



TECHNICAL RESPONSE

ARTHROPOD EVOLUTION

Response to Comment on “The lower Cambrian lobopodian *Cardiodictyon* resolves the origin of euarthropod brains”

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Budd *et al.* challenge the identity of neural traces reported for the Cambrian lobopodian *Cardiodictyon catenulum*. Their argumentation is unsupported, as are objections with reference to living Onychophora that misinterpret established genomic, genetic, developmental, and neuroanatomical evidence. Instead, phylogenetic data corroborate the finding that the ancestral panarthropod head and brain is unsegmented, as in *C. catenulum*.

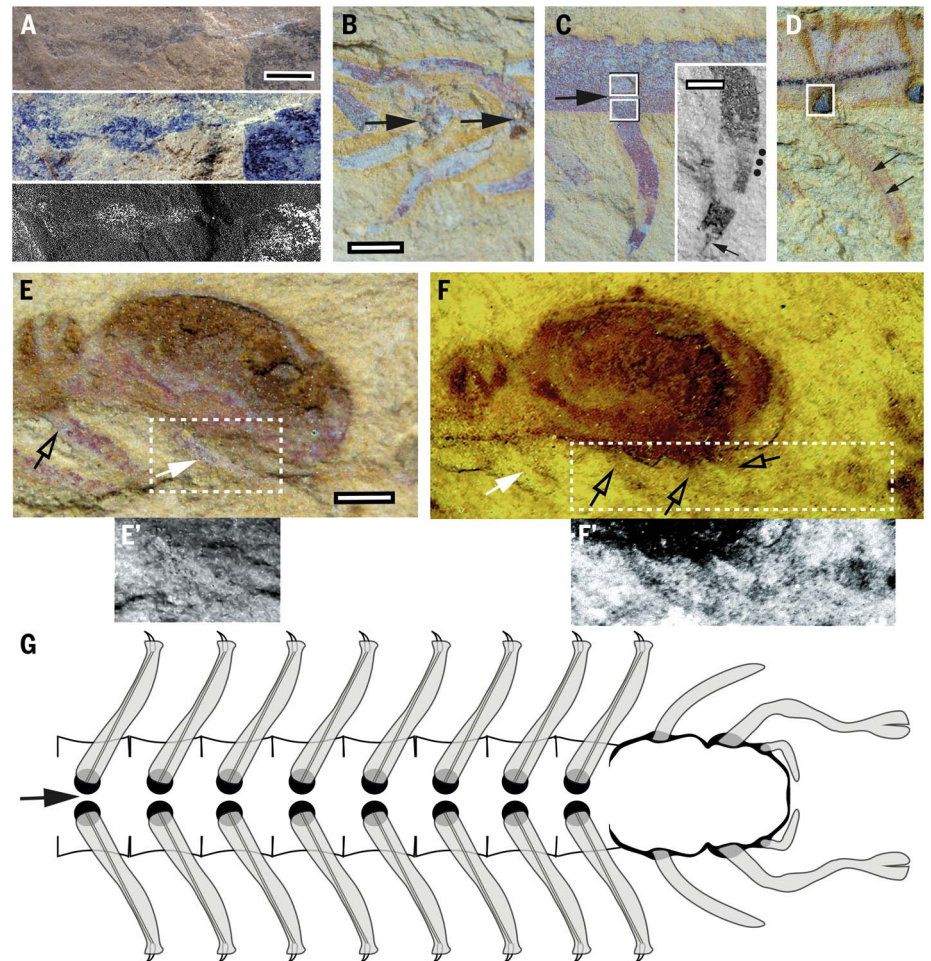
Contemporary genetics and genomics can inform paleontological observations for comparing traits across taxa. Using this combined approach to compare cerebral arrangements across panarthropods, including the lower Cambrian lobopodian *Cardiodictyon catenulum*, resolves a common ground pattern of organization (1). Without

new evidence, Budd *et al.* (2) resort to two lines of argumentation to dispute this finding. The first challenges our characterization of the *Cardiodictyon* nerve cord and brain. The second argues for a segmented head and brain of Onychophora. Neither argument counters evidence reported by Strausfeld *et al.* (1).

Budd *et al.* (2) dispute that neural collaterals extend from the *C. catenulum* ventral nerve cord [figure 2, A to D, in (1)], claiming that they might instead represent body wall annulation or, failing that, then musculature. The decay of neural tissue faster than muscle appears to favor iron deposition and hence carbon (3), the latter demonstrated by chromatic filtering and energy dispersive x-ray spectroscopy (Fig. 1A). Micro-annulation is absent for the *C. catenulum* trunk but is resolved for trunk limbs (Fig. 1C). Sparse muscles conjectured by Budd (4) for stilt-legged lobopodians would bear no correspondence to branched collaterals of the ventral nerve cords revealed by chromatic filtering [figure S4 in (1)]. If in *C. catenulum* there existed

Fig. 1. Chromatic filtering and energy dispersive x-ray spectroscopy reveal fossil neural traces.

(A) Chromatic filtering reveals the retina, optic tract, and neuropils of the radiodontan *Lyrarapax unguispinus* (Cong *et al.* *Nature* 513:538–542). Upper panel: raw image taken with white light; middle panel: suppression of spectra other than blue showing additional evidence of structures supporting energy dispersive x-ray spectroscopy of carbon deposits (lower panel). Scale bar = 1mm. (B–G) Trunk limbs of *Cardiodictyon catenulum* are distinct from its cephalic appendages. (B) Paired trunk limbs extend laterally from loci immediately adjacent to the trunk's midline (arrows). (C) Trunk rotated counterclockwise by about 15° around the anterior-posterior axis shows empty limb socket (upper box) paired with socket (lower box) of the intact contralateral limb. Whereas the trunk lacks microannulation, the enlargement (inset) shows microannulations (three indicated by dots) typical of trunk limbs, which are the only appendage with a terminal claw (inset, arrow). (D) Absent the epidermis, the trunk reveals limbs and their articulation points (spherical density, boxed) from which rod-like elements (double arrows) extend to the limb's claw. (E) Longitudinally split fossil reveals the ce3 cephalic appendage seemingly unattached (white arrow, boxed area high contrast in E'), contrasting with a trunk limb attached to its point of articulation (open arrow). (F) Oblique top-down view of the head showing one seemingly unattached limb extending from under the trunk (white arrow), whereas three cephalic appendages originate from points of articulation from beneath the head's margin (open arrows, box refers to high contrast image in F'). (G). Summary diagram comparing dispositions of segmental trunk limbs and three unique pairs of appendages from the asegmental head. Scale bar for inset to C = 50µm. B, D = 200µm; E, F = 250µm.



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body wall muscles within segments and longitudinal muscles extending between segmental sclerites, neural processes extending ventrodorsally would be consistent with both organizations.

Budd *et al.* misdirect in asserting we identify a tripartite brain in *C. catenulum*. We emphasize a continuum of trace deposits, interpreted as neuropil resolved in an unsegmented head, in which three domains align with three unique (Fig. 1, E to G) appendage pairs, distinct from trunk appendages (Fig. 1, B to D), and seriate features of the foregut—the suggestion being that these domains further evolved as neuromeres in crownward panarthropods (1). Budd *et al.*'s objection to deposits partially outlining the foregut ignores the brains of extant euarthropods, notably chelicerates, which are perforated by the foregut.

Placing *Cardiodictyon* basal within an abbreviated ecdysozoan phylogeny but outside the panarthropod crown group is justified [see supplemental material in (1)], as is attributing in *Kerygmachela* apical traces to a prosocerebrum (ce1) distant from the endomesodermal interface. Budd *et al.* object that loss of ce2 and ce3 requires their re-evolution in more crownward stem euarthropods. This is demonstrably wrong since loss of ce2 and ce3 applies only to the branch providing *Kerygmachela* as shown in figure S8 of (1). The same applies to trunk ganglia in Onychophora: evolved loss of ganglia but longitudinal expansion of synapse-rich nerve cords is a trait mapped by us exclusively onto the onychophoran trajectory (1).

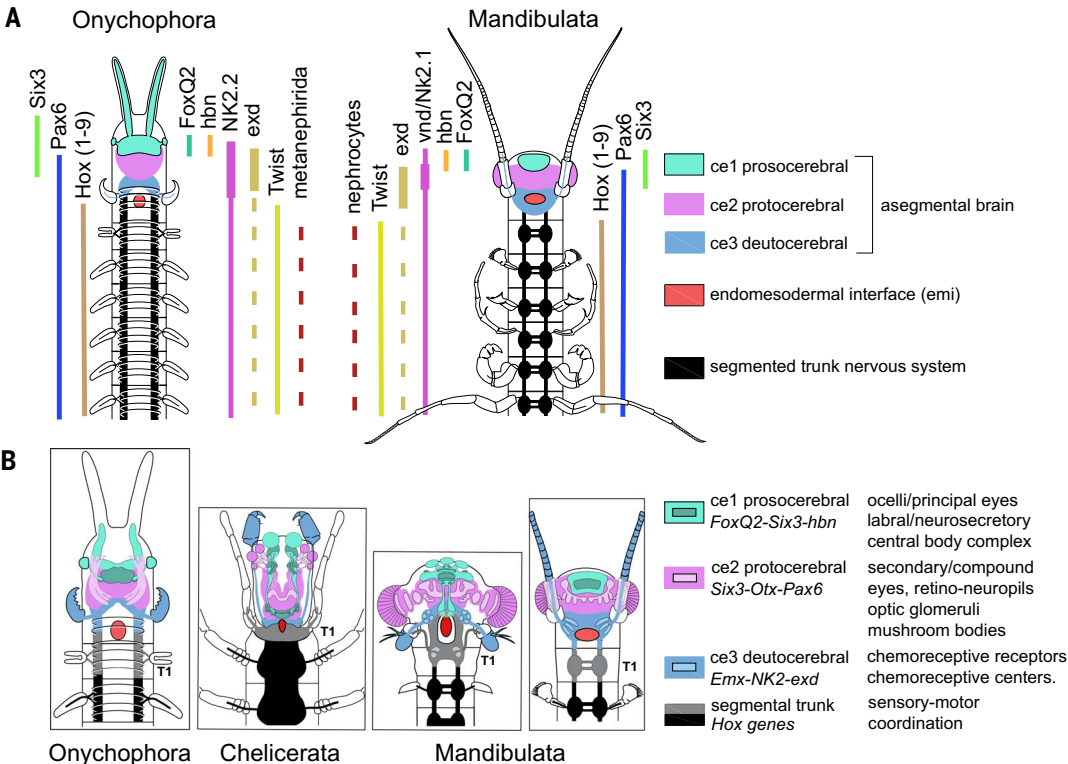
Budd *et al.*'s second line of argument focuses on living onychophorans, evoking supposed ancestral traits to challenge interpretations of *C. catenulum*. Onychophora is, however, an extant taxon allowing alignment of its brain, neuropil centers, and unique cephalic appendages with those of other panarthropods (Fig. 2) (1). Budd *et al.* refers to what are described as transitory coelomic cavities of “negligible volume” (5) and a possible nephridium (6) to claim head mesoderm is segmental thus indistinguishable from trunk segmentation despite the absence of mesoderm-specific homeotic (Hox) gene expression. In contrast, neither early embryonic development nor expression patterns of developmental control genes (Fig. 2A) support Budd *et al.*'s claim. Budd *et al.* lean heavily on Manton's 1949 schematics of onychophoran development. These included recognition of rostral pre-stomodeal “optic rudiments” in Crustacea appearing before the specification of trunk segments [figure 6 in (7)]. A corresponding feature in Onychophora would not have suited Manton's polyphyletic Arthropoda. Yet her sketches of the developing onychophoran *Peripatopsis balfouri* [figure 7, K to M, in (7)] suggest tissue rostral to the stomodeum prior to the first appearance of segmentation. Manton reported that mesoderm migrates rostrally from a post-oral position thereby populating the head [see plate 38, figs. 79 to 84, in (7)]. However, there is no genetic evidence for mesoderm-related coelomic cavities in the onychophoran head, consistent with the absence of Hox gene expression

(Fig. 2A). Manton (7) also described the post-oral progression of growth caudally, accordant with an onychophoran-specific role of Notch-Delta in posterior growth but not in segmentation (8). The gene *Twist* is an evolutionarily conserved key regulator for mesoderm specification (9), the expression of which is restricted to the trunk in Onychophora (10), as it is in euarthropods (Fig. 2A) and in other phyla (9). The genetic network for mesoderm-related nephridia formation applies to the onychophoran trunk (11), but not to its head (Fig. 2A). Together these data establish that head mesoderm in Onychophora is no indicator for segmentation unless one would postulate non-Twist-non-Hox mesoderm, for which there is no evidence.

Correspondingly, there is no genetic evidence for brain segmentation in Onychophora. Early transient, anterior *engrailed* expression is consistent with its ancestral role in neural fate specification, but not segmentation (12). Likewise, the reported ‘segment-polarity’ gene expression patterns suggest a role in organogenesis but not segmentation (13). This also applies to homologs of the conserved patterning genes *extradenticle (exd)* and *ventral nervous system defective (vnd)/NK2.1/NK2.2* (14, 15), with corresponding expression across euarthropods (16–18) (Fig. 2A). In Onychophora, neurogenesis is characterized by “massive irregular segregation of neural precursors” (19), in contrast to the regular, neuromere-related segregation typical for euarthropod trunk neurogenesis. Expression of the patterning genes *FoxQ2* and

Fig. 2. Corresponding genetics and brain center organization in Onychophora and Euarthropoda.

(A) Alignment of onychophoran and mandibulate with reference to their endomesodermal interface (emi) demonstrates corresponding expression patterns of homologous genes, the combination of which define the brain as three cerebral domains. Note that homeotic (Hox) gene expression defining mesoderm-related segmentation of the trunk does not extend anterior to the emi (1). (B) Each cerebral domain ce1-ce3 is further characterized across panarthropods by corresponding arrangements of circuits, the development of which depend on specific combinatorial expression patterns of homeobox transcription factors (listed to the right of the relevant domain). For Euarthropoda, these domains are referred to as the pro-, proto-, and deutocerebrum (1). T1 indicates the first segment of the trunk.



homeobrain (*hbn*) unambiguously identifies the ce1 domain across total Panarthropoda (18, 20, 21), consistent with *Six3*, *Pax6* and *non-Hox* expression territories that together demarcate ce2 and ce3, which are genetically distinct from the trunk (Fig. 2A).

Developmental genetics is consistent with and regulates the formation and functions of domain-specific brain centers that correspond across panarthropods, including Onychophora (Fig. 2B): ce1-specific *Six3*, *FoxQ2* and *hbn* define prosocerebral rostral visual pathways and the central body; ce2-specific *Otx* and *Pax6* define protocerebral optic and memory processing neuropils; ce3-specific *Emx*, *Nk2* and *Exd* determine deutocerebral chemosensory integration centers (1, 18, 21). Taken together, we conclude that arguments offered by

Budd *et al.* comprehensively fail to refute any of the findings reported in Strausfeld *et al.* (1).

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