Three-dimensionally preserved minute larva of a great-appendage arthropod from the early Cambrian Chengjiang biota

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A three-dimensionally preserved 2-mm-long larva of the arthropod *Leanchoilia illecebrosa* from the 520-million-year-old early Cambrian Chengjiang biota of China represents the first evidence, to our knowledge, of such an early developmental stage in a short-great-appendage (SGA) arthropod. The larva possesses a pair of three-fingered great appendages, a hypostome, and four pairs of well-developed biramous appendages. More posteriorly, a series of rudimentary limb Anlagen revealed by X-ray microcomputed tomography shows a gradient of decreasing differentiation toward the rear. This, and postembryonic segment addition at the putative growth zone, are features of late-stage metanauplii of eucrustaceans. *L. illecebrosa* and other SGA arthropods, however, are considered representative of early chelicerates or part of the ancestor of Euarthropoda. The larva of an early Cambrian SGA arthropod with a small number of anterior segments and their respective appendages suggests that posthatching segment addition occurred in the ancestor of Euarthropoda.

Results

The specimen was discovered by separating two opposing slabs (YKLP 11088a, b; Fig. 1, Insets). Slab a (YKLP 11088a) exhibits the specimen from a ventral perspective, but, because the two slabs separated from each other along the dorsal side of the specimen, it shows details of the dorsal morphology (Fig. 2). The SGAs are preserved only on this slab (cf. Figs. 3 and 4), where two fingers of each SGA are exposed on the surface (Fig. 2 A–C). Fluorescence microscopy revealed spine-like armatures on one finger (Fig. 2 C and D) and setae along the outer edge of the exopod of the first post-SGA appendage on the left side of the animal (Fig. 2 C and E). A third finger, hidden inside the slab, was resolved with microcomputed tomography (micro-CT) (Fig. 2F). Body segmentation is indicated by the insertion of the appendages (Fig. 2 A and B). In addition to the segments bearing the eye and SGA, 14 post-SGA segments are identified (Fig. S2). The outline of the anterior portion of the body (from eye segment to fourth post-SGA segment) is somewhat oval, whereas the posterior portion (fifth to 14th post-SGA segment) is much narrower (Fig. S2C).

Significance

Understanding the nature of the Cambrian radiation involves knowing not only the morphologies of adult animals but also their developmental pathways. However, fossil evidence of early larvae is rare. Here we describe a well-preserved 2-mm-long larva of the short-great-appendage arthropod *Leanchoilia illecebrosa* from the early Cambrian Chengjiang biota. The exceptional 3D preservation has allowed our microcomputed tomography analyses to resolve a series of rudimentary limb Anlagen in the posterior portion of the larva—an arrangement resembling that in late-stage eucrustacean metanauplii. *L. illecebrosa* is considered an early representative of either chelicerates or of euarthropods as a whole. Therefore, this discovery provides fossil evidence that posthatching segment addition is a feature rooted in the ancestor of Euarthropoda.


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Slab b (YKLP 11088b) exhibits the animal from a dorsal perspective (Figs. 3 and 4). The anteriormost structure of the specimen shown on this slab is a somewhat rounded, 150-μm-long hypostome (Figs. 3B and 4 C and F). Although the exopods of the first four pairs of biramous appendages (following the SGA) are well preserved on the surface of slabs a and b (Figs. 2E and 3D), the endopods of these appendages are concealed within slab b and revealed by micro-CT (Fig. 4). Around seven podomeres (sensu 13), each 50–60 μm long, are evident in the endopod of post-SGA appendages 2–4 (Fig. 4 E and F). Post-SGA appendages 5 (left and right) and 8 (left) are incomplete, each exhibiting a basipod and posteriorly directed endopod consisting of several podomeres (see Fig. 4F and Fig. S3). Exopods of these appendages were probably lost during fossilization, as were both branches of post-SGA appendage 6 left and right, 7 left, and 8 right (Fig. S3). All of the more posterior appendages are much less differentiated than the first eight post-SGA appendages; each of post-SGA appendages 9–13 appears as a single flap-like rudimentary Anlage, their length decreasing toward the posterior, suggesting a decreasing gradient of differentiation (Fig. S3). The rear end of the specimen is represented by a telson whose anterior part is an enlarged area (Fig. S3) that is thought to have accommodated the...
Fig. 3. Minute larva of *L. illecebrosa* (slab b, YKLP 11088b). Dorsal perspective. (A) Macrophotograph documenting four pairs of appendages (l1–l4, r1–r4) in the oval-shaped anterior part of the larva and a much narrower posterior part ending in a dagger-like telson (te). (B) SEM additionally revealing the hypostome (hy; see Fig. 4 C and F) and the setae or seta-like armatures on the exopods (see C and D) and the telson (see F). (C) Fluorescence microscopy showing details of the setae or seta-like armatures. (D) Close-up showing setae or spines in the exopod (ex) of post-SGA appendages 3 (r3) and 4 (r4) on the right side of the larva. The basipod (bas) and endopod (en) of r4 are also shown. (E) Close-up showing several setae along the margin of the basipod of r4. (F) Close-up showing several setae along the margin of the telson. [Scale bars: 0.5 mm (A–C); 0.1 mm (D and F); 0.05 mm (E).]

Discussion

Tiny specimens such as that reported here are rare in the early Cambrian Chengjiang biota, which is dominated by flattened arthropod fossils in the size range of several centimeters (10). We interpret the specimen as a larva (*sensu* 12) of *L. illecebrosa* based on (i) the occurrence of adult specimens at the same locality (see Methods Summary); (ii) the spine-like armatures on the three-fingered SGA (Fig. 2 C and D)—a diagnostic character of the larval form of *L. illecebrosa* (12); and (iii) the overall morphology, including the telson with a dagger-like terminal end piece whose margin bears several setae (Figs. 3 C and F and 4). However, another diagnostic feature of this species—the short, pointed rostrum that forms the front end of the head shield (10)—is absent in the larva specimen and may not have developed by this ontogenetic stage (Fig. 2 A–C).

By combining the information from both slabs (see Results), we conclude that the first four pairs of biramous appendages of the larva are well developed and, most likely, fully functional. An oval space enclosed by the hypostome and the basal parts of post-SGA biramous appendages 1–4 would have played a role in feeding by gathering food items trapped by the SGAs (12) (Figs. 3B and 4). The paddle-like exopods (Fig. 2E) were probably used for swimming, as in the adult (13). Although information about post-SGA appendages 5–8 is very limited due to the preservation of the specimen, an elongate endopod with several podomeres is evident in these appendages (Fig. S3). This suggests that post-SGA appendages 5–8 are also developed as limbs but not to the same extent as the more anterior ones (Fig. 4F and Fig. S3). A significant size decrease is evident in post-SGA appendages 9–13, each of which is preserved as a small, unbranched flap (Fig. S3). Post-SGA appendage 14 is represented by just a pair of flattened triangular structures that are associated with a segment emerging from a putative growth zone located anterior to the anus. Such a strong size gradient is not observed in the appendages of the adult (10, 13) but is considered to be real, as all of the Anlagen are revealed by two or more different 3D softwares such as Drishti (Fig. 4) and Fiji (Fig. S3), and are presumably preserved in the same manner. Adults of *L. illecebrosa* have 11 trunk segments, each bearing a pair of biramous appendages (10, 13). Although previous interpretations identified three pairs of biramous appendages posterior to the SGA in the adult head (13), our recent investigation of new material suggests four pairs (Fig. S4). This gives a total of 15 pairs of biramous appendages in the adult, and further suggests that one additional appendage-bearing segment remained to emerge from the growth zone in the larva.

The developmental mode revealed by the minute larva of *L. illecebrosa* is similar to that in many extant eucrustaceans. Eucrustaceans (e.g., prawns, lobsters, brine shrimps, and barnacles) are so diverse morphologically that they share just a few specific and unique characters—one of which is the nauplius larva (14). Right after hatching, the nauplius (15) bears three pairs of functional appendages (14, 15). In subsequent stages, further segments and their appendages emerge from a growth zone in the posterior portion of the body (15, 16). The minute larva of *L. illecebrosa* resembles the late-stage metanauplius of certain eucrustaceans, especially anostracans (17), other branchiopods, and cephalocarids (14), in several aspects. First, the...
anterior (post-SGA segments 1–4) and posterior (post-SGA segments 5–14) portions of the body differ in outline (rounded vs. narrow and elongated; Fig. 3 and Fig. S2). This is not a result of flattening: The outline of soft-bodied fossils is not subject to distortion as they collapse during decay (18). Second, the anterior and posterior portions of the body also differ in the stage of development of the appendages (well developed and presumably fully functional vs. less developed (post-SGA appendages 5–8) and even rudimentary (post-SGA appendages 9–14); see Figs. 3 and 4 and Fig. S3.

The SGA arthropods are considered either as early representatives of chelicerates (11, 12, 19, 20) (e.g., sea spiders, horseshoe crabs, spiders, scorpions, and mites) or stem lineage representatives of all euarthropods (21) (chelicerates, myriapods, and others).
crustaceans, and insects). Therefore, this discovery represents the oldest example of well-developed appendages preserved in the larva of an early nonmandibulate arthropod—more than a Cambrian Konservat-Lagerstätte and 4) or Fiji (5), which became Henningsmoenicaris scutula from the late Cambrian (6).

The studied specimens (YKLP 11087, YKLP11088a, b, YKLP 11089, 11094) are housed at the Yunnan Key Laboratory for Palaeobiology, Yunnan University, Kunming, China. Both specimens were collected from the Yu'anshan Member of the Chiungchussu Formation (Cambrian Series 2, Stage 3) at Mafang Village, Haikou, Kunming, Yunnan Province, China.

**Imaging.** To observe as many details as possible, we used a combination of microphotography (MP), scanning electron microscopy (SEM) and fluorescence microscopy (FM) to reveal the structures on the surface of the fossil slabs (Figs. 2 and 3). We also used X-ray micro-CT to resolve structures hidden inside the slabs (Figs. 2F and 4). Digital MP images were captured with an MP-E 65-μm macro objective attached to a Canon EOS Rebel T3i digital camera. For SEM analyses, a Leo 1430 VP was used with 100-μm aperture, acceleration voltage of 30 kV, chamber pressure between 2 pa and 16 pa, and four quadrant back-scattered electron (QBSE) detector. Fluorescence photographs were generated as a composite fluorescence image with a Keyence BZ-9000 fluorescence microscope. A stacked holographic method was used to record each specimen and fused as a composite image with Microsoft Image Composite Editor or the Photomerge function of Adobe Photoshop CS3. For micro-CT scanning, each slab was mounted on a holder made of tightly fitting plastic vials. A Phoenix Nanotom (GE Sensing & Inspection Technologies) cone beam CT scanner located at the Bavarian State Collection of Zoology, Munich, was used at a voltage of 140 kV and a current of 120 μA (Fig. 2F) and 100 kV/70 μA (Fig. 4 and Fig. S3) for 47 min each. A total of 1,440 radiographs were registered in each scan and saved as TIFF stacks for further processing with either Drishti (Figs. 2F and 4) or Fiji (Fig. S3). The figures were arranged in Canvas Draw.

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**Methods Summary**

**Materials.** The studied specimens (YKLP 11087, YKLP11088a, b, YKLP 11089–11094) are housed at the Yunnan Key Laboratory for Palaeobiology, Yunnan University, Kunming, China. Both specimens were collected from the Yu'anshan

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