

An armoured Cambrian lobopodian from China with arthropod-like appendages

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Cambrian fossil Lagerstätten preserving soft-bodied organisms have contributed much towards our understanding of metazoan origins^{1–3}. Lobopodians are a particularly interesting group that diversified and flourished in the Cambrian seas. Resembling ‘worms with legs’, they have long attracted much attention in that they may have given rise to both Onychophora (velvet worms)^{4–6} and Tardigrada (water bears)^{7,8}, as well as to arthropods in general^{9–12}. Here we describe *Diania cactiformis* gen. et sp. nov. as an ‘armoured’ lobopodian from the Chengjiang fossil Lagerstätte (Cambrian Stage 3), Yunnan, southwestern China. Although sharing features with other typical lobopodians, it is remarkable for possessing robust and probably sclerotized appendages, with what appear to be articulated elements. In terms of limb morphology it is therefore closer to the arthropod condition, to our knowledge, than any lobopodian recorded until now. Phylogenetic analysis recovers it in a derived position, close to Arthropoda; thus, it seems to belong to a grade of organization close to the point of becoming a true arthropod. Further, *D. cactiformis* could imply that arthropodization (sclerotization of the limbs) preceded arthropodization (sclerotization of the body). Comparing our fossils with other lobopodian appendage morphologies—see *Kerygmachela*^{9,10}, *Jianshanopodia*¹³ and *Megadictyon*¹²—reinforces the hypothesis that the group as a whole is paraphyletic, with different taxa expressing different grades of arthropodization.

Lobopodia Snodgrass, 1938

Xenusia Dzik & Krumbiegel, 1989

Diania cactiformis gen. et sp. nov.

Etymology. *Dian* represents a linguistic abbreviation in Chinese of the Yunnan province, where the Chengjiang fossil Lagerstätte is located; *cactiformis* refers to its overall cactus-like shape, which resulted in its nickname ‘walking cactus’.

Holotype. ELI-WT006A,B (Fig. 1a–d), part and counterpart of the complete specimen, which was collected in 2006 from the Jianshan section, Haikou, Yunnan, southwestern China. ELI is an abbreviation of the Early Life Institute, Northwest University, Xi’an, China.

Locality. Yu’anshan (Heilinpu) Formation (*Wutingaspis*–*Eoredlichia* Zone).

Horizon. Cambrian Stage 3.

Diagnosis. Armoured lobopodian with ten pairs of appendages. Trunk region with nine segments, bearing rows of transverse annulations each with some tubercles. Each region possesses a pair of robust and sclerotized spiny appendages with primary articulation. Anterior is extended, probably forming a proboscis. Posterior region bears a protrusion.

Diania cactiformis is known from the part and counterpart of three complete specimens, and about 30 other incomplete specimens (see Methods). The complete specimens are about 6-cm long, with a proboscis-like structure bearing some wrinkles, unlike those of *Xenusion*¹⁴ or *Microdictyon*⁶. The proboscis of *Diania cactiformis* is slightly

expanded compared to the trunk and shows no sign of becoming thinner towards the end. No mouth can be observed (Figs 2a–e and 3). A small projection occurs at the posterior end of the body (Figs 2a–c and 3). The main body trunk is sub-circular in outline and composed of nine segments, each comprising five rows of sub-parallel transverse annulations alternating with five rings of tubercles with tiny spines (Fig. 1a, b); each with 10–12 tubercles. The tiny spines are not well-preserved, possibly as an artefact of taphonomy, and in most cases only the tubercles of these spines are observable (Figs 1a, b and 2b–c).

A pair of strongly armoured appendages protrudes from the ventrolateral region of each segment, each limb attached robustly at its base to the otherwise slender trunk. Indeed, there is usually a hard, disc-like structure observed in the middle of each trunk segment, with only one such disc per appendage pair (Figs 1a, b and 2a–c, g); different from, for example, the paired spine bases seen in *Hallucigenia*. Some filamentary lines occur within these disc-like structures. These lines are interpreted here as the remains of muscle tissue associated with the limb attachment area (Figs 1a, b and 2a–c, g). The attachment area is a little wider than the adjacent trunk segments. Most appendages are incompletely preserved, although in the holotype the seventh right appendage is very clear and is composed of some annulations at the base and sixteen strongly sclerotized segments (Fig. 1a, b). It is worth noting that adjacent segments show a ‘joint-like’ morphology (Figs 1a, b and 2f, h, i–l) and that each segment bears two spines on both lateral sides and two spines on the dorsal side (Figs 1a, b and 2h–k). Further, the appendages terminate in two spines (Fig. 2j). These spines, especially the two dorsal spines with their rounded bases (Figs 1a, b and 2i, k), robustly attach to the appendages. However, probably owing to dorsoventral flattening, the two dorsal spines are usually compressed and can appear shorter than the two lateral spines. These are thorn-like with a maximum height of 2.2 mm and a basal width of 1.1 mm. These spines seem rather stiff and conical in shape, as implied by their triangular profile with a cone angle of approximately 30° (Fig. 2h–k). There is a fold-like structure between the bottom of the triangle and the rim of the appendage (Figs 1a, b and 2j, l), which indicates that the lateral spines also have a solid attachment to the trunk. In particular, the angle between the appendages and trunk differs going from front to back. The angle between the appendages and the trunk in the holotype increases from ~5° (first pair of appendages) to ~10° (second pair) to ~20° (third pair) to ~40° (fourth pair), and then increases abruptly such that nearly all are at right angles (Figs 1a, b and 3). This implies that the appendages of the anterior and posterior body regions may have differed in their function. The anterior appendages were more ventrally orientated and thus more suitable for grasping/feeding from the substrate, whereas the posterior appendages were more laterally orientated and may have served more for locomotion. In specimen ELI-WD001A, a light black band is observed running from the base of the appendage towards the tip. We suggest that this was an extension of the body cavity or gut caeca (Fig. 2f), and that corresponding to each

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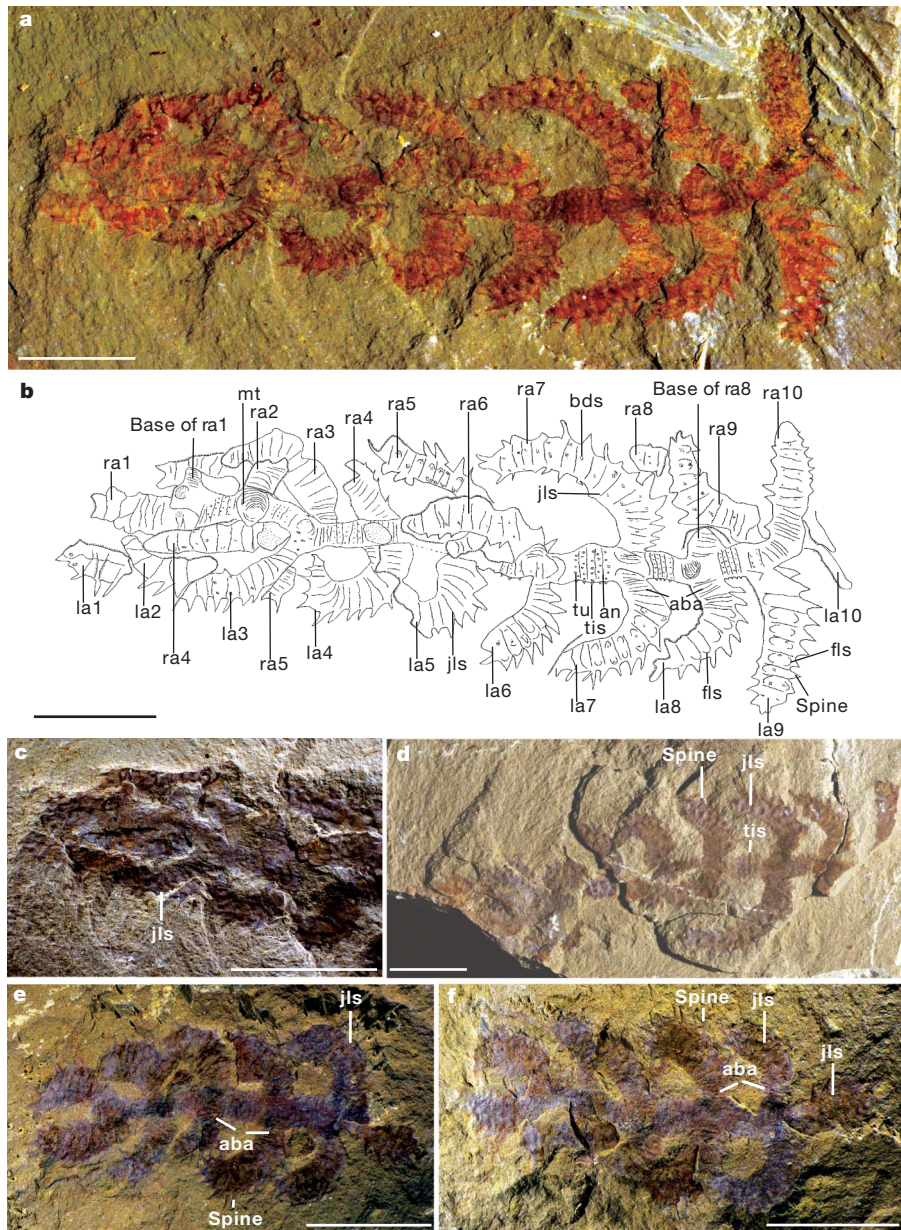


Figure 1 | The early Cambrian lobopodian *Diania cactiformis* gen. et sp. nov. from the Chengjiang fossil Lagerstätte, Kunming, Yunnan, China.

a–d, Holotype ELI-WD006A,B. **a**, Part of holotype photographed under ethanol, revealing almost all morphological features; note that the specimen is twisted towards the anterior end and the appendages are consequently slightly disordered, especially the fourth right appendage protruding under the trunk and appearing on the left side and the fifth right appendage protruding above the trunk and appearing again on the left side. **b**, Camera lucida drawing of **a**. **c**, Detail of the anterior part of **a**, which shows that the appendages are not

segment making up the appendage this cavity or gut caeca is divided into several small parts by joint-like structures (Fig. 2i–l), similar to that of Euarthropoda. In specimen ELI-WD0026A,B, a 2-mm-long and 1-mm-wide trunk extension is observed on the posterior-most body segment (Figs 2a–c and 3), similar to that of *Miraluolishania*¹¹ and *Hallucigenia fortis*⁶. We interpret this structure as a posterior protrusion as well.

Recent Onychophora (velvet worms) are frequently considered to be an outgroup of arthropod phylogeny, sharing with them numerous synapomorphies (as reviewed in ref. 15). Given that fossil lobopodians are almost certainly related in some way to velvet worms^{5,6,16}, they represent a particularly valuable group for understanding the transition from soft-bodied ecdysozoans to fully sclerotized arthropods.

preserved in the same layer; probably the specimens are to some extent three-dimensional. **d**, Counterpart of holotype. **e**, **f**, Specimen ELI-WT002A,B; although the specimens are incomplete, the appendages have clear annulations at the base and joint-like structures. **aba**, annulations of the base of appendages; **an**, annulations; **bds**, base of the dorsal spines; **dls**, disc-like structure; **fls**, fold-like structures; **jls**, joint-like structures; **la**, left appendage; **mt**, muscle tissue; **pr**, proboscis; **ra**, right appendage; **pp**, posterior protrusion; **tis**, tiny spine; **tu**, tubercle; **wr**, wrinkle. Scale bars, 10 mm.

Key innovations towards the Arthropoda condition (Euarthropoda in the sense of ref. 17) are eyes, sclerotized bodies and sclerotized, jointed limbs—whereas crown-group Arthropoda can be defined by, for example, a distinct head and biramous limbs with an endopod composed of relatively few articles.

How close Cambrian lobopodians are to the ground plan of the arthropod common ancestor remains a point of debate^{10,18}. Yet it is worth reiterating that in living Onychophora it is the claws at the tips of the legs that are fully sclerotized, whereas numerous fossil lobopodians preserve plates or spines ornamenting the trunk⁶. This begs the question whether the transition towards the euarthropod condition began primarily with the limbs (so-called arthropodization) or through sclerotization of the body (arthrodization), with subsequent tagmosis

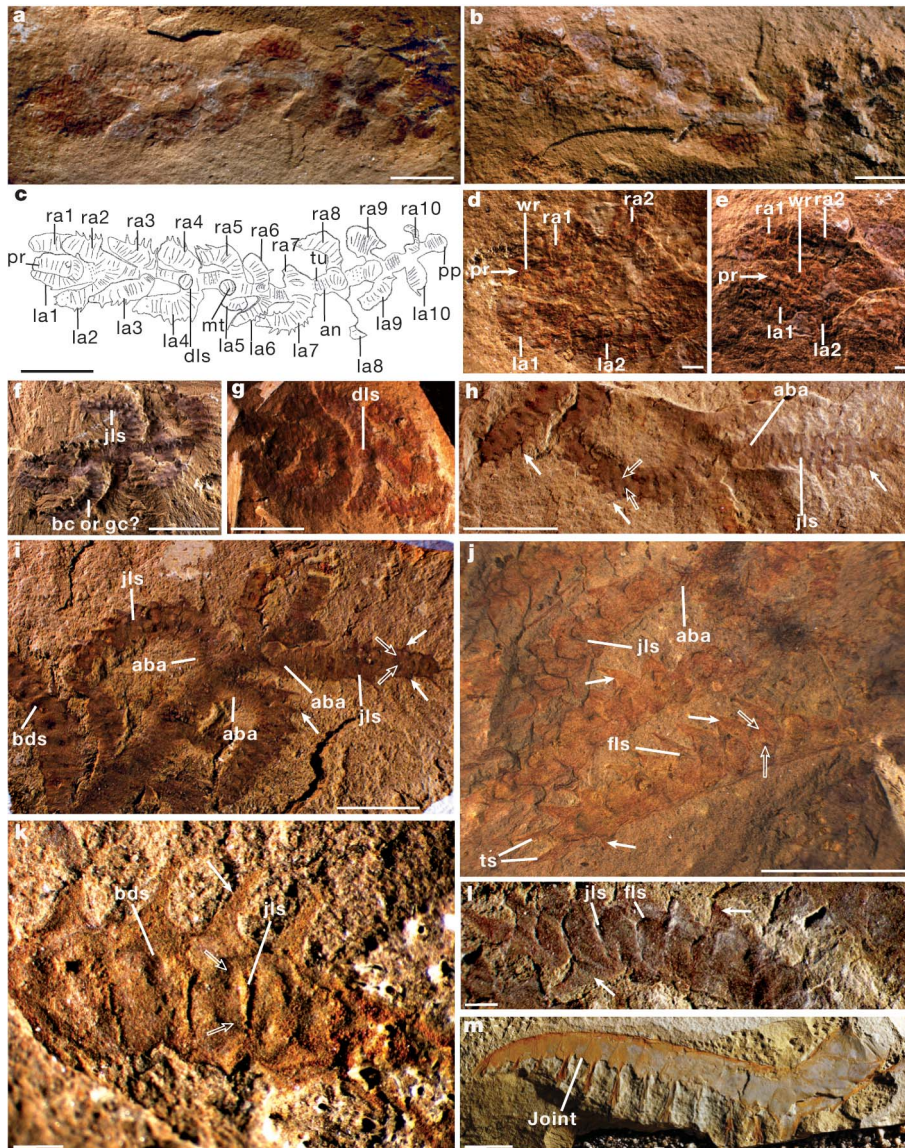


Figure 2 | Further examples of *Diania cactiformis* and comparison of the limbs to the frontal appendages of *Anomalocaris*. **a, b**, The complete specimen ELI-WD0026A,B; note the proboscis, the small protrusion and the ten pairs of appendages. **c**, Camera lucida drawing of **b**; this specimen is not twisted, thus the proboscis and the small protrusion are clearly visible. **d, e**, Enlargement of anterior part of **a** and **b**, showing the proboscis with wrinkles and the anterior two pairs of appendages, which in most cases overlapped the proboscis. **f**, ELI-WD001A, showing extension of the body cavity or gut caeca into the appendages and body cavity or gut caeca divided into several small parts by joint-like structures. **g**, S1, showing the disc-like structures on the trunk and the angle between trunk and appendages. **h–i**, ELI-EJ08-0690A,B; note the annulations at the appendage bases and the succeeding joint-like structures. **j**, S2B photographed under ethanol, appendages revealing

and cephalisation at a later date¹⁹. Some authors have specifically argued that arthropodization should have preceded arthropodization²⁰.

Our new fossils cannot resolve this question in its entirety, but they do demonstrate that appendage morphology was more diverse among Cambrian lobopodians than is sometimes realized. Even among these early faunas there may have been evolutionary grades ranging from the short, stubby but still essentially soft limbs ending in paired claws—as seen in Recent Onychophora and, for example, the Burgess Shale *Aysheaia*—through to somewhat longer, apparently still soft, but annulate ‘lobopodian’ limbs in taxa such as *Hallucigenia* and *Microdictyon*. Our *Diania* fossils further indicate the presence of lobopodians with rather robust and heavily ornamented limbs, probably finely annulated

annulations at the base followed by joint-like elements (limb articles?), the dorsal spines with round bases, the lateral spines with fold-like structures and the appendages terminating in two spines. **k**, Specimen ELI-WD008B, showing well-preserved structures resembling articulation points comparable with the condition in **k**; implying that the appendages of *Diania cactiformis* have indeed also undergone arthropodization. bc, body cavity; dls, disc-like structure; gc, gut caeca; ts, terminal spine. Unfilled white arrows point to dorsal spines, filled white arrows indicate the lateral spines. For other abbreviations see Fig. 1. Scale bars of **a–c**, **f–j** and **m** are 10 mm; those of **d, e, k** and **l** are 1 mm.

at their bases (Figs 1a, b and 2h–j), but with good evidence (see earlier) that much of the limb was divided into discrete, ring-like elements. This is evidently closer to the arthropod condition and not dissimilar to interpretations of the limb rod in ‘primitive’ arthropods and/or members of the arthropod stem-lineage such as *Fuxianhuia*²¹, or the frontal appendages of *Anomalocaris*²² (Fig. 2m) in particular.

Whether the limb ‘articles’ observed here in *Diania* were fully sclerotized and/or articulated against each other via explicit condyles like modern arthropod limbs is hard to determine from the material available although, significantly, slight projections resembling articulation points are evident (Fig. 2k, l) and reinforces their interpretation as truly jointed appendages. Nevertheless, we reiterate here that, to our

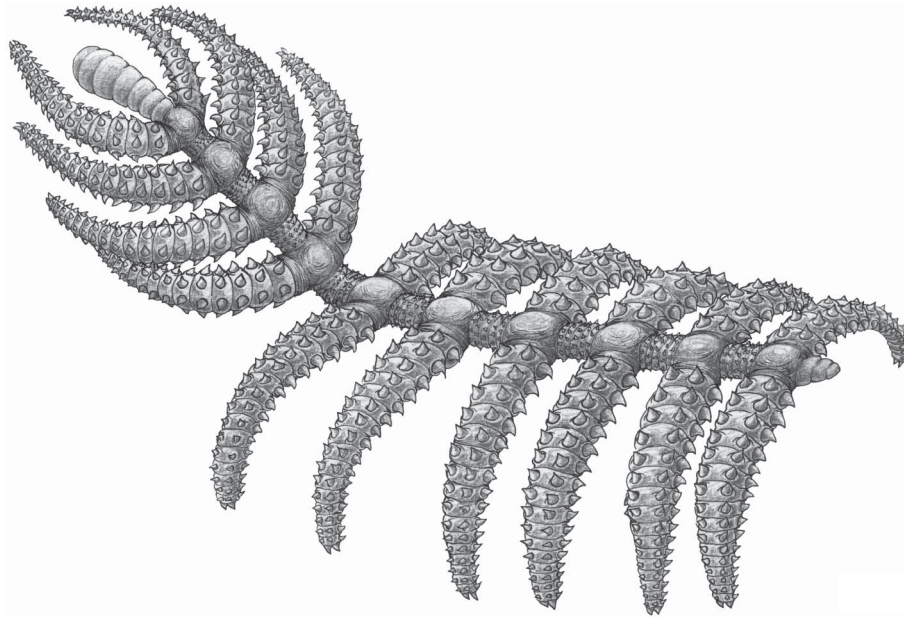


Figure 3 | Reconstruction of *Diania cactiformis* in dorsolateral view.

knowledge, *Diania* has the most robust and arthropod-like limbs found in any lobopodian until now. Given that the trunk shows little evidence for tergites or sternites (or indeed clear segments of any sort), a possible inference would be that arthropodization actually preceded arthrodization; at least within this branch of the lobopodians.

Does this 'leg-first' scenario also apply to Arthropoda in general? In part, this comes back to the problem of whether lobopodians are a clade or a (paraphyletic) grade^{10,18} in which some lineages are closer to arthropods than to modern velvet worms. Cladistic analysis of lobopodians and other stem-group arthropods (Fig. 4) does indeed resolve lobopodians as paraphyletic, with one lineage leading towards Onychophora and another towards Arthropoda. *Diania* is recovered here in the strict consensus tree as the sister-taxon of Arthropoda, on the basis of the putative apomorphy of jointed trunk appendages. Our outgroups to (*Diania* + Arthropoda) are an assemblage of fossils largely equivalent to the Dinocaridida in the sense used in refs 23 and 18, which encompasses the unusual 'gilled' lobopodians *Kerygmachela* and *Pambdelurion* as well as putative stem-group arthropods such as *Opabinia* and the Radiodonta group of *Anomalocaris* and similar forms. However, we should caution that dinocaridids, *Diania* and other potential stem-arthropods typically express mosaics of arthropod-like characters, which makes resolving a single, simple tree of arthropod origins problematic. Indeed, the position recovered here for *Diania* between Radiodonta and the ostensibly similar-looking *Schinderhannes*²⁴ is surprising. *Diania*, *Schinderhannes* and the remaining Arthropoda all share the putative apomorphy of jointed trunk appendages, and yet the trunk limbs of *Diania* resemble the frontal appendages of *Anomalocaris* and other radiodontans, which themselves lack trunk limbs entirely. If this is a secondary reduction in fossils like *Anomalocaris*, then *Diania* may in fact occupy a more basal position with respect to Radiodonta, *Schinderhannes* and Arthropoda; a scenario that would be more consistent with their fairly simple body morphology (see Supplementary Information for discussion).

Further, *Kerygmachela* and *Pambdelurion* have—compared to *Diania*—more typically lobopodian and less obviously jointed walking limbs¹⁰, but share with *Opabinia* and Radiodonta the apomorphy of lateral lobes. One possible scenario^{9,10} involves these (gill?) lobes contributing to the eventual outer branch of the arthropod biramous limb, and there is no evidence for such lobes in *Diania*. Productive areas for future research may therefore be to investigate: (1) whether leg sclerotization really did precede body sclerotization; and (2) the timing of the appearance and evolution of the endopod limb branch relative to the exopod.

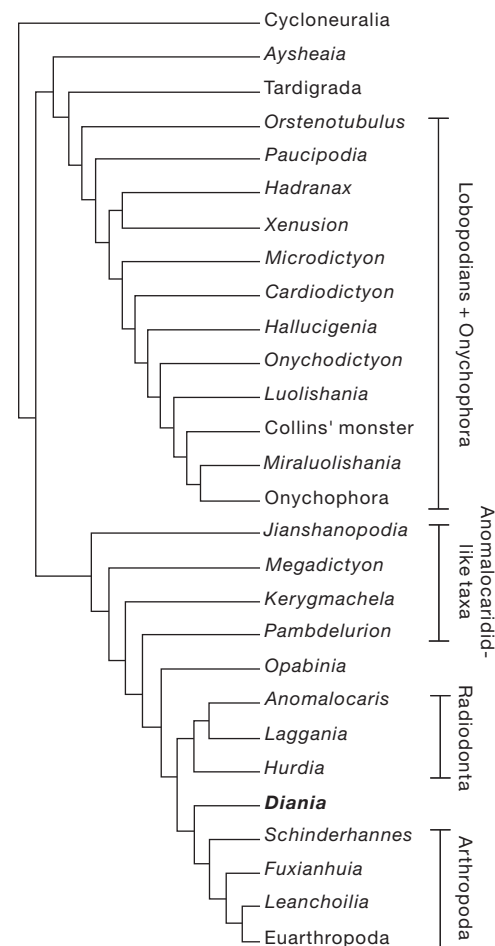


Figure 4 | Cladistic analysis of all Cambrian lobopodians and some arthropod stem group taxa. This is a strict consensus of three trees found using a branch and bound search under implied weights ($k = 2$). Tree length 130; consistency index (CI) 0.61; retention index (RI) 0.52. Tree rooted by taking Cycloneuralia as an outgroup. See Supplementary Fig. 1 and Supplementary Table 1.

Irrespective of its exact position, *Diania*, with its stout and spiny limbs attached to a slender, vermiform body, remains a highly unusual creature. It is hard to envisage it as the progenitor of any modern arthropod group, yet it may derive from a grade of lobopodian that acquired a key evolutionary innovation—and the name-giving character for Arthropoda—whereby sclerotized, jointed appendages began to fully develop.

METHODS SUMMARY

The holotype and other figured specimens with the prefix ELI are held in the Early Life Institute, Northwest University, Xi'an, China. Additional figured specimens with the prefix S are held in the collections of the Department of Earth Sciences of the Freie Universität Berlin. Most fossils were photographed with a dhs Microcam 3.3 through the ocular of a Leica Stereomicroscope MZ75; others were photographed (see figure legends for details) with a Nikon D300 specifically under 100% ethanol. Drawings were made with a camera lucida on a Leica MZ75 Stereomicroscope. The figures were prepared with Adobe Photoshop 7, Coral Draw 9 and Adobe Illustrator Artwork 13.

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- Hou, X.-G., Ramsköld, L. & Bergström, J. Composition and preservation of the Chengjiang fauna—a lower Cambrian soft-bodied biota. *Zool. Scr.* **20**, 395–411 (1991).
- Shu, D.-G. *et al.* Lower Cambrian vertebrates from south China. *Nature* **402**, 42–46 (1999).
- Chen, J.-Y. & Zhou, G.-Q. Biology of the Chengjiang fauna. *Bull. Natl Mus. Nat. Sci.* **10**, 11–115 (1997).
- Whittington, H. B. The lobopod animal *Aysheaia pedunculata* Walcott, Middle Cambrian, Burgess Shale, British Columbia. *Phil. Trans. R. Soc. B* **284**, 165–197 (1978).
- Hou, X.-G. & Bergström, J. Cambrian lobopodians—ancestors of extant onychophorans? *Zool. J. Linn. Soc.* **114**, 3–19 (1995).
- Ramsköld, L. & Chen, J.-Y. in *Arthropod Fossils and Phylogeny* (ed. Edgecombe, G.) 77–93 (Columbia Univ. Press, 1998).
- Delle Cave, L. & Simonetta, A. M. Notes on the morphology and taxonomic position of *Aysheaia* (Onychophora?) and of *Skania* (undetermined phylum). *Monit. Zool. Ital.* **9**, 67–81 (1975).
- Budd, G. E. Tardigrades as “stem-group arthropods”: the evidence from the Cambrian fauna. *Zool. Anz.* **240**, 265–279 (2001).
- Budd, G. E. A Cambrian gilled lobopod from Greenland. *Nature* **364**, 709–711 (1993).
- Budd, G. E. The morphology and phylogenetic significance of *Kerygmachela kierkegaardii* Budd (Buen Formation, Lower Cambrian, N Greenland). *Trans. R. Soc. Edinb. Earth Sci.* **89**, 249–290 (1999).
- Liu, J.-N., Shu, D.-G., Han, J. & Zhang, Z.-F. A rare lobopod with well-preserved eyes from Chengjiang Lagerstätte and its implications for origin of arthropods. *Chin. Sci. Bull.* **49**, 1063–1071 (2004).
- Liu, J.-N., Shu, D.-G., Han, J., Zhang, Z.-F. & Zhang, X.-L. Morpho-anatomy of the lobopod *Megadictyon cf. haikouensis* from the Early Cambrian Chengjiang Lagerstätte, South China. *Acta Zool.* **88**, 279–288 (2007).
- Liu, J.-N., Shu, D.-G., Han, J., Zhang, Z.-F. & Zhang, X.-L. A large xenosiid lobopod with complex appendages from the Chengjiang Lagerstätte (Lower Cambrian, China). *Acta Palaeontol. Pol.* **51**, 215–222 (2006).
- Dzik, J. & Krumbiegel, G. The oldest ‘onychophoran’ *Xenusion*: a link connecting phyla? *Lethaia* **22**, 169–181 (1989).
- Edgecombe, G. D. Arthropod phylogeny: an overview from the perspectives of morphology, molecular data and the fossil record. *Arthropod Struct. Dev.* **39**, 74–87 (2010).
- Maas, A., Mayer, G., Kristensen, R. M. & Waloszek, D. A Cambrian micro-lobopodian and the evolution of arthropod locomotion and reproduction. *Chin. Sci. Bull.* **52**, 3385–3392 (2007).
- Waloszek, D., Chen, J.-Y., Maas, A. & Wang, Y.-Q. Early Cambrian arthropods—new insights into arthropod head and structural evolution. *Arthropod Struct. Dev.* **34**, 189–205 (2005).
- Ma, X.-Y., Hou, X.-G. & Bergström, J. Morphology of *Luolishania longicuris* (Lower Cambrian, Chengjiang Lagerstätte, SW China) and the phylogenetic relationships within lobopodians. *Arthropod Struct. Dev.* **38**, 271–291 (2009).
- Willmer, P. *Invertebrate Relationships: Patterns in Animal Evolution* 273–296 (Cambridge Univ. Press, 1991).
- Wang, X.-Q. & Chen, J.-Y. Possible developmental mechanisms underlying the origin of crown lineages of arthropods. *Chin. Sci. Bull.* **49**, 49–53 (2004).
- Bergström, J., Hou, X.-G., Zhang, X.-G. & Clausen, S. A new view of the Cambrian arthropod *Fuxianhuia*. *GFF* **130**, 189–201 (2008).
- Hou, X.-G., Bergström, J. & Ahlberg, P. *Anomalocaris* and other large animals in the Lower Cambrian Chengjiang fauna of southwest China. *GFF* **117**, 163–183 (1995).
- Collins, D. The “evolution” of *Anomalocaris* and its classification in the arthropod class Dinocarida (nov.) and order Radiodontia (nov.). *J. Palaeont.* **70**, 280–293 (1996).
- Kühl G., Briggs, D. E. G. & Rust, J. A Great-Appendage arthropod with a radial mouth from the Lower Devonian Hunsrück Slate, Germany. *Science* **323**, 771–773 (2009).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions J.L. collected most of the fossils, described them and wrote the paper with the other authors; M.S. collected material and was involved in the phylogenetic analysis; J.A.D. contributed to the discussion; H.K. and D.S. were involved in technical aspects of the analysis; Q.O. provided three specimens. J.H. and Z.Z. contributed to fieldwork; X.Z. was involved in the analysis.

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