

New evidence on the anatomy and phylogeny of the earliest vertebrates

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We report the discovery of a new agnathan specimen from the Lower Cambrian Chengjiang Lagerstätte of China and thereby provide new evidence on the myomeres (V-shaped), the branchial apparatus (gill filaments and arches), the dorsal fin and the gonads (24–26) of the earliest vertebrates. The new specimen and the co-occurring *Myllokunmingia fengjiaoa* and *Haikouichthys ercaicunensis* represent a single species, which is a primitive member of the crown group craniates (vertebrates) and post-dates the origin of the myxinoids (hagfish). The origin of the vertebrate clade is at least as old as Early Cambrian.

Keywords: vertebrates; fish; Cambrian; China; Konservat-Lagerstätte

1. INTRODUCTION

The fossil record of agnathan vertebrates prior to the Ordovician Period is very patchy and mostly controversial. Almost all of the known fossils are either scattered phosphatic elements of feeding apparatuses, primarily those of the conodonts and of putative conodont ancestors (Donoghue *et al.* 2000), or probable fragments of dermal skeletal plates (Bockelie & Fortey 1976; Smith *et al.* 1996; Young *et al.* 1996). The most significant fossils are two specimens described recently from the Early Cambrian Chengjiang biota of Yunnan Province, South China (Shu *et al.* 1999). These show the almost complete remains of soft-bodied chordates, which have been interpreted as two distinct monotypic genera, one (*Haikouichthys*) tentatively related to the lampreys (petromyzontids) and the other (*Myllokunmingia*) considered possibly to represent the sister group of the lampreys plus all the skeletonized vertebrates (Janvier 1999; Shu *et al.* 1999). We report the discovery of a third comparable specimen from the Chengjiang Lagerstätte, which provides new information on the characters and relationships of these earliest vertebrates and indicates that all three specimens belong to a single species.

2. LOCALITY AND MATERIAL

The three specimens are from the Lower Cambrian Helinpu (formerly Qiongzhusi) Formation of a single locality at Haikou, Kunming, and are preserved as nearly flat impressions on the soft mudstone surfaces. Part and counterpart are known for two of the specimens (ELI-0000201, holotype of *Myllokunmingia fengjiaoa* Shu, Zhang & Han (in Shu *et al.* 1999); and RCCBYU-00195, the new specimen), but only the part has been reported

for the other specimen (HZ-f-12-127, holotype of *Haikouichthys ercaicunensis* Luo, Hu & Shu (in Shu *et al.* 1999)). Several features are particularly well displayed on the new specimen; some of these corroborate observations made on the previous material, but others add new information or indicate a reinterpretation of structures. Taken together, the three specimens provide a fairly complete picture of the anatomy of these early chordates.

3. ANATOMY

(a) *Notochord and myomeres*

The chordate nature of the first two described fossils was based on the identification of two chordate synapomorphies, a notochord and a series of myomeres. We can find no evidence of a preserved notochord on the new specimen (figure 1), nor are we firmly convinced by the faint traces tentatively identified as representing the notochord on the other two fossils (Shu *et al.* 1999). However, myomeres are evident on all three specimens. From the new specimen it is clear that these are V-shaped, rather than sigmoidal. On RCCBYU-00195 they are represented by faint dark bands with the apices dorsal of the midline and directed forwards; these dark bands can be traced to the dorsal and ventral margins of the fossil and there is no evidence that they change direction. On specimen HZ-f-12-127, structures ventral to the midline were interpreted as portions of the myomeres that created a second, posteriorly directed, set of apices (Shu *et al.* 1999), but similar structures on the new specimen are clearly cross-cut by the dark traces of the myomeres, which are un-deflected. The existence of the myomeres is in itself sufficient to demonstrate a chordate affinity and to permit the interpretation of other structures within this context.

(b) *Gonads*

A series of dark, curved imprints near the ventral margin of the new specimen occupy shallow depressions, and lie

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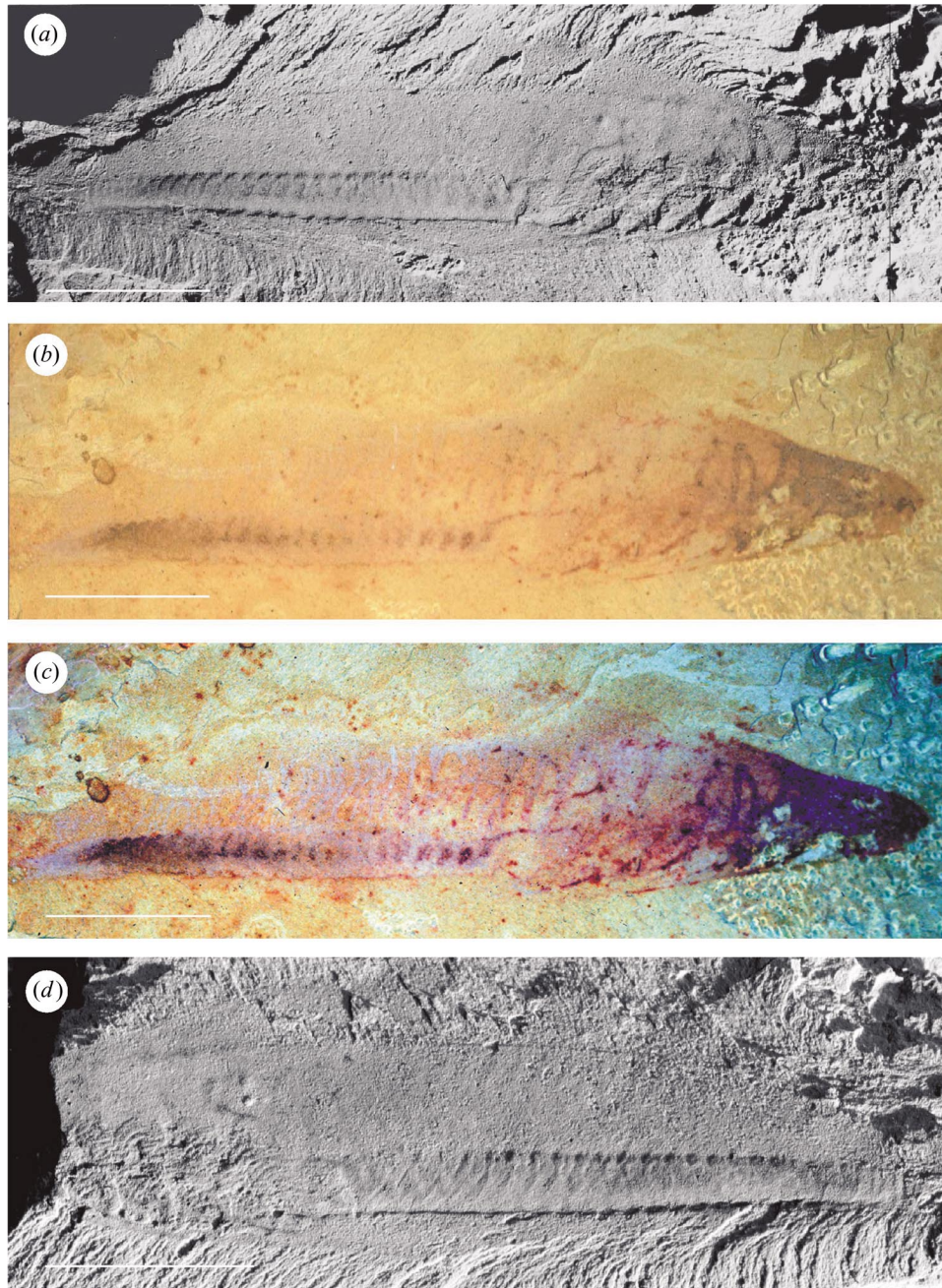


Figure 1. *Myllokunmingia fengjiaoa*, specimen RCCBYU-00195 (Yunnan Research Centre for Chengjiang Biota, Yunnan University, China). (a,d) Part and counterpart; images produced using macrophotographic apparatus on black and white film with low angle light. (b,c) Image of the part produced using macrophotographic apparatus on colour film under polarized light, with image (c) computer enhanced using ADOBE PHOTOSHOP. Scale bars (a–d), 5 mm.

dorsal to a complementary series of connected crescent-shaped ridges; these structures are interpreted as the gonads. Of these, 24 can be clearly counted (figures 1 and 2), and it is possible that there are one or two more; they appear to correspond one-to-one to the traces of the myomeres. Gonads were also tentatively labelled in a similar position on specimen HZ-f-12-127 (Shu *et al.* 1999), and we can confirm that identification, although the number present on that specimen is not clear.

(c) *Dorsal fin*

A dorsal fin is clearly present on all three specimens; although the posterior termination is not clear, the morphology of the preserved portion of the fin indicates that

it is a distinct structure. In the previously described material, an important distinction was made between the fin on specimen ELI-0000201, which is smooth, and that on HZ-f-12-127, which has what were reported to be fin-radials, anomalously inclined forwards (Shu *et al.* 1999). The new specimen also shows a set of fine linear structures in the fin (figure 1d); these are only apparent on the counterpart, where they have a similar spacing and anterior inclination to the lines in HZ-f-12-127. However, these lines are clearly part of a more extensive fabric that continues across the body of the fossil and also beyond its dorsal margin (figure 1d). This appears to be a sedimentary or structural fabric that is not expressed in the part, where the dorsal fin is smooth. This observation appears

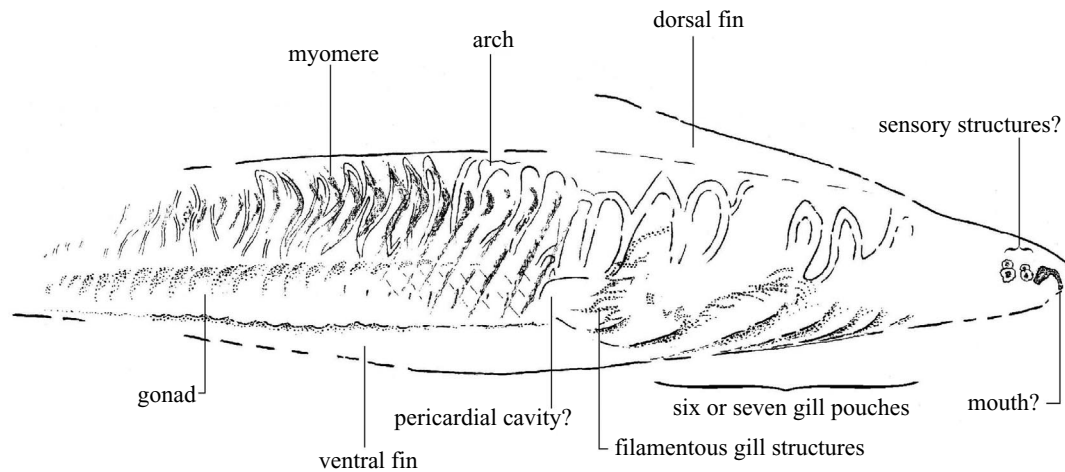


Figure 2. Composite camera lucida drawing to show interpretation of features, based primarily on the part, but with the myomeres added mostly from the counterpart, where they are clearer. Scale bar, 5 mm.

to refute identification of these structures as fin-radials in both the new specimen and in HZ-f-12-127, where the published photographs and diagram also seem to show them extending faintly into the body of the animal. It is just possible, however, that a structure in the fossil is controlling the surface fabric of the rock where it has split; if this is the case, the effect on RCCBYU-00195 is restricted to the counterpart.

(d) *Ventral fin*

A smooth, narrow ventral fin-fold, gradually tapering posteriorly, is evident on the new specimen; it lies flat and there is no indication that it is paired. A pairing of the ventral fin was interpreted for both previous specimens (Shu *et al.* 1999), either on the basis of a tilting of the fin relative to the trunk (specimen ELI-0000201) or by a 'rather abrupt scarp' (specimen HZ-f-12-127). We are unable to confirm these interpretations, which have also been questioned elsewhere (Holland & Chen 2001).

(e) *Gut*

A darkened area with some positive relief in the ventral portion of ELI-0000201 has been interpreted as a gut trace, and there is a more equivocal suggestion of the preservation of some of the gut in HZ-f-12-127 (Shu *et al.* 1999). There is no evidence of a preserved gut on the new specimen.

(f) *Branchial basket, gill pouches and gill filaments*

A set of four to six gill pouches with hemibranchs was identified on ELI-0000201, and six to nine 'rods' of a branchial basket on HZ-f-12-127, where no gills or filaments were distinguished (Shu *et al.* 1999). The 'rods' have subsequently been interpreted as elements of a visceral arch skeleton (Holland & Chen 2001). On the new specimen, both the 'rods' of the visceral arches and the pouches are clearly visible on the part and counterpart (figures 1 and 2); there are certainly six, and maybe as many as seven pouches. The interiors of the pouches display filamentous gill structures with feather-like terminations, which appear to comprise bilateral sets of filaments branching from an axis (figures 1*a,d* and 2). In addition,

we identify here for the first time, to our knowledge, a much more extensive development of the arched structures. These arches begin immediately behind the head and extend posteriorly for the total length of the preserved specimen; dorso-ventrally they span from near the dorsal margin to the upper limit of the gonads. They are conjoined, with rounded to slightly angular tops and acute V-shaped lower connections (figures 1*b,c* and 2). They are clear on both part and counterpart and portions of at least 25 can be counted, although they fade posteriorly. These structures are present, although much less clearly displayed, on both the previously described specimens, and were sketched, but not commented on, in the published descriptions (Shu *et al.* 1999). The posterior and dorsal extension of these arches is similar to that shown by juvenile specimens of amphioxus.

(g) *Pericardial cavity*

A possible pericardial cavity was identified on both previous specimens (Shu *et al.* 1999), and an equivalent structure is apparent in the same position on the new specimen (figures 1*b,c* and 2).

(h) *Structures of the head*

A head is clearly differentiated at the anterior of all the specimens as a region of darker preservation; the posterior limit of the head coincides with a distinct change in slope of the dorsal profile of the new specimen. A suite of possible cartilages was identified on HZ-f-12-127 (Shu *et al.* 1999) but, although there are several areas of differential colouring or relief, we are hesitant to identify these as distinct structures on the new specimen. There is, however, a noticeable, very dark patch near the anterior in the area where a mouth would be expected, and two pairs of small rounded structures situated immediately posterior of this possibly represent optic and otic capsules (figures 1*b* and 2). The more posterior pair is in an almost identical position to features interpreted as eyes on the Carboniferous lamprey *Hardistiella* (Janvier & Lund 1983), and on the Carboniferous hagfish *Myxiniakela* (Bardack 1991).

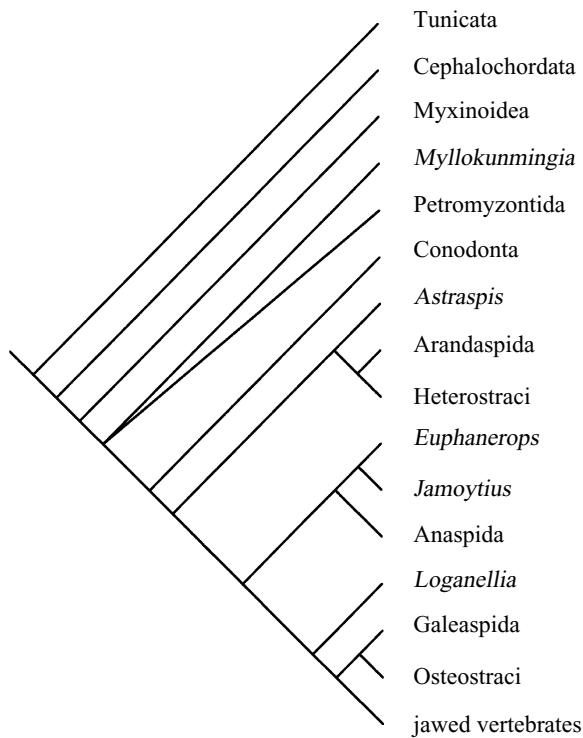


Figure 3. Fifty per cent majority rule consensus tree based on morphological characters, equally weighted, of living and fossil vertebrates, including *Myllokunmingia*. Tunicates and cephalochordates are outgroups.

4. CONSPECIFIC NATURE OF THE MATERIAL

The previously described specimens were assigned to separate taxa primarily on the basis of the more prominent dorsal fin with fin radials in HZ-f-12-127 (Janvier 1999; Shu *et al.* 1999). Other differences highlighted included the more slender form of HZ-f-12-127 and the contrasts in the gill structures. The new specimen shows that the gill features preserved separately in the two previous specimens actually occur within the same animal. More importantly, the demonstration that the apparent fin rays are almost certainly the expression of a lithological fabric and that in any event they can be preserved only on the part (or on the counterpart) of a single specimen precludes their use in discriminating between taxa. The differences in shape between the three specimens are trivial and, therefore, we regard them as conspecific. The first taxon named was *M. fengjiaoa* Shu, Zhang & Han (in Shu *et al.* 1999), and, as first revisers, we consider this to be the appropriate name for all three specimens. The name given to specimen HZ-f-12-127, *H. ercaicunensis* Luo, Hu & Shu (in Shu *et al.* 1999), becomes a subjective junior synonym.

5. EVOLUTIONARY SIGNIFICANCE

The reinterpretation of characters of *Myllokunmingia* necessitates a reconsideration of its phylogenetic position and significance. The animal is clearly a chordate, and the possession of probable paired sensory structures in the head indicates that it is a vertebrate. In terms of more advanced features, we can discard the possession of fin radials, but add the presence of filamentous gills. Other characters, such as the V-shaped myomeres and the series

of gonads (interestingly close in number to the 26 present in the cephalochordate *Branchiostoma*) are plesiomorphic for vertebrates, while the presence of a distinct dorsal fin indicates a more derived condition than in the hagfish. Pairing of the ventral fin-fold, if confirmed, is an important character, and it would be of significance if this were demonstrated by these fossils to be a primitive feature (Shu *et al.* 1999); however, we cannot find evidence of pairing on the new specimen.

We have included *Myllokunmingia* in a phylogenetic analysis using MACCLADE (Maddison & Maddison 1992) and PAUP (Swofford 1998), based on the most recently published data matrix for extant and fossil agnathans (Donoghue *et al.* 2000). The results give 10 equally parsimonious trees, five of which show *Myllokunmingia* as the sister group to the lampreys plus skeletonized vertebrates, and five of which show *Myllokunmingia* as sister group to the lampreys. A 50% majority rule consensus tree of the 10 trees is shown in figure 3. Whichever the position favoured, this analysis indicates that *Myllokunmingia* is a primitive member of the crown group craniates (vertebrates according to most authors), that it post-dates the origin of the myxinooids and that the origin of the vertebrate clade is at least as old as Early Cambrian.

Additional resolution to these relationships may come from molecular data, which increasingly seem to indicate that hagfish and lampreys form a monophyletic group, the cyclostomes (Mallat & Sullivan 1998; Mallat *et al.* 2001), with an estimated divergence time for the hagfish and lampreys at 499 ± 38 Myr ago in the Late Cambrian (Blair Hedges 2001). *Myllokunmingia* pre-dates this suggested divergence, so, if the morphological evidence that it is more derived than the hagfish is sustained, then *Myllokunmingia* may represent the most primitive of the stem-group gnathostomes.

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