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1 Interpreting fossilized nervous tissues

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22 **Abstract**

23 Paleoneuroanatomy is an emerging subfield of paleontological research with great potential
24 for the study of evolution. However, the interpretation of fossilized nervous tissues is a
25 difficult task and presently lacks a rigorous methodology. We critically review here cases
26 of neural tissue preservation reported in Cambrian arthropods, following a set of
27 fundamental paleontological criteria for their recognition. These criteria are based on a
28 variety of taphonomic parameters and account for anatomical complexity. Application of
29 these criteria shows that firm evidence for fossilized nervous tissues is less abundant and
30 detailed than previously reported, and we synthesize here evidence that has strong support.
31 We argue that the vascular system, and in particular its lacunae, is likely central to the
32 understanding of many of the fossilized peri-intestinal features known across Cambrian
33 arthropods. In conclusion, our results suggest the need for caution in the interpretation of
34 evidence for fossilized neural tissue, which will increase the accuracy of evolutionary
35 scenarios.

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39 **Keywords**

40 Paleoneuroanatomy, Cambrian, Burgess Shale-type, Taphonomy, Arthropoda,
41 Lagerstätten, Morphoanatomy

42

44 **1. INTRODUCTION: EVIDENCE FOR FOSSILIZED BRAINS IN CAMBRIAN**
45 **ARTHROPODS**

46 In the past decade, there has been a surge of studies presenting evidence of fossilized
47 nervous tissues, mainly in fossil arthropods from Cambrian Konservat-Lagerstätten^[1–10],
48 but also, more rarely, from more recent deposits^[11]. The question of whether such labile
49 tissues—that is, prone to rapid decay—as ganglia and nerves can be preserved in settings
50 of BST deposits has been discussed at length before^[12–14]. The possibility of direct
51 fossilization of nervous tissues (as opposed to, e.g., skull endocasts^[15]) rests on three key
52 observations: (1) the experimental demonstration that nervous tissues in invertebrates can
53 be persistent to decay, all other conditions being equal^[14], (2) the demonstration that the
54 timing of decay observed under controlled experimentation does not correspond to the
55 range of preservation seen in fossils^[12], particularly in BST deposits^[16], and (3) the well-
56 established presence in these fossils of other labile tissues, such as muscles, digestive tracts,
57 and even eggs^[17].

58 Some paleoneurological evidence has also been criticized on interpretative and
59 taphonomic grounds^[18–20]. Criticism has stemmed largely from inconsistency of either the
60 fossil structure with its expected morphology or with the characteristics of preservation of
61 compression fossils, or both. Insufficient consideration of such complexities in some
62 studies has led to the reconstruction of the CNS in numerous Cambrian arthropods, which
63 when integrated into synthetic reconstructions and evolutionary scenarios^[3], must remain
64 open to question. Despite efforts to disambiguate observable tissues, fundamental aspects
65 of BST preservation and of the internal morphoanatomy of arthropods have been
66 overlooked.

67 Considering morphoanatomical features surrounding the gut is crucial because they
68 are closely associated with neural tissues. Some common and long-known structures, such
69 as “tonguelets”^[2,21,22], defined as sub-intestinal triangular projections, are particularly
70 important in this context, but have rarely been considered when studying the CNS,
71 specifically, the ventral nerve cord (VNC) that runs along the trunk. For this reason, it is
72 important to reassess the peri-intestinal anatomy of Cambrian arthropods^[19] based on
73 robust criteria and clear methodology. Although fossilized nervous tissues have been
74 discussed in a few non-arthropod BST fossils^[23,24], here we focus specifically on
75 morphoanatomy and paleoneuroanatomical evidence in fossil arthropods. Our conclusions
76 and methodological criteria, however, apply to all preserved nervous tissues.

77

78 **2. BURGESS-SHALE TYPE PRESERVATION AND TAPHONOMY**

79 *Implications of the fossilization process.* The leading question in the identification of
80 fossilized CNS, as for other tissues, should be: What unambiguously constrains the identity
81 a feature of interest in its specific taphonomic context? Below, we discuss the nature of
82 BST preservation and caveats related to compression that must be accounted for when
83 making interpretations. It should also be emphasized that a definitive interpretation is *not*
84 always possible. In epistemological terms, uncertainty is a positive criterion. To consider
85 uncertainty as valid information given the data is not trivial: it was historically important
86 during the redescription of Burgess Shale fossils led by Harry Whittington^[25,26], and
87 different conceptual views in more recent years—translating uncertainty into absence—
88 have strongly impacted our view of early arthropod evolution^[27].

89 The taphonomy of exceptional preservation is governed by specific spatial and
90 temporal parameters^[28–30]. The defining characteristic of BST deposits is that soft-bodied
91 organisms are preserved primarily as organic remains^[31,32]. Fossils are strongly
92 compressed parallel to bedding, however preparation and uneven splitting^[e.g., 33] as well as
93 tomographic techniques^[e.g., 34], where possible, reveal that a slight three dimensional
94 expression of organic features persists. Over geologic time, organic matter comprising BST
95 fossils is transformed into kerogen^[32]. As a consequence, most individual tissues become
96 indistinguishable compositionally and visually, although, under certain conditions, they
97 can still be differentiated topologically (see also Supplementary Discussion). In
98 unweathered BST fossils, original morphoanatomical features rich in carbon typically
99 appear as reflective, compositionally homogeneous patches (Figs 1-6).

100 Although organic tissues are primarily preserved as carbon films, mineralization
101 may also play a role, either as infill of anatomical cavities, as primary replacements of soft
102 tissue during fossilization or as secondary products related to subsequent geologic history,
103 specifically metamorphism and weathering. These phenomena have led to the precipitation
104 of aluminosilicate minerals across organic remains of fossils from the Burgess Shale^[31]
105 and Sirius Passet^[41], and to the deposition of iron oxides within Chengjiang fossils^[80]. We
106 discuss these aspects in more detail in Supplementary Discussion, but, in the context of
107 this study, it is particularly important to emphasize that elements of the arthropod digestive
108 system, including the intestine, are often preserved as a combination of phases, with carbon
109 representing the gut wall surrounding a mineralized fluorapatite filling of the intestinal
110 cavity. Thus, the intestine of a BST fossil visible after a perfect coronal split of the
111 specimen would display a central phosphatic tract bordered by two parallel carbon lines.

112 Typically, however, the split occurs along the surface of the relief of the gut infill. In
113 addition, in a majority of cases, the carbonaceous gut wall is not distinct because it is
114 included in a broader peri-intestinal film. In deposit feeders, the formation of intestinal
115 apatite is prevented when the gut is filled, yielding instead a composition close to that of
116 the matrix (see e.g., *Burgessia*^[2]); heavy weathering, as in the case of early Cambrian BST
117 deposits of China, is also likely responsible for the replacement of fluorapatite
118 permineralization by aluminosilicates^[35].

119 Tonguelets commonly occur in a variety of BST arthropods and are dominantly
120 preserved as carbon, although in the Burgess Shale aluminosilicate films occur in
121 association. In certain groups, such as leaichoiliids, tonguelets co-occur with distinct
122 midgut glands^[21,35]. This evidence speaks against a digestive identity of these features. The
123 vascular morphology of tonguelets in certain taxa^[22] and their broad association with a
124 circum-intestinal carbon film^[2,21] suggest that these features are derived instead from
125 hemocoelic cavities that played a role in gas and nutrient distribution.

126 It has been proposed that serial peri-intestinal ramifications, including tonguelets,
127 could be formed as artefacts of bacterial activity during early stages of decay^[36,18]. Limited
128 autolysis under reducing conditions would favour colonization of tissues by invading
129 bacteria and it is possible that microbial biofilms could also be preserved as organic carbon
130 remains. The documented microbial colonization of the peri-intestinal cavity during early
131 stages of decay^[36] highlights the role that bacteria may play in the taphonomy of peri-
132 intestinal tissues, specifically in the formation of various artefacts such as blackened
133 cuticular linings and stains (Figs 1, 2). However, patterns of microbial replication require
134 an original biological substrate, which is why it is important to stress that microbial

135 proliferation cannot be used to explain the entirety of internal features preserved in BST
136 fossils as artefacts^[18]. Fossils have been used to show that a wide range of symmetrical
137 patterns indeed may be attributable to decay^[18], but such decay merely tends to transform
138 original symmetrical biological features into irregular shapes with indistinct boundaries. In
139 the specific case of *Fuxianhuia*, decay is clearly visible in imperfectly preserved tissues
140 associated with the circum-digestive area and the masticatory elements of mandibles.

141 One critical implication of these observations for BST fossils is that there is, as of
142 yet, no established tissue-specific pathway for mineralized preservation outside of the
143 digestive system and muscles. Tonguelets and perhaps other structures (gills, see below)
144 can potentially also be identified via their 3D replication in phosphate, but a better
145 disambiguation is required. The identification of all other tissues, including the CNS,
146 depends almost exclusively on visual separation, topological (physical) separation and
147 morphological consistency with the interpreted structure (Figs 1, 2). Given that BST fossils
148 are strongly compressed, the difficulty lies in demonstrating such isolation, after
149 considering the presence of all other fossilized morphological features and their
150 taphonomic variations.

151 *Contextual taphonomy.* Taphonomy is also determined by parameters related to
152 entombment and splitting. When reconstructing the morphoanatomy of BST animals, the
153 main contextual aspects that must be considered are: orientation of the fossil relative to
154 bedding, superimposition of features, artefacts related to compression and/or to the
155 mudstone matrix (such as slickenides), degree of decay and incomplete preservation of
156 features, unevenness in the plane of splitting that passes through fossils and may create
157 positive and negative features between part and counterpart, and the layered, three-

158 dimensional distribution of features intersected by the plane of splitting^[26,37,38]. In the latter
159 case, we call three-dimensionality the degree of vertical distribution of fossil features
160 across the matrix, perpendicular to the plane of splitting.

161 Although there are exceptions, these parameters imply that our ability to constrain
162 feature identity or morphology ideally necessitates a sample of multiple specimens
163 covering a range of orientations and taphonomic variations. This is also true for fully three-
164 dimensional, shelly fossils, which are rarely complete and unaltered. A good understanding
165 of taphonomic parameters for a particular species, in addition to the context of the entire
166 deposit, is crucial to constructing a methodology of identification, because it informs signal
167 versus possible noise (i.e., artefacts) in fossils. As a general rule, the more that fossils are
168 altered, the more parameters must be considered to constrain the identity of internal
169 features observed.

170 Obviously, accurate fossil interpretation also requires a coherent understanding of
171 morphoanatomy between extant and fossil taxa. The distinction and reconstruction of
172 internal features in BST material is a particularly complex task owing to (1) the
173 preservation of most tissues as carbonaceous remains, (2) the merging of various tissues
174 into a single kerogen film, and (3) the complexity and diversity of arthropod internal
175 morphoanatomy. This is particularly evident in the head and peri-intestinal regions where
176 the overlap of the eyes, brain tissues, esophagus, gut and gut glands, hemolymph cavities
177 as well as limbs, segments and other cuticular outlines (Fig. 3A-C) may create an entangled
178 assemblage of traces that are difficult to discriminate. In the cephalic region of extant
179 arthropods, the CNS consists of three main units (protocerebrum, deutocerebrum,
180 tritocerebrum) that have extensions into various sensory organs and head appendages.

181 Posteriorly, the body is innervated by the ventral nerve cord (VNC) and its numerous
182 extensions (towards trunk organs and abdominal appendages), in close association with
183 other organs systems, typically the circulatory system. Overlap patterns of these tissues
184 when compressed, combined with potential decay prior to the stabilization of organic
185 tissues, can lead to a disparity of shapes that do not accurately reflect the original biological
186 configuration and could potentially yield incorrect interpretations (Fig. 2).

187 A prominent issue affecting research in systematic paleontology is also that fossil
188 documentation in the literature is severely biased. Most of the time, only the best specimens
189 of a given taxon are illustrated, which makes learning to interpret fossils based only on
190 literature very arduous, if not impossible. Eye training is a long and demanding process
191 which is critical to direct fossil observation but also to the interpretation of modern imagery
192 techniques. Without background training providing knowledge of artefact ranges and
193 taphonomic variations, or when interpretations are not critical enough, exceptional fossils
194 can easily be overinterpreted.

195 *Three fundamental criteria and redundancy.* In consideration of the above, we propose to
196 use three fundamental criteria for the identification and interpretation of fossilized nervous
197 tissues. The first is *topological disambiguation*, the ability to visually distinguish the
198 structure of interest and constrain it as biological in origin. The second is an *explicit*
199 *taphonomic context*, whereby the conditions surrounding fossilization, including
200 compaction, are known and favorable to a positive identification of the structure of interest.
201 The third is *morphoanatomical consistency*, requiring that the identified feature be
202 consistent in shape and location with the overall morphoanatomy of the fossil species and
203 its group. Additionally, owing to the fact that taphonomic variations condition our

204 understanding of fossils, an interpretation will be stronger when there are replicates of the
205 evidence in other specimens—which we call here *redundancy* of the evidence.

206

207 **3. CASE ANALYSES**

208 **3.1. “Gilled lobopodians” and radiodontans**

209 Radiodontans, iconic early Paleozoic predators, are terminologically regarded as the
210 earliest arthropods^[27] or as early stem euarthropods^[39]. Nomenclatural differences
211 notwithstanding, the phylogenetic placement of radiodontans within Panarthropoda is
212 consensual^[40]. Their prominent frontalmost, arthrodized appendages been compared to and
213 considered homologous with the antennae of onychophorans (velvet worms), which are,
214 with tardigrades (water bears), the only extant sister groups to arthropods. Owing to the
215 protocerebral innervation of antennae in onychophorans, some authors have concluded that
216 the frontalmost appendages of radiodontans may not be homologous with the topologically
217 equivalent limbs—chelicerae or antennulae—of true arthropods^[39], because the
218 frontalmost appendages arguably connect to the deutocerebrum^[41]. This hypothesis
219 conflicts, however, with existing fossil and phylogenetic evidence suggesting structural
220 homology between the frontalmost appendages of radiodontans and euarthropods^[19]—
221 termed the cheirae^[27]—, and lacks support from direct fossil evidence.

222 In 2012, *Lyrarapax unguispinus*, from the Chengjiang Lagerstätte, China, was
223 presented as preserving brain tissues demonstrating the protocerebral nature of radiodontan
224 cheirae. There have been concerns^[19,20], however, that the key specimen *Lyrarapax*
225 *unguispinus* YKLP13305 had been misinterpreted^[9]. Originally, the authors described a
226 large central brain mass from which two frontal lobes protruded, purportedly innervating the
227 cheirae. The neural identification of the tissue is notably supported by the quality of the

228 specimen, isolation of the relevant carbon trace from other remains (mainly gut and
229 appendages), morphology, and consistent counterpart. The frontal lobes have clear
230 margins, are likely part of the CNS by exclusion (but see section 4 below) and connect to
231 the central mass lying on the same plane, supporting interpretation as a brain, and, in the
232 specific case of the frontal lobes, the protocerebrum (Fig. 4F).

233 However, the location, connection and identity of these features conflict with other
234 aspects of the morphoanatomy of the fossil, which is particularly clear in YKLP13305. As
235 confirmed by all other specimens of *Lyrarapax unguispinus*, the eye stalks in this animal
236 are long, bent, and insert very fronto-laterally^[9,42] (Fig. 4F). The frontal, arguably
237 protocerebral lobes overlap the base of the eye stalks (as opposed to the misinterpreted base
238 of the cheirae) and partially follow their bending. Lateral nerves posterior to these lobes
239 and projecting from the central brain mass were originally interpreted to innerve the eyes.
240 However, this is anatomically impossible, because these lateral nerves do not connect with
241 the eye stalks, but instead project towards the lateral margins of the head, clearly delimited
242 by cracks in the sediment caused by relief of lateral head margins (Fig. 4F). If indeed they
243 are nerves, these extensions would be bound to the deutocerebral neuropil, and would
244 connect to the bases of the cheirae, which in YKLP13305 are most likely located below
245 them.

246 This reexamination aligns with the recent description of similar structures in
247 another radiodontan, *Stanleycaris hirpex*^[43]. This taxon satisfies the best scenario criteria
248 for the identification of a fossilized brain for similar reasons as *Lyrarapax unguispinus*
249 does, with the help of multiple, particularly well-preserved specimens. Although it is not
250 entirely clear whether part of the foregut or esophagus could be associated with the remains

251 described as the CNS, the structures identified as brain, optic tracts and appendicular nerves
252 form a coherent ensemble characterized by dense kerogen remains. Importantly,
253 *Stanleycaris* also offers exceptional redundancy of evidence compared to other cases of
254 fossilized CNS: many of the specimens taken alone would be insufficient to determine the
255 morphology of these internal organs, and the wide range of variations in preservation
256 illustrates the impact of taphonomy on the interpretation of such features.

257 A crucial aspect of taphonomy in this case is three-dimensionality, in the context
258 of strong compression. In *Lyrarapax* and other paleoneuroanatomical examples (see
259 below), anatomical interpretations are made almost always based on the information visible
260 on the plane of splitting. Irrespective of whether the fossil itself retains some three-
261 dimensional relief, original three-dimensionality strongly affects fossil structure—and, in
262 mature living arthropods, brains have generally pronounced three-dimensional
263 expression^[44]. When strictly comparing with extant arthropods, a frontal, appendicular
264 nerve inserted on the ventral side of the brain would identify it as deutocerebral, rather than
265 protocerebral^[43]; any other, plesiomorphic configuration would otherwise have to be
266 justified. This is why a robust consideration of original three-dimensionality is generally
267 critical to the accurate interpretation of head organs and resolving this type of detail is only
268 possible with the highest fidelity of preservation, allowing for clear feature isolation.

269 The “gilled lobopodian” *Kerygmachela kierkegaardi* from the early Cambrian
270 Sirius Passet Lagerstätte of Greenland was described with head and CNS entombed parallel
271 to the sediment and showing, by contrast, limited relief. The “gilled radiodontans” are stem
272 arthropods that lack arthrodization and resolve more basally than radiodontans, implying
273 that their brain configuration might be both different and simpler. *Kerygmachela* MGUH

274 32049^[10] (Fig. 5C, D) likewise presents a strong case for nervous tissue preservation: the
275 size and shape of the anterior neuropil and its nerves are consistent with that of
276 panarthropods in dorsal view and, importantly, are visually and compositionally distinct
277 from the underlying pharynx, which shows relief and preservation in phosphate. In this
278 instance, frontal appendages are spread out fronto-laterally and do not cause ambiguity
279 with respect to the nerve extensions through them. Problematically, however, insertions of
280 these nerves at the front of the putative brain can be interpreted as protocerebral, conflicting
281 with the radiodontan evidence discussed above, because these enlarged frontal appendages
282 are likely homologous between these groups. Implications are discussed further in section
283 5.

284

285 **3.2. Megacheirans**

286 Three-dimensionality and topological consistency are also crucial factors for
287 interpretations of putative CNS in megacheirans, mostly leanchoiliids. Leanchoiliid
288 megacheirans are early euarthropods, possibly stem chelicerates^[27], characterized by
289 flagellate and multi-chelate cheirae. It has been well documented that, in megacheirans,
290 cheirae insert very anteriorly, close to the insertion of the eyes^[19,21,45]. The eyes of certain
291 leanchoiliid specimens, preserved in dorso-ventral compression, can appear to be ventral,
292 but accounting for the much larger sample of specimens preserved in a variety of different
293 positions, the eyes are more accurately fronto-lateral with the great appendages inserted
294 almost underneath them^[21,46].

295 In several recent anatomical reconstructions based on remains interpreted as
296 CNS^[3,4,6], cheirae are associated with a deutocerebral nerve but are placed in a more

297 posterior location. In these reconstructions, the designated “protocerebrum” lies at the level
298 of the known frontalmost appendage insertion, while the “deutocerebrum” encompasses
299 two post-frontal cephalic limbs (Figs 3D, E, 4B, D). The reconstructions are further
300 complicated by the fact that the purported paleoneuroanatomical evidence itself in
301 leanchoiliids does not account for critical taphonomic criteria.

302 Lan and colleagues^[3] recently presented a detailed reconstruction of leanchoiliid
303 brains based on two specimens of a *Leanchoilia*-like taxon from the middle Cambrian
304 (Wuliuan stage) Kaili biota, arguing that a separate, asegmental part of the forebrain of
305 arthropods called the prosocerebrum was expressed in these taxa. Relative to other BST
306 fossil specimens for which CNS remains have been inferred, both Kaili leanchoiliid
307 specimens are significantly altered by weathering and/or original decay, affecting
308 observable features. Significant alteration complicates the interpretation of unconstrained
309 patterns. The absence of counterparts further complicates the possibility of a strong
310 interpretation. Suggested nervous tissues are not explicitly discriminated from surrounding
311 tissues, perhaps because this is not possible given preservation quality, or perhaps because
312 the distinction is based on morphoanatomical misinterpretation. Critically, the three-
313 dimensionality, orientation and convex boundaries of purported nerves in GRCP15007 are
314 consistent in size and shape with underlying basipods (Fig. 3D), opposing mesially, as
315 previously documented in dorsally-preserved specimens of *Leanchoilia* and similar
316 arthropods^[47]. A pair of swellings located just behind the eyes (Fig. 3D) matches with the
317 expected location of the insertion of cheirae^[21] (Fig. 3A, B, E-F), often expressed in dorsal
318 view by folds, markings and outlines associated with the base of the appendages^{[48, pl. 13,49,}
319 fig. 3B]. Impressions of frontal appendage insertions are known in *Leanchoilia*^[47] and a

320 variety of other BST arthropods^[50], and in specimens in which these appendages do not
321 project anteriorly, they are likely folded under the body^[21], thus potentially leaving paired
322 imprints in the post-buccal area (Fig. 3).

323 The eye tracts illustrated in detail in GRCP 15007 are not visible on the higher-
324 resolution image in (Fig. 3D, E). A small inter-ocular swelling, in the same specimen,
325 might correspond to the location of the leanchoiiliid labrum (putative in adults), but may
326 also represent another feature located at the junction of all optic tracts. Consequently, the
327 trapezoidal outline situated behind the insertion of the frontalmost appendages in GRCP
328 15007 (Fig. 3E) appears consistent with the shape of the mouth in these taxa^[51]. There is a
329 half-ringed area encompassing the space behind the eyes and overlapping the insertion of
330 the frontalmost appendages (Figs 3D-F, 4A) that has been documented before^[47] (plate 1,
331 fig. 1, 3). The well-defined boundaries of this seemingly internal structure suggest it is also
332 cuticular, rather than neural, and therefore could represent a large apodeme.

333 Elemental mapping data^[3, fig. 2] show that the axial area in leanchoiiliid sp. GRCP
334 15007 and 15009 is comprised of a polymorph of calcium phosphate (most likely
335 fluorapatite as in other BST fossils) surrounded by carbon, even if large central chunks of
336 intestinal apatite are missing in GRCP 15009. This is what is expected from the
337 preservation of the internal aspect of the gut by mineralized infill and of the gut epithelium
338 as kerogen (Figs 1, 2): the plane of splitting cuts through the gut, in cross-section showing
339 the interior of the gut tube bound by two parallel lines. GRCP 15009 is severely altered,
340 and the central lacuna of apatite was most likely created by loss at the time of splitting.
341 Nodes and tracts preserved in carbon anterior to the remains of the gut tract repeat the serial
342 pattern of latero-ventral tonguelets. Given these considerations, it appears likely that only

343 the anteriormost section at the level of eye insertion could represent parts of the CNS, but
344 such an interpretation is speculative (see below).

345 ROMIP 57655 is comparable to GRCP 15007 in its angle of burial and in the
346 features visible, though it is much more complete and detailed (Fig. 4A). This specimen
347 likewise displays the eyes without any tract, as well as the underlying half-ringed structure
348 underneath them, which seems to be internal. ROMIP 57655 also documents more clearly
349 the complex peri-intestinal and ramifying kerogen film, showing how various remains of
350 this film overlap in the medial area of GRCP 15007. Peri-intestinal ramifications mostly
351 represent sub-intestinal tonguelets, but they can appear cut, flared out, filamentous or they
352 can be fused altogether with the cuticular margins of limbs as a result of differences in the
353 plane of splitting through specimens and in the extent of partial decay. ROMIP 57655 thus
354 represents a typical general example of internal, peri-intestinal tissue preservation in BST
355 fossils, and shows how variations on these patterns could recreate the appearance of a CNS
356 (Fig. 6). More generally, patterns of folding in compressed cuticle or other carbon-rich
357 tissues can easily produce structural shapes that are not readily interpretable. In *Actaeus*
358 *armatus*, for instance, the manipulation of certain brightness and contrast settings can help
359 isolate elements resembling the brain (Fig. 4G), but the distribution of carbon in the head
360 of this very well-preserved specimen shows much greater complexity (Fig. 4H), notably
361 cuticular collapse and folding. Manipulating these light settings can change the visible
362 amount of carbon (or carbon-coated) film and therefore can reproduce lesser preservation
363 or a different plane of splitting. Cheirae in this specimen could be easily interpreted as
364 protocerebral if one were to disregard other fossil structures, or if the specimen was more
365 poorly preserved.

366 To investigate *Alalcomenaeus* YKLP 11075—another leanchoilid—, Tanaka and
367 colleagues interestingly combined two X-ray-based techniques: one based on X-ray
368 fluorescence and used for elemental analysis, here of Fe, the other being tomographic—
369 MicroCT scanning^[4]. Comparing different sources of visualization could be a way to help
370 disambiguate different structures in fossils. However, the authors interpreted overlapping
371 signals from both techniques as remains of the CNS, without providing a taphonomic
372 rationale as to why this should be the case. As explained earlier, there is, as of yet, no
373 established tissue-specific mineralization pathway outside of some phosphate-based cases.
374 A tomographic investigation sensitive to Fe will provide an incomplete tridimensional map
375 of both internal and external remains, as is now shown for several Chengjiang taxa^[51,52],
376 whereas elemental mapping of Fe will reveal a partly similar pattern, depending on
377 differences in sensitivity. Should the CNS be preserved or secondarily coated in part by
378 Fe-compounds, this approach is insufficient to discriminate it from all other existing
379 internal and external structures. In addition, original imagery in Tanaka et al.^[4] shows that
380 both techniques largely recover 1) incompletely overlapping regions of the same structures,
381 implying that the overlapping signal from both methods is an incomplete representation of
382 the same feature, and 2) structures that are clearly separate; for instance, guts and
383 appendage traces in both cases are confounded with tergo-pleural boundaries, thus ruling
384 out the isolation of a discrete CNS. The patterns shown for *Alalcomenaeus* YKLP 11075
385 therefore correspond to an overlap of the digestive system, the peri-intestinal cavity,
386 appendages, and segment boundaries, with deformation resulting from partial decay and
387 compaction of soft tissues against the cuticle. The “vertebrae”-like central structure in
388 particular, resembling sternites, is likely the result of the impression of the gut

389 superimposed upon, and emphasizing segment boundaries. Interestingly, the features
390 interpreted as neural in *Alalcomenaeus* were considered chelicerate-like in this study, but
391 were instead presented as consistent with an ancestral brain configuration^[3]: this
392 emphasizes the uncertainty of interpretations based on insufficiently clear fossilized
393 biological patterns.

394 A lack of feature discrimination also impacts interpretations of *Alalcomenaeus*
395 MCZ IP-197956 and KUMIP 204782^[6]. MCZ IP-197956 is poorly preserved, but with a
396 general pattern commonly observed in megacheiran specimens^[47,46,45,21], namely a likely
397 phosphatic gut trace surrounded by an originally carbon-rich peri-intestinal trace with
398 ventral lobations and inconsistent filamentous remains—not limited to the ventral side and
399 not consistently projecting into limbs, for instance as in *Surusicaris*^[22]—which are signs
400 of loss to decay. In KUMIP 204782, which is also poorly preserved, filamentous traces are
401 serially repeated, but they are again similar to common linear artefacts in BST arthropods
402 (Fig. 6E-H), and it is not possible to disambiguate them from tonguelets and cuticular lines,
403 especially segment boundaries and appendage outlines, or, considering the quality of the
404 specimen, even possible deformation of tissue margins through decay^[36,18]. The anterior
405 part of the head shows the impression of cheirae, and the “hypostome” is misinterpreted as
406 a displaced part of the foregut (Fig. 4D).

407 Sub-intestinal tonguelets, or taphonomically-separated distal tips of them, were
408 also annotated as nerves in the basal megacheiran-like *Kylinxia*^[53], without argument for
409 this interpretation.

410

411 **3.3. Extant lineages**

412 **3.3.1. Mandibulata**

413 Among other euarthropods, fuxianhuiids have been a central focus of
414 paleoneuroanatomical studies^[5,8,13]—a conjunction of specimen availability, evidence and
415 a long-standing view that these animals represent an ancestral stem euarthropod body
416 plans^[5,54], even though there is evidence that they are in fact mandibulates^[55]. The two
417 cases are the description of optic neuropils and brain in *Fuxianhuia* on the one hand, and
418 that of a VNC in *Chengjiangocaris* on the other.

419 Despite peculiar taphonomic conditions, under which no other soft internal tissue
420 is preserved, the VNC of *Chengjiangocaris* YKLP 12026 benefits from a clear topological
421 isolation—the split of the fossil passes through the nerve cord—and yields fine
422 morphological details. Notwithstanding evolutionary implications for the number of
423 interganglionic peripheral nerves^[5,55], YKLP 12026 satisfies the conditions of topological
424 disambiguation and explicit taphonomic context described above, with each putative
425 ganglion corresponding to a somite on the polypodous body (in which tergites correspond
426 to the dorsal fusion of several segments). Nevertheless, YKLP 12324, preserved in dorsal
427 aspect, and also interpreted to display a VNC, calls for caution. At cursory inspection, the
428 chain-like medial structure is similar, but each successive node is in strong relief,
429 suggesting greater sclerotization, each forms a single unit incompatible with paired ganglia,
430 and, most importantly, each connects rigorously with the base of each adjoining limb.
431 Shape and three-dimensionality would therefore be more consistent with a series of
432 sternites (see, e.g., ref.^[33]). This would not be exclusive of the presence of a VNC *per se*,
433 because the carbonaceous trace observed on fossils and radiating filaments could
434 correspond to the nerve cord overprinted onto the sternites. The morphology of the VNC

435 in YKLP 12026 suggests that taphonomic conditions leading to the preservation of this
436 feature preserve an aspect close to the original VNC. The recognition that the VNC could
437 preserve as poorly-defined trace under the best conditions (see also, e.g., ref.^[23]) can
438 facilitate disambiguation of the VNC from other structures^[14].

439 In *Fuxianhuia* YKLP 15006^[8] and similar specimens^[13], optic neuropils are clearly
440 laid out within the eye stalks, and are present in similar shapes on the counterpart. Thus,
441 the evidence is redundant as well^[13]. However, the post-ocular neural morphology of
442 *Fuxianhuia* can only be partially disambiguated from the rest of the morphoanatomy,
443 because the key specimens presented may have other internal tissues or appendages in the
444 same area that are not distinguishable. For example, in YKLP 15006^[13], there is a broad
445 darkening of the antennules clearly representing more than a single nerve outline, which
446 points to a more complex taphonomic history and possibly the overlap of different tissues
447 in the compression fossil.

448 The brain morphoanatomy of *Fuxianhuia* may be better constrained by looking at
449 another early mandibulate, *Waptia*^[2] (Fig. 5G). ROMIP 64293 and USNM 138231 show
450 the same configurations of branching kerogen tracts, which are comparable to those
451 documented in *Fuxianhuia*, and are of brain-like morphology: an anterior bridge
452 connecting with optic tracts is also connected by lateral tracts to a posterior transversal
453 trace, forming a roughly trapezoidal fenestra which, according to the known anatomy of
454 the head, is located above the mouth. This opening is therefore a strong candidate for the
455 stomodaeum^[2]. As in *Fuxianhuia*, multiple overlapping elements indicate that other
456 tissues—mostly the gut and appendages—are preserved together with the putative neural
457 tissues, but the latter are distinct, consistent with expected morphoanatomy, and the fidelity

458 of preservation is relatively good, although redundancy proves to be crucial here (ROMIP
459 64293 is in poor condition and would only be inconclusive on its own). Although we
460 consider the evidence ambiguous in this specimen, our own tracing of GRCP15009 (Fig.
461 5H, I) suggests that a comparable very anterior stomodaeum might be present in
462 leanchoilids, which would also conform to their morphoanatomy, in particular the
463 insertion of the cheirae.

464 Ma and colleagues revised their interpretation of the morphoanatomy of
465 *Fuxianhuia*'s brain based on the counterpart of YKLP 15006 and two additional
466 specimens^[13], preferring to consider the central "fenestrum" as a part of the brain that was
467 taphonomically removed. Based on the configuration in *Waptia*, and the well-defined
468 anterior margins of the deutocerebrum in YKLP 15006 (labelled "PRO" in ref.^[13]), the
469 fenestrum interpretation is well-grounded and the observed non-lacunar pattern YKLP
470 11357 corresponds in fact to a more ventral layer, below the stomodaeal aperture, where
471 the brain forms a compact mass. It is also possible that the fenestrum is an artefact caused
472 by the compression of a thick protocerebrum with any frontalmost organs (see, e.g.,
473 combined patterns of protocerebrum and naupliar ocellus in *Artemia*^[56]), and therefore that
474 the stomodaeum is more posterior, closer to the brain configuration of extant crustaceans,
475 but this would make the location of the deutocerebrum and the innervation of frontalmost
476 appendages less plausible.

477 In a way similar to some examples cited above, *Waptia fieldensis* ROMIP 64295
478 also exhibits a cephalothoracic peri-intestinal tonguelet pattern that could, without context,
479 be mistaken for a diagnostic chelicerate-like condensed CNS^[57], and other specimens (e.g.,
480 ROMIP 64287^[2]) show intestinal features that mimic ganglia and nerves (Fig. 5A, B).

481 Additional specimens (such as USNM 114259, see^[2, SEM S17]) confirm the occasional rosary
482 aspect of the gut based on location and calcium phosphate infill. This illustrates how a
483 proper taphonomic sampling is essential to an accurate interpretation.

484

485 **3.3.2. Chelicerata**

486 The middle Cambrian euarthropod *Mollisonia*, recently reinterpreted as the earliest bona
487 fide chelicerate^[1], was presented with three pairs of optic neuropils—a surprisingly
488 “derived” condition suggesting in fact that a complex neuropil arrangement was ancestral
489 in euarthropods, and that many extant lineages experienced secondary simplifications.
490 Similar to *Fuxianhuia*, the outline of these neuropils is distinct, and their location makes
491 an overlap with non-neural tissues unlikely, although the full evidence is provided by only
492 a single specimen.

493 Two *Mollisonia* specimens were later reexamined in that context, with description
494 of nervous tissues^[7]. The interpretations of *Mollisonia* MCZ 1811 and USNM 305093^[7]
495 exhibit issues similar to leancholiid examples discussed before. USNM 305093 is a poorly
496 preserved specimen with generic patterns of preservation of internal tissues. Only a central
497 internal tube is visible with reflective ventral ramifications as well as segment boundaries.
498 This central tube is the gut, surrounded by the typical carbon-rich peri-intestinal cavity
499 (Fig. 4E), whose impression connects with the generally carbon-rich eyes, and ventral
500 ramifications. The ventral ramifications correspond to the dorsal margin of the two main
501 diverging rami of the limbs, as evidenced for instance by ROMIP 65015, showing likely
502 further decay (Fig. 6G, H), and in other taxa. MCZ 1811 is of better quality, but the
503 specimen contains only indistinct carbon traces anteriorly and a typical circum-intestinal

504 carbon patch. Features interpreted as a “segmental nerve” and “gut diverticula” are
505 identified by elemental mapping where C, P and Ca overlap, although neither of these
506 features can be discriminated or confirmed by the rest of the fossil evidence^[1]. Consistent
507 with the antero-posterior configuration, the “gut diverticulum” in dorsal view is a tonguelet
508 that the authors interpret as nerve in lateral view.

509 Interpretative consistency is also paramount, even when known morphoanatomy is
510 thoroughly considered, especially in the case of well-studied taxa. For instance, ROMIP
511 57655 illustrates that the alleged esophageal foramen in *Alalcomenaeus* sp. YKLP 11075^[4]
512 (Fig. 4B) can preserve as reflective patch (Fig. 4A), a fenestra (Fig. 4B) or a three-
513 dimensional gut tract swelling (Fig. 4E). This is incompatible with an interpretation of all
514 the reflective traces as neural^[3,4], in which case the foramen itself would not be reflective
515 or swollen, but instead is consistent with an hemocoelo-digestive hypothesis. Given the
516 evidence, this area more likely represents a differentiated foregut, perhaps a stomach.

517

518 **3.4. Other panarthropods**

519 Recent claims were made about the discovery and identification of a brain in the
520 lobopodian *Cardiodictyon*, presented as a tripartite protocerebrum, as well as a VNC^[58].
521 Lobopodians being the paraphyletic sister lineage to arthropods, the presence of a tripartite
522 protocerebrum in these animals would have important implications for head evolution in
523 the entire group^[59]. Here again, however, we find nothing convincing in terms of
524 taphonomy, morphoanatomical consistency, or disambiguation power. Taphonomically,
525 the magenta-to-purple Fe-oxide stains on which the argument is based are well known to
526 be present throughout Chengjiang fossils, with no demonstrated organ affinity (see, e.g.,

527 their fig. S1). They represent Fe-stains derived from oxidation of original pyrite, however,
528 it is not clear that these stains track the original distribution of pyrite, or that pyrite
529 originally replicated the structures in question. In the absence of crystal molds or
530 pseudomorphs that provide evidence for the original distribution of pyrite, as conclusively
531 demonstrated before^[14,60], the colored patches may be entirely secondary in origin, in
532 which case their morphoanatomical significance would be non-specific—as suggested by
533 their uneven distribution and overlap with other oxidized parts of the fossil (Supp. Fig. 3).
534 In general, the distinctly colored phases picked out by chromatic filtering represent the
535 complex post-Cambrian weathering history of Chengjiang fossils^[61]. A careful taphonomic
536 study and model are required to establish the structures as biological in origin.

537 Correlatively, the colored patches isolate neither brain nor nerve cord specifically. We
538 conducted a similar “chromatic isolation” (consisting in selective isolation of color ranges
539 from an image) on the main specimen presented with nerve tissues (YKLP11422) as well
540 as YKLP11418 and YKLP11419 (Supp. Fig. 3). Coloration occurs clearly over the entire
541 surface of the fossil, and patterns—especially in the head—are influenced by cracks or
542 fractures in the matrix (Supp. Fig. 3). Density of this particular stain increases over limbs
543 and in the sclerotic interspace, as well as on the contour of the head. There is neither
544 tripartite consistency nor clear internal topology that would identify any part of these traces
545 as a separate CNS. Fig. S5^[58] in the original publication shows the extent of image
546 manipulation required, including deletion of all surrounding elements, to produce a vague
547 tripartite patch of color. A thorough investigation of these fossils is warranted.

548

549 **4. NATURE AND DISPARITY OF LATERO-VENTRAL ‘TONGUELETS,’ AND**
550 **OTHER POSSIBLE MANIFESTATIONS OF THE LACUNAR CIRCULATORY**
551 **SYSTEM**

552 As we have shown (Figs 1, 2, 4A-E, 5A, B, H, I, 6), latero-ventral tonguelets are of central
553 importance in the disambiguation of the CNS in BST arthropods. The term ‘tonguelet’
554 itself is assigned broadly to all latero-ventral extensions of the peri-intestinal area,
555 encompassing a variety of shapes and possibly tissular identity. As primary distinctions,
556 tonguelets occur with well-defined boundaries as single lobes (Figs 5A, B, 6D) or
557 ramifying structures^[22], but also as variations of those types with more diffuse boundaries
558 (Figs 4A-E, G, H, 5A, 6A, E, G).

559 The ramification of tonguelets into two or three branches is common in BST
560 arthropods (Figs 4A, C, D, 6E-G). Based on taphonomic processes discussed above and a
561 possible hemocoelic affinity advocated previously^[2,22,62,63], such ramifications arguably
562 replicate an original subdivision of the cavity when present across somites in well-
563 preserved specimens. In *Surusicaris*^[22], where tonguelets are arguably consistent with an
564 original biological feature, branching of the carbon film is specifically associated with the
565 exopods. In *Opabinia*, tonguelets often connect with the base of gill blades, and they are
566 also particularly well-developed inside the shaft of respiratory appendages of *Waptia* (Fig.
567 5A, B). This evidence points to a relationship of these tonguelets with a respiratory and
568 more generally gas and nutrient-exchange function^[2,21]. In the context of the peri-intestinal
569 hemocoel model, tonguelets would therefore specifically represent cavities connecting
570 permeable organs with the main hemolymphatic system—the ventral hemolymph
571 reservoirs of the circulatory lacunar system (Supp. Fig. 1).

572 In *Alalcomenaeus* sp. ROMIP 62968 from the Marble Canyon locality (Fig. 6B-D),
573 typical tonguelets expand into the proximalmost bundle of short lamellae. This
574 configuration is consistent with recent tomographic information from Chengjiang fossils
575 demonstrating the presence of proximal lamellate features in arachnomorphs^[52]. This
576 additional evidence further suggests that latero-ventral tonguelets are hemocoelic
577 connections between the peri-intestinal cavity and respiratory appendages, and,
578 occasionally, the gills themselves. The exact correspondence between the circulatory
579 system and tonguelets is beyond the scope of this study but is currently under investigation
580 by the authors.

581 In any case, the similarity in topology and morphology with the ventral lacunar
582 system^[64], as well as the body of evidence showing that ramifications are tightly associated
583 with gills and other gas exchange areas, constrains the identity of sub-intestinal tonguelets
584 as hemocoelic cavities. Not only does this have clear consequences for the interpretation
585 of all comparable tonguelet elements across BST arthropods, but this also calls into
586 question the accuracy of reconstructed brains. If, as tonguelets currently suggest, the
587 lacunar circulatory system is preserved, then this system would also potentially preserve in
588 the cephalic region. In certain cases, such as in horseshoe crabs, the enclosing lacunar
589 circulatory system can replicate the gross morphology of the brain, while the vascular
590 system, closely surrounding the prosomal synganglion, is virtually identical in shape^[65].
591 The recently documented syncerebron of *Euproops*^[11] shows clearly that there was no
592 original mineralization of nervous tissues, but instead a mold of a central cavity which, in
593 xiphosurans, is indistinguishable from the cavity of the circulatory system tightly enclosing
594 the brain^[65]. However, cephalic lacunar systems are poorly known in general, in part due

595 to technical difficulties in keeping them intact experimentally. Derived pancrustaceans
596 have shown so far a great complexity of cavities connected to cephalic organs, as well as
597 eye stalks (Torben Göpel, pers. comm.), which could create patterns interpretable as
598 neuropils and commissures that do not accurately replicate the CNS.

599 Therefore, the preservation of a cardiovascular system in *Fuxianhuia protensa*
600 YKLP 11336 would represent preservation of a unique type of tissue among BST fossils.
601 Similar to our assessments of putative neural remains, it should be noted that the specimen
602 is poorly preserved, (1) not allowing for clear disambiguation with other tissues, and (2)
603 increasing the likelihood of deformation via decay. Darkening of the intermandibular area
604 is quite common^[18,55], whereas serial lobate patterns could correspond to outlines of the
605 limb bases, peculiarly-preserved tonguelets, or a combination of both. Based on the same
606 validation criteria we put forward for brain tissues (see below), the evidence shown in
607 YKLP 11336 should be considered incomplete.

608 Broader support for the identification of peri-intestinal traces as hemocoelic
609 cavities also comes from additional evidence often overlooked in this context. This
610 evidence is comprised of traces of biological origin contrasting body cavity with cuticular
611 margins and in particular doublures, and of long traces inside tailpieces or other extended
612 cuticular features. Interpretations of the thin reflective filament throughout the long telsons
613 of *Burgessia* or *Molaria* (Fig. 6F) have only been tentative, yet this trace is equivalent in
614 size, extent and location to the xiphosuran caudal artery^[65]. This correspondence also led
615 some authors, almost two decades ago, to interpret the peri-intestinal trace and its long
616 ramifications into the cephalic spines of *Marrella* as remains of the circulatory system^[62].
617 Distinctly, but complementarily, broad internal patterns single out the extent of the body

618 doublure, for instance in naraoids^[63, fig. 4], or the doublure of the tailpiece, as in *Waptia*^{[2,}
 619 fig. 18]

620

621 **5. WHAT DO WE REALLY KNOW ABOUT NERVOUS SYSTEMS IN EARLY**
 622 **ARTHROPODS?**

623

624

Genus	Specimen	TD	MAC	ETC	R
<i>Alalcomenaeus</i>	MCZ IP-197956	N	N	N	NA
	MCZ IP-197956	N	Y	N	NA
	YKLP 11075	N	N	N	NA
<i>Chengjiangocaris</i>	YKLP 12024, 12026	Y	P	Y	?
<i>Fuxianhuia</i>	YKLP 15006, YKLP 11357, YKLP 11358	P	Y	P	Y (3)
<i>Kerygmachela</i>	MGUH 32048- 57	Y	Y	Y	Y (10)
<i>Kylinxia</i>	NIGP 171304	N	Y	N	NA
Leanchoiliid indet.	GRCP15007	N	P	N	NA
	GRCP15009	N	P	N	NA
<i>Lyrarapax</i>	YKLP13304-6	Y	P	Y	P
<i>Cardiodictyon</i>	YKLP11434	N	N	N	NA
<i>Mollisonia</i>	MCZ 1811	N	P	N	NA
	USNM 305093	N	P	N	NA
	ROMIP 62978	Y	Y	Y	P (3)
<i>Stanleycaris</i>	ROMIP 65674, 65756-59, 65761, 65763, 65777	P	Y	Y	Y (8)
<i>Waptia</i>	USNM 138231, ROMIP 64293	Y	Y	Y	Y (2)
<i>Yawunik</i>	ROMIP 63067	Y	P	P	P

625

626 **Table 1. Assessment of key specimens discussed in this study and used as**
 627 **paleoneuroanatomical references, based on our methodological criteria.** These criteria

628 are assessed relative to the evidence at hand and the interpretation put forward: in some
629 cases, they evaluate the entire brain or CNS, in others, only parts of it. Numbers under the
630 Redundancy column indicate the total number of specimens replicating the relevant
631 evidence. Abbreviations: ETC, Explicit taphonomic context; MAC, Morphoanatomical
632 consistency; N, no; NA, non-applicable (whenever one or more criteria are not fulfilled);
633 P, partial; R, redundancy of evidence (that is, replication in other specimens); TD,
634 topological disambiguation; Y, yes; See also main text and Fig. 7.

635

636 In light of these reassessments, we used the three criteria explicated earlier in this
637 specific context (Fig. 7), as well as redundancy of evidence, to evaluate the robustness of
638 the arthropod paleoneuroanatomical evidence published thus far from Cambrian fossils
639 (Table 1, Supplementary Discussion). Additionally, the outcome of our revision is
640 summarized diagrammatically in Fig. 8, using a phylogenetic framework recovered from
641 recent cladistic analyses^[1,55,66].

642 When a more rigorous approach to tissue interpretation is applied and various
643 extrapolations are subtracted, paleoneurological evidence provides a far less complete
644 scenario than depicted by a number of recent studies, especially regarding the
645 arachnomorph CNS^[3,6,7]. In addition, we present here ideal situations where cephalic
646 lacunae were not misinterpreted as nervous tissues, but this possibility should be
647 considered as well.

648 *Stanleycaris* together with a reexamined *Lyrarapax* provide some reliable anterior
649 CNS morphological evidence in radiodontans (Table 1) that is important both in
650 documenting brain organization before the rise of euarthropods, and in connecting with the

651 condition of *Kerygmachela*. The simple brain of *Kerygmachela*, bearing similarities with
652 the dorsal commissure and possibly the frontal nerve ring of tardigrades as seen from dorsal
653 view^[67] (but see [72]), contrasts with the antero-posterior differentiation of the putative
654 radiodontan brains, which appear to have a separate deutocerebrum and well-defined
655 stomodaeum. This lends support to the view that the pre-radiodontan brain was a
656 metaneuropil that later subdivided^[27,53], as the separate identity of pre-gnathal segments
657 from posterior ones would suggest^[68].

658 By contrast, any brain morphoanatomies remain to be clearly demonstrated in both
659 leanchoilids and mollisoniids (Table 1), outside of the optic neuropils in *Mollisonia*
660 *plenovenatrix*^[1]. *Yawunik kootenayi* ROMIP 63067^[21] (Fig. 5E, F) distinctly shows all four
661 eyes with optic tracts connecting to a central rectangular structure at the very front of the
662 animal, without overlap with the gut, circum-digestive structures or appendages. The
663 central rectangular element could be part of the protocerebrum, but it is also very similar
664 to the inter-ocular element in relief in GRCP15007 that we compared topologically with
665 the labrum (Fig. 3D, E). Hence, at this time, the identity of this feature remains uncertain.

666 Within total-group Mandibulata, optic neuropils and possible brain
667 morphoanatomies would also benefit from more robust evidence. Apart from the arguable
668 nerve cord documented in *Chengjiangocaris*, the post-frontal CNS remains elusive, and
669 additional evidence based on the methodological criteria developed above will be needed
670 to make firm interpretations.

671 It is also important to reexamine existing interpretations in light of the total
672 evidence. In *Fuxianhuia* YKLP 11358^[13], for instance, the proximal part of the eye
673 connectives, including the first pair of optic neuropils, appears short and round (without

674 more distal neuropils) because of the angle of splitting, and yields a morphology very
675 similar to the frontal lobes of *Lyrarapax* (Fig. 4F), which also insert into the base of the
676 eye stalks. This would therefore support an optic neuropil identity for the radiodontan
677 ‘frontal lobes,’ with the base and first neuropil possibly lying on a slightly different plane
678 than the distal neuropils. Based on *Stanleycaris*, it was recently proposed that these lobes
679 could be related to a large ancestral median eye^[43], but the connection between the median
680 eye and the rest of the protocerebrum in this taxon is not clearly preserved as it is in the
681 anterior lobes of *Lyrarapax* or the proximal optic neuropils of *Fuxianhuia*.

682 One of the strongest outcomes of arthropod paleoneuroanatomy is therefore that
683 complex visual systems based on three separate nested optic neuropils either convergently
684 evolved in mollisoniids and fuxianhuiids, or, more likely, were present at the origin of
685 Euarthropoda^[1,27]—and perhaps even Arthropoda^[69]. Our reinterpretations also suggest
686 that a deutocerebrum was already present in radiodontans, in line with recent evidence^[43].
687 There is also evidence for a very anterior stomodaeum in early mandibulates, and a more
688 three-dimensionally shaped brain accommodating a more ventral deutocerebrum, but this
689 was possibly already the case in radiodontans. The location and shape of the stomodaeum
690 in leanchoiliids might be similar to what is observed in early mandibulates (Fig. 5H, I), but
691 this is conjectural. Based on the condition seen in *Kerygmachela*, it appears nonetheless
692 that the origin of Arthropoda involved a significant structural change cumulating in the
693 arthrodization of the frontalmost appendages, the sclerotization of the mouth, the
694 development of compound eyes and, as perhaps would be expected, the physical expansion
695 of the brain.

696

697 **6. CONCLUSIONS**

698 Although considerable effort and progress have been made to consolidate the scientific
699 basis of paleoneuroanatomy^[14], a careful approach to such evidence, more inclusive of the
700 diversity of preservation states and taphonomic variations, and subject to specific criteria
701 of validation, is essential. A critical analysis of the existing evidence in this area and the
702 contribution of additional material constrains a less detailed but more robust basis for
703 understanding paleoneuroanatomical evidence in early arthropods. The evolutionary
704 aspects that we consider here to be well documented are: the structural development of the
705 brain, possibly associated with an early ventralization of the deutocerebrum at the origin
706 of Arthropoda, and the likely presence of a complex visual system involving three distinct
707 optic neuropils occurring with the rise of euarthropods, and perhaps even closer to the
708 origin of compound eyes itself.

709

710 **Institutional abbreviations.** BST, Burgess Shale-type; CNS, central nervous system;
711 GRCP, Museum of Guizhou Research Center for Palaeobiology; ROMIP, Royal Ontario
712 Museum Invertebrate Palaeontology; VNC, ventral nerve cord; YKLP, Yunnan Key
713 Laboratory for Palaeobiology.

714

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717

718 **Conflict of interest.** The authors declare no conflict of interest.

719

720 **Data availability.** All data used in this study are provided upon publication.

721

722 Author contributions. CA designed the study, wrote the first draft of the manuscript and
723 made the figures; all authors contributed to the critical discussion and to the final version
724 of the manuscript.

725

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915

916

917 **Figure captions**

918 **Figure 1.** Explanatory diagrams for peri-intestinal preservation in Burgess-Shale type
919 arthropods. Top: Transverse cross-sections representing different preservation types and
920 two different original conditions for the ventral lacunar system (left and right). Bottom:
921 Coronal sections in dorsal view corresponding to Top diagrams. A. Specimen close to time
922 of entombment, unflattened, with phosphate-filled gut and distinct internal features,
923 including heart (he), gut (gt), gut epithelium (ge), ventro-lateral hemocoelic cavities, part
924 of the lacunar circulatory system (tg for “tonguelets”), ventral nerve cord (vnc) and ventral
925 vascular system (vvs). Contrary to the vascular network, the lacunar circulatory system is
926 not bounded by membranes and is shown in dotted lines. On the right side, intra-
927 appendicular channels (tg) are not well developed; on the left, hemolymph channels branch
928 out from the main lacunar cavity into endopod and exopod rami, and a third, additional
929 cavity projects into a separate gill. On the dorsal view (bottom), depending on orientation
930 and plane of observation, 1, 2 or 3 tonguelets might be visible. In this same view, the ventral

931 nerve cord is here represented by transparency, but not expected to be visible. B. Specimen
932 with limited deformation and minimal to no decay. Membranes and cavities now uniformly
933 preserved as kerogen films, mediated by limited microbial activity. Theoretically, in some
934 conditions, remains of the ventral nerve cord may remain isolated from the peri-intestinal
935 carbon film (right side), although the evidence for this is debatable in fossils. Phosphate
936 formation sometimes invades tonguelets (right side, asterisk). C. Specimen with significant
937 decay and diagenetic alterations. The combination of these alterations produces a variety
938 of peri-intestinal shapes (right side) that can secondarily mimic genuine branching of the
939 hemolymph system (left side), or, to an extent, ganglia and nerves.

940

941 **Figure 2. Explanatory diagrams for general preservation in Burgess-Shale type**
942 **arthropods.** Top: Sagittal sections representing different preservation types of a fictional
943 *Fortiforceps*-like megacheiran. Bottom: Coronal sections in dorsal view corresponding to
944 Top diagrams. A. Specimen close to time of entombment, with phosphate-filled gut and
945 distinct internal features shown as outlines. Nervous tissues in green, apatite in blue,
946 cuticular and gut boundaries in black, sub-intestinal tonguelets in orange. Phosphate
947 formation sometimes invades tonguelets. B. Specimen with limited deformation and
948 minimal to no decay. Membranes and cavities now uniformly preserved as kerogen films,
949 mediated by limited microbial activity. C. Specimen with significant decay and diagenetic
950 alterations. Note merging of carbon films from anterior cephalic area posteriad. Disparity
951 of tonguelet shapes is exaggerated to show range of possible shapes, a number of which is
952 illustrated in this study: BR, branching type (here, secondary); DI, diffuse type; MCa,
953 merged cuticular, appendicular type (peri-intestinal trace merged with cuticular outlines at

954 base of limbs); MCt, merged cuticular, tergo-pleural type (peri-intestinal trace merged with
955 cuticular outlines of tergo-pleurae); SP, split type. Early taphonomic phases are also
956 commonly associated with a ventralization of the gut tract, not shown here. See Figure 1
957 for abbreviations; other abbreviations: ey, eye; fa, frontal appendages; mo, mouth; os,
958 ocular sclerite.

959

960 **Figure 3. Leanchoiliid taphonomy and morphoanatomy.** A-C, Diagrammatic cross-
961 sections of a generalized leanchoiliid; nervous system in green, eyes in dark blue,
962 simplified peri-intestinal lacunar vascular system in light blue, digestive system in red,
963 heart in purple. A, Section at the front of great appendage insertion, showing main brain
964 mass and optic tracts; shape of protocerebrum follows P-S; dotted line represents possible
965 protocerebral fenestra, see text for explanations. B, Section at the back of great appendage
966 insertion, showing looping foregut and mouth opening. C, Section through trunk, with large
967 ventral hemocoelic system connecting to gills and exopods. D, E, *Leanchoilia* sp.
968 GRCP15007, close-up of cephalic area (see Lan et al.^[3] fig. 1A, B). Underlying horseshoe
969 structure as in E in dashed line; circular swellings marking likely frontalmost appendage
970 insertion in dotted lines. F, *L. superlata* ROMIP 57655, close-up of cephalic area, grayscale
971 and inverted lightness. Post-ocular semi-circular structure as in D, E. Double arrow indicate
972 width of branching peri-intestinal carbon pattern. See also Fig. 3A for full specimen.
973 Picture courtesy of Jean-Bernard Caron. G, H, *Leanchoilia* sp. GRCP15009. G, Picture
974 provided from Supplementary Material in Lan et al.^[3] H, Tracing of main carbon linings
975 in G superimposed onto the main feature signal presented in the specimen in Lan et al. fig.
976 2A. Arrowheads mark in: D, E, pyritised cuticular outlines; sides of the post-ocular

977 structure overlapping frontalmost appendage insertion. Asterisks mark in: D, E, small
978 swelling of interocular, pre-oral structure, possibly labral; F, G, areas in relief from each
979 side of the buccal region, possibly related to frontalmost appendages folded under the body.
980 Colour coding: In A-C, nervous system in green, eyes in dark blue, dorsal artery in purple,
981 lacunar circulatory system and peri-intestinal hemocoelic cavity in light blue, digestive
982 system in red; in H, morphological feature lining in red, cuticular folds in red dashed line,
983 cracks in sediment layer in yellow dashed line, oesophagal/post-oesophagal area in purple.
984 Abbreviations: ba, basipod; fai, insertion of frontal appendage; fg, foregut; mo, mouth.
985 Scale bars: 2 mm.

986

987 **Figure 4. Taphonomic variations in the preservation of internal structures among**
988 **Cambrian arthropods.** A, *L. superlata* ROMIP 57655, photo courtesy of Jean-Bernard
989 Caron. Full specimen, wet, polarized light. The branching reflective pattern (typically
990 highly carbonaceous) surrounds the entire digestive tract, including midgut glands and is
991 not limited to ventral pairs of closely associated swellings, like ganglia. B, C,
992 *Alalcomenaeus* sp. YKLP 11075. B, Cephalic area from Tanaka et al.^[4] Extended Data fig.
993 1, with expected location of appendages based on leaichoiliid literature. C, Elemental and
994 tomographic imagery methods capture parts of the same peri-intestinal system, which
995 largely overlap, but do not isolate any structure identifiable with a VNC. D, *Alalcomenaeus*
996 sp. KUMIP 204782 cephalic area from Ortega-Hernández et al.^[6] fig. 2a, showing expected
997 insertion of anterior appendages. Anterior hexagonal piece is a displaced fragment of the
998 foregut. E, *Alalcomenaeus* sp. MCZ IP-197956 trunk section from Ortega-Hernández et
999 al.^[6] fig. 1a. Gut tract likely apatitic with broad peri-intestinal patch and subtriangular

1000 extensions (“tonguelets”). F, *Lyrarapax unguispinus* YKLP13305 anteriormost head
1001 region from Cong et al.^[9] fig. 2h. Internal frontal lobes lie on top of the base of the eye
1002 stalks, and lateral extensions of putative brain are not optical nerve tracts. Double red lines
1003 indicate connection of the eyes with the eye stalks. G, H, *Actaeus armatus* holotype USNM
1004 155597. G, Anteriormost portion of the cephalon, wet specimen with cross-polarized
1005 lighting. Note cuticular collapse in right frontalmost appendage and widespread fold lines.
1006 H, Same as P with low brightness and enhanced contrast. Arrowheads mark in: A, peri-
1007 intestinal tonguelet traces with various shapes; E, probable patches of apatite; G, cuticular
1008 collapse. Asterisks mark in: E, circum-intestinal carbon trace. Abbreviations: *cn*, cephalic
1009 appendage *n*; *es*, eye stalk; *fai*, insertion of frontal appendage; *fl*, frontal lobe; *gt*, gut tract;
1010 *le*, lateral eye; *PC*, protocerebrum; *tg*, sub-intestinal tonguelet; *vr*, various remains. Scale
1011 bars: 2 mm.

1012

1013 **Figure 5. Disambiguation between latero-ventral tonguelets and putative nervous**
1014 **remains.**

1015 A, B, G, *Waptia fieldensis*. A, ROMIP 64287, latero-oblique view, close-up of
1016 cephalothoracic area. B, ROMIP 64295, dorsal view, close-up of thoracic area. C, D,
1017 *Kerygmachela kirkegaardi*, from the lower Cambrian Sirius Passet, frontal area showing
1018 phosphatized muscular pharynx and distinct nervous tissues. C, MGUH 32048, high-angle
1019 polarized lighting. D, MGUH 32049, wavelength dispersive X-ray elemental map of
1020 carbon. E, F, *Yawunik kootenayi* ROMIP 63067. E, Close-up of cephalic area, inverted
1021 brightness. F, Inset in R. G, USNM 138231, close-up of frontal area, with putative brain
1022 remains. H, Tracing of main carbon linings in the frontal area of GRCP15009

1023 superimposed onto the main feature signal presented in the specimen in Lan et al. fig. 2A
1024 ^[3] I, Separate interpretative drawing, showing conjectural neural configuration. Line colour
1025 coding: In H, morphological feature lining in red, cuticular folds in red dashed line, cracks
1026 in sediment layer in yellow dashed line, oesophagal/post-oesophagal area in purple; in I,
1027 digestive tract in blue, gut epithelium in magenta, speculative neural tissues in green, with
1028 denser carbon masses as disks or ellipses, and tonguelet/lacunar system in orange, cuticular
1029 folds in grey. Arrowheads mark in: A, filaments projecting from an intestinal swelling; F,
1030 putative optical nerve tracts; G, H, separate rami of the appendages. Asterisks mark in: G,
1031 I, stomodaeum or protocerebral foramen. Abbreviations: DC, deutocerebrum; fo, frontal
1032 organ; gt, gut; le, lateral eye; me, median eye; nv, nerve; PC; ph, pharynx; oe, oesophagus;
1033 tg, sub-intestinal tonguelet; vs, visual surface. Scale bars: A, B, 1 mm; C, 5 mm; D-H, 2
1034 mm.

1035

1036 **Figure 6. Circum-digestive preservation and anatomical identity.** A, E, *Alalcomenaeus*
1037 *cambricus*. A, ROMIP 45613, Close-up of middle section of trunk, showing circum-
1038 intestinal black pattern with dorsal limb extensions. B-D, *Alalcomenaeus* sp. ROMIP
1039 62968. B, D, Close-ups of middle section of trunk with parallel (B) and perpendicular (D)
1040 polarization, showing carbonaceous preservation of proximal gill bundles (white reflective
1041 areas in D). The gut tract is preserved as apatite (black in D, tridimensional), and additional
1042 apatitic subintestinal elements may represent distal extensions of the hemocoelic cavities
1043 invading the exopods. C, Close-up of gill bundle in B. E, ROMIP 45616, photograph with
1044 inverted brightness, showing web pattern of denser kerogen or kerogen-coated features,
1045 including eyes, gut outline, possible gill bundles, tergite boundaries, and long, ramified

1046 filamentous elements invading the endopods. Arrows point to diffuse reflective areas inside
1047 endopods. Asterisk indicates endopod whose reflective lining clearly represents cuticular
1048 boundaries. F, *Molaria spinifera* USNM 57688, photographed with perpendicular
1049 polarization, showing patterns similar to E. Here, an outstanding carbonaceous trace also
1050 runs through the long telson, and ends relatively widely. G, H, *Mollisonia plenovenatrix*
1051 ROMIP 65015, wet specimen; see Aria and Caron^[1] for details of the counterpart. G, Cross-
1052 polarized light, showing broad peri-intestinal reflective area with bifurcating ventral
1053 tonguelets. H, Direct light. Arrowheads point to in: A, tonguelet extensions on the dorsal
1054 margin of exopods; B, D, apatitic fragments, likely from distal portions of tonguelets; C,
1055 finger-shaped gill bundle elements; E, F, various carbonaceous linings, G, H, tonguelet
1056 bifurcations corresponding to split appendicular lobes. Abbreviations: gt; gut tract; pic,
1057 peri-intestinal cavity; tg, tonguelet; xpl, primary lobe of exopod; xtl, tertiary lobe of
1058 exopod. Scale bars: A, G, H, 2.5 mm; B, D, 1 mm; C, 0.25 mm; E, F, 5 mm.

1059

1060 **Figure 7. Synthetic diagram of proposed methodological criteria for fossilized CNS**
1061 **disambiguation.** Abbreviations: IDed, identified.

1062

1063 **Figure 8. Revised summary of arguable paleoneurological evidence among early**
1064 **arthropods.** An expanded brain appears to emerge with Arthropoda. A complex visual
1065 system involving three optic neuropils could be present at least in the common ancestor of
1066 euarthropods. The identity of the main brain mass in *Kerygmachela* relative to more
1067 derived taxa is uncertain, as it may correspond to a “metaprotocerebrum”^[70,27,68].
1068 Phylogenetic relationships based on ref.^[55], nomenclature based on ref.^[71]. On brain

1069 configuration diagrams (top): protocerebral structures in light green, deutocerebral
1070 structures in dark green, stomodaeum in blue and magenta. On fossil diagrams (middle):
1071 Nervous tissues in green, digestive tract in blue-grey, appendicular outlines in grey, all
1072 other cuticular outlines in black; numbers represent optic neuropils. Curved arrows denote
1073 cases where frontal appendage innervation is likely affected by topology and runs beneath
1074 the rest of the brain, potentially creating compression artefacts. See Supplementary Figure
1075 2 for enlarged diagrams. Abbreviations: APc, anterior protocerebrum; da, deutocerebral
1076 appendage; Dc, deutocerebrum; n, optic neuropil; pc, main protocerebral commissure;
1077 MPc, median protocerebrum; so, sensory organ; st, stomodaeum; T.-G., total-group
1078 (evolutionary group including extinct and extant representatives of a lineage).

1079

1080 **Supplementary Discussion.** Complementary comments on BST mineralization and
1081 recommendations regarding interpretations of fossilized putative nervous systems.

1082

1083 **Supplementary Figure 1. Anatomical complexity of organ systems exemplified by**
1084 **extant crustaceans.** A, B. Histological sections through the anterior part of *Vargula*
1085 *hilgendorffii* (Crustacea, Ostracoda) showing the circulatory and nervous (peri-oesophageal
1086 ring) systems in A and part of the hemolymphatic network in B (see sections location in
1087 C). C. Lateral view of an adult female of *Vargula hilgendorffii*. D. Idealized section
1088 showing hemolymph circulation (blue arrows). E, F. Lateral view of *Nebalia bipes*
1089 (Crustacea, Leptostraca) and details of foliaceous thoracopods (carapace removed). G-I,
1090 *Dahlella caldarensis* (Crustacea, Leptostraca), thoracopods in transmitted light showing
1091 afferent (red arrows) and efferent (blue arrows) hemolymph canals and sinuses running

1092 through thoracopods. A and B are microtome paraffin sections stained with hematoxylin
1093 and eosin. F is an SEM image. Extant arthropods have an extensive network of vessels and
1094 internal hemocoelic cavities filled with hemolymph. Their “open” circulatory system
1095 consists of the vascular system, made of dorsal heart and arteries (e.g. aorta) that deliver
1096 hemolymph to various body regions, and of the relatively understudied lacunar system^[64].
1097 The cardiovascular unit pumps hemolymph into the lacunar system that bathes all tissues
1098 and organs and supplies them with oxygen and nutrients. Oxygen is transported by the
1099 metalloproteins hemocyanin or hemoglobin circulating in the hemolymph from gas
1100 exchange areas, such as gills in marine taxa, or from direct aerial openings in terrestrial
1101 tracheate arthropods, to the heart. The supramillimetric size of most Cambrian arthropods
1102 implies the presence of a comparable circulatory system without which the distribution of
1103 oxygen and nutrients to tissues would be impossible. Abbreviations are as follows: ac,
1104 afferent channel; ao, aorta; ap, appendage; ec, efferent channel; ft, foliaceous thoracopods;
1105 hc, hemocyte; he, heart; mu, muscle; my, myocardium; nt, neural tissue; oe, oesophagus;
1106 por, peri-oesophageal ring. Scales bars: A, B, H, 100 μm ; C, E, 1 mm; F, 500 μm ; G, 200
1107 μm ; H, 100 μm ; I, 50 μm .

1108

1109 **Supplementary Figure 2. Early arthropod diagrams with arguable neural tissue**
1110 **evidence.** A, *Kerygmachela*. B, Generalized anomalocaridid. C, Leanchoiliid. D,
1111 *Mollisonia*. E, Waptiid. F, Fuxianhuiid. Nervous tissues in green, digestive tract in blue-
1112 grey, appendicular outlines in grey, all other cuticular outlines in black; numbers represent
1113 optic neuropils. Numbers count optic neuropils. Abbreviations: pc, main protocerebral
1114 commissure; st, stomodaeum.

1115

1116 **Supplementary Figure 3. Anterior region of *Cardiodictyon catenulum* (from**
1117 **Strausfeld et al.^[58]).** A-C, YKLP11419. D-F, YKLP11418. G-I, YKLP11434. A, D, G,
1118 original plain light photographs. B, E, H, same as A, D, G with color desaturation, except
1119 for the magenta-purple range. C, F, I, isolated magenta-purple coloration from B, E, H on
1120 black background. Yellow dashed lines represent cracks or major relief over the fossil.
1121 Other arrows and annotations are from original publication. Note broad variations in
1122 staining patterns within and among specimens, and how patterns in the head are affected
1123 by cracks and relief. The “eye” is another crack through the superficial oxidized layer and
1124 not a clear biological feature. Scale bars in A and G: 0.5mm. Scale bar for YKLP11418
1125 missing in original publication.

1126