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An early Cambrian pelago-benthic acorn worm and the origin of the hemichordate larva

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ABSTRACT

Enteropneusts (acorn worms) are hemichordates, the sister group to echinoderms. Together they form the clade Ambulacraria, which is closely related to chordates. All three groups appear in the lower Cambrian, but their interrelationships remain problematic, which impedes the understanding of early deuterostome evolution. Enteropneusts are also extremely rare in the fossil record, only a few species are known from Lagerstätten-type deposits. Here, we describe the earliest known enteropneust, *Cambrobranchus pelagobenthos* gen. et sp. nov. based on soft-bodied specimens, including tornaria larvae and juveniles, from the lower Cambrian (Epoch 2, Stage 3) Haiyan Lagerstätte, Chengjiang biota, of China. The enteropneust larvae and post-metamorphic juveniles are the first reported in the fossil record and provide direct evidence for a pelago-benthic lifestyle in a Cambrian deuterostome animal, bolstering the hypothesis that an indirect development is primitive to the enteropneusts and maybe the hemichordates or whole of Ambulacraria.

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INTRODUCTION

A complex, biphasic life history including a benthic, reproductive adult and a pelagic, feeding larva is phylogenetically widespread among extant marine taxa (Thorson, 1950; Mileikovsky, 1973; Levin, 2006; Cowen and Sponaugle, 2009; Dyachuk and Odintsova, 2013). Larvae contribute to the survivorship of species by diversifying both larval and adult life forms and thereby the habitat and resources available. The larval stage is usually morphologically distinct from the adult and the similarities in larva between some phyla are generally attributable to either shared ancestry or to convergence due to small size, mechanical, and developmental constraints (Haug, 2020). For these reasons, larvae provide an overlooked role in unlocking metazoan ecology, evolution, and diversification. The origin of larvae is widely debated and at the root of our understanding of animal evolution. The debate entails whether Cambrian animals were holobenthic and the planktotrophic larva added later, or pelago-benthic with a distinct larval stage (Signor and Vermeij, 1994; Peterson et al., 2005; Wolfe, 2017).

We describe the previously unknown lower Cambrian acorn worm, *Cambrobranchus pelagobenthos*, based on 39 specimens from the Haiyan Lagerstätte of China. Juveniles, adults, and tornaria larva fossils are preserved, providing direct evidence for a pelago-benthic life history early in hemichordate evolution (Figures 1–3, Appendices 1 and 2). This exceptional finding from one of the premiere fossil localities in the early Cambrian, the Haiyan Lagerstätte of the Chengjiang biota (Yang et al., 2021), shows that animals were exploiting benthic and pelagic Cambrian habitats at different life stages.

MATERIALS AND METHODS

Terminology

We follow the morphological terminology used in previous studies of fossil and extant enteropneusts (Urata and Yamaguchi, 2004; Caron et al., 2013; Urata et al., 2014; Nanglu et al., 2016, 2020a; Gonzalez et al., 2017, 2018).

Studied Material

The specimens reported here were collected as part of a broader study of the Haiyan Lagerstätte (Yang et al., 2021), a 40 cm thick interval of alternating event and background mudstone bed sets, near the bottom of the Yu'anshan Member, Chiungchussu Formation (Cambrian, Epoch 2, Stage 3), on the eastern shore of Dianchi Lake, near Kunming, ~24 km northwest of Chengjiang County, China. The enteropneusts were recovered from the lowermost 5 cm of the Lagerstätte interval. To minimize bias (Whitaker and Kimmig, 2020), sampling efforts collected all visible, (principally) complete fossils.

Close-ups and microphotographs were taken with a Leica DFC 500 digital camera mounted on a Leica M205-C stereoscope. Macrophotographs of the fossils were taken with a Canon EOS 5D digital SLR camera equipped with a Canon 50 mm macro lens and cross-polarized lights.

Scanning Electron Microscopy and Energy-Dispersive X-Ray Spectroscopy Analyses

The backscattered electron (BSE) imaging and energy-dispersive X-ray spectroscopy (EDX) of uncoated specimens was conducted with a FEI Quanta 650 FEG field emission scanning electron microscope (SEM) at the Yunnan Key Laboratory for Palaeobiology, Institute of Palaeontology, Yunnan University, Kunming, China (YKLP). All imaging analyses were conducted with the following operating conditions: 14 mm ± 1 mm working distance (minor differences to allow for variation in sample thickness or topography) for basic imaging and EDX, 20 keV beam accelerating voltage, 10 nA beam current, 20 Pa chamber pressure (low vacuum), 50 µm aperture for imaging, and 40 µm aperture for EDX analysis.

X-Ray Tomographic Microscopy

One specimen (YKLP14443) was scanned using an Xradia MicroXCT-520 Versa system (Carl Zeiss XRM) at YKLP. The scan was conducted with the source operating at 70 kV, 6 W, pixel size 6.95 µm over 206º sample rotation (–103º to 103º), with no filter, for 204 minutes. The exposure time of each projection was 4 seconds. Geometric and optical magnification settings were chosen to collect projections with XY pixel dimensions of 6.95 µm. During the scan,1572 radiographs were registered and saved as TIFF stacks for 3D reconstruction with Drishti 2.4 software.

Phylogenetic Analysis and Ancestral State Estimation

We performed phylogenetic analyses using a modified version of the morphological character matrix published in Nanglu et al. (2020a). The data matrix consists of 29 taxa and 113 characters (Appendix 3). The Bayesian phylogenetic analysis was performed using MrBayes 3.2.6 with the following search parameters: 10 million generations, sampling every 1000 generations, 25% burn-in, using an mkv model with a gamma distribution. Additionally, we performed a parsimony analysis, the strict consensus tree for which is presented in the Appendix 1. The Appendixes 3 and 4 includes a detailed list of characters used in both analyses based on Cameron (2005) and Nanglu et al. (2020a). To test the hypothesis that a bi-phasic lifestyle is ancestral for both Hemichordata and Ambulacraria, we used MrBayes to empirically estimate ancestral states for nodes of interest, as detailed by Huelsenbeck and Bollback (2001). This approach allowed us to account for uncertainty in tree topology and all other parameters. For this, and Bayesian phylogenetic analysis, Tracer 1.7.1 (Rambaut et al., 2018) was used to confirm that runs had converged (i.e., effective sample size for all parameters > 200; average standard deviation of split frequencies < 0.01; estimates for all parameter values were ~ normally distributed). Results are detailed in the Appendix 1.

Repositories and Institutional Abbreviations

Types, figures, and other specimens examined in this study are deposited in the following institution: Yunnan Key Laboratory for Palaeobiology, Institute of Palaeontology, Yunnan University, Kunming, China (YKLP).

SYSTEMATIC PALEONTOLOGY

Phylum HEMICHORDATA Bateson, 1885 (emend. Fowler, 1892)
Class ENTEROPNEUSTA Gegenbaur, 1870
Genus *CAMBROBRANCHUS* gen. nov.

zoobank.org/7176AE24-7C5C-407A-BB93-E1B7FFC637E7

Type species. *Cambrobranchus pelagobenthos* gen. et sp. nov.

Cambrobranchus pelagobenthos gen. et sp. nov.
Figures 1A-F, 2A-D, 3A-M, 4A-P, 5

zoobank.org/A0088DD9-1F6F-4666-BFF6-5430FF782D87

Type material. Holotype, YKLP14443 (Figure 1A-C, Appendix 1) from the Yu'anshan Member, Chiungchussu Formation, Cambrian, Epoch 2, Stage 3, *Wutingaspis–Eoredlichia* Biozone, on the eastern shore of Dianchi Lake, near Kunming, ~24 km northwest of Chengjiang County, China (Repository: YKLP). Paratypes, YKLP14529–14537, 14543, 14544 (Figures 1D-F, 3A-H, Appendix 1).

Other specimens. YKLP14538–14540, 14542, 14545–14568 (Figure 3M, Appendix 1).

Diagnosis. An enteropneust possessing a tripartite vermiform body consisting of proboscis, collar, and trunk. Proboscis approximately two times longer than wide; collar approximately two times longer than wide. A series of gill and tongue bars (GB+TB) accounting for 1/3–1/2 of the length of the trunk. The trunk is usually U-shaped or sinuous. Gut is straight with a post-anal organ structure. Indirect development with a tornaria larva.

Etymology. Genus name *Cambro*, for the period (Cambrian) in which the fossils are found; *branchus* for gills. Species name *pelagobenthos* (Latin), for the lifestyle.

Type locality and horizon. Bottom 5 cm of the Haiyan Lagerstätte (Yang et al., 2021), Yu'anshan

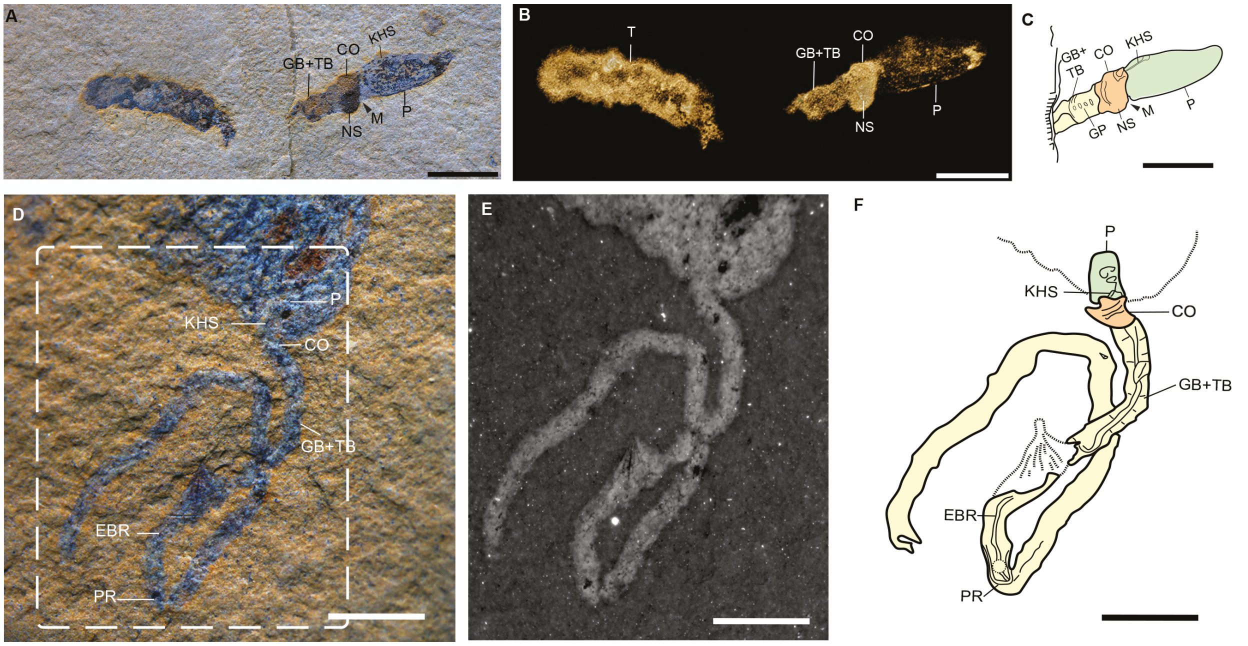

FIGURE 1. Adult specimens of *Cambrobranchus pelagobenthos* from the Haiyan Lagerstätte of China. **A**, Part of the holotype, YKLP14443. **B**, X-ray tomographic image of YKLP14443. **C**, Interpretative drawing of the anterior part of A. **D**, A well-preserved individual attached to an uncertain sclerite, YKLP14529. **E**, Fluorescent microscopy image of the white dashed area in C. **F**, Interpretative drawing of C. Scale bars, 2 mm (A–C, G), 1 mm (D–F). CO, collar; EBR, epibranchial ridge; GB+TB, gill and tongue bars; GP, gill pore; KHS, kidney–heart–stomochord complex; M, mouth; NS, nuchal skeleton; P, proboscis; PR, parabranchial ridges; T, trunk.

Member, Chiungchussu Formation, Cambrian, Epoch 2, Stage 3, *Wutingaspis–Eoredlichia* Biozone, on the eastern shore of Dianchi Lake, near Kunming, ~24 km northwest of Chengjiang County, China.

Description. Specimens are entirely soft-bodied and typically preserved as two-dimensional compressions. Body is tripartite, divided into proboscis, collar, and trunk. Specimens range from 500 μm to 33 mm in total length. The outer surface of the trunk preserves GB+TB, along with gill pores. There is an ovoid post-anal organ. The extension of GB+TB extends to one third or half of the trunk (Figure 1D-F, Appendix 1). The post-anal organ of a few specimens is adhered to unidentified biological fragments (Appendix 1). In some cases, individuals are found in clusters on a single slab (Appendix 1), co-occurring with tornaria larvae (Figure 3A, C) and numerous juveniles (Figure 3E-H, M).

The proboscis is well-preserved in almost all specimens, excluding tornaria, and is usually contracted, the exception being the YKLP14443 and YKLP14534 (Figure 1A, Appendix 1) where it is extended. The proboscis is conical to cylindrical in shape (Figures 1A-F, 2A-D, 3E-H, M, Appendix 1). There is an axial zone within the proboscis coelom (Figures 2A, 3M, Appendix 1) and at its anterior, an irregular elliptical region with a slight bulge, usually darker or more reflective than its surroundings (Figure 3M, Appendix 1). The length of this major axis varies from 0.1 mm to 0.4 mm. The structure is herein interpreted as kidney–heart–stomochord complex (KHS) (Figures 1A-F, 2A-D, 3E-G, M, Appendix 1), as it is in the same area as the KHS in extant enteropneusts (Balser and Ruppert, 1990) and fossil species from the Cambrian Burgess Shale (Caron et al., 2013; Nanglu et al., 2016, 2020a).

A 360-degree collar annulation with a raised outline attaches to the anterior of the trunk. The holotype collar is twice as long as wide (Figure 1A-C), and the length to width ratio of the proboscis, collar, and trunk are 2.5, 1.25, and 9, respectively (Appendix 1). Collar ridges are discernable at the anterior and posterior margins (Appendix 1), and many transversally corrugated bands are preserved. There are at least six rows of corrugated bands in specimen YKLP14529. The bands can also be found in some of the juvenile specimens (Figure 3E-H, M).

The dorsal branchial-pharynx region of the trunk is comprised of thick and thin gill bars alter-

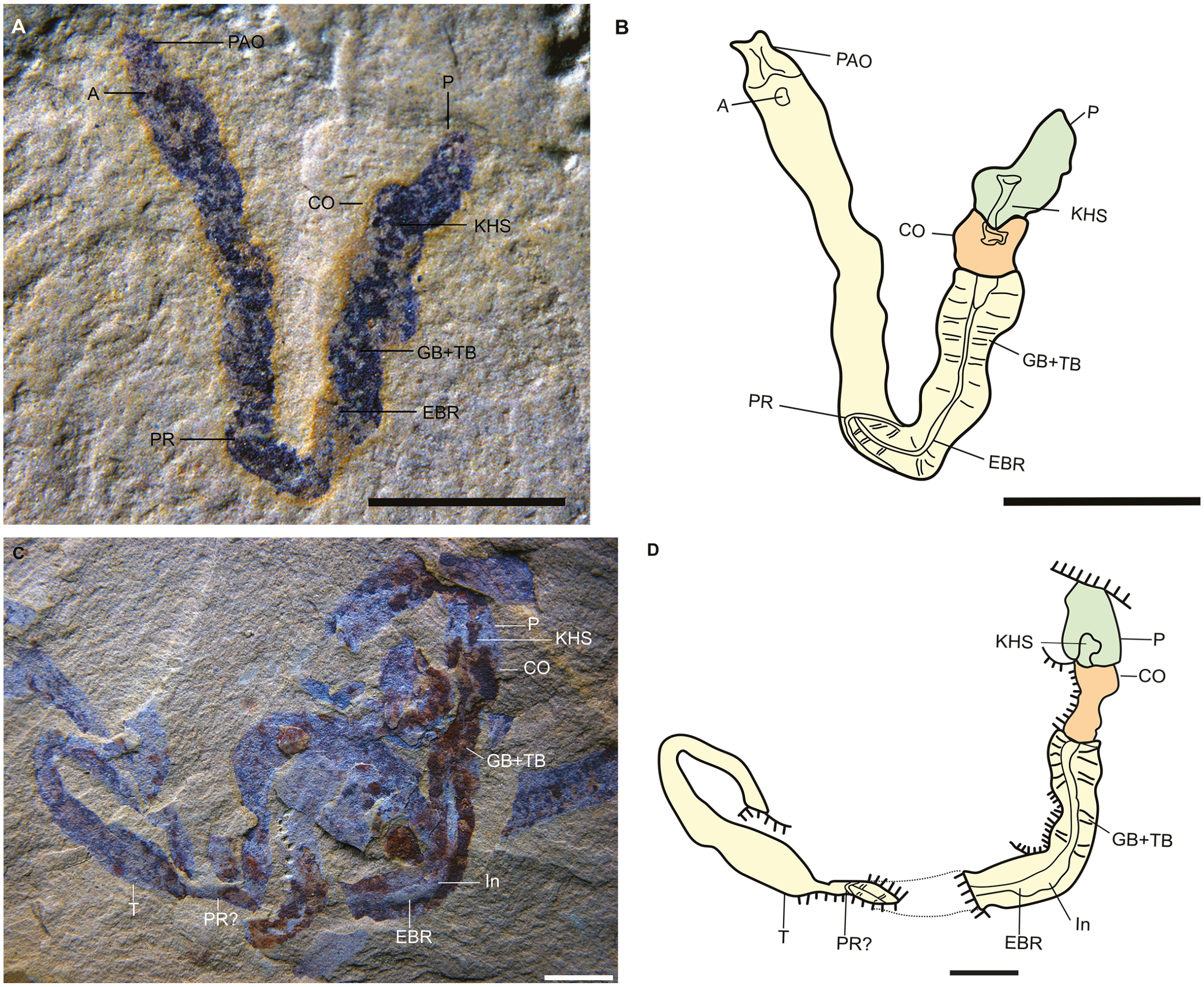

FIGURE 2. Adult specimens of *Cambrobranchus pelagobenthos* from the Haiyan Lagerstätte of China. **A**, U-shaped individual with preserved post-anal organ, YKLP14530. **B**, Interpretative drawing of A. **C**, Nearly complete curved specimen, co-occurring with some incomplete specimens, YKLP14531. **D**, Interpretative drawing of C. Scale bars, 1 mm. A, anus; CO, collar; EBR, epibranchial ridge; GB+TB, gill and tongue bars; In, intestine; KHS, kidney–heart–stomochord complex; P, proboscis; PAO, post-anal organ; PR, parabranchial ridges; T, trunk.

nating, herein interpreted as the GB+TB. Few of the gill slits have been completely exposed due to the taphonomy and possible ecology of the animals, but in specimen YKLP14545 (Appendix 1), a laterally compressed adult, there are about five sets of GB+TB for nearly every 500 μm (Appendix 1). The GB+TB are paired and symmetrically arranged on either side of an epibranchial ridge (EBR) (Figure 1D-F, Appendix 1). The gill slits are defined by gill bars and connect to the outside gill pores by atrial cavities. At least two to four gill pores can be identified in our collection (Figure 3H, L, Appendix 1). The parabranchial ridges (PR) merge posteriorly (Figure 1D-F, Appendix 1). A single pair and serial pairs of GB+TB are well-preserved in YKLP14568 and YKLP14545, respectively (Appendix 1). Some relatively well-preserved GB+TB can be discerned in YKLP14562–14563, 14565–14567 (Appendix 1). The post pharynx region is a simple tube and best preserved in YKLP14565–14566 (Figure 1D-F, Appendix 1).

Digestive tract: The digestive system of *C. pelagobenthos* consists of a mouth, an enlarged pharyngeal area, an oesophageal organ (OO), a posterior intestine, and an anus. The mouth is ventrally located near the most anterior part of the collar (Figures 1A, 3M, Appendix 1). The pharyngeal region extends from one third to almost half the length of the trunk (Figures 1D-F, 2A-B, Appendix 1) and preserves an end-pharyngeal pouch (PH) at the anterior end (Appendix 1). A post-pharyngeal

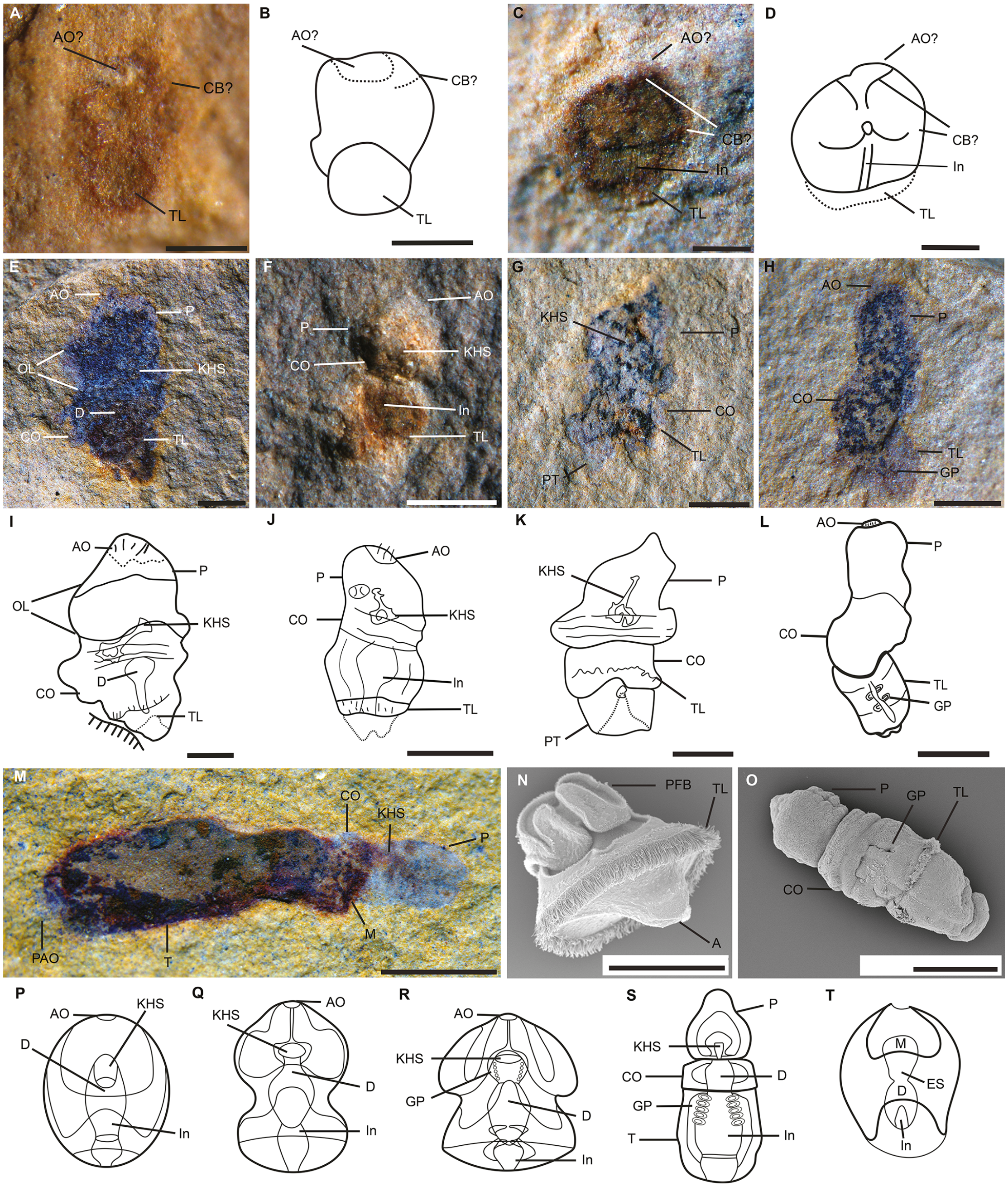

FIGURE 3. Larvae and juvenile specimens of *Cambrobranchus pelagobenthos* from the Haiyan Lagerstätte of China, and comparison to extant hemichordate and echinoderm larvae. **A**, Post-hatched early stage tornaria larva, YKLP 14532. **B**, Interpretive drawing of A. **C**, Early to middle stage larva, YKLP14540. **D**, Interpretive drawing of C. **E**, Juvenile specimen, YKLP14533. **F**, Juvenile specimen, YKLP14536. **G**, Juvenile specimen, YKLP14548. **H**, Juvenile specimen with at least two pairs of preserved oval-shaped gill pores, YKLP14549. **I**, Interpretive drawing of E. **J**, Interpretive drawing of F. **K**, Interpretive drawing of G. **L**, Interpretive drawing of H. **M**, Juvenile specimen, YKLP14551. **N**, SEM micrograph of an extant tornaria larva of *Schizocardium* sp. from the coast of Texas. **O**, SEM micrograph of an extant early stage juvenile of *Schizocardium* sp. from the coast of Texas. **P–S**, Interpretive drawings of the developing stages of an indirect developing modern enteropneust (modified from Gonzalez et al., 2017). **P**, Early stage tornaria larva. **Q**, Middle stage tornaria larva. **R**, Late stage tornaria larva. **S**, Post metamorphic juvenile. **T**, Interpretive drawing of an echinoderm (Holothuroidea) larva (modified from Dyachuk and Odintsova, 2013). Scale bars, 500 μm (A–L, N, O), 1 mm (M). AO, apical organ. CB, ciliary band; CO, collar; D, digestive tract; ES, esophagus; GP, gill pore; In, intestine; KHS, kidney–heart–stomochord complex; M, mouth; P, proboscis; PAO, post-anal organ; PFB, perioral feeding band; PT, post telotroch; T, trunk.; TL, telotroch.

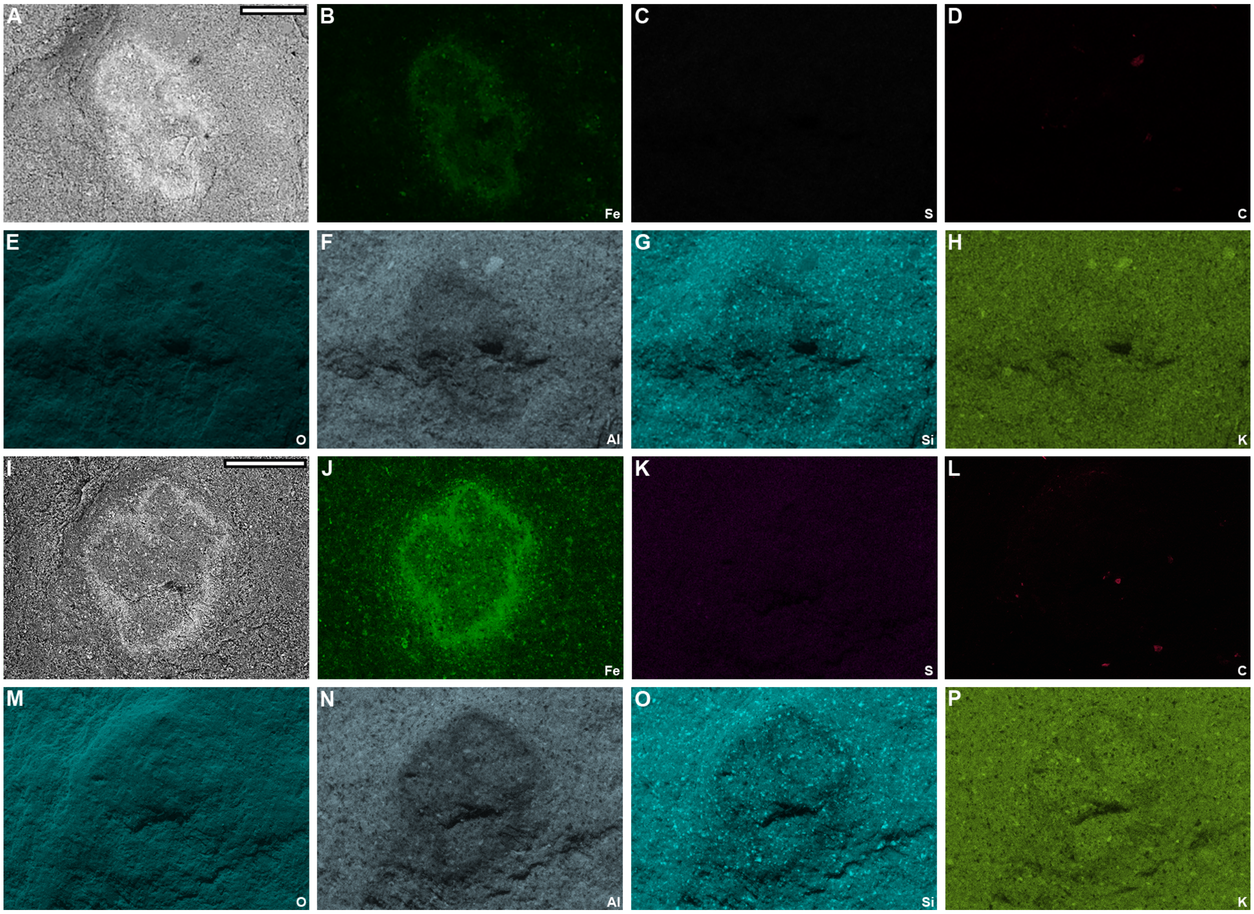

FIGURE 4. SEM and SEM-EDX analyses of *Cambrobranchus pelagobenthos* from the Haiyan Lagerstätte of China. **A–H**, Soft-bodied tissue preservation of YKLP14532. **A**, A SEM micrograph showing the larva. **B–H**, Individual elemental maps. **B**, Iron. **C**, Sulfur. **D**, Carbon. **E**, Oxygen. **F**, Aluminum. **G**, Silicon. **H**, Potassium. **I–P**, Soft-bodied tissue preservation of YKLP14540. **I**, A SEM micrograph showing the larva. **J–P**, Individual elemental maps. **J**, Iron. **K**, Sulfur. **L**, Carbon. **M**, Oxygen. **N**, Aluminum. **O**, Silicon. **P**, Potassium. Scale bars equal 200 μm.

ovoid structure is visible in several specimens (Appendix 1) that is here interpreted as the OO, like the one present in other Cambrian and extant enteropneusts (Caron et al., 2013; Nanglu et al., 2016, 2020a). The OO is followed by the intestine, which terminates in the anus. The anus is rarely preserved but appears to be non-terminal, followed by a post-anal organ (PAO) of roughly ovoid shape. The length of the post-anal organ ranges from 57 μm in juvenile specimens to 454 μm in the largest adult specimen. It is present in several specimens (Figures 2A-B, 3M, Appendix 1) and is sometimes preserved with three-dimensional relief (Figure 2A-B, Appendix 1).

Ontogeny and larval development: Two potential larval specimens have been identified (Figures 3A-D, 4), based on their morphology and compared with modern tornaria. There are other potential larvae present, but the preservation quality of these specimens prevents assignment. The specimens co-occur with juvenile, sub-adult, and adult specimens (Appendix 1), which is common among extant long-lived planktotrophic larva, including tornaria, which routinely settle in the adult sedimentary habitats (Flint and Kalke, 1986; Nielsen and Hay-Schmidt, 2007). The first (Figure 3A-B) is oval shaped, 1.5 mm long, showing the posterior ciliated telotroch swimming band. An outline of the larval body appears above the telotroch, potentially showing a ciliated feeding band and dark apical organ (Figure 3A). The second (Figure 3C-D) is 1.6 mm in length, in anterior view, with a telotroch and feed bands, gut, and a retracted apical organ comparable to extant analogues that characterize the tornaria of modern acorn worms, including *Schizocardium* (Figure 3N), *Glandiceps*, and ptychoderid species (Urata and Yamaguchi, 2004; Urata et al., 2014; Gonzalez et al., 2017, 2018).

Four additional fossils are interpreted as recently metamorphosed early juvenile develop-

FIGURE 5. Reconstruction of *Cambrobranchus pelagobenthos* from the Haiyan Lagerstätte of China: Artistic reconstruction of larvae, juveniles, and an adult specimen by C. McCall.

mental stages (cf. Urata and Yamaguchi, 2004) (Figure 3E-H). The specimens show differentiation into a tripartite body with an elongated conical proboscis, collar, and trunk. The trunk is comparatively short with respect to that of adult specimens. YKLP14549 (Figure 3H) represents an early juvenile specimen with at least two pairs of preserved oval-shaped gill pores (GP1 and GP2). Its length is 1.8 mm, and the position of the gill pores is like that of modern acorn worms (Urata and Yamaguchi, 2004; Urata et al., 2014).

Another seven specimens represent juveniles (Figure 3M, Appendix 1). They are fully developed and morphologically comparable to extant juvenile specimens. The PAO is comparable to extant harrimaniid acorn worms (Figure 3M), a probable homologue to the pterobranch stalk. The trunk of the juveniles has a length of ~1.6 mm in YKLP14551 (Figure 3M). Those of juvenile worms to adults range from 2.2 mm to 33 mm long (Figure 1A-F, Appendix 1). A reconstruction of the different metamorphic stages can be seen in Figure 5.

Remarks. Maletz (2019) reported the presence of pterobranchs from the Fortunian (Stage 1; Epoch 1). This suggests that pterobranchs and enteropneusts were already differentiated at that point, and that *Cambrobranchus pelagobenthos* may not represent the most basal enteropneust.

DISCUSSION

Preservation

All specimens of *Cambrobranchus pelagobenthos* were collected from the lowermost 5 cm of the Haiyan Lagerstätte. They are preserved in finely laminated mudstones, featuring the typical alternations of event and background layers known from the Chengjiang biota (Zhao et al., 2009). All fossils are found in the event layers, which are represented by thin, yellowish-grey layers interbedded in greyish mudstones. The event beds are suggested to have formed by accumulation of suspended mud from periodical storms, tsunamis, or turbidites (Zhao et al., 2009). Temporary high particle suspension aiding preservation in the Chengjiang biota has also recently been suggested (Saleh et al., 2022). As the enteropneust larvae appear to have been planktonic, similar to their modern equivalents (Gonzalez et al., 2018), they must have been killed off in the water column shortly after they hatched or once they settled in the adult sedimentary habitats, possibly by anoxia or a toxic gas release, which supports a 'boom-and-bust' environment (Yang et al., 2021). Additionally, modern enteropneust larvae are very delicate (Nielsen and Hay-Schmidt, 2007), supporting the idea that the specimens were quickly buried by suspended mud.

The fine external details that were preserved through initial pyrite replacement, later weathered to iron oxides, and the intact preservation of internal organs and structures suggest that the *C. pel-*

agobenthos specimens were rapidly buried, but not in a high energy event, as they would have either decayed relatively quickly on the oxygenated ocean floor or within the uppermost oxic- to suboxic layers of sediment, given their infaunal nature (Butler et al., 2015; Sansom, 2016). This can be seen in other deposits where specimens were exposed (Kimmig and Pratt, 2016; Leibach et al., 2021). Additionally, evidence of scavenging would likely be observed, unless the whole animal was consumed.

Some of the less well-preserved internal organs, likely underwent rapid decay, as has been observed for non-cuticular interior organs in decay experiments (Sansom, 2016). These include the KHS, which is preserved as a darker mass between the base of the proboscis and the collar, which corresponds to its position in all modern hemichordates (Cameron et al., 2010) and has been inferred in Cambrian hemichordates (Caron et al., 2013; Nanglu et al., 2015, 2016).

Pyrite or its iron oxide- and/or oxyhydroxide-weathering are the main form of preservation of the fossils (Figure 4, Appendix 1), including the internal organs, with rare patches of organic carbon, similar to fossils from other Chengjiang deposits (Gabbott et al., 2004; Saleh et al., 2020; Yang et al., 2021). While this form of preservation can initially be thought of as inept for the preservation of internal and fine external structures, it has shown to preserve remarkable details in the Chengjiang biota (Liu et al., 2014, 2016; Hou et al., 2017). While oxidized pyrite preservation is observationally evident by a rusty red-orange coloration of the fossils, this mode of preservation is confirmed by the presence of iron-bearing framboidal textures indicating weathering-induced pseudomorphs after the original pyrite (Appendix 1). Iron-bearing mineral concentrations usually follow the margins of the specimens and have also replaced the internal organs (Appendix 1).

While the idea of preserving planktotrophic larvae adjacent to adults of the same species might be counterintuitive, long lived extant planktotrophic larva, including tornaria, routinely settle in the adult sedimentary habitats (Flint and Kalke, 1986; Shanks et al., 2003; Nielsen and Hay-Schmidt, 2007; Shanks, 2009; Freckelton et al., 2022). This has also been observed in the fossil record, i.e., the Ordovician of Siberia, where *Isotelus*-like protaspides (planktonic larvae) have been found in the same beds as their interpreted adult stages (Lerosey-Aubril and Laibl, 2021) and the Ordovician of Canada where *Isotelus* protaspides are found together with benthic juveniles (meraspides) and adults (holaspides) (Chatterton, 1980). The mechanism, inferred for most marine planktotrophic larvae, is that settlement and metamorphosis are induced by local chemical cues. The best studied examples include biofilms and adult pheromones (Zimmer-Faust and Tamburri, 1994; Browne and Zimmer, 2001; Dobretsov and Rittschof, 2020).

When considering the abundance of larval and juvenile fossils found in the Haiyan Lagerstätte, we also must consider its interpretation as a ‘boom-and-bust deposit’ (Yang et al., 2021), which increases the likelihood of any planktotrophic larvae to be preserved adjacent to adult specimens as we are dealing with rapidly deposited event beds, with each layer representing a single burial event. The layers preserve relatively short periods of time compared to time-averaged deposits hosting many other fossils, suggesting that planktotrophic larvae would be trapped during such events. This also indicates that deposition in the Haiyan Lagerstätte was likely not ecologically selective (Saleh et al., 2021), at least in certain event beds, as endobenthic, nektobenthic/epibenthic, and nektonic/planktonic taxa are preserved (Chen, 2004; Yang et al., 2021).

The Differences between the Chengjiang and the Burgess Shale Specimens

There are 114 species of extant enteropneusts in four crown group families: Harrimaniidae, Spengelidae, Torquaratoridae, and Ptychoderidae (Jabr et al., 2018) and seven fossil species (Cameron, 2018). The previously earliest fossil specimens are from the Burgess Shale of Canada and include *Spartobranchus tenuis* Walcott 1911 and *Oesia disjuncta* Walcott 1911. These two fossil body plans are harrimaniid-like in that they lack gill bar synapticula, genital wings, and hepatic sacs (Caron et al., 2013; Nanglu et al., 2016), but unlike any extant forms in that they are tubicolous and possess posterior grasping appendages. The remaining fossil species have been put into crown group families by Cameron (2018) and include *Mazoglossus ramsdelli* Bardack 1997 and *Saccoglossus testa* Cameron 2016 from the Carboniferous Mazon Creek of Illinois, USA, *Megaderaion sinemuriense* Arduini et al., 1981 and *Ptychodera callovianum* Alessandrello et al., 2004 from the Lower and Middle Jurassic of northern Italy and southern France, respectively, and *Mesobalanoglossus buergeri* Bechly and Frickhinger in Frickhinger 1999 from the Upper Jurassic Solnhofen Limestones of southern Germany.

Cambrobranchus pelagobenthos gen. et sp. nov. is the first hemichordate exhibiting enteropneust traits from the early Cambrian Chengjiang biota and predates all other known enteropneust fossils. Its body plan is like the Burgess Shale *S. tenuis* and *O. disjuncta* in that it has a tripartite body composed of a proboscis, collar, and trunk. The three species preserve gill bars, tongue bars, a post-anal organ (or bulb), and some evidence of a KHS (Caron et al., 2013; Nanglu et al., 2016). Also noted are external gill pores (GP) and a well-preserved collar with circumferential collar bands (Figure 1), features that are lacking from the Burgess Shale specimens. The histology of circumferential collar bands is well known: The anterior lip can be pliable and exploratory or stiff and immotile, and the subsequent regions characterized by dense cilia, abundant mucous cells, or dark staining secretory mulberry cells (Benito and Pardos, 1997). The functional biology of this complex collar is largely unknown, but it is ancient. For a detailed comparison of *C. pelagobenthos* with the Burgess Shale acorn worms see Appendix 2.

The new taxon differs ecologically from the Burgess Shale enteropneust species, which were tubicolous. *Spartobranchus tenius* was probably a facultative filter feeder and deposit feeder, whereas *Oesia disjuncta* was a suspension feeder (Caron et al., 2013; Nanglu et al., 2016). *Cambrobranchus pelagobenthos* lacks a tube and is therefore interpreted here as an epibenthic deposit feeder because several specimens have well-preserved fragments of shells and cuticle in the mouth, pharynx, oesophageal organ, and intestine (Appendix 1). While this does not preclude the possibility that *C. pelagobenthos* facultatively filter fed like some modern harrimaniids (Cameron, 2002), the gut contents suggest that it was primarily a deposit feeder. Like the priapulid worm *Ottoia,* which has an excellent record of intestinal residues (Vannier, 2012), the low nutritional value of hyolithids and brachiopods was likely offset by the intake of tissues from carcasses. We cannot rule out that it is also suspension fed, akin to some modern acorn worms. Evidence of gut contents in *C. pelagobenthos* demonstrates that deposit feeding evolved early in the enteropneust line. When considered together with *Spartobranchus, Oesia,* and the stem-group hemichordate *Gyaltsenglossus*, there is increasing evidence that hemichordates had come to occupy several marine niches by the early to middle Cambrian. Extant acorn worms that are epibenthic include the deep-sea rock dwelling *Saxipendium coronatum* Woodwick and Sensenbaugh 1985, the Alaskan kelp holdfast dwelling *Harrimania maculosa* Ritter 1900, and most members of the deep-sea family Torquaratoridae. The remainder are burrowers (Jensen et al., 1992; Holland et al., 2012). We cannot rule out burrowing in *C. pelagobenthos* either because trace fossils are rarely preserved in the Haiyan Lagerstätte due to the soft nature of the substrate.

Phylogeny

The results of our Bayesian phylogenetic analysis (Figure 6) resolve *Cambrobranchus* as a total-group hemichordate with high support, but its exact relationship with the extant Classes is uncertain. More specifically, it is placed in a polytomy with pterobranchs, total-group Enteropneusta (including the fossils *Oesia* and *Spartobranchus*), and the tentaculate *Gyaltsenglossus*. To further investigate the most supported positioning for *Cambrobranchus*, we considered the number of trees in the posterior distribution (n = 20,001) in which Hemichordata and Enteropneusta were monophyletic, as both are unambiguously supported by all recent molecular phylogenies (Cannon et al., 2014; Kapli et al., 2021). This yielded 14,096 total trees, 51% of which supported *Cambrobranchus* as a stem-group enteropneust, 43.44% of which supported *Cambrobranchus* as a stem-group hemichordate, and 5.56% of which supported *Cambrobranchus* as a stem-pterobranch. The posterior distribution of trees is available in Appendix 4.

The most important result of these analyses is that *Cambrobranchus* provides direct fossil evidence of indirect development as present among middle Cambrian hemichordates and, when considered with the distribution of taxa with biphasic lifestyles in our analysis, supports metamorphosis as an ancestral character to both Hemichordata and Ambulacraria (Figure 6). Among the included taxa, the harrimaniid enteropneusts are the only direct developers, suggesting that this is a derived trait within this clade of acorn worms and not the plesiomorphic condition. It is also likely that other Cambrian hemichordates were indirect developers with planktonic larvae as well given their largely sessile habits (Caron et al., 2013; Nanglu et al., 2016, 2020a; Maletz, 2019). However, these juvenile forms have yet to be discovered, further highlighting the significance of the discovery of *Cambrobranchus*.

As we discuss in greater detail below, the origin of a pelago-benthic life history is debated, and similarities between echinoderm and hemichordate

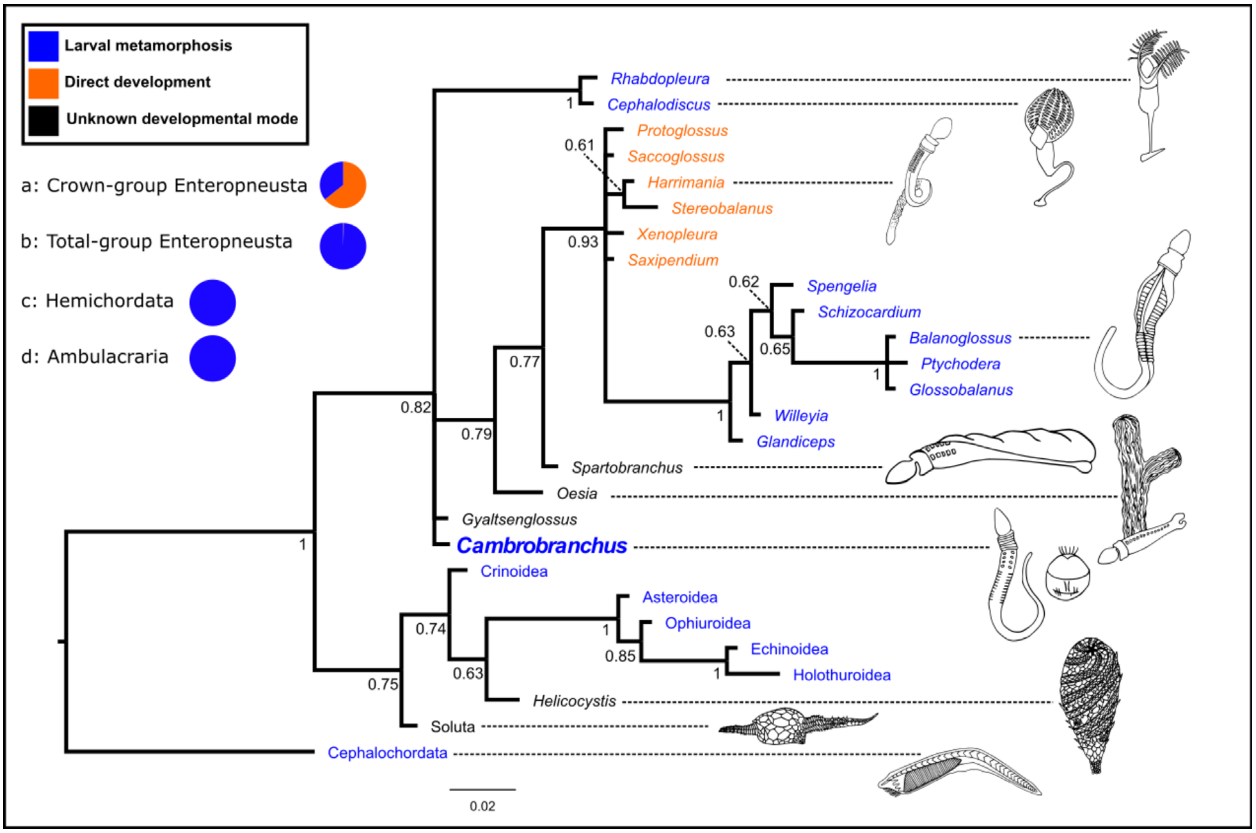

FIGURE 6. Inferred phylogenetic relationships: Phylogenetic position of *Cambrobranchus pelagobenthos* and other Cambrian ambulacrarians using majority-rules Bayesian analysis of 113 characters and 27 taxa. Numbers at nodes represent posterior probabilities. Pie charts indicate the results of an ancestral state reconstruction.

larvae have historically been used to recognize the relatedness of these two disparate phyla (Nezlin, 2000; Röttinger and Lowe, 2012). Moreover, the direct *vs*. indirect development of hemichordates has factored heavily into theories about the eco-morphology of the last common ancestor of the deuterostome (Romer, 1967). The discovery of *Cambrobranchus*, and its larvae, also re-affirms recent discoveries that enteropneusts, which until 2013 had a fossil record dating only to the Carboniferous (Caron et al., 2013), were a significant part of Cambrian ecosystems filling a wide array of marine niches. An ancestral capacity for larval dispersal may also explain the wide biogeographic distribution of Cambrian tube building hemichordate fossils from Laurentia, Canada (Kimmig and Pratt, 2015; Nanglu et al., 2016, 2020b; Nanglu and Caron, 2021) and Utah (Conway Morris and Robison 1988; Kimmig et al., 2019; Foster et al., 2022), South China, Yunnan (Hu et al., 2018), and Siberia (Nielsen, 1998).

Evidence for an Early Pelago-Benthic Life Cycle

The origin of larvae is widely debated and at the root of our understanding of animal evolution. The debate encompasses whether early animals existed initially as holobenthic organisms, adding pelagic larvae later (late Cambrian–Early Ordovician) due to predation pressure (Signor and Vermeij, 1994; Peterson et al., 2005) or the availability of planktonic food (Nützel et al., 2006; Kroeck et al., 2022; Laibl et al., 2023), or if they had a pelagic larva from the beginning (i.e., a pelago-benthic life history) (Peterson et al., 2005). More controversially, it has been suggested that early animals were holopelagic larvae-like organisms (Zhang and Pratt, 1993). Here, we provide the first direct fossil evidence for a Cambrian deuterostome larva. Cambrian acorn worm adults, juveniles, and larvae were found on the same rock slab, supporting the hypothesis that early animals had a pelago-benthic life cycle (Figures 3, 5). The only other record of Cambrian soft-bodied larvae are the Orsten-type arthropod faunas from Sweden and China (Müller and Walossek, 1986; Andres, 1989; Waloszek and Dunlop, 2002; Steiner et al., 2004). These findings have significant macroevolutionary implications because the larva is often the dispersal phase of animal life cycles that permits the establishment of new populations in spatially separated habitats, which in turn maintains genetic diversity. Multiple distant populations mean that a species develops resistance to extinction following a disaster of a local population. A further macro-evolutionary consequence is the longevity of a species (Hansen, 1978, 1980). Populations that overlap genetically due to a widely dispersed larva are expected to have low speciation rates compared to equivalent populations that lack larvae (Jablonski, 1986). In this context it has also been shown that metamorphosis was likely ancestral for crown arthropods and not direct development, and a planktonic ecology may have evolved in the Ediacaran, or earlier (Wolfe, 2017). The same study also shows that, under a variety of phylogenetic hypotheses, metamorphosis is supported as the most likely ancestral state for ecdysozoans and euarthropods, suggesting it was a more common trait than the fossil record shows. Additionally, larval stages might not have preserved well in the fossil record, or not have been collected because of a variety of collections biases (Whitaker and Kimmig, 2020).

The discovery of *C. pelagobenthos* tornaria is the first fossil evidence of a hemichordate larva (Figure 3A-D) and suggests that the modern enteropneust larva (Figure 3J-O) evolved early in the history of the clade (Urata and Yamaguchi, 2004; Urata et al., 2014; Gonzalez et al., 2017). When fossils, including these new larval forms, and the predominant developmental modes among echinoderms are considered, we can reject the hypothesis that direct development, like that seen in the familiar *Saccoglossus*, is plesiomorphic to this Hemichordata (Sly et al., 2002, 2003; Raff and Byrne, 2006; Raff, 2008; Gonzalez et al., 2017). This finding is further supported by ancestral state estimation, which shows that a bi-phasic lifestyle is ancestral for Hemichordata and for Ambulacraria more broadly (Figure 6). Surprisingly, metamorphosis and juvenile morphology reflect those of extant acorn worms (Figure 3J-O). Metamorphic and early juvenile specimens are predominantly proboscis, with a distinct collar, and trunk with a post-anal organ. This mode of development appears to have been highly conserved since at least 525 million years ago (Jablonski and Lutz, 1983; Strathmann, 1985, 1990). The best evidence that direct developing acorn worms, like *Saccoglossus*, have evolved from an indirect developing ancestor is that early developmental stages have a ciliated telotroch band: This vestigial structure is a larval feature.

CONCLUSIONS

Here, we have described a new acorn worm, *Cambrobranchus pelagobenthos* gen. et sp. nov., from the early Cambrian Haiyan Lagerstätte, Chengjiang biota, of China. In addition to adult specimens, we also report juvenile specimens and evidence for the first tornaria larvae in the fossil record. The most important result of this study is that *Cambrobranchus* provides direct fossil evidence of indirect development as present among middle Cambrian hemichordates and, when considered with the distribution of taxa with biphasic lifestyles in our analysis, supports metamorphosis as an ancestral character to both Hemichordata and Ambulacraria.

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APPENDIX 1.

Supplementary figures. Available in zipped format for download at
https://palaeo-electronica.org/content/2024/5163-origin-of-the-hemichordate-larva

APPENDIX 2.

Supplementary tables. Available in zipped format for download at
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APPENDIX 3.

Data matrix. Available in zipped format for download at
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APPENDIX 4.

Posterior distribution file. Available in zipped format for download at
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