

Vermiform animals from the Lower Cambrian Chengjiang Lagerstätte

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by

Ma Xiaoya
BSc & MSc (Yunnan University, China)

Department of Geology
University of Leicester

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Abstract

The exceptional preservation of the Lower Cambrian Chengjiang Lagerstätte provides a unique insight into the early evolution of vermiform animals. This study presents new vermiform taxa, describes their morphological features, hypothesizes possible modes of life and discusses phylogenetic relationships among early metazoan phyla.

Morphological features are re-assessed in the Cambrian lobopodian *Luolishania longicruris*, and *Miraluolishania haikouensis* is considered to be its junior synonym. Evidence indicates that *L. longicruris* may have had a filter feeding lifestyle. Cladistic analysis suggests that Cambrian lobopodians are paraphyletic or even polyphyletic, and that *L. longicruris* with well developed sensory structures ('antennae', eyes and setae) and tagmosis (a distinct head and two trunk sections) may be an important representative of the stem lineage leading to arthropods.

A new fossil priapulid *Eximipriapululus globocaudatus* is reported and described on the basis of specimens that reveal exquisite morphological details. Possible internal fertilization is suggested and a putative juvenile is described. Evidence indicates that the animal was an active burrower using a double-anchor strategy. Cladistic analysis resolves *E. globocaudatus* as one of the most derived Cambrian stem priapulids.

The eyes of *Hallucigenia fortis* and *Cardiodictyon catenulum* are reported, along with a re-description of eyes from *L. longicruris*. Three visual units are found within the eyes of *H. fortis* and *L. longicruris*, suggesting that they resemble arthropod lateral visual organs and appear to represent the primitive visual systems of arthropods.

Three new vermiform taxa, *Acanthipos torquatus*, *Hamuscolex bosolveri*, and *Palaeomyzon discus* are described. Comparison with extant taxa suggests that they may be stem group representatives of three separate phyla of extant parasitic worms. The oral disc of *Palaeomyzon discus* indicates a parasitic lifestyle. This study extends both the biodiversity and ecological diversity of known Early Cambrian ecosystems.

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Chapter 1

Introduction

This PhD project is focussed on the vermiform animals of the Chengjiang Lagerstätte, southwest China, with the aims of enhancing understanding of the biodiversity of this fossil assemblage and of the evolution of early metazoans.

1. Chengjiang Lagerstätte

The Chengjiang Lagerstätte is one of the oldest exceptionally well-preserved animal assemblages known, discovered in 1984 at Maotianshan Mountain in Chengjiang County, eastern Yunnan, southwest China (Hou *et al.*, 1999, 2004b; also see Fig. 1A-C). The lagerstätte occurs in the lower part of the Yu'an-shan Member of the Lower Cambrian Heilinpu Formation (Zhang & Hou 1985; also see Fig. 2A, B for localities and stratigraphy) and is of lower to middle Lower Cambrian age, circa 530 million years ago (ma) (Hou & Bergström 1997; Yang *et al.*, 2007; Chang *et al.*, 2007). Therefore, it falls between the Precambrian Ediacaran Lagerstätten (542-635 ma) and the Middle Cambrian Burgess Shale Lagerstätte (515 ma). The Chengjiang Lagerstätte, representing part of the major biotic radiation known as the 'Cambrian Explosion', provides a unique insight into the origin and evolution of early metazoan animals. For three main reasons, this fossil assemblage is considered to be one of the most significant palaeontological discoveries of the 20th Century:

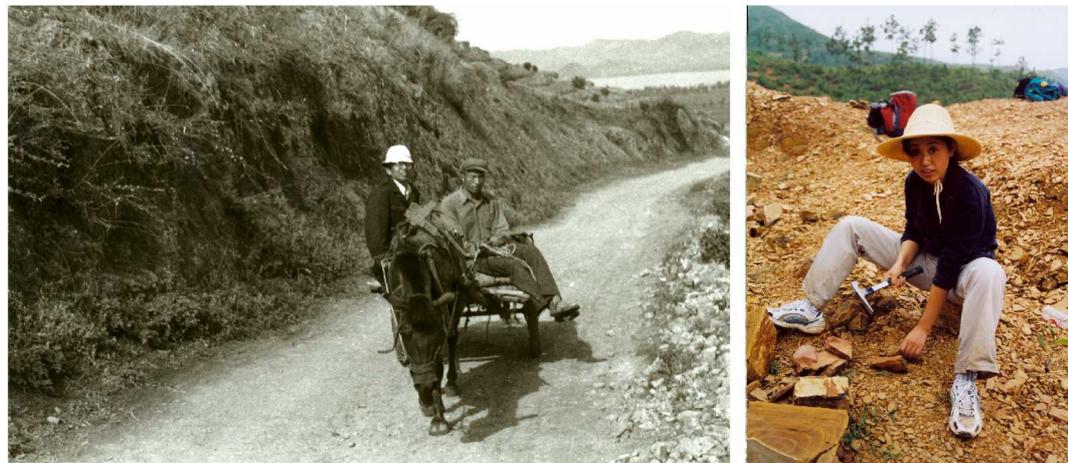


Fig. 1. (A) Maotianshan mountain, the locality where the Chengjiang Lagerstätte was discovered (after Hou *et al.*, 2004b). (B) Prof. Hou Xian-guang in his field trip in 1984, during which he discovered the Chengjiang Lagerstätte (after Hou *et al.*, 2004b). (C) Myself collecting specimens in the Anshan Section, near Mafang village (image taken by Prof. David Siveter).

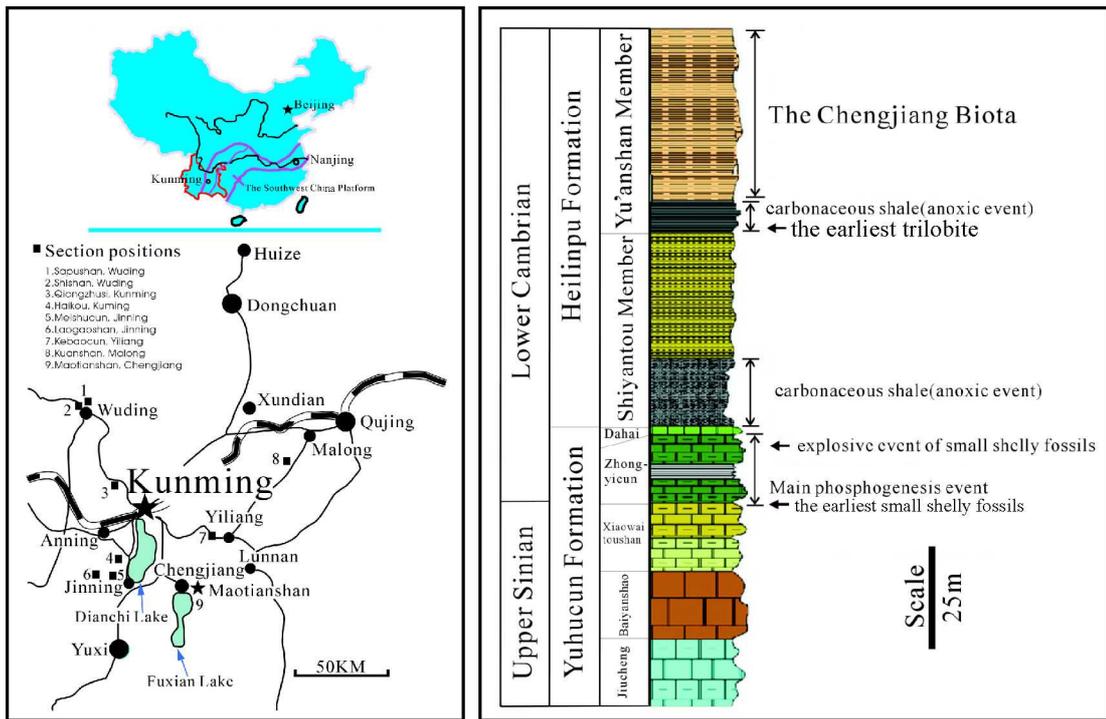


Fig. 2. (A) Geological map of the Kunming to Chengjiang area, showing some of the localities yielding the Chengjiang fossils. (B) The stratigraphic location of the Chengjiang Lagerstätte and other faunal events within the Lower Cambrian of eastern Yunnan Province, China (After Hou *et al.*, 2005).

1) It is one of the earliest metazoan biotas in the world. The Ediacaran biotas are older, but contain few unequivocal representatives of extant phyla. However, the earliest records of many extant phyla are from the Chengjiang Lagerstätte.

2) The Chengjiang Lagerstätte shows exceptional preservation of soft-bodied organisms. It preserves not only hard skeletal or shelled parts of animals (which is typical of most sedimentary deposits), but also preserves in exquisite detail non-biomineralised soft parts (unusual in most sedimentary deposits). Therefore, the Chengjiang fossils make an important contribution in revealing detailed structures of soft-bodied organisms, including providing a unique insight into the earliest vermiform animals.

3) It contains a remarkable biodiversity, a feature considered by many to represent a major radiation in the evolution of life. Over 150 animal species have been reported from this locality, mostly referable to more than ten extant phyla, including Porifera, Cnidaria, Ctenophora, Nematomorpha, Priapulida, Arthropoda, Phoronida, Brachiopoda, Chaetognatha, Echinodermata and Chordata; some are referable to extinct phyla and enigmatic groups, including Hyolitha, Lobopodia, Anomalocarididae, Vetulicolia and un-named groups (Hou *et al.*, 2004b; also see Fig. 3A-G). It is undisputed that the Chengjiang Lagerstätte is exceptional in providing a comprehensive and very early window on the origin and evolution of metazoan animals.

2. Vermiform animals

“Vermiform” (from the Latin *vermes* meaning worm) is an adjective meaning worm-like; the “vermiform animals” are a large group, commonly referred to as “worms” or “worm-like animals”. Although both “vermiform animal” and “worm” are



Fig. 3. A selection of important Chengjiang fossils. (A) *Archisaccophyllia kunmingensis*. (B) *Amplectobelua symbrachiata*. (C) *Naraoia spinosa*. (D) *Cricocosmia jinmingensis*. (E) *Yunnanozoon lividum*. (F) *Myllokunmingia fengjiaoa*. (G) *Luolishania longicruris* (C, E and F after Hou *et al.*, 2004b). Scale bars= 0.3 mm (A); 2 cm (B); 1 cm (C, E, F); 0.5 cm (D, G).

not taxonomic terms, the vermiform body plan has certain common zoological features: bilaterally symmetrical, soft-bodied, and more or less cylindrical with a length greater than width, and generally with a head or anterior sensory region. According to these criteria there are 16 extant animal phyla considered to consist of vermiform animals, including Platyhelminthes (flatworms), Nemertea (ribbon worms), Gnathostomulida (jaw worms), Gastrotricha (no common name), Nematoda (round worms), Nematomorpha (horsehair worms), Priapulida (penis worms), Acanthocephala (spiny-headed worms), Annelida (segmented worms), Echiura (spoon worms), Sipuncula (peanut worms), Tardigrada (water bears), Onychophora (velvet worms), Phoronida (horseshoe worms), Chaetognatha (arrow worms), Hemichordata (acorn worms and pterobranchs) (Fig. 4A-Q).

The vermiform animals are widely distributed and inhabit all kinds of environment with different modes of life (Ruppert & Barnes 1994). You can find them “burrowing in sand and mud, lying under stones and in crevices of rocks, concealed in various types of tubular cases which are free or attached to stones or shells, crawling over the ground or seaweeds, swimming free or attached to other animals” (Arnold, 1968).

In recent years, more and more attention has been paid to the phylogeny of these vermiform taxa using diverse approaches, including morphological, molecular, neurobiological and palaeontological (Wallace *et al.* 1996; Giribet *et al.* 2000; Nielsen 2001).

3. The significance of this project

Vermiform animals, as typical soft-bodied animals, are very rarely preserved, and much of their fossil record comprises trace fossils or fragmented body fossils in poor

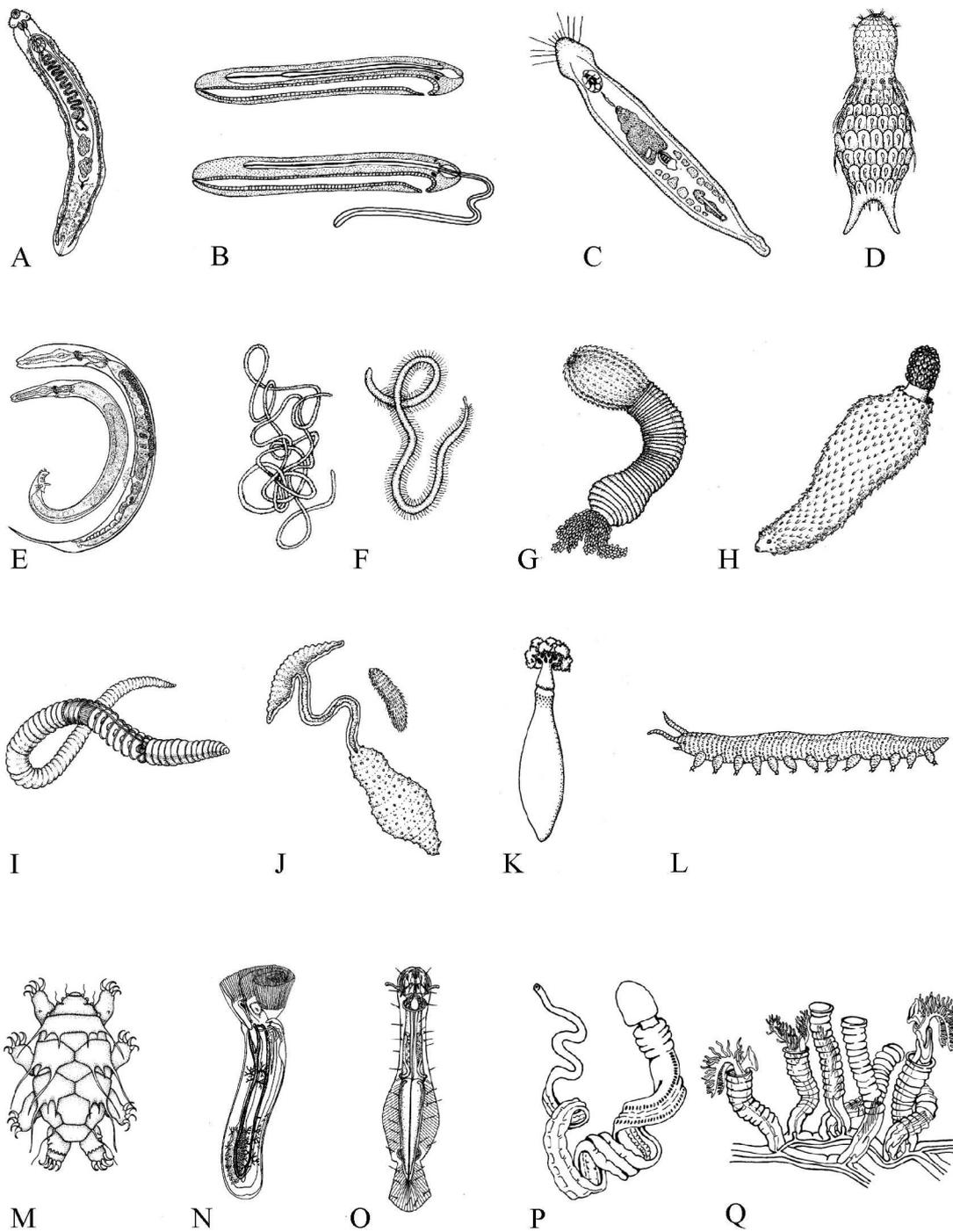


Fig. 4. Animals from 16 vermiform phyla. (A) platyhelminth. (B) nemertean. (C) gnathostomulid. (D) gastrotroch. (E) nematode. (F) nematomorph. (G) priapulid. (H) acanthocephalan. (I) annelid. (J) echiuran. (K) sipunculan. (L) onychophoran. (M) tardigrade. (N) phoronid. (O) chaetognath. (P, Q) Hemichordata. (P) acorn worms. (Q) pterobranchia. (All images come from the BIODIDAC website: <http://biodidac.bio.uottawa.ca>; not to scale)

Table 1. Body Fossil Records of all vermiform phyla (based on Valentine, 2004; Hou *et al.* 2004a; Briggs, *et al.* 1994).

Phylum Name	Number of Occurrences	Earliest Fossil Record	Chengjiang Lagerstätte	Note about the Chengjiang Records
Platyhelminthes	0	-----	No	-----
Nemertea	1	Carboniferous	No	-----
Gnathostomulida	0	-----	No	-----
Gastrotricha	0	-----	No	-----
Nematoda	6	Early Carboniferous	No	-----
Nematomorpha	2	Early Cambrian	Yes?	Three genera reported from Chengjiang, but their assignments have been challenged
Priapulida	16	Early Cambrian	Yes	Abundant, but not all of them are well-accepted
Acanthocephala	0	-----	No	-----
Annelida	6	Early Cambrian	Yes?	A single specimen of a possible annelid reported from Chengjiang
Echiura	1	Late Carboniferous	No	-----
Sipuncula	1	Early Cambrian	Yes	Only a single confirmed body fossil found from Chengjiang in 2004
Onychophora	6	Early Cambrian	Yes?	Fossil lobopodians have been suggested to be their possible ancestors
Tardigrada	2	Early Cambrian	Yes?	
Phoronida	1	Early Cambrian	Yes	Two specimens of one possible species reported from Chengjiang
Chaetognatha	2	Early Cambrian	Yes	A single specimen found in Chengjiang in 2002
Hemichordata	many	Late Cambrian	No	

condition. Complete body fossils are occasionally reported, but few of these have preserved internal structures, which are very important for classification. Given this general paucity of fossil evidence, the phylogenetic study of vermiform animals is still principally based on morphological and molecular research on living animal species.

The fossil record list for each vermiform phylum is presented in Table 1. Of the 16 phyla, four have no body fossil record; another three have no body fossil record before the Carboniferous; one has the earliest fossil record in the Late Cambrian; and the other eight may have their earliest fossil records in the Chengjiang Lagerstätte, although some of these are still debatable. The fossils of the Chengjiang biota are preserved in yellow-weathering mudstone; the very fine-grained nature of the rock has allowed the details of the fossils to be preserved with remarkable fidelity (Hou *et al.* 2004a). One of the most important features of Chengjiang fossils is that different structures of the animals have been preserved in different colours, making details visible, especially some exquisite internal structures of vermiform fossils. Therefore, the Chengjiang biota offers unparalleled insights into the evolution of the earliest vermiform animals.

4. Aims and objectives

The main aims of this PhD project were to:

- increase our knowledge of the biodiversity of vermiform animals in the Lower Cambrian
- increase the understanding of the origin and evolution of early vermiform phyla and their affinities with other animal groups
- explore the significance of vermiform animals in early evolutionary history

- add to our knowledge of the processes of soft tissue preservation in the Chengjiang Lagerstätte

To achieve these aims, my PhD is focussed on the following objectives:

- report and describe new vermiform fossil species from the Chengjiang Lagerstätte
- revise some vermiform taxa using new material
- research the taxonomy and phylogenetic significance of vermiform animals
- study the taphonomy of Chengjiang vermiform fossils

5. Layout of thesis

The following chapters present new results and interpretations. They have been prepared in a format suitable for submission as papers where possible for specific journals, and follow the instructions to authors for those journals.

Chapter 2—Morphology of *Luolishania longicruris* (Lower Cambrian, Chengjiang Lagerstätte, SW China) and the phylogenetic relationships within lobopodians.

This chapter is developed from my master's project, in which I carried out primary research on the Cambrian lobopodian *Luolishania longicruris*. By that stage, I had recognized that *Miraluolishania haikouensis* is a junior synonym of *L. longicruris* and revised some morphological features of this animal. During this PhD project, I used new material to further reinterpret certain important morphological structures, discussed the

mode of life, and used cladistic analysis to explore the evolutionary significance of this animal as well as the phylogenetic relationships among panarthropods. This chapter is a multi-author paper in collaboration with Prof. Hou Xian-guang who contributed all the fossil material and Prof. Jan Bergström who discussed aspects of the mode of life and phylogenetic relationships within panarthropods. The paper has been accepted for publication by the journal *Arthropod Structure and Development* and has already been published on-line (16th March, 2009).

Chapter 3—A new, exceptionally preserved stem priapulid from the Early Cambrian Chengjiang biota, Yunnan, China.

This chapter investigates a new priapulid fossil species from Chengjiang and discusses its possible systematic position. This work was completed by myself, written in the appropriate style for publication in the *Proceedings of the Royal Society B* and is ready for submission.

Chapter 4—The eyes of Lower Cambrian lobopodians and their evolutionary significance for arthropod visual systems.

This chapter describes the detailed structure of the visual organs of three Cambrian lobopodian genera, and discusses their evolutionary significance for the development of visual systems. The work was completed by myself. For the time being, the chapter is written in the appropriate style for publication in *Arthropod Structure and Development*. However, with further modification, I aim to submit it to a higher profile journal.

Chapter 5— Evidence of possible parasitic worms from the Lower Cambrian
Chengjiang Lagerstätte.

This chapter reports three new vermiform species from the Chengjiang Lagerstätte, which are suggested to be stem groups of extant parasitic worms. Certain morphological features described lead to the conclusion that parasitism by metazoans had evolved as a mode of life by the Lower Cambrian. All research and interpretation was carried out by myself. At the moment this chapter is in the appropriate style for publication in *Arthropod Structure and Development*. However, as this work does not focus on arthropods and was written in this style to be consistent with other chapters, this paper will eventually be submitted elsewhere and be reformatted accordingly.

Chapter 2

Morphology of *Luolishania longicruris* (Lower Cambrian, Chengjiang Lagerstätte, SW China) and the phylogenetic relationships within lobopodians

Abstract

New material of the lobopodian *Luolishania longicruris* has been recovered from the Lower Cambrian Chengjiang Lagerstätte, southwest China. The specimens throw new light on several morphological features of the species, including the paired antenniform outgrowths, eyes, head shield, setae and other cuticular projections, as well as the differentiated sclerites, appendages, claws, and lobopod interspaces. *L. longicruris* shows well developed tagmosis: a distinct head and a trunk divided into two sections. The new data allow a revised comparison with other lobopodians. *Miraluolishania haikouensis* Liu *et al.*, 2004 is considered to be a junior synonym of *L. longicruris* Hou and Chen, 1989. Evidence from gut filling and specialized morphological characters indicates that *L. longicruris* may have had a filter-feeding lifestyle. A new cladistic analysis suggests that fossil lobopodians are paraphyletic or even polyphyletic and *L. longicruris* may be an important representative of the stem lineage leading to arthropods.

Keywords: Cambrian lobopodian; antenniform appendages; tagmosis; *Miraluolishania haikouensis*; cladistics; arthropod origins

1. Introduction

Phylum Lobopodia were originally considered to include some extant terrestrial vermiform animal groups with unarticulated lobopods, notably onychophorans and tardigrades (Snodgrass 1938). Since the first onychophoran-like fossil was described from the Middle Cambrian Burgess Shale (Walcott, 1911; Whittington, 1978), “lobopodians” have also been used to include the extinct groups included in the Class Xenusia by Dzik and Krumbiegel (1989). Most fossil lobopodians are marine animals and are primarily known from the Lower Cambrian Chengjiang Lagerstätte of southwest China.

In 1984, the Chengjiang Lagerstätte was discovered at Maotianshan in Chengjiang County, Yunnan Province. The biota occurs in the lower part of the Yu’anshan Member of the Lower Cambrian Heilinpu Formation, circa 530 Ma (Zhang and Hou, 1985). Successive studies have not changed this approximation (Yang *et al.*, 2007; Chang *et al.*, 2007). The great antiquity and the excellent soft-body preservation in the Chengjiang Lagerstätte have provided a unique insight into the origin and evolution of early metazoan animals. *Microdictyon sinicum* (Chen *et al.*, 1989) and *Luolishania longicruris* (Hou and Chen, 1989) were the first two lobopodians described from the Chengjiang Lagerstätte. Since then, six more lobopodian species have been reported from this fauna, indicating their considerable diversity and disparity here. During this time, several published Chengjiang lobopodians have been re-described and revised (Ramsköld, 1992; Hou and Bergström, 1995; Ramsköld and Chen, 1998; Bergström and Hou, 2001; Hou *et al.*, 2004b; Liu *et al.*, 2008a).

L. longicruris was first described and named by Hou and Chen (1989), based on a single dorsoventrally preserved specimen from Chengjiang county. All subsequent

papers (Ramsköld, 1992; Hou and Bergström, 1995; Ramsköld and Chen, 1998; Hou *et al.*, 1999; Hou *et al.*, 2004b) were based on the same specimen and little new information has been added. Recently, 42 new specimens of this species were collected by staff of the Yunnan Key Laboratory for Palaeobiology. These specimens are preserved in different orientations and in exquisite detail, revealing many important new morphological characters.

2. Material and methods

2.1. Material

The holotype specimen of *Luolishania longicuris* was collected from the Maotianshan section at Chengjiang (Hou and Chen, 1989), Yunnan Province. In May 1997, this site was formally protected as a Nature Reserve and further collecting is not permitted. The specimens of *Miraluolishania haikouensis* were collected from a similar horizon at the Jianshan section at Haikou (Liu *et al.*, 2004), about 30 km south of Kunming and about 50 km northwest of the Maotianshan. Our new specimens of *Luolishania longicuris* were collected from the Haikou area (see Hou *et al.*, 2004b for location). All the specimens (YKLP 11271 to YKLP 11312) are deposited in the Yunnan Key Laboratory for Palaeobiology, Yunnan University, Kunming, China.

2.2. Preservation

As with other Chengjiang lobopodian fossils, the specimens of *L. longicuris* are preserved in fine-grained, yellow-weathering mudstone and are strongly flattened,

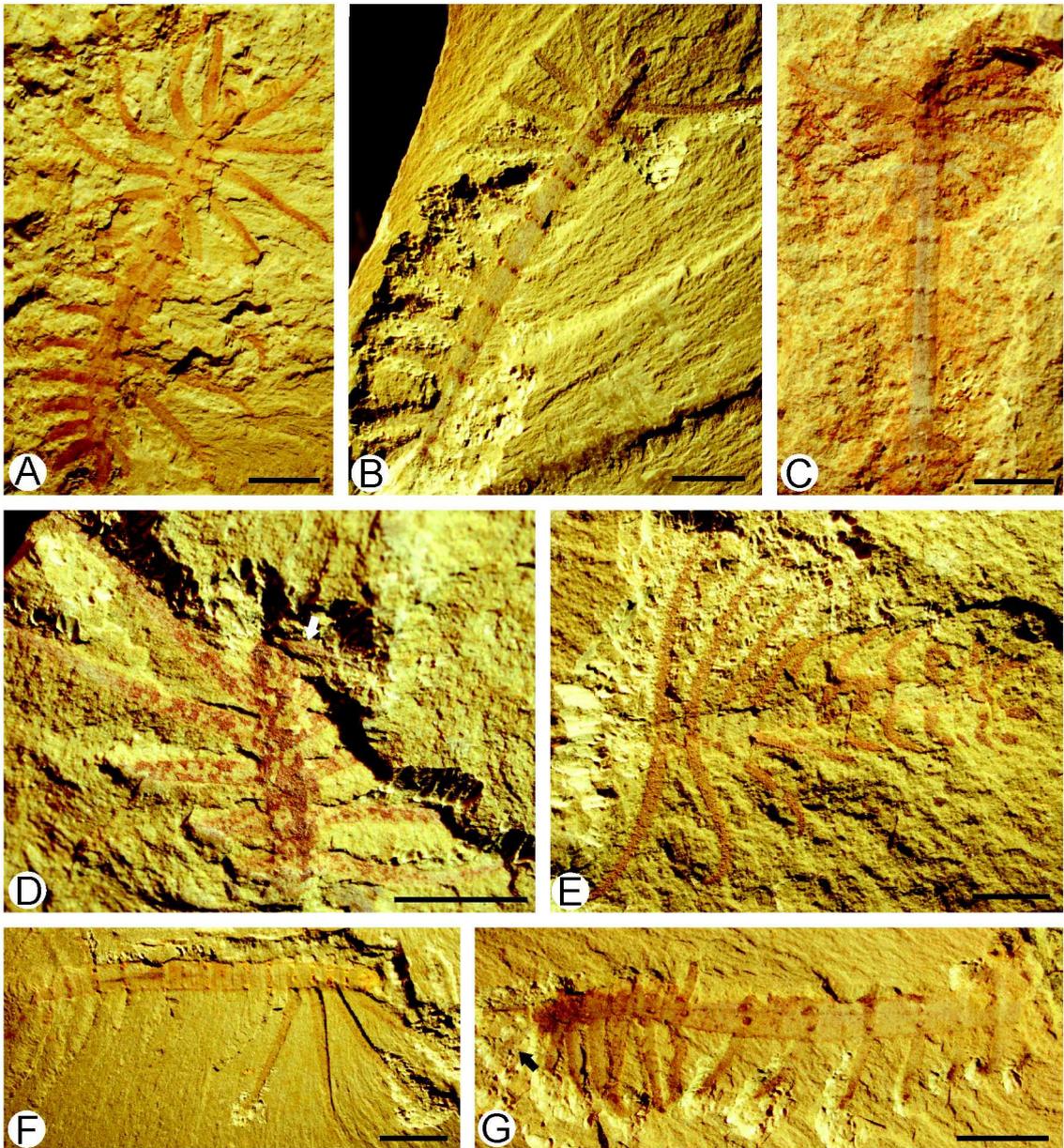


Fig. 1. Dorsoventrally preserved specimens of *Luolishania longicuris* Hou and Chen. (A) RCCBYU 10242, three individual specimens preserved overlapping each other indicating possible social behaviour. See Fig. 2B for explanation. (B) YKLP 11271, showing 14 completely exposed lobopods. See Fig. 2A for explanation. (C) YKLP 11275, showing node-shaped sclerites and the anterior part of the body preserved on a higher lamina. (D) YKLP 11276, arrow indicates an antenniform outgrowth. See close-up in Fig. 3K. (E) YKLP 11280, showing well-exposed appendages and claws. See close-ups in Fig. 10C-F. (F) YKLP 11281, showing trunk annulations and two types of claw. See close-ups in Fig. 10G, H, K, L. (G) YKLP 11283, showing a possible antenniform outgrowth indicated by arrow and setae on the trunk (see close-up in Fig. 9G). Scale bars = 2 mm.

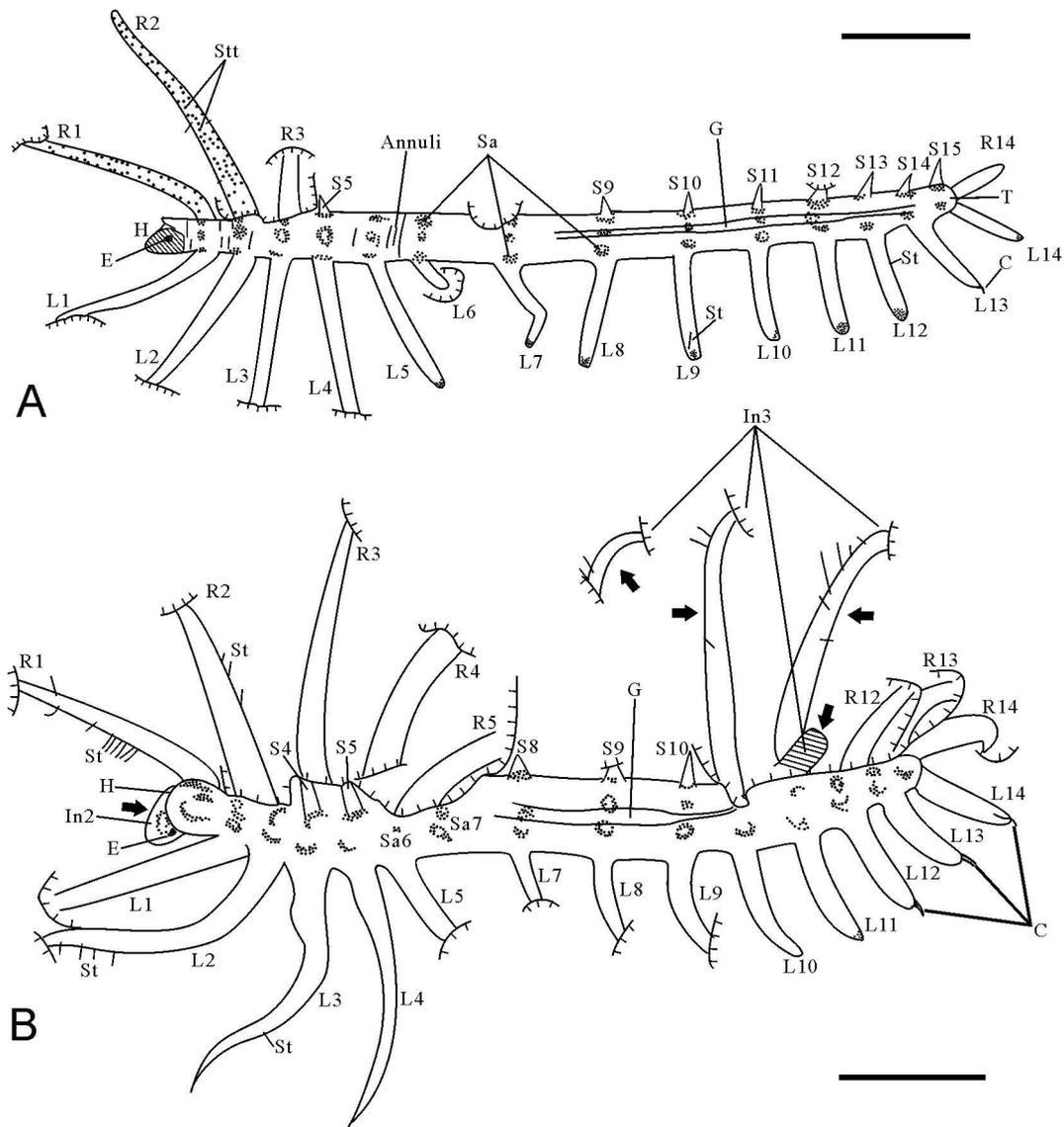
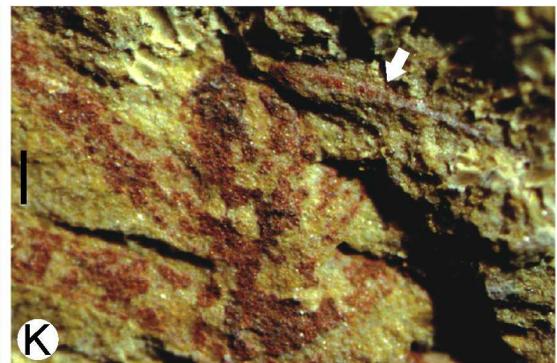
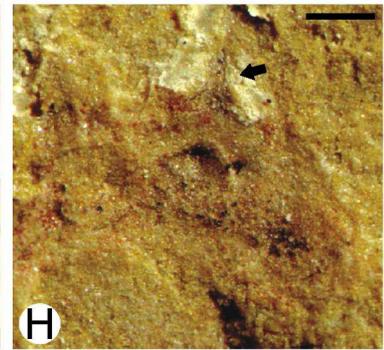
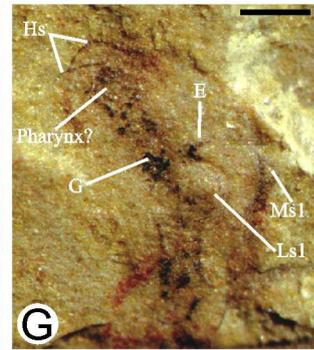
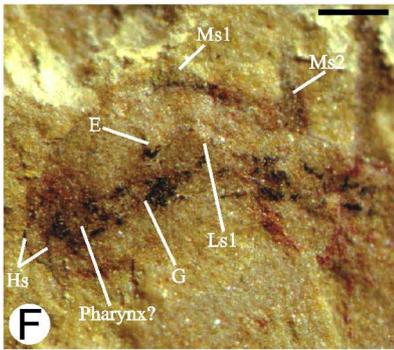
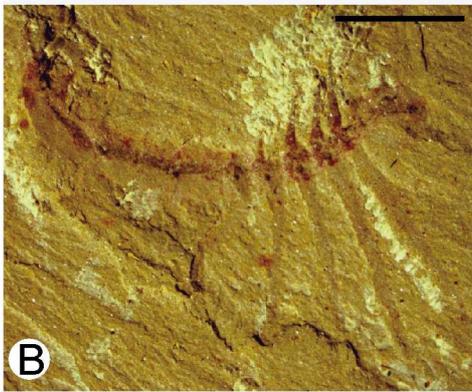
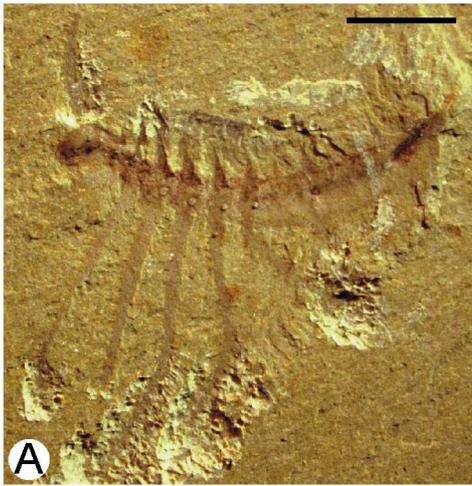


Fig. 2. Camera lucida drawings of *Luolishania longicruris* Hou and Chen. (A) YKLP 11271. (B) RCCBYU 10242; one individual plus small fragments of two others indicated by arrows. Scale bars = 2 mm. C, claw; E, eye; G, gut; H, head; In2, belong to the second individual; In3, belong to the third individual; L, left appendages, counted as L1 to L14 from anterior to posterior; R, right appendages, counted as R1 to R14 from anterior to posterior; S, set of sclerites, counted as S1 to S15 from anterior to posterior; Sa, sclerite attachment; St, seta/setae; Stt, seta traces; T, tail.

Fig. 3. *Luolishania longicruris* Hou and Chen. (A-E) Lateral preserved specimens. (A, B) The part and counterpart of YKLP 11272, showing important details in the head (see close-ups below Fig. 3F-H), specialized long trunk sclerites and long anterior appendages. (C) YKLP 11285, showing right side long trunk sclerites in sets S4 and S5. See close-up in Fig. 4H. (D) YKLP 11278, showing the band where the sclerites attached. (E) YKLP 11279, showing long trunk spines in sets S3-S5, barb-shaped projections set between two neighboring sets of sclerites and annuli on lobopod. See close-up in Fig. 4F. (F-K) Detailed structures of head. (F, G) The part of the head of YKLP 11273, showing the head shield, head sclerites, eye, gut, pharynx and the second sclerite S2. The relief of head sclerite and eye is better shown in (G). See Fig. 6D for explanation. E, eye; G, gut; Hs, head shield; Ls1, left side sclerites of S1; Ms1, middle dorsal sclerites of S1; Ms2, middle dorsal sclerites of S2. (H) The counterpart of YKLP 11273, the better exposed mid-dorsal head sclerite indicated by arrow. (I) YKLP 11272, showing proboscis, head sclerites, paired eyes indicated by arrows. See Fig. 6A for explanation. (J) YKLP 11277, showing paired eyes indicated by arrows, protruding proboscis, head sclerites. See Fig. 6C for explanation. (K) YKLP 11276, a complete antenniform outgrowth indicated by arrow. See Fig. 6B for explanation. Scale bars = 2 mm (A-E); 0.3 mm (F-K).



although some structures retain a low three-dimensional relief. Because of the compaction, it is essential to take into consideration the original orientation when reconstructing the animals. Twenty-one newly collected specimens are dorsoventrally preserved, displaying similar characters to the holotype of *L. longicuris*. Another twenty-one specimens are laterally preserved, displaying new characters similar to those of *M. haikouensis* (Liu *et al.*, 2004). Some of these specimens appear straight, while others are more or less curved or twisted.

2.3. Method

The terminology used in this paper follows that of Hou and Bergström (1995). The observation, preparation and camera lucida drawings of specimens were made under a Nikon SMZ-10A Microscope. Pictures were taken using a Nikon SMZ1000 photomicroscope. SEM EDX (Scanning Electron Microscope Energy Dispersive X-rays) has been used for element mapping.

3. Results

3.1. Systematic palaeontology

Phylum LOBOPODIA Snodgrass, 1938

Order ARCHONYCHOPHORA Hou and Bergström, 1995

1995 Order Scleronychophora ord. nov., Hou and Bergström, pp. 14; non Eoconchariidae.

1995 Order Paronychophora ord. nov., Hou and Bergström, pp. 17.

Diagnosis (emended from Hou and Bergström 1995). Lobopodians with a distinct head, trunk tagmosis and differentiated appendages.

Type family. Luolishaniidae Hou and Bergström, 1995.

Other families. Cardiodictyidae Hou and Bergström, 1995; Hallucigeniidae Conway Morris, 1977a; Onychodictyidae Hou and Bergström, 1995; Collins' monster (unnamed; Collins, 1986, photograph p. 39; Delle Cave and Simonetta, 1991; Collins, 2001, *Abstract* in "The 3rd International Conference on Trilobites and their relatives", Oxford University, pp. 9; Simonetta, 2004). Hou and Bergström (1995) placed various Cambrian lobopodian families into different orders, as follows: Luolishaniidae, Order Archonychophora; Hallucigeniidae and Cardiodictyidae, Order Scleronychophora, which also includes the Eoconchariidae; Onychodictyidae, Order Paronychophora.

Family LUOLISHANIIDAE Hou and Bergström, 1995

Diagnosis (emended from Hou and Bergström 1995). Multi-segmented lobopodians with thorn-shaped sclerites, arranged in sets of three per segment. Numbers of annuli between each set of sclerites decreasing towards both ends of the animal.

Type and only genus. *Luolishania* Hou and Chen, 1989

Remarks. The Luolishaniidae is the only family possessing segmental sets of three sclerites, rather than a pair of sclerites as is common in other lobopodians.

Genus *Luolishania* Hou and Chen, 1989

2004 *Miraluolishania* Liu & Shu gen. nov.; Liu *et al.*, p. 1063

Diagnosis (emended from Hou and Chen 1989). Head elliptical in profile and covered by a thin shield, possessing a pair of antenniform outgrowths and eyes. Fifteen

sets of sclerites arranged along the body, divided into three types: first set of sclerites on the head with large basal area and short spine; third to fifth sets with notably long spines; remaining 11 sets shorter and thorn-shaped. Between each set of sclerites three barb-shaped projections similarly arranged in middle of each segment. Fourteen to sixteen pairs of lobopods ventrolaterally beneath the trunk, each possessing setae and four distal claws. Lobopods and claws differ along trunk: anterior lobopods long, slender, spiny, with thin, straight claws; posterior lobopods shorter, thicker, with thick, curved claws.

Type and only species. *Luolishania longicuris* Hou and Chen, 1989

Discussion. The revised reconstruction of *Luolishania* shows many more similarities to the genus *Hallucigenia* (Walcott, 1911; Conway Morris, 1977a; Hou and Bergström, 1995) than previously understood. These genera share: an elongate trunk; an expanded and oval-shaped head region, including a pair of eyes (unpublished finding in *Hallucigenia* by author); dorsal sclerites; specialised anterior appendages; and a trunk extending beyond the last pair of lobopods. The differences between these two genera are that no paired antenniform outgrowths have been found in *Hallucigenia*, which has two pairs of specialised anterior appendages in front of the first pair of sclerites; only two dorsal sclerites are present above each pair of lobopods in *Hallucigenia*, but three are present in *Luolishania*; *Hallucigenia* has seven pairs of sclerites and nine pairs of appendages, but *Luolishania* has 15 sets of sclerites and 14 or 16 pairs of lobopods; there are two terminal claws on each lobopod in *Hallucigenia*, but four in *Luolishania*; and unlike in *Luolishania*, posterior to the last pair of lobopods the trunk is deflected dorsally in *Hallucigenia fortis* (Hou *et al.*, 1999, p. 72-73, figs. 91-93) or is deflected ventrally in *Hallucigenia sparsa* (Briggs *et al.*, 1994, p. 139, fig. 91).

We also notice the similarities between *Luolishania* and two undescribed Burgess Shale lobopodians, one of them known as “Collins’ monster” (Collins, 1986,

photograph p. 39; Delle Cave and Simonetta, 1991; Collins, 2001, *Abstract* in “The 3rd International Conference on Trilobites and their relatives”, Oxford University, pp. 9; Simonetta, 2004). *Luolishania* and these two Burgess Shale lobopodians all have some spiny arms and ‘anchoring’ rear lobopods, indicating that they may even have shared a similar mode of life.

Species *Luolishania longicruris* Hou and Chen, 1989

1989 *Luolishania longicruris* gen. et. sp. nov., Hou and Chen, pp. 207-213, pl. 1, figs. 1, 2.

1992 *Luolishania longicruris*; Ramsköld, pp. 443-460.

1995 *Luolishania longicruris* Hou & Chen, 1989; Hou and Bergström, pp. 3-19.

1998 *Luolishania longicruris*; Ramsköld and Chen, pp. 112-113, figs. 3.4A-C, 3.5B.

1999 *Luolishania longicruris* Hou et Chen, 1989; Hou *et al.*, p. 69, figs. 85, 86.

2004b *Luolishania longicruris* Hou & Chen, 1989; Hou *et al.*, pp. 82-83, fig. 14.1.

2004 *Luolishania longicruris* Hou & Chen 1989; Chen, p. 239, figs. 368, 369.

2004 *Miraluolishania haikouensis* Liu & Shu gen. et sp. nov., Liu *et al.*, pp. 1063-1071, figs. 1, 2, 3.

2008b *Luolishania longicruris* Hou et Chen 1989; Liu *et al.*, p. 278, fig. 1i.

2008b *Miraluolishania haikouensis* Liu et Shu 2004; Liu *et al.*, p. 278, fig. 1h.

Material and locality. There are 42 specimens (22 with counterparts) in total in the collection of the Yunnan Key Laboratory for Palaeobiology. Additional material has been described by Hou and Chen (1989), Ramsköld and Chen (1998), Hou *et al.* (2004a) and Liu *et al.* (2004). Specimens illustrated herein are YKLP 11271-11287 and RCCBYU10242 (Figs. 1, 2, 4, 9, 10). Specimens referred to but not illustrated are YKLP 11288-11292. All specimens are from the Anshan section, Mafang village,

Haikou, Kunming, except for specimens YKLP 11291-11299 which are from the Jianshan section.

Emended diagnosis. As for the genus.

Holotype. A complete specimen consisting of part and counterpart, collected from the Lower Cambrian Heilinpu Formation, at Maotianshan, Chengjiang, East Yunnan. The holotype, Cat. No. 108741, is stored in the Nanjing Institute of Geology and Palaeontology, Academia Sinica.

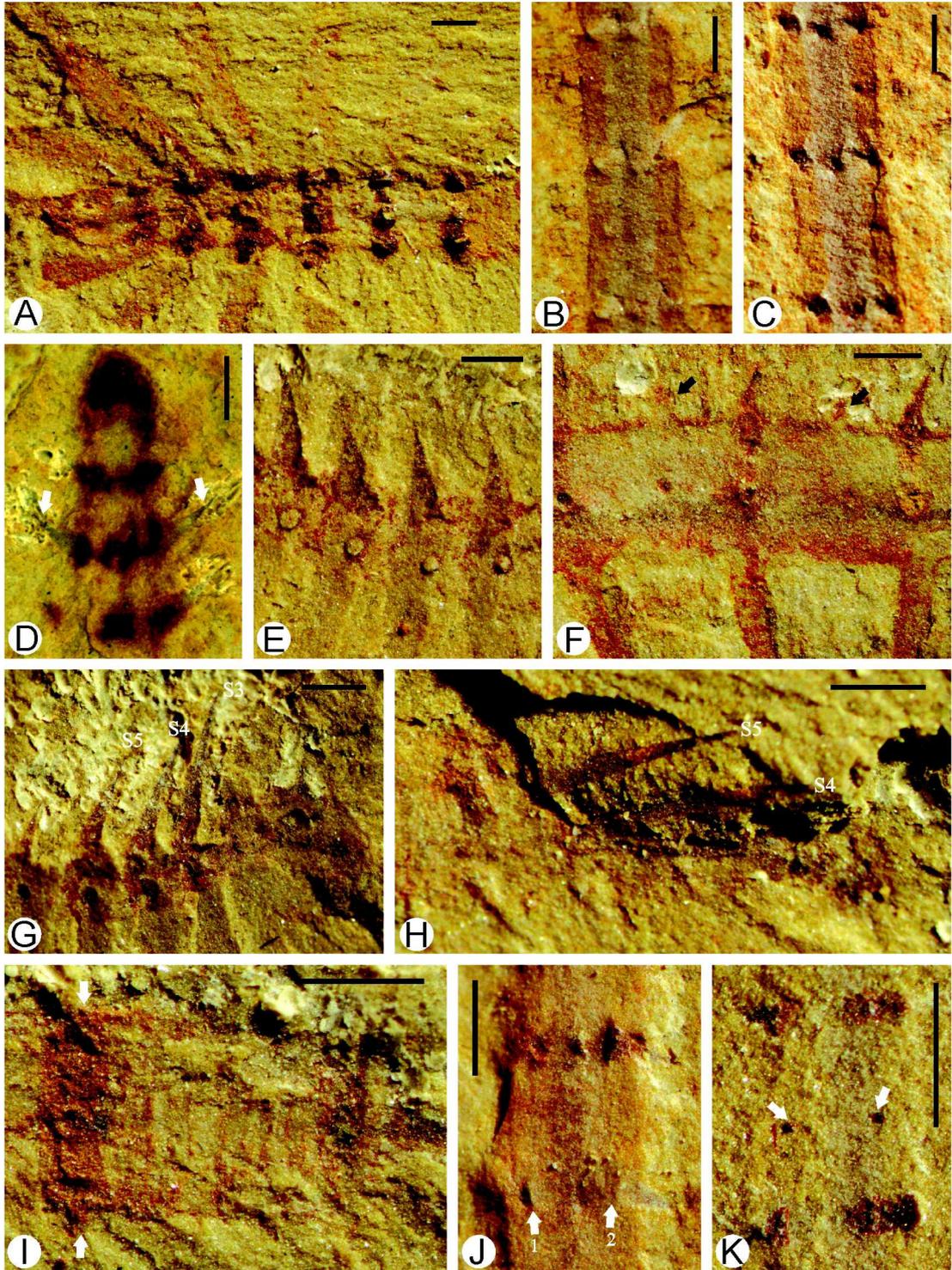
3.2. Description

The body is long and slender, divided into a slightly expanded head (from the anterior end to in front of the first pair of lobopods) and a trunk tapering towards the posterior end. Complete specimens are from 8.5 mm to 14.3 mm long, with an average length of 11.6 mm. The width is up to 0.9 mm. The dimensions of the holotypes of *L. longicruris* and *M. haikouensis* fall within the range of the newly collected material. The greatest width of the trunk is the same in laterally and dorsoventrally compressed specimens, indicating a round transverse section.

3.2.1. Sclerites

L. longicruris possesses 15 sets of sclerites along the body, each set composed of three individual spine-shaped sclerites arranged transversely (one dorsal, two lateral). One of these sets is on the head and the others are each above a pair of lobopods (Figs. 1A-C, 2A and 4A-D). For convenience, the sets of sclerites are numbered S1-S15 from anterior to posterior (see Fig. 2A, B and 6A-D). This interpretation of sclerites differs from previous authors. Hou and Chen (1989) described *L. longicruris* as carrying three

Fig. 4. Preservation and morphology of sclerites. (A) The anterior portion of YKLP 11274, showing three sclerites in a set. (B, C) Trunk of the part and counterpart of YKLP 11275, showing sclerites preserved as nodes and pits. (D) The anterior portion of dorsoventrally preserved YKLP 11287, showing two lateral spine-shaped sclerites indicated by arrows. (E) Trunk of YKLP 11273, showing sclerite morphology in laterally preserved specimen. (F) Trunk of YKLP 112279, arrows point to barb-shaped projections between two neighboring sets of sclerites. (G) The anterior part of YKLP 11273, showing the specialized long trunk spines in sets S3-S5. (H) The anterior part of YKLP 11285, showing the right side long trunk spine in sets S4 and S5. (I) Trunk of YKLP 11274, showing trunk annulations and the rusty coloured band at sclerites attached area (indicated by arrows). (J) Trunk of YKLP 11272, showing only two sclerites preserved within a set (indicated by arrows). (K) Trunk of YKLP 11284, showing barb-shaped projections in dorsoventrally preserved specimen (indicated by arrows). Scale bars = 0.5 mm.



tubercles transversely arranged on each trunk segment just above a pair of lobopods, while *M. haikouensis* was stated to have a pair of dorsal spines in the same position (Liu *et al.*, 2004). These differences in descriptions can be explained by the preservation of sclerites (see below).

3.2.1.1. Sclerite preservation. In most dorsoventrally compressed specimens, the sclerites tend to be preserved as small, seemingly round features corresponding to small pits in the counterpart (Figs. 4A-C). Similar preservation is also seen in the holotype, and this is why these structures were also described as domes (Ramsköld, 1992) or small, rounded bumps (Hou *et al.*, 2004b). However, the lateral sclerites preserved in outline in specimen YKLP 11287 (Fig. 4D) and YKLP 11271 (Figs. 1B and 2A) clearly show that these sclerites are thorn-shaped spines. The node-shaped appearance could be caused by compression, however, it is interesting to note that these nodes are narrower than the base of the mid dorsal spines preserved in outline (Fig. 4E). The nodes therefore may represent internal moulds of hollow spines. Reexamination of the holotype has shown that the right lateral sclerite S10 is also spine-shaped, as is also evident from a published color image (Chen, 2004, p. 239, fig. 368).

Most dorsoventrally preserved specimens show three sclerites in each set, one median and two lateral (Figs. 1A-C, 2A and 4A-D). Compared with the good preservation of lateral sclerites, some of the mid-dorsal sclerites are preserved as faint traces or are even invisible (Figs. 1A, 2B, 4J, K and 9A), causing the false impression that there are only two sclerites on each trunk segment (cf. Ramsköld, 1992; Hou *et al.*, 2004b; Liu *et al.*, 2004). The poor preservation of the mid-dorsal sclerites may be the result of two factors: 1) the major part of the mid-dorsal sclerites being buried in the overlying matrix, which has been removed when the specimen was exposed; 2) twisting

of the trunk may obscure features, leaving only two sclerites visible. The transverse belt in which the three sclerites are situated shows a distinct rusty-coloured band (Figs. 3D and 4A, I). The rusty colour is iron oxide reflecting pyrite (Gabbott *et al.* 2004), and it is often associated with cuticle or cuticular structures in Chengjiang fossils. Therefore, the colour could be the result of possibly thicker epidermis in this area, or of weathering of the sclerites and epidermis.

In laterally compressed specimens, the mid-dorsal sclerites are well preserved, while the lateral sclerites of one side are visible as nodes or traces and those of the other side are invisible (Figs. 3A-E and 4E-H).

3.2.1.2. Sclerite morphology and differentiation. The lateral morphology of the sclerites and their circular traces indicate that the sclerites are thorn-shaped, with a wide base and a slightly curving process. The laterally preserved mid-dorsal sclerites usually display the sclerite morphologies better and reveal a differentiation of sclerites into three types (Figs. 5A-C).

The first type is represented by the specialised spines of the head (S1). Liu *et al.* (2004) described *M. haikouensis* as possessing a pair of “horns” posteriorly on the head. Most of our dorsoventrally preserved specimens also show a pair of bumps posteriorly on the head, corresponding to a pair of pits in the counterpart (Figs. 3I, J and 6A, C). However, the exquisite preservation of specimen YKLP 11273 (Figs. 3F-H, and see Fig. 6D) reveals that this head structure is actually a set of three specialised sclerites on the posterior part of the head. In specimen YKLP 11273, the left lateral sclerite is preserved as a knob in low relief, with a smooth round margin and a diameter of 0.3 mm (about half of the head width). The mid-dorsal sclerite is completely exposed in the counterpart (Fig. 3H), clearly showing the spine shape with a height of 0.3 mm. As the specimen is

laterally compressed, the right lateral sclerite is obscured. The traces of the three head sclerite bases can also be observed in the dorsoventrally preserved specimens YKLP 11276 (Figs. 3K and 6B) and RCCBYU 10242 (Figs. 1A, 2B and 9A). In summary, the first type of sclerite, represented by a single set in the head, has a large disc-shaped base and a short spine (Fig. 5A).

The second type of sclerite is represented by the third to fifth sets of spines (S3-S5), which are very long and situated on the trunk segments two to four. During the preparation of specimen YKLP 11273, the mid-dorsal spines in sets S3-S5 were well exposed, with a length of 1.5 mm, 1.3 mm and 1.0 mm respectively (Figs. 3A, B and 4G). Compared with the other trunk sclerites (about 0.5 mm long), these three spines are especially long (Fig. 5B). The long mid-dorsal spines in sets S3-S5 are also well preserved in the laterally preserved specimen YKLP 11279A (Fig. 3E) as well as in *M. haikouensis* of Liu *et al.* (2004, figs. 2a-d). Specimen YKLP 11285 (Figs. 3C, 4H) shows that the right lateral sclerites in sets S3 and S4 have the same morphology as the mid-dorsal ones, indicating that the long trunk spines of S3 to S5 are uniform within a transverse set. On all specimens these long spines are slightly inclined towards the anterior end, and the tip of each is slightly curved and sharply pointed.

The third type of sclerite is represented by the posterior sets of thorn-shaped trunk spines S6-S15 and by S2. Sclerites of S6-S15 show a uniform morphology towards the posterior end, with a height of 0.5 mm (Figs. 2A, B, 3D, E, 4E, F and 5C). The S2 sclerites, situated on the first trunk segment, are notably different from their neighbours on the head and on the second trunk segment. As seen in lateral view, they are about 0.4 mm high and morphologically similar to the posterior trunk sclerites (Figs. 3A, F and 6D).

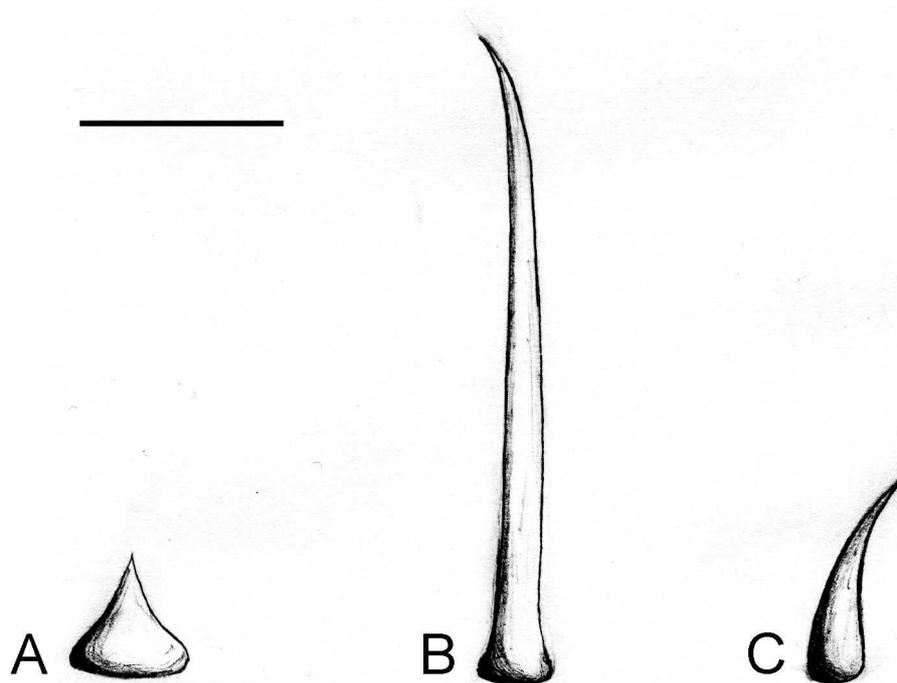


Fig. 5. Reconstruction drawings of three types of sclerites. (A) head sclerites, S1. (B) Long trunk spines, S3-S5. (C) Posterior trunk sclerites, S2 and S6-S15. Scale bar = 0.5 mm.

3.2.2. Barb-shaped projections

Specimen YKLP 11279 displays a regular set of three barb-shaped projections situated mid-way between two neighbouring sets of sclerites (Figs. 3E and 4F). In this laterally compressed specimen, the left projections are preserved as small pits, while the mid-dorsal projections are barb-shaped with a height of 0.26 mm (about half the size of the normal trunk sclerites). Sclerites are often weathered and preserved in a rusty colour. However, the projections are preserved in the same pinkish colour as the pliable trunk tissue with a smooth orange margin caused by the preservation of an epidermis. They are not distinctly demarcated from the adjacent trunk tissue. The barb-shaped projections are also seen in specimen YKLP 11273 (Fig. 3A). In dorsoventrally preserved specimens (Figs. 4B, C, K), the projections are seen as small round nodes, very similar to neighbouring sclerites. Ramsköld (1992) found the same structure in the holotype of *L. longicruris* and described it as a pair of annular nodes, the apparent shape of which would be influenced by preservation, just as in specimen YKLP 11284 (Fig. 4K). This type of projection can only be seen with certainty in the middle part of the trunk from the third to the thirteenth walking lobopods; this could be preservational, but it is also possible that they were not developed in the distal segments of the animal. Similar triangular projections are also found along the trunk of *Aysheaia* (Whittington 1978, p. 177, plates 3-5).

3.2.3. Head

The head extends from the anterior end of the animal to in front of the first pair of lobopods. In dorsoventrally flattened specimens (Figs. 1A, B, D, 2A, B, 3I-K, 4A, D and 6A-C), the head appears to be rounded and slightly elongate; this is also apparent in the holotype (Hou and Chen, 1989, Fig. 2; Hou *et al.*, 2004b, Fig. 14.1). In lateral

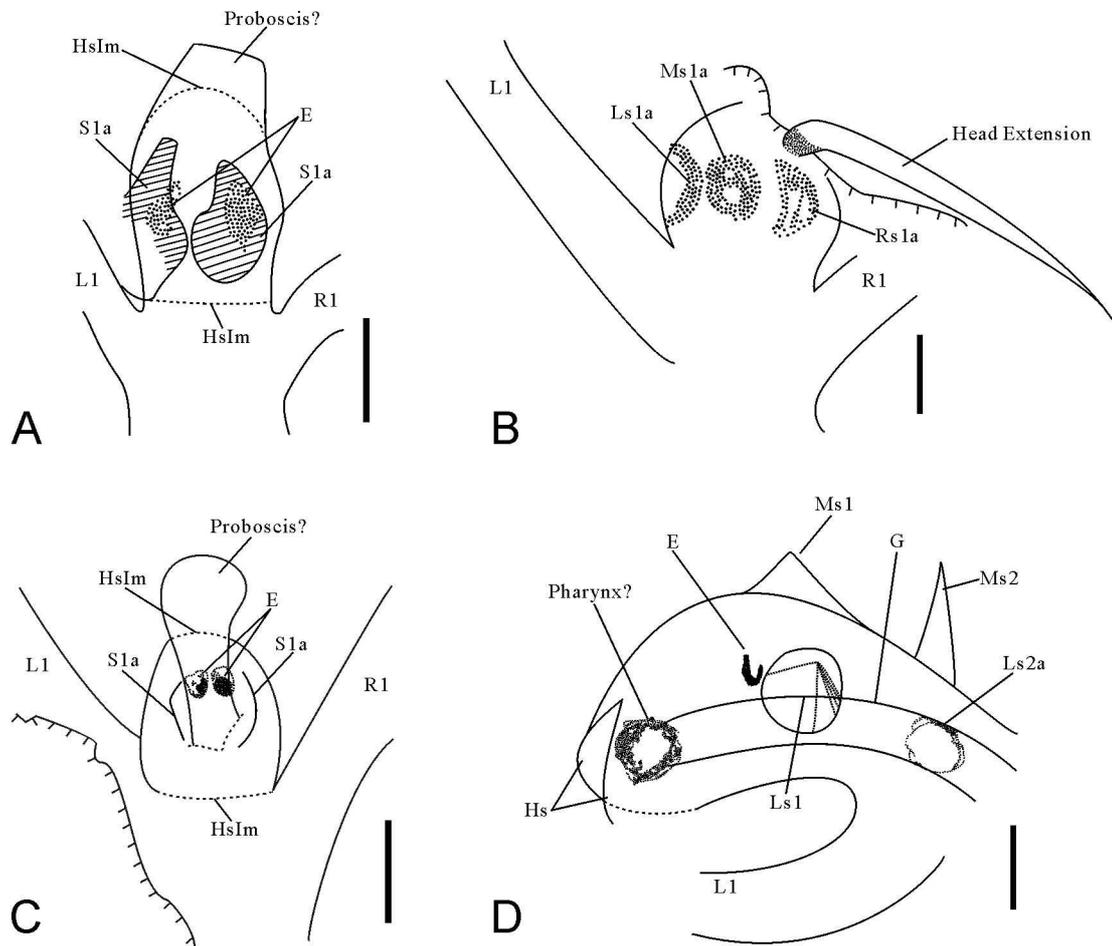


Fig. 6. Camera lucida drawings of the detailed head structures. (A) YKLP 11272. (B) YKLP 11276. (C) YKLP 11277. (D) YKLP 11273. Scale bars = 0.3 mm. E, eye; G, gut; Hs, head shield; HsIm, impression of head shield; L1, the first left lobopod; Ls1, left side sclerites of S1; Ls1a, the attachment of left side sclerites of S1; Ls2a, the attachment of right side sclerite of S2; Ms1, middle dorsal sclerites of S1; Ms1a, the attachment of middle dorsal sclerites of S1; Ms2, middle dorsal sclerites of S2; R1, the first right lobopod; Rs1a, the attachment of right side sclerites of S1; S1a, the attachment of sclerites in set S1.

preservation, the head has an oval shape (Figs. 3A, B, D, F-H and 6D, cf. *M. haikouensis* of Liu *et al.*, 2004). Behind the head, there is a slight constriction indicating its delineation from the trunk.

3.2.3.1. Paired antenniform outgrowths. Specimen YKLP 11276 is a dorsoventrally preserved specimen displaying the dorsal side. During preparation, a complete antenniform outgrowth was exposed from the right side of the head. The structure extends backwards, upwards and to the right and is preserved at a higher level than the head (Figs. 1D, 3K and 6B). This characteristic outgrowth is long and slender, tapering towards the distal end, and is about 2 mm long with a base width of 0.1 mm (one-fifth of the width of the first lobopod). No annulations or setae have been found on this structure. The attachment of this extension is at the very anterior part of the head (Figs. 3K and 6B), rather than at the posterior as suggested by Liu *et al.* (2004). In the holotype, a pair of tubercles (found at the very anterior part of the head) has been previously been suggested to indicate the presence of head appendages (Ramsköld and Chen, 1998). In specimen YKLP 11283, a rod-like structure extends forwards and to the left from the anterior region of the head (Fig. 1G). This antenniform outgrowth is similar to the long trunk spines, but: 1) the sclerites have a wider base, shorter length and a triangular shape, while the outgrowth is longer and more slender; 2) the sclerites are transversely arranged in a set of three, but the outgrowths appear as a pair; 3) the sclerites are distinctly stiff, but the attitude in which the outgrowth is preserved suggests more flexibility; 4) the outgrowth is preserved in a similar condition and colour to the body and lobopods, indicating that it is soft tissue.

3.2.3.2. *Eyes*. Eight of the dorsoventrally preserved specimens show a pair of round black spots in the middle of the head (Figs. 3I, J); a similar structure was reported from *M. haikouensis* where it was interpreted as a pair of eyes (Liu *et al.*, 2004).

The eyes are situated dorsolaterally on the head well behind the paired frontal extensions. The laterally preserved specimen YKLP 11273 shows that the eye is just in front of the head sclerite (Figs. 3A, F-H and 6D). In dorsoventrally preserved specimens the two structures often seem to overlap (Figs. 2A, B and 3I, J). Therefore, the eyes probably lie at mid-length on the head. In laterally preserved specimens, only one eye is exposed. It is often seen just above the gut trace (Figs. 3F, G and 6D), which indicates that the eye is situated laterally on the side of the head. Overall, the original position of the two eyes was apparently dorsolateral.

3.2.3.3. *Head shield*. In the laterally preserved specimen YKLP 11273, there are two purplish red smooth lines preserved at the anterior edge of the head (Figs. 3F, G and 6D). These lines converge at an anterodorsal point, a quarter of the length of the head from in front. No hinge line has been found behind this point. The two lines extend and curve anteroventrally and were preserved crossing each other at the anterior end, as seen in lateral view. Behind this point, they are not well preserved. A faint trace occurs ventrally beyond this point, but its relationship to the two lines is not clear. These lines represent the anterior outside margin of the head, but two distinct smooth lines could not be the result of simple compression of the head. Therefore, these lines may indicate that there is a defined structure covering the head with an opening at the anterior end (Figs. 3F, G and 6D). Herein we call this structure the “head shield”. Although this structure is not apparent in other specimens of *L. longicuris*, similar head shields have been found in two other Cambrian lobopodian species, *Cardiodictyon catenulum* (Hou

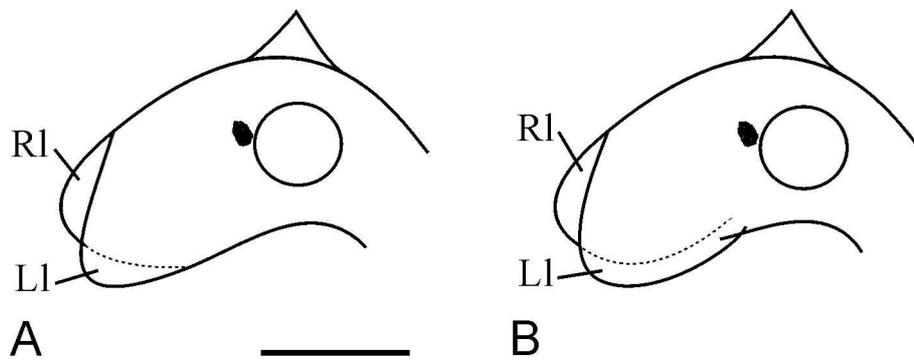


Fig. 7. Complementary drawings to show two possibilities of the structure of the head shield. (A) The two lateral lobes converge ventrally at a point corresponding to their dorsal convergence. (B) The two parts of the head shield are totally separated ventrally. Scale bars = 0.5 mm. LI, the left lobe; RI, the right lobe.

et al., 1991) and *Hallucigenia fortis* (Hou and Bergström, 1995). The head shield of *L. longicruris*, if present, appears to be thin, transparent and to extend directly from the trunk epidermis. In front of the dorsal convergence point the head shield divides into a pair of lateral lobes and forms an anterior opening, but its ventral morphology is not clear. There are two possibilities: 1) the two edges of the lateral lobes converge ventrally at a point corresponding to their dorsal convergence (Fig. 7A); 2) the two parts of the head shield are totally separated ventrally (Fig. 7B).

3.2.3.4. Mouth. In specimens YKLP 11273 and YKLP 11291 the alimentary canal goes through the head and terminates at its anterior end (Figs. 3F-H and 6D), indicating that the mouth is situated anteriorly rather than antero-ventrally as suggested by Liu *et al.* (2004). A similar position for the mouth can also be observed in *Hallucigenia fortis* (Hou *et al.*, 2004b, p. 89, fig. 14.7). Specimen YKLP 11273 shows that there is a circular, slightly expanded structure at the anterior end of the alimentary canal (Figs. 3F, G and 6D). This may be a pharynx similar to that of *Paucipodia inermis* (Hou *et al.*, 2004a). Several dorsoventrally preserved specimens display a round structure in the anterior gap of the head shield (Figs. 3 I, J and 6A, C). In specimen YKLP 11277, this structure is clearly seen extending from inside of the head shield (Fig. 3J and 6C), suggesting a proboscis-like extension of the gut. This structure is not visible in all specimens, indicating that it might be able to protrude and withdraw from the mouth.

3.2.4. Lobopods

In the newly collected material, 14 pairs of lobopods, designated R/L 1 to R/L14 (R for right, L for left) from anterior to posterior, are arranged ventrolaterally along the

trunk. Each pair corresponds in segmental position to a set of sclerites. The morphology of the lobopods varies from anterior to posterior.

3.2.4.1. General morphology. Measurements of the lobopods in eight complete specimens are given in Table 1. The first five pairs of lobopods are notably long (Fig. 8A), all more than 4 mm in length. The length decreases dramatically in the sixth and seventh lobopods to attain a constant length of 1.55 mm in the eighth to the fourteenth lobopods. A similar variation of lobopod length was reported from *M. haikouensis* by Liu *et al.* (2004), but their description and division do not equate with our measurements. Although the lobopod length varies, the width of the proximal part is the same in all lobopods (about 0.4 mm). Therefore, the first five pairs of lobopods appear long, slender and slightly tapering towards the distal end, while the other lobopods appear relatively short and thick.

The lobopods are finely annulated, with about 12-13 annuli per millimetre. In the centre of each lobopod, there is a whitish coloured strip about 0.2 mm wide, extending along the entire length of the lobopod and connecting with the trunk area (Fig. 9J). The same structure can be seen in almost every Chengjiang lobopodian. It has been suggested to be a central canal that may have functioned as a hydroskeleton (Hou *et al.*, 2004b).

3.2.4.2. Setae on lobopods. Specimens RCCBYU 10242 and YKLP 11276 show that there are some spine-like setae densely arranged on at least the first five pairs of lobopods (Figs. 1A, 2B and 9A, D); these setae are more often preserved as red-spot traces in other specimens (Figs. 2A and 9B). On lobopod R5 of specimen YKLP 11276, a round, red base can be clearly seen at the proximal end of the setae, indicating that the

Table 1

Measurement (in mm) of lobopod length in *L. longicruris*

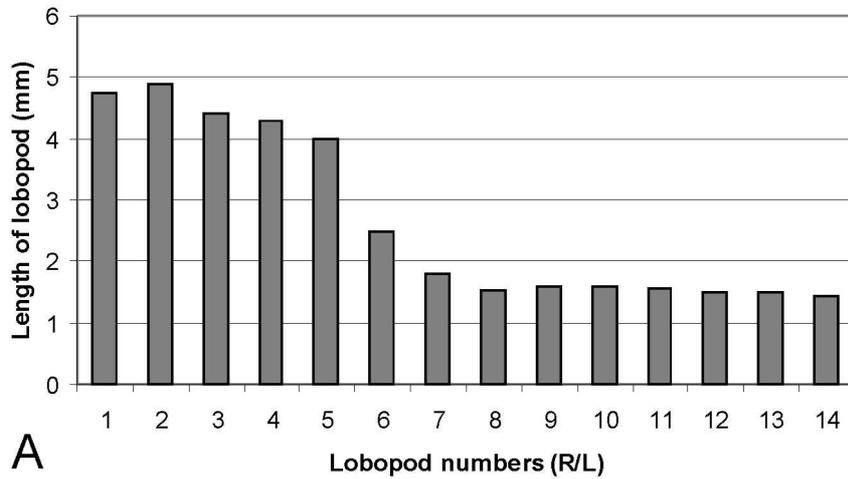
Lobopod numbers (R/L)	Specimen numbers (YKLP)								Average
	11271	11273	11276	11279	11280	11281	11285	10242	
1	>3.6	—	>5.5	—	4.9	4.5	4.1	>3.3	4.75
2	>3.9	4.6	6.4	—	4.8	4.6	4	>3.8	4.88
3	>2.5	>3.6	5.8	>1.8	4	4.3	4.2	3.8	4.42
4	>2.8	5	>2.4	>1.9	>2.9	4.3	4.1	3.8	4.30
5	>3.3	>4.3	>3.0	>2.4	>2.5	—	>3.5	>2.6	≈4.00*
6	>0.8	2.5	>2.2	>1.3	2.5	—	>2.0	—	2.50
7	>1.6	—	>1.8	>1.6	1.8	—	—	—	1.80
8	1.6	>1.1	>1.0	1.6	1.4	—	—	>1.5	1.53
9	1.6	—	>1.2	>1.2	—	—	—	1.6	1.60
10	1.5	—	>1.2	>1.5	—	—	—	1.7	1.60
11	1.6	—	—	1.5	—	—	—	1.6	1.57
12	1.5	—	—	>1.1	—	—	—	1.5	1.50
13	1.6	—	—	>0.8	—	—	—	1.4	1.50
14	1.5	—	—	—	—	—	—	1.4	1.45

*The fifth lobopod is incompletely preserved in all specimens. However, the better preservation in specimen YKLP 11273 and YKLP 11285 shows that it should have a similar length as the lobopods in front.

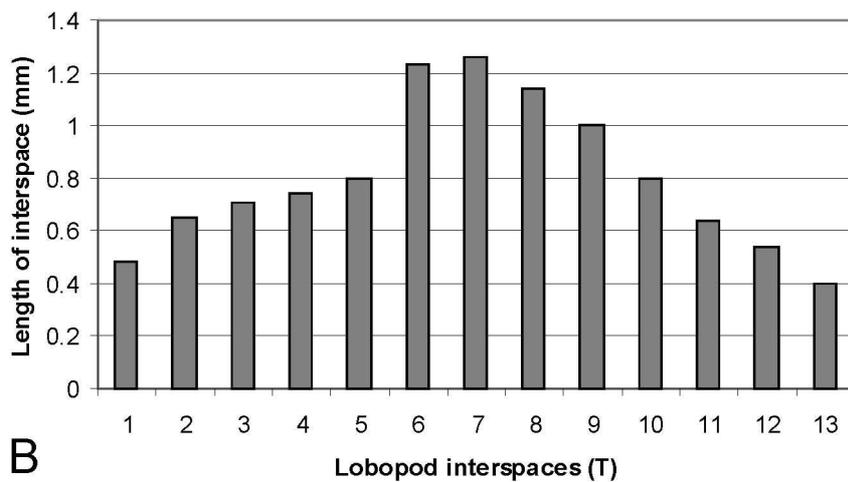
Table 2

Measurement (in mm) on the length of lobopod interspaces

Interspace numbers (T)	Specimen numbers (YKLP)									Average
	11271	11272	11275	11276	11284	11288	11289	11290	10242	
1	0.5	0.5	0.5	0.6	—	0.5	0.5	0.6	0.5	0.48
2	0.7	0.7	0.5	0.7	—	0.6	0.5	0.7	0.8	0.65
3	0.7	0.7	0.7	0.7	—	0.7	0.7	0.7	0.8	0.71
4	0.8	0.8	0.8	0.7	0.6	0.7	0.7	0.9	0.7	0.74
5	0.8	0.8	0.9	0.7	1	0.8	0.5	0.9	0.8	0.80
6	1.3	1.2	1.3	1.5	0.9	1.3	1	1.5	1.1	1.23
7	1.5	1.3	1.4	1.5	0.9	1.2	1	1.3	1.2	1.26
8	1.3	1.2	1.1	1.6	0.7	1	0.8	1.3	1.2	1.14
9	1.2	1.1	1	1.2	0.6	0.9	0.8	1	1.2	1.00
10	1	0.9	0.8	0.7	0.6	0.7	0.8	0.9	0.8	0.80
11	0.7	0.7	0.7	—	0.5	0.7	0.5	0.6	0.7	0.64
12	0.6	0.6	0.5	—	0.5	0.5	0.5	0.6	0.5	0.54
13	0.4	0.4	0.4	—	0.5	0.3	—	0.4	0.4	0.40



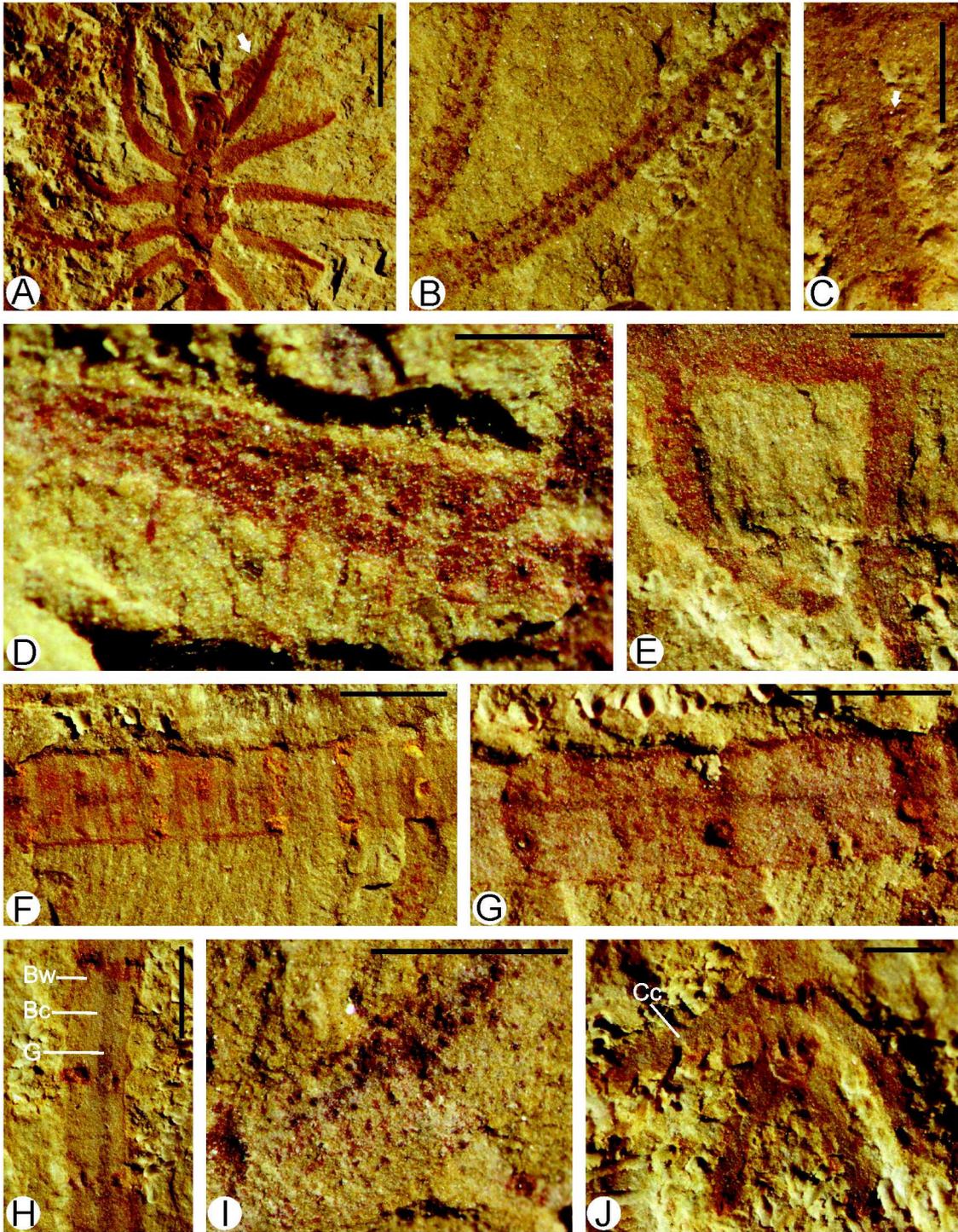
A



B

Fig. 8. The division of trunk tagmata in *Luolishania longicruris*. (A) The variation of lobopod length through the trunk, showing that the first five pairs of lobopods are significantly long. (B) The variation of the length of lobopod interspaces, showing that the trunk appears to be divided into two parts between the fifth and sixth lobopod interspaces.

Fig. 9. Detailed structures on body surface. (A-E) Setae and annulations on appendages. (A) The anterior portion of RCCBYU 10242, arrow indicates long and dense setae on an anterior lobopod. (B) Lobopods R1 and R2 of YKLP 11271, showing the red spots as traces of setae. (C) Lobopod L12 of YKLP 11271, arrow points to a seta attached to it. (D) Lobopod R5 of YKLP 11276, showing setae and their bases. (E) Lobopod R8 and R9 of YKLP 11279, showing annulations and red seta traces on lobopod. (F-J) Detailed morphology of trunk. (F) Trunk of YKLP 11281, showing annulation. (G) Trunk of YKLP 11283, showing red spots along annuli, which indicating the existent of setae on trunk. (H) Trunk of YKLP 11272, showing internal anatomical structures by different-colour longitudinal bands. Bw, body wall; Bc, body cavity; G, gut. (I) Trunk of YKLP 11282, showing crowded nodes arranged along the annuli. (J) Posterior portion of YKLP 11272, showing a small round trunk extension after the last pair of lobopods, the whitish bands in these lobopods indicating the central canal of lobopods. Cc, central canal. Scale bars = 2 mm (A); 1 mm (B, F-I); 0.5 mm (C-E, J).



red spots are the bases (Fig. 9D). Three rows of red spots are clearly shown on lobopod R2 of specimen YKLP 01a (Figs. 2A, 9B), indicating that there are at least three rows of setae along the lobopod rather than two rows as described by Liu *et al.* (2004). Since we cannot see the opposite side of the lobopod, it is possible that there is also a fourth row of setae. Setae on posterior lobopods have also been found in several specimens (Fig. 2A, 9C), but they appear to be much scarcer than those on the anterior legs, causing the false impression that only lobopods R/L 1-6 carry setae (cf. Liu *et al.*, 2004, p. 1066).

3.2.4.3. *Claws*. The claws on the posterior lobopods are not as well exposed as those in front. The anterior-lobopod claws are preserved in a yellow to brown colour, and are long, thin, straight, and needle-shaped (Figs. 10A-H). Four claws fan out at the end of lobopod L1 in specimen YKLP 11277 (Fig. 10A, B), indicating that the number of claws on each lobopod may be four, which matches the original description of “four or five claws” on the holotype (Hou and Chen, 1989). The report of only one claw in *M. haikouensis* (Liu *et al.*, 2004, p. 1064) may be explained as preservational bias, such as in our specimens YKLP 11280 and YKLP 11281 (Figs. 10E-H). Lobopod R2 of specimen YKLP 11280 also shows four claws at its end, the two most posterior ones preserved at slightly different levels. In this specimen, it is noticeable that the most anterior claw seems to be a little further away from the other three claws and to protrude from the lateral side of the lobopod rather than being terminal (Figs. 10C, D). However, such an arrangement of the claws is not well supported by the other specimens.

In specimen YKLP 11286, because the body is slightly twisted towards the posterior, several posterior lobopods become upturned and preserved at a higher level above the trunk. Several well preserved claws occur at the end of these lobopods (Figs. 10I, J). These claws are different from the claws on the anterior lobopods. They are preserved

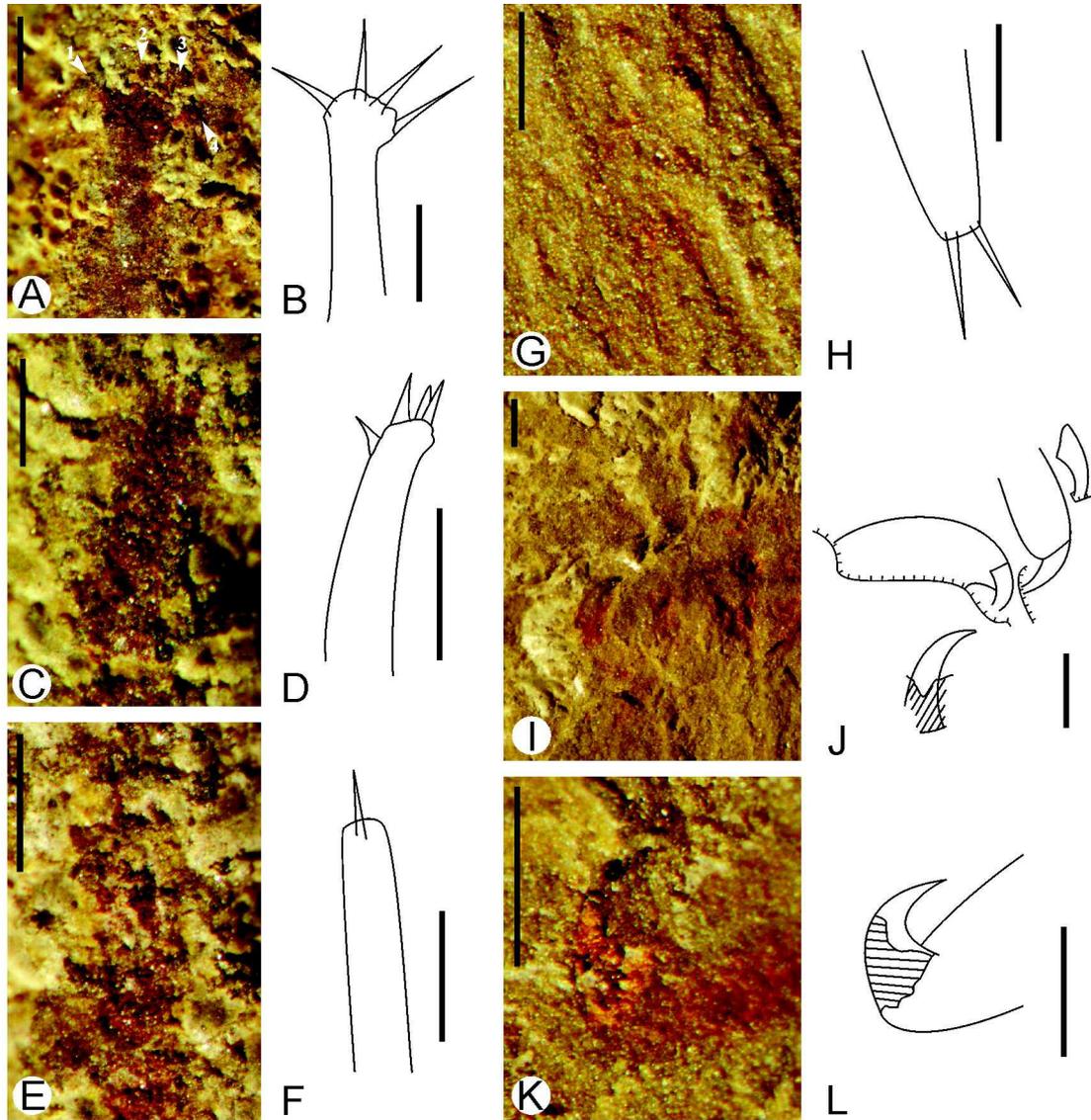


Fig. 10. Claw morphology. (A, B) Claws on lobopod L1 of YKLP 11277, showing four claws indicated by arrows in A. (C, D) Claws on lobopod R2 of YKLP 11280, showing four claws and the anteriormost claw is on the lateral side of the lobopod. (E, F) Claw on lobopod R1 of YKLP 11280, only one needle-shaped claw exposed. (G, H) Claws on lobopod R3 of YKLP 11281, showing two needle-shaped claws. (I, J) Claws on posterior lobopods of YKLP 11286, showing the hook-shaped thick claws. (K, L) Claw on lobopod R13 of YKLP 11281, showing the claw morphology on posterior lobopod, the base part weathered as rusty colour. Scale bars = 0.3 mm.

in a grey to black colour, and they are large, curved and hook-shaped, with a wide proximal end which is often mineralised and preserved in a rusty colour. The same type of claw was also found on lobopod R13 of specimen YKLP 11281 (Figs. 10K, L). The claws on the posterior lobopods are very similar in both shape and colour to the claws of *Onychodictyon ferox* (Hou *et al.* 1991). The number of claws on each posterior lobopod is not visible on our specimens, but Hou and Chen (1989) described “four or five claws” on each of the last four posterior lobopods of the holotype.

In summary, it appears that all the lobopods probably carry four distal claws, which are differentiated into an anterior and a posterior type, being distinct in their morphology and possibly also in composition. However, there is no evidence for a boundary between the two types of claw, because the claws on the middle lobopods are poorly preserved. Given that the lobopods are divisible into two morphological groups, it may well be that the claw types are distributed accordingly. Thus, the first five pairs of lobopods are long and slender, with three or four longitudinal rows of dense setae, and probably carry needle-shaped claws; the rest of the lobopods are short and thick, with sparse setae, and probably possess hook-shaped claws.

3.2.5. *Trunk*

The lobopod-bearing portion of the animal (RL1 to RL14) is a cylindrical and elongated trunk.

3.2.5.1. Lobopod interspaces. The trunk is divided into lobopod interspaces, measured as the distance between the centres of each lobopod attachment site. We designate them T1 (the first interspace: between lobopods RL1 and RL2) to T13 (lobopods RL13 to RL14). Table 2 displays the measurement of leg interspaces in nine relatively complete

specimens (all flattened dorsoventrally except YKLP 11289 preserved laterally), and the leg interspaces show similar variation in all measured specimens. The average distance of lobopod interspaces (Fig. 8B) steadily increases from T1 (0.48 mm) to T5 (0.80 mm), then sharply increases at T6 (1.23 mm) and is greatest at T7 (1.26 mm). From T8 backwards, each successive interspace is 0.14 mm shorter than the preceding one, until it reaches its smallest distance at T13 (0.40 mm). Therefore, it appears that the trunk is actually divided into two parts, between T5 and T6. The anterior trunk part consists of lobopod interspaces T1 to T5 which are relatively short and crowded; T6 to T13 represent a posterior trunk part.

3.2.5.2. Anatomical structures. Anatomical structures of the trunk are often displayed as different-coloured longitudinal bands, as shown in specimen YKLP 11275 (Fig. 9H).

The outer matrix-coloured band is the body wall, about 0.3 mm thick. On the outside of the body wall, there is a thin orange margin, indicating the epidermis. A black band in the centre of the trunk is the alimentary canal, with a diameter of 0.15 mm. The gut is flattened; straight, simple and extends through the entire length of the body; no sediment filling has been found. The whitish coloured band between the body wall and the gut is interpreted as the body cavity, with a diameter of 0.3 mm. It is shown in many specimens that the body cavity is connected with the central canal of the limbs.

3.2.5.3. Annuli, setae and papillae. The trunk surface carries fine annulations, about 6-7 annuli per millimetre (Figs. 1F, 2A and 9F). The density of the annulations is very constant throughout the body, so the number of annuli within each lobopod interspace varies with the change of interspace distance. There is no annulation in the circular belts where limbs and sclerites are attached; this band is smooth. In addition to the

annulations, some red spots (similar to the traces of setae on the limbs) and seta-like structures are also found on the trunk surface (Fig. 9G), indicating that the trunk carries some setae like those on the lobopods. Specimen YKLP 11283 and 11282 show that the red spots are arranged along the annuli (Figs. 9G, I).

Some different raised papillae are present on both the trunk and lobopod of specimen YKLP 11282 (Fig. 9I). They seem to be set closer together and are also arranged along the annuli. A similar structure was described also from the holotype, and it was suggested that many annuli carried symmetrically set nodes (Ramsköld, 1992). However, we did not observe any symmetrical pattern in our specimens.

3.2.6. Tail

The body tapers slightly towards the posterior end, and there is a small, bluntly rounded projection behind the last pair of lobopods (Figs. 2A, B and 9J), herein termed the “tail”.

3.3. Reconstruction

The result of this study is visualised in a new reconstruction (Fig. 11).

4. Discussion

4.1. The synonymy of *L. longicuris* and *M. haikouensis*.

Liu *et al.* (2004) reported *M. haikouensis* as a new genus and new species, and presented five differences between this species and *L. longicuris*. However, the

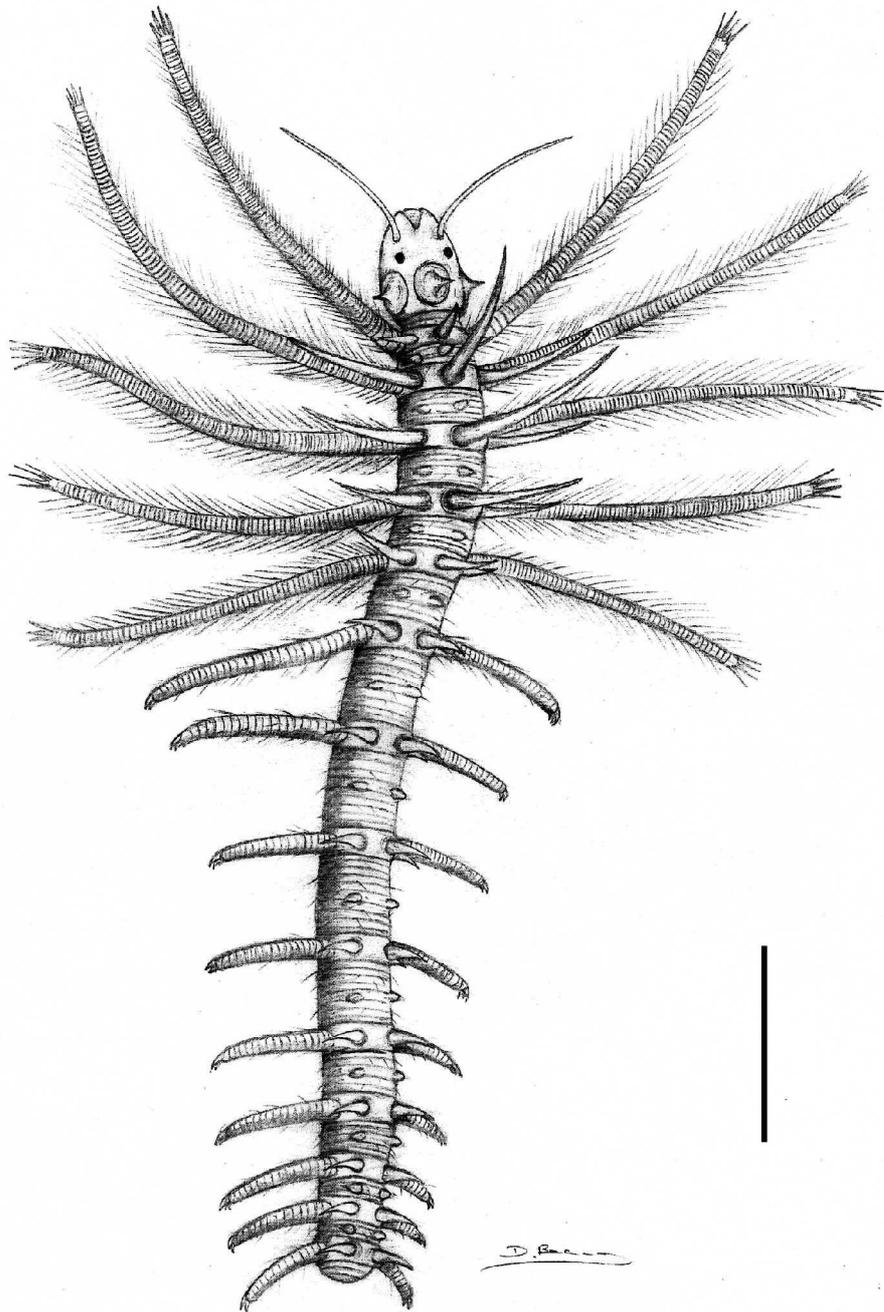


Fig.11. Reconstruction of *Luolishania longicuris*. Scale bar = 2 mm.

evidence herein from newly obtained specimens indicates that these differences can be explained by taphonomic conditions. The result suggests that *M. haikouensis* is a junior synonym of *L. longicruris*.

It was reported that the holotype of *L. longicruris* has 16 pairs of lobopods (Hou and Bergström, 1995), while both the holotype of *M. haikouensis* and our additional specimens have only 14. The difference was suggested as a diagnostic character to distinguish *M. haikouensis* from *L. longicruris* (Liu *et al.*, 2004), but there are alternative explanations.

The description of the holotype of *L. longicruris* may be incorrect concerning the number of lobopods and segments, as it is poorly preserved and difficult to interpret. Most of the lobopods are not exposed, and counting them hinges on the recognition of the sets of sclerites. In the original paper (Hou and Chen, 1989), the number was given as 15. This includes an anterior structure that is seemingly too narrow to be a lobopod and might correspond to our antenniform outgrowth. This would make 14 pairs of lobopods, matching *M. haikouensis* and our newly obtained specimens. Successive interpretations (Hou and Bergström, 1995; Ramsköld and Chen, 1998) changed the number of lobopods to 16 pairs, but counting remains problematic. Unfortunately, in dorsoventrally preserved specimens the morphology of sclerites and the barb-shaped projections is too similar for us to distinguish between them (Ramsköld, 1992). This could adversely affect the number of inferred pairs of lobopods. It is also possible that the holotype is to some degree composite, since some of our specimens show animals preserved on top of each other (Figs. 1A and 2B).

Even if the holotype does have 16 pairs of lobopods, this could reflect intraspecific variation and is therefore not to be considered as a diagnostic character. Furthermore, in extant onychophorans, it is common that the males and females of individual species

have different numbers of lobopods (Tait, 2001; Mayer, 2007). Ontogenetic variation may also cause a different appendage number.

Herein, we leave the interpretation open and describe *L. longicruris* as having 14 to 16 pairs of lobopods.

The original reconstructions of *L. longicruris* (Hou and Bergström, 1995) and *M. haikouensis* (Liu *et al.*, 2004) at first sight seem to be very different. However, many of the characteristics said to be unique to *M. haikouensis* are present in our specimens and the holotype of *L. longicruris* (see above). Other features of *M. haikouensis* are slightly different from those seen in *L. longicruris*. However, as mentioned previously, these differences are mainly the result of taphonomic processes (see sections on sclerites and appendages in the Description).

Thus *M. haikouensis* is considered to be conspecific with, and a junior synonym of, *L. longicruris*.

4.2. Tagmosis

L. longicruris is unusual among Cambrian lobopodians in that it displays comprehensive segmental differentiation not only between head and trunk, but also within the trunk (Fig. 11). The lobopods are differentiated into anterior (R/L 1-5) and posterior (R/L 6-14) types by length, claw morphology, and density of setae. Furthermore, the body sclerites are differentiated into three types, and the trunk is differentiated into two sections by the gap between lobopod interspaces T5 and T6. Thus, we can distinguish three tagmata in *L. longicruris*. The first tagma consists of the head with its unique set of characteristics: head shield, paired antenniform outgrowths, eyes, uniquely shaped sclerites, and lack of ventral limbs. The second tagma is from the

head end to the front of the sixth pair of lobopods. Here, the lobopod interspaces are crowded and the lobopods are very long, provided with rows of comparatively tightly set setae and with fairly straight terminal claws. In the third tagma (from the sixth pair of lobopods to the posterior end), the lobopods are short and have fewer setae and strongly curved terminal claws. The other characters tend to make the boundary between tagmata two and three less distinct. Thus, the long spines are confined to only the middle three of the five segments in the second tagma, and it is the first six, rather than five, pairs of lobopods that are tightly crowded. It could be argued that the boundary between the second and third tagma may come after the sixth pair of lobopods. However, the sixth pair of lobopods is significantly shorter than the fifth and morphologically it resembles the seventh pair, thus the boundary between the second and third tagma is in front of the sixth pairs of lobopods.

Cisne (1974) measured the degree of arthropod limb tagmosis using the Brillouin expression (Brillouin, 1962), an equation first conceived for general applications in information theory. The coefficient of limb tagmosis (h) is given by:

$$h = \frac{1}{N \ln 2} \ln \frac{N!}{N_1! N_2! \dots N_i!}$$

where N is the total number of limb pairs, and N_i is the number of limb pairs of the i th type. Wills *et al.* (1997) further discussed the pros and cons of this equation and used it for a large number of fossil and recent arthropods.

They suggested that the mean Brillouin value for Cambrian arthropods is 0.92 (Wills *et al.*, 1997, p. 58). Herein, we apply this method to the Cambrian lobopodians whose limb arrangements are relatively clear. The mean Brillouin value for these lobopodians appears as 0.34 (Table 3). With an h of 0.99, *L. longicruris* has a significantly higher degree of tagmosis than any other Cambrian lobopodian and is much closer to the average tagmosis value of Cambrian arthropods.

Table 3

Brillouin values (h) for Cambrian lobopodians.

Genus	Limb formula	<i>h</i>
<i>Paucipodia</i>	9	0
<i>Microdictyon</i>	10	0
<i>Onychodictyon</i>	2, 9	0.53
<i>Cardiodictyon</i>	2, 21/23	0.35/0.33
<i>Hallucigenia</i>	2, 8	0.55
<i>Luolishania</i>	1, 5, 9	0.99
<i>Aysheaia</i>	1, 10	0.31
<i>Xenusion</i>	20	0
Mean		0.34

4.3. The development of sense organs and their evolutionary significance

Compared with other Cambrian lobopodians, *L. longicruris* possesses relatively advanced sense organs, including possibly the paired antenniform outgrowths, eyes and sensory setae.

4.3.1. Paired antenniform outgrowths

The frontal paired antenniform outgrowths of *L. longicruris* might have been sensory in function. Possibly, if this animal was raptorial, the frontal sensory organs would detect sudden changes of water pressure which would confer an advantage in prey-detection. Specialised appendages situated anteriorly are also present in two other Chengjiang lobopodians, *H. fortis* and *C. catenulum*. In *H. fortis*, two pairs of long and slender limbs are located immediately behind the head and in front of the first pair of sclerites (Hou *et al.*, 2004a, p. 88-89, figs. 14.6 and 14.7a). In *C. catenulum*, there are two or three pairs of slender ventral appendage-like structures in the head region in front of the most anterior pair of sclerites (Hou *et al.*, 2004a, p. 86-87, figs. 14.4 and 14.5). Possibly, in *H. fortis* and *C. catenulum*, respectively, two pairs of anterior lobopods have become highly specialised as sensory organs and become displaced from the trunk towards the head, but are still positioned ventrally.

In contrast, the paired antenniform outgrowths of *L. longicruris* are located on the anterodorsal part of the head and in front of the eyes. This position indicates that these structures are innervated in front of or at the same level as the eyes, which in arthropod terms implies a protocerebral innervation. We know from recent studies that the onychophoran first appendage pair is innervated from the protocerebrum (Budd, 2002), whereas the antennules of all extant crustaceans, myriapods, and hexapods are

deutocerebral (Boxshall, 2004) and the second antennae of crustaceans belong to the tritocerebral segment. Therefore, the paired antenniform outgrowths from *L. longicruris*, if they are modified lobopods, are likely to be homologous to the first lobopod pair of homonomous lobodians and to the first appendage pair of onychophorans. They are not to be considered homologous of antennules.

Like the first pairs of lobopods in homonomous lobopodians, such as *Xenusion* or *Paucipodia*, the first appendage pair of extant onychophoran is strikingly lobopod-like. This contrasts with the paired antenniform outgrowths of *L. longicruris*, which are not similar to any of the lobopods. If the antenniform outgrowths of *L. longicruris* are modified lobopods, then they would be expected to reveal annuli or at least vestigial annuli. That annuli are not visible may be explained as follows:

- 1) The lack of annulations on these outgrowths is caused by imperfect preservation; annulations are also invisible elsewhere in the same specimen YKLP 11276.

- 2) The antenniform appendages of *L. longicruris* do not have a lobopod origin and are a structure unique to this animal.

4.3.2. Eyes

L. longicruris is the first Cambrian lobopodian reported to have eyes (Liu *et al.*, 2004). Although the lobopodian eye seems simple, it perhaps would better enable *L. longicruris* to detect light variations and movements of prey/predator. The high light levels in the shallow marine shelf conditions of the Chengjiang environment may have favoured the evolution of more complex eyes.

4.3.3. *Setae and nodes*

The many seta-like structures distributed on the body surface of *L. longicruris* are comparable with the setae on onychophorans and arthropods, and are suggested to be sensilla involved in mechanoreception (Ruppert and Barnes, 1996; pp. 601, Fig. 12-3; pp. 607; pp. 819, Fig. 15-13B). As *L. longicruris* is an aquatic animal, it is possible that these sensilla were used for sensing water pressure change caused, for example, by the movement of a predator or prey. As mentioned above, the setae on the long anterior appendages are much denser than on posterior appendages, and these long anterior appendages are suggested to be specialised for feeding (see below). These sensilla may thus have played a key role in food location or tactile recognition. Possibly some may have possessed chemosensitive attributes although present techniques are not yet able to distinguish pores in what are quite well-preserved sensilla. In addition to the setae, there are some raised papillae arranged on the body surface of the animal, the function of which is unknown. Perhaps these nodes were used for sensing water chemistry and/or temperature.

4.4. *Development of the head in lobopodians*

Determining anteroposterior orientation is difficult in some of the Cambrian lobopodians, such as *Paucipodia inermis* (Hou *et al.*, 2004b). This is because of the lack of a morphologically discrete head with a distinct boundary between head and trunk. In contrast, one of the key features of *L. longicruris* is that it possesses a discrete head, with eyes and paired antenniform outgrowths, and is protected by a head shield. The sclerites on the head of *L. longicruris* are considered to be homologous with the

sclerites on the trunk, but in all other Cambrian lobopodians the sclerites are always confined to the trunk.

It is possible to distinguish some stages in the development of a head in the Cambrian lobopodians. *Paucipodia* lacks a distinct boundary between the head and the trunk, has no apparent external specialisation of the anterior end and probably represents a basal stage. In *Hallucigenia* and *Cardiodictyon* there is possibly a head shield and a few ventral limbs are specialised and appear to be associated with the head. *Luolishania* appears not to have incorporated any lobopod segments into the head (unless lobopods have been lost or the antenniform outgrowths have a lobopod origin), and its specialised anterior lobopods form a distinct second tagma behind the head.

4.5. Mode of life

Cambrian lobopodians were soft-bodied, marine animals, with an elongated trunk equipped with clawed lobopods. As their appendages tended to be soft and slender, they seem functionally ill adapted for swimming or burrowing; therefore, these lobopodian animals were probably epifaunal. However, the diverse morphology of Cambrian lobopodians also suggests that different species were adapted to different lifestyles. *Paucipodia inermis* is in some respects the simplest Cambrian lobopodian, without any sclerites, setae, projections or differentiated appendages, and has been suggested to be an omnivorous deposit feeder (Hou *et al.*, 2004b). In contrast, Hou and Chen (1989) suggested that *L. longicruris* is not a mud-eater but may have climbed and preyed on sponges like *Aysheaia*. Our research indicates that *L. longicruris* may feed on suspended food particles as a filter feeder (see below for further discussion).

4.5.1. Direct evidence from the gut

The gut of *L. longicruris* is flattened and black in colour. No sediment infilling has been found. This type of gut preservation in Chengjiang animals has been suggested to be indicative of a carnivorous feeding habit (Hou and Bergström, 1997). Our SEM EDX element mapping results also show high carbon concentration in the gut area, indicating that it is rich in organic matter. Dark-stained matter seemingly squeezed out from the gut in some Burgess Shale pan-arthropods (but not in others) is probably also an indication of food specialisation (e.g. *Aysheaia pedunculata*, Whittington, 1978, Figs. 4, 5, 10; *Marrella splendens*, Whittington, 1971, Fig. 22; Garcia-Bellido and Collins, 2006, Figs. 4, 6).

4.5.2. Evidence from morphological characters

As mentioned above, the concentration of sense organs of *L. longicruris*, such as the eyes, paired antenniform outgrowths and the abundant setae on the long frontal lobopods, were likely to have been employed in detecting the animal's environment and may also have played a key role in food acquisition.

Furthermore, the distinct morphological differences in the anterior and posterior lobopods of *L. longicruris* indicate a functional difference, with the anterior lobopods probably used in foraging activities. This is suggested by them being very long, invested with many setae and spines, and provided with needle-shaped claws. A number of dorsoventral specimens show that the anterior lobopods spread horizontally in different directions (Figs. 1A-E, 2A, B, 4A and 9A). The angle between each pair of anterior lobopods and the mid-axis of the anterior body increases from L/R1 (about 20 degrees) to L/R 5 (about 120 degrees). This is consistent with what is seen in laterally preserved specimens. The laterally preserved anterior lobopods are positioned under the ventral

side of the animal (Figs. 3A-E), indicating that these lobopods have moved inwards from the horizontal position. These features, combined with their long length, indicates that these lobopods could cover a wide area while moving around, forwards to backwards and inwards to outwards. In addition, the anterior lobopods commonly show curvature indicating their flexibility. All of these features support the idea that the anterior lobopods were highly mobile sensors and possibly specialised in foraging.

In contrast, the posterior lobopods are much shorter, with fewer setae, but equipped with strong hook-like claws. Rather than being used for capturing prey, they were probably used for locomotion and as holdfasts. The stouter morphology of the posterior lobopods seems more suited for walking and/or climbing. However, the difference in length between anterior and posterior lobopods makes it unlikely that the animal was an efficient walker and the hook-like claws seem adapted for climbing and holding onto a protrusion rather than walking.

The short distance between the last pair of feeding lobopods (R/L 5) and the first pair of locomotory lobopods (R/L 6) could have been helpful in supporting the anterior part during feeding.

4.5.3. Evidence from taphonomy

The majority of specimens show better exposure of the anterior lobopods as opposed to the posterior ones. Some specimens expose both the anterior and posterior lobopods, but they tend to be preserved on slightly different bedding planes. For example, the dorsoventrally preserved specimen YKLP 11275 (Fig. 1C) clearly shows that the anterior part of the animal is rising up. This mode of preservation indicates that the anterior and posterior parts of the body were probably held in different positions in life. The long appendages were, perhaps, held more horizontally (relative to the body

orientation) and therefore tend to be better preserved in the cleavage plane, whereas the short appendages, hanging below the axis of the body, are hidden in the sediment.

4.5.4. Evidence from other fossils

Some fragments of a sponge fossil were found beside specimen YKLP 11292, indicating a possible ecological association. Similar associations have also been reported for other lobopodian groups. For example, Whittington (1978) found that many *Aysheaia* specimens are associated with sponges and suggested a climbing, symbiotic, or parasitic mode of life. The association between Cambrian lobopodians and sponges are also observed by Chen and Zhou (1997).

Based on the evidence above, we suggest that *L. longicruris* led a filter feeding lifestyle. Evidence from the gut indicates that this animal was clearly not a mud eater, but fed on rich organic matter. However, *L. longicruris* was also unlikely to have been an active predator. Although it possesses some specialised anterior appendages, their morphology, especially the needle shaped claws, does not show evidence of adaptation for catching or grasping prey. Also this animal was probably not an efficient walker or swimmer, which would be a disadvantage in hunting prey. *L. longicruris* could climb sponges or other animals and suck mucus or bacteria from the surface; however, this lifestyle does not offer an interpretation for the function of the specialised anterior lobopods. A more satisfactory explanation is that the anterior lobopods were used in filtering organic food particles from the water. Their extreme length facilitates the search for food within a relatively wide area; the setae on these lobopods may have acted as sensory structures as well as a filter; the stout posterior legs with strong hook-shaped claws help to anchor the posterior two-thirds of the body firmly to a suitable

structure (e.g. sponge), enabling the anterior body and lobopods to move around freely in the search for food. This mode of life exposes the animal to potential predators. The morphology of the sclerites and the presence of a head shield may have evolved as protection from predators.

If *L. longicruris* fed on organic particles and fragments, how could the food be transferred into the mouth? Due to the lack of firm evidence, the question remains unresolved, as with *Marrella* (Briggs and Whittington, 1985; Garcia-Bellido and Collins, 2006). Herein we suggest two possibilities for *L. longicruris*: 1) the filtering function of the anterior lobopods increases the density of food particles in front of the animal; at the same time, their sweeping movements create water currents that bring the food nearer to the mouth whereby they are picked up by the protruding proboscis of the animal; 2) the long setae on the anterior lobopods trap food particles, then the highly flexible anterior appendages are passed in front of the mouth to feed the animal.

4.6 Phylogenetic position and significance of Luolishania

Despite Cambrian lobopodians being widely used in discussions about the origin of arthropods, the phylogenetic relationships within Cambrian lobopodians and between them and extant panarthropods are still poorly understood. A few phylogenetic analyses of fossil lobopodians have been published (Hou and Bergström, 1995; Budd, 1996; Ramsköld and Chen, 1998; Bergström and Hou, 2001; Liu *et al.*, 2004, 2007); however, with the updated knowledge of new species and new/amended characters in recent years, a new comprehensive phylogenetic investigation of Cambrian lobopodians and allied animals is required. Herein a cladistic analysis has been undertaken using two software packages, MacClade 4 (Maddison and Maddison 2000) and PAUP 4.0 (Swofford 2002).

4.6.1. Taxa analysed

In total 22 taxa are included in this analysis: 1) 12 genera of Cambrian lobopodians known from soft-body preservation: *Paucipodia* Chen *et al.*, 1995 (see also Hou *et al.*, 2004b), *Microdictyon* Bengtson *et al.*, 1981 (Bengtson *et al.*, 1986; see also Chen *et al.*, 1989), *Onychodictyon* Hou *et al.*, 1991, *Cardiodictyon* Hou *et al.*, 1991, *Hallucigenia* Conway Morris, 1977, *Luolishania* Hou and Chen, 1989, *Jianshanopodia* Liu *et al.*, 2006, *Megadictyon* Liu *et al.*, 2007, *Aysheaia* Walcott, 1911, *Xenusion* Pompeckj, 1927, *Hadrax* Budd and Peel, 1998 and Collins' monster (see Collins, 1986); 2) four genera of dinocaridids (Collins, 1996): *Anomalocaris* Whiteaves, 1892, *Parapeytoia* Hou *et al.*, 1995, *Opabinia* Walcott, 1912 (see also Whittington, 1975) and *Kerygmachela* Budd, 1993; 3) two genera of arthropods: *Fuxianhuia* Hou, 1987 and *Eoredlichia* Zhang, 1951; 4) Onychophora; 5) Tardigrada; 7) two cycloneuralians chosen as outgroups: Priapulida and Nematoda.

4.6.2. Characters selected

Character selection is a crucial but difficult step in all cladistic analyses, especially when dealing with a large range of diverse fossil taxa. The great disparity of Cambrian lobopodians and the limited information on most taxa make it difficult to identify possible homologous characters shared by two or more taxa. Previous phylogenetic research on lobopodians and dinocaridids (Bergström and Hou, 2003; Budd, 1993, 1996, 1997, 1999; Hou and Bergström, 1995, 2006; Hou *et al.*, 2006; Liu *et al.* 2004, 2007; Ramsköld and Chen, 1998) has incorporated a number of widely-accepted characters that are also used here. However, some debated but uncertain characters have been

avoided or modified, with some additional ones included on the basis of recent research.

The following characters are used here:

Limbs

1. Locomotory limbs: absent (0); present (1).
2. Length of ventral lobopods gradually decreasing along body towards anterior and/or posterior end: absent (0); present (1). This is applicable only for lobopodians that lack apparent tagmosis/a set of differentiated limbs, such as *Paucipodia inermis* (Hou *et al.*, 2004b).
3. An anterior set of specialised, uniramous ventral limbs: absent (0); present (1). This refers to two or more pairs of limbs situated on the anterior part of the trunk and possibly on part of the head, that are clearly differentiated from the more posterior limbs.
4. Grasping limb: absent (0); present (1). This refers to the most anterior unique appendages with spines or plates clearly indicating a grasping function. This limb is not preceded by an extension from the head.
5. Sclerotized grasping appendages: absent (0); present (1). If sclerotized, they are segmented rather than annulated.
6. Grasping appendages held together at least basally, united by median plate or fused: absent (0); present (1). The grasping appendages in *Anomalocaris*-type dinocaridids tend to be united even when dissociated from the head; in *Opabinia* their shared base is greatly elongated and proboscis-like.
7. Lateral long-based limb flap: absent (0); present (1). There is a distinctly long basal portion, in cases with 5 limb endites or segments, before limb branches occur in dinocaridids.

8. Lateral short-based limb flap: absent (0); present (1). There is a short basal portion, typically consisting of one segment, before limb branches are distinct (arthropods). Margins may be fused for another two segments.
9. Locomotory limbs with spinules/papillae: absent (0); present (1). This character is applicable only to lobopodians and soft lobopod-bearing dinocaridids.
10. Terminal claws on limbs: absent (0); present (1).
11. More than two claws on limbs: absent (0); present (1).
12. Pre-ocular appendage-like structures: absent (0); present (1). This refers to paired antenniform structures in front of or aligned with the eyes and presumably functioning as a sensory organ. We noticed that a pair of probable sensory appendages were reported from *Onychodictyon* (Liu *et al.*, 2008a); however, due to the lack of firm evidence, we coded this character in *Onychodictyon* as “?”.
13. Post-ocular limbs developed as antennules: absent (0); present (1). The term antennules refer here only to modified limbs in the first post-ocular segment.

Mouth

14. Mouth position: terminal (0); ventral (1).
15. Mouth with sclerites forming a more or less radial cone: absent (0); present (1).
This is the type of mouth found in a number of dinocaridids. In some of them, it is known to be dissymmetric (An object that is not superimposable on its mirror image).
16. Mouth cone directed ventrally and posteriorly: absent (0); present (1). This character is applicable only to the dinocaridids.

Eyes

17. Lateral eyes: absent (0); present (1). There are several distinct structural and functional types of lateral eyes, but they are considered homologous in that they represent photoreceptors innervated from the same region of the brain (Wills *et al.* 1998). However, Mayer (2006) recently suggested that the eyes of Onychophora are not homologues of arthropod lateral eyes but are instead homologous with arthropod median eyes. As there are opposing views, and the issue unresolved (Strausfeld *et al.*, 2006a, b), we coded this character in Onychophora as “?”.
18. Compound eyes: absent (0); present (1). The compound eyes are made up of repeating units, each of which functions as a separate visual receptor. Their number and position may vary.

Body

19. Segmented body: absent (0); present (1).
20. Body notably tapering: absent (0); present (1). The pleural fold is not included in the body width. An earthworm or millipede can be taken as examples of animals with an untapering body. When tapering, the body typically tapers posteriorly, but in some cases also anteriorly.
21. Body with segmented exoskeleton: absent (0); present (1).
22. Wide pleural folds: absent (0); present (1). Except for *Kerygmachela*, which has a soft-bodied trunk axis where its pleural folds are inserted individually (Budd 1999, Figs. 4, 5), a pleural fold is a direct continuation of a dorsal sclerite

(tergite). It is fundamentally different from an exopod/lateral appendage flap.

Both conditions are scored “1” here.

23. Extended posterior end: absent (0); present (1). This means that the last pair of appendages is not terminal.
24. *Opabinia* type tail: absent (0); present (1). This tail is formed by three pairs of dorsolaterally directed pleural extensions.

Tagmosis

25. Distinct head: absent (0); present (1). This means that a head of whatever length is clearly distinguishable from a trunk. It may have or lack sclerites.
26. Head shield: absent (0); present (1). This refers to the possibly bivalved sclerites covering the head, applicable only to lobopodians.
27. Two long tagmata expressed in the appendages: absent (0); present (1). Each tagma includes at least 5 segments.

Surface of body

28. Isolated sclerites/nodes: absent (0); present (1). This category includes sclerites surrounded by soft integument, with or without spines.
29. Spine-shaped sclerites: absent (0); present (1). The length and shape of the spine varies.
30. One pair of limbs behind the last pair/set of sclerites: absent (0); present (1).
31. Rows of lanceolate blades: absent (0); present (1). These are the “gills” identified by some authors in dinocaridids, but their function is unknown. As shown by Bergström (1986), they covered the dorsal side in segmentally arranged transverse rows. These rows extend over the midline in some genera (e.g., *Anomalocaris*; for illustrations, see Whittington & Briggs, 1985 Fig. 34;

Whittington, 1985, Fig. 4.80; Bergström, 1986; Hou *et al.*, 1995, Figs. 17A-B), but may be confined to the pleural area in others (possibly *Opabinia*; cf. Bergström, 1986, Fig. 1, and Zhang & Briggs, 2007, Fig. 4; and also *Kerygmachela*, see Budd, 1999, Figs. 29-30 – but this is questionable).

32. Papillae/setae on trunk annuli: absent (0); present (1).

4.6.3. Results

Heuristic analysis of the complete dataset (Table 4) yielded three most parsimonious trees (MPTs) of length 58 steps (CI=0.5517, RI=0.7374). All characters in the analysis are parsimony-informative except character 16. A further analysis was carried out by deactivating character 16, producing the same three cladograms this time of length 57 steps (CI=0.5439, RI=0.7374). The strict consensus of these MPTs leaves the relative relationships among lobopodians *Paucipodia*, *Microdictyon*, *Jianshanopodia*, *Aysheia*, Onychophora, Tardigrada and *Xenusion* + *Hadranax* unresolved (Fig. 12A).

Successive rounds of *a posteriori* reweighting yield a single most parsimonious tree in which all the equivocation among the trees derived from the unweighted dataset is resolved (CI=0.8015; RI=0.9026; RC=0.7234). In the cladogram (Fig. 12B), all the ingroup taxa are grouped in one large clade (“Clade A”). The significant feature of the tree is that most Cambrian lobopodians fall into two distinct paraphyletic clades (“Clade D” and “Clade K”). The single exception is *Aysheia*, which occupies the most basal branch of the ingroup and is separated from other Cambrian lobopodians by the Tardigrada. Onychophora groups as sister to *Megadictyon* (“Clade H”) and forms the crown group of “Clade D”. Dinocaridids (“Clade P”) is well defined as a monophyletic group and is positioned as sister group to arthropods (“Clade S”). Although the analysis is still preliminary, the result reflects the current state of knowledge, and includes some

Table 4

Data matrix used in cladistic analysis

Characters	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3
Taxa	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2		
<i>Paucipodia</i>	1	1	0	0	-	-	0	0	0	1	0	0	0	0	0	-	0	-	1	1	0	0	1	0	0	0	0	0	0	-	-	0	0	
<i>Microdictyon</i>	1	0	0	0	-	-	0	0	0	1	0	0	0	0	0	-	0	-	1	1	0	0	1	0	0	0	0	1	0	1	0	0		
<i>Onychodictyon</i>	1	0	1	0	-	-	0	0	1	1	0	?	0	0	0	-	0	-	1	0	0	0	0	0	?	?	0	1	1	1	0	1		
<i>Cardiodictyon</i>	1	-	1	0	-	-	0	0	0	1	?	0	0	0	0	-	1	?	1	0	0	0	1	0	1	1	0	1	0	0	0	0		
<i>Hallucigenia</i>	1	-	1	0	-	-	0	0	0	1	0	0	0	0	0	-	1	?	1	1	0	0	1	0	1	1	0	1	1	1	0	0		
<i>Luolishania</i>	1	-	1	0	-	-	0	0	1	1	1	1	0	0	0	-	1	?	1	0	0	0	1	0	1	1	1	1	1	1	0	0	1	
<i>Jianshanopodia</i>	1	1	0	1	0	0	0	0	0	0	-	0	0	0	0	-	0	-	1	1	0	0	1	0	0	0	0	0	0	-	-	0	0	
<i>Magadictyon</i>	1	?	0	1	-	-	0	0	1	0	-	0	0	1	?	-	?	-	1	?	0	0	?	0	?	?	?	0	0	-	-	0	0	
<i>Aysheaia</i>	1	0	0	1	0	0	0	0	1	1	1	0	0	0	0	-	0	-	1	0	0	0	0	0	0	0	0	0	0	-	-	0	1	
<i>Xenusion</i>	1	1	0	0	-	-	0	0	1	0	-	0	0	0	0	-	0	-	1	1	0	0	?	0	0	0	0	1	?	0	0	0		
<i>Hadrax</i>	1	?	?	?	-	-	0	0	1	0	-	0	0	?	?	-	?	-	1	?	0	0	?	0	?	?	?	0	1	0	?	0	?	
Collins' monster	1	-	1	?	-	-	0	0	1	?	-	0	0	?	?	-	?	-	1	?	0	0	?	0	?	?	?	1	1	1	?	0	?	
<i>Anomalocaris</i>	1	-	1	1	1	1	1	0	-	0	-	0	0	1	1	?	1	1	1	1	1	1	?	?	?	1	1	-	0	0	-	-	1	0
<i>Parapeytoia</i>	1	-	1	1	1	1	1	0	-	1	0	0	0	1	1	1	1	1	1	1	1	1	?	?	?	?	1	-	0	0	-	-	1	0
<i>Opabinia</i>	?	-	-	1	0	1	0	0	-	-	-	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	-	0	0	-	-	1	0	
<i>Kerygmachela</i>	1	-	0	1	0	?	0	0	0	?	-	0	0	?	1	0	?	-	1	0	-	1	?	0	1	-	0	?	-	-	?	0		
<i>Fuxianhuia</i>	1	-	0	0	-	-	0	1	-	0	-	0	1	1	0	-	1	1	1	0	1	1	1	1	0	1	-	1	0	-	-	0	0	
<i>Eoredlichia</i>	1	-	0	0	-	-	0	1	-	1	0	0	1	1	0	-	1	1	1	1	1	1	1	1	0	1	-	0	0	-	-	0	0	
Onychophora	1	1	0	0	-	-	0	0	1	1	0	1	0	1	0	-	?	0	1	1	0	0	1	0	0	0	0	0	0	-	-	0	1	
Tardigrada	1	0	0	0	-	-	0	0	1	1	1	0	0	0	?	-	1	0	1	0	-	0	0	0	0	0	0	0	0	-	-	0	0	
Priapulida	0	-	-	0	-	-	-	-	-	-	-	0	0	0	0	-	0	-	0	0	0	0	-	0	0	-	0	0	-	-	0	1		
Nematoda	0	-	-	0	-	-	-	-	-	-	-	0	0	0	0	-	0	-	0	1	0	0	-	0	0	-	0	0	-	-	0	1		

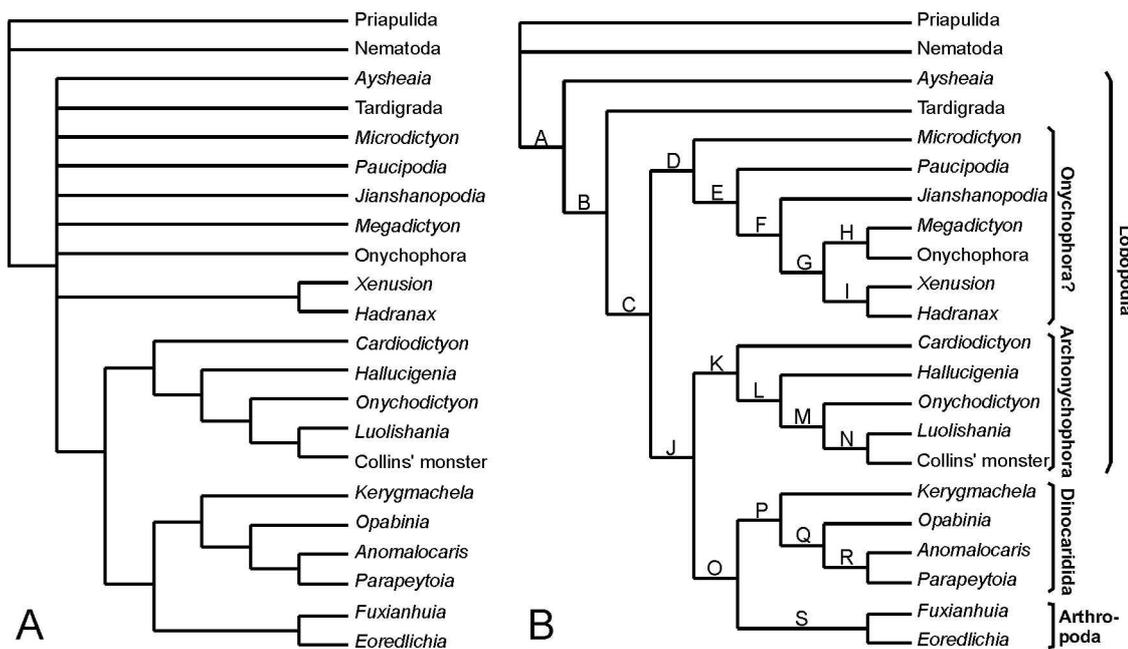


Fig. 12. Phylogenetic trees resulting from analyses of the morphological dataset in Table 4. (A) The strict consensus of three most parsimonious trees derived from the unweighted dataset. (B) The most parsimonious tree resolved after reweighting the dataset; letter A-S identifies individual clades.

relatively robust branches. It provides a basis for further investigation that might lead to better systematic and phylogenetic resolution. The robustness and synapomorphies of each clade will be further discussed below.

4.6.4. Discussion

4.6.4.1. *Aysheaia*. In the cladogram (Fig. 12B), *Aysheaia* is positioned at the most basal branch of the ingroup. In previous studies, although a placement of *Aysheaia* varies slightly, it is also often resolved as a relatively basal lineage among Cambrian lobopodians (Hou & Bergström, 1995; Budd, 1996; Liu *et al.*, 2004, 2007). There seem to be insufficient similarities to link *Aysheaia* with other Cambrian lobopodians, and in the current cladogram this placement renders the Cambrian lobopodians polyphyletic. Therefore, it is possible that *Aysheaia* is not derived from an Early Cambrian lobopodian, but is a direct descendant of a panarthropod-cycloneuralian stem animal. Herein we leave the conclusion open because of the limited evidence. However, *Aysheaia* has often been selected as a representative of Cambrian lobopodians (e.g. Wills *et al.*, 1998; Budd 1997) and we advise caution in this respect. Further research into the phylogenetic position of *Aysheaia* is needed.

4.6.4.2. *Tardigrada*. Our analyses resolve the position of Tardigrada in a branch one node up from *Aysheaia* (Fig. 12B). During the experiments, Tardigrada also occasionally resolved as the sister group to *Aysheaia*. The basal position of tardigrades is supported by the latest molecular and neurobiological research which suggests tardigrades are closely related to cycloneuralians (Dunn *et al.*, 2008; Zantke *et al.*, 2008).

4.6.4.3. *Lobopodians in “Clade D” (Fig. 12B)*. The lobopodian taxa *Microdictyon*, *Paucipodia*, *Jianshanopodia*, *Megadictyon*, *Onychophora*, *Xenusion* and *Hadranax* form a distinct clade that occupies a sister position to the lobopodians in “Clade K” (Fig. 12B) + dinocaridids (“Clade P”) + arthropods (“Clade S”). Synapomorphies of “Clade D” are the presence of limbs shortening towards one end of the body (character 2, state 1) and a tapering body shape (character 20, state 1). Most members of this clade also share large body size, a character not employed in the analysis. Throughout the experiments “Clade D” showed a high level of instability, caused principally by the unstable positions of *Microdictyon* and *Onychophora*. However, there are still some robust relationships within this clade: the sister group of *Xenusion* and *Hadranax* is always well supported, as indicated by Budd and Peel (1998); the linkage between *Jianshanopodia*, *Megadictyon* and *Xenusion* + *Hadranax* accords with the similarities recognised by Liu *et al.* (2007, p. 6); the basal position of *Paucipodia* is consistently stable.

4.6.4.4. *Onychophora*. The association between onychophorans and Cambrian lobopodians has been discussed for decades, and many authors have accepted that Cambrian lobopodians are marine relatives of modern onychophorans (Hutchinson, 1930; Tiegs and Manton, 1958; Manton, 1977; Thompson & Jones, 1980; Robison, 1985; Dzik & Krumbiegel, 1989; Ramsköld & Hou, 1991; Hou & Bergström, 1995; Ramsköld & Chen, 1998), although some papers have challenged this hypothesis (Bergström 1978, 1981; Budd, 1993; Bergström & Hou, 2001).

In our analysis, onychophorans resolve as sister taxon to *Megadictyon* in a derived position in “Clade D” (Fig. 12B) by the presence of a ventral mouth (character 14, state

1). As stated above, the placement of Onychophora is highly unstable, but some signals seem clear:

1) Onychophorans almost certainly originated from a Cambrian lobopodian, but their exact phylogenetic position is unresolved. There seem to be close affinities between extant onychophorans and the Cambrian lobopodians in “Clade D” (Fig. 12B). In addition to the synapomorphies of “Clade D”, some of these taxa (Onychophora, *Xenusion* and *Hadranax*) also have strongly convex annuli (Robison, 1985), as is also the case with *Aysheaia*.

2) Onychophorans appear to be more derived than tardigrades but less derived than the Early Cambrian lobopodians in “Clade K” (Fig. 12B).

3) Cambrian lobopodians seem to be paraphyletic or even polyphyletic, and therefore we support Budd’s (1993) conclusion that the usage of the name Onychophora should not be extended to include all Cambrian lobopodians. If the original cladogram (Fig. 12B) is correct in placing Onychophora as a crown group of “Clade D”, then we could name this whole clade the Onychophora.

4.6.4.5. *Cambrian lobopodians in “Clade K” (Fig. 12B).* The Cambrian lobopodians *Cardiodictyon*, *Hallucigenia*, *Onychodictyon*, *Luolishania* and Collins’ monster form a distinct clade (“Clade K” in Fig. 12B) which is positioned as a sister group to dinocaridids (“Clade P”) + arthropods (“Clade S”). In contrast with the instability of “Clade D” (Fig. 12B), this Cambrian lobopodian clade is much more stable and remains the same through all the experiments mentioned above (Figs. 13A-D). It is supported by two diagnostic synapomorphies: presence of an anterior set of specialised, uniramous ventral limbs (character 3, state 1; this synapomorphy also supports *Anomalocaris* + *Parapeytoia*) and presence of a head shield (character 26, state 1). Within “Clade K”,

the grouping *Hallucigenia* + *Onychodictyon* + *Luolishania* + Collins' monster ("Clade L") is supported by the presence of spine-shaped sclerites (character 29, state 1); *Onychodictyon* + *Luolishania* + Collins' monster ("Clade M") is supported by the presence of spinules/papillae on locomotory limbs (character 9, state 1) and the presence of papillae/setae on trunk annuli (character 32, state 1); *Luolishania* + Collins' monster ("Clade N") is supported by the presence of two long trunk tagmata (character 27, state 1).

The robustness of "Clade K" (Fig. 12B) supports the idea that all members of this clade should be placed into the single order Archonychophora (see Systematic Palaeontology above). The Archonychophora appear as the sister group of dinocaridids ("Clade P") + arthropods ("Clade S"). These three group together as "Clade J" (Fig. 12B), supported by the presence of compound eyes (character 18, state 1) and the presence of a distinct head (character 25, state 1). Of special relevance to our study is the altered phylogenetic position of the genus *Luolishania*. In previous studies, *Luolishania* was often placed at a relatively basal position within Cambrian lobopodians (Hou & Bergström, 1995; Budd, 1996) because of the lack of accurate information. However, our analyses show that *Luolishania* was actually a derived archonychophoran.

4.6.4.6. *Dinocaridids*. Throughout our experiments, *Kerygmachela*, *Opabinia*, *Anomalocaris* and *Parapeytoia* are consistently resolved as a monophyletic group ("Clade P" in Fig. 12B), which has been called Dinocaridida (Collins, 1996). Although *Kerygmachela* shares some striking similarities to Cambrian lobopodians (Budd, 1993; 1999), it is united with dinocaridids by the presence of a pair of grasping limbs (character 4, state 1), the presence of a ventral mouth cone (character 14 and character 15, state 1) and the presence of wide pleural folds (character 22, state 1). During Bremer

support (decay analysis), *Opabinia* + *Anomalocaris* + *Parapeytoia* (“Clade Q” in Fig. 12B) was the most robust clade in the cladogram.

4.6.4.7. Re-evaluation of characters. New morphological data and cladistic analysis have allowed a re-evaluation of the phylogenetic significance of characters and their polarisation. All characters were unweighted and unordered in preliminary analyses of the dataset, but during experiments it became clear that some characters are more important in defining the cladogram. For the benefit of future discussion, we select some reliable morphological characters within lobopodians based on receiving higher weights under successive approximations weighting and indicate their primitive and advanced states below:

1. Locomotory limbs: decreasing towards one or both ends → subequal.
2. An anterior set of specialised ventral limbs: absent → present.
3. Grasping limbs: present → absent.
4. Spinules/papillae on limbs: present → absent.
5. Terminal claws: present → absent; more than two claws → two claws.
6. Mouth position: terminal → ventral.
7. Eyes: simple eye spots → onychophoran complex eyes → compound eyes.
8. Body shape: notably tapering → untapering.
9. Extended posterior end: absent → present.
10. A distinct head: absent → present.
11. Head shield: absent → present.
12. Trunk tagmosis expressed by limb differentiation: absent → present.
13. Sclerites: without sclerites → with sclerites → spine-shaped sclerites.
14. One pair of limbs behind the last pair/set of sclerites: absent → present.

5. Conclusions

In the interpretation of two-dimensionally preserved fossils it is essential to take taphonomy into consideration. The newly collected material of *L. longicruris* has revealed significant features, including the paired antenniform outgrowths, eyes, head shield, setae and other cuticular projections, as well as the differentiated sclerites, appendages, claws, and lobopod interspaces. This has improved our interpretation and understanding of this species, and has led to the conclusion that *M. haikouensis* is a junior synonym of *L. longicruris*. Evidence indicates that *L. longicruris* was possibly a filter feeder, and its well developed sensory organs may have been adaptations to this mode of life. The development of head and trunk tagma link *Luolishania* with *Cardiodictyon*, *Hallucigenia*, *Onychodictyon* and Collins' monster in a single, robust clade; we put them into the same order Archonychophora. Fossil lobopodians are paraphyletic or even polyphyletic. Archonychophorans represent the most derived lobopodian group and are a stem-group of arthropods. Tardigrada and Onychophora derived from different clades of fossil lobopodians.

Chapter 3

A new, exceptionally preserved stem priapulid from the Early Cambrian Chengjiang biota, Yunnan, China

Abstract

A fossil priapulid, *Eximipriapulus globocaudatus* gen. et sp. nov., is described from the Lower Cambrian Chengjiang biota of Yunnan, China. The exceptional preservation of the animal reveals exquisite morphological details and allows direct comparison with extant priapulids. The animal is divisible into a partially eversible pharynx, a smooth collar, a scalid-bearing introvert, a neck with triangular scalids, an unsegmented trunk with annulations and a distinctly expanded post-trunk region. Several specialized regions of its alimentary canal are recognised: pharynx (lined by a cuticle), oesophagus, midgut (lined by a mesentery), hindgut with a possible rectal pouch (lined by a cuticle) and terminal anus. A possible ventral nerve cord is also identified. Possible internal fertilization is suggested and a putative juvenile is described. Evidence indicates that the animal was an active burrower using a double-anchor strategy, and it is suggested to be both a deposit feeder and a carnivore. Cladistic analysis resolves *Eximipriapulus* as one of the most derived Cambrian stem priapulids and that it forms a clade with *Corynetis* and has similarities to *Ottoia*. *Eximipriapulus* also has potential affinities with the extant priapulids *Tubiluchus* and *Meiopriapulus*, as well as some similarities to *Priapulus*.

Keywords: Early Cambrian; priapulid; Chengjiang; exceptional preservation.

1. INTRODUCTION

The phylum Priapulida comprises marine benthic vermiform animals; the adults have a cylindrical body, divisible into at least two regions: an anterior invaginable introvert bearing longitudinal rows of scalids and an unsegmented posterior trunk with an annulated cuticle. Only 19 extant species are known (e.g. Shirley & Storch 1999; Todaro & Shirley 2003), but the phylum displays a high morphological disparity.

As priapulids are essentially soft-bodied, they are only rarely preserved as fossils. Body fossils of priapulids are mainly known from the Lower Cambrian Chengjiang biota (ca. 525 mya) of Yunnan Province, southwest China and the Middle Cambrian Burgess Shale (ca. 505 mya), British Columbia, Canada. Evidence from both localities indicates that priapulids were vastly more abundant in Cambrian communities in terms of number and diversity than they are today (Wills 1998). Conway Morris (1977b) described eight priapulid species from the Burgess Shale (see also Briggs *et al.* 1994), which provided the first insight into the Cambrian priapulids. Following the discovery of the Chengjiang biota, 14 worms from that Lagerstätte were placed within the Phylum Priapulida (Chen & Zhou 1997; Hou *et al.* 1999; Huang *et al.* 2004a, b; Han *et al.* 2004). However, the classification and the systematic position of these Cambrian worms are still hotly debated (Adrianov & Malakhov 1996; Wills 1998; Hou *et al.* 2004a; Huang *et al.* 2006; Han & Hu 2006; Maas *et al.* 2007).

In recent years, priapulids have drawn attention from both biologists and palaeontologists, as they are widely considered to be model organisms for understanding the evolution of the Ecdysozoa (Webster *et al.* 2006). The phylum Priapulida is allied with the Nematoda, Nematomorpha, Kinorhyncha and Loricifera in a clade called the Introverta (Nielsen 1995) or Cycloneuralia (Ahlrichs 1995). Together with the Panarthropda, the Introverta are generally considered to comprise the

Ecdysozoa. Recent molecular studies have suggested that the Priapulida is basal within the Ecdysozoa (Garey 2001; Webster *et al.* 2006), and morphologists have also pointed out that priapulids have retained numerous plesiomorphic features (Adrianov & Malakhov 1996). However, there is still a need for reliable information on the relationships between the major groups of the Introverta and their relationships to the Panarthropoda (Telford *et al.* 2008). Detailed research on Cambrian priapulids can, thus, be of significance in understanding the early evolution of ecdysozoans (Webster *et al.* 2006; Maas *et al.* 2007).

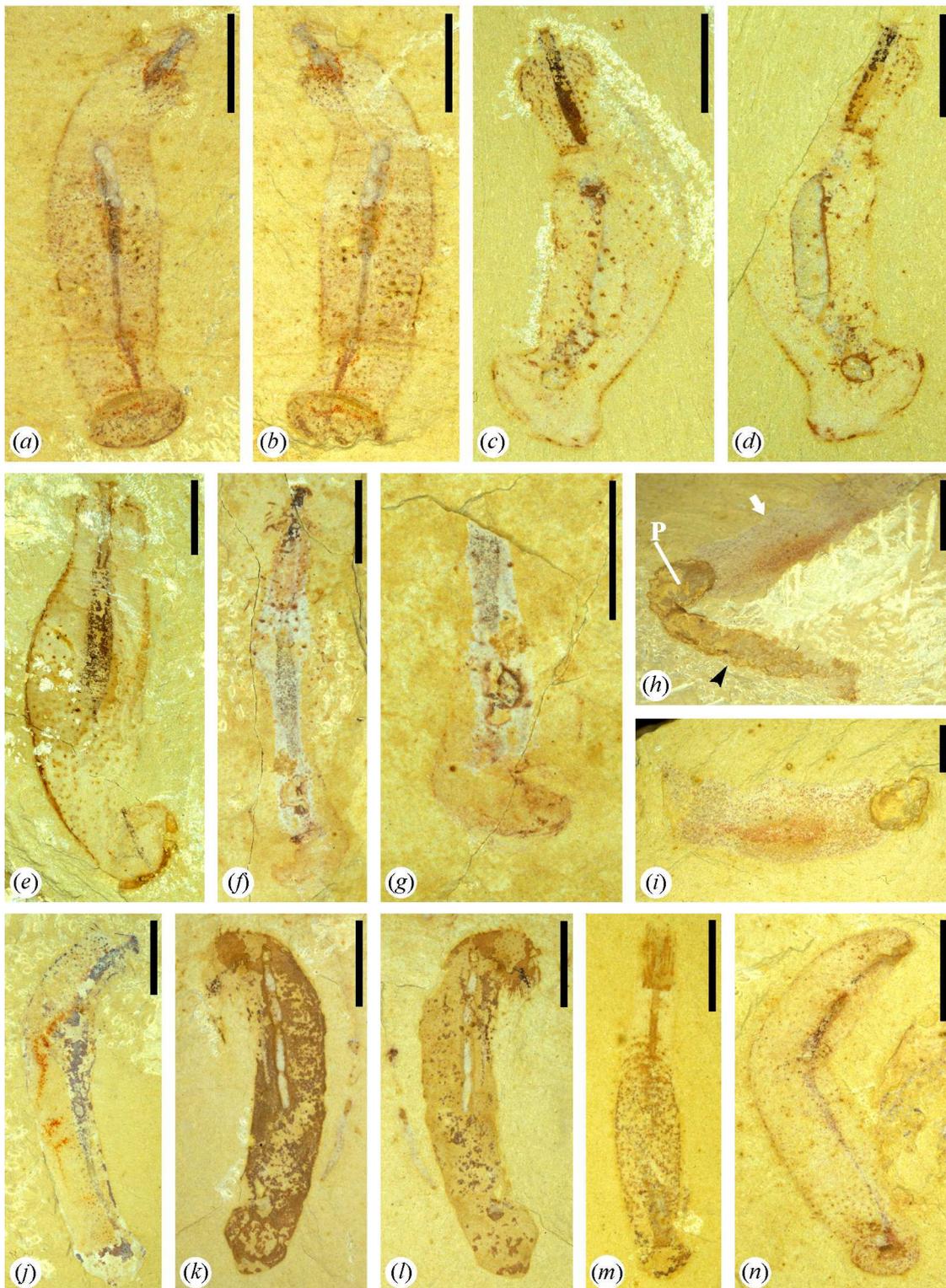
In this chapter, a new stem-priapulid genus and species is reported from the Chengjiang biota, with anatomical details preserved with exceptional fidelity. These specimens provide new insights into the early evolution and the mode of life of Cambrian priapulids.

2. MATERIAL AND METHODS

The new species is represented by nine specimens (five with counterparts), all of which are from the *Eoredlichia* Biozone, Yu'an Shan Member, Lower Cambrian Helinpu Formation, south Yunnan Province. One specimen was collected from the Maotianshan section near Chengjiang, the remainder are from the Anshan section near Mafang village in the Haikou region (see Hou *et al.* 2004a for locations). All the specimens are deposited in the Yunnan Key Laboratory for Palaeobiology (YKLP), Yunnan University, Kunming, China: YKLP 11263 to YKLP 11271 (figures 1a-n; 2a-l).

The observation, preparation and camera lucida drawings of specimens were made under a Wild Heerbrugg Plan 1X Microscope. The light for photographing was used in two ways: polarizing light to increase the colour contrast between different structures,

Figure 1. *Eximipriapulus globocaudatus*, photos taken using polarizing light. (a), (b) Part and counterpart of complete specimen YKLP 11263, showing important details of internal anatomy. See Fig. 4A for explanation. (c), (d) Part and counterpart of holotype YKLP 11264, showing the body divisions, especially at the anterior. See Fig. 4B for explanation. (e) Complete specimen YKLP 11265 (part only); the posterior end is preserved with a twist. (f), (g) Part and counterpart of YKLP 11266; the animal decayed considerably before being preserved as fossil. See Fig. 6C for explanation. (h), (i) Part and counterpart of incomplete specimen YKLP 11267; (h) shows the specimen is connected with another individual at their posterior ends. White arrow indicates specimen YKLP 11267, black arrow indicates another individual. P, post-trunk region. (j) Complete specimen YKLP 11268 (part only), showing detailed morphology of pharyngeal teeth, scalids and setae. See close-ups in Fig. 5c, d. (k), (l) Part and counterpart of almost complete specimen YKLP 11269, showing a possible retractor muscle or ventral nerve cord; the anterior end is bent and decayed considerably. See Fig. 6A, B for explanation. (m) Complete specimen YKLP 11270 (part only), putative juvenile. See Fig. 6D for explanation. (n) Incomplete specimen YKLP 11271 (part only), showing regularly arranged papillae/spinules on the poster part of the trunk; the anterior end is not preserved. Scale bars = 3 mm.



so that it is easier to distinguish some weak-colour structures (figure 1*a-n*); normal light with low angle to highlight some structures preserved in low relief or depression (figure 2*a-l*). Some specimens were also photographed uncoated in a variable pressure SEM (Hitachi S3500N) and elemental mapping was undertaken using an Oxford Instruments EDX (figure 3*a-k*).

3. PRESERVATION

All specimens are two-dimensionally preserved on the bedding surfaces of the host mudstone. However, in some specimens the mid-gut is preserved three-dimensionally (figure 2*a-d,f,h,i*) and most of the scalids are also preserved in slight relief (figure 2*a-e,k*). Cuticle and cuticular structures, such as scalids and spines, are highly mineralized and preserved in a rusty colour, caused by a concentration of iron oxides (figure 3*a,d,g,h-k*). However, elemental mapping (SEM EDX) reveals that the dark colour inside the alimentary canal represents a high concentration of carbon (figure 3*b,c,e,f*). Current models suggest that the Chengjiang fossils consist of recalcitrant organic carbon films, with coatings of iron oxides which are pseudomorphs after pyrite (Gabbott *et al.* 2004).

4. SYSTEMATIC PALAEOLOGY

Genus *Eximipriapul* gen. nov.

Derivation of name: Latin *eximius*, extraordinary, with reference to the exceptional preservation, and Priapulida.

Diagnosis: cylindrical body divided into an anterior part, trunk and an expanded post-trunk region. Collar non retractable, with a smooth surface; bulbous introvert completely invaginable, with long spine-like scalids arranged in approximately 30

Figure 2. *Eximipriapulus globocaudatus*, photos taken using low-angle light. (a), (b) Part and counterpart of YKLP 11263, showing midgut content, scalids and trunk seta traces preserved in relief. (c), (d) Part and counterpart of holotype YKLP 11264, showing introvert scalids preserved in relief and mid-gut preserved three-dimensionally. (e) YKLP 11265 (part only), showing annulations on the trunk. (f) YKLP 11266 (part), arrows indicate two uninterpreted tubular structures at the anterior part of the body. (g) YKLP 11268 (part only), showing foregut preserved in a relief. (h), (i) Part and counterpart of YKLP 11269, showing a three-dimensionally preserved gut, some spinules/papillae arranged in longitudinal rows on the posterior end, and possible muscle fibres or cuticular folds. (j) YKLP 11267 (part), showing the two individuals preserved on different bedding laminae. (k) YKLP 11270 (part only), showing scalids preserved in relief, a smooth neck contrasting with the roughness of the trunk. (l) YKLP 11271, showing regularly arranged papillae/spinules on the posterior portion of the trunk, preserved in low relief. Scale bars = 3 mm. Gc, gut content; Is, introvert scalids; Mf, muscle fibres; P, post-trunk region; Rp, regularly arranged papillae; Ts, trunk seta.

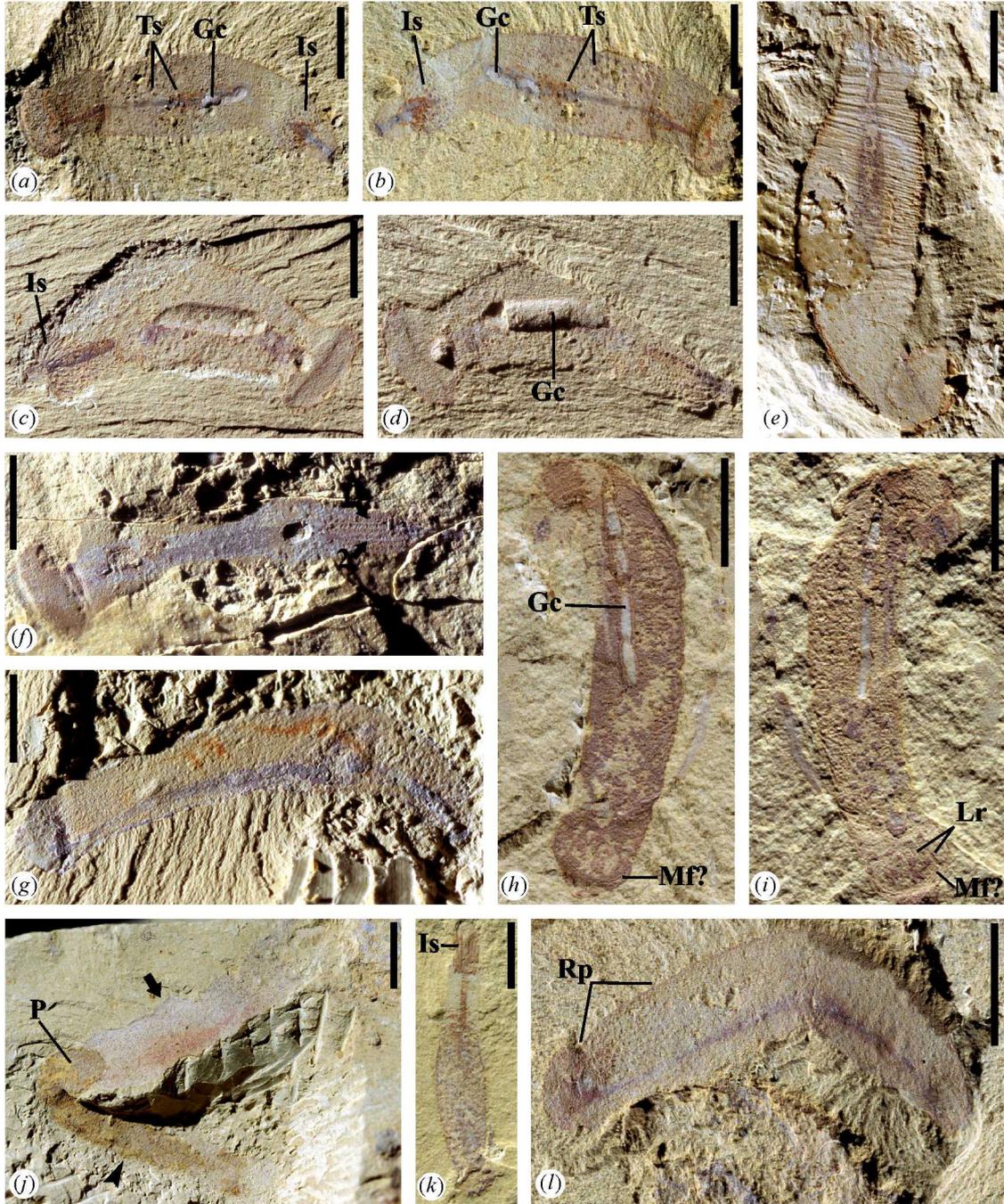
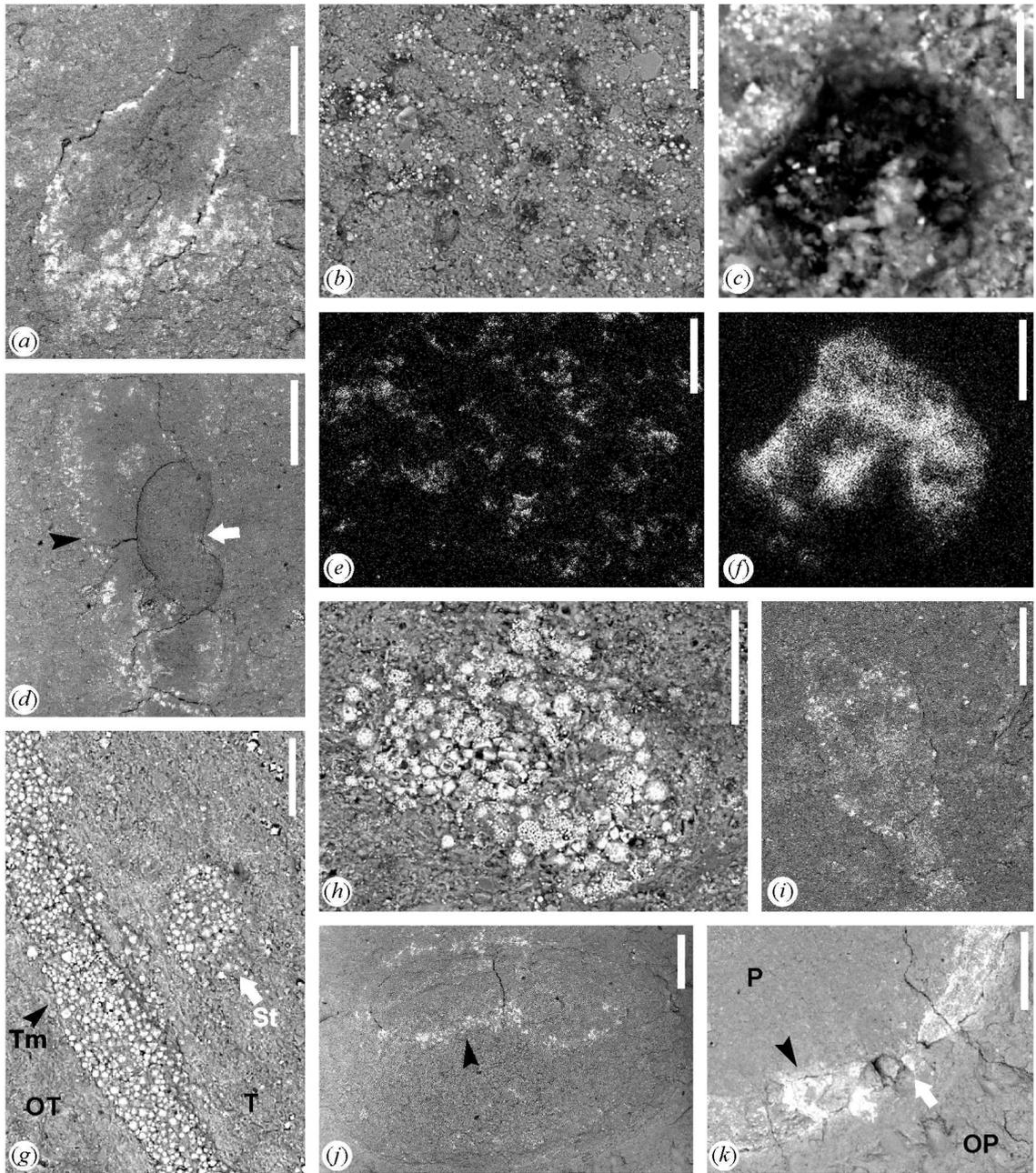


Figure 3. SEM and EDX images of *Eximipriapulus globocaudatus*. (a) Backscatter image of the pharynx of YKLP 11263, showing an iron rich margin (bright). (b), (e) A small area in the anterior part of the pharynx of YKLP 11264. (b) Backscatter image showing some evenly distributed black spots in this area. (e) EDX carbon map of the same area as (b) showing the carbon composition of black spots. (c), (f) Backscatter image (c) and EDX carbon map (f) of a selected black spot in the gut of YKLP 11264, showing areas of carbon richness (dark). (d) Backscatter image of midgut of YKLP 11263, black arrow indicates the iron-rich margin of the gut, white arrow indicates the three-dimensionally preserved contents. (g) Backscatter image of middle trunk area of YKLP 11265, black arrow indicates the trunk margin (Tm), white arrow indicates a seta trace on the trunk; both structures are iron-rich relative to the rock. (h) Backscatter image of a seta trace (St) on the trunk of YKLP 11264, showing concentration of iron oxide pseudomorphs after pyrite. (i) Backscatter image of the possible rectal pouch or urogenital organ of specimen YKLP 11263, showing an iron rich outline. (j) Backscatter image of the post-trunk region of YKLP 11263, arrow indicates a possible septum which is rich in iron. (k) Backscatter image of the posterior end of YKLP 11265, black arrow indicates an iron rich margin, white arrow indicates the anus. P, post-trunk region; OP, outside post-trunk region. Scale bars=500 μm (a), (d), (i)-(k); 50 μm (b), (e), (g), (h); 10 μm (c), (f). OT, outside trunk; St, seta trace; T, trunk; Tm, trunk margin.



indistinct longitudinal rows with 9 scalids per row forming circles around the introvert; neck region bearing conical to triangular scalids arranged in approximately 30 indistinct longitudinal rows with 13 scalids per row forming circles around the neck. Annulated trunk stout, cylindrical; spinules arranged along annuli, more regular in circles on posterior part of trunk; larger seta-like projections scattered around mid part of trunk. Post-trunk region distinctively expanded into a globe, separated from the body cavity by a septum. Mouth opening at anterior end, leading to a muscular, partially eversible pharynx lined with pharyngeal teeth, and thence to a straight intestine; mid-gut lined with mesentery; hindgut with a possible rectal pouch; anus terminal.

Species *Eximipriapulus globocaudatus* gen. et sp. nov.

Derivation of name: Latin *globus*, ball and *cauda*, tail; alluding to the distinctively expanded post-trunk region.

Diagnosis: as for genus.

Material: Holotype, YKLP 11264 (figures 1*c,d*; 2*c,d*; 4*b*; 5*a,b*), part and counterpart, from the Anshan section near Haikou. Paratypes: YKLP 11263 (figures 1*a,b*; 2*a,b*; 4*a*; 5*g,h*), YKLP 11267 (figures 1 *h,i*; 2*j*), YKLP 11269 (figures 1*k,l*; 2*h,i*; 6*a,b*), each having part and counterpart; and YKLP 11265 (figures 1*e*; 2*e*), YKLP 11268 (figures 1*j*; 2*g*; 5*c,d*), YKLP 11270 (figures 1*m*; 2*k*; 6*d*) and YKLP 11271 (figure 1*n*; 2*l*), part only; all from the type locality. Paratype YKLP 11266 (figures 1*f,g*; 2*f*; 6*c*), part and counterpart, from the Maotianshan section near Chengjiang.

Description:

(i) *Size and overall form*

Presumed mature animal 13.3-15.8 mm in length, mean 14.4 mm; trunk width at

widest part 2.4-4.5 mm, mean 3.3 mm. Body stout, cylindrical (figures 1*a-d*; 4*a,b*); body proportions vary between specimens (Table 1). Most specimens are curved to some degree, possibly indicating the body's attitude in life.

(ii) *Anterior structures*

The anterior part of the animal has four subdivisions, here provisionally labelled Stages I-IV, from anterior to posterior (figures 1*a-d*; 4*a,b*; 5*a-h*).

There is no clear boundary between Stages I and II, which form a cone-shaped structure at the most anterior part of the animal. The size and shape of the cone varies, and is on average about 0.8 mm high and 1.1 mm wide. Stage I is represented by a small dome-shaped projection in front of Stage II in specimen YKLP 11263 (figures 1*a,b*; 2*a,b*; 4*a*; 6*g,h*), whereas it is a dark rusty-coloured anterior region of the cone in specimen YKLP 11268 (figures 1*j*; 5*c,d*); it is not visible in other specimens. Stage II is a cone-shaped structure immediately following Stage I or at the most anterior part of the body when Stage I is not visible (figures 1*a-c,j*; 2*a-c*; 4*a,b*; 5*a-d,g,h*). This structure is apparent in all specimens and its surface is smooth with no sign of any cuticular projections.

Stage III follows Stage II with a clear boundary, only fully visible in specimen YKLP 11264 (figures 1*c,d*; 2*c,d*; 4*b*; 5*a,b*), but also evident in specimen YKLP 11268 (figures 1*j*; 5*c,d*). Shape oblate, 1.7 mm high, 2.5 mm wide at widest part, with the structure fully extended; surface covered with profuse long, spine-like scalds arranged in indistinct longitudinal rows and transverse circles: estimates from specimens YKLP 11264 and YKLP 11268 are approximately 30 longitudinal rows, with 9 scalds per row forming circles around Stage III. Spines 0.6-0.8 mm long, preserved at different angles

Table 1. Measurements (in mm) on *Eximipriapulus globocaudatus*.

<i>Body Divisions</i>	<i>Specimen Number (YKLP)</i>								<i>Mean*</i>
	<i>11263</i>	<i>11264</i>	<i>11265</i>	<i>11266</i>	<i>11268</i>	<i>11269</i>	<i>11270</i>	<i>11271</i>	
Body Length	13.25	13.75	15.81	15.30	15.71	14.87	11.90	11.81	14.36
Stage I + II Length	1.00	0.63	0.71	0.75	0.90	-	-	-	0.80
Stage I + II Widest Width	1.58	0.92	0.87	0.80	1.33	-	-	-	1.10
Stage III Length	-	1.67	-	-	0.81	-	-	-	1.24
Stage III Widest Width	-	2.46	-	-	1.86	-	-	-	2.16
Stage IV Length	1.36	1.63	1.05	-	1.71	-	-	-	1.44
Stage IV Widest Width	2.33	2.71	2.71	-	2.19	-	-	-	2.49
Trunk Length	7.85	7.13	10.48	12.45	10.67	12.62	8.73	10.36	10.22
Trunk Widest Width	3.46	4.08	4.52	3.30	2.95	2.43	2.15	2.68	3.35
Post-trunk Region Length	2.04	2.25	3.05	2.10	1.62	2.25	1.26	1.45	2.11
Post-trunk Region Widest Width	3.08	3.88	3.43	3.10	2.57	2.35	1.94	2.07	2.93

* The average of all listed specimens except YKLP 11270, as it is a putative juvenile.

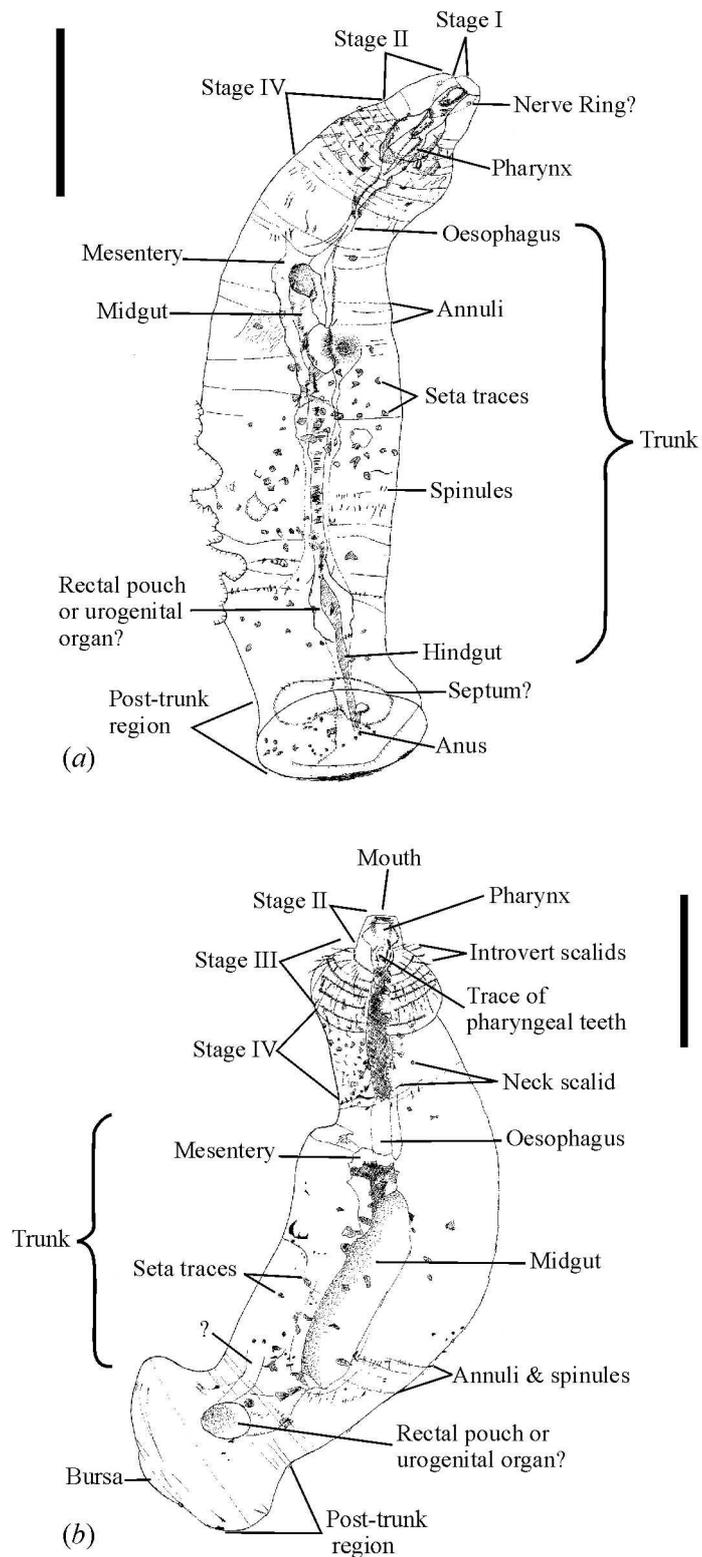
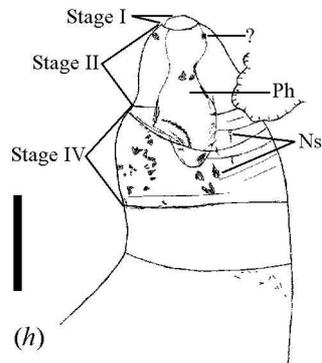
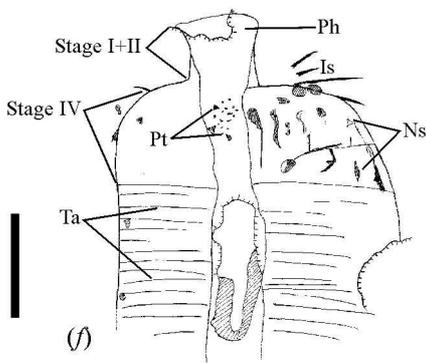
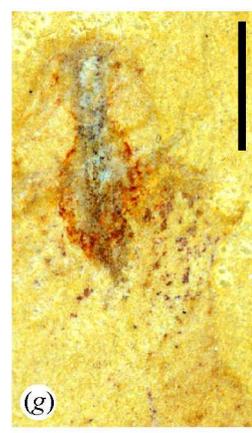
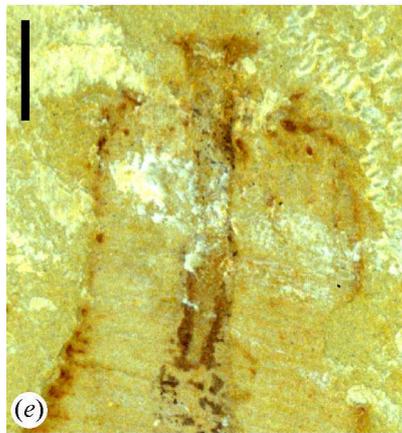
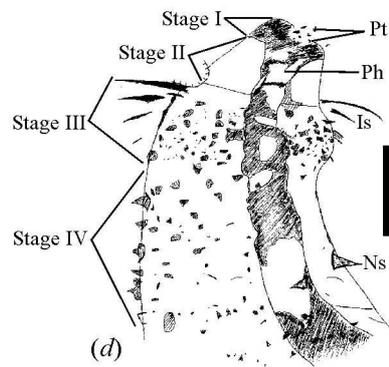
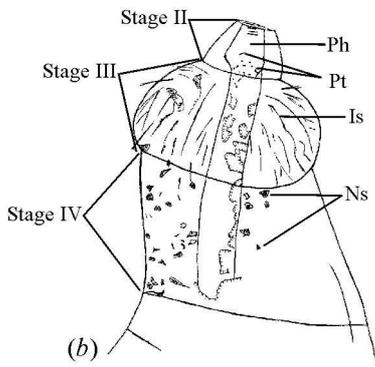
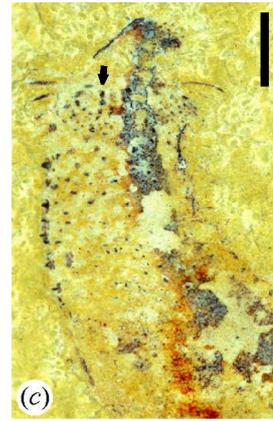
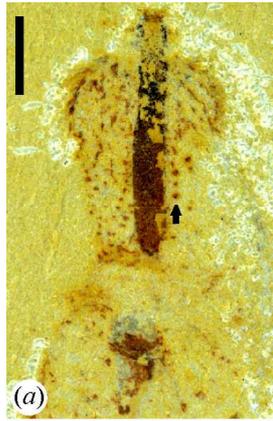


Figure 4. Interpretative camera lucida drawings of *Eximipriapulus globocaudatus*. (a) YKLP 11263, Stage III is hidden. (b) YKLP 11264, Stage I is hidden. Scale bars = 3 mm.

Figure 5. (a), (b) Anterior portion of YKLP 11264. (a) Photo taken using polarizing light. Arrow points to a longitudinal row of neck scalids. (b) Camera lucida drawing of the same specimen. (c), (d) Anterior portion of YKLP 11268. (c) Photo taken using polarizing light. Arrow indicates a longitudinal row of introvert scalids. (d) Camera lucida drawing of the same specimen. (e), (f) Anterior portion of YKLP 11265. (e) Photo taken using polarizing light. (f) Camera lucida drawing of the same specimen. (g), (h) Anterior portion of YKLP 11263. (g) Photo of the part using polarizing light. (h) Camera lucida drawing of the counterpart. Scale bars=1 mm. Is, introvert scalids; Ns, neck scalids; Ph, pharynx; Pt, pharyngeal teeth; Ta, trunk annulations.



in different specimens (see Remarks).

Stage IV is cylindrical, 1.1-1.7 mm long, 2.2-2.7 mm wide, distinguished from Stage III by its narrower shape and a different type of scalids (figures 1*a-d,j*; 2*a-d*; 4*a,b*; 5*a-d*). In most specimens, the scalids of Stage IV are preserved as rusty-coloured spots arranged in both longitudinal rows and circles; as far as can be determined more than 30 rows and about 13 circles. The scalids preserved along the body edge of YKLP11268 show a stout conical to triangular shape with a large base (figure 5*c,d*), which is distinctly different from the scalids of Stage III.

(iii) *Trunk*

The trunk is demarcated from the anterior stages by an increase in girth behind Stage IV. The trunk is stout, cylindrical, slightly tapering towards both ends, with no discernible subdivisions; comprises 2/3 of body length (7.1-12.6 mm long), annulated with average width of each annulus about 0.1 mm. Annuli preserved in strong relief in YKLP 11265 (figures 2*e*; 5*e,f*), perhaps due to contraction of body. Three types of cuticular ornamentation can be recognized on the surface of the trunk. 1) Small spinules/papillae preserved along annuli as scattered rusty dots (figures 1*a-e,j,n*; 4*a,b*); specimen YKLP 11271 displays papillae that appear more regularly arranged in circles on the posterior part of the trunk (figures 1*n*; 2*l*); on YKLP 11269 longitudinal rows of spinules can be seen posteriorly with low-angle illumination (figures 2*h,i*; 6*a,b*). 2) Larger rusty spots irregularly scattered around the mid trunk (figures 1*a-g*; 2*a,b,e*); a few of these are preserved laterally on the edge of the mid trunk, displaying an outline of a triangular base and a seta-like prolongation. 3) The trunk surface of specimen YKLP 11268 is endowed with a collection of stout triangular setae pointing anteriorly,

arranged in a longitudinal strip along the body (figure 1j).

(iv) *Post-trunk region*

The expanded structure forming the posterior part of the body is separated from the trunk by a constriction; has a distinct margin, which is often strongly mineralized (figures 1a,b,e; 2a,b,e; 3k). Shape oblate, surface smooth, 1.6-3.0 mm long (up to one fifth of body), 2.4-3.9 mm wide at the widest point, with some variation in shape and size in different specimens (Table 1; figures 1a-n; 4a,b; 6a-c). Specimens YKLP 11264, 11268 and 11269 display a small projection at the posterior terminal end (figures 1c,d,j-l; 2c,d,h,i; 4b; 6a,b), similar to the bursa in *Ottoia* (see Conway Morris, 1977b) hence interpreted as such.

(v) *Body cavity*

The body wall of specimen YKLP 11266 decayed considerably before preservation, resulting in clear representation of the body cavity. The preserved body wall is close in colour to the surrounding yellow matrix; the gut is preserved in black. Between the body wall and the gut, a distinct whitish to grey area indicates the body cavity (figures 1f,g; 6c). The body wall is about 1.1 mm thick; diameter of gut at widest part is 0.8 mm. Body cavity, varying in width along specimen, widest at anterior part of mid-gut where distance between gut and body wall reaches 0.9 mm, but much narrower at (0.2 mm) along posterior part of gut. Body cavity terminates in front of post-trunk region.

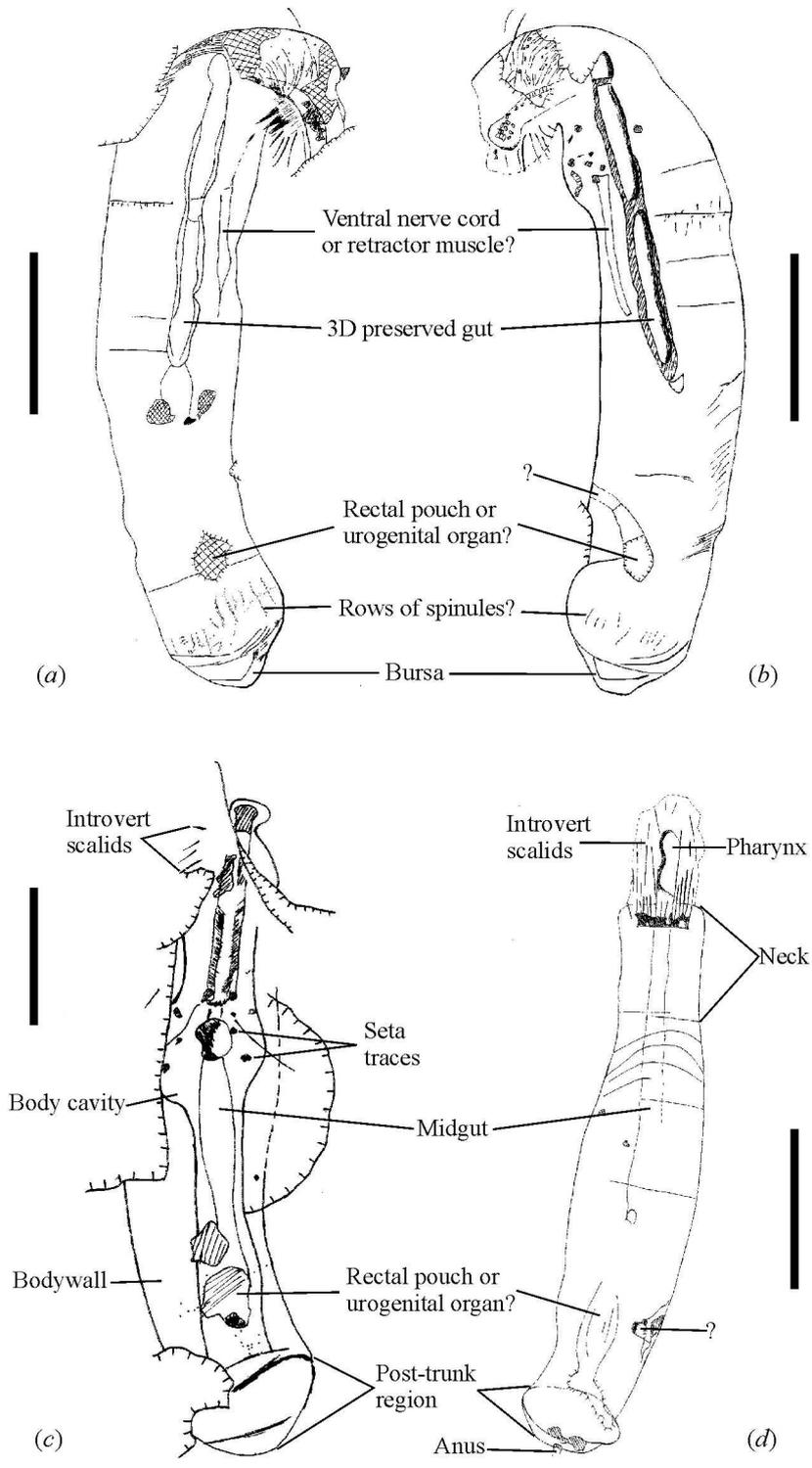


Figure 6. Interpretative camera lucida drawings of *Eximipriapulus globocaudatus*. (a), (b) Part and counterpart of YKLP 11269. (c) YKLP 11266. (d) Possible juvenile specimen YKLP 11270. Scale bars=3 mm.

(vi) *Alimentary canal*

The alimentary canal runs along the longitudinal axis of the body, and is composed of a terminal mouth, a muscular pharynx lined with teeth, oesophagus, midgut and hindgut.

The pharynx is preserved as a straight, dark rusty coloured tube in most specimens. Specimen YKLP 11264 shows the pharynx retracted inside the body and present from the mouth opening to the posterior end of Stage IV (figures 1*c,d*; 2*c,d*; 4*b*; 5*a,b*). However, specimen YKLP 11263 displays an oval shaped muscular pharynx which may be the result of contraction (figures 1*a,b*; 2*a,b*; 4*a*; 5*g,h*). In this specimen, the pharynx is outlined by a red wavy margin that is locally fractured and shows iron enrichment (figure 3*a*); this margin may indicate a myoepithelium. The anterior part (Stage I) of the pharynx is partially everted in specimen YKLP 11263 and 11268 (figures 1*a,b,j*; 4*a*; 5*c,d,g,h*). Rusty coloured mineralized elements within the pharynx may indicate the presence of pharyngeal teeth (figures 1*c,d,j*; 4*b*; 5*a-d*). Some evenly spaced black dots can be recognised in the pharynx (figure 5*a,b*), reflecting higher carbon content (figure 3*d,e*) and perhaps indicating the attachment positions of pharyngeal teeth. The pattern of arrangement of pharyngeal teeth is unclear. In specimen YKLP 11268, evagination of the pharynx exposes several pharyngeal teeth at the top of the mouth opening (figure 5*c,d*); these appear conical, but their morphology is indistinct.

A short oesophagus connects the pharynx to the midgut (figures 1*a-d*; 4*a,b*). The midgut is the widest part of the alimentary canal and is often dark grey to black, due to the concentration of carbon (EDX analysis; 3C, G). Some specimens have three-dimensionally preserved mid-guts filled with sediment, whitish to grey in colour (figures 1*a-d,k,l*; 2*a-d,h,i*; 3*d*). Specimen YKLP 11263 has a thin whitish layer surrounding the mid-gut and attached to the body wall (figures 1*a,b*; 2*a,b*; 4*a*); this is

interpreted as mesentery that retains the gut in the centre of the trunk. The midgut margin is often preserved in a rusty colour and shows iron enrichment (figure 3*d*), which indicates that the midgut is lined with cuticle.

The hindgut is much narrower than the midgut, passing through the post-trunk region to a terminal anus (figures 1*a,b,e,n*; 3*k*; 4*a*). Specimen YKLP 11263 has an expanded structure outlined in red in the middle of the hindgut; it appears to represent a thick, pleated cuticle with a muscular layer (figures 3*i*; 4*a*). A similarly round structure is also present at the same location in other specimens (figures 4*b*; 6*a-c*). A reconstruction of *E. globocaudatus* is given in figure 7.

Remarks: Interpretation of anatomical features

E. globocaudatus resembles scalidophorans, especially priapulids, in the general division of body sections, anterior structures, surface ornament and internal anatomy. Direct comparison with priapulids has led to further interpretations of the structures of *E. globocaudatus*.

(i) *Anterior structures*

Stage I of *E. globocaudatus* corresponds to that previously referred to as an “anterior pharynx” (Calloway 1975), “everted pharynx” (Ruppert & Barnes 1996), “mouth cone” (Nielsen 1995, 2001; Storch *et al.* 1989; Wills 1998) and “introvert Zone III” (Conway Morris 1977*b*; Wills 1998). Herein, the term “everted pharynx” is preferred. The everted pharynx of *E. globocaudatus* is only a small portion of the anterior pharynx which could be everted and retracted into the mouth.

Stage II of *E. globocaudatus* corresponds to that previously referred to as a “buccal

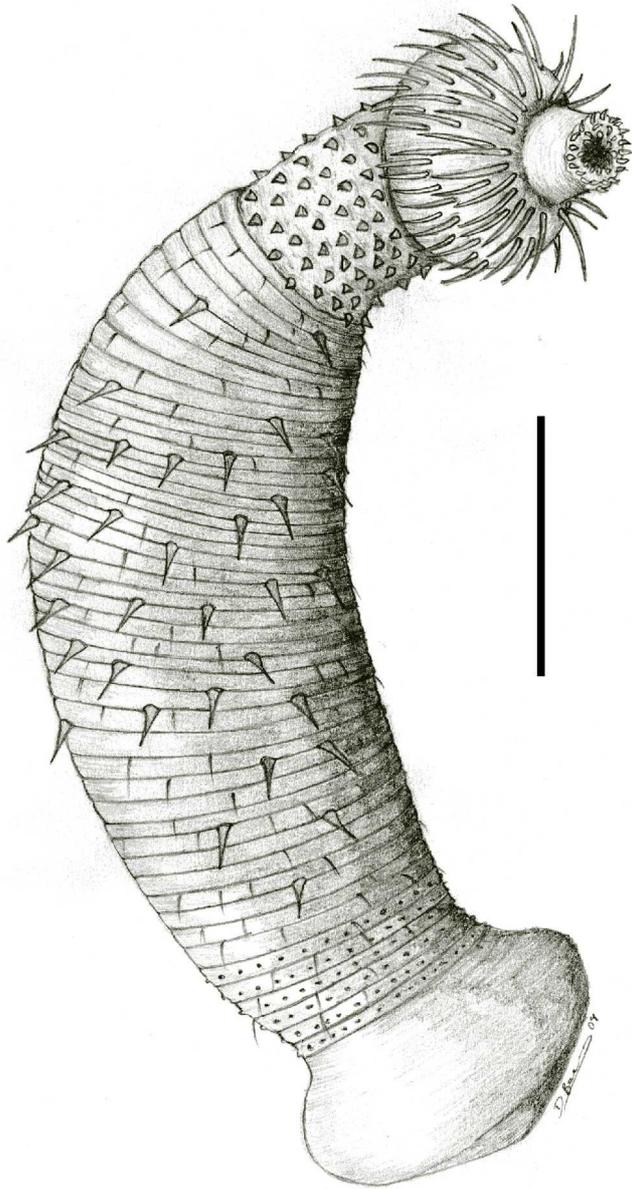


Figure 7. Artist's impression of *Eximipriapulus globocaudatus*. Scale bar=3 mm.

tube” (Calloway 1975; Wills 1998; Todaro & Shirley 2003), “collar” (Conway Morris 1977b; Stachowitsch 1992) or “introvert Zone II” (Conway Morris 1977b; Wills 1998). If the pharynx of priapulids corresponds to the mouth cone of loriciferans (Wills 1998), then their “buccal tubes” should be positionally non-homologous. Therefore, the term “collar” is used here to avoid any confusion. The collar of *E. globocaudatus* is present in all specimens with well-preserved anterior ends, indicating that it is non-retractable. The variation of its size and shape may be due to the movement of the associated pharynx.

Stage III of *E. globocaudatus* corresponds to that previously referred to as an “introvert” (Land 1970; Calloway 1975) and “introvert Zone I” (Conway Morris 1977b; Wills 1998). According to the literal meaning, the term “introvert” is only applied to the anterior structure which is capable of inversion into the body, rather than the whole anterior proboscis. Therefore, herein the term “introvert” is only used for Stage III.

Stage IV of *E. globocaudatus* may correspond to: 1) a part of the introvert which bears a second type of introvert scalids, on the basis that the boundary between Stage III and Stage IV is not always clear and that Stage IV also seems to be able to partially invert into the body (figures 1e; 2e; 5e,f); 2) the anterior trunk part with triangular scalids, such as in *Meiopriapulius* (Morse 1981); 3) the neck region, such as in *Tubiluchus* (Calloway 1975; Todaro & Shirley 2003), which can be seen as a narrowing or constriction between the introvert and the trunk; although the folds of Stage IV are not clear, its cuticular modifications arranged in rings resemble the papillae on the neck of *Tubiluchus* (Todaro & Shirley 2003). The slightly varied size and shape of Stage IV in different specimens may indicate the contraction of a neck, therefore the third interpretation is preferred.

(ii) *Post-trunk region*

The post-trunk region of *E. globocaudatus* is distinctive and does not correspond to any type of posterior termination shown by extant priapulids.

Most extant genera, including *Priapulites*, *Priapululus*, *Acanthopriapululus*, *Priapulopsis* and *Tubiluchus*, possess one or two caudal appendages, also called a tail. The tail is a cylindrical post-anal extension of the abdomen and differs from the latter only in the relative abundance of cuticular structures (Land 1970). However, the post-trunk region of *E. globocaudata* is principally a pre-anal structure and appears to have been able to contract to some degree. Thus it is not just a cuticular structure and cannot be regarded as a tail.

Two extant genera, *Meiopriapululus* and *Maccabeus*, lack the tail, but the trunk has a posterior pre-anal subregion bordered with hooks (Morse 1981; Adrianov & Malakhov 1996). The post-trunk region of *E. globocaudatus* lacks comparable hooks; it also differs in its larger size and spherical shape from the pre-anal subregion in *Meiopriapululus* and *Maccabeus*. Importantly, the post-trunk region of *E. globocaudata* appears to be separated from the body cavity by a septum and is not, therefore, a subregion of the trunk; *Meiopriapululus* and *Maccabeus* show no such separation between the trunk and the pre-anal subregion (Storch *et al.* 1989; Por 1983). Specimen YKLP 11266 shows the body cavity terminating anterior to the post-trunk region (figures 1f,g; 2f; 6c), and YKLP 11268 shows differential preservation of trunk and post-trunk region (figures 1j; 2g). Furthermore, there is an orange ring at the trunk/post-trunk boundary of YKLP 11263 (figures 1a,b; 2a,b; 4a), the preservation of which is consistent with a cuticular structure (figure 3j); this may indicate a septum separating the post-trunk from the body cavity and allowing passage only of the hindgut.

One extant genus, *Halicryptus*, has neither a tail nor a pre-anal region, but possesses a pair of setae at the anal position (Shirley & Storch 1999). The Chengjiang specimens examined here show no evidence of anal setae.

Overall, the post-trunk region of *E. globocaudatus* seems to be anatomically unique. However, its pre-anal position and separation from the trunk may indicate that it represents a transitional stage from a pre-anal subregion of trunk to a caudal appendage.

(iii) *Rectal pouch or urogenital organ?*

The oval shaped structure in the hindgut may be a rectal pouch, responsible for tight closure of the anus, such as in *Tubiluchus* (Storch 1991); however, a rectal pouch is normally located very close to the anus, whereas the oval shaped structure in *E. globocaudatus* seems too distant from the anus. Alternatively, it may be a urogenital organ, as a possible diverticulum is suggested to one side of the body in the posterior trunk part (figures 1*c,d,k,l*; 2*c,d,h,i*; 4*b*; 6*b*). The urogenital system of extant priapulids consists of two elongated ducts, each suspended in a mesentery beside the posterior region of the intestine and joined anteriorly by a protonephridial tubule and laterally by the gonads; both ducts open to the exterior on either side of the anus (Ruppert & Barnes 1996). However, a similar structure has neither been found in the exceptionally preserved *E. globocaudatus* nor *Ottoia* (Conway Morris 1977*b*). As there are no other candidates for a urogenital organ preserved in this animal, perhaps it is represented by the oval shaped structure in the hindgut. A long, thin, whitish structure preserved beside the body of YKLP 11269 may be connected to the oval shaped structure, as there is a similarly coloured trace preserved in this area (figures 1*k,l*; 2*h,i*). A similar structure is also seen in specimen YKLP 11270 (figure 1*m*).

(iv) *Possible nervous system?*

Specimen YKLP 11263 shows a pair of small orange structures which are evident at the top of the collar (figures 1*a,b*; 4*a*; 5*g,h*). A pair of dots can also be seen at a similar position in specimen YKLP 11264 (figures 1*c,d*; 4*b*; 5*a,b*). If the structure is real, its position may suggest the presence of the buccal nerve ring.

Within the body of specimen YKLP 11269, a long, thin, whitish band is preserved parallel to gut on the concave side of the body (figures 1*k,l*; 2*h,i*; 6*a,b*); this may represent a retractor muscle, perhaps preferentially preserved through contraction during curving of the specimen, or it may be a ventral nerve cord (the body normally curves towards the ventral side; see Conway Morris 1977b). As several sets of retractor muscles would be expected and as this structure appears to extend further along the body than would be expected for retractor muscles, the latter interpretation is favoured.

5. DISCUSSION

(i) *Reproduction and ontogeny*

YKLP 11265 shows two individuals connected at their posterior ends (figures 1*h*; 2*j*). Although most extant large-bodied priapulids employ external fertilization, internal fertilization occurs in *Tubiluchus* (Alberti & Storch 1988) and probably in other meiofaunal species (Ruppert & Barnes 1996). The two individuals in question could possibly represent a mating couple. If so, these animals had evolved internal fertilization by the Early Cambrian.

Little is known of the ontogeny of Cambrian priapulids. *E. globocaudatus* appears to be represented by at least one juvenile specimen (YKLP 11270; figures 1*m*; 2*k*; 6*d*). It is smaller than the other co-occurring specimens (about 12 mm long and 2 mm wide) and differs slightly in morphology. Nevertheless, it shows most of the important characters of *E. globocaudatus*, including a distinct post-trunk region, several long spine-like scalids around the introvert, a straight intestine from mouth to terminal anus and a pouch-like structure at the hindgut. The distinct, smooth neck-region is typical of priapulid juveniles (Land 1970; Adrianov & Malakhov 1996), and the absence of cuticular ornamentation on the neck (see Stage IV description and remarks) appears to be an early developmental feature. Approximately fifteen long spine-like scalids are clearly evident on the introvert, but more may have been present, as the preservation is two-dimensional. These scalids are arranged in a circle, with their bases aligned at a position lower than the anterior boundary of the neck, and with a narrower diameter (figure 6*d*). This suggests that the introvert has inverted somewhat into the neck, causing the scalids to point anteriorly. The pharynx can be seen running through the middle of the introvert scalids, with a rough surface indicating the presence of pharyngeal teeth. A lorica larval stage remains to be identified for *E. globocaudatus*.

(ii) *Mode of locomotion and feeding*

The size and shape of the introvert and the post-trunk region of the animal varies between specimens, and this may indicate the mode of locomotion.

(a) *Invagination of the introvert*

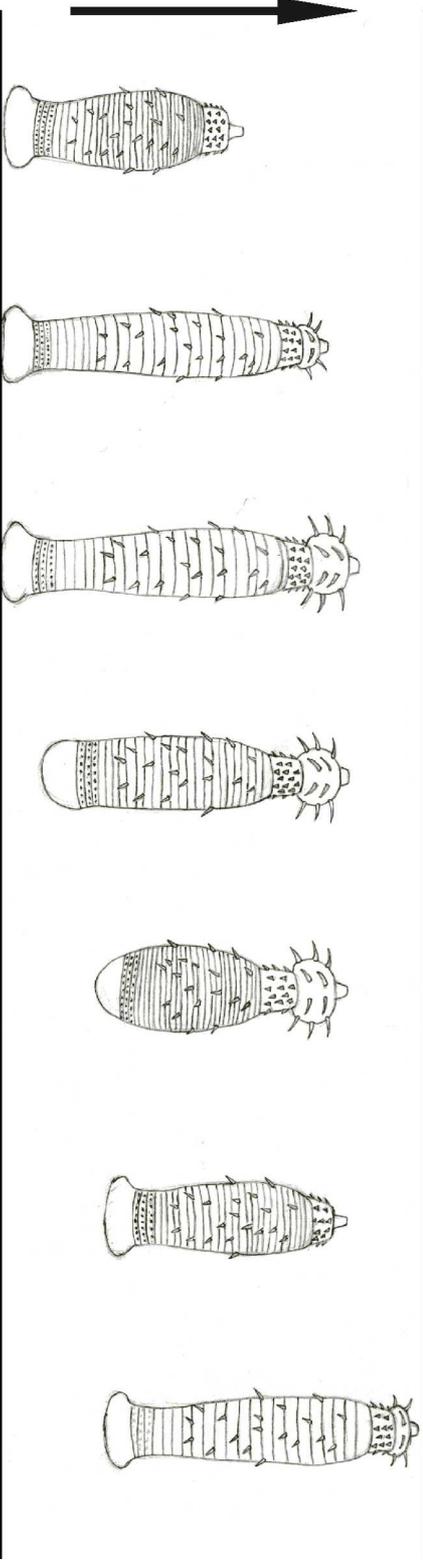
Four stages of movement of the introvert are recorded in *E. globocaudatus* (figure 5*a-h*). 1) In YKLP 11264, the introvert is completely visible, with an oblate shape, the

surface covered by long spine-like scalids that point posteriorly. The angle between the longitudinal axis of the body and the long spines ranges from 110° to 170° away from the anterior end (figures 1*c,d*; 2*c,d*; 4*b*; 5*a,b*), indicating a fully everted introvert with the retractor muscles relaxed. 2) In YKLP 11268, the introvert is still visible but has a short cylindrical shape with the long spines pointing outwards at an angle of 90-130° to the body axis (figures 1*j*; 5*c,d*), indicating that the introvert is partly retracted into the body, presumably through contraction of the retractor muscles. 3) YKLP 11265 displays a further stage of retraction, with the introvert being almost invisible, but some long spines are apparent at the base of the collar at an angle of 50-80° to the body axis (figures 1*e*; 2*e*; 5*e,f*). 4) In YKLP 11263, the introvert is totally invisible, and long scalids are not seen (figures 1*a,b*; 2*a,b*; 4*a*; 5*g,h*); complete invagination has presumably occurred.

(b) *Contraction of the post-trunk region*

The post-trunk region is much wider than the posterior trunk in some specimens (figure 1*c,d*), whereas it is more slender in others (figure 1*j-l*), indicating that the post-trunk region is contractible. There is no fossil evidence as to whether contraction of the post-trunk region was controlled by retractor muscles, or hydroskeleton, or a combination of both. However, by comparison to the operation of the posterior trunk musculature in *Priapulius caudatus* (Hammond 1970), what may happen in *E. globocaudatus* is that the wider post-trunk region represents the situation when the posterior retractor muscles are contracted, changing its morphology from slender to oblate. At the same time, the muscular septum contracts and opens the aperture between the body cavity and the lumen of the post-trunk region, allowing fluid to pass from the body cavity to the post-trunk region; the increased fluid pressure helps to maintain the oblate shape of the post-trunk region.

Figure 8. Model of the interpreted locomotion of *Eximipriapulus globocaudatus*. Arrow indicates the direction of animal movement. I, animal body in the most compact position: the introvert fully invaginated, the trunk contracted, and the post-trunk region expanded and anchoring the body in the sediment. II, the trunk begins to elongate and the introvert begins to evert. III, the trunk reaches its maximum extension and the introvert is fully evaginated; these movements drive the animal forward into the sediment. IV, the introvert becomes anchored in the sediment, the post-trunk region contracts to trunk width, and the trunk shortens and moves forward. V, the trunk reaches its maximum contraction and the post-trunk region is pulled forward together with the trunk. I, the introvert is invaginated into the body and the post-trunk region expanded to serve as an anchor; completion of cycle.



I

II

III

IV

V

I

II

Table 2. Coding of *Eximipriapulus* according to the scheme of Donoghue *et al.* (2006) including both multistate and absence/presence characters. We are able to determine 48 out of the 88 characters; those not determined (coded as “?”) are not listed.

<i>State</i>	<i>Character definitions</i>
1	1. Introvert invaginable: absent (0), present (1)
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)
1	3. Zone I: unarmed (0), armed (1)
1	4. Arrangement of zone I armature into discrete parallel longitudinal rows: absent (0), present (1)
1	6. Morphology of zone I armature: papillae (0), simple spines (1), hooks or spinose hooks (2), conical scalids (3), telescopic scalids (4), curved scalids and dentoscalids (5), complex scalids (6), glandular scalids, trifid spines, sensory spines, and double, tentaculite scalids (7), scalids with pectinate hood (8), spinoscalids and clavoscalids (kinorhynch and loriferans) (9)
2	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).
0	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)
0	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 (kinorhynch): absent (0), present (1)
0	10. Zone II: unarmed (0), armed (1)
1	12. Zone III: unarmed (0), armed (1)
3	13. Number of circlets of zone III armature: one to four (1), six to eight (2), 16 or more (3)
1	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)
1	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)
1	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)
0	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)
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)
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)
1	23. Surface of trunk cuticle: smooth and unannulated (0), annulated (1)
2	24. Number of trunk annuli: 7–11 (0), 30–50 (1), 90–120 (2), 160 or more (3)
1	25. Trunk spines, fine spines, or setae: absent (0), present (1)
1	26. Trunk papillae: absent (0), present (1)
0	27. Trunk sclerites: absent (0), present (1)
0	28. Trunk tumuli: absent (0), present (1)
0	30. Flosculi, N-flosculi or sensory spots: absent (0), present (1)
0	31. Posterior hooks: absent (0), present (1)
1	32. Posterior ring papillae: absent (0), present (1)
1	33. Eversible bursa: absent (0), present (1)
0	34. Position of the anus: anus terminal, whether within a bursa or otherwise (0), anus in posterolateral or posteroventral surface of the abdomen (1)
0	35. Posterior tubuli or setae: absent (0), present (1)
0	36. Caudal appendage(s): absent (0), present (1)
0	39. Polythyridium: absent (0), present (1)
1	45. Scalids (nonspecific and sensu lato, including both scalids and presumed scald derivatives): absent (0), present (1)
1	47. Terminal mouth: absent (0), present (1)
1	48. Mouth cone: absent (0), present (1)
0	49. Noninversible mouth cone: absent (0), present (1)
1	50. Division of the body into a distinct proboscis and abdomen in juvenile/larva: absent (0), present (1)
1	51. Division of the body into a distinct proboscis and abdomen in adult: absent (0), present (1)
1	52. Introvert: absent (0), present (1)
1	55. Circumoral structures: absent (0), present (1)
1	57. Ventral nerve cord unpaired throughout its length: absent (0), present (1)
0	58. Ventral nerve cords merge caudally: absent (0), present (1)
0	71. High aspect ratio of body length to width in adult: absent (0), present (1)
1	78. Voluminous primary body cavity: absent (0), present (1)
1	79. Movement by peristaltic movement of the pharynx/introvert: absent (0), present (1)
0	82. Introvert 30–50% of body length: absent (0), present (1)
0	86. Paired, lateral, locomotory appendages: absent (0), present (1)
0	87. Anterior branched frontal appendages: absent (0), present (1)
0	88. Annulation type: homonomous (0), heteronomous (1)

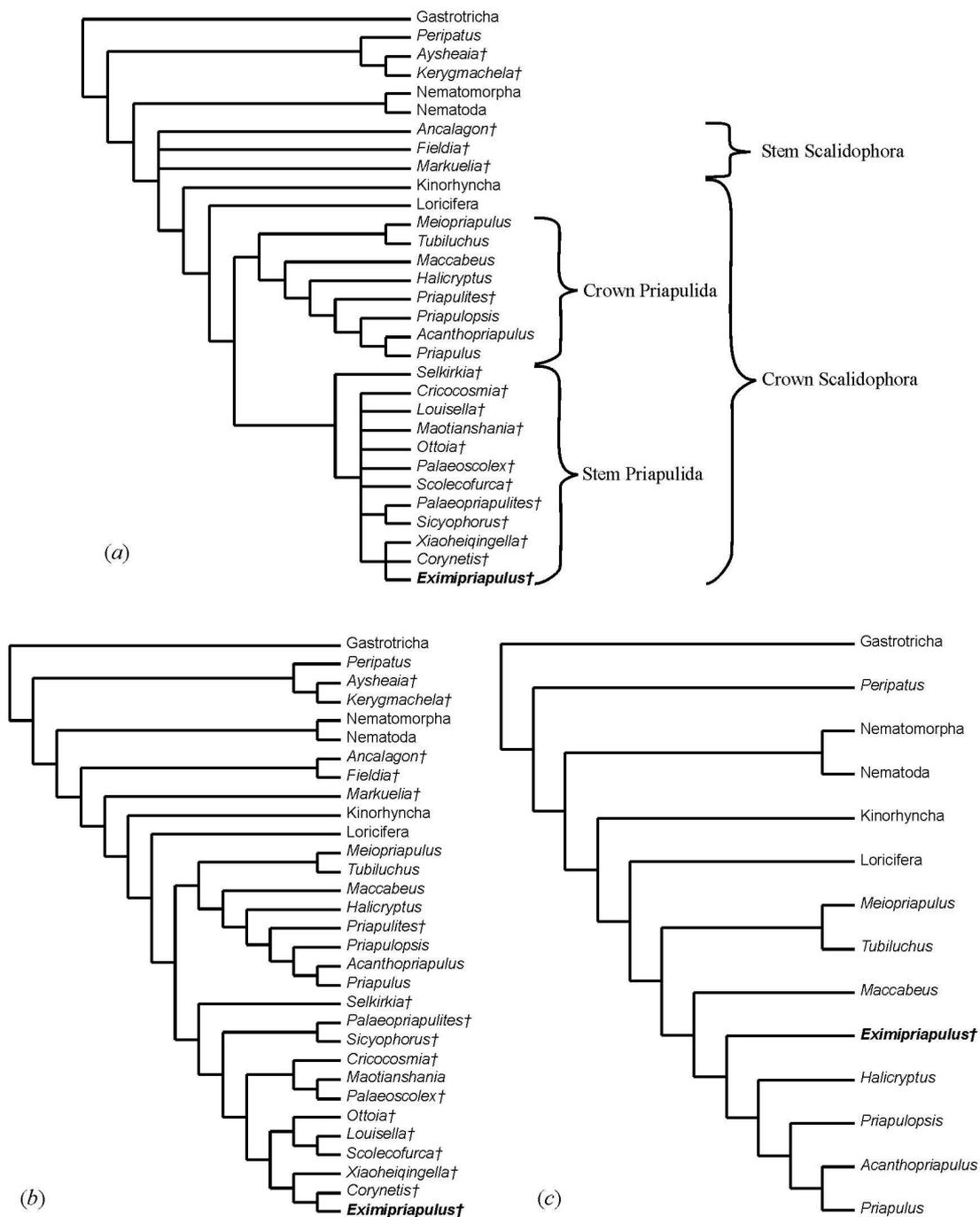


Figure 9. Phylogenetic trees resulting from analyses of the dataset of Donoghue *et al.* (2006) with *Eximipriapulust* added (see Table 2). † represents fossil taxa. (a) The strict consensus of 32 most parsimonious trees derived from the unweighted dataset. (b) The most parsimonious tree resolved after successive reweighting of the dataset. (c) The most parsimonious tree yielded by successive reweighting after excluding all fossil taxa except *Eximipriapulust* from the initial dataset.

All the evidence above suggests that *E. globocaudatus* was an active burrower (figure 8), perhaps using a double-anchor strategy as in *P. caudatus* (Hammond 1970; Ruppert & Barnes 1996): first, the post-trunk region anchored the posterior part of the body in the sediment, then the anterior part of the body extended forwards by a combination of body wall peristalsis and eversion/retraction movements of the introvert. When the body reached its maximum extension, the introvert with its long spine-like scalids served to anchor the anterior part of the body into the sediment, followed by contraction of the post-trunk region, which was pulled forward by the trunk through contraction of the longitudinal body wall muscles. A model of this interpretation of the locomotion of *E. globocaudatus* is illustrated in figure 8.

The bodies of most specimens are curved to some degree to one side of the body, which could indicate burrowing activity. In a study of extant priapulids, Hammond (1970) hypothesized that the trunk moves forward and becomes curved, imparting a lateral force on the posterior end, thereby increasing the efficiency of the anchor. The curvature of the animal may also aid turning in the sediment. A possible alternative interpretation for the curved appearance of the specimens is that more muscles insert on the ventral surface. This is shown in preserved extant priapulids which are often flexed ventrally due to contraction of these muscles (Morse 1981).

In most specimens, parts of the alimentary canal are preserved three-dimensionally, especially around the mid-gut area (figures 1*a-d,k,l*; 2*a-d,h,i*). Other parts of the intestine are completely flat and preserved in a dark colour (figures 1*a,b,e*; 2*a,b,e*). Chengjiang fossils with three-dimensionally preserved guts have been interpreted by some authors as sediment ingesting deposit feeders (Bergström *et al.* 2007; but see Butterfield 2002, for an alternative interpretation), while those with flat, dark coloured guts have been considered to be carnivores (Hou and Bergström 1997). The SEM EDX

element mapping results also show high carbon concentration in the gut area, indicating that it is rich in organic matter. The combined features in *E. globocaudatus* may indicate that it was both a deposit-feeder and a carnivore, consuming organic material from the sediment during burrowing, but at other times preying on small animals using the everted pharyngeal teeth. A similar duality has been suggested for other early Cambrian priapulids (Huang *et al.* 2004a).

(iii) *Systematic position and evolutionary significance*

(a) *Phylogenetic analysis*

The first comparative study covering fossil and extant priapulids with an accompanying cladistic analysis was done by Wills (1998). Later, Dong *et al.* (2004, 2005) and Donoghue *et al.* (2006) presented a more comprehensive and rigorous phylogenetic synthesis, by redefining outgroup and ingroup taxa, adding new fossil taxa and including more characters, following the work of Lemburg (1999). The coding scheme and the phylogenetic conclusions of this work have not been accepted uncritically, as omitting one fossil taxon (such as *Ottoia*) can have a great impact on the topology of the tree (Cobbett *et al.* 2007). However, the dataset of Donoghue *et al.* (2006) provides the only available up-to-date cladistic framework within which *Eximipriapulidus* can be analysed; hence the work of these authors has been followed herein, with the objective of determining the phylogenetic position of *Eximipriapulidus*, not as a means of resolving the interrelationships of extant ecdysozoans/Scalidophorans.

Except for adding *Eximipriapulidus* into the original data matrix (Table 2), there are a few other modifications: 1) the removal of *Yunnanpriapulidus*, as it was suggested by Han *et al.* (2004) to be synonymous with *Xiaoheiqingella* and their coding in the data matrix is almost identical; 2) the revision of the coding for character 26 in

Xiaoheiqingella, from “?” to “1”, by combining additional information from *Yunnanpriapulid*. With the modified data matrix, two phylogenetic analyses were undertaken.

First analysis: including all fossil taxa

A heuristic analysis was run using the original procedure (Donoghue *et al.* 2006), which yielded 32 equally most parsimonious trees (MPTs) of length 201 steps (CI=0.5572; RI=0.7201, RC=0.4013). The strict consensus of these MPTs is shown in figure 9a. Most Cambrian taxa (except *Ancalagon*, *Fieldia* and *Markuelia* which resolve as basal to scalidophorans) and extant priapulids respectively form two distinct clades that are positioned as sister groups to each other. Although the relative relationships of Cambrian stem priapulids are unresolved, *Eximipriapulid* clearly falls into the Cambrian stem priapulid group and forms a subclade with *Xiaoheiqingella* and *Corynetis*.

Successive rounds of *a posteriori* reweighting yield a single most parsimonious tree (figure 9b; 86.99650 steps; CI=0.7875; RI=0.8757; RC=0.6896). In this tree, all clades are resolved and the general topology of the tree is consistent with Figure Supp3 in Donoghue *et al.* (2006). However, there are some changes in the relative positions of some Cambrian priapulids: 1) *Cricocosmia* separates out from *Ottoia* + *Louisella* + *Scolecifurca* and resolves as a sister taxon of *Maotianshan* + *Palaeoscolex*. The *Cricocosmia* + *Maotianshan* + *Palaeoscolex* clade is supported by the presence of posterior hooks (character 31, state 1; this character is also shared by Nematomorpha, *Markuelia*, *Meiopriapulid* and *Maccabeus*). 2) The Chengjiang taxa *Xiaoheiqingella* + *Corynetis* + *Eximipriapulid* form a monophyletic clade that is positioned as a sister group to the Burgess Shale taxa *Ottoia* + *Louisella* + *Scolecifurca*. The two clades are

grouped together by the presence of an eversible bursa (character 33, state 1). 3) *Eximipriapul* resolves as a sister taxon of *Corynetis*, and the grouping is supported by the presence of trunk spines or setae (character 25, state 1; this character is also shared by Loricifera, *Meiopriapul*, *Tubiluchus*, *Halicryptus* and *Louisella*); *Xiaoheiqingella* + *Corynetis* + *Eximipriapul* is supported by the presence of posterior ring papillae (character 32, state 1; this character is also shared by *Halicryptus*, *Priapulopsis*, *Acanthopriapul* and *Priapul*).

It is interesting to note that the groupings of Cambrian stem priapulids presented in the cladogram (figure 9b) match the four different body-form types suggested by Maas *et al.* (2007): 1) *Selkirkia* is positioned at the most basal branch of the Cambrian stem priapulid clade, and it has a distinct tubiform trunk which suggests a possible semi-sessile life habit (Conway Morris 1977b; Hou *et al.* 1999, 2004a); 2) *Palaeopriapulites* + *Sicyophorus* (= *Protopriapulites*) are positioned one node up from *Selkirkia*, and both possess a dumb-bell body shape with a distinct loricate trunk region in the adults (loricate trunks also occur in some extant priapulid larvae); 3) *Cricocosmia* + *Maotianshan* + *Palaeoscolex* have a long, worm-shaped form, which has historically led to their assignment to Phylum Nematomorpha (Hou *et al.* 1999, 2004a); 4) *Ottoia* + *Louisella* + *Scolecifurca* + *Xiaoheiqingella* + *Corynetis* + *Eximipriapul* are all suggested to have a sac-like body shape, and extant priapulids also share the same/similar body design (Maas *et al.* 2007).

Second analysis: excluding all fossil taxa except *Eximipriapul*

Many Cambrian priapulids lack detailed morphological information and a number of systematic positions are hotly debated. These questions result in a relatively large number of undetermined characters in Cambrian taxa. The “missing” coding may

contribute to their basal placement within the clade relative to extant priapulids for which more characters are determined. Therefore, a further analysis that excludes all fossil taxa except *Eximipriapulius* was undertaken, to investigate the relationship between *Eximipriapulius* and extant priapulids.

The analysis generated three equally most parsimonious trees that differ in the relative relationships of *Eximipriapulius*, *Maccabeus* and *Halicryptus* (134 steps; CI=0.7463; RI=0.7834; RC=0.5847). Successive rounds of *a posteriori* reweighting yield one most parsimonious tree (figure 9c; CI=0.9082; RI=0.9283; RC=0.8431). Contrary to expectations from the previous analysis that *Eximipriapulius* should be located basal to crown priapulids, it is positioned within the crown group and appears to be more derived than *Meiopriapulius* + *Tubiluchus* and *Maccabeus*, but less derived than *Halicryptus*, *Priapulopsis* and *Acanthopriapulius* + *Priapulius*. The grouping of *Eximipriapulius* + *Halicryptus* + *Priapulopsis* + *Acanthopriapulius* + *Priapulius* is supported by two synapomorphies: the presence of 16 or more circles of zone III armature (character 13, state 1) and the presence of posterior ring papillae (character 32, state 1).

The phylogenetic position of *Eximipriapulius* is very different in the two cladistic analyses undertaken. Although it is still debated whether data from fossils should be included in parsimony analysis, a recent comprehensive study on this subject strongly recommended the inclusion of fossils (Cobbett *et al.* 2007). On this basis, the result generated from the first cladistic analysis is favoured here; thus *Eximipriapulius* is considered as a stem priapulid and it appears as one of the most derived Cambrian taxa. Both of the cladograms (figure 9b,c) reveal interesting relationships between *Eximipriapulius* and other fossil and extant taxa, and further morphological comparisons

are discussed below.

(b) *Affinities with fossil priapulids*

E. globocaudatus is distinct from other Cambrian priapulids, especially in its unique expanded post-trunk region. According to the cladistic analysis (figure 9b), *Eximipriapululus* shows close affinities with another Chengjiang taxon *Corynetis brevis* (= *Anningvermis multispinosus*). Both taxa have a cylindrical, sac-like body, an unarmed collar (Zone II), an annulated trunk covered by spines or setae with posterior ring papillae and a posterior end with an eversible bursa but lacking in caudal appendages/hooks. These two genera are easily distinguished from each other as *E. globocaudatus* has a distinct anterior introvert but *C. brevis* does not; *C. brevis* has a completely eversible pharynx, but the pharynx in *E. globocaudatus* is only partially eversible; the base of the collar of *C. brevis* is crowned with a circle of long spines, which are absent in *E. globocaudatus*; and *E. globocaudatus* has a distinct post-trunk region, but *C. brevis* does not.

In general morphology *E. globocaudatus* has a greater similarity to *Ottoia prolifica* (Conway Morris 1977b) than to *C. brevis*. Both *E. globocaudatus* and *O. prolifica* have a stout body shape, a bulbous introvert covered by spinose scalids, a posterior end with an eversible bursa, a spacious body cavity and a mid-gut lined with mesentery. *E. globocaudatus* differs from *O. prolifica* in having only a partially eversible pharynx, a neck region covered by triangular trunk scalids, setae or papillae on the trunk surface and an expanded post-trunk region. Furthermore, *O. prolifica* has a circle of posterior hooks at the end of the trunk, but these are absent in *E. globocaudatus*.

(c) *Affinities with extant priapulids*

Similarities with *Meiopriapulus* and *Tubiluchus*:

In the cladistic analyses *Meiopriapulus* and *Tubiluchus* are grouped together as sister taxa and positioned at the most basal branch of the priapulid crown group (figure 9a-c). The synapomorphic characters of these two meiobenthic genera are body length up to 2 mm; cone-like protrusible pharynx; pectinate pharyngeal teeth; trunk tumuli and trunk tubuli; and a foregut with polythyridium.

It is the anterior morphology of *Eximipriapulus* that most resembles *Meiopriapulus* and *Tubiluchus*. All three genera have a bulbous introvert. *Eximipriapulus* also seems to have a cone-like protrusible pharynx, as in *Tubiluchus* and *Meiopriapulus*. Both *Eximipriapulus* and *Tubiluchus* have a distinct cone-shaped collar and the introverts of *Eximipriapulus* and *Tubiluchus* bear a single series of conical furry scalids. Furthermore, immediately posterior to the introvert *Tubiluchus* possesses a well developed neck region carrying papillae. *Meiopriapulus* has an anterior trunk part bearing triangular scalids, which is also inferred to be the neck (pp. 229, Adrianov & Malakhov 1996), and *Eximipriapulus* also has a comparable anterior region Stage IV covered by triangular scalids. The post-introvert regions in these three genera may, therefore, be homologous.

Eximipriapulus also shares a few other characters with *Tubiluchus* and *Meiopriapulus*: 1) the triangular seta-like prolongations on the trunk surface of *Eximipriapulus* possibly are tubuli/setae as in *Tubiluchus* and *Meiopriapulus*; 2) the densely packed tubuli and setae on the male ventral surface in *Tubiluchus* may be reflected in the longitudinal strip of triangular setae on the trunk surface of *Eximipriapulus* specimen YKLP 11268 (figure 1j); 3) both *Eximipriapulus* and *Meiopriapulus* lack a tail; 4) *Eximipriapulus* possesses a preanal post-trunk region and

Meiopriapulus has a preanal trunk subregion.

Overall, the results of the cladistic analyses and the similarities among *Eximipriapulus*, *Tubiluchus* and *Meiopriapulus* indicate that *Tubiluchus* and *Meiopriapulus* could derive directly from a primitive *Eximipriapulus*-like Cambrian ancestor. It is likely that the ancestor of *Tubiluchus* and *Meiopriapulus* was macrobenthic and their micro-body size today may be an evolutionary result of interspecific competition and adaptation to certain ecological niches (both these two taxa live in coarse sand, while other extant priapulids are often found in muddy sediments).

Similarities with *Priapulus*:

In the cladistic analysis, *Priapulus* resolves as the most derived extant priapulid (figure 9a-c). There are a few characters shared between *Eximipriapulus* and *Priapulus*: 1) macrobenthic taxa; 2) spacious body cavity; 3) posterior trunk with ring papillae; 4) the contractible/expandable posterior body part serving as an anchor during burrowing activity. As mentioned above, the contraction of the post-trunk region in *Eximipriapulus* may be controlled by a similar posterior musculature to that of the posterior trunk of *Priapulus*, indicating possible homologies. However, this shared character also could be a result of convergent evolution.

Chapter 4

The eyes of Lower Cambrian lobopodians and their evolutionary significance for arthropod visual systems

Abstract

Evidence of fossil visual systems is crucial for understanding the origin and evolution of eyes. This study investigated the rare visual organs of Cambrian lobopodians from the Chengjiang Lagerstätte, Kunming, China, using a new technique, Infinite Focus Microscopy, which can reproduce 3D topographic information. The eyes of *Hallucigenia fortis* and *Cardiodictyon catenulum* are reported for the first time, and the morphological details of the eye of *L. longicruris* are revised. The eyes of *H. fortis* and *L. longicruris* are composed of three visual units, each of which has a pigment cup and a ‘lens’. The eye of *C. catenulum* only has a single visual unit with a deeply sunk ‘lens’. Comparison between Cambrian lobopodians suggests that their visual systems may have evolved from an eyeless condition to a single-unit eye, tri-unit eye and finally a stalked eye. Comparison with panarthropods indicates that the eyes of Cambrian lobopodians are not similar to the eye of tardigrades and onychophorans, but resemble arthropod lateral visual organs, especially myriapod eyes. The eyes of Cambrian lobopodians appear to represent the ancestral visual systems of arthropods and gave rise to the two major types of arthropod lateral eyes. By calculating the focal distance, the eyes of *L. longicruris* and *H. fortis* appear capable of forming an image; the eye of *C. catenulum* was probably unable to do so.

Keywords: Cambrian lobopodian eye; *Luolishania longicruris*; *Hallucigenia fortis*; *Cardiodictyon catenulum*; ancestral visual systems of arthropods.

1. Introduction

The evolution of the eye has been a matter of much debate ever since the publication of Darwin's *Origin of Species* (1859). Critics of evolution often attack the theory on the grounds that seemingly perfect biological organs, such as the eye, could not have arisen by natural selection. Darwin devoted an entire chapter to the problem and postulated a simple and imperfect prototype eye, from which the more perfect visual organs might have arisen gradually, by variation and by natural selection. The prototype eye can be reconstructed by a structural and molecular comparison with extant eyes such as the simple pigment-cup eyes found in many invertebrate groups, the arthropod compound eye and the camera eyes of cephalopods and vertebrates.

Despite the fact that debate about the evolution of the eye has occurred for over 150 years, a definition for a "real eye" was only coined in the last decade by Land and Nilsson (2002): "an organ for spatial vision, in which an eye should have at least two light sensitive cells shielded so that they do not pick up light from exactly the same direction". Although all eyes provide similar information for vision and for other purposes such as navigation and temporal awareness, they occur in a great variety of shapes, sizes, optical designs and locations on the body. This diversity has led evolutionary biologists to question whether eyes are monophyletic or polyphyletic. Based on comprehensive studies of the anatomical structures of various types of eye and photoreceptor cell, Salvini-Plawen and Mayr (1977) concluded that eyes have independently evolved 40-65 times. However, with the discovery of a single, well

conserved 'master' gene, *Pax6*, that can initiate eye construction in diverse species, Gehring and Ikeo (1999) proposed that eyes must have arisen from a single ancestor. This debate is still ongoing due to a lack of direct evidence, particularly evidence from the fossil record.

Most records of fossil invertebrate eyes are based on arthropods. Whether arthropod optic organs are homologous plays an important role in the discussion of arthropod phylogeny (Paulus 1979, 2000; Bitsch and Bitsch, 2005). A long-debated question is whether the photoreceptors of the various Euarthropoda evolved only once in the ancestral group or from several independent sources. Fossil evidence is vital to address this question. The paired compound eyes of trilobites are the best researched of all fossil visual systems. They commonly occur as a well-preserved lens-bearing surface, but the sublensar structures remain unknown (Clarkson *et al.*, 2006). The earliest fossil records of eyes in non-trilobite arthropods are from the Lower Cambrian Chengjiang biota (circa 525 mya) of China and the Middle Cambrian Burgess Shale (circa 515 mya) of British Columbia. Within these two Cambrian lagerstätten, eyes were already highly developed in arthropods as well as in other advanced animals and appear in a great diversity of morphologies and positions: stalked/un-stalked; dorsal/frontal; large/small; and as a pair or even more in number (e.g. *Opabinia*; Budd, 1996). However, due to the limits of preservation as well as the lack of detailed investigation, almost nothing is known about the ultrastructure of these Cambrian visual systems.

The visual organs of Cambrian arthropods were already highly derived and divergent, so they still cannot provide the ultimate clue as to the origin of arthropod eyes. To answer this question, we would have to investigate the visual systems that had developed prior to arthropod eyes. Lobopodian animals (including extant onychophorans and tardigrades and extinct fossil lobopodians) are ideal candidates for

this purpose, as they are considered to have a close but antecedent phylogenetic association with arthropods (Budd and Telford, 2009). Some detailed investigation has been undertaken on the visual systems of the extant onychophorans (Balfour, 1883; Dakin, 1921; Eakin and Westfall, 1965; Mayer, 2006; Strausfeld, 2006a, b) and tardigrades (Kristensen, 1982; Dewel *et al.*, 1993; Greven, 2007). However, knowledge about fossil lobopodian eyes was lacking until a pair of eyes was reported from the Cambrian *Miraluolishania haikouensis* from the Chengjiang biota (Liu *et al.* 2004). This taxon is now considered to be a junior synonym of *Luolishania longicruris* Hou and Chen, 1989 (Ma *et al.*, in press). Schoenemann *et al.* (2008) further observed the structures of the eye from this Cambrian lobopodian and concluded that it is a *simple eye* and resembles those of onychophorans. However, their research was entirely based on a few dorsoventrally preserved specimens and took no account of the likely taphonomic effects on eye morphology. They were also unable to make detailed analyses of the eye structures (Schoenemann *et al.*, 2008, p. 6), so their observations and conclusions were rather speculative.

In this study, the detailed structure of the eye of *L. longicruris* is redescribed by the application of new techniques. Also for the first time, visual organs are reported from two other Chengjiang lobopodian taxa, *Hallucigenia fortis* and *Cardiodictyon catenulum*. The evolutionary significance of these eyes for the origin and evolution of arthropod visual systems and visual systems in general is discussed.

2. Material and methods

All Chengjiang lobopodian specimens in the collections of the Yunnan Key Laboratory for Palaeobiology, Yunnan University, Kunming, China were examined.

Visual organs are confirmed in 18 specimens, 16 *Luolishania longicruris*, one *Hallucigenia fortis* and one *Cardiodictyon catenulum*. All specimens are from the Anshan section, near Mafang village, Haikou, Kunming. Material described by Liu *et al.* (2004) and Schoenemann *et al.* (2008) is also considered herein on the basis of their published information.

Preparation and camera lucida drawings of specimens were made using a Nikon SMZ-10A Microscope. Scanning Electron Microscopy (SEM), and associated Energy Dispersive X-ray analysis (EDX) was used for micro-image capture and element mapping. An Alicona Infinite Focus Microscope (IFM) was used for surface analysis, 3D measurements and micro-image capture.

3. Preservation

All the eyes are preserved as distinct black structures against the yellowish background of the animal's head. Elemental maps using SEM EDX show that these structures coincide with a very high concentration of carbon compared to the rest of the fossil and the sediment. Calcium was not detected and so they are composed of carbon (Figs. 1C, D, 2C, D and 6E, F). Under the IFM, these dark structures comprise thin films (approximately 3µm thick) of material that shows a cracked appearance (Figs. 1B; 2B). Thus, these structures in lobopodians from the Chengjiang have a composition and texture similar to many fossils from the Burgess Shale (e.g. see Page *et al.* 2008). The carbon film preservation along with the position and morphology of the structures are interpreted to indicate the presence of ommochrome (visual pigments), especially melanin, remains from the original eyes. In living animals ommochrome molecules contain many atoms of carbon, and melanin has been suggested as a very stable

Fig. 1. Eye of the laterally preserved specimen YKLP 11291 *Luolishania longicruris*. (A) Head region. Arrow indicates the position of the eye. (B) IFM image close up of the eye, showing the structure of three visual units indicated by black arrows. White arrow indicates a lens-like structure preserved in a darker tone to the surrounding matrix. (C, D) SEM and EDX analysis of the eye. (C) Backscatter image of the eye. Arrow indicates the margin of the lens-like structure in (B). (D) EDX carbon map, showing the visual units as areas of carbon enrichment. (E, F) IFM digital elevation model (DEM) image of the eye (E) and contoured DEM (F), showing the visual units 1 and 2 (v1 and v2) as elevated structures. A depression can be seen in visual unit 1 (v1) corresponding to the interior of the pigment cup. a, anterior; d, dorsal; p, posterior; v, ventral; v1-3, visual units 1-3.

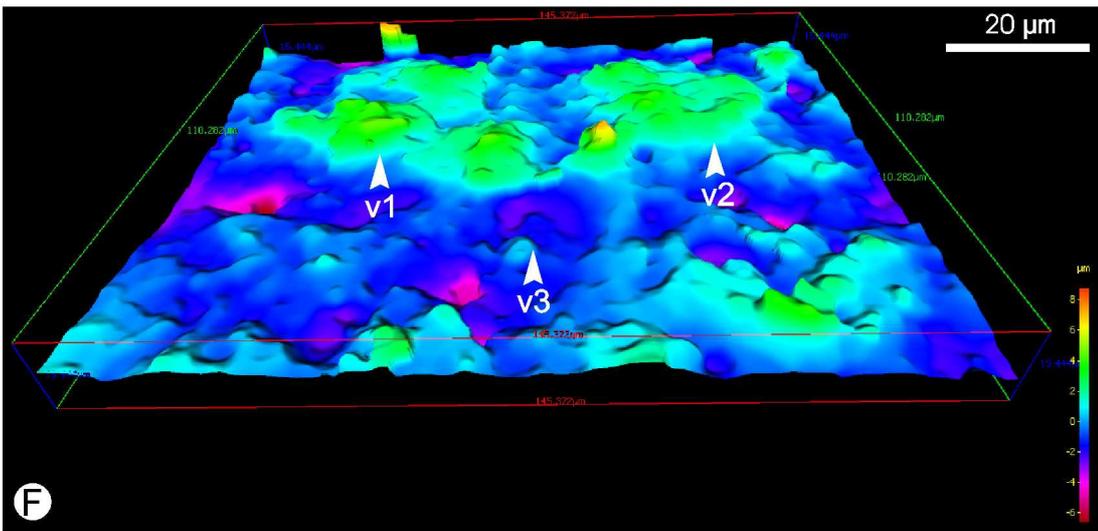
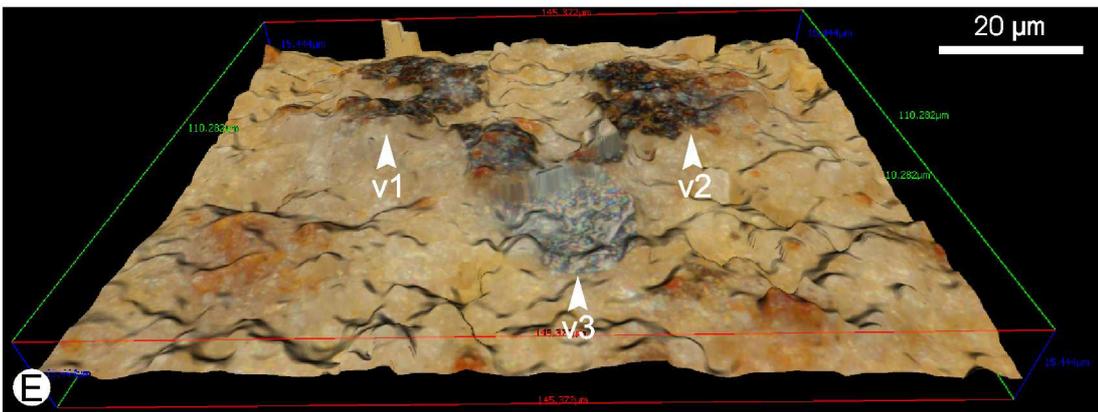
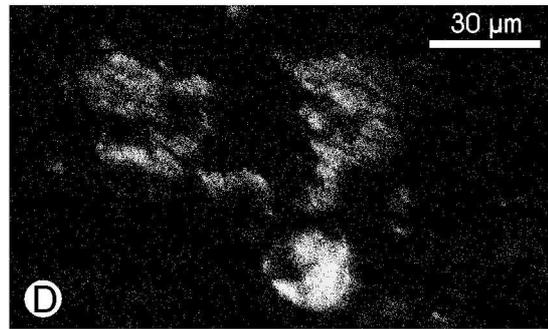
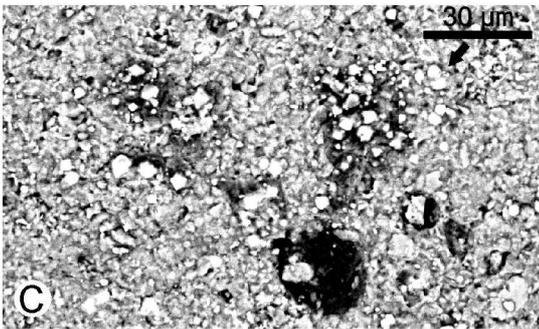
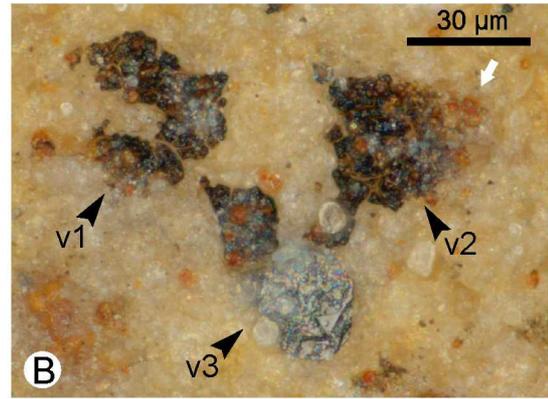
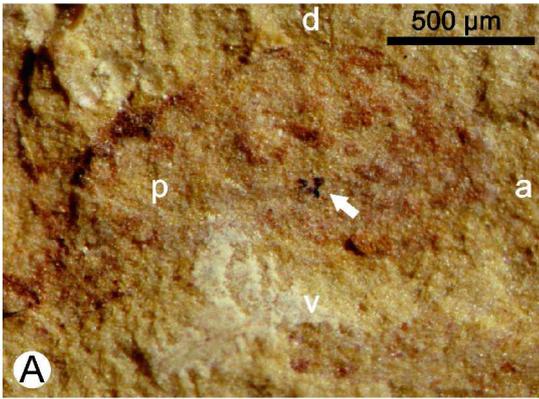
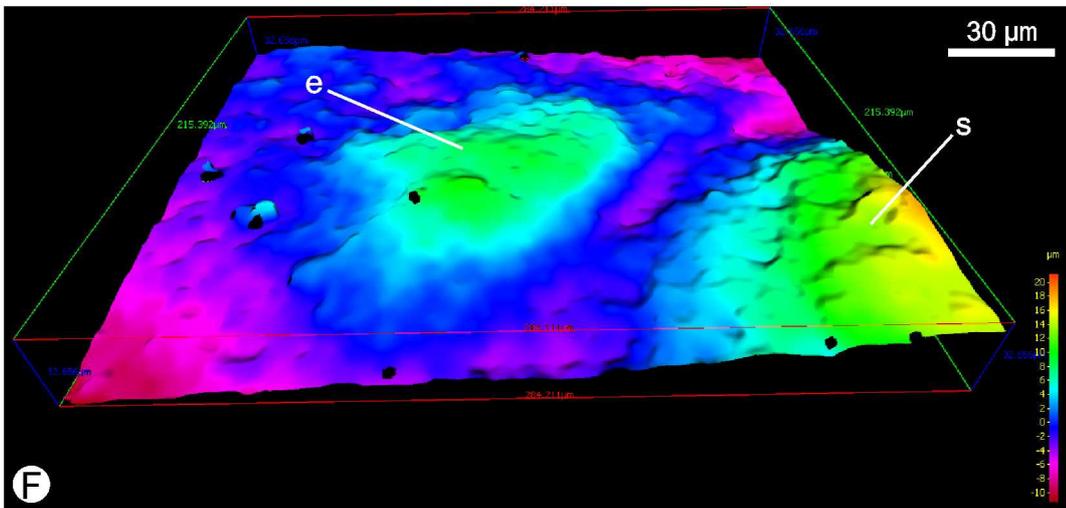
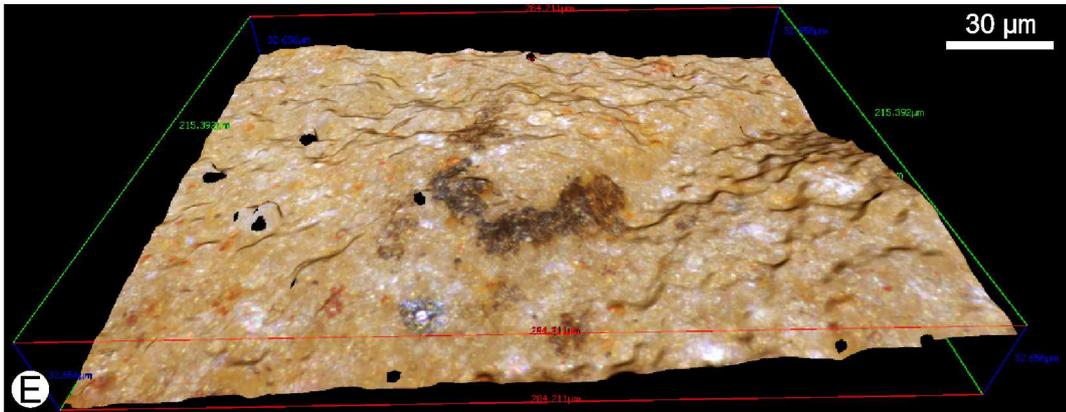
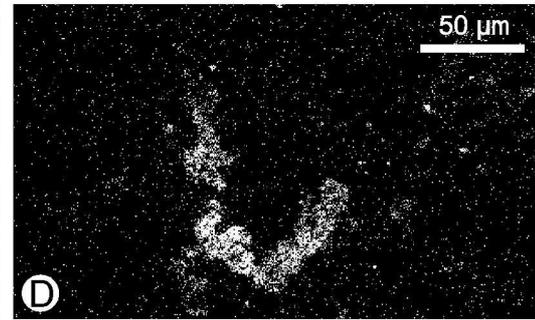
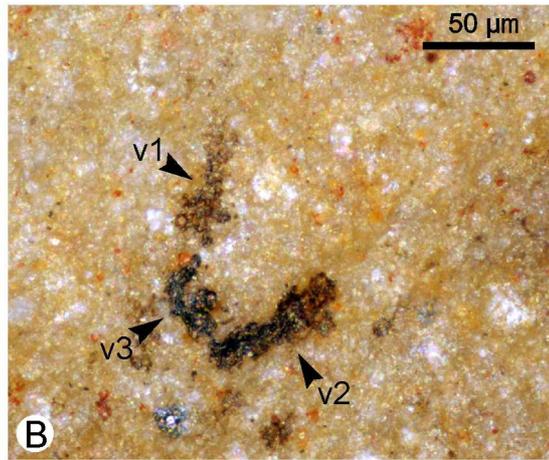
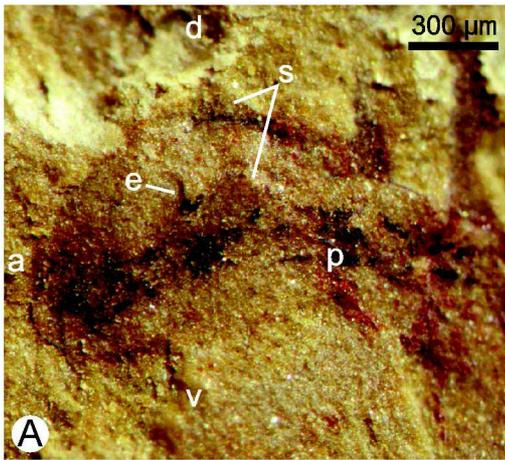


Fig. 2. Eye of the laterally preserved specimen YKLP 11273 *Luolishania longicruris*. (A) Head region, showing the position of the eye. (B) IFM image close up of the eye. Arrows indicate three visual units. (C, D) SEM and EDX analysis of the eye. Backscatter image (C) and EDX carbon map (D), showing the eye as an area of carbon enrichment. (E) IFM DEM of the eye, image data overlaid on the topography. (F) IFM contoured DEM of the same region as (E), showing the eye and a nearby head sclerite as elevated structures. a, anterior; d, dorsal; e, eye; p, posterior; s, sclerite; v, ventral; v1-3, visual units 1-3.



macromolecule which can be preserved during the fossilisation process (Beyermann and Hasenmaier, 1973; Cheun 2004). A similar preservation also occurs in the eyes of Chengjiang arthropods such as *Isoxys auritus* (Fig. 16.15, pp. 117, Hou *et al.*, 2004a) and *Canadaspis laevigata* (Fig. 16.11, pp.113, Hou *et al.*, 2004a), as well as in the visual organs of other groups of fossils at other stratigraphic horizons (e.g., Janvier and Lund, 1983; Lund and Janvier, 1986; von Bitter *et al.*, 2007). Although the eyes appear to lack relief when viewed with a light microscope, IFM imaging reveals that they are actually preserved with some 3D details (see below).

4. The Visual System of Cambrian lobopodians

4.1. The eyes of Luolishania longicuris

Of 42 specimens of *L. longicuris* examined, 16 show distinct black spots at a similar location on the head, indicating the presence of a genuine structure (numbers YKLP11271-11281, 11288, 11289). In 11 dorsoventrally preserved specimens, the black ovoid shaped spots are preserved as a pair (Figs. 3A, D and 4A). The repeated position, regular shape, paired morphology and richness of carbon present indicates that these structures represent the visual organs of *L. longicuris*.

4.1.1 Position of eyes

In all specimens, the eyes lie at a mid point along the longitudinal axis of the head. In dorsoventrally preserved specimens, the eyes often overlap with sclerites on the head (Figs. 3A, D and 4A), but it is clear in the laterally preserved specimens that the eyes are positioned just in front of this structure (Figs. 2A and 5A). However, their dorsal or

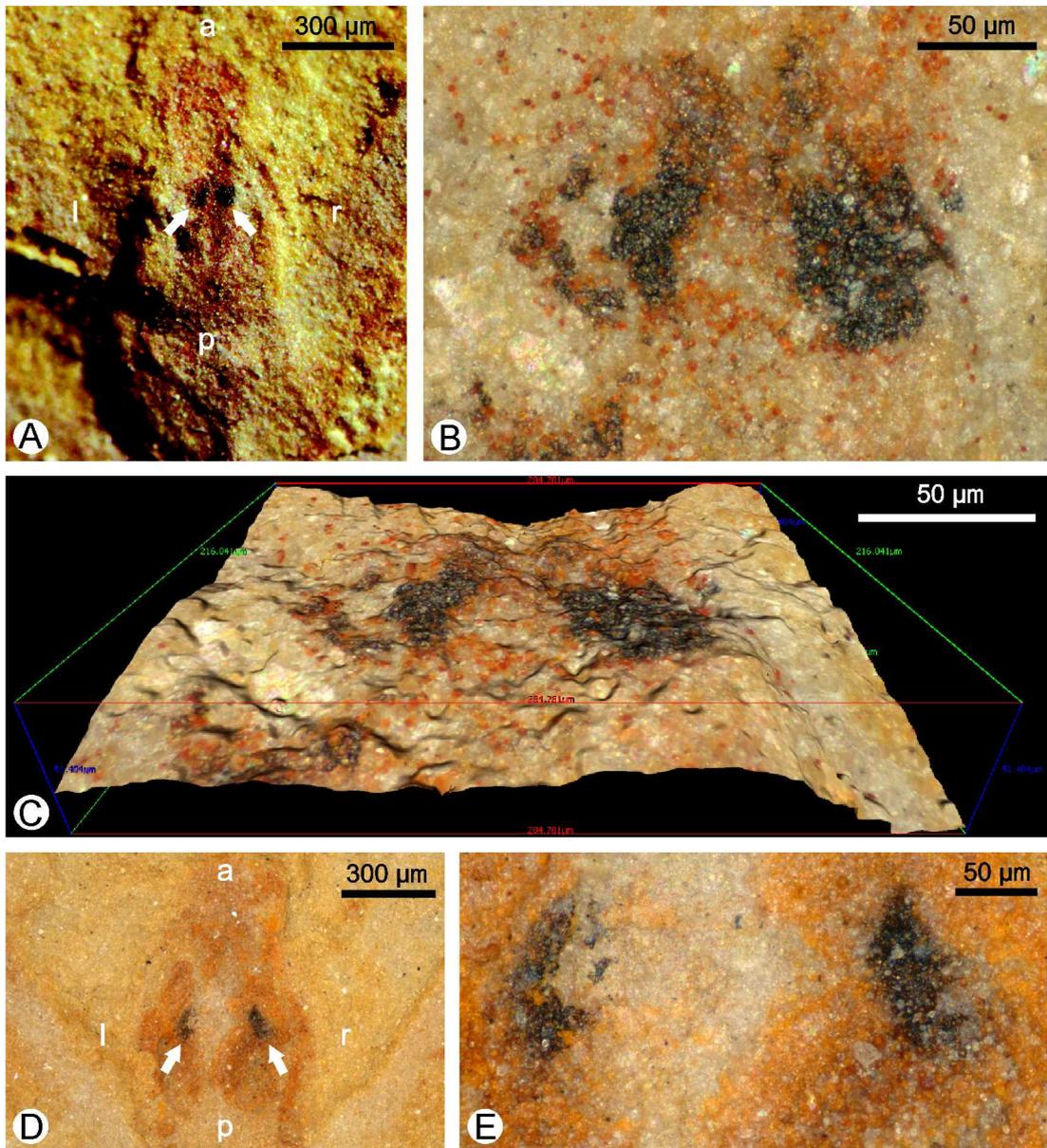


Fig. 3. Eye of dorsoventrally preserved specimens of *Luolishania longicruris*. (A-C) Specimen YKLP 11277. (A) Head region, showing the paired eyes indicated by arrows. (B) IFM image close up, showing the uneven distribution of dark staining around the left eye, while the right eye is compressed into a single dark area. (C) IFM DEM image of the eye. (D, E) Specimen YKLP 11272. (D) Head region, showing the paired eyes indicated by arrows. (E) IFM image close up, showing the uneven distribution of dark staining around the left eye resulting in a cup shape, while the right eye is compressed into a single dark area. a, anterior; l, left; p, posterior; r, right.

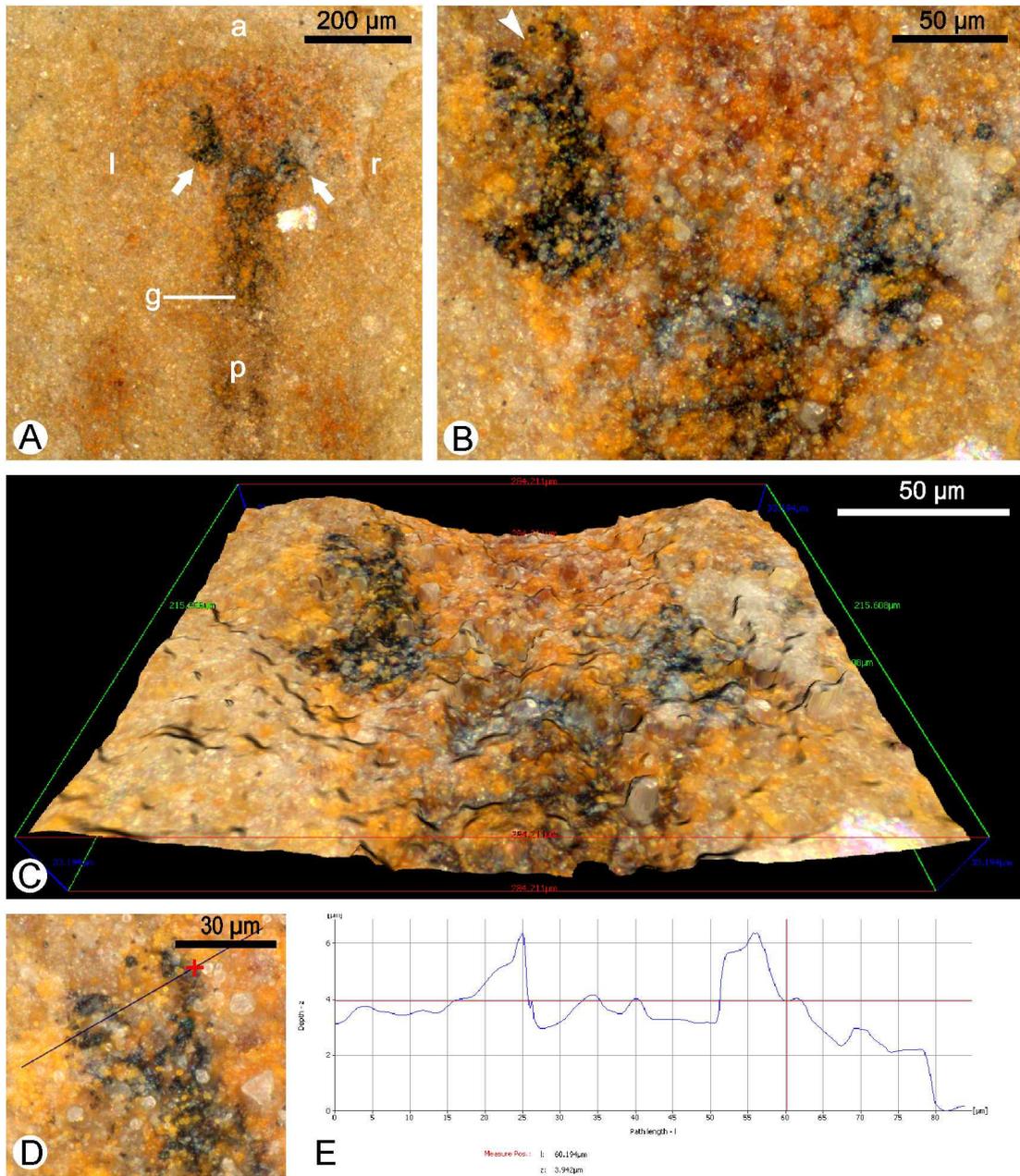


Fig. 4. Eye of the dorsoventrally preserved specimen YKLP 11288 *Luolishania longicruris*. (A) Head region, arrows indicate position of paired eyes. The eyes are preserved very close to the gut trace. (B) IFM image close up of the eyes. The left eye shows a cup-shaped trace, but a real eye cup is found towards its anterior edge indicated by arrow. (C) IFM DEM image of the eyes. (D) IFM image close up of the pigment cup of the left eye indicated in (B). (E) Topographic profile along the line shown on image (D). Red cross in (D) indicates the position of cross hairs on the profile (E). a, anterior; g, gut; l, left; p, posterior; r, right.

lateral position is not very clear. In dorsoventrally preserved specimens, they are preserved as a pair of black spots that can be very close to each other, indicating that they are situated dorsally on the head (Figs. 3A, B). However, in laterally preserved specimens, only one eye is exposed. It is often seen just above the gut trace (Figs. 1A and 2A), which indicates that the eye is situated laterally on the side of the head. The dark stains are only the remaining pigment cups, not the whole eye. Therefore, they may be more representative of the position of the pigment cups inside the head, rather than the external morphology of the eye, which explains the difference in eye position as indicated by dorsoventrally and laterally preserved specimens. Overall, the evidence indicates that the original position of the two eyes was possibly dorsolateral (Fig. 5A).

4.1.2 Structure of the eye

Schoenemann *et al.* (2008) described the eye of *L. longicruris* from four dorsoventrally preserved specimens. Based on SEM and BEM (backscattered electron microscopy) images, they suggested that the black structure of the eye is an outer eye cup and the surface structure immediately in front of it is an inner lens. However, this conclusion is not consistent with the observations herein. In dorsoventrally preserved specimens, the eyes are generally preserved with an oval shaped outline. Specimen YKLP 11277 shows that the length of the oval is ~120 μm and the width is ~75 μm . Some dark stains are often preserved around the edge, leaving either one side or the central part of the eye the colour of the unstained matrix (Figs. 3A, B, D, E). In some cases, the black parts of the eyes resemble a cup shape as Schoenemann *et al.* (2008) indicated (Figs. 3D, E and 4A, B), but when viewed under high magnification the black region of the eye is not well constrained to a definite shape and is not continuous but is composed of a number of elements (Figs. 3B, E and 4B, D). In other cases, these black

elements overlap and are compressed to form a simple black spot (Figs. 3A-E). The unevenly distributed black material indicates that there are more detailed structures inside the margin.

Although only five laterally preserved specimens show eye spots (YKLP 11273, 11285, 11289, 11291, 11292), two of them reveal exquisite details of the eye structure (YKLP 11273 and YKLP 11291). In specimen YKLP 11291, there is a faint round margin in the area of the eye, and within it there are three black spots (Fig. 1A). More detailed information concerning this structure has been revealed using SEM photography, SEM EDX element mapping and IFM analysis (1B-F). EDX analysis indicates that the black-coloured material is a concentration of carbon that produces a clear carbon map which reproduces the shape of the structure (Fig. 1D). Therefore, the three separate round-shaped black spots are interpreted to represent three pigment cups, indicating the existence of three visual units within the eye of *L. longicruris*. These are arranged as a triangle, with two units dorsolateral and one ventromedial (according to the orientation of the animal head). For convenience of description, herein these three visual units are designated *v1* to *v3* (see Fig. 1B).

The black pigment cups of dorsolateral units *v1* and *v2* appear slightly larger with a diameter of ~35 μm ; the smaller pigment cup of ventromedial unit *v3* is ~26 μm in diameter, with a more regular round shape. The size difference could be a genuine feature, but also could be caused by differential preservation. Two pigmented narrow structures respectively connect the dorsolateral visual units (*v1* and *v2*) with the ventromedial one (*v3*), but there is no direct connection between the units *v1* and *v2*. In a high resolution IFM image (Fig. 1B) these structures are preserved in different colours: the two dorsolateral pigment cups and the narrow structures connecting them with the ventromedial cup are preserved as a cracked black carbon film, while the ventromedial

cup is preserved in a slightly lower sediment layer and is a dark silver colour. The reason for this differential colour preservation is not clear, but it may be that carbon from the pigment cup has been preserved in a slightly different condition. IFM images also show that the connecting structure between the visual unit $v1$ and $v3$ is thicker than that between $v2$ and $v3$, which may be a bias caused by imperfect preservation (Fig. 1B).

In the dorsolateral visual unit $v1$, an indentation is present and the pigment cup is preserved in a crescent shape (Fig. 1B). The IFM digital elevation model (DEM; Fig. 1E) and the contoured DEM (Fig. 1F) show that the indentation is $\sim 2.24 \mu\text{m}$ deep. In the dorsolateral visual unit $v2$, a hemispherical structure protrudes from its anterior side and is preserved in a darker tone than the surrounding matrix (Fig. 1B). Its hemispherical margin is also can be seen in the SEM backscatter image (Fig. 1C). The shape, colour and location of this structure indicate that it may be a lens or lens-like structure within the visual unit.

In specimen YKLP 11273, the eye is preserved in oval-shaped relief (Fig. 2A, B, E, F), with a length of $\sim 127 \mu\text{m}$ and a width of $\sim 69 \mu\text{m}$. A 3D projection suggests that the eye relief is $\sim 8 \mu\text{m}$ higher than the surrounding matrix (Fig. 2F). The lower half of the oval is stained in a dark colour (Fig. 2B, C), which is a concentration of carbon according to EDX analysis (Fig. 2D). Using IFM, three visual units can also be recognised within the black colour region, respectively corresponding to the visual unit $v1$ to $v3$ in specimen YKLP 11291 (Fig. 2B). The upper half of the oval-shaped relief area has a smooth, faintly preserved margin marked by a very fine yellowish line (Fig. 2A, C).

In summary, the visual system of *L. longicuris* is interpreted to consist of the following:

- 1) a pair of lateral eyes situated in a dorsolateral position on the head;

- 2) within an eye, there are at least three visual units arranged in a triangle, two dorsolateral and one ventromedial;
- 3) the two dorsolateral visual units seem to be connected to the ventromedial one by pigmented structures;
- 4) each visual unit is composed of a pigment cup plus a lens-like structure;
- 5) the three visual units reside within an ovoid cuticular structure with dome-like relief.

4.1.3 Further taphonomic interpretation

Knowledge of the preserved orientation of the animal can provide better understanding the structures of the visual system. Based solely on dorsoventrally preserved specimens, Schoenemann *et al.* (2008) interpreted the whole black region of the eye as a single pigment cup. Similar preservation can also be found from some dorsoventrally preserved specimens considered here (Figs. 3D, E and 4A, B). However, after discovering three separate visual units from lateral specimens, it is clear that a false impression is caused by the three units being compressed dorsoventrally and coming together. As these units are arranged in a triangle, it seems logical that they appear to form a crescent shape along one side of the round margin of the eye when they are compressed from certain angles. Thereby, in specimen YKLP 11273, the three recognisable visual units connect to each other and form a “v” shape (Fig. 2B). Further evidence is also seen in specimen YKLP 11288. The left eye of this specimen shows a cup-shaped trace, but a real eye cup is found towards its anterior edge (Figs. 4A-D). A topographic profile across this eye cup shows a distinct indentation (Fig. 4E), which is similar to the indentation in visual unit *v1* of specimen YKLP 11291.

In specimen YKLP 11291 (Fig. 1B), the lens-like structure is situated on the anterior side of unit v2, possibly indicating a position in life, pointing slightly towards the anterior. The other unit v1 is preserved laterally as a cup-shaped structure with the opening pointing posteriorly, therefore the 'lens' should presumably also point in that direction. As mentioned above, the ventromedial visual unit is preserved in a lower layer of sediments, with a distinctly more rounded shape and slightly different colour. One possible interpretation is that there was a lens-like structure situated on top of the pigment cup pointing outwards and the two structures were compressed together during preservation. The lens-like structure could be hard enough to leave a depression at this site. The round shape results from the vertical compression of the pigment cup. The combined preservation of the lens-like structure and the pigment cup may also contribute to the distinct colour of this unit.

The indentation within the pigment cups (Fig. 1E, F and 4D, E) may indicate the existence of relatively hard or thick structure that were lost during fossilisation, such as the vitreous body or multilayered rhabdom.

There are only a few traces of the preserved original cuticular structure that covered the entire eye, shown by the oval to round shaped smooth outline and the dome-like relief preserved in specimen YKLP 11273 (Fig. 2A, E, F). Therefore, this structure either could be a very thin part of the head shield (for this structure see Ma *et al.*, in press), or represent a transparent cuticular lens.

Interpretative illustrations of the eye structures of *L. longicuris* are given in Fig. 5A-C.

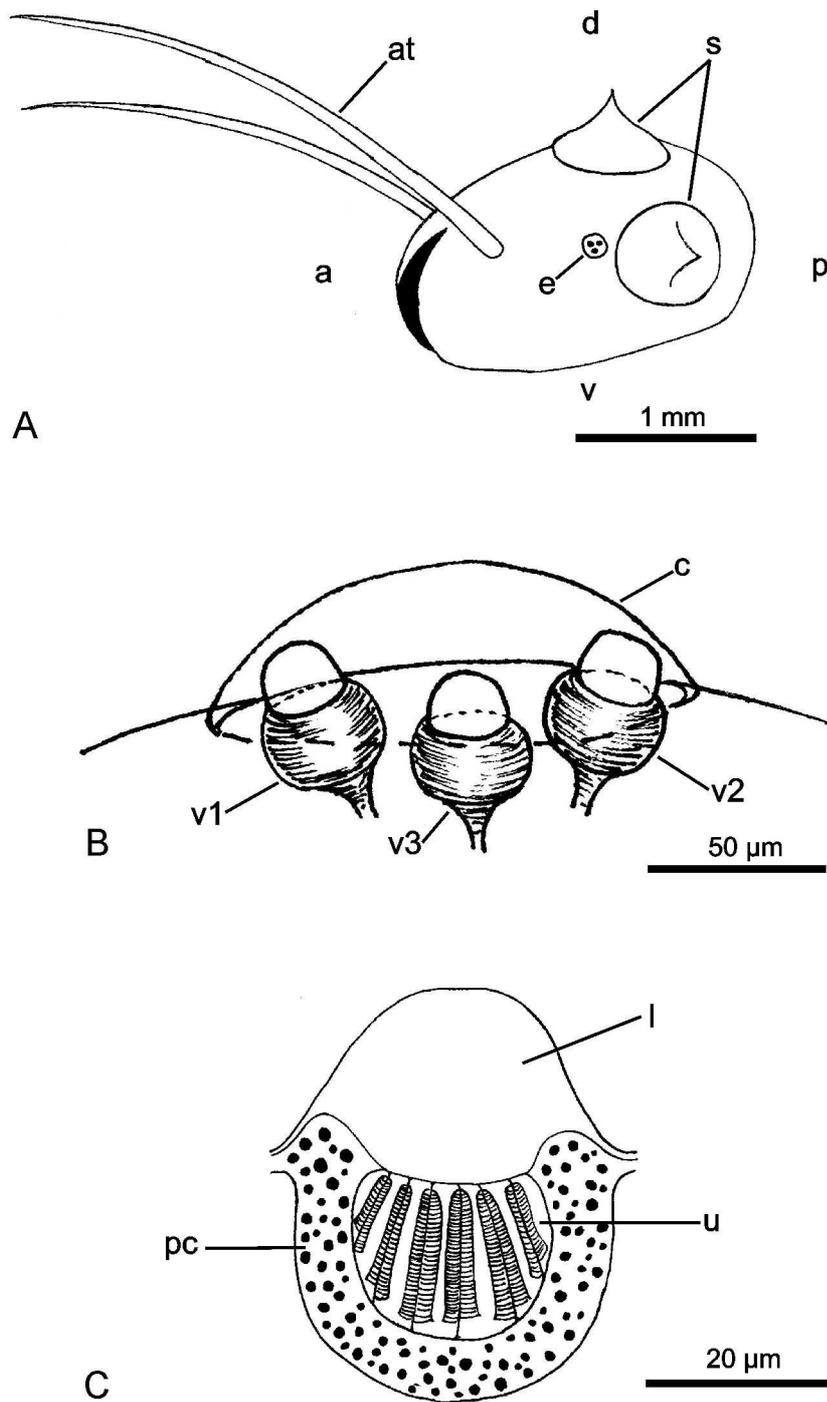


Fig. 5. Interpretative illustrations of the eye of *Luolishania longicuris*. (A) Head region, showing the eye position. (B) Horizontal schematic view of the eye structure, showing the three pigment cups and the covering cuticular structure. (C) Speculative longitudinal section of the visual unit, based on Paulus (1979, fig. 6.25C). a, anterior; at, antenniform outgrowth of *L. longicuris*; c, cuticular structure; d, dorsal; e, eye; l, lens or lens-like structure; p, posterior; pc, pigment cup; s, sclerite; u, unknown but possible rhabdomeric structure; v, ventral; v1-3, visual units 1-3.

4.2. *The eyes of Hallucigenia fortis*

SEM and IFM examination of other Chengjiang lobopodian specimens has indicated that a figured laterally preserved specimen of *H. fortis* (RCCBYU 10248, Hou *et al.* 2004b, p. 89, Fig. 14.7c) has a black spot at the mid-anterior part of the head, just above the gut (Fig. 6A). The position, colour and composition of this structure resemble the eye of *L. longicruris*. The eye of *H. fortis* is currently only known from this single specimen, (*H. fortis* is a very rare Chengjiang lobopodian with few specimens having a well-preserved head region).

There is a faint round margin in the area of the eye, with a diameter of ~223 μm (Fig. 6A, B). Within the outline, three distinct black spots are arranged in a triangle: two larger, ventrolateral, oval shaped with a length of ~100 μm ; and a much smaller dorsomedial spot, with a diameter ~38 μm . As in *L. longicruris*, these black spots are interpreted to represent visual units within the eye, and their size difference could represent a genuine feature, but also could be a factor of the different orientations in which the pigment cups are preserved. These three black spots are clear in SEM (Fig. 6E), and the SEM EDX analysis show significantly higher carbon concentration in these areas (Fig. 6F). In high magnification IFM image (Fig. 6C), both the ventrolateral black spots (*v1* and *v2*) show a distinct cup shape, while the morphology of the dorsomedial pigment cup (*v3*) is more irregular (see interpretative illustration in Fig. 6D). There is a faint smooth margin protruding from the left side of the ventrolateral pigment cup *v1*, indicating the presence of a possible lens-like structure (Fig. 6C, D). Two black string-like structures extending out from the side of each ventrolateral pigment cup may represent the same connection structures as in the eye of *L. longicruris*.

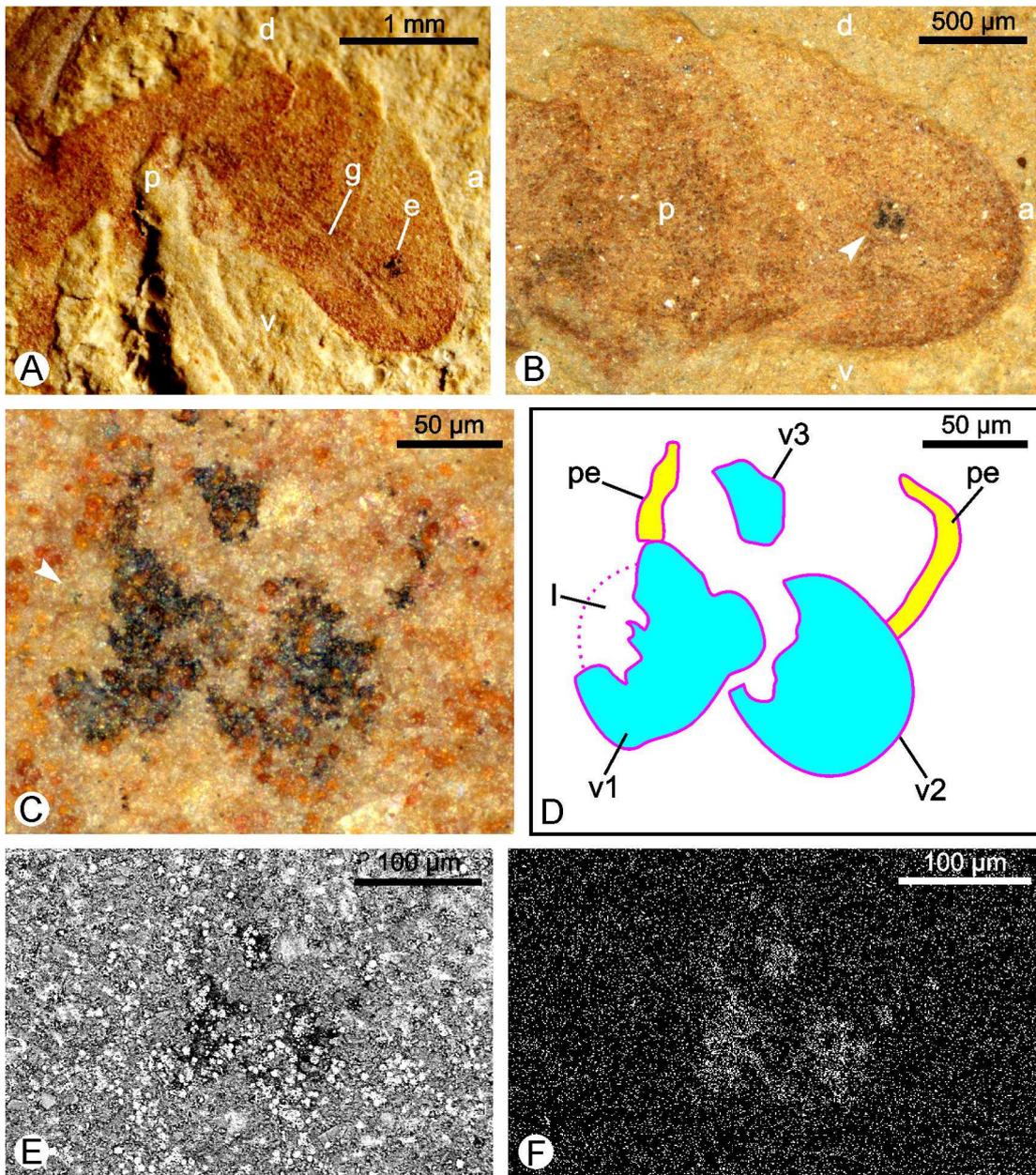


Fig. 6. Eye of specimen RCCBYU 10248 *Hallucigenia fortis*. (A) Head region, showing eye position. (B) IFM image close up of the head. Arrow indicates the margin of the cuticular structure. (C) IFM image close up of the eye, showing three visual units. Arrow indicates a faint margin of a possible lens-like structure. (D) Diagram interpreting the visual unit structure in (C). Visual units *v1* and *v2* show an image (E) and EDX carbon map (F), showing the eye as an area of carbon enrichment. a, anterior; d, dorsal; e, eye; g, gut; l, lens or lens-like structure; p, posterior; pe, pigmented extension; v, ventral; v1-3, visual unit 1-3.

4.3. The eyes of *Cardiodictyon catenulum*

A black spot on the head has also been identified in a specimen of *C. catenulum*, in which the head shield is exfoliating (Fig. 7A). This presumed eye is apparently smaller than the eyes found in *L. longicruris* and *H. fortis*, but could not be differentiated in SEM and EDX analysis. However, some interesting details of this eye have been revealed using IFM.

The eye appears to be round in shape with a diameter of ~60 μm (Fig. 7B). There seems to be only a single unit in the eye of *C. catenulum* in contrast to the eyes of *L. longicruris* and *H. fortis*, each of which are composed of three visual units. The detailed structures of the eye can be further recognised by colour differences: the black coloured pigment cup shows an opening pointing towards the anterior and a posterior stalk-like extension; a round whitish coloured structure covering the whole eye region may indicate the presence of a cuticular covering or a cuticular lens; a hemispherical structure in front of the pigment cup is preserved in a darker tone to the adjacent matrix (Fig. 7B), similar to the colour of the lens-like structure found in *L. longicruris* (Fig. 1B). A IFM DEM cross section reveals that the eye is preserved as a slight depression to the surrounding matrix, and the lens-like structure is preserved as a deep concavity, with a width of ~30 μm and a maximum depth of ~13 μm (Fig. 7C, D).

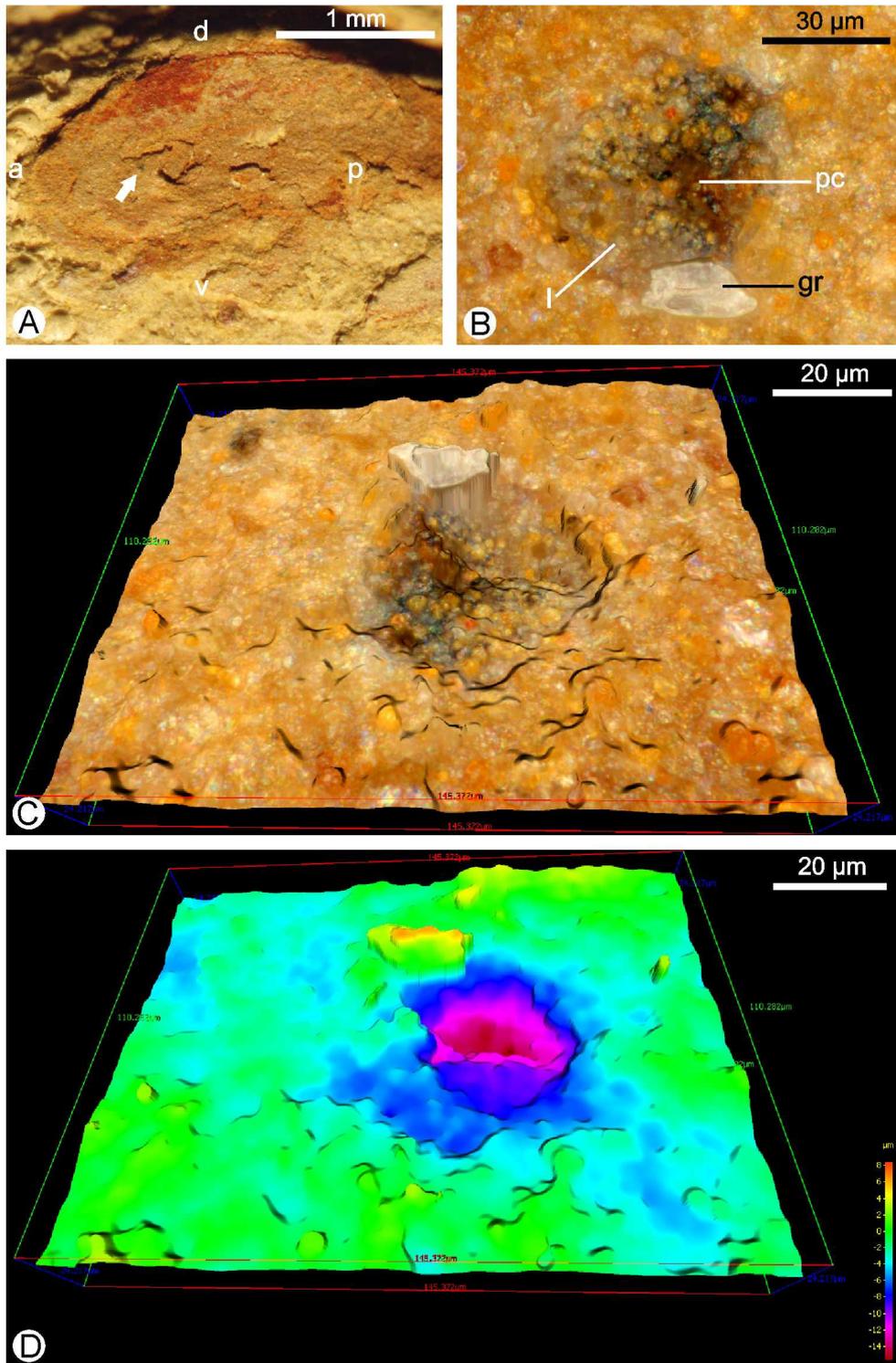


Fig. 7. Eye of specimen YKLP 11293 *Cardiodictyon catenulum*. (A) Head region. Arrow indicates the eye position. (B) IFM image close up of the eye, showing the black pigment cup and a possible 'lens' preserved in a darker tone than the surrounding matrix. (C, D) IFM DEM image of the eye (E) and contoured DEM (F), showing the whole eye preserved as a depression; a deep sunk structure corresponds to the 'lens' indicated in (B). a, anterior; d, dorsal; gr, a grain, not a structure belonging to the eye; l, lens or lens-like structure; p, posterior; pc, pigment cup; v, ventral.

5. Discussion

5.1. Origin and evolution of Cambrian lobopodian eyes

Re-examination of all lobopodians from the Chengjiang Lagerstätte has revealed the presence of visual organs in *L. longicruris*, *H. fortis* and *C. catenulum*. No visual organs have been found in *Paucipodia inermis*, *Microdictyon sinicum*, *Onychodictyon ferox*, *Megadictyon haikouensis* or *Jianshanopodia decora*. Visual organs may indeed be absent in the latter lobopodian species: they lack a distinct head (see Ma *et al.*, in press), so they may have a relatively simple brain structure and the optic centre in the brain may not have developed; Alternatively visual organs may have been present in these animals, but are not preserved, perhaps because they are very small.

In a cladistic analysis, *L. longicruris*, *H. fortis* and *C. catenulum* fall into the derived Cambrian lobopodian clade Archonychophora (Ma *et al.*, in press). Within this clade (Fig. 12B, Ma *et al.*, in press) *C. catenulum* is positioned on a more basal branch to *H. fortis* and *L. longicruris*. Therefore, the single-unit eye found in *C. catenulum* could represent an earlier evolutionary stage of Cambrian lobopodian eye development. Land and Nilsson (2002) suggested two fundamentally different ways by which spatial vision can evolve from a shielded photoreceptor unit: 1) more photoreceptors are added to exploit the same pigment shield; 2) or the visual organ grows by the multiplication of individual visual units. The development from the single-unit eye found in *C. catenulum* to the tri-unit eye of *H. fortis* and *L. longicruris* suggests that Cambrian lobopodian eye development followed the latter evolutionary pathway.

Recently, a pair of stalked (perhaps also compound) eyes has been found in a Middle Cambrian lobopodian called “Collins’ monster” (Collins, 1986, photograph p.

39; Delle Cave and Simonetta, 1991) from the Burgess Shale Lagerstätte (Dr. Greg Edgecombe personal communication). This animal is positioned in the most derived branch of the Archonychophora clade (Fig. 12B, Ma *et al.*, in press), so its stalked (compound) eye may indicate an evolutionary development from the Chengjiang lobopodian eyes.

5.2. Affinities with panarthropod eyes

Cambrian lobopodians are purported to be closely related to extant panarthropods (Tardigrada, Onychophora and Euarthropoda). Therefore, to better understand Cambrian lobopodian eyes and their evolutionary significance, it is important to compare them with different types of eye found in panarthropods.

5.2.1. Comparison with the eyes of tardigrades

Tardigrades possess only a pair of simple eye spots located within the head. Each eye is composed of a single cup-like pigment cell, filled with microvilli. The whole eye is ~65 μm in diameter. No lens has been found in tardigrade eyes (for a more detailed description see Greven, 2007). As the eyes of *H. fortis* and *L. longicruris* are composed of three visual units, they do not resemble the tardigrade eye. The single-unit eye of *C. catenulum* is a similar size to tardigrade eyes, but their pigment cup shape seems to be different and as described above the eye of *C. catenulum* is likely to possess a lens-like structure within the visual unit.

5.2.2. Comparison with the eyes of onychophorans

In onychophorans, paired eyes are situated dorsolaterally on the head behind the antennae. Their size varies in different species, between 100-300 μm depending on body size. Each onychophoran eye (ocellus) is simply-constructed and composed of a cornea, an optic cavity containing a vitreous body or 'lens', retinal cells ('rod layer') and a pigment cup (Eakin and Westfall, 1965; Mayer, 2006). Despite onychophoran eyes having been intuitively regarded as a starting point in the evolution of visual organs in arthropods (Paulus, 1979), Mayer (2006) recently proposed the challenging hypothesis that onychophoran eyes are homologous to euarthropodan median eyes, suggesting that lateral compound eyes are an autapomorphy of Euarthropoda. Mayer (2006) also suggested that the last common ancestor of arthropods only had one pair of ocellus-like visual organs, and claimed that his hypothesis was supported by the morphology of Cambrian lobopodian eyes based on the initial report from Liu *et al.* (2004). Schoenemann *et al.* (2008) also suggested that the eyes of *L. longicruris* resembled the eyes of onychophorans.

However, each eye of *H. fortis* and *L. longicruris* contains at least three visual units; therefore, they are fundamentally different from the ocellus-like eyes of onychophorans. The eye of *C. catenulum* only has a single unit, but its size (~60 μm) is much smaller than even the smallest onychophoran eye. Therefore, it seems that all the Cambrian lobopodian eyes described herein are very different from the eyes of onychophorans, and the hypothesis and conclusions given by Mayer (2006) and Schoenemann *et al.* (2008) should be reconsidered.

According to the latest cladistic analysis of lobopodians (Ma *et al.*, in press), Onychophora is the crown taxon of a monophyletic clade *Microdictyon* + *Paucipodia* + *Jianshanopodia* + *Xenusion* + *Hadranax* + *Megadictyon* + *Onychophora*, and this clade

is suggested to be designated “Onychophora”. As no visual organ has been reported from any of the Cambrian lobopodians in this clade, Onychophora could have evolved from a Cambrian lobopodian ancestor that was eyeless or only had a primitive eye spot. The lobopodian clades “Onychophora” and “Archonychophora” form a paraphyletic group, with clade “Onychophora” more basal. Therefore, the eye development in onychophorans and in archonychophorans (the latter including *C. catenulum*, *H. fortis* and *L. longicruris*) is likely to be independent, following different evolutionary trajectories. In contrast to *H. fortis* and *L. longicruris*, the eye of onychophorans may have evolved from a simple eye spot by adding more photoreceptors to exploit the same pigment shield (Land and Nilsson, 2002).

Concerning Mayer’s hypothesis (2006) that onychophoran eyes are homologous with arthropod median eyes, opinions differ according to neuroanatomical studies on the architecture of onychophoran brains and possible median eyes are also reported from Onychophora (Strausfeld, 2006b). The origin of arthropod visual organs will be discussed below.

5.2.3. Comparison with arthropod visual organs

Arthropod visual organs present a considerable diversity in number, position and structure. According to their position on the cephalic region and their connection with the brain, these visual organs can be divided into two major types: median eyes and lateral eyes (see reviews by Paulus, 1979, 2000; Bitsch and Bitsch, 2005). Median eyes (also called frontal eyes/ocelli/organs, anterior eyes, median ocelli and naupliar eyes) are ocellus-type simple eyes, occupying a median position on the head and innervated from a median neuropil of the protocerebrum, the so-called ocellar centre. In contrast, lateral eyes occupy a lateral position on the head and are innervated from the optic

centres located laterally on each side of the protocerebrum. Arthropod lateral eyes occur as two different types: the simple-lens eye with a cup-shaped retina and the typical faceted eye, composed of many ommatidia. The origin and evolution of these different arthropod eye types is still a matter for discussion (Paulus, 1979, 2000; Bitsch and Bitsch, 2005; Harzsch and Hafner, 2006; Harzsch *et al.*, 2007). Although we still do not know the exact ultrastructure of Cambrian lobopodian eyes due to the limits of fossil preservation, the new data on their morphology and architecture revealed in this study has offered some insight into the ancestral visual organs of arthropods.

5.2.3.1. Comparison with arthropod median eyes. Superficially, the triangularly-arranged visual units in the eye of *L. longicruris* and *H. fortis* somewhat resemble the frontal ocelli of Hexapoda (e.g. ocellar optics in bees, Warrant *et al.*, 2006) and the nauplius eyes of Crustacea (e.g. ostracod nauplius eyes, Reimann and Richter, 2007) which can also show a triangular pattern. However, Cambrian lobopodian eyes occur as a pair, each of which is located on the lateral side of the head, indicating possible innervations from lateral sides of the protocerebrum. Therefore, Cambrian lobopodian eyes seem fundamentally different from arthropod median eyes. However, because Mayer (2006) suggested a homology between onychophoran eyes and the median eyes of euarthropods, it appears necessary to further compare the morphology of Cambrian lobopodian eyes with arthropod median eyes.

The frontal ocelli of Hexapoda are isolated organs and clearly separated from each other by a considerable distance (Warrant *et al.*, 2006), while the three visual units in Cambrian lobopodian eyes are arranged closer to each other, even connected by string-like structures, and are covered under a single cuticular structure. Therefore, the three

visual units of Cambrian lobopodians clearly belong to a single visual organ and are distinctively different from the frontal ocelli of euarthropods.

The nauplius eyes, present in the larvae of most groups of Crustacea, and in some adults, correspond to other types of median eyes (Bitsch and Bitsch, 2005). In the ostracod *Vargula graminicola*, for example, the nauplius eye is composed of three contiguous eye cups, two dorso-lateral and one ventral. These three eye cups are separated from each other by pigment cells which display a “Y” shape (Huvard, 1990, fig. 3). In this case, the black coloured pigment granules do not form a round cup-shape and only appear as three thin lines, so this type of nauplius eye looks different from Cambrian lobopodian eyes. However, in another ostracod *Notodromas monachus*, the three cups of the nauplius eye are widely separated and formed by four pigment cells, in cross section forming a “Y” with a cup at the end of each arm (Andersson and Nilsson, 1981, fig. 1). Therefore, this type of nauplius eye looks similar to the eye of *L. longicruris* (Figs. 1B-D): they are all composed of three pigment cups arranged in a triangle; some pigmented structures connect the cups to each other; the cups are equipped with a lens or lens-like structure. However, Cambrian lobopodian eyes are still significantly different from this type of nauplius eye: 1) they occur as a pair, located on the lateral side of the head; 2) in *L. longicruris*, the pigmented connections between the eye cups look indistinct, rather than forming a clear “Y” shape; 3) the tri-unit eye of *H. fortis* looks very different from the nauplius eye: the dorsoventral arrangement of the eye cups are oriented the opposite way and the string-like structures leading from the two larger pigment cups do not form a “Y” shape, with no evidence that they connect with the smaller dorsal pigment cup; 4) the three pigment cups in both *L. longicruris* and *H. fortis* are covered beneath a single round cuticular structure.

5.2.3.2. *Comparison with arthropod lateral eyes.* The lateral position of paired Cambrian lobopodian eyes is comparable with arthropod lateral eyes, which come in two forms.

Compound eyes, well developed in most arthropod groups, are typically composed of many repeated ommatidia, each ommatidium appearing as a highly differentiated visual unit (Bitsch and Bitsch, 2005). The eyes of *L. longicuris* and *H. fortis* are composed of three similar visual units, and in this respect have a similarity to the compound eye of arthropods. However, compared with the typical compound eye, the three visual units in Cambrian lobopodian eyes are not contiguous, but clearly separated from each other; although there appear to be lens-like structures in each visual unit, there are no typical facets in Cambrian lobopodian eyes. The visual units in these eyes seem relatively simple, unlike highly developed ommatidia; each pigment cup of Cambrian lobopodian eyes shows a distinct cup-like shape, but the distribution of pigment granules in the ommatidia of arthropod compound eyes show a more linear arrangement (Paulus, 1979, fig. 6.7).

Arthropod lateral eyes also occur as simple eyes, such as the stemmata of myriapods. Stemmata are most often composed of a single retinula whose structure is little different from that found in the ommatidia of compound eyes. The number of visual units on each side of the head varies depending on species and they may be well separated or contiguous (Bitsch and Bitsch, 2005). Cambrian lobopodian eyes show striking similarities to the stemmata of myriapods: 1) visual units are separate but still closely associated; 2) in contrast to the ommatidia of compound eyes, the visual units of myriapod and Cambrian lobopodian eyes can be of variable size (for myriapod eye, Paulus, 2000, fig. 5; for Cambrian lobopodian eyes, see above description and Figs. 1B-D and 6B-D); 3) compared with the tube-shaped distribution of pigment granules in

ommatidia, the visual units of myriapods are much stouter, with pigment granules distributed in a typical cup shape (e.g. Paulus, 2000, fig. 4), which is similar to the pigment cup of Cambrian lobopodian eyes (Figs. 1B, 4D and 6C, D). The triangular pattern of Cambrian eye evolution is also supported by the evidence from the developmental biology of eyes in myriapods (Harzsch *et al.*, 2007). However, the cuticular structure covering the visual units of Cambrian lobopodian eyes is not found in myriapods.

To summarise, the eyes of Cambrian lobopodians differ from the eyes of tardigrades and onychophorans, but resemble some arthropod visual organs. This similarity is consistent with cladistic analysis indicating their close phylogenetic relationship to arthropods. That the eyes of the Cambrian lobopodians *L. longicruris* and *H. fortis* are perhaps visual organs similar to myriapod eyes, representing the ancestral visual system of arthropods.

The evolutionary relationship between simple lateral eyes and compound eyes has puzzled evolutionary biologists. The more commonly accepted traditional hypothesis is that simple lateral eyes were derived from compound eyes by disintegration of the latter into single ommatidia and subsequent fusion of several ommatidia to form multicellular ocelli (Paulus, 1979, 1986, 2000; Spies, 1981; Bitsch and Bitsch, 2005). However, this opinion has been challenged recently by Harzsch *et al.* (2007), as they found that during eye growth in Myriapoda new visual units are added to the side of the eye field to form a pattern closely resembling that in horseshoe crabs (Chelicerata) and Trilobita. Therefore, they suggested that the trilobite, xiphosuran, diplopod and chilopod mechanism of eye growth represents the ancestral euarthropod mode of visual-system formation.

The similarities between Cambrian lobopodian eyes and myriapod eyes clearly support the latter hypothesis. Further, it is possible to propose that Cambrian lobopodian eyes represent an ancestral visual system that gave rise to both types of arthropod lateral eyes. From Cambrian lobopodian eyes that are composed of a few separate visual units and covered by a single cuticular structure there are perhaps two evolutionary pathways to arthropod lateral eyes: 1) The separate visual units come together until they are contiguous; at the same time, they are further modified into highly developed ommatidia; this pathway eventually leads to the typical compound eye. 2) The single cuticular structure becomes divided into individual cornea for each visual unit, which is also modified, becoming a more independent multicellular ocellus; this pathway leads to simple lateral eyes.

5.3. *The possible function of the Cambrian lobopodian eye*

The performance of eyes depends on their sensitivity to light and ability to resolve images (Land and Nilsson, 2002). Schoenemann *et al.* (2008) utilised formulae taken from Land (1981) and Land and Nilsson (2002) to determine the visual performance of the eye of *L. longicruris*. As the interpretation of Cambrian lobopodian eye morphology herein differs from that of Schoenemann *et al.* (2008), the methodology has been applied here using the revised data.

According to Land and Nilsson (pp. 72, 2002), the focal length of the surface is given by the formula:

$$f = r/(n_2 - n_1)$$

where f is focal length, r is the radius of curvature of a surface, n_2 is the refractive index inside the eye, and n_1 is the refractive index outside the eye. Because difference

of refractive indices is higher in air than in seawater, Land and Nilsson (2002) suggested that for a cornea in air $f \approx 3r$, but for a lens in water $f \approx 2.5r$. A possible spherical lens was found in specimen YKLP 11291 of *L. longicruris* (Fig. 1B, C), with a diameter $\sim 25 \mu\text{m}$. Therefore, an estimate of the focal length for the eye of *L. longicruris* is about $f \approx 2.5r \approx 31.25 \mu\text{m}$. The depth of the pigment cup of *L. longicruris* is $\sim 32.94 \mu\text{m}$ (measured from the dorsolateral pigment cup *vl* in Fig. 1B). Therefore, it is possible that the lens of *L. longicruris* could form an image onto the retina. However, as we do not know the quality of the image (sharp or coarse) or if the brain was capable of dealing with the information, it is still difficult to judge how important the eye was in the animal's life.

The eye of *H. fortis* is very similar to the eye of *L. longicruris*, so it may also have been able to form images. However, the eye of *C. catenulum* is relatively simple. A calculation using the above formula indicates that the eye of *C. catenulum* could be over-focused (focal length is too short to reach the retina). As the head shield of *C. catenulum* is apparently thicker than those of *H. fortis* and *L. longicruris*, it may also have limited the function of the eye.

Chapter 5

Evidence of possible parasitic worms from the Lower Cambrian Chengjiang Lagerstätte

Abstract

Three new vermiform genera and species, *Acanthipos torquatus*, *Hamuscolex bolsoveri*, and *Palaeomyzon discus* are described from the Chengjiang Lagerstätte. They possess distinct morphological features from other reported fossil worms and comparison with extant vermiform animals indicates that they may be stem group representatives of three separate phyla of extant parasitic worms. The morphological evidence also suggests that these new vermiform taxa may have led a parasitic lifestyle, extending both the biodiversity and ecological diversity of known Early Cambrian ecosystems.

Keywords: the Chengjiang Lagerstätte; parasitic worms; *Acanthipos torquatus*; *Hamuscolex bolsoveri*; *Palaeomyzon discus*

1. Introduction

The fossilisation of vermiform animals is extremely rare due to their soft bodies and they only occur in some exceptionally preserved fossil assemblages, such as the Lower Cambrian Chengjiang Lagerstätte (circa 530 mya) and the Middle Cambrian Burgess Shale (circa 515 mya). Despite the great diversity of vermiform species recovered from the Chengjiang Lagerstätte, almost all of them are assigned to the Phylum Priapulida, which led a burrowing/crawling and deposit feeding/predatory lifestyle (Chen, 2004; Hou *et al.*, 2004a; Maas *et al.*, 2007). Therefore, there is still little known about the early evolution of other vermiform phyla and their lifestyles.

This study reports and describes three new non-priapulid vermiform taxa from the Chengjiang Lagerstätte. The fossils are very rare, as only one specimen has been discovered for each species in 25 years of collecting. However, the specimens show distinct features that distinguish them from other reported fossil worms and also allow direct comparison with extant taxa. They also occupied an unreported ecological niche within the Chengjiang Lagerstätte ecosystem.

2. Material and Methods

The three new taxa are each represented by a single specimen (one with counterpart). All material is from the Anshan section near Mafang village, Haikou region, Yunnan Province; geologically in the Yu'an-shan Member, Helinpu Formation, *Eoredlichia* Biozone, Lower Cambrian. All the specimens are deposited in the Yunnan Key Laboratory for Palaeobiology (YKLP), Yunnan University, Kunming, China: YKLP 11294 to YKLP 11296.

The observation, preparation and camera lucida drawings of specimens were made under a Wild Heerbrugg Plan 1X Microscope. Scanning Electron Microscopy (SEM) and an Alicona Infinite Focus Microscope (IFM) were used for micro-image capture.

3. Preservation

All specimens are two-dimensionally preserved on the bedding surfaces of the host mudstone. Specimen YKLP 11294 is preserved in a whitish to yellowish colour close to the surrounding matrix, with some cuticular structures mineralized and preserved in a rusty colour (Fig. 1A-C, E, F). Specimen YKLP 11295 is dark reddish in colour, with various structures preserved in strong black, rusty or red coloration (Fig. 4A-H). Specimen YKLP 11296 is brownish to black, with some internal structures preserved in low relief (Fig. 6C, E); SEM backscatter images show an unusually thick carbon film with cracked features concentrated at the anterior end of this animal, while the body wall and gut are iron enriched (Fig. 6D, F, H).

4. Systematic Palaeontology

Genus *Acanthipos* gen. nov.

Derivation of name: Greek *akanthos*, thorn, and *ipos*, a kind of worm.

Diagnosis: Cylindrical body divided into a proboscis, neck, collar and trunk.

Proboscis covered with cuticular features, possibly hooks. Neck smooth with anterior end equipped with short spines. Collar marked by long spines around posterior end of neck. Trunk long, cylindrical and with a scattering of small spines. Set of four spine-like structures situated near posterior end of trunk, pointing anteriorly.

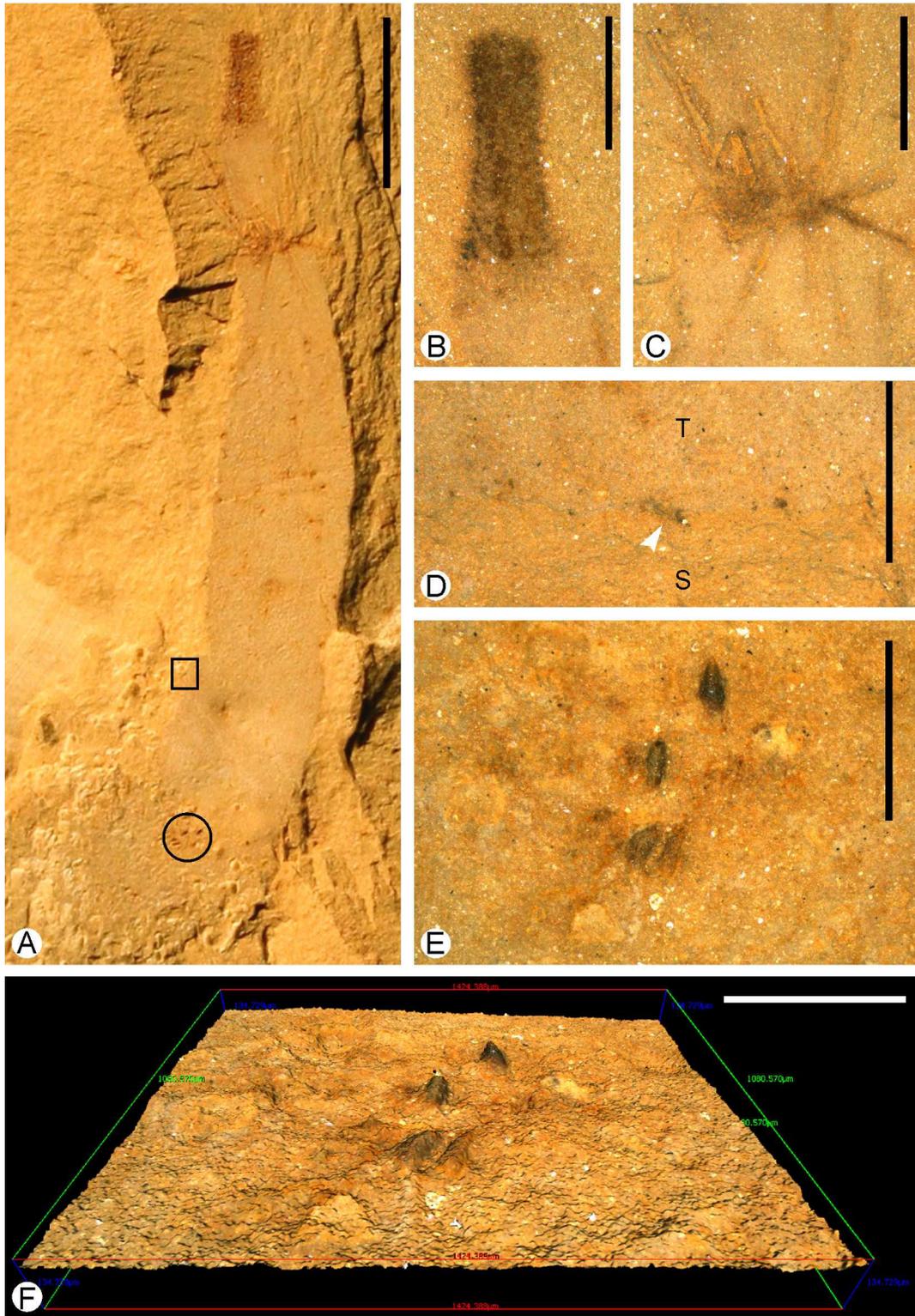


Fig. 1. *Acanthipos torquatus* gen. et sp. nov. Holotype YKLP 11294. (A) Whole specimen, showing its general morphology and body divisions. (B) Probocis. (C) Collar, showing long spines. (D) Mid trunk edge (square area in A), showing the trunk spine (arrow). T, trunk; S, sediment. (E) A set of four spine shaped structures on the posterior trunk (circle area in A). (F) IFM digital elevation model of the structures in (E), showing their three-dimensional morphology. Scale bars = 3 mm (A); 1 mm (B-D); 0.5 mm (E); 0.3 mm (F).

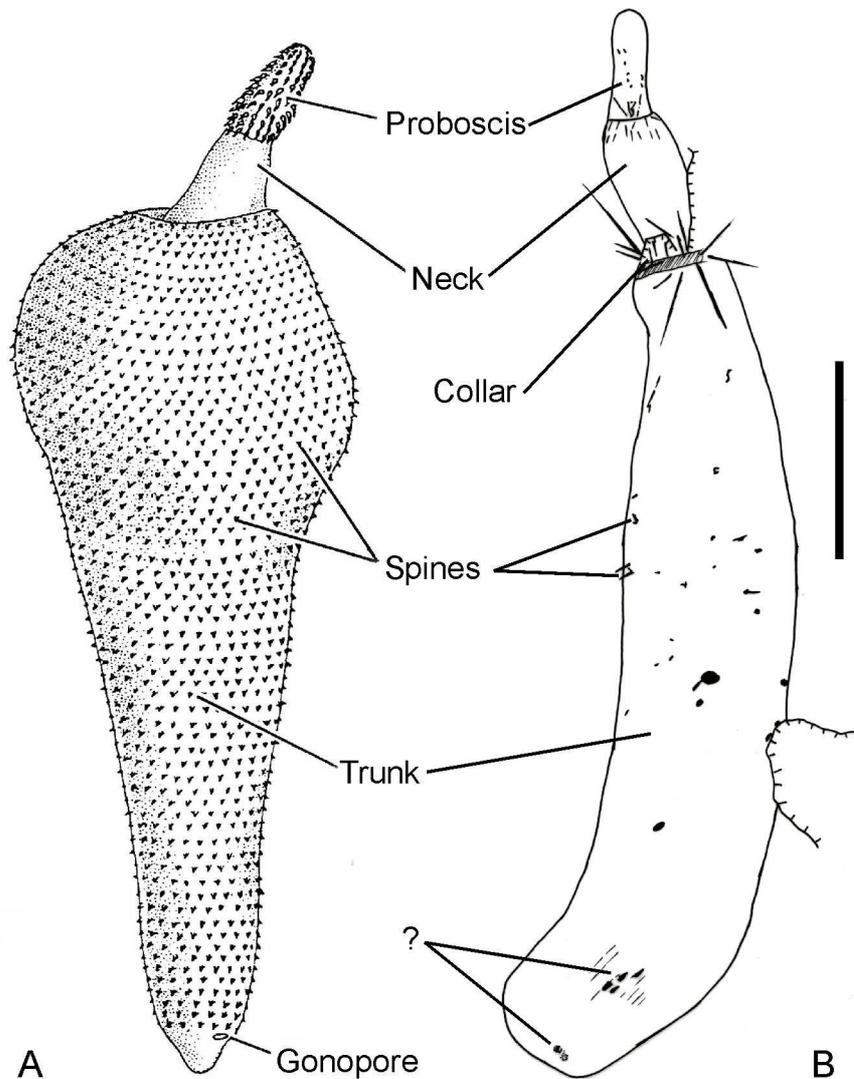


Fig. 2. Comparison between: (A) reconstruction drawing of *Corynosoma*, a palaeacanthocephalan found in aquatic birds and seals (image adapted from Brusca and Brusca, 2003, pp. 369, fig. 12.29B; not to scale); (B) Camera lucida drawing of holotype YKLP 11294 *Acanthipos torquatus* gen. et sp. nov. Scale bar = 0.3 mm.

Species *Acanthipos torquatus* gen. et sp. nov.

Derivation of name: Latin *torquatus*, necklace, collar.

Diagnosis: as for genus.

Material: Holotype and only specimen, YKLP 11294 (Fig. 1A; 2B), from Anshan section near Mafang village, Haikou region, Yunnan Province, China. The holotype is part only, complete and well preserved.

Description: Body ~15 mm long. Everted proboscis ~1 mm long and ~0.5 mm wide (Fig. 1B; 2B). Fossil preserved in a rusty colour, with a rough surface. This type of preservation in Chengjiang fossils often indicates the existence of cuticular structures on the surface. Dots arranged in a longitudinal row along the axis of the proboscis (Fig. 1B; 2B) may indicate the attachment of cuticular structures such as spines or hooks on the surface of the proboscis. At the junction between the proboscis and the neck, a darker band supports short spine-like structures (Fig. 1B; 2B); this could be an independent feature or an extension of the proboscis. Neck ~2.2 mm long and ~1.1 mm wide, with a smooth surface and ovoid shape. Immediately behind the neck, a distinct collar is marked by a set of long spines pointing in different directions (Fig. 1C; 2B). A rusty band around the collar may result from preservation of the spine attachment area. Trunk long, cylindrical with no evident subdivision. Trunk surface generally smooth with no annulations, but bears some sparsely scattered rusty coloured spots. High magnification of the trunk margin (Fig. 1D) shows a dark spine, suggesting the rusty spots may indicate spine attachment points. A set of four spine-shaped structures is situated axially on the trunk near the posterior end (Fig. 1E, F; 2B). These differ from the spine-shaped cuticular ornamentation on the trunk surface, in being strongly mineralized with a dark rusty colour, and preserved in three-dimensions; they are close to each other rather than

sparsely scattered and all point anteriorly. A pair of small dots is preserved at the posterior end and seems to connect to each other (Fig. 1A; 2B). There is no preserved gut or other internal organ.

Discussion: *Acanthipos torquatus* is distinguished from other reported vermiform fossils from the Chengjiang Lagerstätte by its distinct body divisions, characteristically spiny collar and absence of annulation and gut. Comparisons with extant vermiform taxa reveal that this animal shares many similarities with the Acanthocephala (Fig. 2A; 3A-E): 1) body divided into proboscis, neck and trunk; 2) the presence of an anterior protrudible proboscis that is usually covered with spiny hooks or similar cuticular structures (the most notable feature of acanthocephalans); 3) neck smooth; 4) trunk bearing spines and 5) gut absent. In addition, the set of four spine-shaped structures near the posterior end of *A. torquatus* seems to correspond to the urogenital structures in acanthocephalans. However, the distinct spiny collar of *A. torquatus* is absent in extant acanthocephalans, while the unique circulatory system (lacunar canal system) of extant acanthocephalans is not recognised in *A. torquatus*, possibly due to preservational bias.

All extant acanthocephalans are obligate parasites, and they often attach to their host's intestinal wall by their proboscis hooks. Nutrients are absorbed through the body wall. The morphological similarities between *A. torquatus* and acanthocephalans indicate that these two groups of animals may have close phylogenetic relationships and/or share a similar lifestyle.

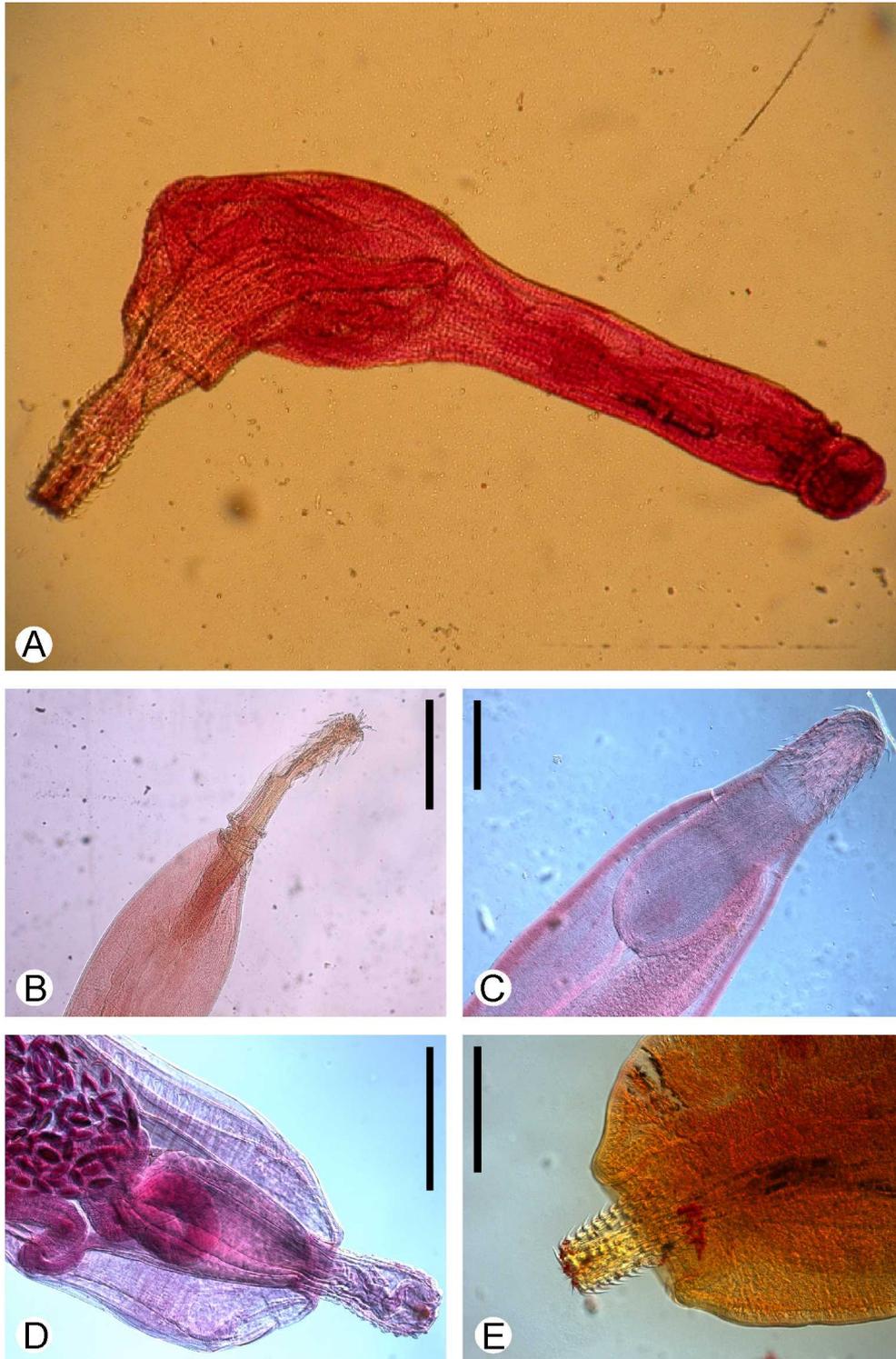


Fig. 3. Extant acanthocephalans. (A) *Corynnosoma wegneri*, showing general morphology and body divisions (image taken by Dr Neil Campbell, University of Aberdeen, Scotland, UK). (B-E) Anterior part of various acanthocephalans (images taken from the samples stored in the Natural History Museum, London). (B) *Acanthocephalus anguillae*. (C) *Acanthocephalus ranae*. (D) *Moniliformis moniliformis*. (E) *Acanthocephalus anthuris*. Scale bars = 1 mm (B, C); 3 mm (D); 2 mm (E).

Genus *Hamuscolex* gen. nov.

Derivation of name: Latin *hamus*, hook, and *scolex*, worm. Gender masculine.

Diagnosis: Body hook-shaped, divided into a trunk, which incorporates a head and a tail. Trunk irregular in shape, with an apparent expansion at its posterior end. A triangular projection at the anterior end of the head is followed by three or four dark round structures. Tail cylindrical, tapering towards posterior end, with an oval structure at the proximal end; distal tip of tail divided into two caudal lobes, with an adjacent lateral projection.

Species *Hamuscolex bolsoveri* gen. et sp. nov.

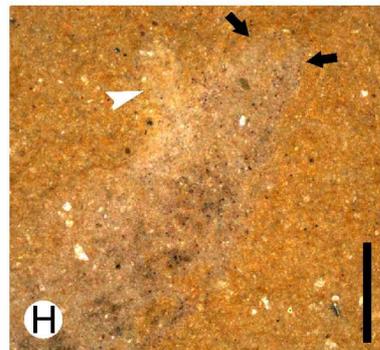
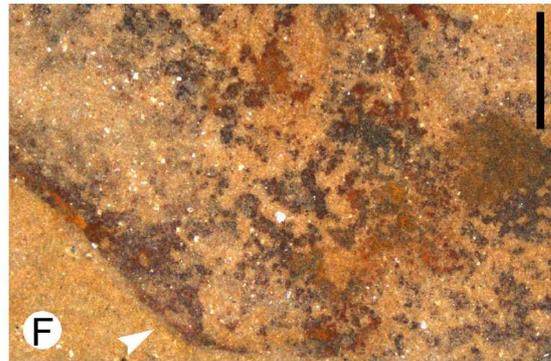
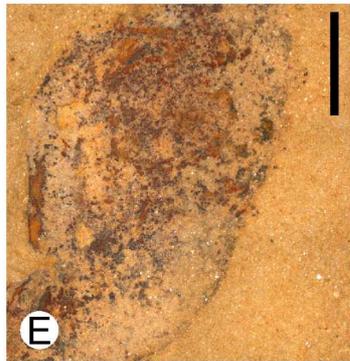
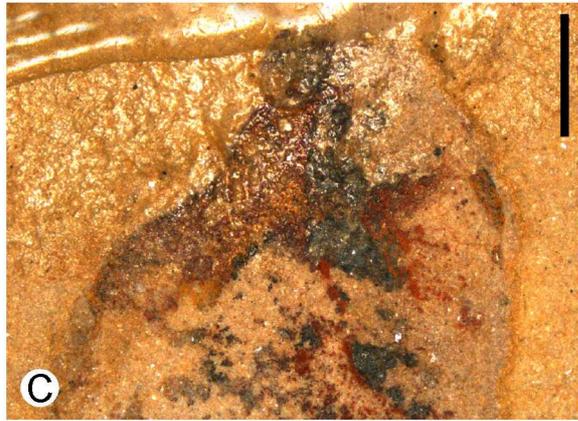
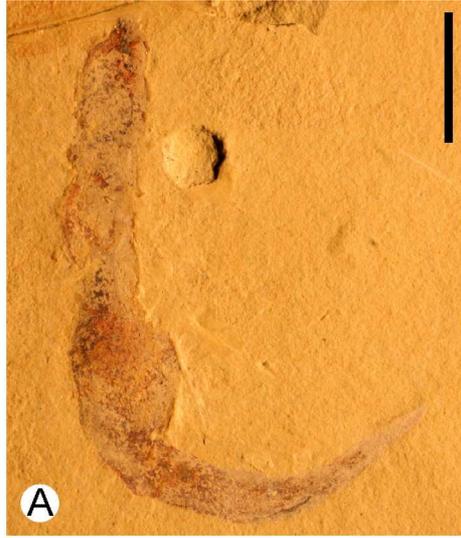
Derivation of name: After Mr Bill Bolsover of Aggregate Industries Ltd, who supported my research.

Diagnosis: as for genus.

Material: Holotype and only specimen, YKLP 11295 (Fig. 4A, B; 5A, B), from Anshan section near Mafang village, Haikou region, Yunnan Province, China. Part and counterpart, complete and well preserved.

Description: Body ~30 mm long, divided into two parts by a constriction, separating a trunk and a tail (Fig. 5A). Trunk cylindrical in general shape but with an irregular margin, superficially divided into three subdivisions (Fig. 5A), with a head at the anterior of the anterior trunk subdivision. A grey triangular projection points anteriorly at the front of the head (Fig. 4B, D; 5B). Behind this are 3~4 round black structures, different in shape on the part and counterpart (Fig. 4A-D; 5A, B); their nature is uncertain. The mid trunk is relatively thinner, with traces of dark internal structures (Fig. 4A, B; 5A, B), which may represent the alimentary canal or the reproductive system. The posterior trunk is strongly rusty in colour, enlarged and rounded, approximately

Fig. 4. *Hamuscolex bolsoveri* gen. et sp. nov. Holotype YKLP 11295. (A-B) Part (A) and counterpart (B) of the specimen, showing general morphology. (C) Head region of the part. (D) Head region of the counterpart, showing a triangular projection in front of the head (arrow). (E) Expanded posterior trunk region. (F) A possible anal region (arrow), situated near the proximal end of the tail. (G) A black stripe-like internal structure indicated by arrow, situated where the tail turns. (H) The posterior end of the tail, showing a spine-like projection on the side (white arrow), and two caudal lobes at the posterior tip (black arrows). Scale bars = 5 mm (A, B); 0.6 mm (C, F, G); 1 mm (D); 2 mm (E); 0.3 mm (H).



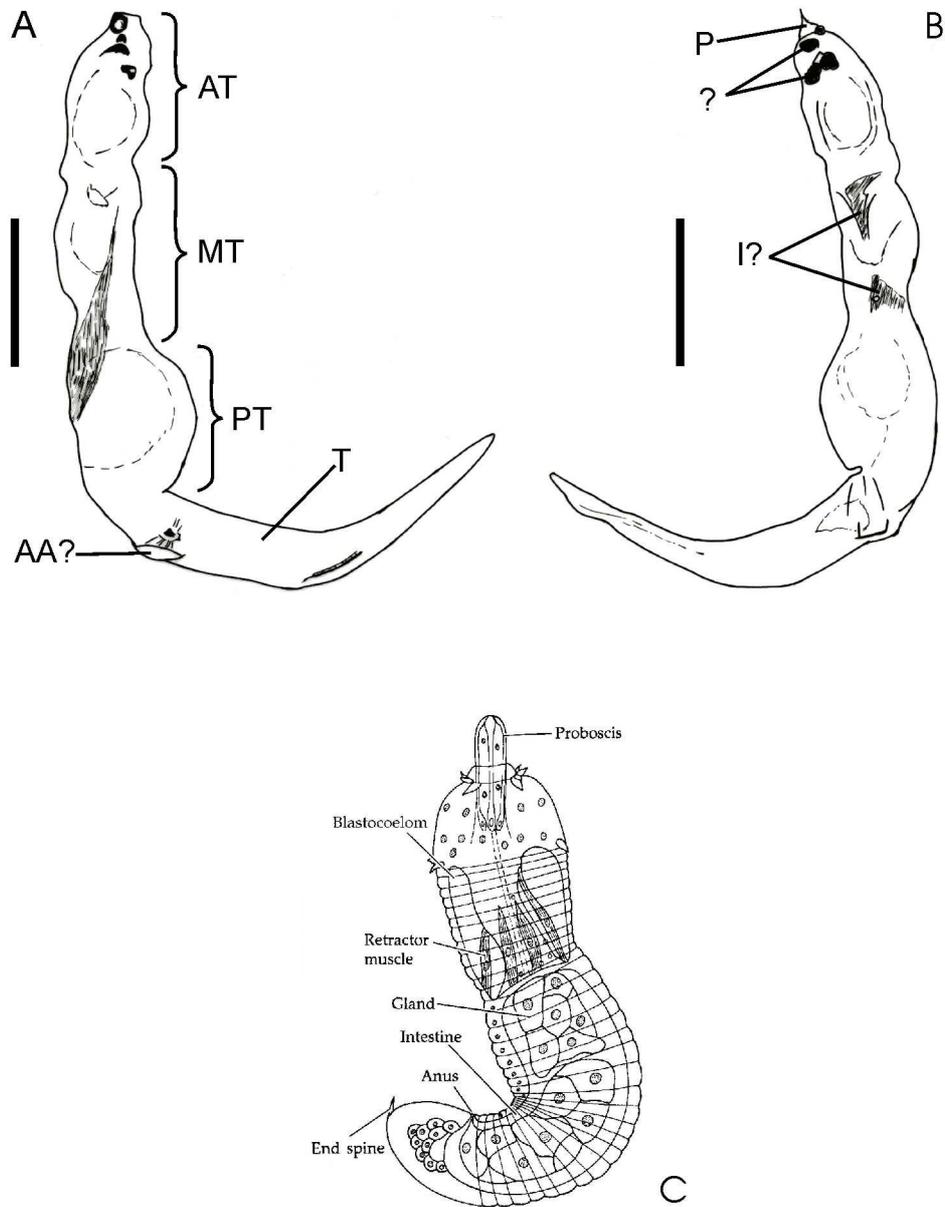


Fig. 5. (A-B) Camera lucida drawings of the part (A) and counterpart (B) of specimen YKLP 11295, explaining body divisions and possible internal structures. (C) Comparison with a nematomorph larva (image taken from Brusca and Brusca, 2003 p. 364, fig. 12.26H; not to scale). Scale bars = 0.5 cm (A, B). AA, anus area; AT, anterior part of trunk; I, intestine; MT, mid part of trunk; P, proboscis; PT, posterior part of trunk; T, tail.

~4.1 mm at its widest part; a trace of the dark internal structures of the mid-trunk apparently extends into this region.

Tail grey, hook-shaped, length ~12.9 mm, with a regular cylindrical shape tapering towards posterior end. Proximally, an oval shaped structure protrudes laterally (Fig. 4A, B, F; 5A, B), with one margin within the tail and the other margin congruent with the tail margin; an adjacent structure is indistinct. These two structures together may represent the anal area or urogenital pore. Posterior of the point of maximum curvature, a black stripe along the tail margin contains a series of lighter blocks (Fig. 4G); this may represent a blood vessel or nervous structure. A spine-shaped projection occurs on the margin near the posterior tip of the tail, which is divided into two caudal lobes (Fig. 4H).

Discussion: *Hamuscolex bolsoveri* is distinguished from other reported fossil worms by its unusual head structures, irregular trunk shape and a distinctly hook-shaped tail. The lack of detailed information of its internal structures and the unusual morphological outline hinder direct comparisons between *H. bolsoveri* and extant vermiform animals, but the closest comparators are the Nematomorpha, especially the larvae (Fig. 5C). The larvae are the dominant stage in the life cycle of nematomorphs (Ruppert and Barnes, 1996), and they superficially share some similarities with *H. bolsoveri*: 1) both have a hook-shaped body outline; 2) the anterior triangular projection of *H. bolsoveri* may correspond to the pointed proboscis in nematomorph larvae; 3) the anus is not terminal; 4) there is an end spine. Furthermore, *H. bolsoveri* appears to have two caudal lobes, a feature which it shares with adult nematomorphs. The short-lived adult nematomorphs are free-living in freshwater, while the longer-lived larvae parasitize arthropods, so the similarities suggest *H. bolsoveri* perhaps also led a parasitic lifestyle. The anterior projection and rounded structures in the head region of *H. bolsoveri* may indicate a parasitic apparatus.

Genus *Palaeomyzon* gen. nov.

Derivation of name: Greek *palaios*, ancient, and *myzo*, suck.

Diagnosis: Body cylindrical. A distinct anterior oral sucker, composed of a series of concentric rings, leads to a wide alimentary tract posteriorly divided into two intestines.

Species *Palaeomyzon discus* gen. et sp. nov.

Derivation of name: Latin, *discus*, flat, circular.

Diagnosis: as for genus.

Material: Holotype and only specimen, YKLP 11296 (Fig. 6A-E; 7A), from Anshan section near Mafang village, Haikou region, Yunnan Province, China. The holotype is part only, incomplete but well preserved.

Description: Only the anterior portion of the body is preserved, ~ 11 mm long (Fig. 6A, C). The specimen shows a degree of decay before fossilization, with the body margin poorly defined and the posterior portion of the specimen fades into the sediment. A dark brown disc-like structure at the anterior end (Fig. 6A-F; 7A) contains a series of concentric rings with a central mouth opening; round structures preserved with a low relief follow the lines of the concentric circles. Ill-defined iron enriched cuticle at the junction of the disc and body may be spines or part of the oral disc. The mouth leads directly to the alimentary tract (Fig. 6D, E), ~0.44 mm wide, which is divided and preserved as several ridges along the longitudinal axis; posteriorly, two stripe-shaped structures are evident (Fig. 6C). Two rows of rounded, sediment-covered structures at the anterior end of the body are arranged along both sides of the alimentary tract; these may be anatomical features or preservational artifacts.

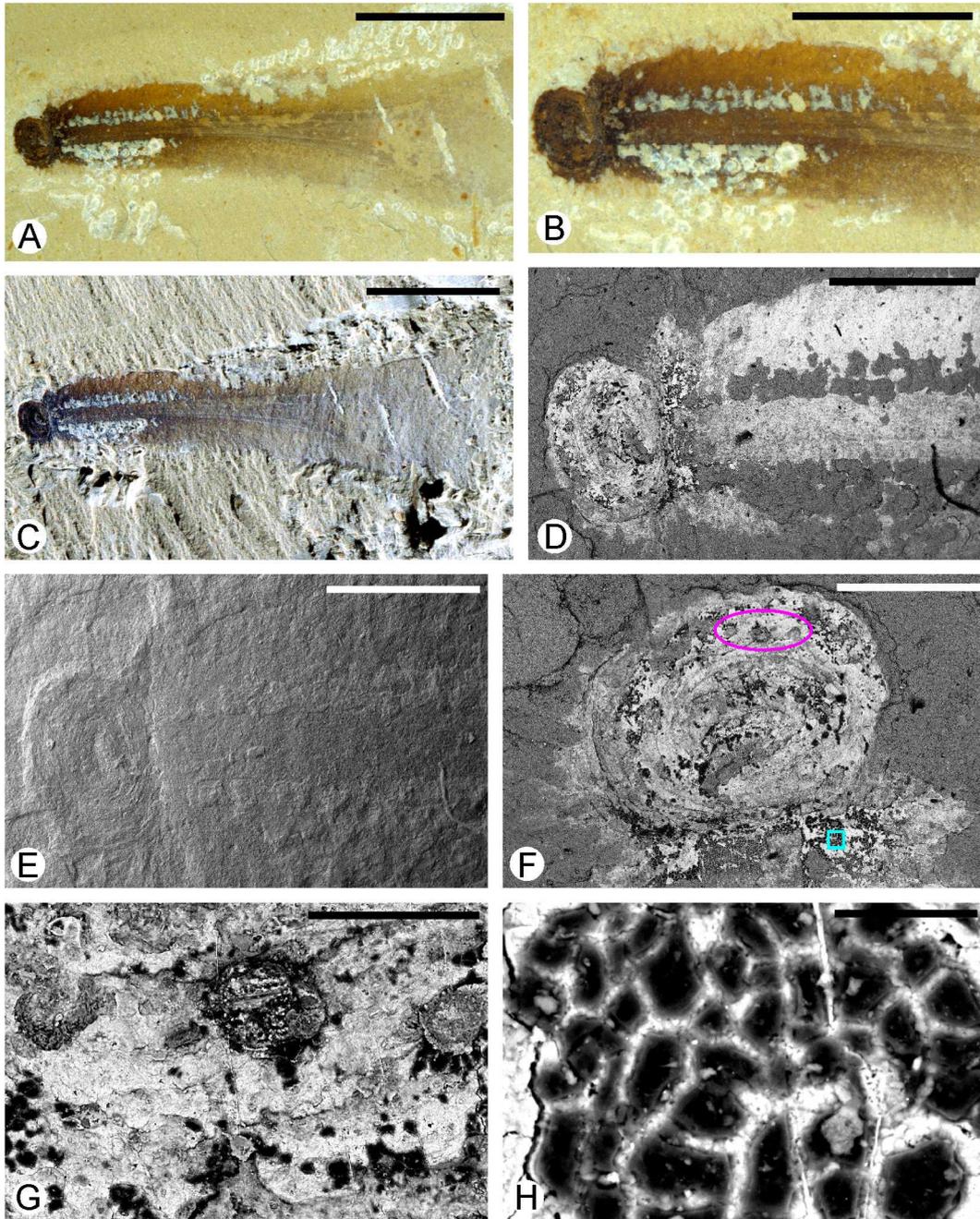


Fig. 6. *Palaeomyzon discus* gen. et. sp. nov. Holotype YKLP 11296. (A-B) The preserved anterior portion (A) and a close-up image of the anterior end (B), both taken under polarized light. (C) The preserved anterior portion taken under low-angle light, showing the intestine preserved as low relief, with two branches. (D) SEM backscatter image, showing the different chemical composition of certain features and highlighting structures. (E) Topographic SEM image, showing the concentric rings of the oral disc and the unknown structures beside the alimentary canal. (F) SEM backscatter image, showing concentric rings of the oral disc and the concentration of carbon film in this area. (G) SEM backscatter image, showing detail of round structures on the surface of the oral disc (oval area in F). (H) SEM backscatter image showing the cracked nature of the carbon film (square area in F). Scale bars= 3 mm (A, B); 2 mm (C); 1 mm (D, E); 500 μ m (F); 100 μ m (G); 20 μ m (H).

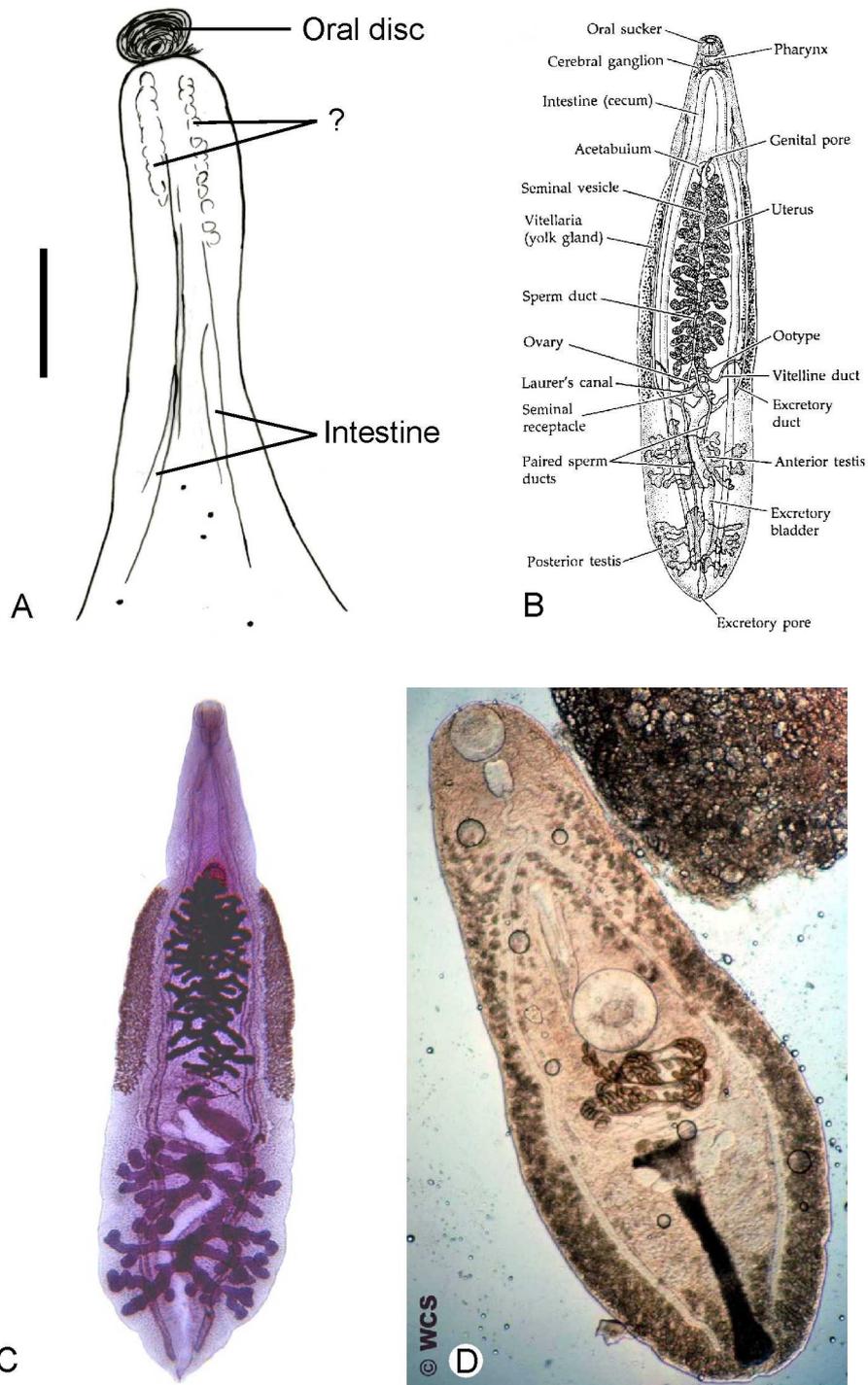


Fig. 7. Comparison between *P. discus* and extant platyhelminths. (A) Camera lucida drawing of specimen YK1p 11296. (B) A reconstruction of *Opisthorchis sinensis*, a digenetic fluke that inhabits human livers (adapted from Brusca and Brusca, 2003, pp 290, fig. 10.3B; not to scale). (C) A photo image of the same species as in (B) (image downloaded from <http://kentsimmons.uwinnipeg.ca>; not to scale). (D) A photo image of *Helicometra* sp. (image downloaded with permission from the Wikipedia website: <http://en.wikipedia.org/wiki/File:Helicometra.jpg>; not to scale). Scale bar = 2 mm (A)

Discussion: Palaeomyzon discus is distinguished from other reported fossil vermiform animals by its unique oral disc-like structure (unlike any structure reported from Lower Cambrian fossils) and divergent alimentary canal. These structures make *P. discus* comparable with some extant taxa, even though the posterior portion of the animal remains unknown. *P. discus* appears to resemble some platyhelminths (Fig. 7B-D), in that: 1) its oral disc-like structure is similar to the oral sucker of flukes (Subclass Digenea); 2) the divergence of the alimentary tract of *P. discus* may correspond to the two blind intestinal ceca that extend posteriorly along the length of the body (Ruppert and Barnes, 1996). Most platyhelminths (except turbellarians) are entirely parasitic with their oral suckers preventing dislodgment and aiding in feeding; *P. discus* may have shared a similar lifestyle.

5. Discussion and Conclusions

The similarities of these vermiform fossils to different extant phyla may indicate genuine phylogenetic relationships. If so, the new taxa reported here are the first and only fossil records for the phyla Acanthocephala and Platyhelminthes, and the earliest record of the Nematomorpha (Valentine, 2004). The addition of these three phyla to the Chengjiang biota would extend the evidence for the origin of most animals' body plans during the "Cambrian Explosion".

Furthermore, similar morphology may indicate similar function. As these three extant phyla mainly lead a parasitic lifestyle, so *A. torquatus*, *H. bolsoveri* and *P. discus* could have shared a similar mode of life. There are several possibilities: 1) the three new worms reported here are stem taxa to their respective extant parasitic phyla, sharing

similar morphological characters, but leading a free-living life; 2) they are stem taxa that also adopted a parasitic ecological niche; 3) the morphological similarities are the result of convergent evolution, which may or may not reflect similar parasitic lifestyles. The first possibility is favoured as it is the most parsimonious explanation. Importantly, possible evidence of a potential guild of parasitic animals in the Early Cambrian further indicates that complex communities and ecological relationships had developed by this time.

However, the limitations of specimen numbers and of preservation of the structures in each fossil mean that the current interpretations are provisional. It is to be hoped that the discovery of more specimens will provide further information to test the important hypotheses advanced here.

Conclusions

This study of vermiform animals from the Chengjiang Lagerstätte has contributed to our knowledge of the Lower Cambrian vermiform taxa in respect of their diversity, morphology, phylogeny, sensory anatomy, ecology and taphonomy.

1. Biodiversity in the Lower Cambrian. In this PhD project, four new genera and species have been reported from the Chengjiang Lagerstätte, *Eximipriapulidus globocaudatus* (stem priapulid), *Acanthipos torquatus* (putative stem acanthocephalan), *Hamuscolex bolsoveri* (putative stem nematomorph) and *Palaeomyzon discus* (putative stem platyhelminth). Two of them are suggested to represent the first fossil records of the Acanthocephala and Platyhelminthes. These discoveries provide further evidence for the origin of most animal phyla during the “Cambrian Explosion”.

2. Accurate morphology of the earliest vermiform species. The morphological features of the Cambrian lobopodian *Luolishania longicruris* are redescribed and reinterpreted. These new data support junior synonymy of *Miraluolishania haikouensis* and allow accurate comparison of *L. longicruris* with other lobopodian fossils and extant panarthropods. A new stem priapulid *E. globocaudatus* is preserved in exquisite detail, showing unique internal structures previously unknown from Lower Cambrian priapulid fossils. Some distinct features preserved in *A. torquatus*, *H. bolsoveri* and *P. discus* are also reported for the first time from Lower Cambrian fossils, which has helped to interpret their phylogenetic positions.

3. Deep phylogeny in the Lower Cambrian. With updated information, cladistic analysis has been used as a tool to explore the deep phylogenetic relationships among different ecdysozoan phyla. Cambrian lobopodians have been suggested to be paraphyletic or even polyphyletic, and a monophyletic group Archonchophora may be an important representative of the stem lineage leading to arthropods. Cladistic analysis has also revealed that *E. globocaudatus* is one of the most derived stem priapulids, which shares many characteristics with extant taxa.

4. Early evolution of sensory structures. During research on *L. longicruris*, some important sensory structures have been described and interpreted. These include a pair of antenniform outgrowths on the head, dense setae along the appendages, and a pair of eyes. The eyes of *Hallucigenia fortis* and *Cardiodictyon catenulum* are also reported for the first time. By applying new techniques, more detailed structures have been revealed within these eyes. Comparison with panarthropods indicates that they are not similar to the eyes of tardigrades and onychophorans, but resemble arthropod lateral visual organs. The eyes of Cambrian lobopodians represent the ancestral visual systems of arthropods and gave rise to the two major types of arthropod lateral eyes.

5. Mode of life and ecological niches of Cambrian vermiform animals. A filter feeding lifestyle has been suggested for the Cambrian lobopodian *L. longicruris*. A double anchor strategy of locomotion has been suggested for the burrowing life of the Cambrian stem priapulid *E. globocaudatus*. A possible parasitic lifestyle has been hypothesized for *A. torquatus*, *H. bolsoveri* and *P. discus*, an ecological strategy that is reported for the first time from the Lower Cambrian.

6. Exceptional preservation of Chengjiang vermiform animals. The detailed and colourful preservation of the Chengjiang vermiform specimens has made the whole project possible. SEM and EDX analysis was used as a key method to explore the chemical composition of Chengjiang vermiform specimens. Some general rules have been formulated: organic carbon is often found within the alimentary tract and within the visual organs; iron enrichment often occurs in the cuticular structures, such as the epidermis, spines, scalids, setae and head shields; some structures are also often preserved with three-dimensional information, such as a gut filled with sediment, cuticular ornamentations, even eye cups (revealed by IFM).

As with most scientific research, to answer one question often leads to many others. After this project, there will still be many puzzles remaining about early vermiform animals: 1) Many specimens are still not described, and these should reveal more morphological data regarding Lower Cambrian vermiform animals and perhaps change our understanding of their classification and phylogenetic relationships. 2) Both priapulids and lobopodians are early ecdysozoans, but what is the relationship between them? When did the first walking apparatus evolve within ecdysozoans? When did the major splits occur within ecdysozoans? 3) Sensory organs have been described in Cambrian lobopodians, but what did their central nervous systems (CNS) look like? How did visual systems evolve from the lobopodians to the arthropods? 4) What were the parasitic worms parasitic upon? Were they internal or external parasites at this stage? What was their ecological role?

All these questions will open a number of different avenues for future research projects. I would like to continue my studies of vermiform animals and hopefully to be able to answer some of these questions in the future.

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