
Supplementary information

Exceptional fossil preservation and evolution of the ray-finned fish brain

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Part A. Phylogenetic placement of †*Coccocephalus wildi*

†*Coccocephalus wildi* has been included in a number of phylogenetic analyses investigating the relationships of early ray-finned fishes^{1–11}. It was originally placed in the actinopterygian crown¹, but all subsequent analyses resolve it on the actinopterygian stem, sometimes within a large polytomy including most other late Palaeozoic ray-finned fishes. A detailed redescription of †*Coccocephalus* based on new μ CT data, in conjunction with an updated phylogenetic analysis, is currently in preparation (S.G., R.F., M.F.), although we do not anticipate this leading to a change in the phylogenetic placement of the taxon.

The cranial morphology of †*Coccocephalus wildi* is comparable to other late Palaeozoic taxa that are well-within the ray-finned fish stem. The clearest comparisons are with the

Carboniferous †*Kentuckia*¹², †*Kansasiella*¹³, and †*Lawrenciella*^{13–15}, all of which preserve information on the endocranium. In common with these taxa, †*Coccocephalus* has a completely enclosed spiracular canal, a posteroventrally-directed hyoid facet, and open vestibular fontanelles continuous with the oticooccipital fissure. As in †*Kentuckia* and †*Lawrenciella*, the aortic canal of †*Coccocephalus* is pierced by a single midline opening, and as in †*Kentuckia* the divergence of the lateral dorsal aortae is not enclosed within the aortic canal and the parasphenoid is flat rather than dorsally inflected below the orbit. †*Coccocephalus* also shares other features in common with a subset of the above taxa, as well as with some Devonian and stratigraphically younger taxa.

†*Coccocephalus* is excluded from the crown by several successive nodes on the basis of the following ambiguous character optimisations (taken from ref. 5):

- Character 42: bone carrying otic portion of lateral line canal extends past posterior margin of parietals
- Character 89: two infradentaries
- Character 94: coronoid process formed by surangular
- Character 100: comineralised palatoquadrate ossifications
- Character 156: braincase comprises single ossification
- Character 171: parasphenoid terminates at/anterior to ventral otic fissure
- Character 178: aortic notch absent from parasphenoid
- Character 43: two pairs of extrascapulars
- Character 69: maxilla contributes to posterior margin of cheek
- Character 153: dorsal aorta not pierced by canal/s for exit of eff.a.1
- Character 175: parasphenoid teeth small

Part B. Potential paths for the fossilization of brain tissues

The delicate soft tissues that form the brain of vertebrates are traditionally considered to be prone to rapid decomposition. However, exceptional examples from the fossil record as well as archaeological evidence and forensic material show that these tissues can survive complete obliteration under certain conditions^{16–18}. The processes leading to fossil preservation comprise a complex system of interacting factors^{19–24} related to physical parameters (e.g., temperature, salinity, oxygen concentration, water composition), the depositional environment (e.g., rate of deposition, depth, granulometry, porosity) and taxon-specific aspects of biology (e.g., composition of tissues, ecology). Thus, dealing with this hyperdimensional data is a challenge for understanding modes of preservation of soft tissue in the fossil record and studies need to focus on specific environments or taxa for minimizing the number of variables involved in the process. In the following paragraphs we explore previously described modes of preservation of neural soft-tissue and compare these to the preservation of the brain of †*Coccocephalus wildi*.

1) *Adipocere formation*

One of the potential mechanisms through which brain material can survive is through the formation of a hard saponified fat coat called adipocere²⁵. Brain preservation through adipocere formation has been recorded for human remains to various degrees¹⁶. Mähler et al.²⁶ state that the preservation of soft tissues like muscles in invertebrates occurs as pseudomorphs—the replacement of original soft tissues with minerals—and that this process might be initiated by

adipocere and biofilm formation. However, examples of adipocere linked to soft-tissue preservation are not yet known described for the vertebrate fossil record, although the three-dimensional iguanodontid dinosaur brain reported by Brasier et al.¹⁷ might be partially explained by adipocere coating. The high concentration of lipidic tissues in the brain of many vertebrates^{27,28} may facilitate the preservation through this process. Unfortunately there is no evidence available that can securely associate the preservation of the brain described herein with adipocere coating.

2) *Burgess Shale-style preservation*

An alternative pathway for the preservation of brain tissues is that exhibited by the Cambrian faunas from the Burgess Shale. Several arthropods from these strata show preservation of flattened bilateral elements associated with the central nervous system^{29,30}. The validity of the interpretation of these elements as central nervous systems has been amply discussed in the literature with opposing hypotheses of these elements being formed by microbial mats^{18,31}. Following the interpretation that these do indeed represent neural elements, proposed preservation pathways tend to be associated with partial decomposition of fats further suppressed by anoxic conditions causing the preservation of carbonaceous or pyritized films²⁹. Although we cannot refute a similar mechanism for the preservation of the elements described herein, especially due to the similar composition of fossilized remains and matrix, it is difficult to explain how this mechanism would allow for a three-dimensional preservation as seen in †*Coccocephalus*.

3) *Calcium phosphate microenvironment*

Finally, the three-dimensional iniopterygian brain described by Pradel et al.^{32,33} is comparable to that of †*Coccocephalus* in the sense that it preserves the brain body and ventricles, some nerves exit the brain body and run toward foramina on the braincase, it is visible in tomographic data as denser material than surrounding matrix, and skeletal remains are still partially articulated indicating constraints on time before burial. This iniopterygian brain seems to have been preserved through partial decay of brain fatty acids in an anoxic condition that favored the precipitation of calcium phosphate, distinguishing the fossil brain from the surrounding calcium carbonate infill of the braincase. Pradel³³ also argues that—since there is no evidence of other soft tissues outside the braincase and that nerves do not continue outside the braincase—the enclosed space of the cranial cavity must have played a role in the preservation of the brain tissues by creating a microenvironment that favored the preservation of the brain. Although the mechanisms that took place for this exceptional iniopterygian brain to be preserved are not clear, it is possible that a similar process permitted the preservation of the brains described here. The tomographic data from Pradel et al.³² show the dense brain recrystallized tissue being surrounded by a thin halo of similar density, especially visible in less dense regions of the brain fossil. This halo is absent in our specimens, indicating that there may be differences in the mode of preservation of our specimens to that of Pradel et al.³².

4) *Bacteria-mediated phosphatization*

In placoderms from the Gogo Formation (~384-382 Ma³⁴) in Australia, there is ample evidence of muscle soft-tissue preservation, which is considered to have been preserved through bacteria-mediated phosphatization^{35–37}. Trinajstić et al.³⁷ report novel occurrences of placoderm

organs preserved in three dimensions that, according to the authors, seem to have been preserved in the same manner as muscle tissue mentioned above. Trinajstić et al. also argues that the presence of uncrushed skeletal elements together with three-dimensional soft-tissue preservation is an indicator of rapid formation and cementation of the nodules containing these fossils. The combination of a chemocline in the water column, photic zone euxinia and bacterial activity played a major role in the preservation of soft tissues in Gogo Formation fossils^{35,37}. However, in none of the placoderm specimens reported with soft-tissue preservation is there any indication of preservation of brain tissues (although skeletal muscle innervation is present in association to muscles in some fossils³⁵). This, together with the higher degree of disarticulation—in comparison to †*Coccocephalus*—of the skeletal elements of the fossils reported by Trinajstić et al.³⁷ indicate that although preservation of internal organs is possible in various environmental conditions, neural tissue requires more precise conditions to be preserved in fossil vertebrates.

Part C. Scanning parameters for newly generated μ CU datasets

Supplementary table 1. Scanning parameters for μ CT datasets generated for this study. MANCH, Manchester Museum, University of Manchester, Manchester, UK; UMMZ, University of Michigan Museum of Zoology, Ann Arbor, USA.

	Head Length (mm)	Energy (kV)	Current (μ A)	Exposure (s)	Filter	Filter (mm)	Voxel (mm)	Frames/Projection	Minimize Ring Artifacts
†<i>Coccocephalus wildi</i> (MANCH: W.12451)	~39	120	125	2.83	Cu	0.5	0.01535	8	YES
<i>Squalus acanthias</i> (UMMZ 253084)	108	160	120	1.42	-	-	0.06243	2	YES
<i>Polypterus senegalus</i> (UMMZ 195008)	28.5	100	172	0.25	-	-	0.01733	16	YES
<i>Acipenser brevirostrum</i> (UMMZ 219456)	50.5	85	200	0.25	-	-	0.03236	16	YES
<i>Amia calva</i> (UMMZ 235291)	58	85	200	0.25	-	-	0.03451	16	YES

Part D. Supplementary References

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