

Biorad AG-1 X8 100–200 mesh column of 2–4 ml, depending on sample size. Matrix elements were removed using ten column volumes (c.v.) of the loading acid and then conditioned with 0.5 c.v. HNO<sub>3</sub> + 0.5 M HF. W was eluted with 7 c.v. 4 M HNO<sub>3</sub> + 0.5 M HF and dried to dryness. Some samples were treated with equal parts H<sub>2</sub>O<sub>2</sub> and 7 M HNO<sub>3</sub> to break down possible organic compounds. We note that several other protocols (for example, eluting in 8 M HCl + 1 M HF) gave poor yields and in some cases, caused artificial anomalies. Samples were analysed in static mode on either of the two Finnigan Neptune plasma ionization multi-collector mass spectrometers (PIMMS) at the University of Bristol. The samples were aspirated through either a Cetac Aridus desolvating nebulizer or an Apex HF nebulizer. The data were corrected for mass bias using <sup>186</sup>W/<sup>183</sup>W = 1.98594 and screened using the corrected <sup>183</sup>W/<sup>184</sup>W for NIST SRM3163. Total analytical blank was 200–300 pg except for the Allende chondrites where a different batch of HF gave a considerably higher blank at 940 pg. The blank contribution was insignificant to all samples (>25 ng W) except the Allende chondrite (~60 ng). Three samples that deviated significantly from SRM3163 in its <sup>183</sup>W/<sup>184</sup>W were rejected. All samples are reported in Table 1 relative to stock SRM3163 using the epsilon notation for both <sup>183</sup>W/<sup>184</sup>W and <sup>183</sup>W/<sup>184</sup>W, and where SRM3163 ε<sup>182</sup>W and ε<sup>183</sup>W are both zero by definition.

Received 8 July; accepted 10 November 2003; doi:10.1038/nature02221.

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**Acknowledgements** We thank A. LeRoex, J. Gurney, K. Westerlund, N. Coe and M. Coetzee at the University of Cape Town who provided and helped us select kimberlite samples. S. Russel at the Natural History Museum, London, donated the meteorite samples. Discussions on the manuscript by E. Hauri, G. Helffrich & B. Wood are appreciated. This work is supported by a EU Marie Curie post-doctoral fellowship.

**Competing interests statement** The authors declare that they have no competing financial interests.

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## Fossil embryos from the Middle and Late Cambrian period of Hunan, south China

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Comparative embryology is integral to uncovering the pattern and process of metazoan phylogeny<sup>1</sup>, but it relies on the assumption that life histories of living taxa are representative of their antecedents. Fossil embryos provide a crucial test of this assumption and, potentially, insight into the evolution of development, but because discoveries so far<sup>2–5</sup> lack phylogenetic constraint, their significance is moot. Here we describe a collection of embryos from the Middle and Late Cambrian period (500 million years ago) of Hunan, south China, that preserves stages of development from cleavage to the pre-hatching embryo of a direct-developing animal comparable to living Scalidophora (phyla Priapulida, Kinorhyncha, Loricifera). The latest-stage embryos show affinity to the Lower Cambrian embryo *Markuelia*<sup>3</sup>, whose life-history strategy contrasts both with the primitive condition inferred for metazoan phyla and with many proposed hypotheses of affinity<sup>3,6</sup>, all of which prescribe indirect development. Phylogenetic tests based on these embryological data suggest a stem Scalidophora affinity. These discoveries corroborate, rather than contradict, the predictions of comparative embryology, providing direct historical support for the view that the life-history strategies of living taxa are representative of their stem lineages.

All of the embryos are preserved in calcium phosphate with varying degrees of fidelity, from extremes in which structures of less than 0.3 μm are preserved, to others in which it is possible to make out only the general outline of the embryos; most specimens show a microspheritic surface texture indicative of bacterially mediated soft-tissue replacement<sup>7</sup>.

The embryos vary in size and in the developmental stages represented. The smallest (diameter 236 μm) and earliest (Fig. 1a, b) stage is a cleavage embryo preserving the surface boundaries between blastomeres. Although not complete, dividing the surface area of the embryos by the average cell area suggests 485 cells. The remaining collection of embryos (for example, Fig. 1c–f) range in diameter from 370 to 411 μm. Three further specimens (for example, Fig. 1c) are later developmental stages but do not fully occupy the volume of the embryo, and much of the surface area preserves undifferentiated tissue, probably yolk.

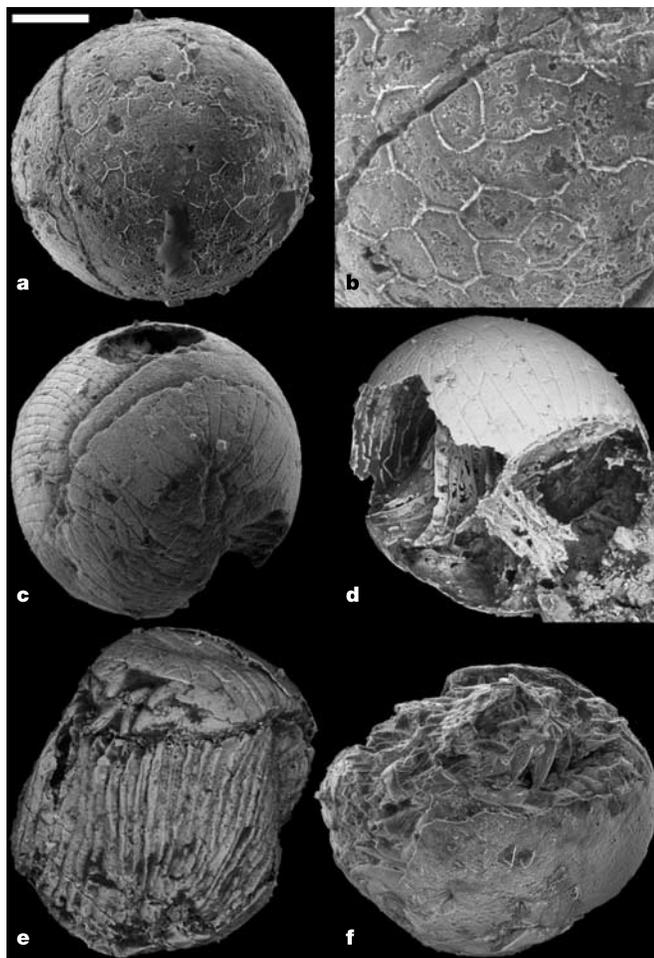
## letters to nature

The latest-stage embryos have a vermiform anatomy coiled into a sphere with head and tail juxtaposed laterally (Fig. 1f). The trunk is coiled in an inverted S-shaped double loop and is annulated throughout (Fig. 1d); individual annulae are 20–25  $\mu\text{m}$  in length and 180–190  $\mu\text{m}$  in width, suggesting that there are  $\sim$ 130 annulae in total. The annulated surface is ornamented by perpendicularly orientated, anastomosing ribs of 0.3–0.5  $\mu\text{m}$ . Annular margins are seen to extend internally in fractured specimens (Fig. 1d) and are, thus, not simply surface undulations. The head and tail are distinguished as terminal, spine-bearing regions in which annulae are less obviously developed. The tail bears six curved spines of roughly equal length (Fig. 1e, f), varying between specimens from 50 to 95  $\mu\text{m}$  in length. These are broadly paired but seem to have been arranged radially about a terminal depression or opening (Fig. 1e). Four spines are positioned marginal to, and oriented away from, this depression, and the remaining two may occur in the depression itself.

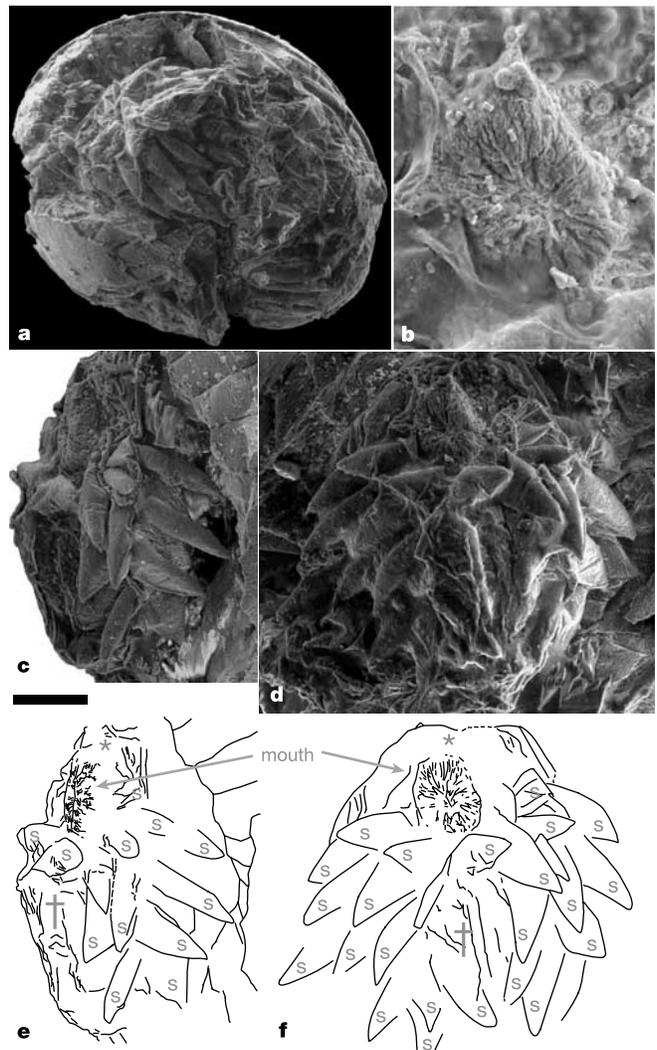
The presumed head is well preserved in only one specimen (Figs 1f and 2a–f), which reveals a terminal mouth of 46  $\mu\text{m}$  in diameter that is surrounded by at least three rows of partially overlapping rows of flat, broadly triangular, posteriorly directed spines of up to 27  $\mu\text{m}$  in

length and 11  $\mu\text{m}$  in width; the spine rows extend around all visible sides of mouth and are, thus, presumably radial (part of the subterminal region is obscured by collapse and diagenetic cement). Some spines show evidence of collapse and folding (Fig. 2c, d), suggesting that they are not solid epicuticular thickenings. Both the posterior and the oral spines show a smooth surface, distinct from the finely ribbed trunk, head and tail, indicating that they are not simple extensions of the body wall and may have been more extensively cuticularized. From anterior to posterior, the reconstructed embryo (Fig. 3a) would have been just over 3 mm in length.

The new material is closely comparable to *Markuelia secunda*<sup>3</sup>, whose embryos are also annulated, vermiform and coiled in a double 'S' loop, with head and tail juxtaposed. It differs by lacking conical trunk protuberances and by showing a broadly radial, rather than bilateral, arrangement of terminal (posterior) spines. But given the reported distinctiveness of *Markuelia* from the embryos of any well-known living taxon<sup>3</sup>, the similarities shared with the new material must reflect close kinship; indeed, we deem it sufficiently



**Figure 1** *Markuelia hunanensis*. **a, b**, Late cleavage embryo with surface margins of blastomeres preserved (GMPKU2007). **c**, Annulated embryo with yolk tissue (GMPKU2008). **d**, Fractured late-stage embryo with internal annular divisions (GMPKU2009). **e**, Late-stage embryo with tail and six terminal hooked spines (GMPKU2011). **f**, Late-stage embryo with head (upper) and tail (lower) juxtaposed (GMPKU2010). Relative scale bar, 58  $\mu\text{m}$  (**a**); 104  $\mu\text{m}$  (**b**); 92  $\mu\text{m}$  (**c**); 27  $\mu\text{m}$  (**d**); 80  $\mu\text{m}$  (**e**); 88  $\mu\text{m}$  (**f**).



**Figure 2** The oral region of the holotype specimen (GMPKU2010). **a**, Whole embryo viewed directly onto the oral region. **b–f**, Detail of the mouth cone (**b**), viewed from the side (**c, e**) and from above (**d, f**). The image in **a** is rotated 90° counter-clockwise from all other images. The position of scalids is shown by s; asterisks indicate the subterminal region of the mouth, which is obscured by diagenetic cement; daggers indicate the area obscured by surface coating. Relative scale bar, 34  $\mu\text{m}$  (**a**); 40  $\mu\text{m}$  (**b**); 11  $\mu\text{m}$  (**c**).

similar to place this new species, *Markuelia humanensis*, in the same genus. Thus, the additional anatomical information provided by *M. humanensis* can be used to constrain not only its own phylogenetic relationships, but also those of the genus *Markuelia*.

The features common to *Markuelia secunda* and *M. humanensis* have hitherto been taken to suggest an affinity with lobopods<sup>3</sup>, annelids<sup>3</sup> and/or halkieriids<sup>6</sup> (a grade of organisms putatively including ancestors and close relatives of the annelids, molluscs and brachiopods<sup>8</sup>); the additional anatomical characters from *M. humanensis* facilitate a test of these hypotheses. The absence of paired appendages, or their anlagen, in these later-stage embryos precludes a close affinity to lobopods and annelids. An affinity with halkieriids was originally proposed on the basis of co-occurrence in geological samples, and the possibility that the trunk spines of *M. secunda* may represent scalid anlagen. The presence of oral spines demands comparison to the putative radulae of halkieriids and *Wiwaxia*. However, the adoral rather than aboral orientation of these spines, their apparent circumoral rather than bilateral disposition and, most significantly, the terminal rather than sub-terminal position of the mouth all contrast with the situation in halkieriids<sup>8</sup> and *Wiwaxia*<sup>9</sup>. Finally, although we would not wish to preclude the possibility of identifying evolution of life-history strategies, the direct mode of development shown by *Markuelia* contrasts significantly with molluscs, brachiopods and most annelids. Thus, the affinities of *Markuelia* lie elsewhere.

The head and mouth of *Markuelia* are potentially more instructive phylogenetically: the terminal position of the mouth is a key feature of cycloneuralians *sensu*<sup>1</sup>, and the radial arrangement of

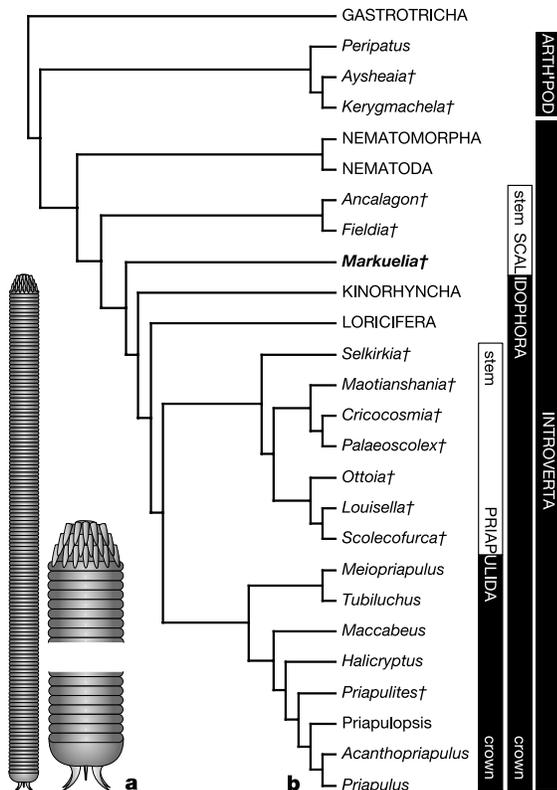
circum-oral spines is directly comparable, both morphologically and topologically, to the scalids of Scalidophora. Circum-oral scalids are present in other cycloneuralians, such as nematodes and nematomorphs<sup>10</sup>, although these may be convergent<sup>11</sup>. Nevertheless, the five phyla share an eversible proboscis (introvert) associated with scalids and are united as Introverta on the basis of this apomorphy<sup>1</sup>. The structure of the mouth in *Markuelia*, although not definitive, is compatible with the presence of a proboscis. The other features shown by *Markuelia*, including annulation, spine-bearing trunk and terminal appendages, are also found among all five introvert phyla.

Thus, the possession of multiple rows of circum-oral scalids suggests a closer affinity of *Markuelia* to Scalidophora than to nematodes, nematomorphs or a combination thereof, or to any more inclusive groupings, although this is contingent on a phylogenetic test of character distribution. To this end, we undertook a phylogenetic analysis of Introverta, which was based on several data sets compiled to resolve the interrelationships of fossil and living introverts<sup>12–14</sup>. This analysis (see Supplementary Information) resolves *Markuelia* as a sister taxon to extant Scalidophora, although in an unresolved position relative to two other stem scalidophorans; with successive weighting, *Markuelia* is resolved as the most derived member of the scalidophoran stem lineage (Fig. 3b and Supplementary Information).

Resolution of the affinity of *Markuelia* provides a basis for determining its significance in understanding the evolution of development and the inferential power of evolutionary embryology. Initial speculation suggested a lophotrochozoan affinity and, thus, evidence that the preponderance of indirect developers undergoing a planktotrophic larval stage may be an artifact of convergence<sup>6</sup>. The placement of *Markuelia* among Ecdysozoa, a clade of animals lacking a planktotrophic larval stage, corroborates rather than contradicts the predictions of evolutionary embryology. Nevertheless, the phylogenetic position of *Markuelia* supports the prediction that stem scalidophorans were direct developers and that the secondary larvae of priapulids and loriciferans reflect a derived, rather than plesiomorphic life-history strategy for Scalidophora and Introverta. This conclusion has previously been contingent on distant outgroups (Arthropoda and/or Gastrotricha) because of conflict in the ingroup, but the placement of *Markuelia* resolves this conflict with respect to both Scalidophora and Introverta without reference to an outgroup.

Suggestions that the similarities between nematoids and scalidophorans are convergent<sup>11</sup> are weakened by the extension of many potential homologies into the scalidophoran stem group. These include the possession of an eversible proboscis and the possession of circum-oral scalids. That the absence of giant fibres in the body wall of extant scalidophorans, a putative synapomorphy of nematoids<sup>11,14</sup>, might be a secondary adaptation to small size seems to be borne out by their presence in palaeoscoleceids (P.C.J.D., unpublished observation), resolved herein among the priapulid stem lineage. Differences in the organizational symmetry of oral structures between nematoids and scalidophorans may well be resolved by further examination of the stem groups of, and to, these super-phyletic clades. Introvert sympleisiomorphies potentially have an even wider distribution, extending to the groundplan of Introverta plus Panarthropoda, given that a subset of these characters are general also to gastrotrichs, a sister taxon to this clade<sup>13</sup>. Significantly, this corroborates the view that a terminally positioned mouth is an arthropod sympleisiomorphy<sup>15</sup>.

The identification of *Markuelia* as a stem scalidophoran provides both direct evidence for the establishment of this lineage in the Lower Tommotian (Lower Cambrian; 538 million years ago<sup>16</sup>) and indirect evidence for the establishment of the nematoid lineage by this time, and fulfils the prediction of a ghost lineage inferred from the earliest fossil record of the introvert sister clade Euarthropoda<sup>17,18</sup>. The reconstruction of a Cambrian stem group to a clade



**Figure 3** *Markuelia* is a stem scalidophoran. **a**, Reconstruction of the embryo unfurled. **b**, Tree summarizing interrelationships among living and fossil introverts and the outgroup, derived from numerical cladistic analysis of 87 morphological characters using branch-and-bound and heuristic search techniques of the unweighted and reweighted datasets in PAUP\* 4.0b10. See Supplementary Information for component analyses and their statistical support. Dagggers indicate extinct taxa.

of three phyla is compatible with the view that the establishment of phyla continued into the Cambrian<sup>19</sup>, and it provides a more complete framework of character evolution in the assembly of their bodyplans for which developmental explanations must now be sought. The placement of fossil-based developmental data in this context facilitates a thorough integration of palaeontology into evolutionary developmental biology in understanding the evolution of development.

Superphylum Introverta Nielsen 1995 (ref. 20)

Genus *Markuelia* Val'kov 1983 (ref. 21)

*Markuelia hunanensis* Dong & Donoghue sp. nov.

**Etymology.** Named for its provenance in the Chinese Province of Hunan.

**Holotype.** Geological Museum of Peking University, Beijing, China: GMPKU2010.

**Stratigraphy and locality.** Middle Upper Cambrian Bitiao Formation in Wangcun, Hunan, south China.

**Diagnosis.** A species of *Markuelia* with six terminal, posterior spines arranged radially and away from a central depression or opening, lacking trunk spines, and showing at least three overlapping rows of posteriorly directed circum-oral scalds.

**Remarks.** *Markuelia* cannot be allocated to existing rank taxa below Introverta without rendering such taxa paraphyletic. The establishment of many hierarchies of new rank taxa, solely to encompass *Markuelia*, will not serve scientific communication. □

Received 4 September; accepted 4 November 2003; doi:10.1038/nature02215.

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Supplementary Information accompanies the paper on [www.nature.com/nature](http://www.nature.com/nature).

**Acknowledgements** We thank W. Guo for field assistance, and S. Bengtson and S. Conway Morris for discussion. This work was supported by grants from the National Natural Science Foundation of China, Laboratory of Paleobiology and Stratigraphy of the Nanjing Institute of Geology & Paleontology, Chinese Academy of Sciences and the Ministry of Education of China (to X.-P.D.); the Royal Society and Natural Environment Research Council (to P.C.J.D.); and the Ministry of Science and Technology of China (to J.-B.L.).

**Competing interests statement** The authors declare that they have no competing financial interests.

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## Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs

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Ecosystems are supported by organic carbon from two distinct sources. Endogenous carbon is produced by photosynthesis within an ecosystem by autotrophic organisms. Exogenous carbon is produced elsewhere and transported into ecosystems. Consumers may use exogenous carbon with consequent influences on population dynamics, predator–prey relationships and ecosystem processes<sup>1</sup>. For example, exogenous inputs provide resources that may enhance consumer abundance beyond levels supported by within-system primary production<sup>2</sup>. Exogenous fluxes of organic carbon to ecosystems are often large, but this material is recalcitrant and difficult to assimilate, in contrast to endogenously produced organic matter, which is used more easily<sup>3,4</sup>. Here we show, by the experimental manipulation of dissolved inorganic <sup>13</sup>C in two lakes, that internal primary production is insufficient to support the food webs of these ecosystems. Additions of NaH<sup>13</sup>CO<sub>3</sub> enriched the <sup>13</sup>C content of dissolved inorganic carbon, particulate organic carbon, zooplankton and fish. Dynamics of <sup>13</sup>C indicate that 40–55% of particulate organic carbon and 22–50% of zooplankton carbon are derived from terrestrial sources, showing that there is significant subsidy of these ecosystems by organic carbon produced outside their boundaries.

In lakes, grazing and microbial–detrital trophic pathways support higher consumers<sup>5,6</sup>. The importance of dissolved and particulate organic matter not derived from primary producers has been recognized<sup>4,7</sup>, but not widely considered in studies of food webs<sup>1</sup>. System respiration exceeds gross primary production in many lakes, implying the metabolism of exogenous organic carbon<sup>8</sup>. Aquatic bacteria use exogenous dissolved organic carbon (DOC), and subsequent consumption of bacteria by predators provides a pathway for transfer into food webs<sup>9,10</sup>. However, efficiencies of bacterial growth and trophic transfer of exogenous carbon are often low, and the importance of this pathway is uncertain<sup>6,11</sup>. Direct use of exogenous carbon by animal consumers is also possible<sup>12,13</sup>, but poorly understood.

Large-scale tests of the importance of exogenous carbon to food