). Although legs are absent in kinorhynchs, these animals are characterized  
306 by a well-marked segmentation and bilateral profile, which may correlate with the  
307 development of paired ganglia and juxtaposed VNC bundles (see transverse sections in  
308 (32)). Likewise, loriciferans display a clear bilateral construction exemplified by their  
309 lorica made of jointed plates with underlying and associated paired muscular and nervous  
310 features (e.g. paired VNC).

311 Leaving the apparent originality of kinorhynchs and loriciferans aside, there is a huge gap  
312 between ecdysozoan worms and panarthropods in terms of neural organization (17), the  
313 latter having a ladder-like paired VNC with segmentally distributed pairs of ganglia  
314 (except for onychophorans that have a lateralized VNC; (55)), each pair being connected  
315 by transverse commissures. How this highly differentiated nervous system may have  
316 evolved from possible ecdysozoan ancestors remains to be elucidated (e.g. (13, 17, 29)).  
317 Based on our results, the unpaired VNC might represent the ancestral condition of  
318 ecdysozoan worms and perhaps even their deeper ancestors (Fig. 6, fig. S2). Furthermore,  
319 it is clear that unsegmented ecdysozoan worms (Priapulida and Nematoida) have an  
320 unpaired VNC, whereas segmental kinorhynchs have evolved ganglionated and paired  
321 VNC, resembling that of Euarthropoda and Tardigrada.

322 This strongly suggests a relation between the presence of a paired VNC, ganglia, and legs  
323 and the development of segments as a necessary condition for coordinating locomotion in  
324 panarthropods (64-66). This assumed major innovation, -i.e. evolution of ganglia and  
325 paired VNC, is likely to have taken place among the basal panarthropods such as  
326 lobopodians that had paired soft-bodied appendages (67, 68). This suggests that the  
327 development of legs in invertebrate animals that most likely occurred during the  
328 Precambrian-Cambrian transition (first lobopodians) is concomitant and inextricably  
329 linked with major transformations of the ventral nerve cord and muscle systems.

## 331 **Materials and Methods**

332 **Fossil material-** *Eopriapulites sphinx* (Specimens ELIXX42-269, ELIXX118-96 and  
333 ELIXX139-256) and *Eokinorhynchus rarus* (Specimen ELIXX140-23) were collected  
334 from the basal Cambrian Kuanchuanpu Formation (Fortunian Stage, Terreneuvian Series),  
335 more precisely from Bed 2, Zhangjiagou Section, Xixiang County, Shaanxi Province (33).  
336 Fossils were obtained by digesting calcareous rock samples in 8–10% acetic acid.  
337 Phosphatic residues were picked up under the binocular microscope. Specimens of  
338 *Mafangsclex yunnanensis* (ELIJS0055) and *Xiaoheiqingella peculiaris* (ELIJS1260)  
339 were both collected from the Chengjiang Lagerstätte (Cambrian Stage 3; see (69) for  
340 details). All fossils are deposited in the collections of the Shaanxi Key Laboratory of Early  
341 Life and Environments collections, Northwest University, Xi'an, China (ELIXX



342 numbers). Specimens of *Eopriapulites sphinx* and *Eokinorhynchus rarus* were mounted on  
343 stubs, coated with gold, and observed under the Scanning Electron Microscope (SEM; FEI  
344 Quanta 400 FEG, high vacuum, secondary electrons) at Northwest University Xi'an. Light  
345 photographs of scalidophoran worms (*Xiaoheiqingella peculiaris* ELIJS1260 and  
346 *Mafangscoplex yunnanensis* ELIJS0055) were taken with a Canon EOS 5DS R. Images of  
347 *Ottoia prolifica* (Burgess Shale) in cross-polarized light were obtained from Jean-Bernard  
348 Caron (Royal Ontario Museum, Toronto, Canada; Desmond Collins' archives), those of  
349 two additional specimens of *Eopriapulites sphinx* (UMCU 14CHD0816-007 and  
350 14CHD0816-008) from Yunhuan Liu, Tiequan Shao, and Jiachen Qin (Chang'an  
351 University, China).

352  
353 **Biological material-** *Priapulites caudatus* was collected near the Kristineberg Marine  
354 Station (Gullmarsfjord, Sweden; University of Göteborg) and the White Sea Biological  
355 Station (WSBS; along the coast of the Kandalaksha Bay, Moscow State University), fixed,  
356 preserved in 70% ethanol, dissected, and desiccated (Critical Point method) before  
357 observations and imaging under the SEM (FEI Quanta 250 FEG; high vacuum,  
358 acceleration voltage 5 KV) at CTμ, University Claude Bernard Lyon 1, France. Images of  
359 specimens immersed in 70% ethanol were taken with a Leica MZ 125 binocular  
360 microscope. Andreas Schmidt-Rhaesa (Museum der Natur Hamburg, Germany) provided  
361 a light photograph of a specimen of *Priapulopsis australis* (accession number 162937  
362 from the National Institute of Water and Atmospheric Research collection in Auckland,  
363 New Zealand).

364  
365 **Phylogenetic analysis.** A dataset with 42 taxa and 120 characters was assembled from (28,  
366 35, 70-72). It contains new neurological characters (see details in data S1 and other  
367 supplementary material). This dataset was analyzed using parsimonious inference with  
368 TNT v.1.6 (73) using new technology search (Driven Search with Sectorial Search,  
369 Ratchet, Drift, and Tree fusing options activated) in default settings under equal weight  
370 and implied weights (74, 75). A strict consensus of the five most parsimonious trees is  
371 presented from equal character weighting (fig. S2A) and clade support was assessed by  
372 jack-knife resampling (76). A strict consensus of the two most parsimonious trees (fig. S  
373 2B) is presented for implied character weighting (using the default concavity constant  $k =$   
374 3) and clade support was assessed by symmetrical resampling (77). Nematoda plus  
375 Panarthropoda were constrained as monophyletic group based on strongly molecular  
376 phylogenetic evidence (e.g. (23, 24)).

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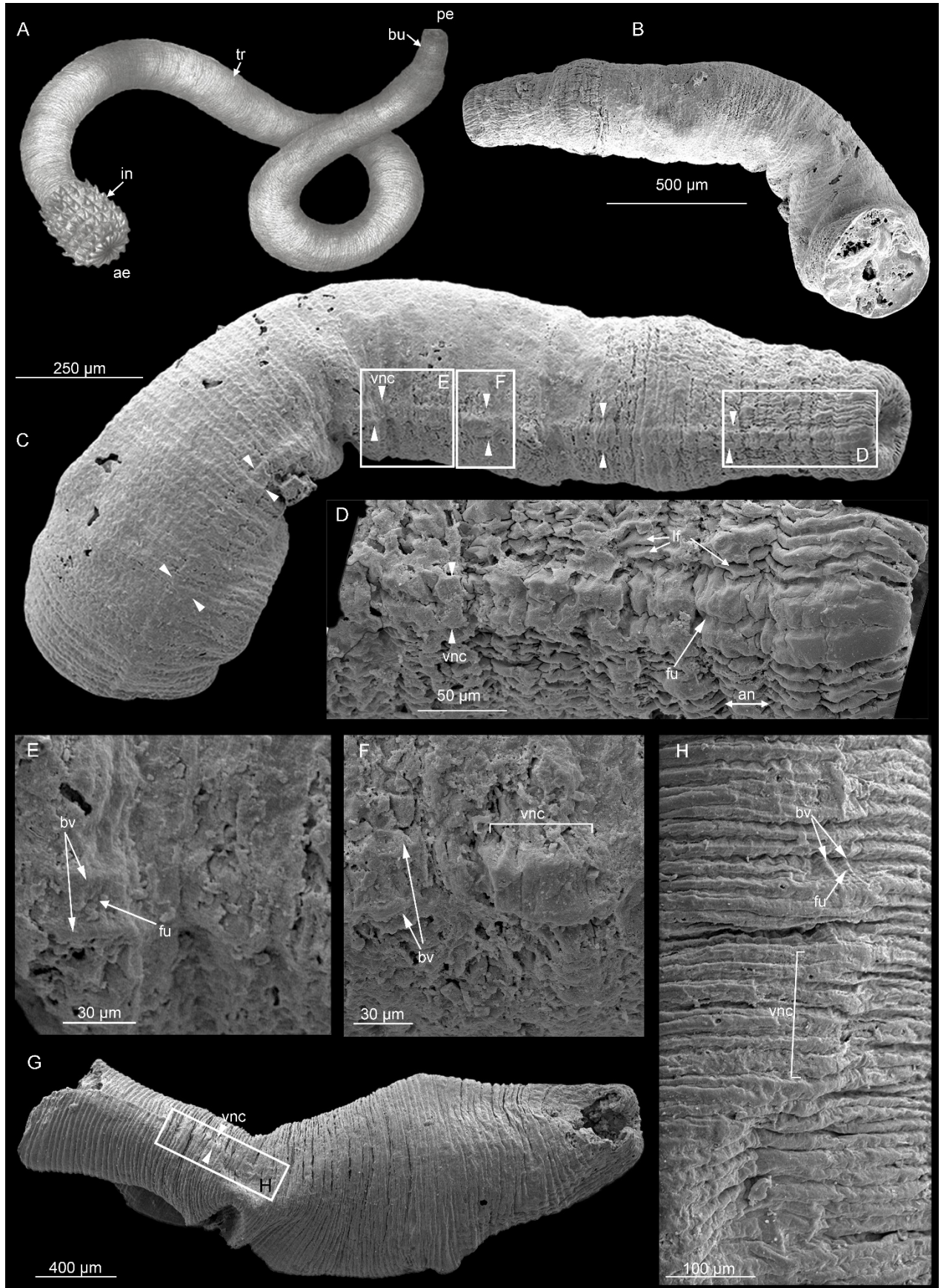
599     Conceptualization: DW  
600     Methodology: DW, JV  
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**Figures and Tables**

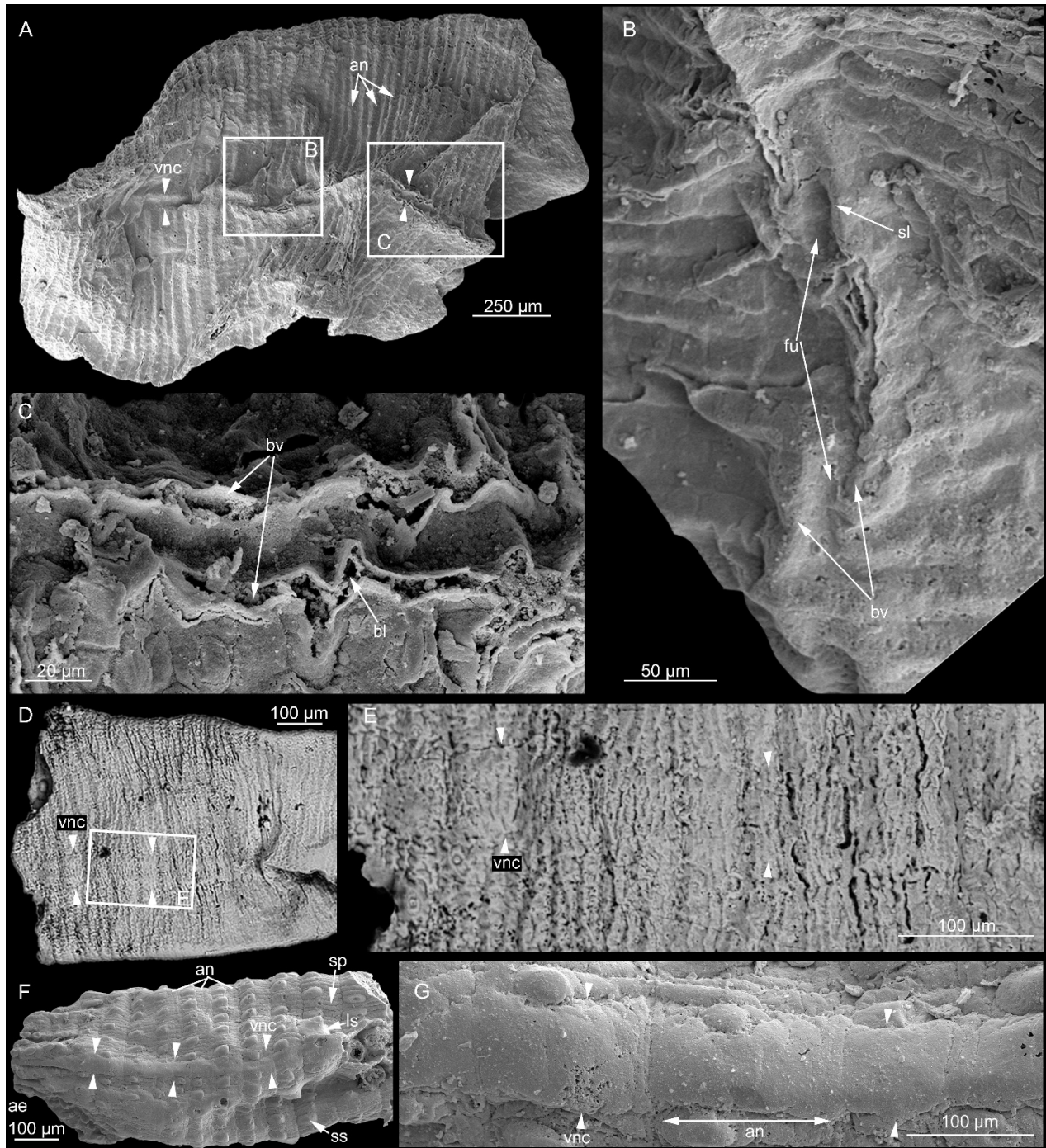


**Fig. 1. Tubular incomplete specimens of *Eopriapulites sphinx* from the early Cambrian Kuanchuanpu Formation, showing their ventral nerve cord (VNC). (A), Reconstruction of *Eopriapulites sphinx*, after (78). (B and C), ELIXX118-96,**

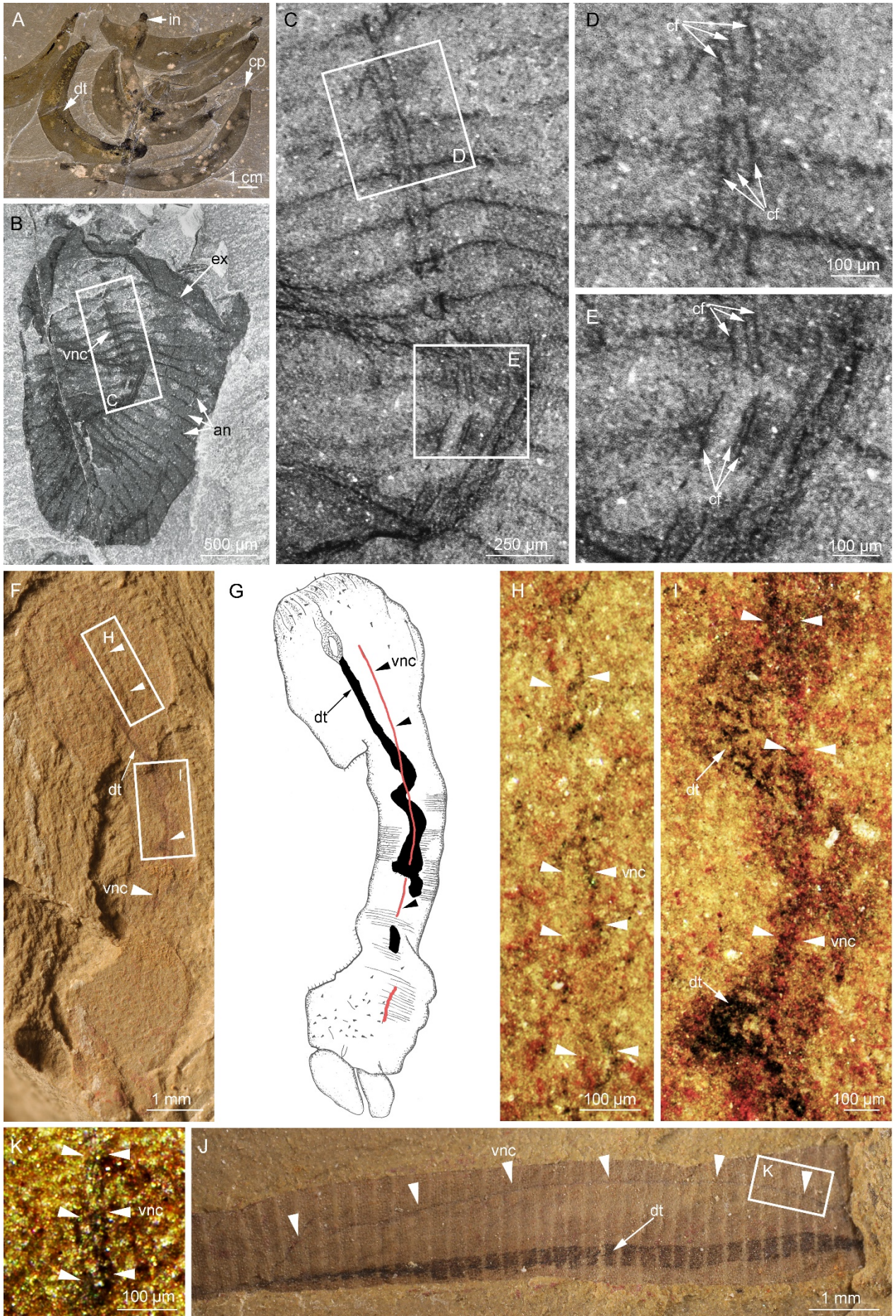
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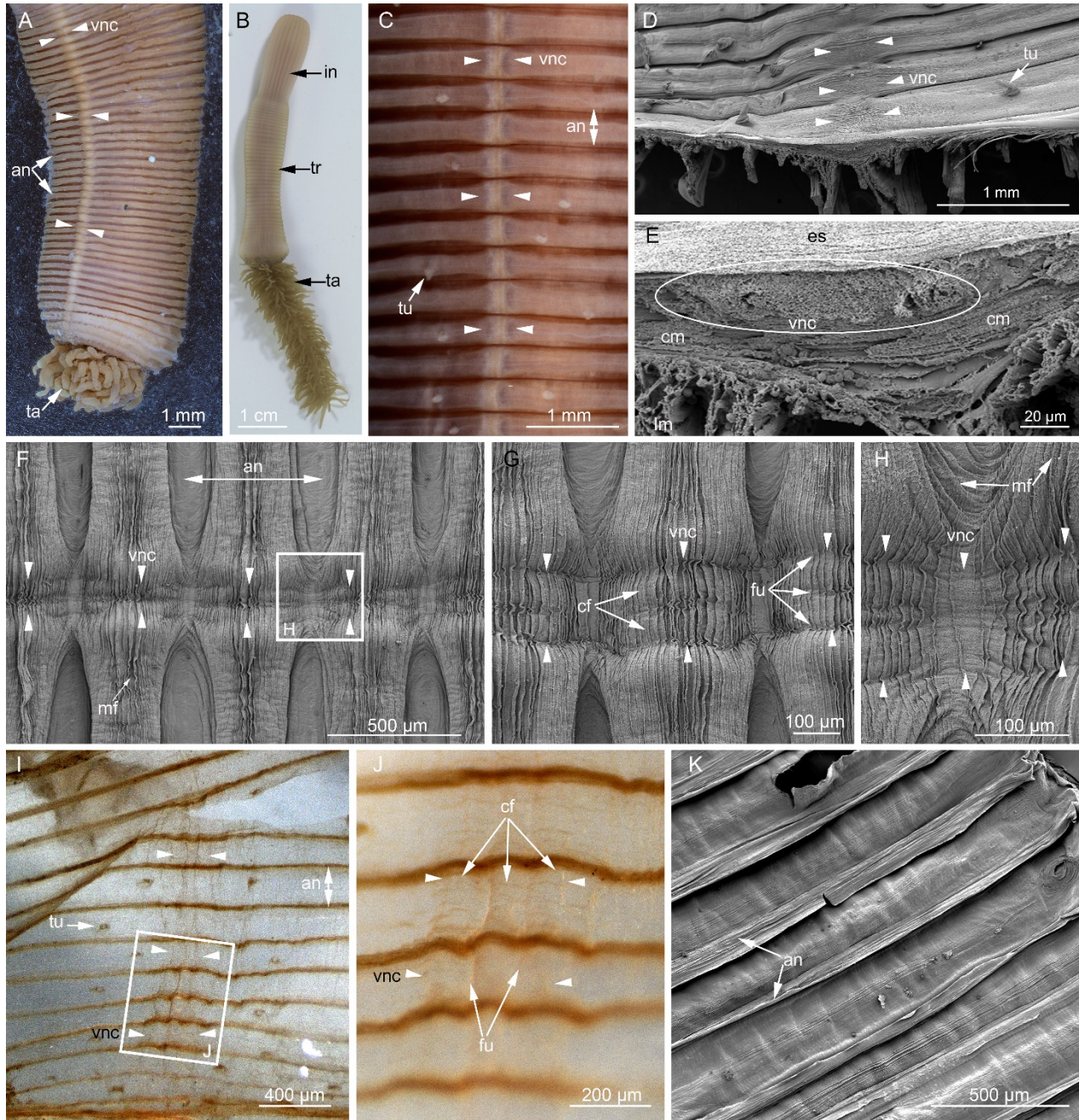
655 incomplete trunk showing both sides of the body (one with unpaired VNC). **(D)**,  
 656 close-up of **(C)** showing unpaired VNC near bursa (white arrowheads),  
 657 longitudinal folds, and bilobate aspect of VNC that vanishes posteriorly. **(E and**  
 658 **F)**, close-up of **(C)** showing bilobate aspect of VNC in the middle part of the trunk.  
 659 **(G and H)**, ELIXX139-256, incomplete trunk showing unpaired VNC on one side  
 660 in low relief and close-up of **(G)** showing faint bilobate feature locally.  
 661 Abbreviations: ae, anterior end; an, annulation; bu, bursa; bv, bilobate VNC; fu,  
 662 furrow; in, introvert; lf, longitudinal folds; pe, posterior end; tr, trunk; vnc, ventral  
 663 nerve cord.



669 **Fig. 2. Flattened incomplete specimens from the early Cambrian Kuanchuanpu**  
670 **Formation, showing VNC. (A–E), *Eopriapulites sphinx*. (A–C), ELIXX42-269**  
671 **showing truncated bilobate VNC with central furrow. (B and C), close-up of (A)**  
672 **showing details of smooth and broken lobes. (D and E), UMCU 14CHD0816-007**  
673 **showing low-relief VNC. (F and G), Tubular incomplete specimens of**  
674 ***Eokinorhynchus rarus*. (F), Fragment of trunk showing small and large sclerites,**  
675 **small plates (without spines), and unpaired VNC. (G), Details of unpaired VNC**  
676 **in positive relief showing transverse folds across annulations. Abbreviations: ae,**  
677 **anterior end; an, annulation; bl, broken lobe; bv, bilobate VNC; fu, furrow; ls,**  
678 **large sclerite; sl, smooth lobe; sp, small plate; ss, small sclerite; vnc, ventral nerve**  
679 **cord.**



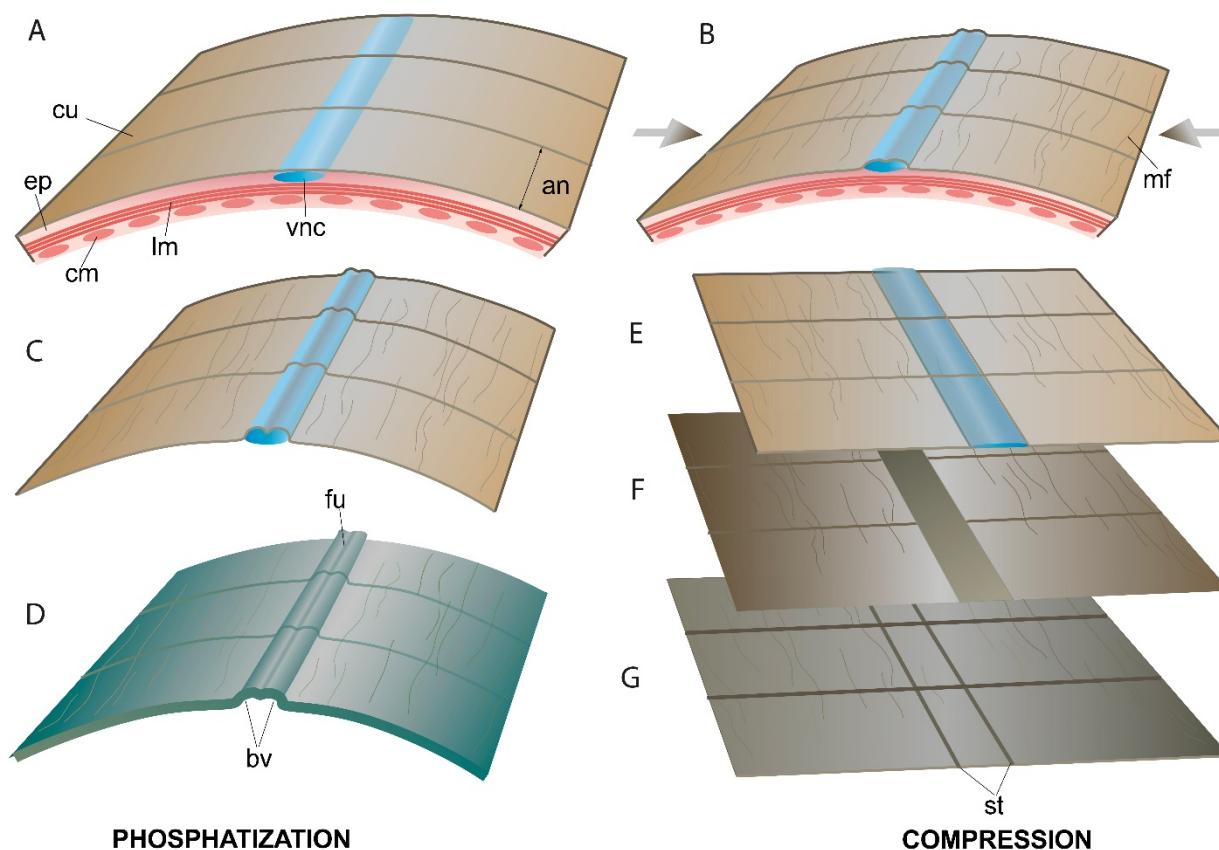
681 **Fig. 3. Burgess Shale-type early to mid-Cambrian scalidophorans with VNC. (A–E),**  
682 *Ottoia prolifica* from the mid-Cambrian (Miaolingian Series, Wuliuan Stage)  
683 Burgess Shale Lagerstätte. (A), ROM 61780a, group of complete worms on the  
684 surface of a rock slab. (B–E), USNM 188635, exuvia showing interconnected  
685 transverse and longitudinal ladder-like features; both representing cuticular folds  
686 and thickenings and cannot be interpreted as fossilized remains of soft nervous  
687 tissues (e.g. paired VNC and circular fibers; see ref. (22)). (D and E), close-up  
688 showing a faint additional linear feature between these two strips. (F–K)  
689 Scalidophorans from the early Cambrian Chengjiang Lagerstätte (Jianshan  
690 County, Yunnan, China). (F–I), *Xiaoheiqingella peculiaris* (ELIJS1260), general  
691 view (gut in black; VNC in red, see white arrowheads) and close-ups of VNC. (J  
692 and K), *Mafangscotlex yunnanensis* (ELIJS0055), general view showing gut  
693 (dark strip) and VNC (thin line with white arrowheads); note tiny longitudinal  
694 wrinkles on VNC. Abbreviations: an, annulation; cf, cuticular folds at the VNC  
695 location; cp, caudal part; dt, digestive gut; ex, exuvia; in, introvert; vnc, ventral  
696 nerve cord.



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**Fig. 4. Ventral nerve cord (VNC) in living priapulids.** (A), *Priapulopsis australis*, posterior part showing annulated body and longitudinal VNC. (B–K), *Priapululus caudatus*. (B), general morphology of *Priapululus caudatus* (live specimen from Sweden). (C–K), *Priapululus caudatus* from Russia (White Sea), light and SEM images of the same newly molted specimen (thin folded cuticle). (C), general view of VNC and body annulation (in 7% ethanol). (D and E), Transverse section through VNC, general view and details. (F–H), External view of the cuticle on both sides of the underlying VNC and details of micro-folded cuticle; note bilobed deformation over VNC. (I–K), Exuvia of *Priapululus caudatus* (Priapulida; extant, White Sea, Russia) showing cuticular annulations and longitudinal traces (white arrowheads) where the VNC was (compare with Figure 3B–E), in transmitted light (brown lines represent the areas where the cuticle is relatively thicker) and under the SEM (boundary between annulations). Abbreviations: an, annulation; cf, cuticular folds at the VNC location; cm, circular muscle; es, external surface; fu,

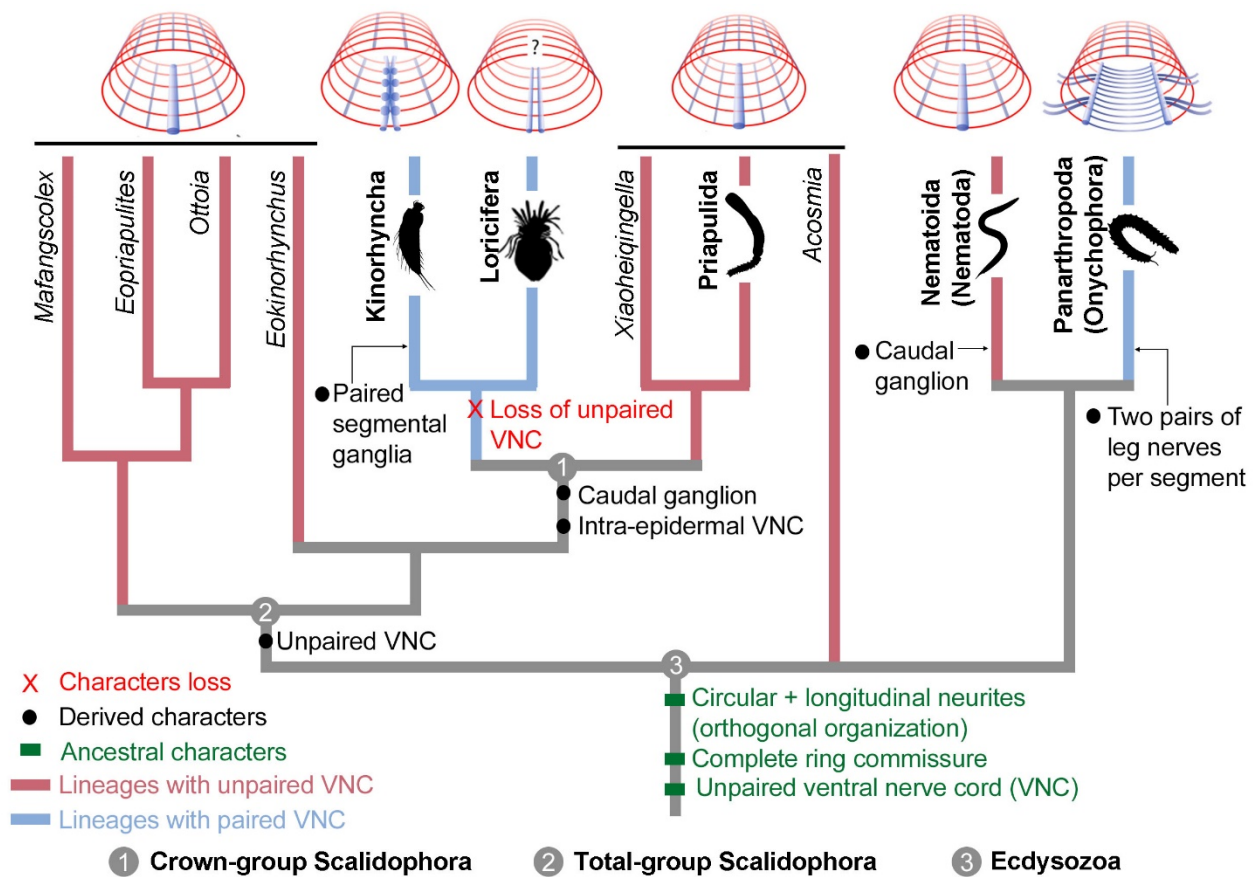
furrow; in, introvert; lm, longitudinal muscle; micro-fold; ta, tail; tr, trunk; tu,  
mf, tubulus; vnc, ventral nerve cord.



**Fig. 5. Taphonomic scenario to explain the preservation of the VNC in Cambrian scalidophorans, in the case of preservation via secondary phosphatization (left) or compression (right).** (A), body wall of living scalidophoran (exemplified by priapulids) showing cuticular, epidermal and muscular (circular, longitudinal) layers in transverse section. (B), post-mortem modification including possible shrinkage and cuticular micro-folding. (C), rapid decay of muscles tissues and epidermal cells (VNC might decay less rapidly, but eventually disappeared). (D), phosphatization of the cuticle that replicates the relief of the VNC (as seen in Kuanchuanpu specimens). (E), compression in sediment. (F), VNC preserved as a carbon film and/or pyrite (as seen in Chengjiang fossils). (G), exuvia (as seen in *Ottoia*); the only organic remains are cuticular micro-folds between annuli and along the VNC resulting in ladder-like features. Not to scale. Abbreviations: an,

annulus; bv, bilobate VNC; cm, circular muscles; cu, cuticle; ep, epidermal layer;  
fu, furrow; lm, longitudinal muscles; mf, cuticular microfolds, st, strip; vnc, ventral  
nerve cord.

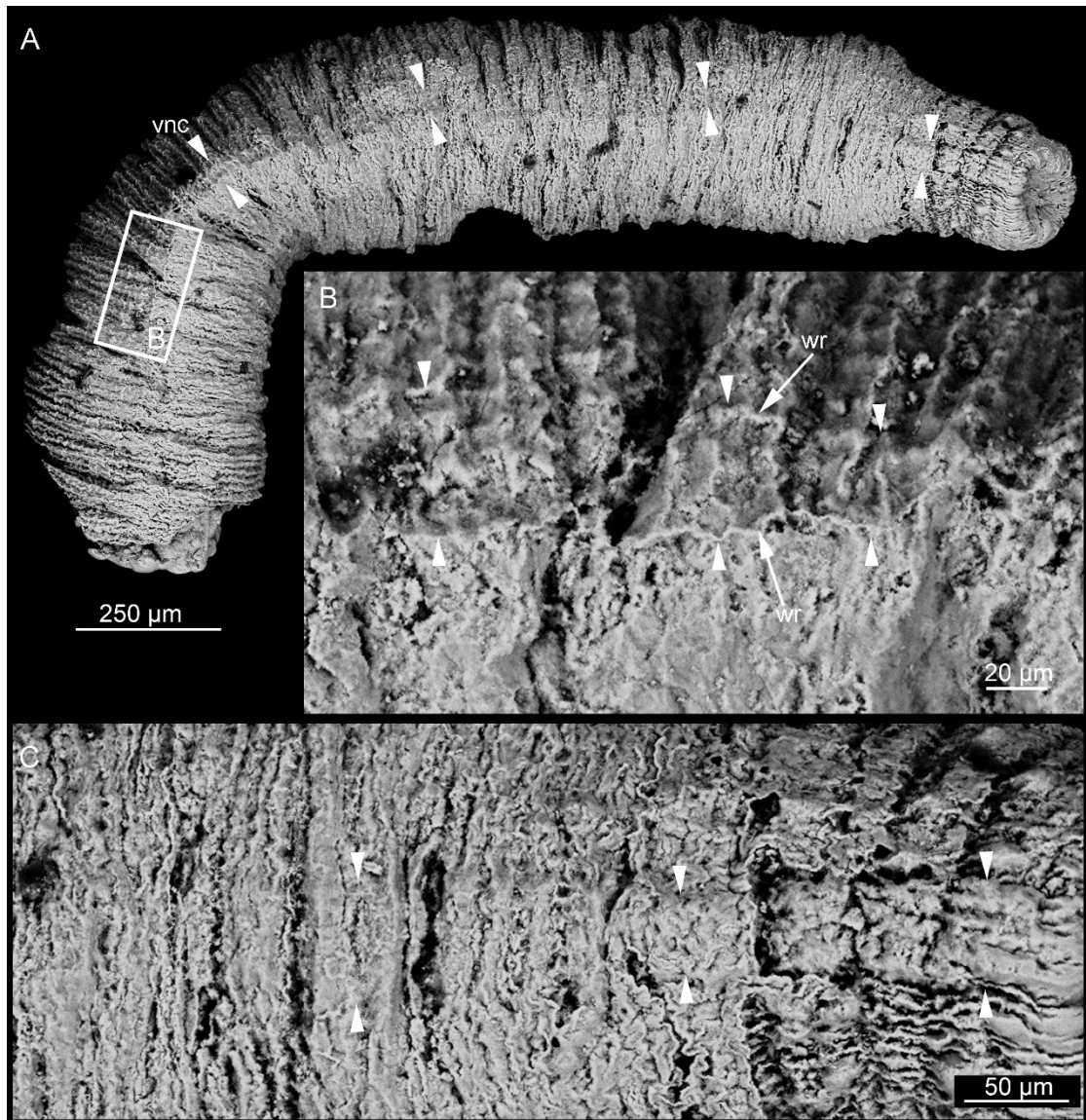
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**Fig. 6. Early evolution of the ventral nerve cord (VNC) in ecdysozoan worms (Nematoida and Scalidophora).** Tree showing the relation between ecdysozoan ingroups (including key species of Cambrian scalidophorans) and their neural organization (upper row of the diagram) with the ventral nerve cord (VNC) and associated features in blue and the peripheral system in red. Nervous system of Cambrian scalidophorans (only VNC known in fossils) is extrapolated from that of extant priapulids. The peripheral system of Cambrian scalidophorans is hypothetical. Key derived and ancestral characters of the nervous system are indicated along the branches of the tree. Reconstruction of VNC in Kinorhyncha, Nematoda, Priapulida, and Onychophora adapted from (13, 21, 32).







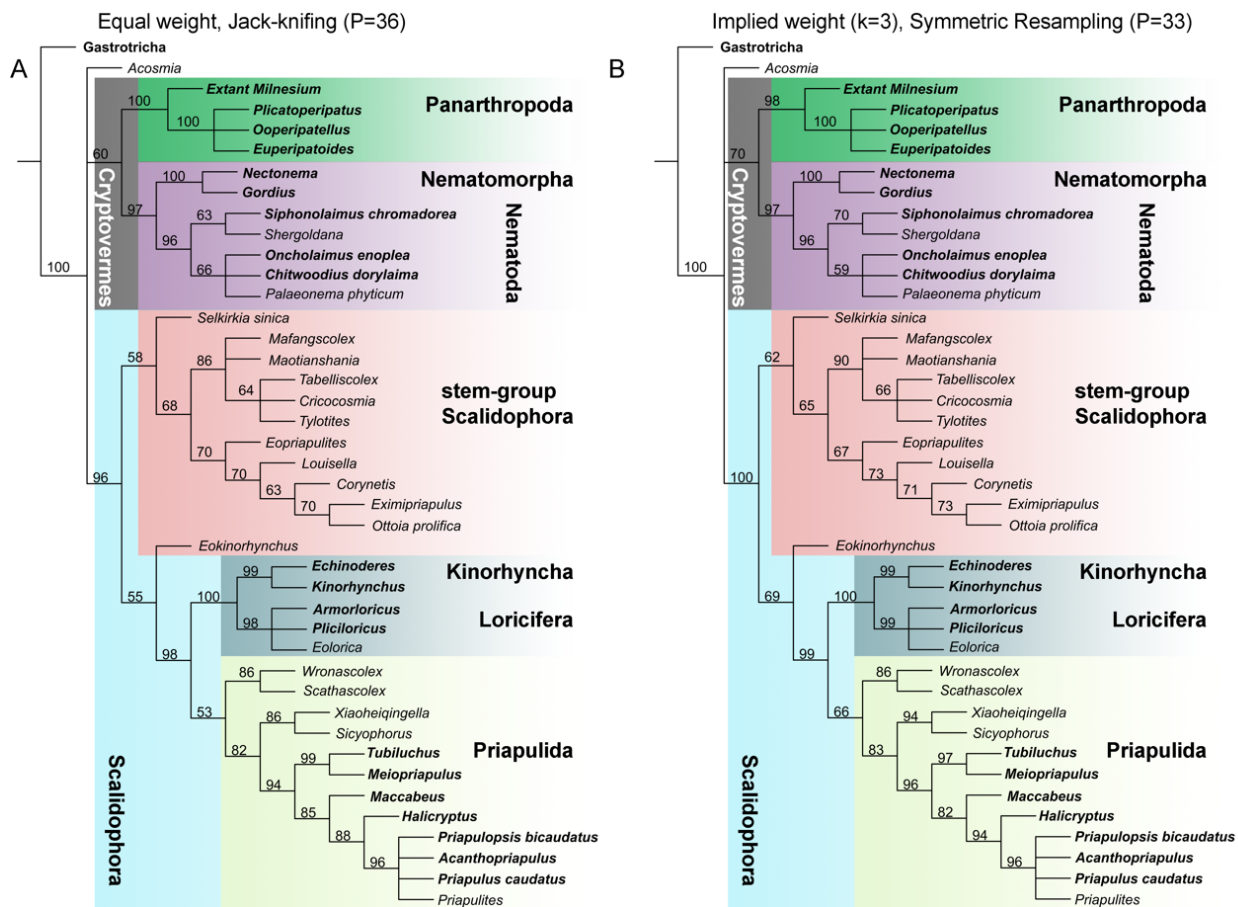
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815 **Fig. S1.**

816 *Eopriapulites* from the early Cambrian of the Kuanchuanpu Formation, China, showing the trace of  
817 its VNC. **A–C**, UMCU 14CHD0816-008. **A**, general view of the specimen preserved in 3D. **B**,  
818 close-ups of cuticular micro-wrinkles on VNC's imprint. **C**, VNC's relief best seen in posterior  
819 part near bursa. White arrows indicate VNC's boundaries. Abbreviation: wr, wrinkle.

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824 Full phylogenetic trees based on parsimony inference under TNT showing *Eokinorhynchus*,  
825 *Eopriapulites*, *Mafangsclex*, and *Ottoia* as stem members of Scalidophora, and *Xiaoheiqingella*  
826 as stem members of Priapulida. **A**, New technology search under equal weight, showing a strict  
827 consensus of six most parsimonious trees, with nodal supports above 50% shown from Jack-knife  
828 resampling under default settings. **B**, New technology search under implied weight (concavity  
829 constant  $k=3$ ), showing a strict consensus of two most parsimonious trees, with nodal supports  
830 above 50% shown from symmetric resampling under default settings. The position of *Acosmia*  
831 *maotiana*, which is a separate branch within Ecdysozoa, is not well resolved in both analyses.  
832 Nematoda and Panarthropoda is constrained based on recent molecular evidence (see Method  
833 and material in main text).

834 **Fig. S2.**

Taxa	Specimen	Preservation	Locality	W <sub>tr</sub> <sup>(a)</sup>	W <sub>vnc</sub>	W <sub>sl</sub>	Nl
<i>Eopriapulites sphinx</i>	ELIXX42-269	2D; phosphatized	Cambrian, Fortunian Kuanchuanpu Formation	800	36-39	9-13	2
	ELIXX118-96	3D; phosphatized		400	33-50	10-14	2/A
	ELIXX139-256	3D; phosphatized		970	42-48	9-13	2/A
	UMCU 14CHD0816-007	2D; phosphatized		620	62-67	A	N
	UMCU 14CHD0816-008	3D; phosphatized		430	43-48	4-5	2/A
<i>Eokinorhynchus rarus</i>	ELIXX140-23	3D; phosphatized		730	75-85	A	N
<i>Xiaoheiqingella peculiaris</i>	ELIJS1260	compression	Cambrian Stage 3, Chengjiang Lagerstätte	1355	40-49	A	N
<i>Mafangscoplex yunnanensis</i>	ELIJS0055	compression		1800	42-58	A	N
<i>Ottoia prolifica</i>	USNM188635	compression exuvia	Cambrian, Wuliuan Burgess Shale Lagerstätte	1700	46-58	10-15 <sup>(b)</sup>	3
<i>Priapulopsis australis</i>	Accession 162937	three-dimension	extant	5230	320-350	A	N
<i>Priapulid caudatus</i>	No collection number	three-dimension	extant	8650	125-178	13-17 <sup>(b)</sup>	3
<i>Priapulid caudatus</i>	No collection number	exuvia	extant	N	390	90-100	3-4

**Table S1.**

VNC measurements (in  $\mu\text{m}$ ) in stem and extant scalidophorans. W<sub>tr</sub>, width of the trunk; W<sub>vnc</sub>, width of VNC; W<sub>sl</sub>, width of lobe (if present). Nl, number of lobes. A, absence. N, inapplicable.<sup>(a)</sup>, in 3D-preserved and compressed specimens W<sub>tr</sub> corresponds to the trunk diameter and maximum trunk width, respectively.<sup>(b)</sup>, data from imprint of cuticle.

## Data S1. Notes on character coding

List of morphological characters used in phylogenetic analysis.

### Head, anterior trunk, proboscis etc.

#### 1. Cephalization:

(0) Proboscis

(1) Distinct head region clearly discriminated

Character 1 in Shi et al. (35)

#### 2. Proboscis invaginable (introvert):

(0) Absent

(1) Present

Modified from character 1 in Harvey et al. (70)

#### 3. Degree to which the introvert can be invaginated:

(0) Partially invaginable (i.e., part of Zone I)

(1) Completely invaginable into the trunk (i.e., to the base of Zone I)

Modified from character 2 in Harvey et al. (70)

#### 4. Introvert representing 30-50% of body length:

(0) Absent

(1) Present

Modified from character 71 in Harvey et al. (70)

### Sensory structures

#### 5. Eyes:

(0) Absent

(1) Present

866 Character 29 in Yang et al. (79)

867 **6. Lateral amphids:**

868 (0) Absent

869 (1) Present

870 Character 17 in Shi et al. (35)

871 **7. Trunk tumuli:**

872 (0) Absent

873 (1) Present

874 Character 25 in Harvey et al. (70)

875 **8. Trunk tubuli:**

876 (0) Absent

877 (1) Present

878 Character 26 in Harvey et al. (70)

879 **9. Flosculi, N-flosculi or sensory spots:**

880 (0) Absent

881 (1) Present

882 Character 27 in Harvey et al. (70)

883

884 **Oral, circumoral and pharyngeal morphology**

885 **10. Mouth opening orientation:**

886 (0) Terminal

887 (1) Ventral

888 Character 21 in Shi et al. (35)

889 **11. One or more pairs of appendages located anteriorly relative to the mouth opening:**

890 (0) Absent

891 (1) Present

892 Character 24 in Yang et al. (79)

893 **12. Mouth cone:**

894 (0) Absent

895 (1) Present

896 Character 42 in Harvey et al. (70)

897 **13. Mouth tube:**

898 (0) Absent

899 (1) Present

900 Character 24 in Shi et al. (35)

901 **14. Subdivided pharyngostome:**

902 (0) Absent

903 (1) Present

904 Character 25 in Shi et al. (35)

905 **15. Metastegosomal teeth:**

906 (0) Absent

907 (1) Present

908 Character 26 in Shi et al. (35)

909 **16. Odontostyle:**

910 (0) Absent

911 (1) Present

912 Character 27 in Shi et al. (35)

913 **17. Radially symmetrical circumoral structures:**

914 (0) Absent

915 (1) Present

916 Character 25 in Yang et al. (79), and character 28 in Shi et al. (35)

917

918 **18. General form of radial circumoral structures:**

919 (0) Scalids – hollow spines with apical pore and sensory cells surrounded by a thin layer of cuticle  
920 (scalidophorans)

921 (1) Hooks composed exclusively of cuticle (nematomorphs, some nematodes)

922 (2) Oral papillae or lamellae (some tardigrades)

923 (3) “Lips” (some nematodes, possibly *Acosmia* and *Shergoldana*)

924 (4) Cephalic setae (some nematodes)

925 (5) Oral elements (onychophorans)

926 Modified from character 26 in Yang et al. (79), and character 40 in Harvey et al. (70)

927 **19. Zone I armature comprises:**

928 (0) Fewer than 3 rings

929 (1) Many rings (i.e. more than 3)

930 (2) 3 rings

931 Character 30 in Shi et al. (35)

932 **20. Arrangement of Zone I armature into discrete parallel longitudinal rows:**

933 (0) Absent

934 (1) Present

935 Modified from character 4 in Harvey et al. (70)

936 **21. Unarmed proximal introvert:**

937 (0) Absent

938 (1) Present

939 “-“ inapplicable if character 19 is coded “0” or “2”.

940 Modified from character 32 in Shi et al. (35)

941 **22. Symmetry of circumoral structures:**

942 (0) Hexaradial

943 (1) Pentaradial

944 (2) Octaradial

945 (3) 28-fold

946 Modified from character 5 in Harvey et al. (70)

947 **23. Anterior ring of anteriorly directed jointed, broad appendages:**

948 (0) Absent

949 (1) Present

950 Character 34 in Shi et al. (35)

951 **24. Sexual dimorphism of clavoscalids:**

952 (0) Clavoscalids all the same

953 (1) Branched clavoscalids in males

954 Character 35 in Shi et al. (35)

955 **25. Rings of segmented, elongated spinose appendages:**

956 (0) Absent

957 (1) Present

- 958 Character 36 in Shi et al. (35)
- 959 **26. Double organ:**
- 960 (0) Absent
- 961 (1) Present
- 962 Character 37 in Shi et al. (35)
- 963 **27. Trichoscalids:**
- 964 (0) Absent
- 965 (1) Present
- 966 Character 38 in Shi et al. (35)
- 967 **28. Number of trichoscalids on proximal part of Zone I**
- 968 (0) 0
- 969 (1) 6
- 970 (2) 14
- 971 Character 18 in Wang et al. (72)
- 972 **29. The number of longitudinal rows of elements in Zone I formed by the fourth and following**
- 973 **circles:**
- 974 (0)  $\leq 20$
- 975 (1) 25
- 976 (2)  $>25$
- 977 Modified from character 7 in Harvey et al. (70)
- 978 **30. Zone II:**
- 979 (0) Unarmed
- 980 (1) Armed
- 981 Modified from character 10 in Harvey et al. (70)
- 982 **31. Number of elements in the first circle of Zone I:**
- 983 (0) Numerous ( $>8$ )
- 984 (1) 8
- 985 (2)  $<8$
- 986 Modified from character 11 in Harvey et al. (70)
- 987 **32. Coronal spines at boundary of Zones I and II:**
- 988 (0) Absent
- 989 (1) Present, undifferentiated
- 990 (2) Present, elongate spines
- 991 Modified from character 13 in Wills et al. (71)
- 992 **33. Zone III:**
- 993 (0) Unarmed
- 994 (1) Armed
- 995 Character 12 in Harvey et al. (70)
- 996 **34. Zone III comprises sclerotized pharyngeal teeth circlets:**
- 997 (0) Absent
- 998 (1) Present
- 999 Modified from character 13 in Smith and Caron et al. (80)
- 1000 **35. Number of circlets of Zone III armature:**
- 1001 (0) 1-4
- 1002 (1) 6-8
- 1003 (2) 16 or more

1004 Modified from character 13 in Harvey et al. (70)

1005 **36. General morphology of proximal circlets of Zone III armature (teeth):**

- 1006 (0) Absent
- 1007 (1) Spines or papillae
- 1008 (2) Multispinose
- 1009 (3) Multispinose but massively reduced
- 1010 (4) Hooks
- 1011 (5) Conical with a fringe of spines
- 1012 (6) Sclerotized trabeculae
- 1013 (7) Pectinate
- 1014 (8) Conical papillae terminating in a long spine (prickle)
- 1015 (9) Placoids

1016 Modified from character 14 in Harvey et al. (70)

1017 **37. Morphology of middle circlets of Zone III armature (teeth):**

- 1018 (0) Absent
- 1019 (1) Spines or papillae
- 1020 (2) Multispinose
- 1021 (3) Pectinate
- 1022 (4) Placoids

1023 Modified from character 15 in Harvey et al. (70)

1024 **38. Morphology of distal circlets of Zone III armature:**

- 1025 (0) Absent
- 1026 (1) Spines
- 1027 (2) Multispinose
- 1028 (3) Pectinate
- 1029 (4) Placoids

1030 Modified from character 16 in Harvey et al. (70)

1031 **39. Number of elements in the first circlet of pharyngeal armature (base on Zone III):**

- 1032 (0) >10
- 1033 (1) 10
- 1034 (2) 5
- 1035 (3) 8

1036 Modified from character 17 in Harvey et al. (70)

1037 **40. Pentaradial symmetry of Zone III armature:**

- 1038 (4) Absent
- 1039 (5) Present

1040 Modified from character 18 in Harvey et al. (70)

1041 **41. Number of proximal pentagonal circlets in Zone III:**

- 1042 (0) None
- 1043 (1) Five
- 1044 (2) Six
- 1045 (3) Seven
- 1046 (4) Three

1047 Modified from character 18 in Harvey et al. (70)

1048 **42. Teeth of second circle of the larvae with very small median denticle:**

- 1049 (0) Absent
- 1050 (1) Present

1051 Character 72 in Harvey et al. (70)

1052 **43. Distal portion of Zone III expanded into a bulb:**

- 1053 (0) Absent
- 1054 (1) Present



- 1055 Modified from character 20 in Harvey et al. (70)
- 1056 **44. Zone III eversibility:**
- 1057 (0) No degree of eversibility
- 1058 (1) Eversible (to any degree)
- 1059 Modified from character 21 in Harvey et al. (70)
- 1060 **45. Eversibility of Zone III:**
- 1061 (0) Completely eversible
- 1062 (1) Incompletely eversible, eversible beyond the proximal teeth
- 1063 (2) Eversible only as far as the proximal teeth
- 1064 Modified from character 21 in Harvey et al. (70)
- 1065 **46. Eversible pharynx:**
- 1066 (0) Absent
- 1067 (1) Present
- 1068 Character 70 in Harvey et al. (70)
- 1069 **47. All Zone III elements:**
- 1070 (0) Of approximately equal size
- 1071 (1) Decreasing regularly in size from the posterior to the anterior
- 1072 Modified from character 22 in Harvey et al. (70)
- 1073 **48. Radial oral stylets in mouth cone:**
- 1074 (0) Absent
- 1075 (1) Present
- 1076 Character 34 in Wang et al. (72)
- 1077 **49. Number of radial oral stylets:**
- 1078 (0) 4
- 1079 (1) 6
- 1080 (2) 8
- 1081 (3) 9
- 1082 Character 35 in Shi et al. (35)
- 1083 **50. Placids:**
- 1084 (0) Absent
- 1085 (1) Present
- 1086 Character 43 in Harvey et al. (70)
- 1087 **51. Number of placids**
- 1088 (0) 8
- 1089 (1) 16
- 1090 Character 44 in Yang et al. (13)
- 1091 **52. Triradial cuticular thickenings ornamenting pharynx:**
- 1092 (0) Absent
- 1093 (1) Present
- 1094 Character 64 in Shi et al. (35)
- 1095 **53. Pharyngeal lumina:**
- 1096 (0) Round
- 1097 (1) Triradiate
- 1098 Character 73 in Harvey et al. (70)
- 1099

- 1100 **Trunk region**
- 1101 **54. Locomotion by peristaltic contraction:**
- 1102 (0) Absent
- 1103 (1) Present
- 1104 Modified from character 68 in Harvey et al. (70)
- 1105 **55. Body musculature:**
- 1106 (0) Circular and longitudinal peripheral musculature
- 1107 (1) Longitudinal peripheral only
- 1108 (2) Metameric skeletal musculature
- 1109 Modified from characters 112-114 in Zhang et al. (45)
- 1110 **56. Circular musculature inside longitudinal musculature:**
- 1111 (0) Absent
- 1112 (1) Present
- 1113 Character 115 from Zhang et al. (45)
- 1114 **57. Annulations:**
- 1115 (0) Absent
- 1116 (1) Present
- 1117 Character 26 in Daley et al. (81)
- 1118 **58. Papillae on annulations:**
- 1119 (0) Absent
- 1120 (1) Present
- 1121 Modified from character 41 in Ma et al. (82)
- 1122 **59. Branching of annular rings:**
- 1123 (0) Unbranched
- 1124 (1) Branched
- 1125 Character 51 in Zhang et al. (45)
- 1126
- 1127 **Serially repeated trunk nodes/sclerites**
- 1128 **60. Trunk with serially repeated paired ventral/ventrolateral structures:**
- 1129 (0) Absent
- 1130 (1) Present
- 1131 Character 77 in Shi et al. (35)
- 1132 **61. Form of serially repeated paired ventral/ventrolateral trunk structures:**
- 1133 (0) Spines/protuberances
- 1134 (1) Moveable limbs producing an overall gait – i.e. paired appendages
- 1135 Character 78 in Shi et al. (35)
- 1136 **62. Serially repeated epidermal specializations:**
- 1137 (0) Absent
- 1138 (1) Present
- 1139 Modified from character 39 in Yang et al. (79)
- 1140 **63. Position of serially repeated epidermal specializations:**
- 1141 (0) Dorsal/lateral/dorsolateral longitudinal rows
- 1142 (1) Complete transverse rings
- 1143 Character 80 in Shi et al. (35)
- 1144 **64. Nature of serially repeated epidermal dorsolateral specializations:**
- 1145 (0) Epidermal depressions
- 1146 (1) Epidermal evaginations

1147 Modified from character 40 in Yang et al. (79), and character 81 in Shi et al. (35).

1148

1149 **Posterior trunk part**

1150 **65. Posterior ring papillae:**

1151 (0) Absent

1152 (1) Present

1153 Character 28 in Harvey et al. (70)

1154 **66. Eversible bursa:**

1155 (0) Absent

1156 (1) Present

1157 Character 29 in Harvey et al. (70)

1158 **67. Caudal appendage(s):**

1159 (0) Absent

1160 (1) Present

1161 Character 31 in Harvey et al. (70)

1162 **68. Division of caudal appendage(s):**

1163 (0) Unpaired

1164 (1) Paired

1165 Modified from character 32 in Harvey et al. (70)

1166 **69. Caudal appendage vesiculate:**

1167 (0) Absent

1168 (1) Present

1169 Character 33 in Harvey et al. (70)

1170 **70. Caudal appendage thin and tail-like:**

1171 (0) Absent

1172 (1) Present

1173 Character 145 in Shi et al. (35)

1174 **71. Terminally posterior spines, hooks, or cones of basal diameter >20% of trunk diameter:**

1175 (0) Absent

1176 (1) Present

1177 Character 93 in Harvey et al. (70)

1178 **72. Number of terminally posterior spines, hooks:**

1179 (0) One

1180 (1) One pair

1181 (2) Three

1182 (3) Two pairs

1183 Modified from character 94 in Harvey et al. (70)

1184 **73. Arc or ring of posterior spines or hooks:**

1185 (0) Absent

1186 (1) Present

1187 Character 95 in Harvey et al. (70)

1188

1189 **Cuticle:**

1190 **74. Cuticle surface with ornament of tessellating polygons:**

1191 (0) Absent

1192 (1) Present

1193 Character 91 in Harvey et al. (70)

1194

1195 **Soft tissue organisation**

1196 **75. Polythyridium:**

1197 (0) Absent

1198 (1) Present

1199 Character 34 in Harvey et al. (70)

1200 **76. Ventral nerve cord throughout its length:**

1201 (0) Paired

1202 (1) Unpaired

1203 Character 48 from Harvey et al. (70)

1204 **77. Ventral nerve cord with paired ganglia:**

1205 (0) Absent

1206 (1) Present

1207 Character 81 in Yang et al. (79)

1208 **78. Brain type:**

1209 (0) Condensed dorsal ganglional

1210 (1) Circumpharyngeal

1211 (2) Dorsal commissure

1212 Character 57 in Harvey et al. (70)

1213 **79. Apical part of the brain composed on perikarya:**

1214 (0) Absent

1215 (1) Present

1216 Character 58 in Harvey et al. (70)

1217 **80. Two rings of retractor muscles attached through the collar-shaped brain:**

1218 (0) Absent

1219 (1) Present

1220 Modified from character 60 in Harvey et al. (70)

1221 **81. Pharyngeal nervous system comprised of numerous tooth ganglia connected by a diagonal nerve net:**

1222 (0) Absent

1223 (1) Present

1224 Character 66 in Harvey et al. (70)

1226 **82. Number of neuromeres integrated into the dorsal condensed brain:**

1227 (0) 1

1228 (1) 2

1229 (2) 3

1230 Character 83 in Yang et al. (79)

1231 **83. Mouth innervation relative to brain neuromeres:**

1232 (0) Protocerebral innervation

1233 (1) Innervation from multiple neuromeres

1234 Character 84 in Yang et al. (79)

1235 **84. Nerve cord lateralised:**

1236 (0) Absent

1237 (1) Present

1238 Character 85 in Yang et al. (79)

1239 **85. Dorsal heart:**

1240 (0) Absent

- 1241 (1) Present  
1242 Character 86 from Yang et al. (79)
- 86. Protonephridia:**  
1243  
1244 (0) Absent  
1245 (1) Present  
1246 Character 52 in Harvey et al. (70)
- 87. Protonephridia flow into the gonoduct and/or are integrated into the gonad (= urogenital system):**  
1247  
1248 (0) Absent  
1249 (1) Present  
1250 Modified from character 53 in Harvey et al. (70)
- 88. Urogenital system attached to the body wall by a ligament:**  
1251  
1252 (0) Absent  
1253 (1) Present  
1254 Modified from character 54 in Harvey et al. (70)  
1255
- Development and reproduction:**
- 89. Developmental mode:**  
1256  
1257  
1258 (0) Direct  
1259 (1) Distinct larval stages  
1260 Modified from character 36 in Harvey et al. (70)
- 90. Loricata stage in life cycle:**  
1261  
1262 (0) Absent  
1263 (1) Present  
1264 Modified from character 37 in Harvey et al. (70)
- 91. Lorica present in larva:**  
1265  
1266 (0) Absent  
1267 (1) Present  
1268 Character 166 from Shi et al. (35)
- 92. Lorica present in adult:**  
1269  
1270 (0) Absent  
1271 (1) Present  
1272 Character 167 from Shi et al. (35)
- 93. Lorica of the larvae dorso-ventrally flattened (at least in older stages), with 6 lateral plates in-  
folded accordion-like:**  
1273  
1274  
1275 (0) Absent  
1276 (1) Present  
1277 Character 63 in Harvey et al. (70)
- 94. Larvae with six long pharynx retractor muscles:**  
1278  
1279 (0) Absent  
1280 (1) Present  
1281 Character 67 in Harvey et al. (70)
- 95. Division of the body into a distinct proboscis and abdomen in the juvenile/larva:**  
1282  
1283 (0) Absent  
1284 (1) Present  
1285 Modified from character 44 in Harvey et al. (70)
- 96. Cloaca in both sexes:**  
1286  
1287 (0) Absent  
1288 (1) Present

- 1289 Character 51 from Harvey et al. (70)
- 1290 **97. Spermatozoa without a flagellum:**
- 1291 (0) Absent
- 1292 (1) Present
- 1293 Character 55 in Harvey et al. (70)
- 1294 **98. Sclerotized tube:**
- 1295 (0) Absent
- 1296 (1) Present
- 1297 Character 70 in Wang et al. (72)
- 1298 **99. Zone I has three subdivisions:**
- 1299 (0) Absent
- 1300 (1) Present
- 1301 Character 11 in Wang et al. (72)
- 1302 **100. Intraepidermal VNC: [NEW]**
- 1303 (0) Absent
- 1304 (1) Present
- 1305 **101. Nerve cord with orthogonal organization:**
- 1306 (0) Absent
- 1307 (1) Present
- 1308 Character 89 in Yang et al. (13)
- 1309 **102. Orthogonal nerve cord with complete ring-commissures:**
- 1310 (0) Absent
- 1311 (1) Present
- 1312 Character 90 in Yang et al. (13)
- 1313 **103. Regularly spaced peripheral nerves running entire length of nerve cord:**
- 1314 (0) Absent
- 1315 (1) Present
- 1316 Character 93 in Yang et al. (13)
- 1317 **104. VNC bifurcates anteriorly or posteriorly: [NEW]**
- 1318 (0) Absent
- 1319 (1) Present
- 1320 Note: The VNC of Kinorhyncha bifurcates anteriorly and posteriorly (38) and that of nematomorph
- 1321 Gordiida (only males) bifurcates anteriorly (56).
- 1322 **105. Caudal ganglion: [NEW]**
- 1323 (0) Absent
- 1324 (1) Present
- 1325 Note: It is only absent in male Gordiida (Nematomorpha) (56).
- 1326 **106. Through-gut**
- 1327 (0) Absent
- 1328 (1) Present
- 1329 Character 67 from Ou et al. (83)
- 1330 **107. Circum-pharyngeal, collar-shaped brain with anterior and posterior rings of perikarya separated**
- 1331 **by a ring-shaped neuropil.**
- 1332 (0) Absent
- 1333 (1) Present
- 1334 **108. Cephalic sensillae:**
- 1335 (0) Absent
- 1336 (1) Present

- 1337 Character 147 from Howard et al. (28)  
1338 *Note: The anterior mechano/chemosensory organs in nematodes, form rings (6 + 6 + 4 pattern) around*  
1339 *the anterior region (57)*
- 1340 **109. Ciliated epidermis.**  
1341 (0) Absent  
1342 (1) Present
- 1343 Character 15 from Vinther and Parry (84)
- 1344 **110. Cuticle predominantly containing collagen:**  
1345 (0) Absent  
1346 (1) Present
- 1347 Character 79 from Harvey et al. (70)
- 1348 **111. Cuticle containing chitin at least in life:**  
1349 (0) Absent  
1350 (1) Present
- 1351 Character 80 from Harvey et al. (70)
- 1352 **112. Distribution of chitin in the cuticle:**  
1353 (0) predominantly within the middle cuticle layer (exocuticle)  
1354 (1) predominantly within the lowermost cuticle layer (endocuticle).
- 1355 Character 81 from Harvey et al. (70)
- 1356 **113. Trilaminar epicuticle in one repeat unit:**  
1357 (0) Absent  
1358 (1) Present
- 1359 Characters 77, 78 from Harvey et al. (70)
- 1360 **114. Cuticle with  $\alpha$ -chitin:**  
1361 (0) Absent  
1362 (1) Present
- 1363 Character 72 from Ou et al. (83)
- 1364 **115. Cuticle moult:**  
1365 (0) Absent  
1366 (1) Present  
1367 **Character 73 from Wills et al. (71)**
- 1368 **116. Body divided with distinct inflated head and neck region:**  
1369 (0) Absent  
1370 (1) Present
- 1371 Character 42 from Vinther and Parry (84)
- 1372 **117. Bipartite gut with cuticular pharynx:**  
1373 (0) Absent  
1374 (1) Present
- 1375 Character 43 from Vinther and Parry (84)
- 1376 **118. Paired lobopods:**  
1377 (0) Absent  
1378 (1) Present
- 1379 Character 75 from Ou et al. (83)
- 1380 **119. Terminal claws on lobopods:**  
1381 (0) Absent  
1382 (1) Present

1383 Character 64 in Yang et al. (79)

1384 **120. Segmented body:**

1385 (0) Absent

1386 (1) Present

1387

1388

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