Report

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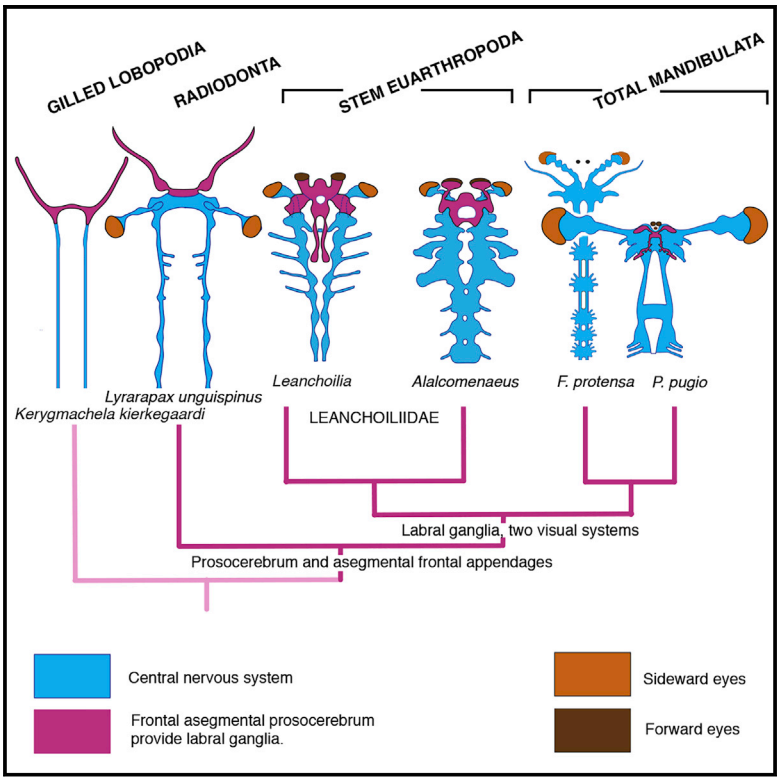
Leanchoiliidae reveals the ancestral organization of the stem euarthropod brain

Graphical abstract



Authors

Tian Lan, Yuanlong Zhao, Fangchen Zhao, You He, Pedro Martinez, Nicholas J. Strausfeld

Correspondence

lantianing@sina.com (T.L.), flybrain@neurobio.arizona.edu (N.J.S.)

In brief

Tian et al. find near-identical stem euarthropod fossils from the Kaili biota with central nervous systems showing unique evidence for the brain's asegmental prosocerebrum, which, as gene and developmental studies of extant arthropods predict, provide the appendicular labral ganglia and one of two ubiquitous visual systems.

Highlights

- Stem-euarthropod fossils from the Kaili biota reveal bilaterally symmetric CNS
- An asegmental prosocerebrum is rostral to segmental proto- deuto-, and tritocerebra
- The prosocerebrum gives rise to paired labral nerves and their ganglia
- Separate prosocerebral and protocerebral visual systems

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Leanchoiliidae reveals the ancestral organization of the stem euarthropod brain

Tian Lan,[1,2,*] Yuanlong Zhao,[1] Fangchen Zhao,[3] You He,[6] Pedro Martinez,[4,5] and Nicholas J. Strausfeld[7,8,*]

[1]Guizhou Research Center for Palaeobiology, Guizhou University, Guiyang, Guizhou, China

[2]Key Laboratory of Karst Georesources and Environment, Ministry of Education, The College of Resources and Environmental Engineering, Guizhou University, Guiyang 500025, Guizhou, China

[3]State Key Laboratory of Palaeobiology and Stratigraphy, Nanjing Institute of Geology and Palaeontology and Centre for Excellence in Life and Palaeoenvironment, Chinese Academy of Sciences, Nanjing, China

[4]Departament de Genetica, Microbiologia i Estadística, Universitat de Barcelona, Barcelona, Spain

[5]Institut Català de Recerca i Estudis Avancats (ICREA), Passeig de Lluis Companys, Barcelona 08010, Spain

[6]Shanghai Synchrotron Radiation Facility, Shanghai Advanced Research Institute, Chinese Academy of Sciences, Shanghai, China

[7]Department of Neuroscience, University of Arizona, Tucson, AZ 85721, USA

[8]Lead contact

*Correspondence: lantianing@sina.com (T.L.), flybrain@neurobio.arizona.edu (N.J.S.)

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SUMMARY

Fossils provide insights into how organs may have diversified over geological time.[1] However, diversification already accomplished early in evolution can obscure ancestral events leading to it. For example, already by the mid-Cambrian period, euarthropods had condensed brains typifying modern mandibulate lineages.[2] However, the demonstration that extant euarthropods and chordates share orthologous developmental control genes defining the segmental fore-, mid-, and hindbrain suggests that those character states were present even before the onset of the Cambrian.[3] Fossilized nervous systems of stem Euarthropoda might, therefore, be expected to reveal ancestral segmental organization, from which divergent arrangements emerged. Here, we demonstrate unsurpassed preservation of cerebral tissue in Kaili leanchoiliids revealing near-identical arrangements of bilaterally symmetric ganglia identified as the proto-, deuto-, and tritocerebra disposed behind an asegmental frontal domain, the prosocerebrum, from which paired nerves extend to labral ganglia flanking the stomodeum. This organization corresponds to labral connections hallmarking extant euarthropod clades[4] and to predicted transformations of presegmental ganglia serving raptorial preocular appendages of Radiodonta.[5] Trace nervous system in the gilled lobopodian *Kerygmachela kierkegaardi*[6] suggests an even deeper prosocerebral ancestry. An asegmental prosocerebrum resolves its location relative to the midline asegmental sclerite of the radiodontan head, which persists in stem Euarthropoda.[7] Here, data from two Kaili *Leanchoilia*, with additional reference to *Alalcomenaeus*,[8,9] demonstrate that Cambrian stem Euarthropoda confirm genomic and developmental studies[10–15] claiming that the most frontal domain of the euarthropod brain is a unique evolutionary module distinct from, and ancestral to, the fore-, mid-, and hindbrain.

RESULTS AND DISCUSSION

The following descriptions refer to specimens (GRCP15007, GRCP15008, GRCP15009, and GRCP15011) retrieved from the Cambrian (Miaolingian Series: Wuliuan stage) Kaili biota (IUGS dated 508 mya),[16] identified as leanchoiliids by their two pairs (sideward and forward) of single-lens eyes located anteriorly at the margin of the cephalic shield, which is followed by an 11-segment trunk terminating in a spinous triangulate telson (Figure S1). Specimens are here viewed ventral side up. Two specimens (GRCP15007 and GRCP15009) reveal exquisitely preserved, near-identical arrangements of bilaterally symmetric asegmental and segmental ganglia and nerve cords (Figures 1 and S2).

UV-evoked bright red fluorescence (Figure S2; STAR Methods) reveals in GRCP15007 and GRCP15009 a bilaterally symmetric brain with four clear divisions (Figures 1A–1F). The first and most rostral of these consists of a heterolateral cerebral bridge, here named the prosocerebrum (see Urbach and Technau[10]), which is unambiguously associated with the paired forward eyes (Figures 1C and 1F). At the prosocerebrum's midline, a small, circumscribed domain, termed the pars medialis, provides in both specimens a pair of extremely thin but clearly defined nerve cords that extend caudally as far as the brain's fourth division, here identified as the tritocerebrum (Figures 1C and 1F). Each nerve cord from the pars medialis terminates as a swollen labral ganglion (denoted as LABG in Figures 1C and 1F) at a level corresponding to the fronto-lateral margin of the stomodeum and abutting the inner margins of the adjacent tritocerebral hemiganglia. The second, third, and fourth divisions of the brain are each distinguished by pairs of serially connected

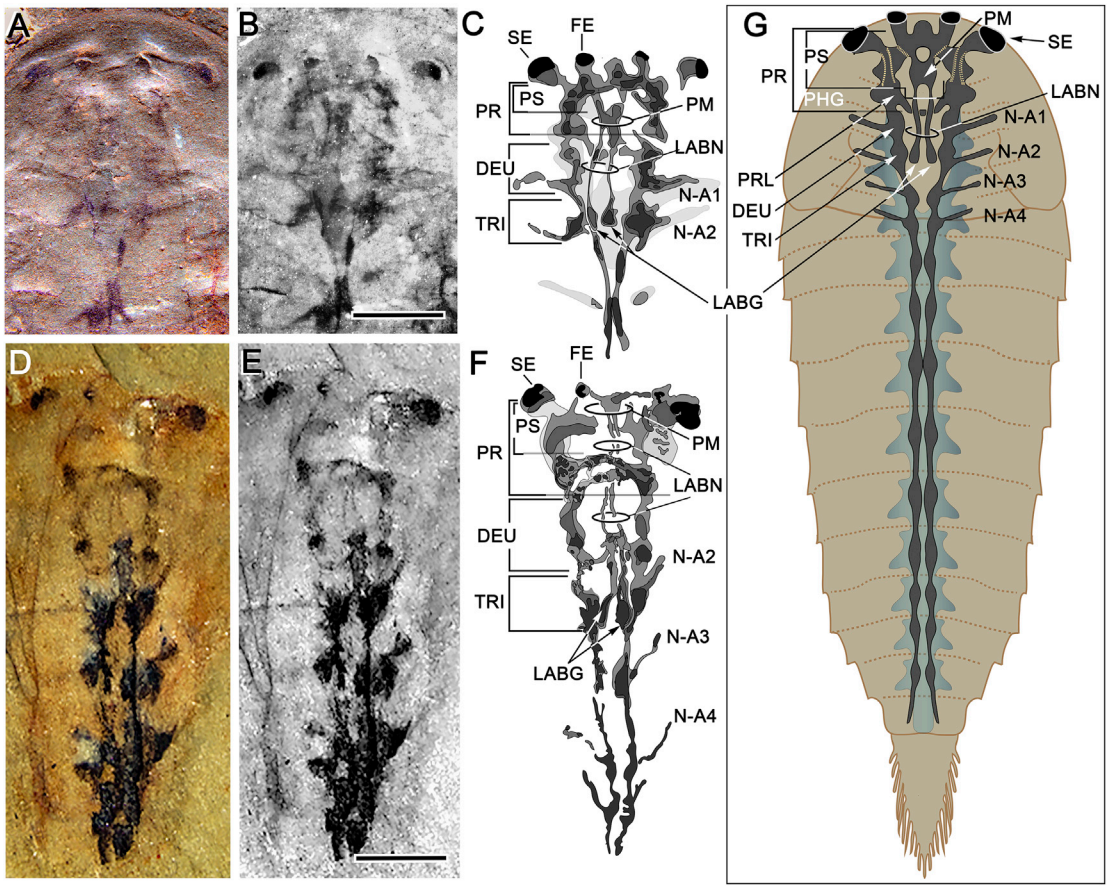

Figure 1. Asegmental and ganglionic structures constitute the ancestral euarthropod brain

(A and D) Specimens GRCP15007 (A) and GRCP15009 (D).

(B and E) Grayscale renditions of red fluorescence images (Figure S2) reveal well-defined bilateral residues of neural tissue.

(C and F) Camera lucida drawings of neural traces. The brain is clearly segmented, with the most anterior part (protocerebrum, PR) comprising an asegmental prosocerebrum (PS) contiguous caudally with the protocerebral hemiganglia (PHG). The bulging protocerebral lobes (PRL) are connected heterolaterally by the protocerebral commissure. Trace neuropil extends antero-laterally from the PR to reach the sideward (lateral) eyes (SE). The paired forward (medial) eyes (FE) arise from the asegmental prosocerebrum. A discrete midline domain, the pars medialis (PM) attached to the prosocerebrum provides paired labral nerves (LABN, circled) to the labral ganglia (LABG), which abut the inner margins of the two tritocerebral (TRI) hemiganglia. DEU, deutocerebral.

(G) Interpretive drawing of the Kaili leanchoiliid nervous system showing, the brain with deutocerebral and TRI segmental nerves (N-A1 and N-A2). Also beneath the head shield are two segmental ganglia with nerves extending toward the locations of the first two motile appendages (N-A3 and N-A4).

GRCP15007 and GRCP15009 and other examples of Kaili Leanchoiliidae are shown in Figure S1. Scale bars in (A)–(C), (E), and (F), 2 mm. See also Figure S1.

hemiganglia. The first pair, situated immediately caudal to the prosocerebrum, is interpreted as the protocerebrum (Figures 1C, 1F, and 1G). Its left and right hemiganglia are connected by a heterolateral commissure. Trace neuropil extending forward and laterally from a protocerebral hemiganglion suggests its contiguity with the paired sideward eyes (Figures 1E–1G). The protocerebral hemiganglia are each linked caudally to a second pair of segmental hemiganglia constituting the deutocerebrum. The two deutocerebral hemiganglia each provides a laterally extending nerve toward the assumed attachment point of the corresponding deutocerebral appendages, which are not resolved in these specimens but would correspond to the prehensile "great appendage," as defined by Haug et al.[17] The third pair of segmental hemiganglia is interpreted as the tritocerebrum. It likewise provides laterally extending nerves to the second post-protocerebral appendage, the morphology of which is described for *Leanchoilia superlata*.[17]

In both GRCP15007 and GRCP15009, the tritocerebral hemiganglia give rise to caudally directed, paired nerve cords, which connect segmentally arranged pairs of hemiganglia (Figures 1G, 2G, and 2H), each of which is assumed to be functionally associated with a similar pair of appendages: two beneath or at the margin of the head shield and a pair in each of the subsequent 11 trunk segments.[17] Although some hemiganglia appear to almost touch at the midline, they show no obvious evidence for complete heterolateral fusion (Figures 2A–2F) such as typifies thoracic-abdominal ganglia in extant euarthropods. However, as occurs in extant Euarthropoda,[18,19] hemiganglia would likely have been connected across the midline by heterolateral neurons mediating bilateral motor coordination.[20]

Intense red fluorescence resolves trunk hemiganglia as unambiguously distinct from other preserved soft tissue, here identified as the gut (Figures 2G and 2H), equipped with segmentally arranged lateral pouches (diverticuli and, possibly, glands). Their

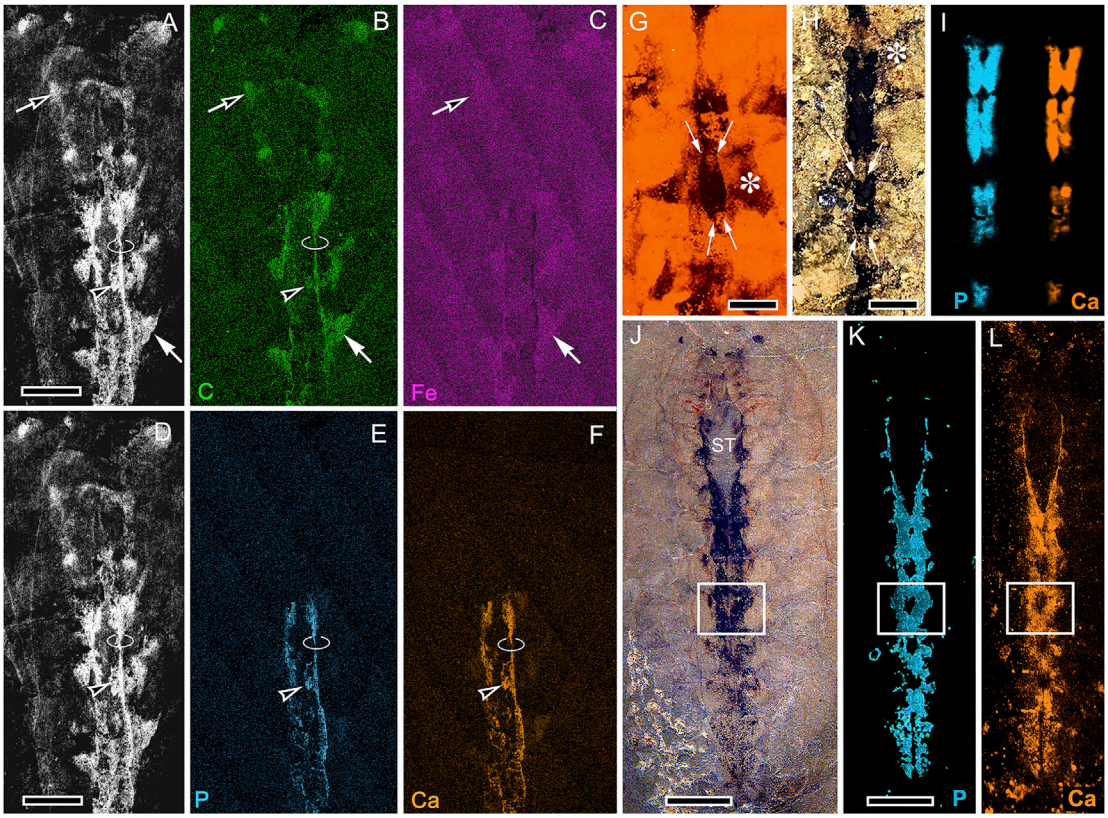

Figure 2. Specimen preservation revealed by elemental analyses

Elemental energy dispersive spectroscopy (EDS) (B, C, E, and F) and micro-X-ray fluorescence (μ-XRF) analyses (I, K, and L) distinguish brain and central nervous system from alimentary canal.

(A and D) Inverted grayscale rendition of specimen GRCP15009 for reference (STAR Methods; Figures S2C and S2D) resolves cerebral neuropil (example indicated by open arrow), segmental ganglia (arrowhead) and nerve cord (circled), and gastric pouches (closed arrow).

(B) EDS carbon scans reveal corresponding features.

(C) EDS iron scans also resolve corresponding features but barely elevated above the background signal from the iron-rich matrix.

(E and F) EDS phosphate and calcium profiles in the thorax resolve paired nerve cords and hemiganglia.

(G) Red fluorescence imaging of the ventral nervous system of specimen GRCP15007 distinguishes faint traces of the gut from the much darker segmental hemiganglia and nerve cords (indicated by arrows). Gastric pouches (one indicated by an asterisk) appear as pale triangulate deposits.

(H and I) Specimen GRCP15011 imaged with white light shows identical arrangements of ganglia and gastric pouches (H), which μ-XRF (I) resolves as phosphate and calcium.

(J–L) μ-XRF of specimen GRCP15008 (J) resolves phosphate (K) and calcium (L) signatures that identify the gut, extending from the stomodeum (ST, in J). No cerebral components are resolved in this specimen, although dark areas at each segment (boxed) suggest overlying midline structures comparable in size to paired hemiganglia.

Scale bars, 2 mm (A–F); 5 mm (G–I); 1 mm (J and K).

arrangements in Figure 2J are consistent with those resolved in other Cambrian euarthropods.[21–23] Carbon signatures obtained by energy dispersive spectroscopy (EDS) resolve cerebral arrangements in the head and trunk ganglia corresponding to those observed under red fluorescence (Figures 2A and 2D). EDS also reveals carbon-delineating gastric pouches in the thorax (Figure 2B). Phosphate and calcium signatures detected by EDS resolve trunk ganglia and their connectives (Figures 2E and 2F) but not cerebral nervous tissue. Although revealing trace neuropil in a Middle Triassic archaeognathan insect,[24] resolution of the nervous system solely as calcium phosphate deposits would be a novel type of neural soft tissue preservation for Cambrian fossils where, to date, calcium phosphate is known only to preserve digestive tissue.[23] Here, we interpret the occurrence of calcium phosphate specifying nerve cords and ganglia (Figures 2D and 2E) as a consequence of phosphates, provided internally from the gut,[25] that become secondarily deposited on neural tissue that is undergoing (or has undergone) unusually early stabilization as a carbonaceous film.[26] Because described specimens are preserved ventral side up, so that the scanned nervous tissue overlies the gut, which lies deeper in the matrix, the absence of an EDS signature indicating gut morphology is thus likely due to the depth of penetration by the incident beam being constrained to 1 μm (STAR Methods). That calcium phosphate does not indicate cerebral tissue, although this is preserved as a carbon film, is likely due to the absence of gut diverticuli within the head region, as shown in Figures 2J–2L, and thus the absence of available phosphate. Micro X-ray fluorescence (μ-XRF) identifying calcium and phosphate in the gut of specimen GRCP15008 demonstrates the gut's origin at the level of

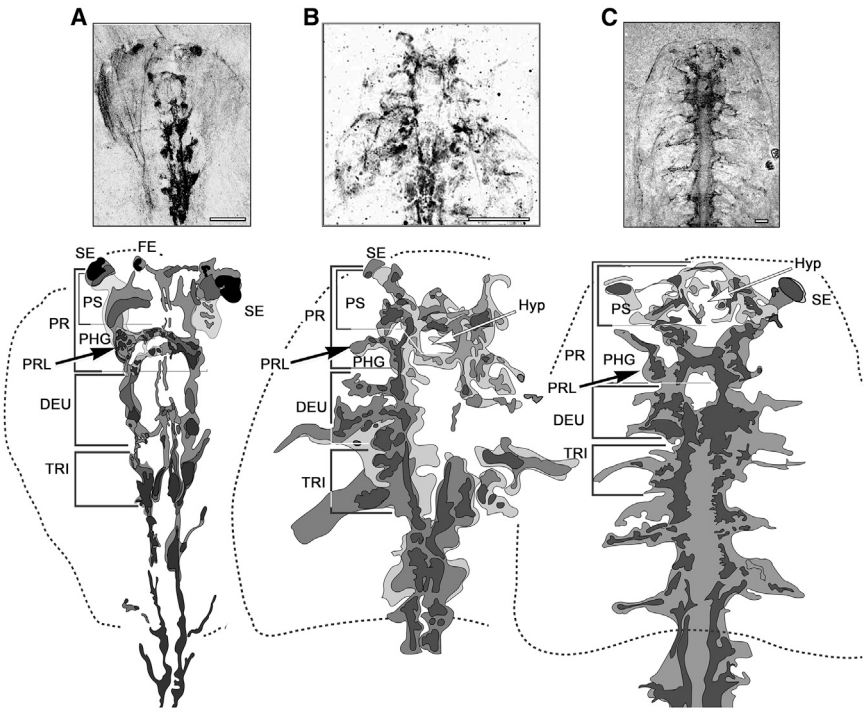

Figure 3. Correspondence of cerebral organization across Leanchoiliidae

Arrangements of the apical asegmental prosocerebrum and the tripartite arrangement of the proto-, deuto-, and tritocerebra (PR, DEU, and TRI, respectively), as seen in Kaili *Leanchoilia* (A), can also be resolved in the condensed cerebral traces of exceptionally preserved specimens of *Alalcomenaeus* sp. (B and C). Enlargements of the upper panels are provided in Figure S3. In each taxon, the traditionally acknowledged protocerebrum comprises a heterolateral, asegmental prosocerebrum (PS) contiguous with paired protocerebral hemiganglia (PHG). The paired forward single-lens eyes (FE) are associated with the prosocerebrum. The paired sideward eyes (SE) crown an elongated rostrolateral extension of the protocerebral lobe (PRL). Whereas in the Kaili specimen, the prosocerebrum provides the paired labral nerves and ganglia (Figure 1G), in *Alalcomenaeus*, traces of neuropil at the level of the hypostome (Hyp) may indicate a more rostral association of the paired labra in that species. (B) A detail of the micro-CT scan used for the interpretative reconstruction by Tanaka et al.[8] The original photograph used for the interpretative reconstruction in (C) was generously provided by Javier Ortega-Hernández et al.[9] Scale bars, 2 mm. See also Figure S3.

the stomodeum (Figure 2J), with the first diverticulum visible in segment 1 of the trunk. The gut's segmental organization is resolvable as far as segment 11 (Figure 2J). Although μ-XRF scans of GRCP15008 do not distinguish neural tissue, segmentally occurring dark regions at the midline (e.g., boxed area in Figures 2J–2L) suggest the presence of overlying pairs of hemiganglia that have not taken up calcium phosphate. Nevertheless, as already mentioned, red fluorescence clearly differentiates ganglia and ventral nerve cord from the gut, as in specimen GRCP15007 (Figure 2G), where the darker paired hemiganglia overlie the paler gut at its midline. Direct illumination reveals the same arrangements in GRCP15011 (Figure 2H), and μ-XRF scans of that specimen resolve calcium and phosphate signals delineating the paired arrangements of GRCP15011's segmental hemiganglia (Figure 2I).

The interpretive reconstruction in Figure 1G of the fossilized brain and ventral nervous system common to GRCP15007 and GRCP15009 derives from the extraordinary fine detail preserved in these specimens also showing bilateral symmetry, one of several criteria defining preservation of soft tissue.[26,27] Rare examples are known of bilateral preservation of the brain. These include *Alalcomenaeus* specimens, one from the Cambrian deposits (Miaolingian Series: Drumian stage; Figure 3) in Utah,[9] another from the Cambrian Chengjiang biota (Yu'anshan Member, Heilinpu Formation; Figure 3),[8] and a brain of the annelid *Canadia spinosa* from the Burgess Shale.[28] Here, the detailed bilateral preservation in the iron-rich sediment typifying the Kaili biota may indicate redox conditions that likely slowed decay,[29,30] thereby preserving even extremely delicate elements of neural tissue—as exemplified by the labral nerves (Figures 1C and 1F)—during deposition of the carbon film. Subsequent pyritization, also characterizing neural tissue preservation in Chengjiang specimens,[26] is in the present Kaili material just resolvable against the extremely high background level of endogenous iron that characterizes the matrix (Figure 2C).

The Kaili specimens are of crucial phylogenetic significance as they provide the first direct fossil evidence for the ancestral euarthropod prosocerebrum and, crucially, its relationship with the paired appendicular labral ganglia located at the mid-level margin of the stomodeum (Figures 1C and 1F), although the actual cuticular labrum may be located further rostrally, as suggested by micro-computer tomographic imaging of Chengjiang leanchoiliids.[31] The distinction of the prosocerebrum (proso-: front) from the segmental protocerebrum (proto-: first) was predicted by developmental genetics,[10–14] showing that in *Drosophila* the front part of the embryonic brain is defined by two neural lineage-specific domains. The more caudal domain (called the "archicerebrum") provides the compound eye's visual system, whereas the more rostral prosocerebrum provides neuron cell lineages relating to integrative centers such as the central complex, mushroom bodies, and the labrum. The labrum was first identified in pancrustaceans as paired preocular asegmental appendages[11,12] and has since been recognized in pancrustaceans and chelicerates as originating from within the head's asegmental *six3/foxQ2* gene expression domain,[12–14] which also designates the paired frontal appendages of Onychophora.[15] The prosocerebrum thus denotes the most rostral domain not just of the euarthropod head but also the onychophoran head and, by comparison with the organization in Radiodonta, the asegmental preocular neuropil serving the radiodontan frontal appendages. That the labral ganglia are identified in the Kaili leanchoiliids provides additional evidence for the existence of labral appendages in total Euarthropoda.[31]

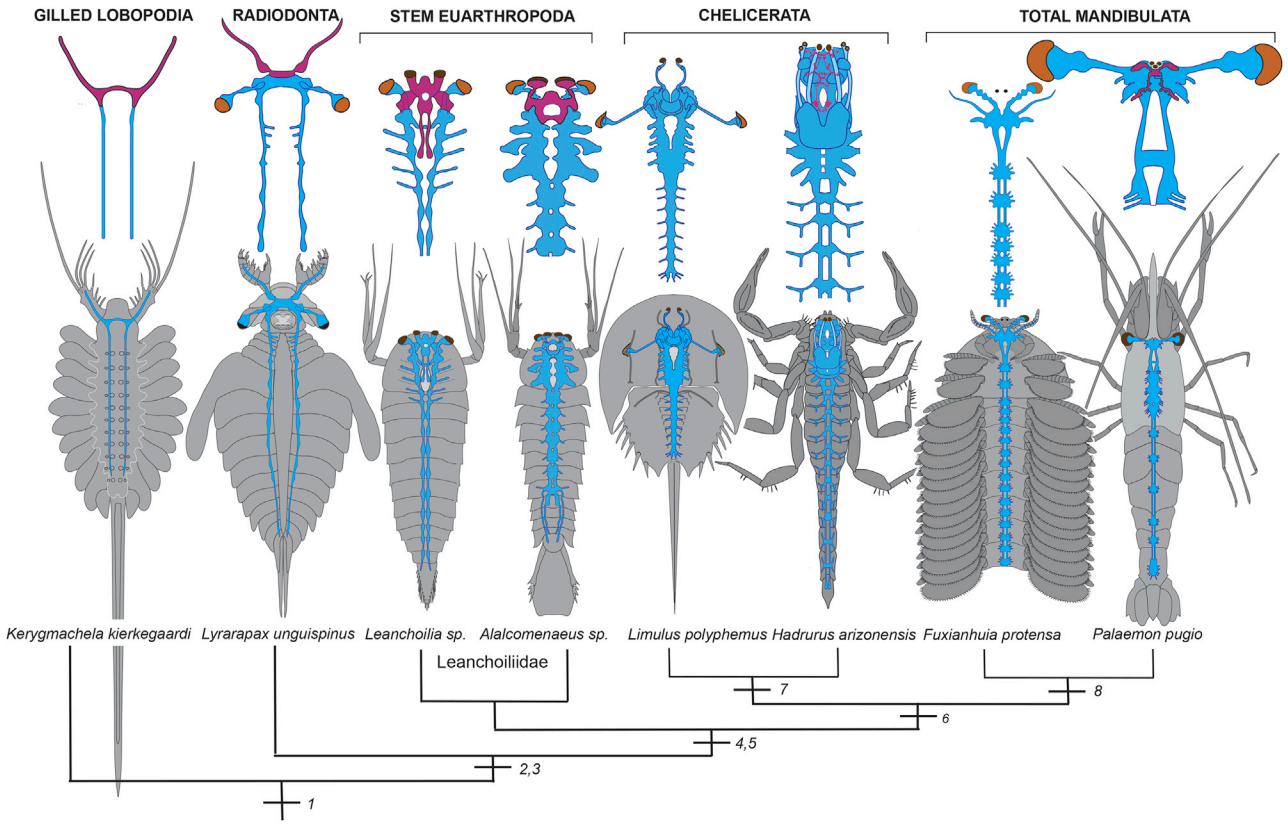

Figure 4. Ground pattern organization of euarthropod brains

Top row: brains. Bottom row: species with disposition of their central nervous systems. Simplified tree topology shows stem Euarthropoda equally related to crown Chelicerata and total Mandibulata. Central nervous system is indicated in cyan. Asegmental prosocerebra of gilled Lobopodia, Radiodonta, and stem Euarthropoda are indicated in magenta, as are identifiable, labral nerve/ganglia and their homologs in *Hadrurus* and *Palaemon*. The single heterolateral neuropil (magenta) that subtends appendicular receptor arrays in the gilled lobopodian *K. kierkegaardi*[6] is interpreted here as the ancestral asegmental prosocerebrum aligned with the paired forward extending appendages (1). In Radiodonta, a segmental protocerebrum equipped with paired compound eyes (2) is disposed immediately caudal to the prosocerebrum (magenta), serving preocular raptorial appendages (3), which are homologs of Euarthropoda labral appendages (4 and 5). The stem euarthropod ground pattern is represented by Leanchoiliidae. Two innovations are the segmental ganglionic organization of the central nervous system (4) and the caudal migration of labral ganglia to reside at the level of the stomodeum (5). Present evidence from the Kaili *Leanchoilia* ascribes protocerebral identity to the sideward (lateral) eye pairs. Data from *Alalcomenaeus* is ambiguous. Forward (medial) eye pairs in both leanchoiliid species are identified as prosocerebral. Brains of Chelicerata and total Mandibulata, including the lower Cambrian *Fuxianhuia protensa*,[35] are typically condensed, obscuring the prosocerebrum and separation of segmental cephalic ganglia (6). Immunostaining with an antiserum raised against the neuropeptide hugin[34] resolves in chelicerate (e.g., *Hadrurus*) and mandibulate (e.g., *P. pugio*) organization of paired labral ganglia (magenta) with their connections to the most rostral domain of the brain, signifying the cryptic ancestral prosocerebrum wholly integrated into the condensed forebrain. Visual systems (7 and 8) evolved convergently in chelicerates and mandibulates, the latter re-evolving the protocerebral compound eyes.

Labral ganglia in extant euarthropods are aligned with the rostral, or rostro-lateral, margin of the mouth. That location supports the prediction from observations of *Lyrarapax unguispinus* that labral ganglia and their paired nerves in Euarthropoda originated from a neuropil that served the paired preocular raptorial appendages in Radiodonta.[5] In *L. unguispinus*, that neuropil is indicated as asegmental, thus prosocerebral, by virtue of its location beneath the asegmental dorsal plate (H-element) situated extreme rostrally. The protocerebral neuropil, which extends out from each side of the head to the radiodontan compound eyes, indicates the first segment of the head,[5] as suggested by the presence of flanking lateral plates or "P-elements" that correspond to the bivalved head shield of stem euarthropods.[32,33] These arrangements in Radiodonta denote a prosocerebrum contiguous with neuropil immediately behind it corresponding to the segmental protocerebrum. As proposed from considerations of the morphology of *Lyrarapax* compared to Onychophora and euarthropods,[5] the paired labral appendages underwent an evolved ventro-caudal shift from their original location flanking the radiodontan mouth to retain their association with the mouth, as in extant Euarthropoda, at the level of the tritocerebral segment.[11,12] The paired labral nerves in GRCP15007 and GRCP15009 exactly reflect that migration (see Figure 4 in Cong et al.[5]). Antibodies against the neuropeptide "hugin" resolve this arrangement across all extant euarthropod lineages,[4,34] two exemplified here by the chelicerate *Hadrurus arizonensis* and the shrimp *Palaemon pugio* (Figure 4). The fossilized pars medialis/prosocerebrum thus provides direct evidence for an asegmental ancestral brain in Arthropoda, likely identical to the fossilized heterolateral neuropil

comprising the rostral brain of the gilled lobopodian *Kerygmachela*. Although referred to as a protocerebrum,[6] its neuropil is more aptly ascribed to an asegmental prosocerebrum (Figure 4).

Interpreting the organization of nerves from the brain as indicative of brain segmentation is historically grounded on observations of cerebral nerves supplying head appendages in extant Euarthropoda.[36] As suggested in their interpretive drawings, previous studies of fossilized brains have likewise inferred their segmental organization from the arrangement of nerves that extend outward to the eyestalks (when present) and postocular appendages.[2,8,9] Nevertheless, claiming that cerebral fusion and condensation are derived attributes of an ancestral segmented organization has been challenged by a more reductive interpretation of Cambrian euarthropods that proposes that their brains may have been unsegmented and, hence, exclusively protocerebral and that segmentation typifying the brains of crown taxa evolved later.[37]

Here, new observations of *Leanchoilia* and *Alalcomenaeus* speak against that interpretation. Morphological correspondences of exoskeletal characters place *Leanchoilia* as sister to *Alalcomenaeus*.[38] The expectation that their brain morphologies would likewise correspond is demonstrated here from revisiting two additional Leanchoiliidae: the Chengjiang *Alalcomenaeus* specimen YKLP 11075[8] and high-resolution depictions of the Miaolingian Series *Alalcomenaeus* KUMIP204782[9] (see Acknowledgments). Density tracings of these specimens (Figures 3 and S3) identify corresponding organization of the prosocerebra and cerebral ganglia demonstrated from the Kaili *Leanchoilia*. Together, these megacheiran species demonstrate the stem euarthropod cerebral ground pattern: a 1+3 arrangement of prosocerebrum with the tripartite composition of subsequent proto-, deuto-, and tritocerebral segmental ganglia, as revealed in extant pancrustaceans by the expression of head gap genes.[3] Although the third and fourth post-protocerebral appendages, which are motile,[17] originate from beneath or at the head shield, their corresponding ganglia are not accorded cerebral roles.

Notably, the two *Alalcomenaeus* specimens demonstrate partial condensation shown as prosocerebral fusion with the segmental ganglia caudal to it. Pronounced cerebral condensation is a condition that pertains to all crown Euarthropoda and is why the existence of a prosocerebrum in extant species can only be inferred by patterns of gene expression.[10,13,14] The suggestion[37] that, in crown Euarthropoda, the paired labral appendages are innervated from the tritocerebrum is mistaken. As shown by anti-hugin immunoreactivity,[34] labral ganglia are distinct from tritocerebral neuropils and are separately connected to the most rostral domain of the brain (Fig. 9.13 in Strausfeld[4]).

The segmental organization of the stem euarthropod brain reflects early developmental stages recognized across living Chelicerata and Pancrustacea, where *Limulus*, crayfish, and insects all demonstrate that the two hemiganglia of the protocerebrum first form a heterolateral commissure before the paired deutocerebral hemiganglia migrate forward to where their commissural outgrowths fuse with that of the protocerebrum.[39,40] This is reflected by the apparent absence of a discrete deutocerebral commissure in specimens GRCP15007 and GRCP15009. In extant Euarthropoda, the third pair of hemiganglia defining the tritocerebrum flank the stomodeum, as they do in the present Kaili specimens. The absence in GRCP15007 and GRCP15009 of tritocerebral sub- and supraesophageal commissures, which are found in extant euarthropods,[41] may suggest their later evolution, concomitant with the morphological divergence, elaboration, and functionality of the biramous tritocerebral appendage.[42]

Evidence provided here demonstrates that, already in the lower mid-Cambrian, the stem euarthropod Leanchoiliidae (comprising *Leanchoilia* and *Alalcomenaeus*) possessed an asegmental prosocerebrum that provided the labral ganglia and was connected to the tripartite segmental organization of the proto-, deuto- and tritocerebral ganglia. The identification of those arrangements defines the stem euarthropod brain ground pattern, and these arrangements are unchanged today. Like the leanchoiliid-defining telson morphologies and 11 trunk segments,[38] the sideward and forward single-lens eye pairs[43] situated at the margin of the head shield are also diagnostic characters of this taxon.[44] The present fossil evidence resolves the paired forward eyes as associated with the prosocerebrum (Figures 1 and 3), whereas the paired sideward (lateral) eyes, which crown a short peduncle (*Alalcomenaeus cambricus*[45]), are associated with the protocerebrum (Figure 1G).

The distinction of prosocerebral and protocerebral eyes in Leanchoiliidae evokes comparisons with crown Chelicerata. In araneans, the anterior median (‘‘principal’’) eyes are developmentally and genetically distinct from the ‘‘secondary’’ single-lens eyes that evolved from the ancestral (plesiomorphic) faceted eye.[46–48] Principal eyes have been proposed as homologs of the paired eyes of Onychophora,[49] as well as of the nauplius eyes of Oligostraca and Multicrustacea and the single-lens ocelli of insects.[50,51] All arise extremely rostrally in the embryonic brain and have central connections to rostral neuropils, including the central complex,[49,52–55] which, in Hexapoda, originates in the asegmental *six3 foxQ2* domain.[14] The disposition and relationship of the forward and sideward eye pairs of Kaili *Leanchoilia* to, respectively, the prosocerebrum and protocerebrum suggest a crucial stage in the evolution of two separate visual systems that are ubiquitous to Chelicerata and Mandibulata. That the sideward (protocerebral) eyes of Leanchoiliidae show no evidence of facets[56] indicates an evolved derivation from the plesiomorphic compound eye typified by Radiodonta.[43,57] That subsequent euarthropod lineages reestablished sideward compound eyes is evidenced by their presence in eurypterids and *Limulus*[58,59] and their repeated appearance during oligostracan and multicrustacean evolution.[60–63]

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Materials Availability
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- EXPERIMENTAL MODEL AND SUBJECT DETAILS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2021.07.048.

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AUTHOR CONTRIBUTIONS

T.L. made the initial identification of preserved nervous system in the Kaili biota fossils discovered and retrieved by Y.Z.[16] T.L., assisted by F.Z. and Y.H., acquired fluorescence, μ-XRF, and EDS data. N.J.S. and T.L. analyzed the data. N.J.S. conceptualized the present article and wrote the manuscript, with suggestions from T.L. and P.M. N.J.S. prepared the figures. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological Samples		
Leanchoilia sp. Fossil specimens	Museum of Guizhou Research Center for Palaeobiology, Guizhou University.	GRCP15006
		GRCP15007
		GRCP15008
		GRCP15009
		GRCP15010
		GRCP15011
		GRCP15012
Deposited data		
EDS data for GRCP15009 LM and μ-XRF data for GRCP15008-15011	This study	Zenodo: https://doi.org/10.5281/zenodo.4679952
	This study	Zenodo: https://doi.org/10.5281/zenodo.4679927
Software and algorithms		
Adobe Photoshop CC	Adobe Systems	N/A
Adobe Illustrator CC	Adobe Systems	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Nicholas J. Strausfeld (flybrain@neurobio.arizona.edu).

Materials Availability

Fossil specimens (GRCP15006 - GRCP15012) used for this study are deposited at the Museum of Guizhou Research Center for Palaeobiology, Guizhou University,

Data and code availability

Original μ-XRF and EDS data for GRCP15008, 15009, 15011 generated for this study is deposited at Zenodo: https://doi.org/10.5281/zenodo.4679952.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mid-Cambrian Fossils. Specimens of *Leanchoilia* sp. were collected from the upper Kaili Formation, Cambrian (Miaolingian Series: Wuliuan Stage), in Guizhou, China.

METHOD DETAILS

Fossil photography

Dry specimens were photographed using a Leica M205C microscope equipped with Fusion Optics, white light and an intense UV illumination source. The wavelength of the excitation spectrum was 560 nm to evoke intense red fluorescence at 650 nm. Images at a final magnification of x16–x25 were collected using a Leica M205C digital camera. Images were processed using Adobe Photoshop CC 2017.

Fossil elemental analysis

Micro X-ray fluorescence (μ-XRF) analysis was performed at the Shanghai Advanced Research Institute, Chinese Academy of Sciences (CAS) and Northwestern University using an M4 TORNADO (Bruker) μ-XRF spectrometer operating at 50 kV, 200 μA, with spot size 20 μm, step 20 μm, and pixel time 8 ms. Energy dispersive spectroscopy (EDS) analysis was performed at Nanjing Institute of Geology and Palaeontology, CAS using a TESCAN MAIA 3 GMU microscope equipped with an Oxford energy spectrometer.

Other material

Schematic of labral ganglia and connections in *Hadrurus* and *Palaeomon* (*Palaeomonetes*) are modified from Strausfeld's[4] Figure 9.13, based on anti-hugin immunostaining made in 2011, following the method description in Melcher and Pankratz.[34]