Cambrian origin of the arachnid brain reveals early divergence of Chelicerata

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Fossils from the lower Cambrian provide crucial insights into the origin of arthropod lineages: Mandibulata, exemplified by centipedes, insects, and crustaceans; and Chelicerata, whose descendants include sea spiders and horseshoe crabs and terrestrial spiders, scorpions, and ticks. One fossil genus claimed as an early Cambrian chelicerate is *Mollisonia*, defined by its clustered limbs beneath a rostral carapace, segmented trunk and pygidium, approximating a prosoma-opisthosoma-like organization. Here, we demonstrate singularly preserved trace neuronal tissues that identify a cerebral organization of *M. symmetrica* uniquely distinct from brains attributed to upper stem euarthopods. The fossilized neural traces reveal a copious asegmental cerebrum lying above contiguous ganglia associated with their clustered appendages. This arrangement exclusively characterizes the central nervous systems of extant arachnids. Comparison with the brains of megacheiran chelicerates illuminates the marine origin of Arachnida at the base of the chelicerate tree of life leading to today's most versatile arthropod predators.

INTRODUCTION

Observations of stem arthropod morphologies and their cognate fossilized nervous systems *(1-5)* have thus far resolved two candidate taxa that suggest their identities as stem Chelicerata. These are the short-bodied Megacheira, *Alalacomeneaus* and *Leanchoilia*, whose proso- and protocerebra are defined by two pairs of unfaceted eyes, and whose deutocerebra are defined by "great appendages," typified by pectinate flagellate chelae *(6,7)*. The organization of their fossilized cerebral and trunk nervous system resolves arrangements comparable to the brain and ganglia of immature merostomes (Xiphosura and extinct sea scorpions) *(2-4)*. Until now, the

analysis of these fossil nervous systems, and those of stem mandibulates *(1, 8),* has assumed that central nervous system organization is linear: a bilateral organization approximately aligned along the anterior-posterior neuraxis, the prosocerebrum being the most rostral of the cerebral domains (hereafter referred to as ce1), followed by the protocerebrum (ce2) and the deutocerebrum (ce3) *(4, 9).* The linear nervous systems of the megacheirans, *Alalacomeneaus* and *Leanchoilia,* closely match those of larval *Limulus*, whose chelicerae are supplied by nerves reaching forward from a deutocerebrum located caudal to the protocerebrum *(2,10)*. Further, the postcephalic organization of these megacheiran species is uniformly repetitive: each true segment – the morphologies of which would be defined by Hox gene expression *(11)* - is identically equipped with a pair of biramous appendages comprising a lamellate exopodite and a clawed telopodite, which shows incipient linear differentiation *(12, 13)*.

The discovery in 1988 of *Sanctacaris uncata* promoted an interpretation of its tagmatal organization, not as a chelicerate *sensu lato,* but as tripartite: a prosoma equipped with six pairs of biramous limbs suitable for benthic locomotion and foraging, an opisthosoma whose appendages were suited for swimming; and a post-anal paddle-like pygidium *(14)*. The arachnid-like tagmatization typifying *Sanctacaris* has since allowed the inclusion into the family Sanctacarididae *(15)* of *Wisangocaris barbarahardyae* from the Emu Bay Shale Lagerstätte *(16)*, *Utahcaris orion (15)* and *Messorocaris magna (17)* from the Utah Wheeler formation, and the Burgess Shale *Habelia optata (18)*. Stem chelicerates having comparable tagmata, albeit with trunk segments showing species-specific elaborations and a segmented pygidium, comprise the eponymous order Mollisoniida, whose members include *Mollisonia plenovenatrix* and *M. symmetrica (19-21)*. *Mollisonia plenovenatrix* has provided detailed information about clustered prosomal appendages specialized for walking and grasping that contrast with trunk appendages modified for swimming *(20)*. From those findings, the expectation would be a corresponding elaboration of its rostral neural organization to include both a cerebrum and segmental ganglia.

Here we describe the prosomal nervous system of the historical specimen MCZ 1811, originally named *Houghtonites gracilis (22),* which has subsequently been formally assigned to Mollisoniida as *M. symmetrica (21)*. We present empirical evidence that identifies a brain morphology that radically departs from the linear cerebral ground pattern and ventral nervous system typifying megacheiran chelicerates. Instead, neural traces in *M. symmetrica* resolve a

reflected rostral cerebrum overlying condensed segmental ganglia. These provide a nervous system typifying extant Arachnida.

RESULTS

In contrast to previous studies *(20, 21)*, we analyzed both the part (a) and counterpart (b) of *Mollisonia symmetrica,* specimen MCZ 1811, curated at the Harvard Museum of Comparative Zoology. Figure 1A,B shows both MCZ 1811a and MCZ 1811b photographed under the same low-light intensity and subject to the same digitally adjusted exposure. Whereas the part reveals potential trace neuropils and tracts, the counterpart appears almost opaque. However, stepwise increase of digital exposure (Fig. S1) of the counterpart reveals many more details of fossilized neuropils and axonal pathways, some of which match structures also resolved in the part (Fig. 1C,D). For example, the isolated nerve tract and its collaterals, marked by an asterisk in Fig. 1A and enlarged in frame 1 of Figure 1C, correspond to neuropil discernable in frame 2 in Fig. 1D. Other traces framed in Fig. 1D are interpreted as trace neuropils that are resolved in detail as shown in frames 2-4, enlarged in Fig. 1E. Also of note is evidence for one of a pair of chelae (bracketed in C) extending forwards medial to the eye.

Published elemental scans of the prosomal area reveal carbon traces converging from the two eyes centrally *(21)*. Their correspondence to backscatter electron microscopy evidence showing the same arrangements has been used to justify carbon signals as identifiers of fossilized neural tissue *(21)*. We evaluated the same energy-dispersive X-ray spectroscopy (EDS) carbon scan at a higher resolution (Fig. S1H) and compared this to the structures obtained by photomicroscopy. Other than one false positive, all carbon signals in the enlarged EDS scan correspond to profiles obtained photographically (Fig. S1G,H). Together, photographic images validated by carbon scans provide compelling evidence of cerebral tissue extending across almost the whole prosoma.

We next traced the boundaries of neuropils and tracts to reconstruct the cerebrum and other neuropils underlying it, noting that the ventral surface and what lies immediately above it is shown in the part, whereas the counterpart provides information of more dorsal arrangements. As a result, neural traces in the part find their corresponding traces in the counterpart (Fig 2A, B). Some asymmetries of trace neuropils common to both sides of the counterpart (Fig. 2C) further indicate that the part and counterpart are split obliquely into two unequal pieces. This tilt enables

examination of layered organization within a volume that has undergone considerable compression. For example, the traces indicated by the three arrows on the right of the counterpart in Fig. 2A correspond to the traces indicated on the right of the part in Fig. 2B. Traces indicated by arrows on the left of Fig. 2A suggest bilateral symmetry with the trajectory of the neural trace on the right.

The images in Figs. 2C and 2D provide the outlines of neuropil and gut diverticuli. The distribution of corresponding features between the part (Fig. 2D), mainly comprising ventral components of the prosoma, and the counterpart revealing more dorsal components of the prosoma (Fig. 2C), indicates a tilted specimen: the left side of the counterpart is more ventral than the right side allowing an interpretation of traces representing two distinct neural ensembles. One, which is shown as white tracings in Fig. 2C, D, is superior to a deeper level outlined in yellow. These mappings across the part and counterpart suggest a segmental series of fused neuropils that we here interpret as relating to six pairs of appendages associated with the first six true trunk segments of this arthropod. Although no appendages are preserved in situ, low-angle, cross-polarized illumination indicates disassociated limbs that occur only in proximity to the prosoma (Fig. S2B). The white profiles in Fig. 2C, D represent the asegmental cerebrum, the outlines of which suggest their forward projection toward and into the chelae.

This organization suggests that the cerebrum (white profiles) is folded back over the underlying trunk segments (yellow profiles) resulting in the forward location of the deutocerebrum. This arrangement distinguishes the mollisoniid brain organization from that typifying *Alalcomenaeus* and instead aligns it with that of extant arachnids (Fig. 2I, J). The two distinct, but obviously functionally integrated, nervous systems of *Mollisonia* are summarized as mirror symmetric in Fig. 2E, with the ventral nervous system shown on its own in Fig. 2F with those gut diverticuli identified in elemental maps of calcium *(21)*. It is notable that the ventral segmental nervous system is highly constrained laterally, as are segmented ganglia of the arthropod trunk *(23)*. Together, this evidence allows the recognition of neural tissues and thence a graphical interpretation of the mollisoniid prosomal nervous system (Fig. 3A,B). This analysis also allows a reconstruction of the likely line of fracture separating the part and counterpart (Fig. 3).

Existing precedents for a backward-folded cerebrum are provided by total Arachnida, examples of which are shown in Fig. 2G-K. The degree to which neural traces dominate the

fossilized prosoma of MCZ 1811 is less surprising in the light of what is known about extant arachnid neuroanatomy. The prosomal nervous system of arachnids is unusually dense and possesses very few long-distance tracts compared with the brains of mandibulates *(24)*. Figure 2G, H shows the dense neuropils of the ce2 domain (protocerebrum) of an aranean cerebrum and the stratified neuropils of its ce1 domain (prososcerebrum). Further evidence that arachnid cerebra are copiously endowed with neuropils is shown in Fig. 2K, where the cerebrum occupies almost the whole extent of the prosoma.

Notably, as shown in Fig. 2H, the nerve connecting the arachnid principal eyes extends extreme laterally to reach the ce1 domain. This trajectory corresponds to the lateral tracts visible in the counterpart and part of *Mollisonia* (arrows on right in Fig. 2A, B). A backward reflected cerebrum is further demonstrated in a pseudoscorpion (Fig. 2I) and orb spider (Figure 2J), the latter shown with its forward projecting nerve tract originating in the ce3 domain (deutocerebrum) and connected to a chela. The consequence of this cerebral inflexion helps to clarify the distinction of cheliceral innervation in *Mollisonia.* In the reflected cerebrum of MCZ 1811b, the deutocerebral ce3 domain is the most rostral domain from which axons reach into the small, paired chelicerae as they do in extant arachnids (Fig. 2D).

This neural arrangement of the *Mollisonia* brain is further supported by the non-neural endomesodermal interface (EMI) between the fore- and midgut. In *Mollisonia* the EMI, indicated by the red oval aligned across Fig. 4 appears to be located in front of the cerebrum (Fig. 4D, E); however, the first three cerebral domains are folded back to lie over the condensed segmental ganglia of the trunk nervous system beneath (Fig. 4D). If the cerebrum would be unfolded, the location of the EMI would indeed be situated where it is in linear brains; namely, immediately in front of or at the anterior boundary of the T1 segment (Fig. 4B,C, compared to 4D,E). In living araneans, the backward folding of the brain is a result of morphogenetic movements during late embryogenesis when the opisthosoma straightens and condenses its trunk segments, whereas the curvature of the embryo around the spherical yolk persists rostrally (*25*). This results in the deutocerebrum becoming located most anteriorly with reference to the body axis. The observed similarities thus identify the 'reflected brain' as a shared derived character of both *Mollisonia* and extant arachnids.

Ganglia serving the six pairs of prosomal appendages, their presumed presence based on their description for *M. plenovenatrix (20)*, are not visible. But fragments of limbs each side of

the prosoma (Fig. S2B) suggests that they lie in the matrix in which MCZ 1811 was encased, alongside the part thus indicating their ventral disposition. The presence of ganglionated trunk nervous system is indicated by a description of another very much smaller specimen of *M. symmetrica* (USNM 305093), which offers a ventral view where each trunk segment is equipped with an identical ganglion *(21)*. However, its prosomal nervous system appears to be undivided and therefore suggested to provide innervation to seven pairs of appendages leading to an interpretation of the brain comprising fused ganglia disposed as a 'synganglion' *(21)*. Our identification of linearly organized ventral segment-like neuropils accords with an interpretation positing that the first six pairs of trunk appendages are associated with the six anterior trunk segmental ganglia situated beneath the cerebrum - as mapped in Figs. 2E, F and reconstructed in Fig. 3A, B - thus providing a neural architecture that is diagnostic of that of extant arachnopulmonates (Fig. 2I, J).

So far unconsidered is the distinction between the visual system of *M. symmetrica* and that of *M. plenovenatrix* whose paired eyes each contains two retinal areas sharing the same dioptric volume and hence two first optic neuropils both of which appear to extend tracts centripetally to a more central neuropil domain *(20)*. The present analysis of *M. symmetrica* suggests in addition to the lateral nerve, a second pathway from the eyes, possibly originating from, or overlying, a small dark ovoid structure at the eye's inner margin, adjacent to or just overlapping the main retinal area. Variations of illumination (Fig. S2A–C) suggest that the dioptric area is more pear-shaped than spherical and thus able to accommodate one large and a second much smaller retinal area, the larger area revealing a hexagonal mosaic organization here interpreted as organized retinotopic units defined by the photoreceptor layer and its first order circuitry. These retinal arrangements conform to the ground pattern of the arachnid visual system, bearing in mind that embryogenesis of the aranean visual system includes intermediate stages in which small principal and a paired large secondary lateral eyes appear to be conjoined *(26)*

DISCUSSION

Elucidating the component organization of the arthropod head has been called "the endless pursuit" *(27)*, in part due to the striking diversity of morphologies, but also because of the assumption that partitions of the brain reflect the organization of a segmental exoskeleton. Yet

early studies on nervous system embryogenesis informs that the embryonic mandibulate cephalon is asegmental *(28)*, with recent studies confirming this both in arachnids and mandibulates *(29, 30)*. Understanding the origin and evolution of these asegmental domains is crucial in defining cephalic traits that distinguish mandibulates from chelicerates and, within the latter, arachnids from merostomes and pygnogonids. In considering these it is useful to bear in mind that precisely timed non-cyclic combinatorial gene expression patterns determine the partitioned brain *(29)* in contrast to the cyclic expression of segmentation genes that determine the segmental trunk *(31,32),* composed of 'repeated units along the anterior-posterior (a-p) axis having the same a-p polarity' *(33)*. The cerebrum is defined by three unique neural domains (ce1-ce3), each specified by the combinatorial activity of highly conserved homeobox genes *(9, 34)*. In crown euarthropods, each cerebral domain is characterized by its unique genetic code and the resulting neuropil with its associated sensory traits (Fig. 4A, B, G). These neural arrangements of extant euarthropods align with identifiable fossilized soft tissue interpreted as neural in the linear cerebrum of *Alalcomenaeus* (Fig. 4C) and in the reflected cerebrum of *Mollisonia* (Fig. 4E) and living Arachnida (Fig. 4F). For example, the prosocerebral ce1 domain is defined by combinatorial *FoxQ2, Six3,* and *hbn* activity, anterior to the rostral limit of Pax6 expression *(35)*. Its suite of sensory and neurological traits, also identifiable in fossils, includes paired labra, the principal eyes of arachnids and their cognate neuropil *(36)*. The protocerebral ce2 domain is likewise specified by a unique combination of conserved homeobox gene expression patterns, as is the deutocerebral ce3 domain (Fig. 4). The correspondences between neural arrangements and underlying genetic code suggest that exceptional fossilized traces relating to appendicular structures are homologs of those belonging to extant taxa irrespective of the character state: linear or reflected brain (Fig. 4).

The present finding that *Mollisonia* possessed a reflected arachnopulmonate cerebrum begs the question whether this taxon can be viewed either as an inclusive upper stem ancestor of total Chelicerata as recently proposed *(21)* or as the exclusive upper stem ancestor of Arachnida. The present findings also bring Megacheira, as represented by *Alalcomenaeus* and *Leanchoilia (2–4)*, to the fore as denoting a divergent merostomatan stem implied by its separate retinal arrangements *(37)* and its linear brain *(2)*. Fossils of crown chelicerates are not informative: the earliest terrestrial arachnopulmonates are lower Devonian *(38,39)*. The first limulus-like fossils are ascribed to the late Ordovician, with a late Carboniferous specimen preserved with its entire

serial central nervous system that demonstrates the posterior location of the cheliceral 'ganglia' *(40)*.

Molecular phylogenetics indicates that Arachnida likely diversified as marine and colonized land well before fossil evidence indicates it *(41)*. The reflected configuration of the arachnopulmonate cerebrum here ascribed to mollisoniids requires a view that diverges from the conventional placement of *Limulus* within Arachnida. A separate status of *Limulus* is supported by its linear cerebral organization, which differs from the reflected cerebral organization of *Mollisonia* and Arachnida (Fig. 5). Pycnogonida, recognized as the sister group of Euchelicerata, possess linear brains. Although in many pycnogonid species the neuraxis is tilted upwards, their chelicerae are innervated by a deutocerebrum situated posterior to the protocerebrum *(42-44)*, as in Xiphosura *(10, 43)*. The linear brain supports the proposition that megacheirans with linear cerebra comparable to the developing limuliid brain *(2)* are best viewed as upper stem euarthropods most closely related to a xiphosuran/pycnogonid grouping with the exclusion of Arachnida (Fig. 5). These distinctions of cerebral organization, linear in fossil *Alalcomenaeus* and extant Pycnogonida and Xiphosura, but reflected in fossil Mollisoniida and extant Arachnida (Fig. 4C-F), resonate with Ballesteros et al.'s recent analysis *(45)* that focuses on the ambiguous status of Merostomata, to which Xiphosura belongs: the question being whether Merostomata is integrated within or is distinct from total Arachnida.

Here we have shown that the cerebral disparities indicate a split between Arachnida and Merostomata had already occurred by the mid-Cambrian. *Mollisonia* as the stem taxon closest to extant Arachnida indicates a marine origin of Chelicerata, *sensu* Ballesteros. The megacheiran *Alalcomenaeus (2)* is the stem taxon closest to Pycnogonida and Xiphosura.

METHODS

Fossil provenance. We thank Dr. Javier Ortega-Hernández of the Museum of Comparative Zoology and the Department of Organismic and Evolutionary Biology, Harvard University, who provided access to specimens MCZ 1811a and MCZ 1811b at the Harvard Museum of Comparative Zoology in which the curated specimens are stored.

Fossil analysis. Files of the counterpart (MCZ1811b) showing elemental distributions acquired by energy dispersive X-ray spectroscopy were provided by Dr. Javier Ortega-Hernandez,

Harvard Museum of Comparative Zoology (HMCZ) who arranged N.J.S. access for documentation of specimen MCZ 1811a and b, which provide data discussed herein. Specimens were photographed wet with cross polarization. Images were taken mainly at medium to low light intensity with exposure times ranging from about 0.5-3 seconds. Images were acquired using a Zeiss Axiocam 208 color camera mounted on a Zeiss Stemi 305 microscope linked to a laptop computer running Zen 3.5 software. A Zeiss CL6000 was the light source for flexible dual branch light guides with twistable polarization filters. Red or green gel Wratten filters were occasionally used for color neutralization. Selected enlargements of high-definition files were processed using Adobe Photoshop 24.6 employing raw filter functions for adjusting color parameters in preparation for conversion to greyscale. Further digital adjustments involved exposure, offset and contrast.

Histological Microscopy. The collection of silver stained nervous systems produced in the N.J.S. laboratory representing major arachnid groups provided comparison with trace neuropils described herein. Images of the reduced silver- or osmium-stained cerebrum of aranean brains were taken using a JenaOptic Progress C3 digital camera fitted to a Leica Diaplan microscope equipped with plan neofluar oil immersion objectives.

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expression data to the described panarthropods. N.J.S. and F.H. wrote the manuscript.

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Author contributions: N.J.S. and F.H. originated the project, analyzed documentation of the specimens. F.H. ascribed published gene expression data to described panarthropods.

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FIGURES AND LEGENDS

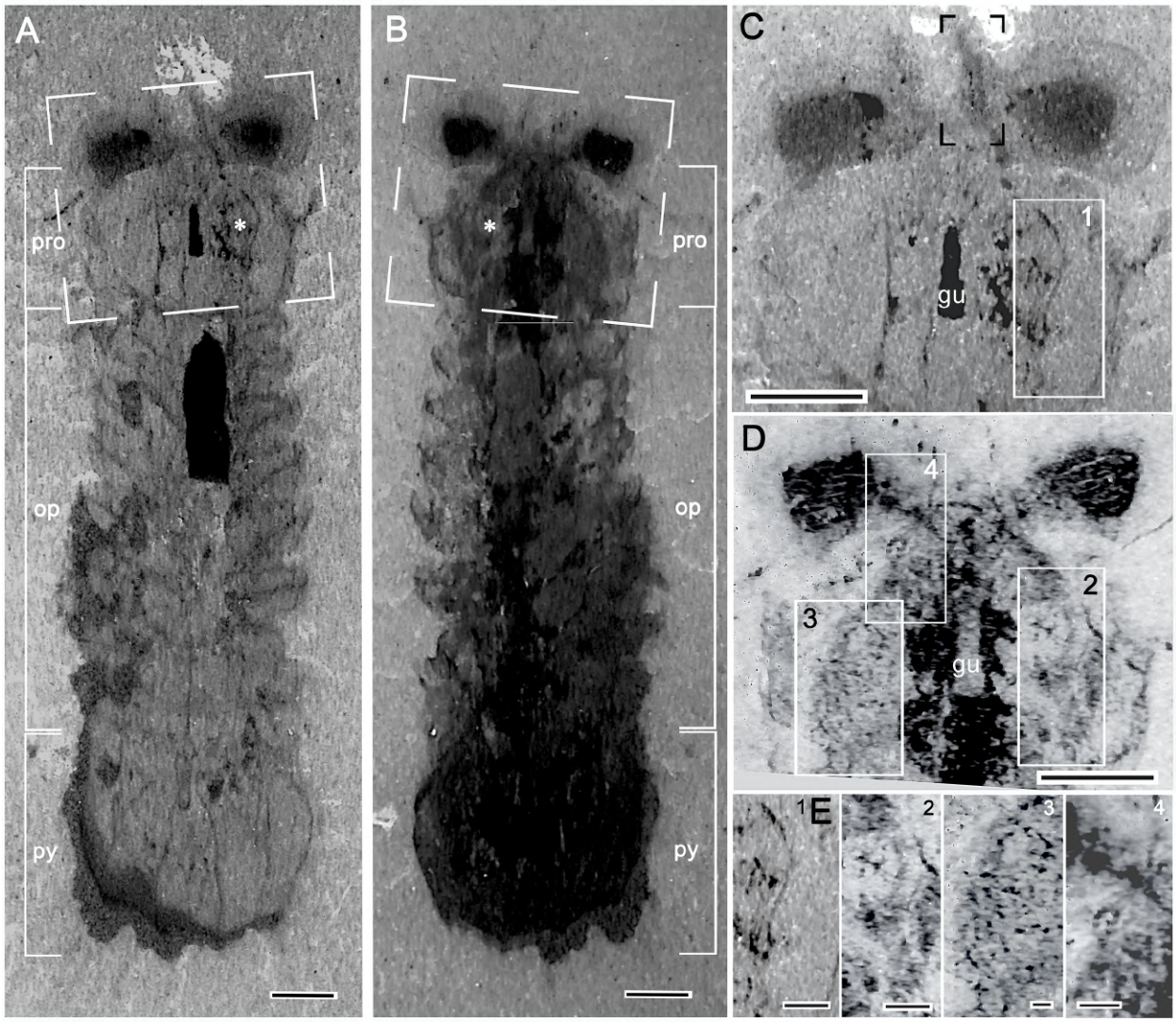

Figure 1. Trace neuronal tissue beneath the prosomal carapace of *Mollisonia symmetrica* (MCZ 1811). (**A,B**) Mirrored part (MCZ 1811a) and counterpart (MCZ 1811b) photographed wet with cross-polarized illumination. Framed areas are enlarged for panels C, D. Asterisks indicate corresponding locations in part and counterpart. The body is divided into three tagmata: prosoma (pro), opisthosoma (op), and pygidium (py). **(C)** Enlargement of framed area in A. One of a pair of frontal chelae (ch) is bracketed. **(D**) Enlargement of framed area in B, flipped to facilitate comparison with c and digitally processed with deep focus and extended illumination (See Methods and Fig. 1S) resolves structured neuropil (2, 3) lateral to the gut (gu). Corresponding traces of discrete neuropil in the part (box 1) and counterpart (box 2) are shown side by side in insets 1,2 of panel **E** , which also shows boxes 3, 4 in D enlarged. Scale bars: A-D = 1mm; E1= 100µm, E2-5 = 250µm.

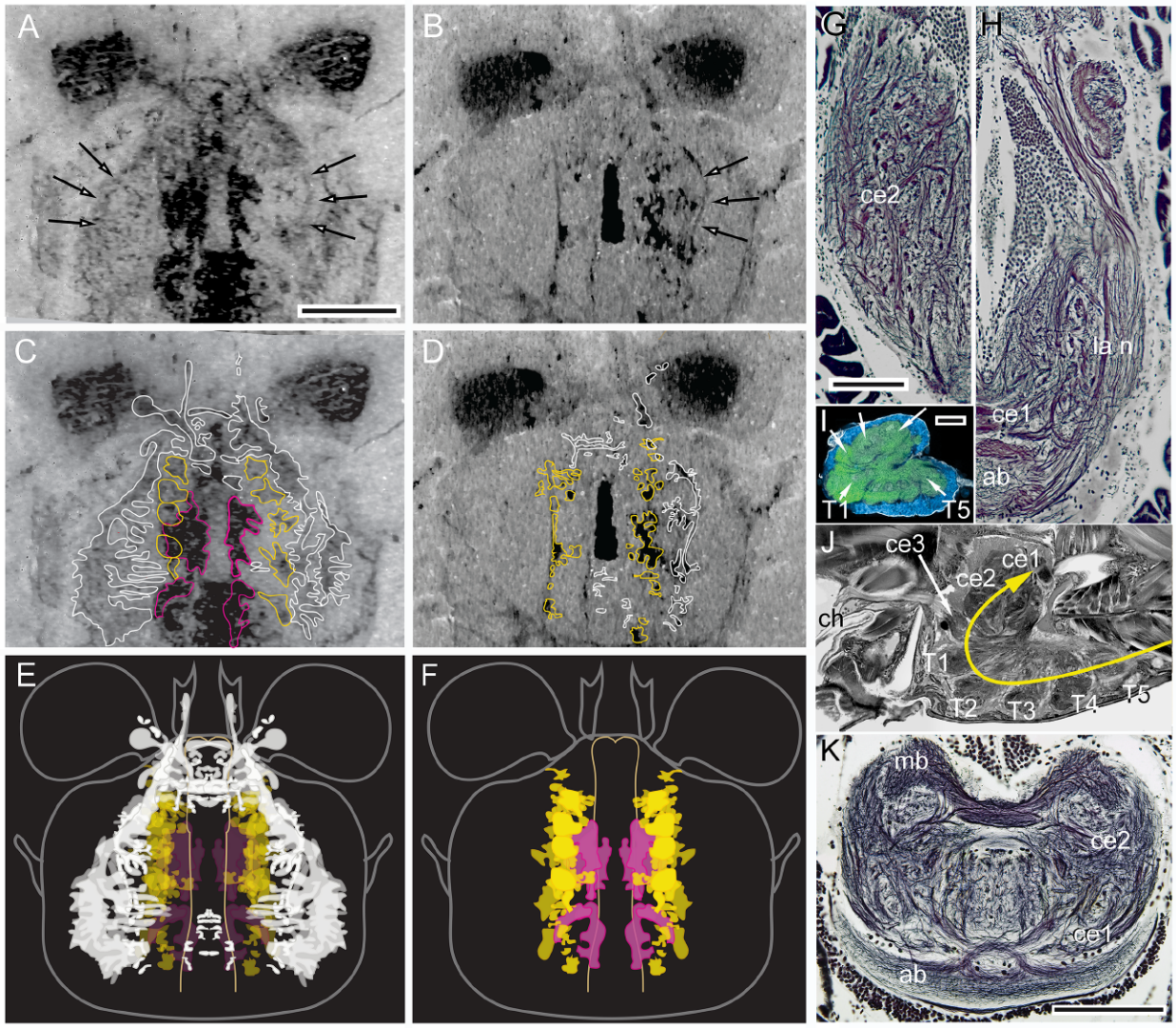

Fig. 2. Fossilized neuropils and tracts of *Mollisonia symmetrica* correspond to extant arachnid brains. (**A**) The counterpart MCZ 1811b is invested with many more neural traces than the part MCZ 1811a (**B**). Nevertheless, neural traces in the counterpart coincide with traces in the part, as indicated by the arrows on the right. Arrows on the left in A indicate contralateral traces at a level deeper in the counterpart than in the part (see also Fig. 3). (**C,D**) Copies of panels A,B show outlines of neural traces (yellow) flanking the gut and its diverticuli (magenta). Widespread bilateral neuropils located above the domains traced in yellow are outlined in white. (**E,F**) Superposition of mirrored and filled white traces interpreted as cerebral onto more ventral neuropil represented by mirrored yellow profiles at 50% opacity. These are shown fully represented, flanking gut diverticuli (magenta) in panel F. Their restriction to narrow elongated territories is interpreted as evidence of fused segmental ganglia. (**G,H**) Distribution and laterality of neuropils of the huntsman spider *Delena cancerides,* typifying the protocerebral (ce2) and prosocerebral (ce1) domains, the latter defined by the arcuate body (ab). The lateral nerve (la n) in panel H corresponds to the lateral pathway indicated by the row of arrows on the right in panels A,B. This pathway is a defining attribute of arachnid cerebra. (**I,J**). Sagittal sections

showing segmental ganglia (T1-T5) under the backward-inflected cerebral domains in the pseudoscorpion *Chelifer cancroides* (I) and in the orb spider *Tetragnatha* sp (J). In panel I, the three unlabeled arrows indicate from left to right: the ce3 domain (aka cheliceral 'ganglion'), the protocerebral domain ce2, and prosocerebral domain ce1. (**J**) Sagittal section of the orb spider prosoma showing its ce3 domain in the most rostral location where it provides axon bundles projecting forward to the chelae (ch). In extant arachnids five prosomal appendage pairs are served by paired segmental ganglia T1-T5. The backwards inflected yellow arrow indicates the neuraxis typifying arachnids. (**k**) Horizontal section across the cerebrum of the ogre spider *Deinopis spinosa*. As in *Mollisonia*, and typical of arachnids, almost the entire prosoma is occupied by the cerebrum. The ce2 domain is dominated by its heterolateral mushroom body (mb), and the ce1 domain is defined by the arcuate body (ab). The ce3 domain, which is at a lower level, is not visible. Notably, although the ce2 and ce1 neuropils are clearly lateralized, they also extend patchwise across the underlying gut. Scale bars: panel A (for A-F) =1mm; panels G,H = 200 μm; panel I = 100 μm; panels J,K =250 μm.

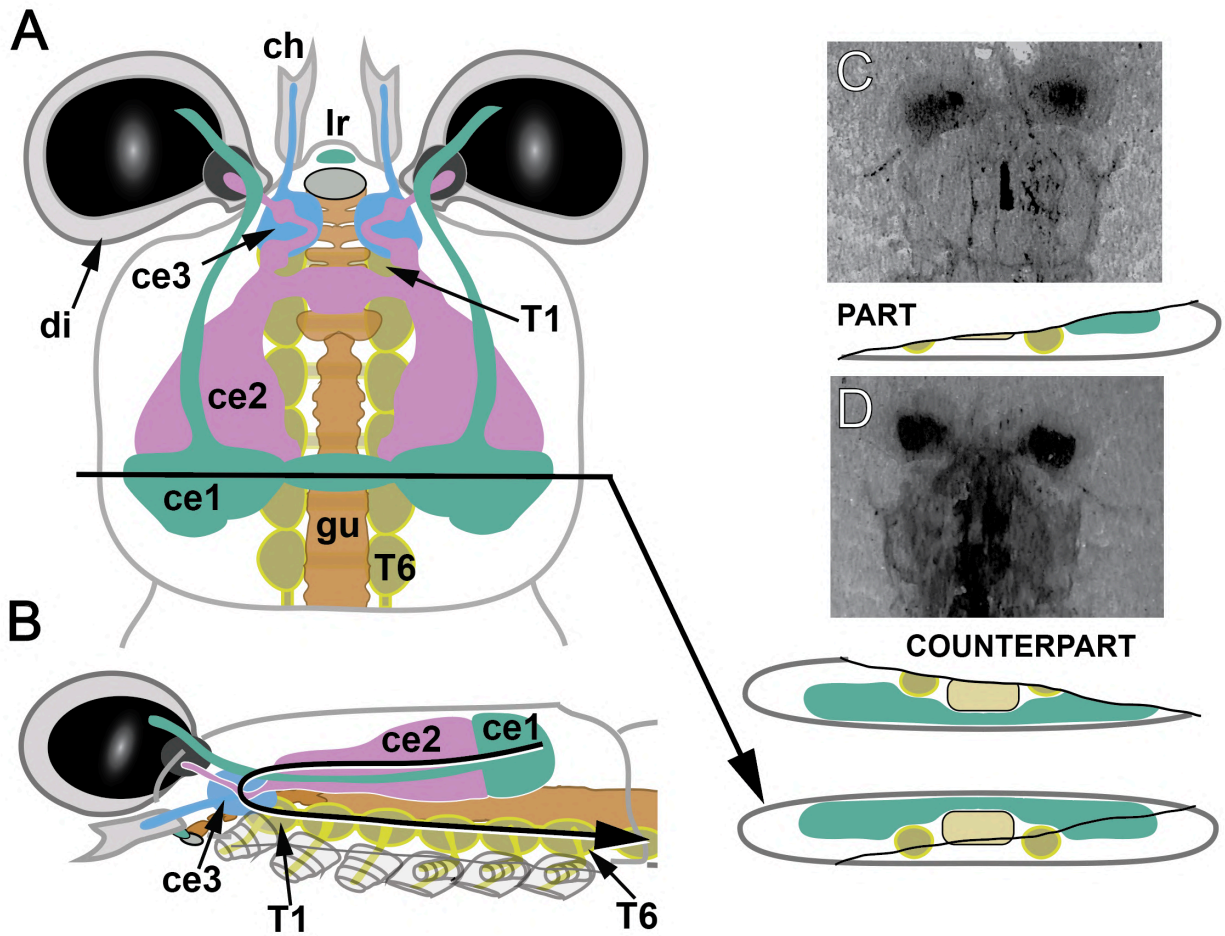

Fig. 3. Reconstructed brain and segmental ganglia of *Mollisonia symmetrica* with reference to part and counterpart contributions. (**A**) Interpretive reconstruction of the prosomal organization of the stem arachnid nervous system. Data provided mainly from the counterpart of MCZ 1811 allows identification of fossilized neural traces of bilaterally symmetric, asegmental neuropils ce1–ce3, which occupy most of the prosomal space beneath the "carapace." These arrangements correspond to those typifying arachnids (see Fig. 2G-K). Neuropil flanking the gut (gu; see Fig. 2F) suggests fused segmental ganglia. (**B**) Interpretative depiction of a side-view of asegmental neuropils ce3-ce1 overlying segmental ganglia T1-T6. The ce3 domain (deutocerebrum) provides short axon bundles to the chelae as in extant arachnids (Fig. 2J). The assumed depth of the prosoma is as found in many arachnids, such as scorpions or pseudoscorpions. Each paired ganglia is assumed to supply a corresponding pair of leg-like segmental appendages. (**C,D**) Image analysis of the fossil's part (C) and counterpart (D), suggest that the fossil was split obliquely, such that the counterpart contains more traces than the part, as suggested by comparisons of paired panels A and B in Fig. 2. The depths indicated in the interpretive drawings for C, D are exaggerated for clarity of explanation.

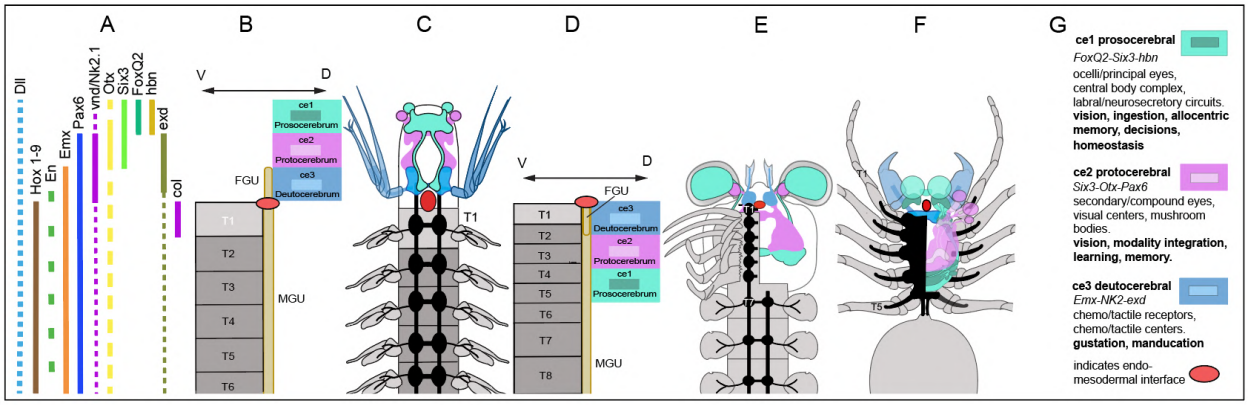

Fig. 4. The linear cerebrum of *Alalcomenaeus* and the reflected cerebrum of Arachnida. (**A**) Combinatorial expression of homologous genes across extant taxa (for source references see *9*) defines the three domains of the asegmental cerebrum (ce1 prosocerebrum; ce2 protocerebrum; ce3 deutocerebrum) with first trunk segment T1 defined by anterior Hox gene expression and *collier (46)*. The junction between the asegmental cerebrum and the segmental trunk coincides with the endomesodermal interface (red ovoid). (**B,C**) Linear brains exemplified here by the megacheiran *Alalcomenaeus*, aligned along the anterior-posterior axis with the cerebrum situated dorsally above the foregut (FGU) and subsequent trunk ganglia beneath the midgut (MGU), as schematically shown with its deutocerebral nerves supplying the chelate appendages that define the most caudal domain ce3. (**D**) Schematic of the reflected arachnid brain and segmental ganglia (gray, T1-T8 etc.). (**E**) Top-down view into the prosoma and first three opisthosomal segments (T7, etc.) of *Mollisonia*, with the carapace and left half of the brain removed to expose the underlying prosomal appendages and their ganglia (T1-T6). The reflected cerebrum, typified by *Mollisonia* and defining extant Arachnida, locates the deutocerebrum (ce3 domain) forward as the most rostral domain from where it supplies nerves to the paired chelicerae. (**F**) The reflected cerebrum is essentially unchanged in extant arachnids, here represented by the brain of a salticid aranean in which the ancestral prosomal appendages are further reduced to paired pedipalps and four pairs of walking legs. (**G**) Genetic code of brain domains, the resulting domain-specific neural centers and associated sensory traits that apply across extant Euarthropoda and Onychophora *(9)*.

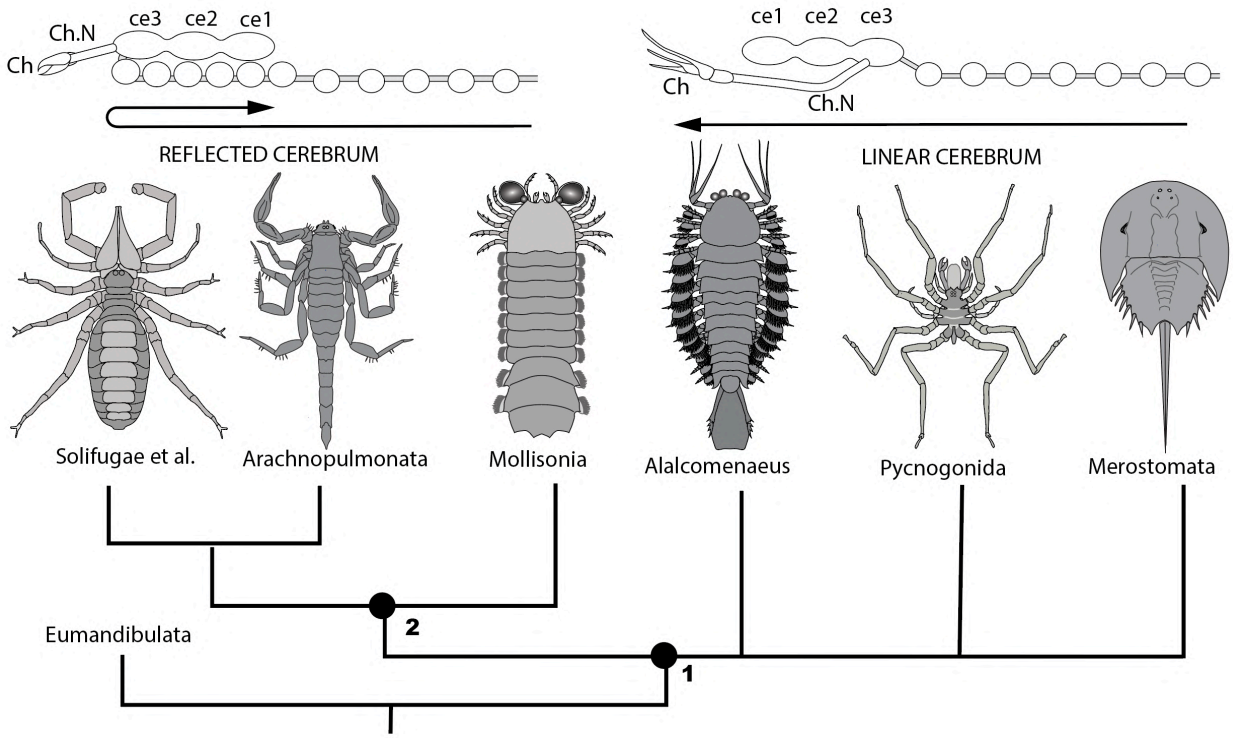

Fig. 5. Divergent evolution of the Chelicerata *sensu lato*. Two fossil total-group chelicerates, *Mollisonia* and *Alalcomenaeus,* exemplify divergent ground pattern organizations of the cerebrum (ce1-ce3), namely, linear or reflected. The lineages *Alalcomenaeus*, Limulidae and Pycnogonida participate in a four-way polytomy (node 1) with the clade total-group Arachnida at node 2. These phylogenetic relationships resonate with recent phylogenomic analyses of all living chelicerate orders (*45,47*). The present data identify the megacheiran *Alalcomenaeus (2)* as the stem taxon closest to Pycnogonida and Xiphosura/Merostomata, and *Mollisonia* as the stem taxon closest to extant Arachnida, thus revealing a marine origin of Arachnida and total group Chelicerata. As part of the reflected brain ground pattern, the ce3 domain lies most anterior with regards to the A-P body axis, its short cheliceral nerve (Ch N) reaching to the chela (Ch). In the linear brain, ce1 is the most rostral brain domain with regards to the A-P body axis, whereas the ce3 is the most caudal brain domain which provides an extended cheliceral nerve reaching its deutocerebral appendages.

SUPPLEMENTARY FIGURES

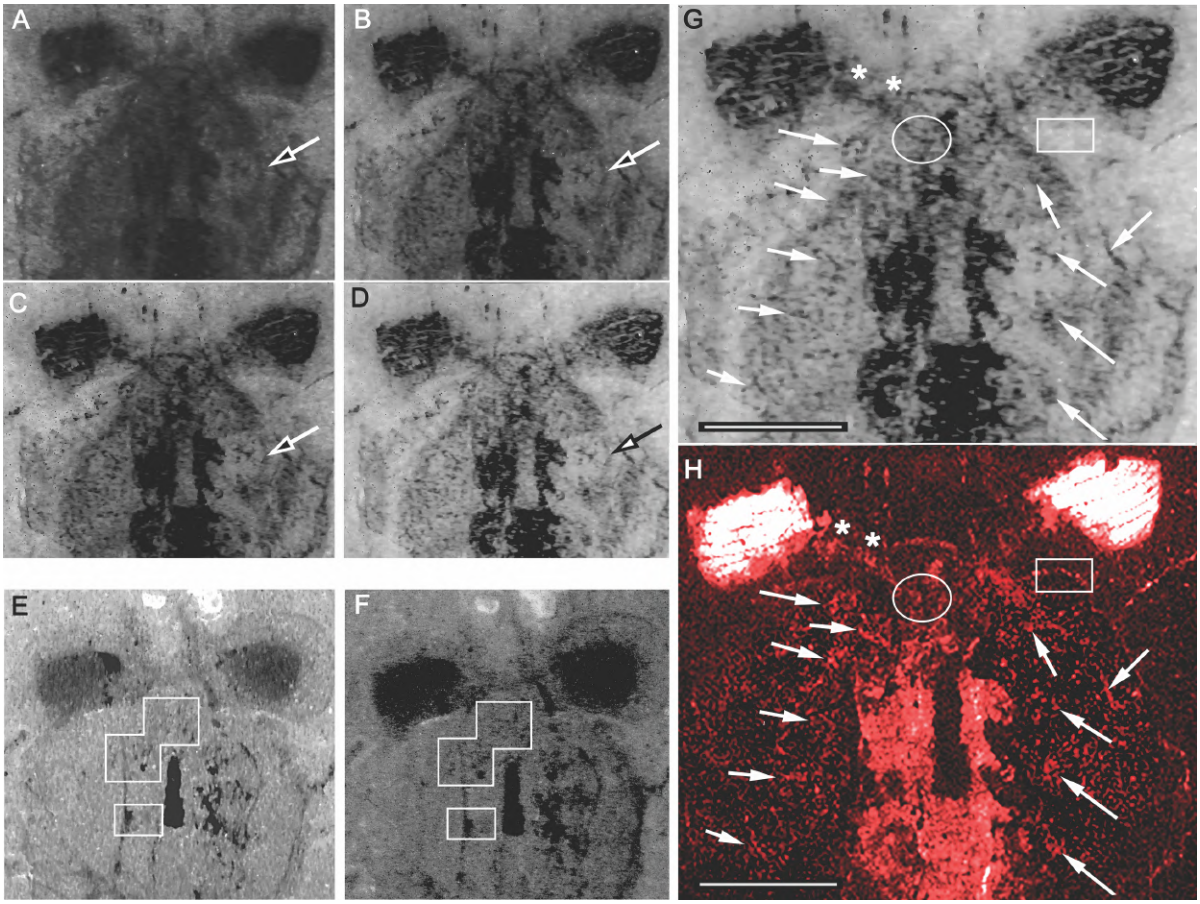

Fig. S1. Resolution of fossilized neuropils and tracts in *Mollisonia symmetrica* (MCZ 1811). (**A**) Counterpart MCZ 1811b: original image achieved using extended exposure and low-light intensity (wet specimen, cross polarization). (**B-D**) Stepwise increasing of digital exposure and offset adjustments resolves finer details as indicated by arrows in A-D that indicate the lateral nerve. (**E,F**) Part MCZ 1811a: Decreasing exposure (F) can resolve ambiguous traces (compare boxed areas in E and F). (**G,H**) Neuropils and tracts in MCZ 1811b resolved photographically (G) compared with the carbon map (H) obtained by energy-dispersed X-ray spectroscopy (EDS). Arrows indicating a selection of corresponding features validate their interpretation as fossilized nervous system. Oval in G illustrates clarity of the photographed image of the optic nerve (asterisks) compared with its 'noisy' carbon image in H. Comparison of G and H also resolves false positives in EDS scans. The rectangle in G shows no obvious structure in the photograph, whereas the EDS carbon scan provides a signal perhaps relating to a crack or fault in the matrix. Scale bar= 1mm.

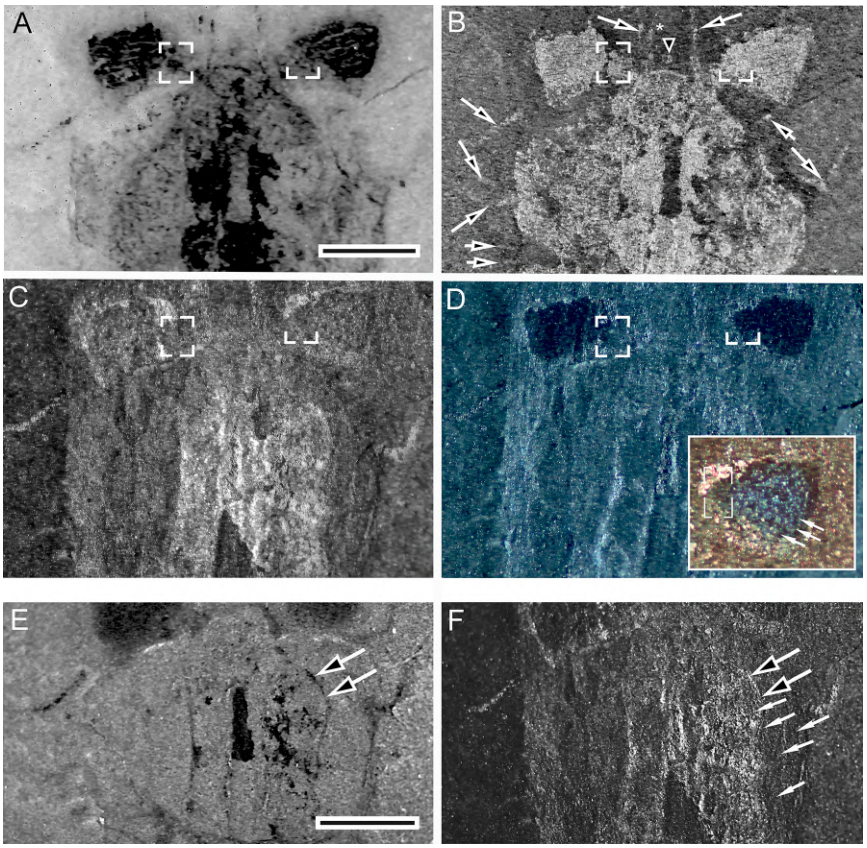

Fig. S2. Resolving ambiguities of exposed soft tissue preservation in *Mollisonia symmetrica*. (**A-D**) Evidence that the present species of *Mollisonia* possesses two receptor/neuropil areas within a shared dioptric structure. (**A**) Neural traces in the counterpart MCZ 1811b, flipped to match orientation of part, include the single large retinal dark area in both eyes and, in one eye (left) a smaller second area (framed). The corresponding contralateral region appears empty (bracketed). (**B**) Low-angle, bilateral cross-polarization of MCZ 1811b reveals reflective features including remnants of the fossil's prosomal appendages (arrowed) with some extending forwards lateral to the chelae (one indicated by asterisk) and labrum (triangle). Both eyes show surface reflections of the dioptric (total eye) areas, including the bracketed area on the right, which appears empty in A. (**C, D**) MCZ 1811a: Bright-light, low-angle illumination of water-immersed part (C) hints at a teardrop profile for both eyes (frames and brackets correspond to those in A, B), a feature further suggested by very low-angle, cross-polarized illumination of surface features (D, water immersion). The inset to panel D is defocused red color layer combined with sharp focus cross- polarization to resolve defined rows of retinotopic units (arrowed) outside the small area (framed). (**E,F**) Micro-irregularities of the matrix have the potential for confusing with neural traces. Here a section of the fossilized lateral neural tract in the part (indicated by double arrows in E) coincides with a parallel intrinsic property of the matrix (double arrows in F). Abundant curved striations (small arrows) suggest this wide-spread patterning of the matrix typifies its broken surface. Comparison of the neural traces in E with surface structures in F resolves this as coincidental. Scale bar in A for panels A-C=1 mm; scale bar in panel E, also for F = 1mm.