Supporting Information

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SI Text

Character Coding. The dataset used for the phylogenetic analysis has been updated from that presented by Yang et al. (36), including the formulation of new neurological characters to resolve large-scale relationships within Panarthropoda. The crown-group euarthropods Limulus polyphemus (Chelicerata, Xiphosura) and Triops cancriformis (Mandibulata, Notostraca) were included as their neurological organization has been extensively studied (1–3, 37) and to appropriately polarize the characters in the analysis. Additional extant taxa were included based on recent studies describing aspects of the brain and VNC organization; these include the eutardigrades Macrobiotus of hannaworthi and Hysipsibius dajuardini (16, 18), the heterotardigrades Echiniscus testudo, Actinurus doryphorus, and Batillipes pennaki (28, 38), the peripatopsid Metaperipatus blainvillii, and the peripatid Epiperipatus biolleyi (12, 13, 15). Fossil and extant taxa are scored according to a single model of head segmental organization that is informed by developmental studies on extant Onychophora, Tardigrada, and Euarthropoda (36).

Characters 1–80 largely follow those of Yang et al. (36); only those with minor modifications are outlined here. Characters 81–86 from Yang et al. (36) have been substantially revised to integrate the paleoneurological data available from C. kunmingensis and better reflect the diversity of VNC morphology in Panarthropoda; these changes are described below. Characters modified from Yang et al. (36).


(0) lobopodous ambulatory limb
(1) lobopodous sensorial limb
(2) lobopodous limb with sclerotized jaw
(3) arthropodized antenniform with distinct podomeres
(4) arthropodized short great-appendage/chelicerae
(−) inapplicable: paired appendages (character 1) absent.

This character is scored for as present for the deutocerebral chelicerae of Limulus (2, 37) under the proposed evolutionary transition from the short-great-appendages of megacheirans into the first appendage of crown-group Chelicera (9).


(0) undifferentiated lobopodous limb
(1) specialized papillae
(2) arthropodized biramous walking leg with distinct podomeres
(3) arthropodized uniramous specialized postantennal appendage
(4) arthropodized biramous second antennae
(−) inapplicable: paired appendages (character 1) absent.

State 4 is introduced to reflect the appendicular morphology of the notostracan Triops (3, 26, 39).

54. Exite organization.

(0) lanceolate dorsal blades
(1) simple oval paddle with marginal spines
(2) bipartite shaft with lamellar setae
(3) numerous podomeres, each bearing a single setae
(4) book gills
(−) inapplicable: trunk exites (character 53) absent.

States 3 and 4 are introduced to reflect the exite morphology of the notostracan Triops (26, 39) and the xiphosuran Limulus (40), respectively (41, character 10).

66. Number of claws on trunk limbs.

(0) one
(1) two
(2) three
(3) four
(4) six
(5) seven
(−) inapplicable: terminal claws (character 64) present.

State 4 is introduced to reflect the presence of six toe-like claws in the heterotardigrade Batillipes pennaki (28).

Cardiovascular and neurological organization.

81. Dorsal heart.

(0) absent
(1) present

See character 86 in Yang et al. (36).

82. Dorsal condensed brain.

(0) absent
(1) present

See character 82 in Yang et al. (36).

83. Number of neuromeres integrated into the dorsal condensed brain.

(0) one
(1) two
(2) three
(−) inapplicable: dorsal condensed brain (character 82) absent.

See character 83 in Yang et al. (36) and also introductory statements above. Limulus and Triops are scored as having three brain neuromeres based on ample neurological data on these model organisms (2, 3, 37).

84. Mouth innervation relative to brain neuromeres.

(0) protocerebral innervation
(1) deutocerebral innervation
(2) tritocerebral innervation
(3) innervation from multiple neuromeres
(−) inapplicable: condensed dorsal brain (character 82) absent.

See character 84 in Yang et al. (36). State 2 has been included to reflect the innervation pattern of Triops, in which the stomatogastric nervous system is associated with the tritocerebral neuromere (3).

85. General organization of VNC.

(0) unpaired
(1) paired

The general organization of the ventral nerve cord in Ecdysozoa can be broadly classified based on whether it is paired or unpaired. Within the scope of the present phylogenetic analysis, an unpaired nerve cord is characteristic of Priapulida (31), whereas a paired nerve cord is found in all members of Panarthropoda (1, 3, 12, 13, 16, 18, 19, 28, 29, 38, 42, 43) (Fig. S5). In fossil representatives, a paired ventral nerve cord has been identified in Alacromenaca sp. (9) and is confirmed for C. kunmingensis in this study (Figs. 1 and 2). A paired VNC is scored as present for L. unguispinus based on the presence of two descending tracts on the anterior trunk region (10). Although Hou et al. (44) have reported the presence of a VNC in the Chengjiang lobopodian Paucipodia inermis, it is not possible to discern whether the organization of this feature is paired or unpaired, and thus it is scored as uncertain pending a formal description of the putative nervous system in this taxon.
86. VNC with morphologically discrete condensed hemiganglia connected by median commissures.

(0) absent
(1) present

Tardigrada and Euarthropoda have a rope ladder-like VNC with morphologically discrete condensed ganglia (1–4, 16, 18, 28, 37, 43) (Fig. S5 B–D), in contrast to the ladder-like VNC of Onychophora (12, 13, 16, 29, 38) (Fig. S5A). The presence of median commissures has been recently demonstrated in Tardigrada (16, 19, 28) and thus suggests that the position of ganglia and transverse commissures represent fundamentally linked neurological features. Priapulida have an unpaired nerve cord associated with a net-like system of neural connectives (31), and thus this character is scored as absent. The present data confirm the presence of condensed ganglia in C. kunmingensis (23). The early Cambrian megacheiran Alalcomenaeus sp. also possesses a VNC with condensed ganglia (9). Condensed ganglia are scored as uncertain in Lyrarapax (10), as the preservation of this taxon does not allow the organization of the VNC to be resolved. The lobopodian P. inermis has been described as possessing condensed ganglia (45). Given that the veracity of these observations has yet to be confirmed by additional study of the material, this character is treated as uncertain in the main analysis (Fig. S6). However, we explored the potential implications of putative ganglia in P. inermis by running a second set of analyses that scored this character as present. Under equal weights and implied weights with k ≥ 20, the analyses produced identical topologies as the original tests (Fig. S6 A and D). However, under implied weights with parameters 20 < k < 0.1, P. inermis was recovered within stem-group Tactopoda (i.e., basal to total-group Tardigrada and total-group Euarthropoda) instead of within stem-group Onychophora. The latter topology ultimately supports a single origin for the condensed ganglia in Panarthropoda, as inferred by our original analyses, and carries the same general implications for the evolution of CNS in this clade (Fig. 3 and Fig. S6).

87. Paired nerve cord lateralized.

(0) absent
(1) present
(–) inapplicable; VNC unpaired (character 85).

This character reflects the organization of the ladder-like paired VNC of Onychophora (12, 13, 15, 16, 29, 38) (Fig. S5A). This character is scored as uncertain in Lyrarapax (10), as the preservation of the postcephalic nervous system does not allow the organization of the VNC to be resolved.

88. Paired nerve cord with median interpedal commissures.

(0) absent
(1) present
(–) inapplicable; VNC unpaired (character 85).

Median interpedal commissures are conspicuous in the ladder-like VNC of Onychophora (12, 13, 16, 29, 38) (Fig. S5A). Recently, Mayer et al. (16) described in detail the presence of transverse interpedal median commissures that fall outside of the condensed ganglia in the nerve cord of Eutardigrada (Fig. S5B), suggesting that this represents a plesiomorphic condition reminiscent of the possible orthogonal organization hypothesized for an ancestral panarthropod. Schulze and Schmidt-Rhaesa (42) and Schulze et al. (28) have reported similar nonganglion associated commissures in the Heterotardigrada, indicating that this represents a widespread condition within Tardigrada. The VNC of Euarthropoda lacks signs of interpedal commissures (1, 2, 16, 37, 46) (Fig. S5 C and D); this character is scored as absent for Alalcomenaeus sp. (9) and scored as uncertain in Lyrarapax (10) and C. kunmingensis (23 and this study), as it is not possible to resolve the preservation of interpedal commissures in the available material.

89. Nerve cord with orthogonal organization.

(0) absent
(1) present

The nerve cord of Priapulida (4, 31) and Onychophora (12, 13, 15, 16) displays a typical orthogonal organization, consisting of several ring-like commissures and peripheral nerves that intersect with additional dorsal and lateral longitudinal nerve strands, forming a distinctive net-like pattern. The nervous system of Tardigrada also displays dorsal and lateral longitudinal nerves that are intersected by transverse peripheral nerves (16, 18, 38, 43), although these organisms lack complete ring commissures (see character 90). Schulze and Schmidt-Rhaesa (28) reported loop-like neurites that extend dorsally in E. testudinaria; this organization is similar to that observed in eutardigrades (16), and thus this character is scored as present. However, an orthogonal-like organization has not been resolved in the heterotardigrades B. pennaki and A. doryphorus (28); pending additional data, this character is scored as uncertain as it is unknown whether this absence of legitimate or whether this aspect of the morphology has not been resolved due to differences in specimen fixation and/or ontogeny. Although the presence of peripheral nerves (see character 93) throughout the VNC of C. kunmingensis may suggest the presence of an orthogon-like organization, as expressed in Onychophora, this character is conservatively scored as uncertain due to the lack of distal preservation of the nerve roots.

90. Orthogonal nerve cord with complete ring-commissures.

(0) absent
(1) present
(–) inapplicable; VNC organization is not orthogonal (Character 90).

This character distinguishes the complete ring commissures of Priapulida and Onychophora (12, 13, 15, 31) from the incomplete peripheral commissures that emerge from the condensed ganglia in the nerve cord of some tardigrades (16, 18, 42). This character is scored as uncertain for C. kunmingensis given the incomplete distal preservation of the peripheral nerves (character 93).

91. Segmental leg nerves shifted anteriorly relative to appendages following parasegmental organization.

(0) absent
(1) present
(–) inapplicable; paired appendages absent (character 1).

The leg nerves emerging from the condensed ganglia in the VNC in Tardigrada and Euarthropoda evince an organization that reflects the position of the parasegmental boundaries in these organisms, resulting in an anterior displacement relative to the actual position of the trunk appendages (16, 28, 42). By contrast, neuronal tracing indicates that this anterior displacement is not expressed in Onychophora (16). Although the orientation of the peripheral nerves in the ganglia of C. kunmingensis could potentially suggest the anterior displacement of the leg nerves relative to the appendages in fuxianhuiids, this character is conservatively scored as uncertain as it is not possible to confirm this condition given the lack of distal preservation of the nerve roots.

92. Paired segmental leg nerves.

(0) absent
(1) present
(–) inapplicable; paired appendages absent (character 1).

This character describes the condition observed in onychophorans and tardigrades, in which each leg is innervated by
two nerves (12, 13, 16, 18, 28, 42, 45); by contrast, a single nerve innervates each leg in Euarthropoda. This character is scored as uncertain for C. kunmingensis as the incomplete preservation of the nerves in the VNC does not allow resolving their precise relationship with the leg innervation.

93. Regularly spaced peripheral nerves running entire length of nerve cord.

(0) absent (Tardigrada, crown-group Euarthropoda)
(1) present (Priapulida, Onychophora, Chengjiangocaris)

Regularly spaced peripheral nerves are characteristic of the orthogonal nervous system of Priapulida (31) and Onychophora (see “interpedal nerves” in refs. 12, 13, 15, and 16) (Fig. SS4). Although the VNC of some crown-group euarthropods displays the so-called intersegmental nerves outside of the segmented ganglia (Fig. S5 C and D), this character is scored as absent as these nerves do not occur regularly throughout the entire length of the corresponding connectives (1, 4, 5, 7, 37, 47). Intersegmental peripheral nerves are entirely absent in Tardigrada (16, 28) (Fig. SS5). The exceptional preservation in C. kunmingensis demonstrates the persistence of segmental and intersegmental peripheral nerves throughout the length of the VNC in representatives of upper stem-group Euarthropoda (Figs. 1 and 2 and Fig. S1).

94. Stomatogastric ganglion.

(0) absent
(1) present

A stomatogastric ganglion associated with the segment bearing the (tritocerebral) second leg pair has been recently described for various species within Eutardigrada, including M. cf. hamsworthi and H. dujardini (16, 18, 19); this structure has been interpreted as potentially homologous with the stomatogastric ganglion of Euarthropoda [2, 3, 46; see refs. in Mayer et al. (19)], and hypothesized as a potential synapomorphy of Tardigrada and Euarthropoda. However, neurological studies of Heterotardigrada have been unable to find evidence for the presence of the stomatogastric ganglion (28, 42); this character is scored as uncertain for Heterotardigrada pending the input of additional neurological data. This character is scored as uncertain for C. kunmingensis and all other fossil taxa.

Additional characters.

95. Cirri.

(0) absent
(1) present
(–) inapplicable; dorsal sclerotized integument (character 33) present.

Cirri are spine-like cuticular projections that represent a distinguishing feature of the anterior region in Heterotardigrada (28).

Comments on the Results of the Phylogenetic Analysis. The results of the cladistic analysis provide strong support for the sister-group relationship of Tardigrada and Euarthropoda (i.e., Tactopoda), to the exclusion of Onychophora (20, 36); this topology is retrieved in the equally weighted analysis (Fig. S6A) and throughout a broad range of homology penalization (Fig. S6 B–D). The stability of Tactopoda allows reconstructing the evolutionary history of the VNC within Panarthropoda in detail (Fig. 3) and indicates that mapping the neurological data (characters 82–94) to match the sister-group relationship between Onychophora and Euarthropoda (21, 22) results in a less parsimonious reconstruction of character transformations (Fig. S7A). More specifically, the latter scenario would require the convergent evolution of several neurological features in Tardigrada and Euarthropoda, namely the presence of condensed ganglia in the nerve cord, the anterior shift of the leg nerves following a parasegmental organization, and the presence of a stomatogastric ganglion (Characters 86, 91, and 94). An alternative scenario in which all these features are symplesiomorphic for Panarthropoda would favor the substantial secondary simplification of the VNC in Onychophora (30) (Fig. S7B); however, the lack of clear developmental or neurological data supporting the loss of all these complex features in the CNS of Onychophora argues against this evolutionary scenario (12, 13, 15).

The variability observed between the different analyses indicates that phylogenetically basal lobopodians within the stem lineages of Onychophora and Euarthropoda represent rogue taxa. Aysheaia pedunculata, Onychodictyon ferox, Jianshanopodia decorra, and Megadictyon haikouensis are variably recovered as either the most basal members of stem-group Euarthropoda (Fig. S6 B and C) or in a less resolved position within stem-group Panarthropoda (Fig. S6 A and D); only at high concavity values is O. ferox resolved as basal within stem-group Onychophora (Fig. S6D). This variability reflects that of previous analyses (20, 36) and indicates that additional material is necessary to better constrain the morphology and phylogenetic affinities of these lobopodians. By contrast, taxa that occupy a more crown-ward phylogenetic position evince a substantial degree of stability within each of the total-groups, even though it is not possible to resolve the internal relationships within Luolishaniidae (stem-group Onychophora) and Radiodonta (stem-group Euarthropoda) with the present dataset. Within stem-group Onychophora, the basal lobopodians Paucipodia inermis, Diania caeciformis, and Onychodictyon gracilis evince some plasticity in their position. It is likely that the formal description of the putative VNC in P. inermis (44) will help to elucidate its precise affinities and further inform the early evolution of the nervous system in early onychophoran ancestors. The internal topology of total-group Tardigrada is stable in all of the analyses and differs from previous studies in that O. ferox was not recovered within this group under any degree of homology penalization. The analyses further allowed to recover a monophyletic Heterotardigrada but were unable to resolve the phylogenetic position of the Orsten Siberian tardigrade (48) relative to the crown-group, despite the overall eutardigrade like appearance of this fossil, including the presence of claws with multiple branches. Finally, the topology within total-group Euarthropoda is largely stable, save for the fluctuating positions of A. pedunculata, O. ferox, J. decorra, and M. haikouensis discussed before. The only additional variation occurred in the position of the gilled lobopodian Pambdelurion whittoni; whereas in most analysis this taxon was recovered as crown-ward relative to Kerygmachela and stem-ward relative to a clade including Radiodonta and Deuteropoda, at low concavity values (Fig. S6B), P. whittoni was resolved as sister-taxon to Radiodonta. The relationships between other members of stem-group Euarthropoda are consistent with previous analyses (17, 20, 49), including the recent finding that Opabinia regalis may occupy a more crown-ward position relative to Radiodonta than previously considered (36).
Fig. S1. Detail of well-preserved VNC in *C. kunmingensis*, specimen YKLP 12026. (A) Preserved VNC with seven sets of condensed ganglia. (B) Magnification of four posterior ganglia and their respective connectives. (C) Magnification of three anterior ganglia and their respective connectives, including the fine preservation of regularly spaced peripheral nerve roots (arrowheads) emerging at either side of the VNC. Abbreviations as in Fig. 1.
Fig. S2. VNC preservation in *C. kunmingensis*. (A) YKLP 12322, complete articulated specimen preserved in dorsolateral view with exposed VNC on the posterior trunk region. (B) YKLP 12324a, articulated specimen in lateral view with exposed VNC on posterior trunk region, showing correlation between condensed ganglia and the walking legs. (C) YKLP 12324b, preservation of VNC on anterior trunk region, showing light coloration due to the advanced degree of weathering (compare with Fig. 2). Abbreviations as in Fig. 1. hs, head shield.
Fig. S3. Morphological reconstruction of C. kunmingensis. (A) Complete exoskeletal morphology in ventral view showing arrangement of walking legs (wl) and their attachment sites to the body (wlas) relative to the preserved VNC (purple) and tergites (Tn); note that only T1–T5 have a one-to-one correspondence with the walking legs. (B) Overall view of the CNS, including the VNC and dorsal brain (the latter extrapolated from Fuxianhuia protensa; sensu 8); the gap between the VNC and brain reflects lack of paleontological data pertaining this region. (C) Magnification of the CNS; note the one-to-one correlation between the ganglia (ga) and T1–T5, and the presence of up to four ganglia on the remaining trunk tergites. (D) Neurological reconstruction of two condensed ganglia. Other abbreviations as in Figs. 1 and 3.
Fig. S4. Raman spectroscopy analysis of VNC in *C. kunmingensis*. (A) Carbon (1,360 cm$^{-1}$) and organic carbon (1,604 cm$^{-1}$) were detected in the dark stripes within the VNC of YKLP 12026. (B) No mineral or organic constituent are detected by Raman spectroscopy in the light areas within the VNC of YKLP 12026. (C) Carbon (1,370 cm$^{-1}$) and organic carbon (1,603 cm$^{-1}$) were detected in the dark stripes within the VNC of YKLP 12320. (D) No mineral or organic constituent detected by Raman spectroscopy in the light areas within the VNC of YKLP 12320.
Fig. S5. Diversity of VNC organization in extant Panarthropoda. (A) E. kanangrensis (Peripatopsidae, Onychophora). VNC confocal micrograph stained with a monoclonal antibody directed toward acetylated tubulin (Sigma). Reprinted with permission from ref. 29. (B) M. cf. harmsworthi (Eutardigrada, Tardigrada). VNC confocal micrograph stained with combined antityrosinated and antiacetylated α-tubulin immunolabeling. Reprinted with permission from ref. 16. (C) S. tulumensis (Remipedia, Euarthropoda). VNC confocal micrograph labeled for acetylated α-tubulin immunoreactivity (TUB-IR, yellow). Reprinted with permission from ref. 5. (D) S. gregaria (Hexapoda, Euarthropoda). VNC confocal micrograph stained with 8b7 immunocytochemistry (46). Abbreviations as in Figs. 1 and 3.
Summary of results from phylogenetic analyses. See SI Text and Dataset S2. (A) Strict consensus of 546 most parsimonious trees (MPTs) under equal weights (178 steps; consistency index (CI) = 0.65; retention index (RI) = 0.88). (B) Strict consensus of 160 MPTs under implied weights ($k = 0.1$; CI = 0.64; RI = 0.87); this topology is stable when $1 \leq k \leq 0.1$, with some variation in the number of MPTs. (C) Strict consensus of 146 MPTs under implied weights ($k = 3$; CI = 0.65; RI = 0.88); this topology is stable when $10 \leq k \leq 3$, with some variation in the number of MPTs. (D) Strict consensus of 58 MPTs under implied weights ($k = 20$; CI = 0.65; RI = 0.88); this topology is stable when $k \geq 20$, with some variation in number of MPTs. Color coding: stem-group Panarthropoda (dark pink), total-group Onychophora (green), total-group Tardigrada (red), total-group Euarthropoda (blue).

Fig. S6. Summary of results from phylogenetic analyses. See SI Text and Dataset S2. (A) Strict consensus of 546 most parsimonious trees (MPTs) under equal weights [178 steps; consistency index (CI) = 0.65; retention index (RI) = 0.88]. (B) Strict consensus of 160 MPTs under implied weights ($k = 0.1$; CI = 0.64; RI = 0.87); this topology is stable when $1 \leq k \leq 0.1$, with some variation in the number of MPTs. (C) Strict consensus of 146 MPTs under implied weights ($k = 3$; CI = 0.65; RI = 0.88); this topology is stable when $10 \leq k \leq 3$, with some variation in the number of MPTs. (D) Strict consensus of 58 MPTs under implied weights ($k = 20$; CI = 0.65; RI = 0.88); this topology is stable when $k \geq 20$, with some variation in number of MPTs. Color coding: stem-group Panarthropoda (dark pink), total-group Onychophora (green), total-group Tardigrada (red), total-group Euarthropoda (blue).
Evolution of the nervous system in Panarthropoda under an alternative phylogenetic hypothesis grouping Onychophora and Euarthropoda as sister taxa. Note that these scenarios for the evolution of the panarthropod nervous system are less parsimonious compared with the topology obtained in the present study (Fig. S6). (A) Evolutionary scenario favoring substantial convergent evolution of the VNC in Tardigrada and Euarthropoda. (B) Evolutionary scenario favoring the secondary simplification of the neurological organization in Onychophora, derived from ancestors with a ganglionated VNC (30). †, fossil taxa; ?, uncertain neurological character polarity within total-group Euarthropoda.
Table S1. Summary of specimens of *C. kunmingensis* (Cambrian stage 3, Xiaoshiba Lagerstätte) with preservation of neurological structures

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Reference/figure</th>
<th>Notes on preservation</th>
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| YKLP-12023       | This study/Fig. 1A  
Yang et al. (23)/figures 1 d and e | Complete articulated specimen in dorsal view. Taphonomic dissection of head shield exposes cephalic appendages, and indicates that the gut overlies the VNC. VNC coloration (reddish/black) indicates moderate weathering. |
| YKLP-12206       | This study/Fig. 2 and Fig. S1 | Complete articulated specimen in oblique view (left side visible). Taphonomic dissection of head shield exposes well-preserved VNC an anterior body region. The VNC comprises seven condensed ganglia and their longitudinal connectives. Exceptional preservation of segmental and intersegmental peripheral nerves revealed by light photograph and fluorescent microscopy. VNC coloration (black) indicates presence of organic carbon films. |
| YKLP-12320       | This study/Fig. 1 B–D and Dataset S1 | Complete articulated specimen in ventral view. VNC exposed throughout entire body length, including condensed ganglia and longitudinal connectives. Clear correlation between individual ganglia and walking leg attachment site. Exceptional preservation of segmental and intersegmental peripheral nerves revealed by fluorescent microscopy. VNC coloration (black) indicates presence of organic carbon films. |
| YKLP-12322       | This study/Fig. S2A | Complete articulated specimen in oblique view (left side visible). Moderately preserved VNC exposed on posterior half of the trunk. VNC coloration (reddish/black) indicates moderate weathering. |
| YKLP-12324a, b   | This study/Fig. S2 B and C | Incomplete articulated specimen in lateral view (right side visible). Part evinces moderately preserved VNC throughout the trunk, in association with walking legs. Taphonomic dissection of head shield in counterpart exposes moderately preserved VNC in anterior region, including seven distinct condensed ganglia. VNC coloration (reddish) indicates moderate weathering. |

Dataset S1. High-resolution fluorescence micrograph composite of *C. kunmingensis*, specimen YKLP 12320

Dataset S1

Arrowheads indicate preservation of peripheral nerves throughout the VNC. Abbreviations as in Fig. 1.

Dataset S2. Character matrix for phylogenetic analysis

Dataset S2