

The anatomy, affinity, and phylogenetic significance of *Markuelia*

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SUMMARY The fossil record provides a paucity of data on the development of extinct organisms, particularly for their embryology. The recovery of fossilized embryos heralds new insight into the evolution of development but advances are limited by an almost complete absence of phylogenetic constraint. *Markuelia* is an exception to this, known from cleavage and pre-hatchling stages as a vermiform and profusely annulated direct-developing bilaterian with terminal circumoral and posterior radial arrays of spines. Phylogenetic

analyses have hitherto suggested assignment to stem-Scalidophora (phyla Kinorhyncha, Loricifera, Priapulida). We test this assumption with additional data and through the inclusion of additional taxa. The available evidence supports stem-Scalidophora affinity, leading to the conclusion that scalidophorans, cyclonerualians, and ecdysozoans are primitive direct developers, and the likelihood that scalidophorans are primitively metameric.

INTRODUCTION

The fossil record is largely a record of adult life and, thus, knowledge of embryological processes and mechanisms in extinct organisms has been restricted to phylogenetic inference—comparative analysis of extant representatives of lineages as a means of inferring the nature of their most recent common ancestor. As comparisons are made between progressively more distantly related organisms, so the reliability of the inferences based upon these comparisons become increasingly tenuous, often indicating incongruent correlations between traits (Strathmann 1993). Concomitantly, our ability to reliably unravel the evolution of development diminishes.

One means of testing developmental explanations for evolutionary events in Deep Time would be through the discovery of a fossil record of early development, and recent years have witnessed a shift in expectations, from impossibility to plausibility. This began, rather inauspiciously, with the description of some rather poorly, although nevertheless remarkably, preserved cleavage stage embryos, attributed to co-occurring trilobites, from the middle Cambrian (Zhang and Pratt 1994; Pratt and Zhang 1995). These discoveries were followed in quick succession by rather more convincing finds, including the complete life cycle of an early Cambrian scyphozoan cnidarian (Bengtson and Yue 1997; Yue and Bengtson 1999; Hua et al. 2004; Steiner et al. 2004a), and late-stage embryos of the early Cambrian bilaterian metazoans, *Markuelia* and *Pseudooloides* (Bengtson and Yue 1997; Steiner

et al. 2004b). Very early cleavage-stage embryos of presumed metazoans and, possibly, bilaterian metazoans, have been recovered from the late Neoproterozoic (Xiao et al. 1998; Zhang et al. 1998; Xiao and Knoll 1999, 2000; Xiao 2002; Yin et al. 2004), along with some rather more dubious records (Chen et al. 2000; Chen et al. 2002) the veracity of which has been seriously questioned (Xiao et al. 2000; Bengtson 2003). Early Cambrian cnidarian-like embryos have also been described (Kouchinsky et al. 1999).

However, excitement surrounding the promise of these new discoveries has been tempered by frustration with the lack of phylogenetic constraint; most of these discoveries preserve stages of development that are simply too early to determine affinity and, thus resolve evolution or conservation in patterns and processes of development. One exception is *Olivoooides*, for which much of the life cycle has been reconstructed from hitherto known but taxonomically discrete stages, linking cleavage embryos to coronate scyphozoan-like cnidarian adults (Bengtson and Yue 1997; Yue and Bengtson 1999). The other exception is *Markuelia*, described by Bengtson and Yue (1997) as an annulated, bilaterally symmetrical vermiform animal represented by only relatively late-stage embryos. Indeed, the embryos are preserved at a sufficiently late stage for viable considerations of affinity. Bengtson and Yue (1997) compared *Markuelia* with arthropods and lophopods (a grade of organisms including the living onychophorans), and especially the annelids, whereas Conway Morris (1998a) plumbed for an affinity with the extinct halkieriids, a

putative grade of lophotrochozoans including members of the mollusc, annelid and brachiopod total groups, including stem members of clades comprising combinations of these phylum level total groups (Conway Morris and Peel 1995). More recently we published a preliminary report on new collections of *Markuelia* from the Middle and late Cambrian of Hunan, south China, which include a greater range of developmental stages than were known hitherto (Dong et al. 2004). These provide further constraint over the affinity of *Markuelia* and we argued for assignment to Scalidiophora, a clade composed of the phyla Kinorhyncha, Loricifera, and Priapulida, and constituting a sister clade to Panarthropoda, possibly including Nematodea (Nematoda plus Nematomorpha). Here we provide a more complete description of *Markuelia*, including data from recently recovered material, and a further consideration of the affinity of *Markuelia* and its significance in uncovering the evolution of development.

MATERIALS AND METHODS

All of the described material is from the middle and late Cambrian of Wangcun, Hunan Province, south China. The embryos are preserved in calcium phosphate and were recovered from limestone using 10% buffered acetic acid. Several hundred embryos have so far been recovered from approximately 8000 kg of rock, together with several thousand more phosphatic spheres of approximately the same size that are identical to the preserved membrane enveloping many of the specimens that are verifiably embryos. The specimens were studied under a scanning electron microscope. The figured specimens are deposited in the Geological Museum of Peking University, Beijing, China (GMPKU) and the Swedish Museum of Natural History, Stockholm, Sweden (NRM).

FOSSILIZED DEVELOPMENTAL STAGES OF *MARKUELIA HUNANENSIS*

Cleavage embryos

The earliest developmental stages present in our collections are cleavage embryos that preserve the surface boundaries between adjacent blastomeres (Fig. 1A) or the blastomeres themselves (Fig. 1B). These embryos are approximately the same size as the late-stage embryos of *Markuelia* and, given that rapidly developing embryos of metazoans invariably maintain a constant volume of cytoplasm and yolk throughout embryogenesis, the attribution of these cleavage embryos to *Markuelia* is reasonable. Cleavage embryos are exceedingly rare, represented by just three out of the collection of several hundred specimens.

Organogenesis

A small number of the recovered embryos include later stages of development in which the adult characters have begun to unfold. These include embryos in which cell boundaries are

not discernable (for developmental or taphonomic reasons) but the surface layers are nevertheless organized into a paired “S” loop (Fig. 1C). In later stages, features of the adult, including a homonomously annulated, or possibly segmented, trunk are present (Fig. 1D). The condition of the head and tail is not revealed in these specimens, although they may have developed by this stage. The full volume of the sphere is not, however, occupied by the embryo, adjacent portions of the characteristically “S”-coiled trunk separated by intervening undifferentiated fields (Fig. 1D), which we interpret as yolk.

Prehatching

The vast majority of embryos represent very late developmental stages in which there are no undifferentiated fields present in the embryo. These embryos range in diameter from 340 to 490 μm (not including specimens that have obviously undergone post mortem shrinkage or other deformation, presumably as a result of dehydration, e.g., Fig. 1E). The embryos are sufficiently tightly coiled into an “S”-shaped loop that the lateral margins of the trunk are directly juxtaposed (Fig. 1F). The trunk varies in width from 150 to 240 μm in well-preserved specimens, to less than 100 μm in specimens that have clearly undergone post mortem shrinkage. Fractured specimens reveal that the trunk was compressed with an ovoid cross-sectional profile (Fig. 1, G and H). However, it is possible that the trunk had a circular cross-sectional profile in vivo and that the ovoid outline is an artifact of close packing.

Transverse annulations (Fig. 1, D and F–I) range in anterior–posterior length from approximately 10 to 25 μm , depending upon the degree of curvature of the trunk. The outer surfaces of the annuli are ornamented with 0.3–0.5- μm anastomosing ribs, which are aligned to the anterior–posterior axis of the animal (Fig. 1I). Annuli exhibit varying degrees of inflation, reflecting differing states of preservation, but fractured specimens indicate that the surface divisions between the successive annulae extend internally as shallow septa with a finite depth of approximately 5 μm (Fig. 1G). The constant depth of these divisions suggests that their extent is a reflection of original anatomy rather than preservational artifact.

In some specimens small spines (approximately 1- μm diameter, 3–5- μm length) emerge from circular pores in the trunk with perpendicular aspect (Fig. 2A). The spines have closed tips and exhibit fine annular banding.

The presumed posterior is characterized by a terminal spine-bearing region surrounding a central depression (Figs. 1, E and F and 2, B and C). There are a total of six hollow curved spines arranged so that their long axes are parallel to, and concave margins and tips are directed away from, the anterior–posterior axis of the animal. The two smaller, straight spines were positioned within the terminal depression and arranged bilaterally. The four larger curved spines are curved approximately the same direction (towards the

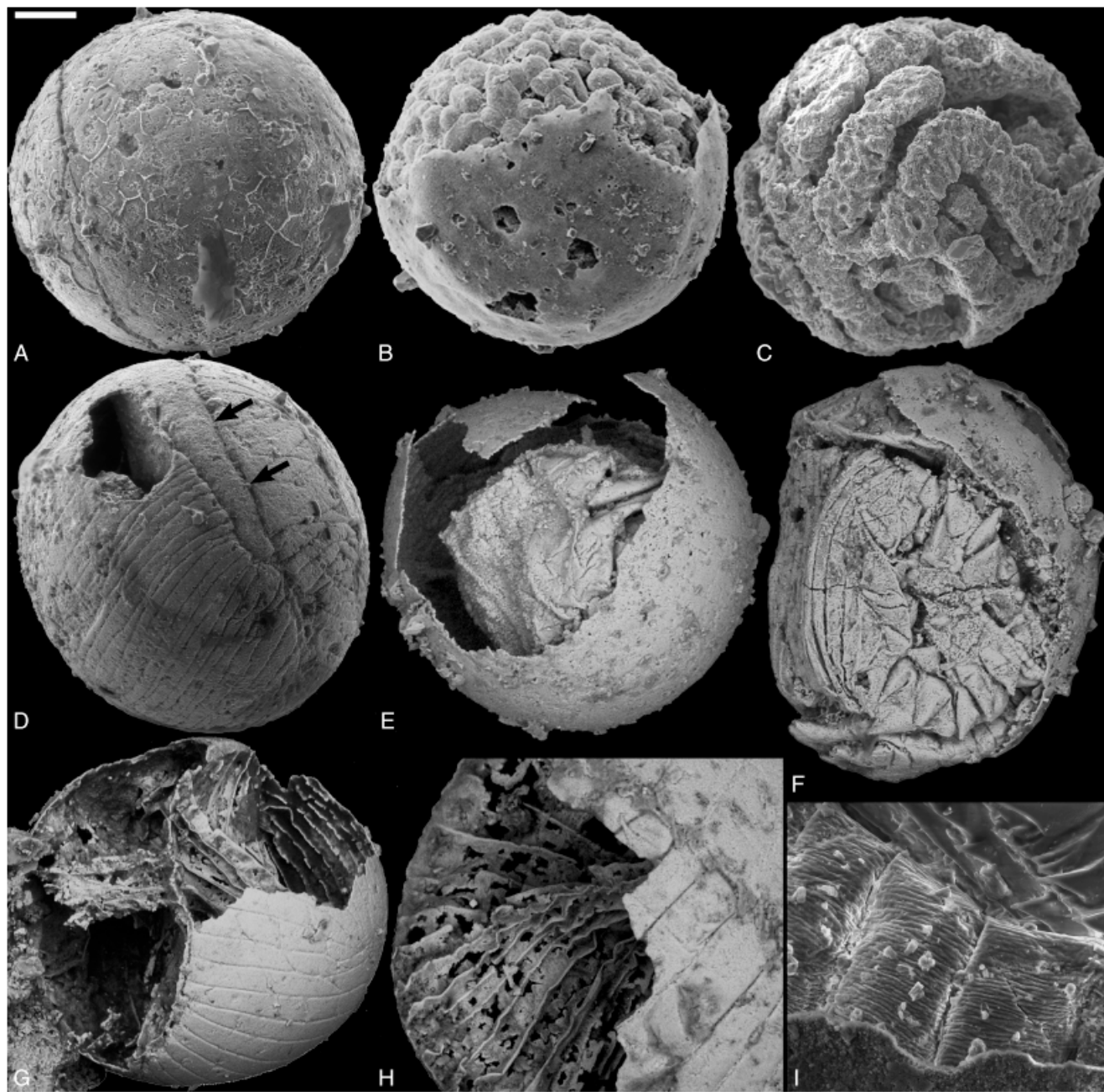


Fig. 1. Embryos of *Markuelia hunanensis* from the middle and late Cambrian Bitiao Formation at Wangcun, Hunan Province, south China. (A) Cleavage-stage embryo exhibiting surface boundaries between blastomeres (GMPKU2007). (B) Cleavage stage embryo in which the blastomeres themselves have been preserved (GMPKU2012). (C) Body outline established (GMPKU2014). (D) Annulation established but yolk (arrowed) still present (GMPKU2008). (E) Embryo enveloped by chorion and exhibiting post-mortem shrinkage (GMPKU2022). (F) Late embryo in which head (center) and tail (lower) are juxtaposed (GMPKU2015). (G) Fractured embryo revealing cross-sectional profile of trunk (GMPKU2009). (H) Septa between annulations extending internally (GMPKU2016). (I) Surface ornamentation of annuli (GMPKU2010). Relative scale bar: (A) 39 μm , (B) 69 μm , (C) 37 μm , (D) 67 μm , (E) 49 μm , (F), 48 μm , (G), 59 μm , (H), 34 μm , (I), 12 μm .

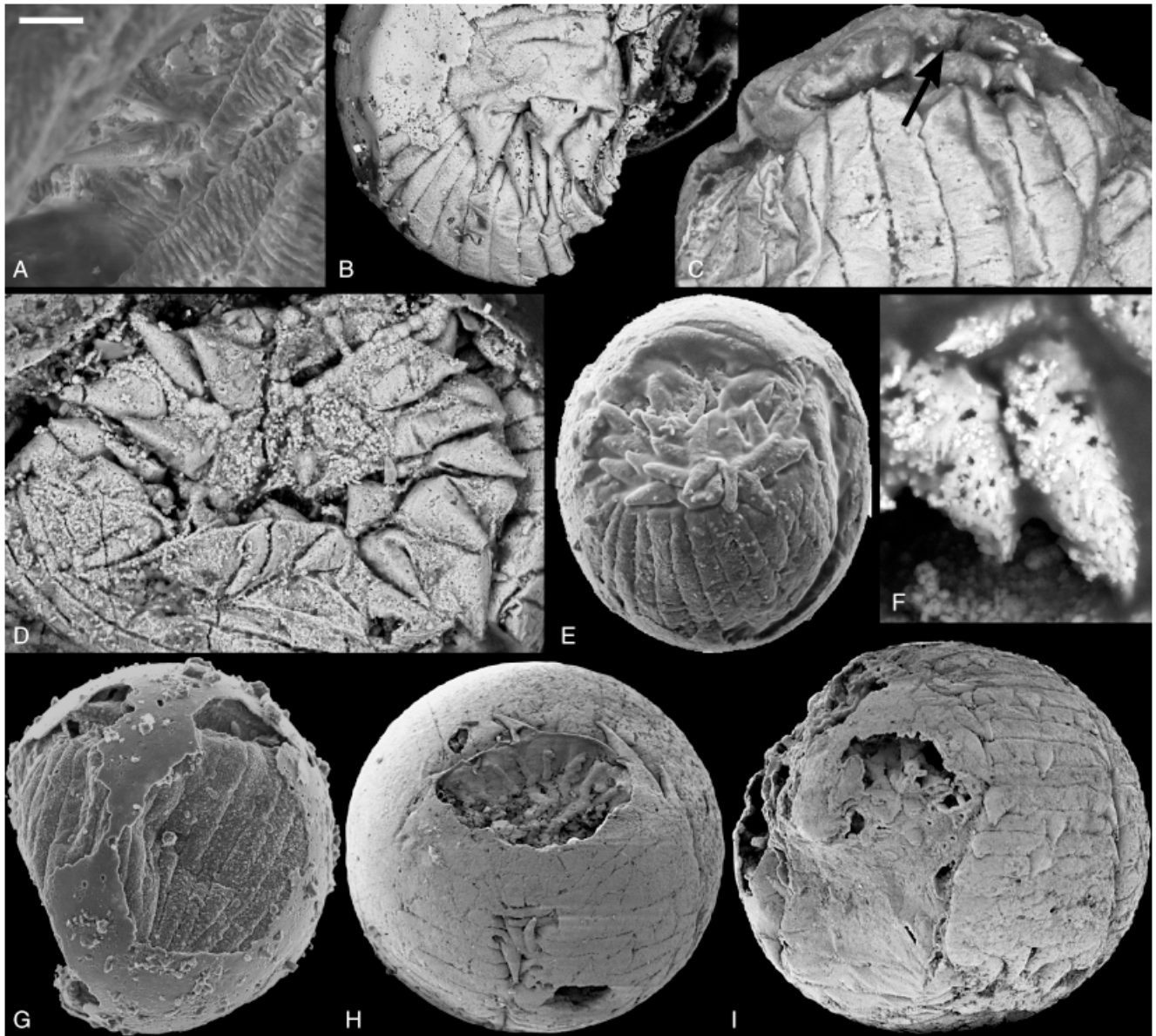


Fig. 2. (A–G) Late-stage embryos of *Markuelia hunanensis* from the middle and late Cambrian Bitiao Formation of Wangcun, Hunan Province, south China. (A) Spine emerging from the surface of the trunk (GMPKU2010). (B) The six terminal spines associated with the posterior (GMPKU2017). (C) View of posterior pole with appendages surrounding central depression (arrowed) (GMPKU2018). (D) Detail of the collapsed anterior pole exhibiting a broadly radial array of multiple circumoral rows of spines (GMPKU2015). (E) Anterior pole exhibiting multiple circumoral rows of spines (GMPKU2019). (F) Detail of spinose scalids (GMPKU2020). (G) Embryo still largely enveloped by chorion (GMPKU2021). (H, I) Embryos of *M. secunda* from the early Cambrian Pestrosvet Formation at Dvortsy, Siberia [reproduced from Bengtson and Yue 1997, with the permission of the authors and publishers]. (H) Fracture revealing serially repeated internal organs in register with surface annuli (NRM X2239). (I) Anterior (left) and posterior (right) extremities juxtaposed; note the conical protuberances emerging from the surface of the trunk (NRM X2240). Relative scale bar: (A) 6 μm , (B) 23 μm , (C) 29 μm , (D) 27 μm , (E) 22 μm , (F) 10 μm , (G) 68 μm , (H) 93 μm , (I) 84 μm .

embryo), rather than arranged radially, as argued by Dong et al. (2004). The spines range in length, from 50 to 95 μm , between specimens and exhibit a smooth surface, distinct from the ribbed surface of the trunk (Figs. 1, E and F and 2, B and C). The portion of the trunk immediately adjacent to the spines is not strongly annulated. This region is interpreted as

the posterior pole of the animal because of the orientation of the spines away from, rather than towards, the animal.

The presumed anterior pole is consistently the least well-preserved aspect of the embryo's anatomy (Figs. 1, F and 2, D–F). It, too, is characterized by a terminal spine-bearing region, although the spines are much smaller than those at

the posterior (ranging 11–40 μm in width and 27–50 μm in length), posteriorly directed, and are arranged radially in a series of successive, partially overlapping rows. The original description (Dong et al. 2004) equivocated over the arrangement of these spines because the anterior pole in the holotype was partially obscured by collapse, diagenetic mineral overgrowth and uneven gold coating. Further specimens preserving this region confirm the radial arrangement of the spines (Figs. 1, F and 2, D and E). It is also now possible to determine that there are many more circlets of spines than were originally described. The precise number remains unclear, but at least six rows are visible in some specimens (Fig. 2E). Similarly, the number of elements present in each circlet is unclear. The spines are hollow and are flattened in cross-sectional profile (Fig. 1, F and 2, D), oriented with the long axis at a tangent to the surface of the trunk. One specimen (Fig. 2F) exhibits spines with surface spinelets; this may represent a later developmental stage of *M. humanensis* or it may represent another species. The successive rows are slightly offset from one another (Figs. 1, F and 2, D and E) but, on the basis of the available data, it is not possible to tell whether spines in different rows are in register or not. The anterior pole itself is characterized by an orifice, preserved in only two specimens where it is 30 and 47 μm in diameter, respectively. The mouth exhibits a radial folding pattern, comparable with the oral cone of extant priapulids (Lemburg 1995) suggesting eversibility. This region is interpreted as the anterior pole of the animal because of the alignment of the spines away from this pole, towards the inferred posterior pole. In all specimens the anterior pole shows evidence of decay-related collapse and, hence, the mouth itself is preserved immediately sub-terminally. However, considering collapse, and the circumorally arranged rows of spines that continue around to the lower, obscured surface, it is clear that in vivo the mouth was positioned terminally.

Chorion

The greater proportion of embryos in our collections are smooth spheres. In some instances, a small portion of the surface has been removed by attrition (presumably during laboratory preparation) revealing an anatomically distinguishable embryo within (Fig. 2G). In other cases, the spheres are empty, or include phosphatic thread-like structures comparable with those seen in embryos from late Neoproterozoic Doushantuo Formation (Xiao and Knoll 1999) and early Cambrian Kuanchuanpu Formation (Yue and Bengtson 1999), where they have been interpreted as filamentous bacteria, fungal hyphae, or decayed organic matter. However, in most instances the sphere is preserved intact and the contents are unknown, or can be determined only by means of oil immersion transmitted light microscopy (Donoghue 1997). We conclude, therefore, that these spheres are chorions,

membranes that enveloped the developing embryo in vivo, also referred to as egg capsules in some of the embryology literature. Thus, the number of embryos preserved in our collections greatly exceeds the few hundred specimens that are demonstrably embryos on the basis of exposed morphology. There is no evidence of division of the membrane.

COMPARISON WITH *M. SECUNDA* AND THE NATURE OF *MARKUELIA*

M. secunda was first described as a fossilized embryo by Bengtson and Yue (1997) although it was first figured and described in abstract terms by Val'kov (1983), and figured many times subsequently (see Bengtson and Yue 1997 for a listing). *M. secunda* is known only from stages equivalent to the prehatching stage of *M. humanensis*, as described above (Fig. 2, H and I). In particular, the long, slender, profusely (and homonomously) annulated body form is a key common feature, as is its size and coiling into an "S"-shaped loop, with head and tail juxtaposed, to comprise the spherical embryo (Fig. 2I). The morphology of the anterior of *M. secunda* is poorly known because this region is not as well preserved as other aspects of anatomy (Fig. 2I), suggesting a common decay pathway for species of *Markuelia*. However, Bengtson and Yue note the presence of a boss-like structure amongst the collapsed remains of the anterior pole that might represent a partially preserved introvert (Fig. 2I). The posterior pole of *M. secunda* also bears curved spines (Fig. 2I), which have been described as occurring in two, bilaterally disposed pairs which, together with trunk spines (Fig. 2I) are the key features distinguishing the two species.

The overwhelming similarity between *M. secunda* and *M. humanensis* fully justifies their referral to the same genus. The combined data from the two known species allow us to further resolve the anatomy of *Markuelia*. In particular, specimens of *M. secunda* figured by Bengtson and Yue (1997; Fig. 2H) preserve internal organs exhibiting repeated organization that is in register with the surface annulations. These are rod-like and are aligned with the surface annuli, although Bengtson and Yue report that in some specimens they are oblique to the annuli and/or detached from the body wall. Their interpretations include gut diverticulae, blood vessels, muscles, nerves, nephridia, or gonads; however they preferred an interpretation as lateral branches of a medial longitudinal nerve chord. In any instance, perhaps the greatest significance of these structures is that they indicate that *Markuelia* possesses a metameric organization, rather than simply a superficial cuticular annulation.

THE AFFINITY OF *MARKUELIA*

Dong et al. (2004) used preliminary data on *M. humanensis* to test between and, ultimately, reject the proposed hypotheses

of close affinity between *Markuelia* and arthropods, lobopods, annelids (Bengtson and Yue 1997), and halkieriids (Conway Morris 1998a, b, 2000). Instead, they drew comparison between the circumoral rows of spines and terminal (possibly eversible) mouth in *Markuelia* and the circumoral scalid-bearing introvert and terminal eversible mouth of the clade Introverta. Introverta is composed of the phyla Nematoda, Nematomorpha, Kinorhyncha, Loricifera, and Priapulida. Some molecular phylogenies suggest that Panarthropoda (Tardigrada, Onychophora, Arthropoda) may be an integral member of this clade (Aguinaldo et al. 1997; Giribet et al. 2000; Garey 2001; Mallatt et al. 2004) although one in which the introvert complex has been lost. Indeed, various basal members of the arthropod stem possess a terminal mouth and in some of these there is evidence for radially arranged appendages (Whittington 1978; Ramsköld and Chen 1998; Budd 2002), a protrusible pharynx (Budd 1999), and/or a pharyngeal armature (Whittington and Briggs 1985; Hou et al. 1995). In addition, the cephalic nervous system of extant priapulids can be interpreted as derived from the circumpharyngeal nerve ring that characterizes this clade (Eriksson and Budd 2000). Thus it is possible that the characters exhibited by *Markuelia* are ecdysozoan symplesiomorphies, and no guide to relationships.

However, it has been argued that an introvert and associated scalids may be a convergent feature. For instance, homology of the introvert and scalids between Scalidophora (Kinorhyncha, Loricifera, Priapulida) and Nematoidea (Nematoda, Nematomorpha) has been disputed on the basis that scalids are innervated, hollow and pentaradially arranged in Scalidophora, whereas in Nematoidea scalids are simple epicuticular thickenings and hexaradially arranged (Schmidt-Rhaesa 1998), although exceptions to this neat dichotomy have recently come to light (Gad 2005). It is not possible to tell whether the introvert was inversible in *Markuelia*. Neither is it possible to determine whether the mouth/pharynx was eversible, as it is in extant priapulids, but not loriciferans nor kinorhynchs. However, the circumoral spines of *Markuelia* were hollow, as evidenced by their collapse (e.g., Fig. 2D), and therefore not simple epicuticular thickenings, suggesting that they are more comparable with the scalids and sensory spines of Scalidophora than the scalids of Nematoidea. The possession of multiple rows of introvert scalids also supports a close relationship between *Markuelia* and Scalidophora; the inferred eversible mouth/pharynx suggests a still closer relationship between *Markuelia* and Priapulida.

However, the direct mode of development exhibited by *Markuelia* contrasts with the indirect developers that characterize Priapulida and Loricifera, which pass through a larval stage (secondary larvae sensu Jägersten 1972) enveloped in all but the introvert by a lorica composed of a small number of cuticular plates. Direct development has been reported in one species of priapulid, *Meiopriapulius fijiensis* (Higgins and

Storch 1991), but its embryo is as morphologically distinct from the adult and, therefore, *Markuelia*, as are loricate larvae. Nematomorphs, too, undergo indirect development, but their larvae bear a strong resemblance to adult priapulids (Hyman 1951; Bohall et al. 1997) and to embryos of *Markuelia*. Like *Markuelia*, kinorhynchs and the vast majority of nematoids undergo direct development, but their embryos and juveniles bear little resemblance to *Markuelia* embryos, although they are known to coil into an “S”-shaped loop, as per *Markuelia*, as the embryo enlarges within the confined space of the chorion (Tadano 1968).

Markuelia clearly exhibits a conflicting suite of characters which suggests that at least some of these characters currently considered synapomorphic for particular clades must be symplesiomorphies—synapomorphies of much more inclusive clades. Which characters have a much wider distribution is potentially resolvable through cladistic analysis of living representatives of the extant clades in combination with *Markuelia*, plus the various extinct taxa that have been allied to these clades. Dong et al. (2004) undertook a preliminary analysis in an attempt to resolve the affinity of *Markuelia*, but did not fully consider the implications of the results of this analysis for character evolution. We have developed this data set and the results of its analysis are presented.

Phylogenetic analysis

The most substantive change to the data set was the inclusion of additional fossil taxa from the Lower Cambrian Chengjiang Lagerstätte. These are *Corynetis brevis* (= *Anningvermis multispinosus*), *Palaeopriapulites parvus*, *Sicyophorus rara* (= *Protopriapulites haikouensis*), *Xiaoheiqingella peculiaris*, and *Yunnanpriapulites halteroformis*, most of which had been described previously, but for which detailed descriptions, or figures, are now available (Han et al. 2004; Hou et al. 2004; Huang et al. 2004a, b); *Acosmia maotiania* remains poorly known and *Paraselkirkia* shows no obvious distinction from *Selkirkia*, which was already present in the data set presented by Dong et al. (2004). *S. rara* is of interest because although it is macroscopic, larger in fact than the adults of many extant priapulid species, it is more comparable with the larval stages of extant priapulids in possessing a lorica, which envelopes approximately half of the animals' posterior anatomy. Although it is possible that *S. rara* represents the larval stages of adults known from the Chengjiang, it differs from most other Cambrian priapulid-grade organisms in exhibiting introvert scalids that are arranged into discrete longitudinal rows. Similarly, the possibility exists that *Markuelia* represents the embryo of one of the known fossil priapulid-grade taxa. However, *Markuelia* exhibits autapomorphic characters that are not readily set aside as juvenile features. In particular, no known fossil adult shows the same arrangement of posterior spines seen in *Markuelia* and so there is justification in considering it a distinct taxon.

Phylogenetic analyses of the data set were performed using PAUP 4.0b10 (Swofford 2002) and rooted on a paraphyletic out-group composed of Gastrotricha, *Peripatus*, *Kerygmachela* and *Aysheia*. Gastrotricha was included because it has traditionally been considered a close relative of the ingroup.

However, molecular phylogenies suggest a much closer relationship between Panarthropoda (Tardigrada, Onychophora, Arthropoda) and extant members of the ingroup. Thus, we included codings for both possible out-groups to constrain for these alternatives. Analysis of the data set using an heuristic

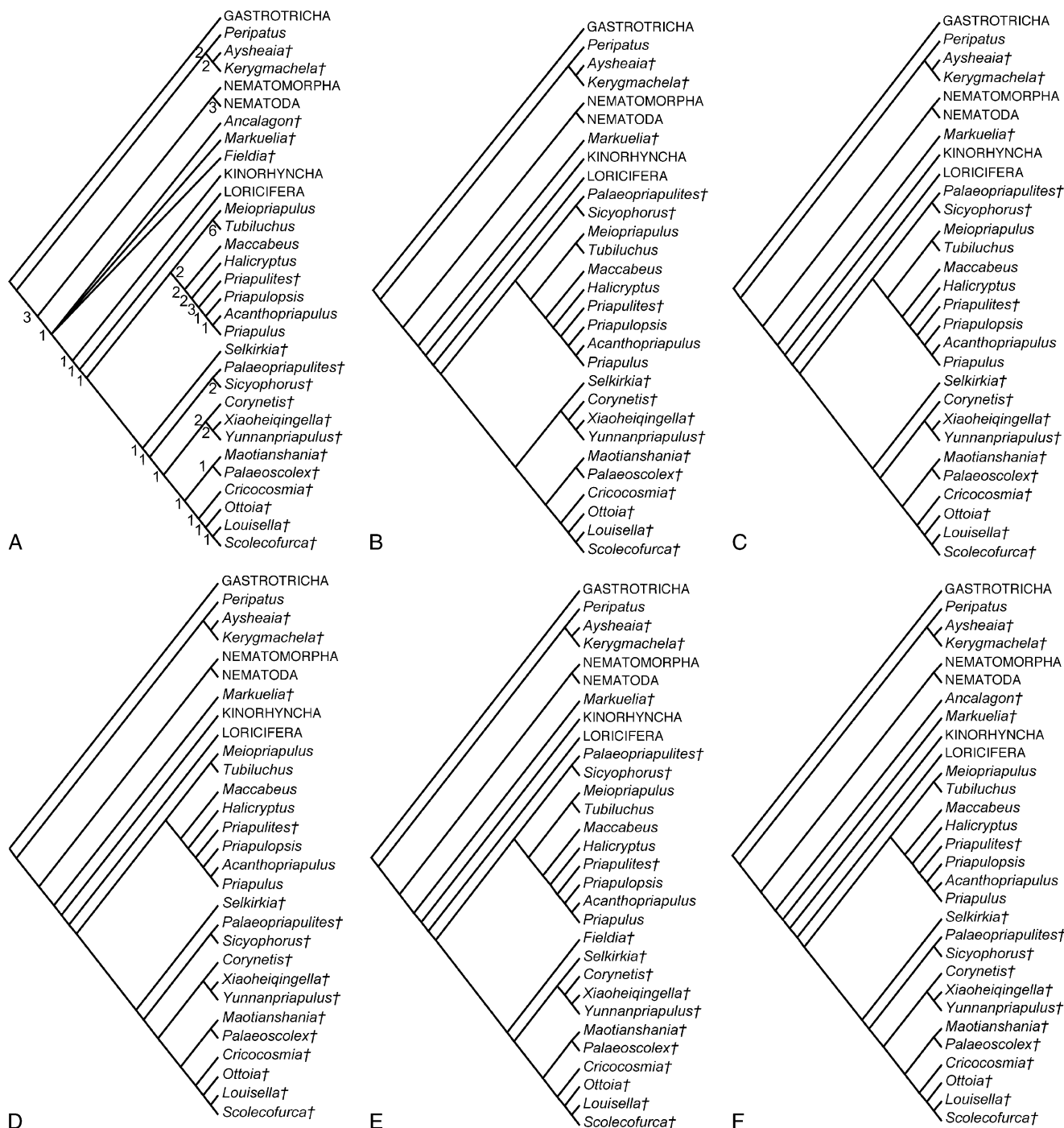


Fig. 3. (A–F) Cladograms arising from the phylogenetic analysis. (A) Strict consensus of three equally most parsimonious trees (MPTs). (B–D) Three MPTs derived from analysis of the data set excluding both *Ancalagon* and *Fieldia*. (E) MPT derived from analysis excluding *Ancalagon*. (F) MPT derived from analysis excluding *Fieldia*.

search (100 replicates of random sequence addition with 10 trees retained at each step) yielded three equally Most Parsimonious Trees (MPTs) (193 steps; CI: 0.5638; RI: 0.7363), the strict consensus of which is presented in Fig. 3A (note: exclusion of *Gastrotricha* from the out-group does not impact upon this or other results). This resolves *Markuelia* as a member of stem-Scalidophora as per Dong et al. (2004). However, the overall topology is extremely sensitive to the inclusion of *Fieldia* and *Ancalagon*, particularly with respect to *Corynetis*, *Palaeopriapulites*, *Sicyphorus*, *Xiaoheiqingella*, and *Yunnanpriapululus*, all of which have recently been considered close relatives of crown-Priapulida (either terminal members of the stem or of the crown itself (Han et al. 2004; Huang et al. 2004a, b). Parsimony analysis of a data set excluding both taxa produces three MPTs (186 steps; CI: 0.5746; RI: 0.7363) in which the clade (*Palaeopriapulites*+*Sicyphorus*) is resolved as the sister-taxon to crown-Priapulida and *Selkirkia* as the sister-taxon to a clade (*Corynetis* (*Xiaoheiqingella*+*Yunnanpriapululus*)) (Fig. 3B), or else *Selkirkia* is resolved as the sister-taxon to this latter clade plus all remaining stem-Priapulida (Fig. 3C), or *Selkirkia* is resolved as the sister-taxon to all these taxa plus (*Palaeopriapulites*+*Yunnanpriapululus*) (Fig. 3D). Exclusion of *Ancalagon* yields a single MPT (190 steps; CI: 0.5676; RI: 0.7342) in which (*Palaeopriapulites*+*Yunnanpriapululus*) are resolved as the sister-group to crown-Priapulida and *Fieldia* as the sister-taxon to an otherwise monophyletic stem-Priapulida (Fig. 3E). Exclusion of *Fieldia* alone yields a single MPT (190 steps; CI: 0.5730; RI: 0.7393) with a topology common to that including both *Ancalagon* and *Fieldia* (Fig. 3F).

The paraphyly of Panarthropoda+Nematoidea agrees with the results of many morphology-based cladistic analyses (Schmidt-Rhaesa et al. 1998; Peterson and Eernisse 2001; Zrzavý 2003), but contrasts with most molecular phylogenetic analyses which resolve a monophyletic (Panarthropoda+Nematoidea) (Aguinaldo et al. 1997; Giribet et al. 2000; Garey 2001). In part, this reflects equivocation over the internal relationships of Ecdysozoa, but also over the validity of Ecdysozoa (Blair et al. 2002; Copley et al. 2004; Telford 2004; Wolf et al. 2004). To test whether acceptance of Nematoidea+Panarthropoda monophyly would have any effect upon the affinity of *Markuelia* we implemented this hypothesis as a backbone constraint tree on the analysis of the data set including only extant taxa plus *Markuelia*. Branch and bound analysis yielded four equally MPTs at 130 steps (three steps longer than the unconstrained analysis) in which *Markuelia* is resolved alternately as the sister-taxon to Scalidophora, to *Peripatus* plus Nematoidea, to *Peripatus*, and to Nematoidea. Heuristic analysis of the entire data set yielded four equally MPTs, but in each instance *Markuelia* was resolved as stem-Scalidophora, although *Fieldia* and *Ancalagon* were resolved as either stem-Scalidophora, or stem-Cycloneuralia (including Panarthropoda).

Although the affinity of *Markuelia* remained unchanged throughout these analyses it is pertinent to consider support for its phylogenetic position. We determined Bremer Support (Bremer 1994) for the primary analysis using RadCon (Thorley and Page 2000). The results, presented in Fig. 3A, indicate that inclusion of *Markuelia* within Cycloneuralia is well supported. However, Double Decay Analysis (Wilkinson et al. 2000), which examines the stability of support for the interrelationships of taxa based on all component subtrees, reveals *Markuelia* to be the least stable taxon within the analysis (leaf stability index of 1.83, compared with an average of 3.47). Undoubtedly, instability relates to the large amount of missing data on the embryology and anatomy of *Markuelia*.

DISCUSSION

Equivocation over the phylogenetic position of *Markuelia* arises not because our knowledge of its anatomy is restricted to embryos, but because crucial aspects of its anatomy remain unknown, in particular, the symmetry of introvert scalid arrangement, and whether or not scalids are present in the pharynx. For instance, if it were assumed that the hexaradial arrangement of the posterior spines in *Markuelia* belies a common arrangement of pharyngeal elements (character 54:0 > 1), equivocation over the phylogenetic position of *Ancalagon*, *Fieldia*, and *Markuelia* would be resolved with *Markuelia* as the most plesiomorphic. Very different hypotheses of affinity may arise with the resolution of further aspects of the anatomy of *Markuelia*, not least in terms of whether it possessed an armored pharynx and, if so, the number, morphology and arrangement of the dental elements. Indeed, in conceiving the stem-group concept Hennig (1981) indicated that stem-membership could be achieved in two ways, either through the correct assignment of fossil taxa as intermediates of two crown-groups, or through the imperfect preservation of fossil remains such that extinct members of the crown group fail to exhibit the full complement of characters diagnostic of membership; in the latter instance, stem-membership is merely a qualified statement concerning ignorance of an extinct taxon's systematic affinity. Further elucidation of the nature of *Markuelia* will provide a means of testing between these two alternatives.

Thus, although equivocation exists, it is limited in scope, and all hypotheses of relationships place *Markuelia* in a basal position within Cycloneuralia (including Panarthropoda). It is significant that, even when the Panarthropoda+Nematoidea is implemented, *Markuelia* is still resolved as a stem-Scalidophoran when analysis of the entire data set is undertaken.

EVOLUTION OF LIFE HISTORY STRATEGIES

Previous suggestions for the affinity of *Markuelia* have been articulated with respect to its significance for understanding

the evolution of life history strategies in Metazoa. Conventionally, metazoans are thought to have evolved from a wholly pelagic ancestor whose descendants adopted a benthic adult-stage phase while maintaining a pelagic larva (Haeckel 1874; Jägersten 1972; Nielsen and Nørrevang 1985; Rieger 1994; Nielsen 2001). This view is supported by the preponderance of planktotrophic larvae among animal phyla (Strathmann 1987) and the homology of the larva's component ciliary bands which are used in locomotion and feeding (Nielsen 1987). It also forms the basis of "set aside theory," the hypothesis which seeks to explain the "Cambrian Explosion" phenomenon by the coincident origin of metamorphosis and an adult stage among the many disparate lineages of metazoan phyla, after their divergence deep within the Proterozoic (Davidson et al. 1995; Peterson et al. 2000). However, although planktotrophic larval stages are widespread, it does not follow that they represent the primitive condition for phyla nor Bilateria. Olive (1985), Emlen (1991), Strathmann (1993, 2000), and Rouse (1999), among many others, have argued that similarities in the topology of the ciliary bands are the product of convergence rather than shared ancestry. This view has been corroborated by histological and molecular data contributing to more rigorous phylogenetic analyses (Haszprunar et al. 1995; Peterson 2004). Although this appears to render set aside theory untenable (Conway Morris 1998a, 2004), lecithotrophic larvae and, thus, indirect development remains the plesiomorphic life history strategy for bilaterians and the majority of bilaterian phyla (Rieger 1994; Haszprunar et al. 1995).

Both Bengtson and Yue (1997) and Conway Morris (1998a, b) have questioned the plesiomorphy of planktotrophy and indirect development largely on the basis of a lophotrochozoan affinity for *Markuelia*. However, as Dong et al. (2004) have argued, such an affinity is untenable, and the direct mode of development exhibited by *Markuelia* is not incongruent with its proposed relationships to ecdysozoans in general and cycloneuralians more specifically. However, although *Markuelia* represents but one lineage among many metazoans, and although its life history strategy is broadly compatible with other ecdysozoans, it does not necessarily follow that it has no impact upon understanding of the evolution of development.

Resolving the life history strategy of the latest common ancestor of crown Scalidophora is complicated by character conflict between Kinorhyncha, which exhibit direct development, and Vinctiplicata (Loricifera+Priapulida), which exhibit indirect development. This can potentially be resolved with reference to out-groups such as Nematodea, Panarthropoda, and Gastrotricha, though, as discussed above, the precise relationships of these taxa to Scalidophora are disputed. The resolution of *Markuelia* as a stem-scalidophoran shifts the balance of character distribution within total-group Scalidophora so leading to the conclusion that the latest common ancestor of the crown-group was a direct developer.

This corroborates the conclusion that Cycloneuralia are also direct-developers plesiomorphically, whether they include Panarthropoda or not.

ANNULATION, METAMERISM, SEGMENTATION

There has been much discussion surrounding the phylogenetic origins and distribution of segmentation throughout Metazoa, not least in light of the discovery of the vicarious function of *engrailed* in establishing metamerism in deuterostomes as well as protostomes (De Robertis 1997; Holland et al. 1997; Davis and Patel 1999; Dewel 2000). The fossil record provides one of the few conceivable tests of the characteristics of such hypotheses, not because fossil taxa are ancestors, but because fossil taxa are in some instances close relatives of the common ancestors of now disparate clades. Such taxa provide a greater insight into the nature of long extinct ancestors than is available from extant relatives, not least because they do not carry the baggage of half a billion years of lineage-specific evolution. Thus, it is increasingly accepted that the last common ancestor of extant bilaterians was not segmented and that the concept of segmentation covers a broad church of conditions and expressions many of which have distinct developmental bases (Budd 2001; Minelli and Fusco 2004). Nevertheless, metamerism, a more generic concept of repeated anatomical patterning, is homologous at a more local phylogenetic scale, and various attempts have been made to resolve the origin(s) of segmentation in groups such as Arthropoda, including comparisons with groups such as the nematoids and scalidophorans that variably exhibit metamerism associated with the cuticle, musculature and/or the nervous system (Budd 2001; Nielsen 2003; Müller et al. 2004; Rothe and Schmidt-Rhaesa 2004).

Comparisons have often been drawn between metamerism in arthropods and scalidophorans, in particular the kinorhynchs. All cycloneuralian phyla, bar Loricifera, exhibit cuticular annulations at the very least, including Nematoda (Wright 1991) which also exhibit metameric character in their nervous system (Johnson and Stretton 1980).

The inclusion of various extinct scalidophoran-grade taxa into the phylogenetic milieu enriches this perspective on the distribution of metamerism among Cycloneuralia because the vast majority of these taxa exhibit cuticular metamerism. Thus, they reinforce the plesiomorphy of this character in Priapulida and Scalidophora, and the extension of these characters deep into the Scalidophoran-stem through the inclusion of *Fieldia*, *Ancalagon* and, possibly, *Markuelia*. This contrasts sharply with the perspective drawn from the extant biota alone.

CONCLUDING REMARKS

Expectations concerning the quality of biological information that may be retrieved from the fossil record have increased

dramatically with the discovery of fossil embryos, although it is only with phylogenetic resolution that it is possible to explore the relevance of these curios of fossil preservation. Palaeoembryological data may be meager in its extent, but debates over the primacy of morphological states and life history strategies are often very finely balanced such that even a minor amount of additional data can lead to the balance of evidence taking a radical shift from received wisdom. In this regard, work on *Markuelia* is not inconsequential: its phylogenetic placement in proximity to the last common ancestor of arthropods and nematoids provides knowledge of this last common ancestor of two of the most popular model organisms in molecular biology, *Drosophila melanogaster* and *Caenorhabditis elegans*. As such, further resolution of the anatomy of *Markuelia* will provide constraint on our understanding of the relevance of vicarious expression patterns of regulatory genes in the search for homology and the evolution of development.

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APPENDIX: CHARACTER DEFINITIONS AND DATAMATRIX

Character definitions

The character descriptions that follow are repeated and augmented from Wills (1998), Lemburg (1999), and Dong et al. (2004), 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 I) (0), completely invaginable into the trunk (i.e., to the base of zone I) (1).
3. Zone I: unarmed (0), armed (2).
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 I 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, trifold spines, sensory spines, and double, tentaculite scalids (7), scalids with pectinate hood (8), spinoscalids and clavoscalids (kinorhynchs and loriferans) (9).
7. Number of elements comprising the first three circlets and, hence, defining the number of longitudinal rows of elements on the introvert: ≤ 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 *Meiopriapulid* where there are 8:9 and then a much greater number comprising the third circlet and those that follow (Adrianov and Malakhov 2001). In loriferans and kinorhynchs there are 20 longitudinal rows.
8. Sequence of zone I 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).
9. Basal circlet of zone I armature separated from more anterior by a constriction (as in loriferans) 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 (more than eight) (0), eight (1), less than eight (2).
12. Zone III: unarmed (0), armed (1).
13. Number of circlets of zone III armature: one to four (1), six to eight (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).
16. 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 (more than 10) (0), first circlet of 10 elements (1), first circlet of 5 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), 90–120 (2), 160 or more (3).
25. Trunk spines, fine spines, or setae: absent (0), present (1).
26. Trunk papillae: absent (0), present (1).
27. Trunk sclerites: absent (0), present (1). Although *Cricocosmia* exhibits a series of sclerites along the trunk these do not resemble the sclerites of palaeoscolecid and it is far from clear whether they are internal or external structures.
28. Trunk tumuli: absent (0), present (1).
29. Trunk tubuli: absent (0), present (1).
30. Flosculi, N-flosculi or sensory spots: absent (0), present (1).

31. Posterior hooks: absent (0), present (1).
32. Posterior ring papillae: absent (0), present (1).
33. Eversible bursa: absent (0), present (1).
34. Position of the anus: anus terminal, whether within a bursa or otherwise (0), anus in posterolateral or posteroventral surface of the abdomen (1).
35. Posterior tubuli or setae: absent (0), present (1).
36. Caudal appendage(s): absent (0), present (1).
37. Division of caudal appendage(s) or tail: undivided (0), pseudosegmented (1).
38. Caudal appendage vesiculae: absent (0), present (1).
39. Polythyridium: absent (0), present (1).
40. Nucleation of "peritoneal" membrane: membrane without nuclei or simply with amoebocytes in association with the surface (0), membrane containing scattered nuclei (1).
41. Developmental mode: direct (0), biphasic (1).
42. Loric stage: absent (0), present (1).
43. Cuticle-containing collagen: absent (0), present (1). *Palaeoscolex* is coded present on the basis of the presence of cross-helicoil fibers underpinning the cuticle of *Gamoscolex*, a taxon that differs from *Palaeoscolex* only on the basis of cuticular plate ornamentation. The position and arrangement of the fibers is entirely consistent with the cuticular collagen of nematomorphs and nematodes.
44. Moulting cuticle: absent (0), present (1).
45. Scalids (nonspecific and *sensu lato*, including both scalids and presumed scalid derivatives): absent (0), present (1).
46. Extent of scalid cuticularization: scalid composed exclusively of cuticle (0), cuticle limited to a thin outer covering (1). Schmidt-Rhaesa (1998) has demonstrated that, in comparison to the scalids of Scalidophora (Kinorhyncha + Loricifera + Priapulida), the scalids of nematoids (Nematoda + Nematomorpha) are composed exclusively of cuticle. The collapse and folding of the scalids in *Markuelia* indicates that they are hollow structures and, thus, not wholly composed of cuticle.
47. Terminal mouth: absent (0), present (1).
48. Mouth cone: absent (0), present (1). The eversion (though not necessarily inversion) upstanding anterior limit of the pharynx, its presence in *Markuelia* is inferred by comparison of the mouth to the mouth cone of priapulids (e.g., Lemburg 1995) which is structurally very similar.
49. Noninversible mouth cone: absent (0), present (1).
50. Division of the body into a distinct proboscis and abdomen in juvenile/larva: absent (0), present (1).
51. Division of the body into a distinct proboscis and abdomen in adult: absent (0), present (1).
52. Introvert: absent (0), present (1).
53. Helicoil collagen in cuticle: absent (0), present (1).
54. Hexaradial arrangement of armature: absent (0), present (1). Characters 5 and 54 are distinguished, rather than reduced to a single binary character, because although it is possible to resolve that *Markuelia* does not possess hexaradial armature, it is not possible to determine whether or not they exhibit a pentaradial arrangement of armature.
55. Circumoral structures: absent (0), present (1).
56. Circular body musculature: absent (0), present (1). Circular body musculature is present in all taxa of nematohelminth grade except nematodes and nematomorphs, and its absence has been considered both secondary and a synapomorphy of Nematoda (Schmidt-Rhaesa 1998).
57. Ventral nerve cord unpaired throughout its length: absent (0), present (1). Living priapulids possess unpaired ventral nerve cords, whereas gastrotrichs, onychophorans 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).
58. Ventral nerve cords merge caudally: absent (0), present (1).
59. Dorsal nerve cord unpaired: absent (0), present (1).
60. Cloaca in both sexes: absent (0), present (1).
61. 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, nematodes and nematomorphs.
62. 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 loriciferans and extant priapulids.
63. Urogenital system attached to the body wall by a ligament: absent (0), present (1). Lemburg (1999) recognized this as a synapomorphy of loriciferans and extant priapulids.
64. Spermatozoa with a flagellum: absent (0), present (1). The presence of a flagellum in spermatozoa is a metazoan sympleiomorphy, but a flagellum is lacking from the spermatozoa of nematodes and nematomorphs (Schmidt-Rhaesa 1998).
65. Locomotory cilia: absent (0), present (1). The presence of locomotory cilia is a sympleiomorphy of the clade, lost in onychophorans, nematodes, nematomorphs, kinorhynchs, loriciferans, and extant priapulids (Nielsen 2001).
66. Endocuticle containing chitin: absent (0), present (1).
67. Circumpharyngeal brain: absent (0), present (1).
68. Brain with anterior–posterior sequence of pericarya–neuropil–pericarya: absent (0), present (1). Lemburg (1999) recognized this as a synapomorphy of Introverta (Nematoda + Nematomorpha + Kinorhyncha + Loricifera + Priapulida).

69. Apical part of the brain composed only of Perikarya (rather than as a sequence of perikarya–neuropil–perikarya): absent (0), present (1). Lemburg (1999) recognizes the presence of this character as a synapomorphy of (extant) Eupriapulida.
70. 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.
71. High aspect ratio of body length to width in adult: absent (0), present (1).
72. Zone I armature arranged in rows aligned diagonal to the anterior-posterior axis of the animal: absent (0), present (1).
73. Lorica of the larvae dorso-ventrally flattened (at least in older stages), with six lateral plates in-folded accordion-like: absent (0), present (1).
74. 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.
75. Single dorso-median caudal appendage: absent (0), present (1).
76. 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.
77. 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.
78. Voluminous primary body cavity: absent (0), present (1). Lemburg (1999) recognizes the presence of this character as a synapomorphy of (extant) Priapulida.
79. Movement by peristaltic movement of the pharynx/introvert: absent (0), present (1).
80. Adult with pectinate pharyngeal scales: absent (0), present (1). Lemburg (1999) recognizes the presence of this character as a synapomorphy of Tubiluchidae (*Tubiluchus*+*Meiopriapulidus*).
81. Cone-like protusible pharynx: absent (0), present (1). Lemburg (1999) recognizes the presence of this character as a synapomorphy of Tubiluchidae (*Tubiluchus*+*Meiopriapulidus*).
82. Introvert 30–50% of body length: absent (0), present (1). Lemburg (1999) recognizes the presence of this character as a synapomorphy of Megaintroverta (*Priapulopsis*+*Acanthopriapulidus*+*Priapulidus*).
83. 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*+*Acanthopriapulidus*+*Priapulidus*).
84. Basal cuticular layer of the adult containing chitin: absent (0), present (1).
85. Pharyngeal lumina: round (0), triradiate (1).
86. Paired, lateral, locomotory appendages: absent (0), present (1).
87. Anterior branched frontal appendages: absent (0), present (1).
88. 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 palaeoscolecid. However, although palaeoscolecids exhibit an alternating pattern of cuticular ornament (Kraft and Mergl 1989; Conway Morris 1997) and although this varies from one body region to another (Müller and Hinz-Schallreuter 1993), adjacent 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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