# Are palaeoscolecids ancestral ecdysozoans?

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**SUMMARY** The reconstruction of ancestors is a central aim of comparative anatomy and evolutionary developmental biology, not least in attempts to understand the relationship between developmental and organismal evolution. Inferences based on living taxa can and should be tested against the fossil record, which provides an independent and direct view onto historical character combinations. Here, we consider the nature of the last common ancestor of living ecdysozoans through a detailed analysis of palaeoscolecids, an early and extinct group of introvert-bearing worms that have been proposed to be ancestral ecdysozoans. In a review of palaeoscolecid anatomy, including newly resolved details of the internal and external cuticle structure, we identify specific characters shared with various living nematoid and

## INTRODUCTION

The discovery of a common toolkit of regulatory genes that directs embryological development across all animals regardless of their morphological disparity has provided a new perspective on comparative anatomy. Vicarious gene expression patterns and gene networks have been used to test homologies grounded in comparative anatomy, and to propose new homologies where comparative anatomy has failed. Collectively, these homologies have been used to infer the nature of deep ancestors, such as the last common ancestor of Bilateria, to gain insights into the relationship between developmental and organismal evolution (Kimmel 1996; De Robertis 1997; Panganiban et al. 1997; Holland and Holland 1999; Tabin et al. 1999; Dewel 2000; Jacobs et al. 2000; Carroll et al. 2001; Cornec and Gilles 2006; Arendt et al. 2008; De Robertis 2008; Hejnol and Martindale 2008). The only conceivable test of these reconstructions is paleontological data, not because it is possible to recognize ancestors in the fossil record-it is not (Nelson 1989), but because some fossil taxa are both taxonomically and temporally proximal to such ancestors, providing better constraint over attempts to infer their nature.

The rich fossil record of early ecdysozoans provides the opportunity to constrain the anatomies of deep ancestors

scalidophoran worms, but not with panarthropods. Considered within a formal cladistic context, these characters provide most overall support for a stem-priapulid affinity, meaning that palaeoscolecids are far-removed from the ecdysozoan ancestor. We conclude that previous interpretations in which palaeoscolecids occupy a deeper position in the ecdysozoan tree lack particular morphological support and rely instead on a paucity of preserved characters. This bears out a more general point that fossil taxa may appear plesiomorphic merely because they preserve only plesiomorphies, rather than the mélange of primitive and derived characters anticipated of organisms properly allocated to a position deep within animal phylogeny.

within a major bilaterian branch (e.g., Budd 2003), and palaeoscolecids have been an emerging focus in this respect. These fossil organisms comprise a group or grade of slender, multiannulated worms that are characterized by a distinctive cuticle ornamentation and are known from the early Cambrian to the late Silurian (c. 520-420 Ma). Tentatively attributed to the annelids in their original description (Whittard 1953), they have more recently been compared with members of Cycloneuralia (sensu Ahlrichs 1995; = Introverta sensu Nielsen 1995; i.e., nematodes, nematomorphs, kinorhynchs, loriciferans, and priapulids). An assignment to this group based on the annulated trunk region (Dzik and Krumbiegel 1989; Conway Morris 1993) has been reinforced by the subsequent discovery of specimens preserving an armoured terminal proboscis (Hou and Bergström 1994; Fig. 1A). However, the precise affinity of palaeoscolecids remains contentious. In addition to being allied with particular ecdysozoan phyla, whether priapulids (e.g., Conway Morris 1997a, b) or nematomorphs (e.g., Hou and Bergström 1994), palaeoscolecids have been proposed to be proximal to the last common ancestor of Ecdysozoa (Cycloneuralia plus Panarthropoda; Budd and Jensen 2000, 2003; Budd 2001c, 2008; Conway Morris and Peel in press) and have also been interpreted more specifically as the priapulid-like ancestors of

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**Fig. 1.** Palaeoscolecid gross anatomy and hypotheses on their place within ecdysozoan evolution. (A) *Maotianshania cylindrica* Sun and Hou (1987), from Maotianshan, Chengjiang, Yunnan (RCCBYU 10225). This specimen shows the characteristic annulation of palaeoscolecids sensu lato but also preserves the armoured proboscis indicating cycloneuralian affinity. This specimen is 22 mm long. (B–E) Hypothetical phylogenetic scenarios in which palaeoscolecids occupy "deep-branching" positions in the ecdysozoan tree. X and Y represent major within-crown lineages of ecdysozoans, equal to Cycloneuralia and Panarthropoda under the hypothesis favoured herein, with the closed triangles indicating their respective crown groups. (B) Palaeoscolecids (p) provide a direct model for the crown ancestor of Ecdysozoa because character polarity is unambiguous. (C) Palaeoscolecids qualify as ancestral to a subset of ecdysozoans including the crown, although the crown ancestor itself need not have shared their morphology. (D) If palaeoscolecids fall within the stems to both of the principal crown-ecdysozoan lineages, parsimony suggests that the crown ancestor shared their morphology. If they belong to the stem of one lineage only, as is the case in (E), then palaeoscolecids are candidate models for ancestral ecdysozoans if no other taxa are more proximal to the crown node, although character polarity remains to be established. Summarized from the hypotheses of Dzik and Krumbiegel (1989), Hou and Bergström (1994), Conway Morris (1997b), and Budd and colleagues (Budd and Jensen 2000, 2003; Budd 2001c, 2008). (F) Ecological and functional scenario for the evolutionary emergence of panarthropods from palaeoscolecid and priapulid-like ancestors, after Dzik and Krumbiegel (1989). Whether explicit or implicit, this is the scenario that underpins hypotheses for the emergence of ecdysozoan phyla from palaeoscolecid ancestors (e.g., Budd and Jensen 2000, 2003; Budd 2001c, 2008).

onychophorans and tardigrades (Dzik and Krumbiegel 1989). Clearly then, palaeoscolecids have the potential to provide constraint over the nature of ancestors deep within Ecdysozoa (Budd 2003).

#### The ancestral ecdysozoan

The last common ancestor of living ecdysozoan groups ("concestor," Dawkins 2004; "crown ancestor," Valentine

2006; "cenancestor," Patthy 2008) presumably possessed the synapomorphies which support the clade (Telford et al. 2008). These are generally considered to include molting of the cuticle controlled by ecdysteroid hormones, loss of epidermal cilia except in receptor cells, trilayered cuticle including a trilaminate epicuticle and  $\alpha$ -chitin in the inner layer, and immunoreactivity in the nervous system to the antihorseradish peroxidase (HRP) antibody (Schmidt-Rhaesa et al. 1998; Haase et al. 2001; Schmidt-Rhaesa

2007). Features that were probably present but may have been inherited from deeper metazoan ancestors include a triradiate pharyngeal lumen and a terminal mouth (Schmidt-Rhaesa et al. 1998; Schmidt-Rhaesa 2007), while a primary larval stage is considered to have been absent, perhaps representing a secondary loss (Peterson et al. 2000). Under an unorthodox scenario which positions Annelida as the sister group to Ecdysozoa, a haemocoel and the partial dissolution of mesodermal sacs are added to the inventory of ancestral ecdysozoan characters (Nielsen 2001). Genomically, the ecdysozoan ancestor is reconstructed as possessing a complement of at least eight Hox genes (de Rosa et al. 1999), while increased sampling is required to determine whether the widespread gene loss characteristic of the model organisms Drosophila melanogaster and Caenorhabditis elegans reflects a shared ecdysozoan history (Telford et al. 2008). Further resolution of the ancestral ecdysozoan requires additional assumptions: the presence of a cycloneuralian brain, for example see Eriksson et al. (2003), along with gross characters of body size and external morphology, are dependent on character polarity among the in-group taxa, and are informed by paleontological observations.

# Why have palaeoscolecids been considered ancestral ecdysozoans?

Palaeoscolecids can only be considered as models (or candidate models) for the ecdysozoan crown ancestor if they occupy a deep branching position within the ecdysozoan tree (see Fig. 1B-E). Support for such a scenario rests on two streams of evidence, although one relies upon the other. Firstly and primarily, palaeoscolecids appear to fulfill expectations based on a consideration of extant ecdysozoans that ancestral ecdysozoans were macroscopic, introvert-bearing, and thus broadly priapulid-like in nature (Budd and Jensen 2000; Budd 2001b, c, 2003; Zrzavý 2003; Webster et al. 2006). Essentially, this expectation arises because the alternatives are seemingly unpalatable: the ancestral ecdysozoan body plan is believed to be heavily overprinted by adaptation to a meiofaunal ecology in kinorhynchs and loriciferans (Dewel 2000; Budd 2001b; Webster et al. 2006; Sørensen et al. 2008), to parasitism in nematodes and nematomorphs (Budd 2003; Webster et al. 2006), and to locomotion using paired appendages in panarthropods (Budd 2001b). Secondly, it has been argued that the similarities between palaeoscolecids and priapulids represent symplesiomorphies of Cycloneuralia (and, by extension, of Ecdysozoa), and furthermore that palaeoscolecids exhibit no synapomorphies that either unite them as a clade, or ally them with any group more exclusive than Cycloneuralia or Ecdysozoa (Budd and Jensen 2000, 2003; Budd 2003). Further support has been

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found for the "deep-branching" hypothesis in the overall similarity between the annulated and papillose body form of palaeoscolecids and extinct onychophoran-like lobopods such as *Aysheaia*, *Kerygmachela*, and *Xenusion*, and in the shared possession of a terminal mouth (Whittard 1953; Dzik and Krumbiegel 1989; Ramsköld 1992; Budd 1999, 2001a, 2008; Ivantsov and Zhuravlev 2005). From this emerged the influential hypothesis that priapulids, palaeoscolecids, lobopods, and onychophorans represent components of an evolutionary transition series of locomotor strategies, from infaunal priapulids and palaeoscolecids locomoting entirely by peristaltic motion, to epifaunal walking lobopods, onychophorans and, ultimately, arthropods (Dzik and Krumbiegel 1989; Fig. 1F).

However, it has also been argued that large body size and possession of an introvert are characters that have been converged upon by scalidophorans, nematodes, nematomorphs and panarthropods, and were therefore absent from the crown ancestor of Ecdysozoa (Schmidt-Rhaesa 1998; Schmidt-Rhaesa et al. 1998). By implication, palaeoscolecids are thus inappropriate candidates for stem- or basal crown ecdysozoans. Furthermore, even if homology of the introvert is accepted, detailed character analyses have suggested a stem-priapulid affinity for palaeoscolecids, which are thus remote from the crown ancestor of Ecdysozoa (Hou and Bergström 1994; Wills 1998; Dong et al. 2004, 2005; Donoghue et al. 2006). It seems clear that a more secure analysis of palaeoscolecid affinities is required before their significance for ecdysozoan evolution can be assessed.

No single palaeoscolecid specimen or taxon exhibits a full complement of characters, and much of palaeoscolecid anatomy is known only from fragmentary microscopic cuticular material that has been described in a correspondingly fragmentary manner in the paleontological literature. The most recent synthesis of these data was provided by Conway Morris (1997b). Therefore, we begin by providing a more comprehensive review in light of our own observations of fossil material in addition to a novel investigation of cuticle microstructure. We then present the results of an extensive cladistic treatment of these data, and explore their implications for early ecdysozoan evolution.

## MATERIAL AND METHODS

In reviewing palaeoscolecid characters, unpublished collections from three localities were studied. Microscopic, three-dimensionally phosphatized specimens of various taxa from the Middle Cambrian of Australia (Müller and Hinz-Schallreuter 1993) and similarly preserved specimens of an unnamed taxon from the Upper Cambrian Bitiao Formation of Wangcun, Hunan Province, China, were examined using scanning electron microscopy (SEM). Macroscopic compression fossils from the Lower Ordovician of Czech Republic (Kraft and Mergl 1989) were examined using reflected light microscopy, and silicone rubber peels of their surfaces were examined using SEM.

To determine the internal cuticle structure of palaeoscolecids, sections were made of isolated cuticular plates belonging to two taxa: Hadimopanella (= Lenargyrion) knappologica Bengtson (1977) (topotype) from the Lower Cambrian of Siberia, and Milaculum ethinclarki Müller 1973, from the uppermost Kimmswick Limestone (latest undatus Zone to late tenuis Zone) from Roasting Ear Creek (Upper Ordovician) of Arkansas. Sections were also made of articulated cuticle belonging to the unnamed taxon from the Upper Cambrian Bitiao Formation of China. Specimens of Milaculum and the unnamed taxon were obtained by acetic acid digestion of limestone samples. For sectioning, specimens were mounted in polyester and epoxy resins, cut with a low-speed lapidary saw, and polished using alumina powder or diamond paste. For SEM, the sections were coated with carbon and imaged using backscattered electrons (BSEM). Figured specimens reposited at the Geology department, Paleontological Collections, Peking University (GMPKU), Palaeontology Museum, Bonn University, Geological Survey, Prague, and the Swedish Museum of Natural History, Stockholm.

## PALAEOSCOLECID CHARACTERS

## Included taxa

Palaeoscolecids have traditionally been recognized on the basis of a distinctive cuticular ornamentation (Whittard 1953; Conway Morris and Robison 1986; Conway Morris 1997b). In our analysis, we draw a distinction between a core group of fossil taxa that unambiguously preserve this character, which we define as an ornamentation of polymorphic tessellating plates (Fig. 2, A and B), and a wider selection of slender, annulated worms for which this character is unproven. We thus focus our discussion on "palaeoscolecids sensu stricto," in which we include the macrofossil genera Palaeoscolex (especially P. piscatorum, early Ordovician, UK; see Conway Morris 1997b), Sahascolex (early Cambrian, Siberia; Ivantsov and Wrona 2004), Protoscolex (partim) (see Conway Morris et al. 1982), Gamascolex and Plasmuscolex (early Ordovician, Czech Republic; Kraft and Mergl 1989), along with the many taxa described from microscopic, three-dimensionally preserved individuals (Cambrian of Australia and China; Müller and Hinz-Schallreuter 1993; Zhang and Pratt 1996; Topper et al. 2010). Of the macrofossil taxa described from the early Cambrian of China, in which preservational resolution of the fine structure is more limited, we include only Palaeoscolex (= Mafangscolex) sinensis (see Hou et al. 2004; Hu 2005) and, more tentatively, Guanduscolex minor (Hu et al. 2008). Similarly, we tentatively include among "strict" palaeoscolecids two macrofossil taxa from the early Cambrian of Greenland

(Conway Morris and Peel in press), although the finest-scale details of ornamentation are not preserved. We also draw on information contained within more fragmentary cuticular remains (e.g., Zhang and Pratt 1996), and the "form genera" of isolated cuticular plates that can unambiguously be assigned to confirmed palaeoscolecids through comparison with more intact material, namely *Hadimopanella*, *Milaculum*, and *Kaimenella* (Cambrian to Silurian, of wide geographic distribution; Hinz et al. 1990; Brock and Cooper 1993; Conway Morris 1997b; Topper et al. 2010).

Among the taxa in which the defining cuticle structure has not to our knowledge been confirmed, but which are often referred to as palaeoscolecids, and share with them a high length:width ratio and a conspicuously uniform body width, are *Louisella* from the middle Cambrian Burgess Shale (Conway Morris 1977) and a number of taxa from the early Cambrian of China, including *Maotianshania, Cricocosmia, Tabelliscolex*, and *Tylotites* (Hou and Bergström 1994; Hu 2005; Han et al. 2007a, b; Maas et al. 2007a). Other early fossil worms have demonstrably divergent body forms (Conway Morris 1977; Maas et al. 2007a) and so are not considered further.

## Overall dimensions and gross morphology

Macroscopic individuals known from compressed body fossils in shales (Fig. 1A) demonstrate that an adult body size of up to several tens of centimeters was attained in at least some palaeoscolecids (e.g., Kraft and Mergl 1989), while millimetric individuals preserved via early postmortem phosphatization-which conceivably represent smallbodied taxa, juveniles, or both-reveal characters on a much finer scale (e.g., Müller and Hinz-Schallreuter 1993; Fig. 2, A,B and E–G). Characters that are common to individuals across this range include a terminal mouth and anus (Conway Morris and Robison 1986; Hou and Bergström 1994); a high length:width ratio (Müller and Hinz-Schallreuter 1993; Maas et al. 2007a); a uniform body width and circular transverse body section (Müller and Hinz-Schallreuter 1993; Hou and Bergström 1994); and a cuticle divided into many (up to several hundred) annuli (e.g., Whittard 1953). Annuli are usually complete, though bifurcations or half-rings are documented (Kraft and Mergl 1989; Müller and Hinz-Schallreuter 1993). The annular divisions do not extend internally (Müller and Hinz-Schallreuter 1993) and there is no indication of body cavity compartmentalization. Annulation is homonomous (sensu Ramsköld 1992), with each trunk annulus essentially similar to its neighbors (Dong et al. 2005), and there is no differentiation into body regions except at the anterior and posterior ends (e.g., Conway Morris and Peel in press). Symmetry varies according to body region: the anterior is radially symmetrical (Fig. 1A), while the trunk and pos-

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**Fig. 2.** Palaeoscolecid fine-scale anatomy. (A–D) Details of palaeoscolecid cuticle ornamentation. (A) Tessellating cuticular plates of various sizes ornamenting two trunk annuli (GMPKU2383; Late Cambrian, China). (B) A contrasting arrangement in which a field of plates adjoins a field of cuticle with a low-relief polygonal ornamentation (GMPKU2384; Late Cambrian, China). (C) Conspicuous circumferential differentiation in the annular ornamentation of *Gamascolex herodes* (early Ordovician, Czech Republic; silicone rubber peel of holotype specimen NM-L 24634, National Museum, Prague). (D) A typical annulus of a specimen cf. *G. herodes* showing two rows of marginal plates and a midline row of pores (lower three arrowed) representing probable sensilla (early Ordovician, Czech Republic; specimen GS-MM 519, Geological Survey, Prague). (E–G) Palaeoscolecid aboral anatomy. (E) Specimen exhibiting a single pair of (incomplete) conical projections (GMPKU2385; Late Cambrian, China). (F) Specimen exhibiting a single prominent pair of conical projections (specimen B7338-1; Middle Cambrian, Australia). (G) Specimen E7414g-1; Middle Cambrian, Australia). (H–J) Probable palaeoscolecid flosculi preserved within isolated cuticular plates (*Milaculum ethinclarki*; Late Ordovician, Arkansas). (H) Dorsal view of plate preserving several radially ornamented pore sensilla; the rectangle indicates the area magnified in (I) (specimen kimD2). (J) Detail of sensillum preserved in an additional specimen (kimD6). (K) Probable palaeoscolecid tubulus (GMPKU2386; Late Cambrian, China). Images are scanning electron micrographs except for (C) which is a reflected light micrograph. Relative scale bar: (A) 50 µm, (B) 60 µm, (C) 820 µm, (D) 470 µm, (E) 170 µm, (F) 175 µm, (G) 110 µm, (H) 195 µm, (I) 35 µm, (J) 17 µm, (K) 6 µm.

terior terminus exhibit bilateral symmetry along the sagittal plane, with the trunk ornamentation frequently expressing some degree of dorso-ventral differentiation (Fig. 2, C–G) (Conway Morris 1977; Kraft and Mergl 1989; Müller and Hinz-Schallreuter 1993).

## Anterior region

Anteriorly, palaeoscolecids bear a introvert characterized by zones of radially arranged spinose structures (Hou and Bergström 1994; Fig. 1A). The anterior region is unknown in the small, phosphatized specimens and so the detailed three-dimensional anatomy has been difficult to ascertain. However, preliminary data from the early Cambrian of China suggest that the introvert/pharynx of the putative palaeoscolecid Cricocosmia bears, from posterior to anterior, a zone bearing longer, perhaps pentaradially arranged scalids, an unornamented region, and a field of much finer, quincunxially arranged pharyngeal teeth (Maas et al. 2007a); the condition in the co-occurring "confirmed" palaeoscolecid P. sinensis, and in two probable palaeoscolecid taxa from Greenland (Conway Morris and Peel in press) appears to be broadly comparable. There is evidence for introvert eversibility in the Greenland taxa, and in the putative palaeoscolecids Louisella and Cricocosmia (Conway Morris 1977; Maas et al. 2007a; Conway Morris and Peel in press).

## Posterior region

Macroscopic specimens with preserved gut traces show the posterior body of palaeoscolecids to taper toward a terminally placed anus (Hou and Bergström 1994). One or two posterior hooks may also be present (Hou and Bergström 1994; Han et al. 2007b; Fig. 1A). In microscopic, three-dimensionally preserved specimens, the terminal opening is seen to be surrounded by paired conical projections of the body which are bilaterally symmetrical about the sagittal plane (Müller and Hinz-Schallreuter 1993). New data from the Middle Cambrian of Australia and from the undescribed Bitiao taxon reveal that either one or two pairs of projections may be present (Fig. 2, E-G). These are sometimes heavily wrinkled or foreshortened through folding of the body wall, suggestive of a degree of eversibility in life. The elongation of the terminal opening in the sagittal plane is reflected in the cuticle ornamentation (Fig. 2G) and is thus unlikely to be an artifact of preservation, or of molting. Occasional specimens preserve an internally directed pocket, continuous with the external body wall (Müller and Hinz-Schallreuter 1993 and personal observations). From these data, we reconstruct the terminal aboral orifice of palaeoscolecids as a dorso-ventrally elongated opening set within an in-folding of the body wall and surrounded by either one or two pairs of conical, flexible, and perhaps eversible projections.

# External cuticle ornamentation and biomineralization

The characteristic ornament of palaeoscolecid cuticle arises from the tessellation of cuticular thickenings of various shapes and sizes (Fig. 2, A and B). The largest thickenings, or "plates," take the form of discrete sclerites, often bearing a surface sculpture that varies among taxa, body regions, and zones within individual annuli (Kraft and Mergl 1989: Müller and Hinz-Schallreuter 1993; Ivantsov and Wrona 2004). Usually, the plates are set among smaller sculpted platelets and yet smaller unornamented microplates (Fig. 2A; following the terminology of Conway Morris 1997b). Alternatively, the surrounding cuticle may be smooth or bear a lowrelief polygonal patterning (e.g., Fig. 2B), or fine wrinkles (Corallioscolex Müller and Hinz-Schallreuter 1993; Sahascolex in Ivantsov and Wrona 2004), though the extent to which differential preservation contributes to this diversity has yet to be addressed. Typically, the plates are arranged along two circumferential rings per annulus, with annulus boundaries marked by a narrow intercalary zone (Fig. 2A), although the anterior and posterior body regions may carry divergent patterns, including a complete absence of annulation (Müller and Hinz-Schallreuter 1993; Zhang and Pratt 1996; Conway Morris 1997a; Fig. 2, E-G). In some specimens, the morphology of plate sculpture varies markedly within a single annulus, defining a strong dorso-ventral differentiation of the cuticle ornament (Kraft and Mergl 1989: Müller and Hinz-Schallreuter 1993; Ivantsov and Wrona 2004; Fig. 2C)

Palaeoscolecid cuticle was at least partially biomineralized in at least some taxa, on the evidence of the three dimensionality of the plates in otherwise flattened body fossils, and the persistence of plates compared with the surrounding cuticle in specimens that appear to record successive stages of decay (from data in Ivantsov and Wrona 2004). Furthermore, the isolated plates that are routinely recovered through acid digestion of limestones (e.g., Hadimopanella, Milaculum) possess discrete and consistent morphologies (Hinz et al. 1990; Figs. 2 and 3), demonstrating that they are not merely mechanically derived remnants of more extensive sheets of diagenetically mineralized cuticle. These isolated plates are consistently phosphatic in composition, and occur in assemblages that show no evidence for phosphatic overprinting, suggesting that the primary biomineral in palaeoscolecids was calcium phosphate (see also Müller and Hinz-Schallreuter 1993). The extent to which platelets and microplates were biomineralized is unclear, not least because of the practical constraints that preclude their recovery from acid etching of limestones. However, evidence for prefossilization flex-



**Fig. 3.** Palaeoscolecid cuticle structure. (A–B) Articulated cuticle in transverse section (GMPKU2387; Late Cambrian, China). (A) Overview of section through an incomplete and distorted specimen. (B) Detail showing a series of bilayered plates overlying a secondarily mineralized layer (from which they are separated by a narrow space). (C–D) Isolated cuticular plates of *Hadimopanella knappologica* (early Cambrian, Siberia). (C) Dorsal view (specimen hadA5). (D) Transverse section; note the thin capping layer and the fibrous basal microstructure (specimen phsA314). (E–H) Isolated cuticular plates of *Milaculum ethinclarki* (Late Ordovician, Arkansas). (E) Ventral (concave) view showing reticulate pattern. (F) Transverse section; note the thin capping layer and the stacked sections of cross-wise fibers basally. (G) Oblique horizontal section; innermost layers to lower left. (H) Detail showing the fibrillar sub-structure of the cross-wise fibers, which are here sectioned horizontally. (I–J) Part of an annulus of *Gamascolex herodes* (early Ordovician, Czech Republic) bearing plates and microplates, and exposing an underlying cuticle layer of cross-wise fibers; inset (J) shows detail (silicone rubber peel of specimen GS-MM 118, Geological Survey, Prague). Images are scanning electron micrographs; (A, B, D, F–H) are backscattered electron images from polished sections. Relative scale bar: (A) 100 µm, (B) 17 µm, (C) 120 µm, (D) 30 µm, (E) 200 µm, (F) 80 µm, (G) 25 µm, (H) 8 µm, (I) 170 µm, (J) 65 µm.

ure and deformation of the cuticle (e.g., Müller and Hinz-Schallreuter 1993) indicates that the entire thickness of the cuticle could not have been mineralized in vivo. Therefore, where mineralization of the cuticle extends beyond the plates into the underlying cuticle, this has occurred postmortem, by mineral replication of unmineralized tissues. By the same token, it is conceivable that some taxa may have lacked biomineralization entirely.

## Sensilla

Fine-scale cuticular structures interpreted as sensilla (sensory and/or secretory organs) are known from various microscopically preserved specimens (Fig. 2, D and H–K). Narrow conical "tubules" (Fig. 2K) measure up to  $35 \,\mu\text{m}$  in length and taper from an expanded, concentrically wrinkled base of 5–10  $\mu\text{m}$  diameter to a distal tip typically 1–2  $\mu\text{m}$  wide (Brock and Cooper 1993; Müller and Hinz-Schallreuter 1993; Zhang and Pratt 1996; see also the "possible seta" of Zhang and Pratt 1996). A hollow tubular part articulates with a cuticular socket, and the former position of tubules in less intact specimens may be inferred from the distribution of appropriately sized cuticular pores (Brock and Cooper 1993; Müller and Hinz-Schallreuter 1993). The tubules and pores are consistently distributed along the circumferential midline of each annulus (Brock and Cooper 1993; Müller and Hinz-Schallreuter 1993; Fig. 2D).

Conical projections of larger size and stouter proportions have also been described ("nipplelike protuberances": Brock and Cooper 1993; Müller and Hinz-Schallreuter 1993; Zhang and Pratt 1996). These may reach 30-90 µm in basal diameter and thus are large in proportion to the annuli of the worms on which they occur, sometimes being accommodated by a local distortion of annulus width (Brock and Cooper 1993; Müller and Hinz-Schallreuter 1993; Zhang and Pratt 1996). Their distribution is often irregular (Brock and Cooper 1993; Müller and Hinz-Schallreuter 1993; Zhang and Pratt 1996), although where they are preserved in situ on more complete specimens they are seen to lie within a longitudinal field and can appear "more-or-less paired... on each third or fourth annulus" (Müller and Hinz-Schallreuter 1993, p. 579).

A third and newly observed type of palaeoscolecid cuticle projection, reported here from isolated plates of *M. ethinclarki*, consists of a central pore surrounded by 8–12 radial lobes which together form an elliptical or circular mound of 15–20  $\mu$ m diameter (Fig. 2, H–J). In light of their pore-bearing morphology, and their apparently scattered distribution across the surface of the cuticle, we suggest that these structures represent pore complexes for sensory or/and secretory organs.

#### Internal cuticle structure

The internal structure of palaeoscolecid cuticle has been described in a cursory manner (Bengtson 1977; Wrona 1982; Peel and Larsen 1984; Dzik 1986; Bendix-Almgreen and Peel 1988; Märss 1988; Brock and Cooper 1993; Müller and Hinz-Schallreuter 1993); it is agreed to be multilayered. In order to reconstruct palaeoscolecid cuticle in greater detail, we produced sections through a diversity of fossil taxa. In specimens of the unnamed Bitiao taxon (Fig. 3, A and B), diagenetic phosphatization preserves articulated cuticle that may or may not have included components that were mineralized in vivo. Here, we interpret internal structure as reflecting differential phosphatization fabrics that originated during diagenesis but were spatially constrained by primary cuticle structures. In contrast, for Milaculum and Hadimopanella (Fig. 3, C-H), which represent isolated biomineralized components of palaeoscolecid cuticle, we suggest that the internal structure reflects

the original cuticle histology at its time of (bio)mineralization in vivo. Biological fabrics are preserved to a submicrometer resolution whereas diagenetic overprinting (see Bengtson and Budd 2004) is not apparent.

Milaculum and Hadimopanella exhibit a division of the cuticle into at least two layers. In both taxa, a thin, finely granular outer layer overlies in sharp contact a layer which grades between a granular upper part and a vertically striated lower part (Fig. 3, F and D). In Milaculum, a third distinct region which is preserved basally contains several (at least four) layers of large cross-wise fibers (Müller 1973; Dzik 1986), where successive layers of parallel fibers cross at angles of  $70-90^{\circ}$ (Fig. 3, E–H). The fibers measure  $2-6\,\mu\text{m}$  in diameter, are of varying size between successive layers but of constant size within layers, and are circular to oval in cross section. Each fiber is constructed from a large number of smaller fibrils, approximately 0.4 µm in diameter, running parallel to the long axis (Fig. 3H). The stacked intersections between fibers in successive layers can appear confluent with (and possibly account for) the striated or columnar fabric observed toward the base of the overlying granular layer (Fig. 3F).

A subsurface cuticle plane preserved in a shale-hosted compression fossil of *Gamascolex* (Kraft and Mergl 1989) identifies the cross-wise fibers preserved on the inner surfaces of *Milaculum* plates as the remnants of a continuous cuticle layer in which large fibers run helically around the body of the worm, with layers running alternately clockwise and anticlockwise with a crossing angle of 60–90° (Fig. 3, I and J). A comparable pattern of cross-wise fibers has also been documented from the inner surfaces of articulated *Kaimenella* cuticle (Brock and Cooper 1993) and isolated plates tentatively referred to *Palaeoscolex* (van den Boogaard 1989).

Sections of secondarily phosphatized and articulated cuticle from the unnamed Bitiao taxon corroborate a broadly three-layered structure (Fig. 3, A and B). Fine details are not discernable in these very small specimens, although the middle cuticle layer, which is positionally equivalent to the graded granular/vertically striated layer in isolated *Milaculum* and *Hadimopanella*, is seen to expand to accommodate the thickness of the plates (Fig. 3B). Cross-wise fibers are not evident in these sections, and have not been observed on the inner cuticle surface of these or similarly preserved worms (Brock and Cooper 1993; Müller and Hinz-Schallreuter 1993; Zhang and Pratt 1996).

## **COMPARISON WITH OTHER GROUPS**

The presence of an anterior armoured introvert (sensu Nielsen 1995) clearly allies palaeoscolecids to cycloneuralian ecdysozoans. The overall habitus of palaeoscolecids is most immediately reminiscent of priapulids, which share a straight gut with terminal mouth and anus, a scalid-bearing introvert

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leading to an eversible toothed pharynx, and a sometimes macroscopic adult body size. However, among extant groups a slender, sinuous body form is more typical of nematoids (nematodes and nematomorphs). Specifically, the uniform body width and the tendency for fossils to be preserved in neatly coiled or S-curved attitudes has been taken as evidence for the absence of circular musculature (Hou and Bergström 1994; Bergström 2001), suggestive of the secondary loss of this character that is considered to be synapomorphic for Nematoida (Nielsen 2001; Schmidt-Rhaesa 2001). That said, the degree to which fossil worms are variously coiled or crumpled is presumably influenced by the degree of stiffness to their cuticles, while purported examples of preserved circular musculature in palaeoscolecids potentially provide direct contradictory evidence (Ivantsov and Zhuravlev 2005; Ivantsov et al. 2005; Han et al. 2007b).

A combination of radial and bilateral symmetry is shared by palaeoscolecids and all noneuarthropod ecdysozoans (Adrianov and Malakhov 2001a, b; Nielsen 2001; see Dong et al. 2005), although some palaeoscolecids (notably Gamascolex; see Kraft and Mergl 1989; Fig. 2C) exhibit conspicuous dorso-ventral differentiation of the cuticle, which is unknown among priapulids of equivalently large body size, and has been suggested to imply an epifaunal rather than burrowing mode of life (e.g., Zhang and Pratt 1996; Conway Morris and Peel in press). The symmetry of introvert elements is of critical importance in resolving the affinities of fossil taxa (Dong et al. 2005). Preliminary analyses of introvert symmetry in compressed specimens of the putative palaeoscolecid Cricocosmia suggest a pentaradial arrangement (Maas et al. 2007a), consistent with the condition in extant scalidophorans.

The aboral end of palaeoscolecids appears to be unique among known ecdysozoans in exhibiting one or two pairs of conical projections arranged around an orifice that is elongated dorso-ventrally. However, the probable posterior invagination is reminiscent of the unannulated, eversible "bursa" present in the middle Cambrian Burgess Shale worms Ottoia and Louisella (Conway Morris 1977). A pair of tail hooks has been considered diagnostic for palaeoscolecids (Han et al. 2007b), although it is unclear whether the proportionally smaller hooks, which are poorly known and described only from compressed macrofossils (Hou and Bergström 1994; Han et al. 2007b), are homologous to the paired conical projections known from the small individuals; the distinction may be ontogenetic, phylogenetic, or taphonomic. In any case, broadly comparable tail hooks are widespread among cycloneuralians, being present in various priapulids, both crown group (e.g., Meiopriapulus, Morse 1981) and stem group (e.g., Ottoia, Conway Morris 1977), and in larval nematomorphs (Hou and Bergström 1994).

The ornamentation of palaeoscolecid cuticle, in which the larger of the tessellating polygons are thickened into sculpted plates with discrete bounding edges, is unique. Furthermore, biomineralization in calcium phosphate is otherwise unknown among cycloneuralians. However, annulation of the cuticle is prominent in priapulids, larval nematomorphs, and various nematodes (Dong et al. 2005), while a finer superimposed ornamentation consisting of tessellating polygons is developed widely in nematomorphs (especially "areoles," e.g., Schmidt-Rhaesa and Menzel 2005) and occasionally (and less conspicuously) in priapulids (Shapeero 1962). Priapulid cuticle may exhibit fine, deep wrinkles (Hammond 1970; Oeschger and Janssen 1991) comparable to the cuticle fabric of certain palaeoscolecid fossils, or may bear closely packed, coneshaped thickenings termed "tumuli" (van der Land 1970; Storch and Alberti 1985) which have been likened to the sculpted plates of palaeoscolecids (Conway Morris 1997b). However, tumuli do not exhibit discrete bounding edges and the resemblance is considered here to be superficial. There is likewise no close similarity between palaeoscolecid plates and the specialized sensory/secretory "warts" of certain priapulids (cf. Kraft and Mergl 1989; Conway Morris 1997b) which are localized to the posterior-most body region and bear a contrasting fine-scale structure consisting of the openings of numerous small tubes (Hammond 1970, Fig. 4).

More compelling comparisons exist for the palaeoscolecid tubules, which are similar in size, shape, socketed articulation, and concentric basal ornamentation to the secretory tubuli of priapulids (Müller and Hinz-Schallreuter 1993; Conway Morris 1997b) (for equivalents in extant taxa see Hammond 1970; Higgins et al. 1993; Lemburg 1995a). The pore sensilla reported here from isolated palaeoscolecid plates are interpreted as flosculi, which are specialized sensilla known from living priapulids, loriciferans and kinorhynchs, and constitute a putative scalidophoran synapomorphy (Ehlers et al. 1996; Nielsen 2001; Schmidt-Rhaesa 2007). Detailed similarities extend to the elliptical to circular outlines, raised central pores, and radial arrangement of "petals," and the fossil structures are morphologically consistent with reconstructions of the plesiomorphic flosculus (Lemburg 1995a).

The larger of the palaeoscolecid cuticular projections, known as "nipplelike protuberances" (Müller and Hinz-Schallreuter 1993; Zhang and Pratt 1996), find fewer comparisons among extant taxa. Despite a superficial similarity both to the posterior paired projections of palaeoscolecids and to the paired appendages of lobopodian panarthropods, the nipplelike projections are inconsistently observed and exhibit an irregular, mostly nonpaired distribution across the body surface. Conceivably they represent sensilla that are developed by a particular taxon or a particular body region; the bacillary band sensilla of some nematodes (compare Gibbons 2002, fig. 2.18) provide at least a superficial comparison. It also seems possible that some of the larger and more irregularly shaped outgrowths are pathological.

#### **Cuticle structure**

The multilayered cuticle structure of palaeoscolecids is consistent with the condition in extant ecdysozoans, which generally possess cuticle with three distinct layers (Schmidt-Rhaesa et al. 1998). However, the trilaminate epicuticle that is considered synapomorphic for Ecdysozoa (Schmidt-Rhaesa

et al. 1998) measures only a few nanometers in thickness (Lemburg 1998) and unsurprisingly has not been resolved in the fossils. The absence of growth lines from palaeoscolecid cuticle is consistent with growth by molting rather than accretion (cf. Lehnert and Kraft 2006), as is a specimen in which old and new cuticles are appear to be superimposed (Müller and Hinz-Schallreuter 1993, text fig. 14C). There is no evidence of the distinctive "ply-wood" construction that is typical of euarthropods, and the following comparisons focus on the remaining ecdysozoan groups, and among these on living representatives. The only fossil examples of early noneuarthropod ecdysozoan cuticle suitable for comparison in this context are the biomineralized plates of the Cambrian lobopod Microdictyon (Conway Morris 1997b), although preliminary observations of their internal structure (Bengtson et al. 1986 and additional unpublished observations) reveal no shared characters beyond a general layered construction.

The outer fine-granular layer of palaeoscolecid cuticle is comparable in texture and position to the exocuticle of various scalidophorans (Lemburg 1998), the areolar layer of some nematomorphs (de Villalobos and Restelli 2001), the exocuticle or cortical zone of some nematodes (Decraemer et al. 2003), and also to particular cuticle layers in some tardigrades (Dewel et al. 1993) and onychophorans (Wright and Luke 1989). The middle region of palaeoscolecid cuticle, which is expanded to accommodate the thickness of the plates, where present, exhibits vertically striated fabrics similar to those present among nematodes (radial striae of various layers; Neuhaus et al. 1996b; Decraemer et al. 2003), tardigrades (Dewel et al. 1993), and priapulids. Among priapulids, the exocuticle III of the larval lorica of Halicryptus spinulosus contains vertical fibers enclosed in canals (Storch and Higgins 1991; Lemburg 1998), while the sclerotized endocuticle of Priapulus caudatus contains irregularly arranged fibers and, interestingly, is restricted in its distribution to stiffened projections (scalids, teeth, etc.; Lemburg 1998). Priapulid sclerotized endocuticle therefore resembles the middle cuticle layer of palaeoscolecids in both its texture and its discontinuous distribution, but differs in being an innermost cuticle layer, while in broader terms the combination in palaeoscolecids of a homogeneous layer underlain by a striated layer is consistent with the suggested plesiomorphic condition for cycloneuralian cuticle (Schmidt-Rhaesa 2007).

However, it is the distinctive fabric of the innermost layer of palaeoscolecid cuticle that is the most informative in terms of structure, chemistry, function and, potentially, phylogeny. A system of large, helically wound cross-wise fibers is restricted among extant ecdysozoans to the cuticle of nematomorphs and some nematodes, which consists largely of the structural protein collagen (Schmidt-Rhaesa et al. 1998)—a putative synapomorphy of Nematoida (Nielsen 2001) (for uncertainties surrounding the precise fiber chemistry in nematomorphs see Brivio et al. 2000; Protasoni et al. 2003). Other ecdysozoans

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exhibit predominantly chitinous cuticles (Ehlers et al. 1996), although collagen has been reported from the basal cuticle layers of some priapulids (Welsch et al. 1992). Also, it is worth noting that fibers of equivalent helical arrangement and proteinaceous (presumably collagenous) composition are believed to have arisen multiple times in cylindrical metazoans, including among annelids, nemerteans, sipunculans, chaetognaths, and even burrowing amphibians (Seymour 1983; Ahnelt 1984; Bresciani 1991; O'Reilly et al. 1997). In each case, the function of the fibers is to constrain the hydrostatic skeleton and prevent bulging and kinking while allowing flexibility during body movement (Wainwright 1988), a mechanism achieved through changes in the crossing angle between successive layers within the stacked structure (Harris and Crofton 1957; Seymour 1983). Conceivably, then, this character arose convergently in palaeoscolecids, as it may have done among nematodes and nematomorphs, possibly as a functional response to increased body size (Schmidt-Rhaesa 1998).

Indeed, a detailed comparison between the large helical fibers of palaeoscolecids and extant nematoids reveals some distinctions despite the overall similarity. The fibers described from nematodes and nematomorphs are generally  $< 1 \, \mu m$  in diameter, of constant size within individuals, and conspicuously closely packed (Brivio et al. 2000; Protasoni et al. 2003), while observed palaeoscolecid fibers reach much greater diameters  $(2-6 \,\mu\text{m})$ , vary in size between successive layers, and are more widely spaced. The internal structure of nematoid fibers is incompletely known, but several discrete arrangements have been reported within nematomorphs, notably stacked platy subunits (Gordius panigettensis; Protasoni et al. 2003), and helically coiled pairs of submicrometer-width fibrils (Parachordodes wolterstorffii; Cham et al. 1983). A possible third arrangement consisting of a large number of uncoiled fibrils aligned parallel to the long axis of the fiber (Gordius villoti; Brivio et al. 2000), if not an artifact of sectioning a platy structure, is most similar to the condition observed in palaeoscolecids. It is unclear to what extent these characters are influenced by biology and taphonomy, although the absence of cross-wise fibers from the larval cuticle of a nematomorph species despite their conspicuous presence in the adult (Jochmann and Schmidt-Rhaesa 2007) indicates a strong ontogenetic control on fiber morphology, and potentially explains the absence of observable fibers in the smaller palaeoscolecid individuals.

Overall, the cuticle of palaeoscolecids is most readily comparable to that of nematoids, although an equivalent complexity of layers overlying the giant cross-wise fibers is unlike the condition in nematomorphs (see Schmidt-Rhaesa and Gerke 2006) and the discontinuous development of a middle striated layer is more reminiscent of priapulids. It is clear that palaeoscolecids exhibit extinct character combinations in their fine-scale as well as gross-level anatomy, and that the phylogenetic status of these characters and the precise affinities of palaeoscolecids require assessment within the context of cladistic analysis.

## PHYLOGENETIC ANALYSIS

To test the phylogenetic position of palaeoscolecids within the context of other extant and extinct ecdysozoans for which adult stages are known, we revised and augmented the dataset presented by Donoghue et al. (2006) with particular emphasis on incorporating the newly resolved details of the cuticle structure. Newly added taxa are Microdictyon, Tabelliscolex, Tardigrada, and *Tylotites*, while *Palaeoscolex* is replaced by a composite taxon labeled as "palaeoscolecids sensu stricto" (see "Included taxa," above). The revised datamatrix and list of character descriptions are provided as an appendix to this article. The dataset was analyzed using PAUP\* 4.0d90dev on an Intel-based MacBook. Gastrotricha was designated the outgroup, in spite of uncertainty surrounding its phylogenetic proximity to Ecdysozoa (see Schmidt-Rhaesa 2007, and discussion below). All characters were unordered and initially set to equal weight, and in each instance a 1000-replicate heuristic search was performed using the random sequence addition option, retaining 10 trees at each step.

Analysis of a dataset including only extant taxa yields a single most parsimonious tree (MPT) at 131 steps (CIe 0.75; RI 0.82; RCI 0.64) in which Nematoida is resolved as the sister taxon to Scalidophora to the exclusion of Panarthropoda (Fig. 4A). The inclusion of the taxon "palaeoscolecids s.s." leads to its unequivocal resolution as the sister taxon to Nematomorpha, but with two MPTs, one resolving panarthropods as the sister to (i) nematoids, and (ii) nematoids+scalidophorans (145 steps; CIe 0.71; RI 0.79; RCI 0.59; Fig. 4B). The inclusion of additional, extinct panarthropods results in 140 equally MPTs at 153 steps (CIe 0.68; RI 0.78; RCI 0.56) the strict consensus of which (Fig. 4C) reveals that conflict is restricted to the interrelationships of Panarthropoda, which are entirely unresolved. Experimental analysis of the dataset revealed that Tardigrada alone is the source of the conflict, and the exclusion of this leaf results in a single MPT that is completely resolved and, aside from the relationships within Panarthropoda, remains compatible with the trees derived from analysis of a dataset that includes Tardigrada (148 steps; CIe 0.70; RI 0.78; RCI 0.57; Fig. 4D).

Parsimony analysis of a dataset that includes a much greater sampling of extinct scalidophorans results in 119 equally MPTs at 205 steps (CIe 0.55; RI 0.72; RCI 0.42), the strict consensus of which is presented in Fig. 4E. In this tree and its derivatives, palaeoscolecids *s.s.* fall within the priapulid stem, forming a clade with some but not all of the palaeoscolecid-like taxa. To assess the strength of the phylogenetic signal within the dataset we undertook a double-decay analysis (DDA; Wilkinson et al. 2000), a

methodological variant of Bremer support (Bremer 1988, 1994) that evaluates support for component trees which are often more highly supported than the overall phylogenetic hypothesis. This occurs because rogue taxa, for which there are disparate equally parsimonious interpretations of affinity, have the effect of diminishing support for otherwise well-supported nodes that intercalate the competing positions on the tree (Wilkinson et al. 2000). We used the RADCON implementation of DDA (Thorley and Page 2000), using the MPTs from the previous analysis as the source tree, and the heuristic search option, running 100 replicate searches, with 10 trees retained at each step for each component backbone constraint tree. We assessed the relative support for the subtrees in terms of the sum of their Bremer support values, which is biased in favor of a greater number of component taxa, but biased against more universal trees that include poorly supported nodes (Wilkinson et al. 2000). The analysis revealed that the sum of the support for the source tree is 31 (topology common to Fig. 4E except that the first plesion within the priapulid stem is collapsed into a polytomy), while there are seventeen trees containing fewer taxa but for which there is higher support. Two trees had a total decay of 37 but these omitted Corynetis, Xiaheiqingella and Yunnanpriapulus, and Xiaheiqingella, Yunnanpriapulus, Maotianshania, and Corvnetis, but they are otherwise consistent with the source tree.

To determine the support for an alternative, stemecdysozoan position for palaeoscolecids, we constructed an experimental tree in which palaeoscolecids s.s. are constrained to resolve as more derived than Gastrotricha but less derived than an unresolved polytomy of the remaining taxa. By implementing a backbone constraint on an unweighted analysis in PAUP, we identified the shortest trees compatible with the stem-Ecdysozoa affinity for palaeoscolecids s.s. while allowing the unconstrained resolution of the remaining taxa. The shortest compatible trees found (six trees at 210 steps; CIe 0.54; RI 0.71; RCI 0.40) were five steps longer than the shortest tree that we derived from unconstrained analysis of the dataset. Subsequent reweighting of the characters using RCI scores from the analysis of the initial unweighted dataset, and using the same backbone constraint tree, produced two MPTs at 86.21 steps (CIe 0.69; RI 0.84; RCI 0.62; strict consensus presented in Fig. 4F). This was among the 28 MPTs recovered from analysis of the unweighted dataset.

## DISCUSSION

The interrelationships of extant taxa within our MPTs and reduced consensus trees are consistent with those recovered previously in parsimony-based analysis of morphological da-

tasets, where nematoids form the sister clade of scalidophorans rather than panarthropods (Lemburg 1999; Dong et al. 2004, 2005; Donoghue et al. 2006). In contrast, molecular phylogenetic analyses of ecdysozoans have usually resolved scalidophorans as the sister clade to nematoids plus panarthropods (Aguinaldo et al. 1997; Ruiz-Trillo et al. 1999; Giribet et al. 2000; Garey 2001; Peterson and Eernisse 2001; Halanych 2004; Mallatt et al. 2004; Baguñà et al. 2008), although this apparent molecular consensus has broken down recently as a result of increased sampling both of taxa and of sequence loci. The first analysis to include data from Loricifera (Park et al. 2006) indicated that kinorhynchs, tardigrades, nematoids, arthropods, and pseudocoelomate priapulids comprise a clade to the exclusion of loriciferans, onychophorans and coelomate priapulids. However, this result may be an artifact of the paucity of data on which it is based. A more sophisticated analysis which included an additional molecular locus resolved a close relationship between Loricifera and Nematomorpha, though the relationship between this clade and nematodes, kinorhynchs, and priapulids was not resolved (Sørensen et al. 2008). More recently, Dunn et al. (2008) provided a dataset that was richer both in taxa and molecular loci (though it omitted Loricifera), and their preferred tree, with rogue taxa excluded, is directly compatible with the morphology-based trees in resolving Cycloneuralia and Panarthropoda as sister clades. Therefore, within the framework of a current consensus of total evidence, we can have some confidence in our character polarity and rooting.

Inclusion of palaeoscolecids within a set of otherwise extant taxa leads to their resolution as the sister taxon to nematomorphs (Fig. 4B), in line with the conclusion of the simple parsimony analysis undertaken by Hou and Bergström (1994) following the discovery of palaeoscolecids with preserved introverts. Nematoida is supported by a number of clear synapomorphies, although palaeoscolecids are only scored for two of these (61: aspect ratio of body length to width; 79: cuticle predominantly containing collagen), and their nematomorph affinity is also supported by just two (albeit unequivocal) characters (91: cuticular ornament of tessellating polygons; 93: terminal posterior spines). Within this scheme, palaeoscolecids exhibit five autapomorphic character states (6: morphology of Zone 1 armature; 26: trunk tubuli; 27: flosculi; 45: division of adult body into proboscis and abdomen; 92: conspicuous trunk sclerites).

Palaeoscolecids remain positioned as the sister taxon to nematomorphs upon the addition of extinct panarthropod taxa (Fig. 4, C and D). However, the inclusion of extinct cycloneuralians resolves an extensive priapulid stem that includes palaeoscolecids (Fig. 4E). This position within Scalidophora is supported by a number of scored synapomorphies (6: morphology of introvert Zone I armature; 14: morphology of introvert Zone III armature; 20: proportions of introvert Zone III; 21: eversibility of Zone

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III; 42: mouth cone; 43: eversibility of mouth cone; 45: division of adult body into proboscis and abdomen; 61: body aspect ratio; 62: alignment of rows of Zone I armature; 68: primary body cavity; 92: conspicuous trunk sclerites; 93: terminal spines). The characters that previously supported a stem-nematomorph affinity for palaeoscolecids are now resolved as homoplasies seen in nematoids and stem priapulids, whereas those identified as palaeoscolecid autapomorphies change their status (some, such as conspicuous trunk sclerites [92] become synapomorphies) and are replaced by three new characters (19: proportions of introvert Zones II and III; 22: size consistency of introvert Zone III elements; 24: number of trunk annuli).

The result of our most inclusive analysis is thus broadly consistent with previous cladistic analyses of the interrelationships of fossil and living cycloneuralians, in which palaeoscolecids have consistently been resolved as stem priapulids (Wills 1998; Dong et al. 2004, 2005; Donoghue et al. 2006; Cobbett et al. 2007). Interestingly, we now resolve the strictly defined palaeoscolecids as grouping with the similarly shaped Tabelliscolex, Cricocosmia and Tylotites in a subclade of the priapulid stem that is characterized by conspicuous, probably biomineralized cuticular sclerites. In contrast, the putative palaeoscolecids Louisella and Maotianshania fall outside this clade (though still within the priapulid stem). This result suggests that although we identified a priori a "core-group" on the basis of a particular cuticle ornament (the validity of which has not been tested in our cladistic analysis owing to the dispersed nature of the fossil data), "palaeoscolecids" as loosely defined are likely to represent a paraphyletic or even polyphyletic assemblage.

None of our analyses provides support for a "deepbranching" position for palaeoscolecids among ecdysozoans (cf. Fig. 1, B-E). However, the analysis in which palaeoscolecids s.s. are forced to resolve as the sister group to all other in-group taxa differs from the unconstrained analysis in resolving a clade of nematoids and panarthropods to the exclusion of extant scalidophorans (Fig. 4F). Also, of the extinct cycloneuralians that are resolved as stem priapulids in the unconstrained analysis, half are resolved in this forced scenario as stem ecdysozoans, and half as crown priapulids. Furthermore, the assembly of palaeoscolecids s.s. with various palaeoscolecid-like taxa in a paraphyletic grade to the crown group is consistent with the hypothesis that the remaining ecdysozoans evolved from a palaeoscolecid-like ancestor (cf. Fig. 1C). This scheme is thus entirely compatible with the scenario for the emergence of ecdysozoans and, more specifically, panarthopods, from broadly priapulid-like ancestors (Dzik and Krumbiegel 1989; Budd 1999, 2001b, c, 2003, 2008; Budd and Jensen 2000, 2003). The five extra steps required to force a stem-ecdysozoan position for palaeoscolecids, given that the tree contains 200 or more steps, do not perhaps appear to represent a substantial obstacle to these hypotheses.

Despite the suggestive topology of the constrained tree, however, it is clear that there is no evidential support for the scenario of palaeoscolecids as ancestral ecdysozoans. Indeed, this scenario requires the rejection of the precious few homology statements that can be made for the fossil cycloneuralians. Importantly, more than 40% of the characters cannot be scored for palaeoscolecids because they are not preserved or could not be known from fossil remains, and in this light it is perhaps surprising that this single leaf has so much impact upon tree length. Indeed, palaeoscolecids are among the more completely known of fossil cycloneuralians-for comparison, 77% of characters cannot be scored for *Tabelliscolex*—and so it is to be expected that the permutation of the position of fossil cycloneuralians within a cladogram imposes little cost in terms of implied character changes. However, such permutations do not merely challenge the homology statements in characters for which the translocated taxa have been scored, but also the homology of characters in their living relatives, for which there is otherwise no structural or phylogenetic evidence. For instance, the result of the constrained analysis implies that scalids are homologous among ecdysozoans and that they have been lost secondarily among panarthropods. However, the homology of scalids among nematoids and scalidophorans has been considered in detail, and has been rejected (Schmidt-Rhaesa 1998). Furthermore, the implied evolution of scalid pattern symmetry becomes more complex: palaeoscolecids and similar fossil worms appear to exhibit pentaradial symmetry, as do kinorhynchs and priapulids, whereas nematoids, loriciferans, and some stem-arthropods exhibit hexaradial symmetry (Schmidt-Rhaesa 1998; Kristensen and Brooke 2002; Maas et al. 2007a). Thus, rather than the independent evolution of pentaradial and hexaradial arrangements of scalid-like structures among ecdysozoans, as has been envisaged previously (Schmidt-Rhaesa 1998), a scenario in which palaeoscolecids are ancestral requires that pentaradiality is plesiomorphic and that hexaradial symmetry has arisen at lease twice independently among nematoids, panarthropods, and loriciferans, within convergent anatomical structures.

Support for a deep-branching position for palaeoscolecids has come from their cylindrical vermiform gross anatomy and the vague similarity of their characteristically ornamented and annulated cuticle to that of the stem-arthropods *Xenusion*, *Aysheaia*, *Hadranax* and *Kerygmachela* (Budd 2003)—a potential synapomorphy that is incompatible with the trees presented here. Indeed, when palaeoscolecids were first identified as a potential evolutionary link between scalidophorans and panarthropods (Dzik and Krumbiegel 1989; Fig. 1F), nothing was known of their anatomy beyond the shape, annulation and ornamentation of their trunk. Within this context they were a classic "stem taxon," in the sense that Hennig (1981) effectively formulated the "stem group" as a taxonomic dustbin for fossil taxa whose membership of a more exclusive crown group could not be determined, because it is not possible to distinguish whether the necessary qualifying synapomorphies were never present in the living animal, or whether they had merely rotted away (Donoghue 2005; Donoghue and Purnell 2009). Thus, as the anatomy of palaeoscolecids has become more completely known (Kraft and Mergl 1989; Müller and Hinz-Schallreuter 1993; Hou and Bergström 1994), they have been promoted to more exclusive clades (Wills 1998; Dong et al. 2004, 2005; Donoghue et al. 2006). This trend, though potentially widespread in the case histories of problematic fossils (Donoghue and Purnell 2009), is not inevitable in the case of palaeoscolecids. Conceivably, comparably detailed and potentially homologous characters relating to cuticle ornament, internal structure and sensilla might have been discovered in early fossil panarthropods, thus adding weight to the hypothesis of a close relationship between palaeoscolecids and panarthropods, but to date they have not, despite the obvious preservational potential (e.g., Bengtson et al. 1986; Maas et al. 2007b).

Meanwhile, a phylogenetic position for palaeoscolecids closer to nematomorphs, as initially suggested by Hou and Bergström (1994), appears to be an artifact resulting from a restricted sampling of stem priapulids. The key potential synapomorphy supporting the close kinship of palaeoscolecids and nematomorphs is the presence of giant fibers in their cuticles (Dzik 2003; Dong et al. 2004), consistent with the view that the collagenous reinforcement of the cuticle is an autapomorphy of nematoids, as distinguished from a suggested alternative strategy of chitinous reinforcement in the cuticle of adult scalidophorans (Nielsen 2001). However, equivalent fibers are also present in the cuticles of large mermithid nematodes (Wright 1991; Lee 2002) and based on both their phylogenetic distribution and histological structure, it has been concluded that they are convergent adaptations to large size (Bresciani 1991; Neuhaus et al. 1996a; Schmidt-Rhaesa 1998). Thus although in a different phylogenetic context the data from palaeoscolecids potentially support a substantial collagenous component to the ancestral ecdysozoan cuticle, in contrast to the prevailing hypothesis (cf. Schmidt-Rhaesa et al. 1998; Schmidt-Rhaesa 2007), the phylogenetic value of the large cross-wise fibers has been brought into question.

A position for palaeoscolecids among stem-priapulids provides insight into the early evolution of a group that is receiving much attention as the potentially most appropriate *extant* model for ancestral ecdysozoans, whether morphologically, developmentally, or genomically (Webster et al. 2006; Telford et al. 2008; Wennberg et al. 2008). Although the premise on which this is based is confounded with the reasoning that accompanies deep-branching hypotheses for palaeoscolecid affinities, there is empirical evidence that the extant *Priapulus caudatus* is slowerevolving than at least the demonstrably idiosyncratic nematodes, and thus inherently better suited to such an

analytic role (Webster et al. 2006). In this context, the wealth of anatomical data now available for stem priapulids-and palaeoscolecids in particular-provides a valuable opportunity to polarize characters present among the disparate crown group. For example, the wide distribution of large body size among stem priapulids suggests that this is the plesiomorphic condition (Dong et al. 2005), despite the opposite character polarity being implied by the small body size shared among a basal grade of living priapulids (Lemburg 1999). Similarly, the symmetry of the trunk is conspicuously more radial in the living Priapulus compared with many palaeoscolecids and other stem priapulids (see Conway Morris 1977; Kraft and Mergl 1989; Hou and Bergström 1994; Han et al. 2007a, b), presumably as a secondary adaptation to deep burrowing (van der Land 1970). This conflicts directly with the hypothesis of Dzik and Krumbiegel (1989), which requires the opposite character transformation in its secondary derivation of trunk bilaterality in panarthropods. Another important implication of our results is that the preponderance of cuticular annulation among stem priapulids, including palaeoscolecids, identifies a plesiomorphic feature that is secondarily absent from the majority of crown priapulids. Annulation as an expression of seriality in the organization of the cuticle is seen conspicuously in the striking patterns of annular ornamentation of many palaeoscolecids, where, suggestively, it is accompanied by a strictly midannulus distribution of certain types of sensilla-a pattern that is shared by the "abdominal papillae" of extant Priapulus (from data in van der Land 1970, and personal observations). Some degree of seriality to the innervating nervous system is therefore implied. Significantly, repetitive structures within the nervous system of the extant priapulid Tubiluchus have been identified (Schmidt-Rhaesa and Rothe in press) and considering also that surface annulation reflects the distribution of underlying circular musculature in extant priapulids (van der Land 1970), the expectation that metamerism has never been a feature of the priapulid body plan (Nielsen 2003; Dong et al. 2005) should perhaps be reconsidered.

## CONCLUSIONS

Our analysis concludes that palaeoscolecids are not ancestral ecdysozoans. Palaeoscolecids instead occupy a stempriapulid position that is remote from the crown ancestor of Ecdysozoa in our most parsimonious scenario, and hypotheses in which they occupy a deeper position arise largely from a paucity of preserved characters, and thus represent an artifactual result which accruing fossil discoveries appear to have corrected. However, this does not require rejection of the hypothesis that the ecdysozoan ancestor was macroscopic, annulated, possessed a proboscis and moved by

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peristaltic locomotion-although it removes the rock solid evidence that fossils might provide this hypothesis. We note, though, that our conclusions are contingent upon the hypothesis of relationships that underlies our evolutionary reconstruction, and it is clear from our experimental analyses of the dataset that the positions of these fossil taxa are highly dependent upon the pattern of character evolution dictated by the interrelationships of their extant relatives. While our morphology-based phylogeny is directly compatible with the most recent and most comprehensive molecular phylogeny (Dunn et al. 2008), attempts to more completely sample scalidophoran diversity (albeit with few loci) have suggested that the taxa conventionally interpreted as crown priapulids are polyphyletic (Park et al. 2006). Given this uncertainty, a rigorous approach to character analysis and a targeted examination of fossil anatomy becomes all the more crucial, and we hope that parallel advances in molecular phylogenetics and evolutionary developmental biology will soon provide the context in which the detailed insights from paleobiology can be applied to their full potential.

More generally, however, our study cautions against the interpretation of incompletely preserved fossil taxa as primitive because they only preserve primitive characters. Organisms such as the ecdysozoan crown ancestor would have possessed the shared primitive characters of its extant descendents, plus further plesiomorphies and apomorphies subsequently lost by derivative lineages. Distinguishing between stem taxa that rightly occupy their phylogenetic position versus stem taxa that lack characters because they have rotted away is not trivial, but it can be achieved by considering whether or not apomorphies of the respective crown group are demonstrably absent from the living organism given the taphonomic history of representative fossils (Donoghue and Purnell 2009). It is alarming that many of the candidates for phylogenetic positions in phylum- or superphylum-level stems are among the most incompletely preserved of fossils (Donoghue and Purnell 2009). We suggest a qualifying test: if a fossil taxon preserves no more characters than would be expected of a particular phylogenetic position—if it fails to inform on the sequence of character evolution-then it should perhaps be assumed that apomorphies diagnostic of a more exclusive clade have merely rotted away. Even if the phylogenetic position of such fossils is correctly resolved, they fail to tell us anything that we did not already know, and until their anatomy is more completely resolved they are of little consequence in attempts to unravel evolutionary history.

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## APPENDIX I: CHARACTER DEFINITIONS AND CHARACTER CODINGS

#### Character descriptions

The character descriptions that follow are repeated and augmented from Wills (1998), Lemburg (1999), Dong et al. (2004, 2005), and Donoghue et al. (2006) in that order.

- 1. Introvert invaginable: absent (0), present (1).
- 2. Degree to which the introvert can be invaginated: partially invaginable (i.e., part of Zone 1) (0), completely invaginable into the trunk (i.e., to the base of Zone 1) (1).
- 3. Zone I: unarmed (0), armed (1).
- 4. Arrangement of Zone I armature into discrete parallel longitudinal rows: absent (0), present (1).
- 5. Pentaradial arrangement of Zone I armature: absent (0), present (1).
- 6. Morphology of Zone 1 armature: papillae (0), simple spines (1), hooks or spinose hooks (2), conical scalids (3), telescopiform scalids (4), curved scalids and dentoscalids (5), complex scalids (6), glandular scalids, trifid spines, sensory spines, and double, tentaculite scalids (7), scalids with pectinate hood (8), spinoscalids and clavoscalids (kinorhynchs and lorificerans) (9).
- 7. Number of elements comprising the first three circlets and, hence, defining the number of longitudinal rows of elements on the introvert:  $\leq 20$  (0), 25 (1), >25 (2). In all extant priapulids there are eight elements in the most proximal circlet of the introvert, their number and position corresponding to innervation derived from the circumpharyngeal brain. The original character in Wills (1998) described the number of elements in the anteriormost circlet. However, it is clear from his coding that he was describing the number of longitudinal rows of elements on the introvert, which is defined not by the anteriormost circlet alone, but in a combination of the first three circlets. In all extant priapulids the first three circlets are composed of 8:9:8 elements establishing the 25 longitudinal rows, except in Meiopriapulus where there are 8:9 and then a much greater number comprising the third circlet and those that follow (Adrianov and Malakhov 2001a, b). In loriciferans and kinorhynchs there are 20 longitudinal rows.

- 8. Sequence of Zone 1 elements: elements as a single series (all elements identical or with differing morphologies) (0), elements organized into two or more transverse bands or series, possibly with different element morphologies within each series, but the sequence of morphologies being comparable between subsequent series (1).
- Basal circlet of Zone 1 armature separated from more anterior by a constriction (as in loriciferans) or by insertion of longitudinal or circular muscles (kinorhynchs): absent (0), present (1).
- 10. Zone II: unarmed (0), armed (1).
- 11. Number of elements in the proximal circlet of Zone II: numerous (>eight) (0), eight (1), <eight (2).
- 12. Zone III: unarmed (0), armed (1).
- Number of circlets of Zone III armature: 1–4 (1), 6–8 (2), 16 or more (3).
- 14. Morphology of proximal circlets of Zone III armature ("teeth"): absent (0), spines or papillae (1), multispinose (2), multispinose but massively reduced (3), hooks (4), conical with a fringe of spines (5), sclerotized trabeculae (6), pectinate (7), conical papillae terminating in a long spine (prickle) (8), oral stylets (9).
- 15. Morphology of middle circlets of Zone III armature (teeth): absent (0), spines (of any length) or papillae (1), multispinose (reduced or otherwise) (2), pectinate (3).
- Morphology of the distal circlets of Zone III armature (teeth): absent (0), spines (of any length) (1), multispinose (reduced or otherwise) (2), pectinate (3).
- 17. Number of elements in first circlet of pharyngeal armature (base of Zone III): first circlet of numerous elements (>10) (0), first circlet of 10 elements (1), first circlet of five elements (2).
- 18. Number of proximal, pentagonal circlets in Zone III of the proboscis: none (0), five (1), six (2), seven (3).
- 19. Width of Zone III relative to Zone II: Zone III less than twice the width of Zone II (0), Zone III equal to or greater than twice the width of Zone II (1).
- 20. Width of the distal portion of Zone III: distal Zone III parallel to proximal Zone III or tapering gradually (0), distal Zone III expanded into a bulb (1).
- 21. Eversibility of Zone III: Zone III completely eversible (0), Zone III incompletely eversible, but eversible beyond the proximal teeth (1), Zone III normally eversible only as far as the proximal teeth (2).
- 22. All Zone III elements of approximately equal size (0), Zone III elements decreasing regularly in size from the posterior to the anterior (anteriormost elements less than half the size of the posteriormost) (1).
- 23. Surface of trunk cuticle: smooth and unannulated (0), annulated (1).
- 24. Number of trunk annuli: 7–11 (0), 30–50 (1), 60–120 (2), 160 or more (3).
- 25. Trunk tumuli: absent (0), present (1).

- 26. Trunk tubuli: absent (0), present (1).
- 27. Flosculi, N-flosculi or sensory spots: absent (0), present (1).
- 28. Posterior ring papillae: absent (0), present (1).
- 29. Eversible bursa: absent (0), present (1).
- 30. Position of the anus: anus terminal, whether within a bursa or otherwise (0), anus in posterolateral or posteroventral surface of the abdomen (1).
- 31. Caudal appendage(s): absent (0), present (1).
- 32. Division of caudal appendage(s) or tail: undivided (0), pseudosegmented (1)
- 33. Caudal appendage vesiculae: absent (0), present (1).
- 34. Polythyridium: absent (0), present (1).
- 35. Nucleation of "peritoneal" membrane: membrane without nuclei or simply with amoebocytes in association with the surface (0), membrane containing scattered nuclei (1).
- 36. Developmental mode: direct (0), biphasic (1).
- 37. Loricate stage: absent (0), present (1).
- 38. Moulting cuticle: absent (0), present (1).
- Scalid-like structures (non-specific and sensu lato, including scalids, presumed scalid derivatives, and anterior "hooks"): absent (0), present (1).
- 40. Extent of cuticularization of scalid-like structures: structures composed exclusively of cuticle (0), cuticle limited to a thin outer covering (1). Schmidt-Rhaesa has demonstrated that, in comparison to the scalids of Scalidophora (Kinorhyncha+Loricifera+Priapulida), the scalid-like structures of nematoids (Nematoda+Nematomorpha) are composed exclusively of cuticle.
- 41. Terminal mouth: absent (0), present (1).
- Mouth cone: absent (0), present (1). The eversible (though not necessarily inversible) upstanding anterior limit of the pharynx, sensu Lemburg (1995a, b).
- 43. Non-inversible mouth cone: absent (0), present (1).
- 44. Division of the body into a distinct proboscis and abdomen in juvenile/larva: absent (0), present (1).
- 45. Division of the body into a distinct proboscis and abdomen in adult: absent (0), present (1).
- 46. Introvert: absent (0), present (1).
- 47. Circular body musculature: absent (0), present (1). Circular body musculature is present in all taxa of nemathelminth grade except nematodes and nematomorphs, and its absence has been considered both secondary and a synapomorphy of Nematoida (Schmidt-Rhaesa 1998).
- 48. Ventral nerve cord unpaired throughout its length: absent (0), present (1). Living priapulids possess unpaired ventral nerve cords, whereas gastrotrichs, onychophorans, tardigrades and loriciferans possess ventral nerve cords that are paired throughout their length, and the ventral nerve cords of nematomorphs and nematodes divide at points along their length (Schmidt-Rhaesa 1998; Brusca and Brusca 2003); the situation in kinorhynchs is unresolved (paired according to Kristensen and Higgins 1991, unpaired

according to Neuhaus 1994). The condition in *Ottoia* is common to extant priapulids (Conway Morris 1977).

- 49. Ventral nerve cords merge caudally: absent (0), present (1).
- 50. Dorsal nerve cord unpaired: absent (0), present (1).
- 51. Cloaca in both sexes: absent (0), present (1).
- 52. Protonephridia: absent (0), present (1). Protonephridia are considered an apomorphy of the Bilateria (Ax 1996) and are present in gastrotrichs, kinorhynchs, loriciferans and extant priapulids, but absent, presumably secondarily, from onychophorans, tardigrades, nematodes and nematomorphs.
- 53. Protonephridia flow into the gonoduct and/or are integrated into the gonad (= urogenital system): absent (0), present (1). Lemburg (1999) recognized this as a synapomorphy of lorciferans and extant priapulids.
- 54. Urogenital system attached to the body wall by a ligament: absent (0), present (1). Lemburg (1999) recognized this as a synapomorphy of lorciferans and extant priapulids.
- 55. Spermatozoa with a flagellum: absent (0), present (1). The presence of a flagellum in spermatozoa is a metazoan symplesiomorphy, but a flagellum is lacking from the spermatozoa of nematodes and nematomorphs (Schmidt-Rhaesa 1998).
- 56. Locomotory cilia: absent (0), present (1). The presence of locomotory cilia is a symplesiomorphy of the clade, lost in onychophorans, tardigrades, nematodes, nematomorphs, kinorhynchs, loriciferans, and extant priapulids (Nielsen 2001).
- 57. Circumpharyngeal brain: absent (0), present (1).
- Apical part of the brain composed of perikarya: absent (0), present (1).
- 59. Brain with anterior-posterior sequence of perikarya-neuropil-perikarya: absent (0), present (1).
- 60. Two rings of introvert retractors attached through the collar-shaped brain: absent (0), present (1). Proposed by Nielsen (2001) as a synapomorphy of kinorhynchs, loriciferans and extant priapulids.
- 61. Aspect ratio of body length to width in adult: <10 (0), 10-20 (1), >20 (2).
- 62. Zone I armature arranged in rows aligned diagonal to the anterior-posterior axis of the animal: absent (0), present (1). This character is inapplicable to taxa lacking an introvert. It is independent of character 4.
- 63. Lorica of the larvae dorso-ventrally flattened (at least in older stages), with 6 lateral plates in-folded accordion-like: absent (0), present (1). This is contingent upon character 37.
- 64. Cuticle of the lorica thickened in dorsal and ventral plates (at least) with sculpture of four to six longitudinal rows of narrow, rectangular fields: absent (0), present (1). Lemburg (1999) recognizes the presence of this character as a synapomorphy of (extant) Eupriapulida. This is contingent upon character 37.

- 65. Caudal appendage single and positioned dorso-medially: absent (0), present (1). This is contingent upon character 31.
- 66. Pharyngeal nervous system comprised of numerous tooth ganglia connected by a diagonal nerve net: absent (0), present (1). Lemburg (1999) recognizes the presence of this character as a synapomorphy of (extant) Priapulida.
- 67. Larvae with six long pharynx retractor muscles: absent (0), present (1). Lemburg (1999) recognizes the presence of this character as a synapomorphy of (extant) Priapulida but it has since been demonstrated that both loriciferans and the larvae of nematomorphs also possess six pharyngeal retractor muscles (Kristensen 2003; Müller et al. 2004). Thus, their presence is likely a scalidophoran or cycloneuralian plesiomorphy.
- 68. Voluminous primary body cavity: absent (0), present (1). Lemburg (1999) recognizes the presence of this character as a synapomorphy of (extant) Priapulida.
- 69. Movement by peristaltic movement of the pharynx/ introvert: absent (0), present (1).
- Cone-like protrusible pharynx: absent (0), present (1). Lemburg (1999) recognizes the presence of this character as a synapomorphy of Tubiluchidae (*Tubiluchus+Meiopriapulus*).
- Introvert 30–50% of body length: absent (0), present (1). Lemburg (1999) recognizes the presence of this character as a synapomorphy of Megaintroverta (*Priapulopsis+Acanthopriapulus+Priapulus*).
- 72. Teeth of second circle of the larvae with very small median denticle: absent (0), present (1). Lemburg (1999) recognizes the presence of this character as a synapomorphy of Megaintroverta (*Priapulopsis+Acanthopriapulus+Priapulus*).
- 73. Pharyngeal lumina: round (0), triradiate (1).
- 74. Paired, lateral, locomotory appendages: absent (0), present (1).
- 75. Anterior branched frontal appendages: absent (0), present (1).
- 76. Annulation type: homonomous (0), heteronomous (1). Both Dzik and Krumbiegel (1989) and Budd (2003) have drawn comparison between the patterns of cuticular ornament in association with the trunk annulae in lobopods and palaeoscolecids. However, although palaeoscolecids exhibit an alternating pattern of cuticular ornament (Kraft and Mergl 1989; Conway Morris 1997b) and although this varies from one body region to another (Müller and Hinz-Schallreuter 1993), adjacent trunk annulae are identical; they exhibit homonomous annulation. This contrasts with the condition in lobopods where adjacent annulae vary both in terms of their axial length and their cuticular ornamentation (Whittington 1978; Budd 1999).

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- 77. Trilaminate epicuticle: absent (0), one repeat unit (1), multiple repeat unit (2).
- 78. Trilaminate epicuticle: juveniles only (0), all growth stages (1).
- 79. Cuticle predominantly containing collagen: absent (0), present (1).
- 80. Cuticle containing chitin: absent (0), present (1).
- 81. Cuticular distribution of chitin: chitin predominantly within middle cuticle layer (exocuticle) (0), chitin predominantly with lowermost cuticle layer (endocuticle) (1).
- 82. Ontogenetic distribution of cuticular chitin: larva/juvenile only (0), not restricted to growth stage (1).
- 83. Extent of cuticular chitin: foregut cuticle only (0), not restricted to foregut cuticle (1).
- 84. Cuticle with homogenous layer at surface/beneath epicuticle: absent (0), present (1).
- 85. Cuticle with fibrous/fibrillar basal layer: absent (0), present (1).
- 86. Cuticle with middle layer of distinct composition: absent (0), present (1).
- 87. Cuticle with radially striated/vertical canal middle layer: absent (0), present (1).
- Cross-wise fibers in cuticle: absent (0), present (1). Fine cross-wise fibers have been reported in *Meiopriapulus* (Storch et al. 1989).
- 89. Large helical fibers in cuticle: absent (0), present (1).
- 90. Construction of helical fibers from: platy subunits (0), unpaired fibrils (1), paired wound fibrils (2).
- 91. Cuticle surface with ornament of tessellating polygons: absent (0), present (1).
- 92. Conspicuous trunk sclerites: absent (0), in two longitudinal rows (1), in annular rings (2). This character describes prominent cuticular sclerites that are likely to have been biomineralized in life, although the style of preservation often leaves this ambiguous.
- 93. Terminally posterior spines, hooks or cones of basal diameter >20% trunk diameter: absent (0), present (1). The pairs of peri-anal structures in *Halicryptus* ("setae") and *Maccabeus* ("tubuli") are much smaller relative to body width (Por and Bromley 1974; Shirley and Storch 1999).
- 94. Terminally posterior spines, hooks or cones of basal diameter >20% trunk diameter arranged in pairs about the sagittal plane: absent (0), present (1). The character definition of "spines, hooks or cones" excludes the posterior body bifucations of *Aysheaia*, *Kerygmachela*, tardigrades, some adult nematomorphs, some kinorhynchs, and gastrotrichs.
- 95. Arc or ring of posterior spines or hooks: absent (0), present (1).

## Character codings

"-"represents inapplicable characters; "n/n" represents polymorphic character states; "?" represents unknown character states.

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## Are palaeoscolecids ancestral ecdysozoans? 199

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