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Brain anatomy provides key evidence for ray-finned fish relationships[1], but two key limitations obscure our understanding of neuroanatomical evolution in this major vertebrate group. First, the deepest branching living lineages are separated from the group's common ancestor by hundreds of millions of years, with indications that aspects of

their brain morphology–like other aspects of their anatomy[2,3]–are specialised relative to primitive conditions. Second, there are no direct constraints on brain morphology in the earliest ray-finned fishes beyond the coarse picture provided by cranial endocasts: natural or virtual infillings of void spaces within the skull[4–8]. Here we report brain and cranial nerve soft-tissue preservation in †*Coccocephalichthys wildi,* a ~319-million-year-old (Myr) ray-finned fish. This oldest example of a well-preserved vertebrate brain provides a unique window into neural anatomy deep within ray-finned fish phylogeny. †*Coccocephalichthys* indicates a more complicated pattern of brain evolution than suggested by living species alone, highlighting cladistian apomorphies[1] and providing temporal constraints on the origin of traits uniting all extant ray-finned fishes[1,9]. Our findings, along with a growing set of studies in other animal groups[10–12], point to the significance of ancient soft tissue preservation in understanding the deep evolutionary assembly of major anatomical systems outside of the narrow subset of skeletal tissues[13–15].

Actinopterygian (ray-finned fish) brains display anatomical innovations not seen in other vertebrates, most notably a forebrain that grows through eversion of the dorsal walls of the telencephalon, rather than evagination of its lateral walls[16,17]. This results in a forebrain formed of two solid hemispheres without a ventricle[18]. Brain anatomy therefore provides important evidence for the monophyly and interrelationships of ray-finned fishes, a major radiation containing roughly half of all vertebrate species[19]. Brain anatomy in living non-teleost ray-finned fishes is limited to a handful of examples, reflecting the low diversity of the deepest extant branches of the ray-finned fish tree of life. Fossil endocasts are thought to provide some

constraints on brain structure deep in actinopterygian phylogeny, although the assumption that they reflect gross neuroanatomy[20] has never been explicitly tested (but see lobe-finned fish examples[21–23]). For over a century, rare natural endocasts[4,24] and a handful of serial sectioning models[5,25,26] provided insight into early ray-finned fish brain structure. Recent application of computed tomography yields more examples spanning the deepest branches of the actinopterygian tree[7] to the teleost and holostean stems[27,28] and groups in between[6,29]. These provide information on gross morphology and represent a source of characters for phylogenetic analysis[6,24]. However, there are significant disconnects between our understanding of neural anatomy in fossil species, based on the endocavity, and living forms, based on the brain itself. This reflects two practical limitations: low preservation potential of brain tissues in the fossil record combined with poor understanding of endocavities in living taxa. Consequently, key evolutionary steps preceding the origin of extant actinopterygian brains remain unknown.

Although rare, there is a growing record of fossil neural tissue. Palaeozoic arthropods provide the most examples[10–12], although a fossil brain is described in a Carboniferous chondrichthyan allied to ratfishes[13]. Here we report an exceptionally preserved brain and associated cranial nerves in the type and only specimen of the Pennsylvanian (Bashkirian; ~319 Myr) ray-finned fish †*Coccocephalichthys wildi*, representing the first known fossil example for actinopterygians. Analyses place this taxon outside the group containing all living ray-finned fish species[28]. Details of brain structure in †*Coccocephalichthys* therefore bear on interpretations of neural morphology during early evolutionary stages in a major vertebrate lineage. Using μCT of fossils in concert with diceCT imaging of extant species[30], we provide a revised picture of brain evolution in bony fishes.

Description

Endocast and otoliths. The endocast of †*Coccocephalichthys*, as in other Palaeozoic actinopterygians, is differentiated into areas appearing to correspond to regions of the brain (Fig. 1a). It agrees most closely with that described for *Lawrenciella*[26,31]. Endocasts of both show a midline olfactory tract, narrow olfactory bulbs, slender cerebellar auricles, and inclined horizontal semicircular canals. A single pair of otoliths, filling the saccular chamber, are preserved (Fig. 1b,d). These are large and teardrop shaped in lateral view, similar to those reported in some other Palaeozoic and early Mesozoic actinopterygians[32]. Their mesial and lateral surfaces are slightly convex and concave, respectively.

Overall preservation of the brain. The cranial cavity contains a symmetrical object that is more radiodense than the surrounding matrix (Fig. 2, Extended Data Figs. 1–8), extending from the level of the orbit to the oticooccipital fissure. It comprises three principal structures: a central, hollow, midline body; ramifications on either side of the central body that in some cases are associated with endoskeletal nerve foramina; and a diamond-shaped sheet that lies posterodorsal to the other elements. The central body includes three regions: a long, narrow anterior extension; a swollen middle region comprising a horizontal plate with two dorsal hemispheres and a ventral outgrowth; and a flattened posterior tube with a slit-like opening on the dorsal midline. Based on this appearance[13] and comparison with neural features in extant fishes (Fig. 2, Extended Data Fig. 1), we interpret the structure as a preserved brain. The three regions described above roughly correspond to the forebrain, midbrain and hindbrain.

Forebrain. The forebrain, comprising the olfactory bulbs, telencephalon and diencephalon, lies anterior to, and is considerably smaller than, the midbrain (Fig. 1). An elongate, slender extension anterior to the telencephalic body represents the olfactory nerve, but the olfactory bulbs are difficult to identify. The olfactory nerve extends to the midpoint of the orbit before dividing anteriorly. A dorsal sheet extends into the pineal chamber posterior to the divergence of the olfactory tract from the telencephalon. This structure may represent the remnants of the velum transversum (Fig. 1D). Thin filaments connect the anterior and posterior margins of this sheet to the endocranial walls, and paired anterior cerebral veins exit from its base. The body of the telencephalon is formed by two small, paired swellings divided by a median septum (Extended Data Fig. 2; Supplementary Video 1). The swellings are moderately expanded laterally, giving the telencephalon an ellipsoidal profile in axial section (Fig. 2; Extended Data Fig. 2). Each swelling is hollow and encloses a large ventricular space, indicating that the forebrain is evaginated as in sarcopterygians and chondrichthyans[16,33–35]. By contrast, all living actinopterygians possess an everted telencephalon[1,16,36] (Fig. 2B). We interpret an additional tissue layer dorsal to the telencephalon as the forebrain meningeal tissue.

No clear boundary divides the telencephalon and diencephalon. A moderate expansion posteroventral to the telencephalon corresponds with an ellipsoidal ventricle within the main body of the brain, indicating the presence of partially developed hypothalamic inferior lobes (Extended Data Figs 2,3). The lobes are visible in cross-section as small ellipsoid structures of a slightly denser material than the matrix, but less dense than the external brain wall. The right lobe is apparent externally on the right side of the brain as a low swelling. A slender and ventrally elongated hypophysis extends from behind the hypothalamus. It leads to a

differentiated distal portion contacting the buccohypophysial canal, and a posterior expansion associated with the saccus vasculosus. The ventricular space within each hypothalamic inferior lobe connects with that of the hypophysis (the diencephalic ventricle) via a narrow canal: the lateral hypothalamic recess[37]. The morphology of this structure in †*Coccocephalichthys* resembles that of *Amia* (Extended Data Fig. 4).

Midbrain. The mesencephalic lobes, the dorsal surfaces of which comprise the optic tectum, are well-developed and oval in dorsal view (Fig. 1). The lobes are connected posteriorly, level with the cerebellar region, diverging anteriorly. Two nerves emerge from the surface of the mesencephalon: a narrow, anterodorsally directed trochlear (IV) nerve; and a stout, anteroventrally directed oculomotor (III) nerve, which bifurcates within the braincase wall and enters the orbit through two foramina. A feature of unclear identity leaves from the anterior margin of the midbrain. The optic chiasma is preserved on the anteroventral surface of the mesencephalon, along with the proximal portions of the optic (II) nerves. These extend and diverge beyond the external margin of the midline optic foramen.

Sections through the midbrain reveal ventricles (Fig. 2, Extended Data Figs. 5–7). The second (mesencephalic) ventricle mirrors the shape of the optic tectum, and is V-shaped axially and U-shaped horizontally. Neither a torus longitudinalis nor torus semicircularis is apparent within the second ventricle. As these intraventricular projections represent denser regions of the brain in living taxa (see Nieuwenhuys et al.[1]), we would expect them to be preferentially preserved relative to other regions of the midbrain, and interpret their absence in †*Coccocephalichthys* as genuine. This is consistent with the distribution of this feature in extant actinopterygians, where

it is absent in cladistians and present in actinopterans. Anteriorly, the mesencephalic ventricles connect to a tube-like ventricle that opens at the roof of the diencephalon. Posteriorly, the mesencephalic ventricles contact the fourth ventricle through a narrow tube-shaped connection.

Hindbrain. Few features of the hindbrain are preserved. The anteriormost portion is developed as small rounded cerebellar auricular lobes, separated by the posterior limits of the mesencephalic lobes (Fig. 1). Posterior to these lies the recessus lateralis of the fourth ventricle, which is continuous with a thin, dorsally extensive rhombencephalic tela choroidea. The cerebellar corpus is barely developed (Extended Data Fig. 7). The fourth ventricle is open dorsally, is anteroposteriorly elongate and circular in axial section, and lies ventral to the mesencephalic ventricle (Fig. 2, Extended Data Figs. 5,6). A cerebral aqueduct connecting the second and fourth ventricles is not apparent. The internal walls of the fourth ventricle lack pronounced ridges, but it is unclear whether this is original or a taphonomic artefact. Two thin, posteroventrally directed branches of the abducens (VI) nerve leave the ventral surface of the brain level with posterior margin of the fourth ventricle. More ventrally, an additional branch extends from the saccular chamber toward the posterior myodome. Due to the position and path of this branch, we identify it as a distally diverging branch of the abducens nerve.

The trigeminofacial nucleus and associated nerves are separated from the body of the hindbrain, presumably a taphonomic artefact (Fig. 1). The trigeminofacial complex on the right of the specimen appears to be associated with the alar wall of the rhombencephalon, which has pulled away from the remainder of the hindbrain. Nerve branches located at the front of this complex are enclosed within skeletal canals and can be identified by comparison with endocasts described

for Palaeozoic actinopterygians[26], although we caution that this nomenclature needs review in comparison to nerve patterns in extant non-teleost actinopterygians. Two stout nerves emerge anterolaterally from the front of this complex, the most anterior of which enters the canal identified as that for the trigeminal (V) nerve, and the more posterior one the lateralis branch of the facial (VIIlat) nerve (Fig. 1, Extended Data Fig. 3). A third nerve, which leaves the complex anteroventrally, enters the canal for the main branch of the facial (VII) nerve. More posteriorly, a series of nerves are associated with the inner ear and otolith, and most likely correspond to branches of the octavolateralis (VIII) nerve (Fig. 1, Extended Data. Fig. 3). The anterior branch of the anterior ramus of the octavolateralis extends dorsally into the anterior ampulla, with the posterior branch of the anterior ramus entering the utriculus. A posteroventral branch contacts the anterior margin of the otolith. Two to three additional rami attach to the medial margin of the otolith, and further branches may be present posteriorly. See Supplementary Annotated Surface File for visualisation of labelled cranial nerves.

A diamond-shaped sheet lies posterodorsal to the brain, in close association with the roof of the endocavity (Fig. 1, Extended Data Fig. 1). This is in a similar position to the meninx primitiva, modified to a cisterna spinobulbularis in *Polypterus*[20,38], and a myelencephalic gland in other early ray-finned fishes[39]. The dorsal surface bears a medially located opening surrounded by a thin layer of tissue that extends as a tube toward the posterodorsal fontanelle of the neurocranium. The vagus (X) nerve lies ventral to this sheet, extending posterolaterally to exit from the braincase via the oticooccipital fissure. Anterior to the vagus nerve root, the glossopharyngeal nerve extends laterally towards the endocranial wall.

Discussion and Conclusions

Correspondence between brains and endocasts. It is widely assumed that there is fidelity between the shape of the brain and the endocast in early ray-finned fishes[4,6,24,20], with many brain regions corresponding with areas of the endocavity hypothesised to accommodate them. For example, the olfactory (I) nerve is housed in the olfactory tract, the tela choroidea extends towards the pineal opening, and the mesencephalon is confined to the region of the optic lobes (Fig. 1, Extended Data Figs. 5,8). This indicates that endocasts can provide some accurate positional information. However, the shape of the brain in †*Coccocephalichthys* does not closely conform to the inner surface of the endocavity (Fig. 1, Extended Data Figs. 5,8). The discrepancy in volume between the endocast and brain raises the possibility that the brain may have contracted during preservation. However, the fact that many cranial nerves connect with the brain itself and extend to their neurocranial foramina suggests a limit to the degree of shrinkage. Taphonomic experiments investigating soft-tissue preservation in bony fishes are in their infancy, and future work will be critical for contextualising these interpretations. Living ray-finned fishes show varying degrees of correspondence between brain and endocast morphology (Fig. 3)[40,41], and perhaps rarely fill the endocavity in a way comparable to lungfishes and some tetrapods[21,42,43]. This does not invalidate endocasts as sources of characters or information about neuroanatomy[6,24], but stresses that the features of brains and endocavities in ray-finned fishes are not interchangeable.

Patterns of brain evolution in bony fishes. The principal actinopterygian lineages show substantial differences in brain and endocavity structure (Fig. 3, Extended Data Fig. 1). Living

members of early-diverging groups like cladistians and chondrosteans provide important clues about primitive brain anatomy but both show morphological specialisations reflecting long independent evolutionary histories. As a stem actinopterygian separated from the common ancestor of all living species by tens—rather than hundreds—of millions of years[3,28,44], †*Coccocephalichthys* provides unique information bearing on primitive ray-fin brain anatomy and sequences of change within the group. Due to the challenges of interpreting soft-tissue preservation we focus on features that are present and most likely reflect true morphological variability, rather than focussing on features that appear to be absent, but which may in fact have been lost during decay and preservation.

Notably, the brain of †*Coccocephalichthys* clarifies neurological synapomorphies of the ray-finned fish total group (i.e. the living radiation and all closely related fossil taxa) and crown group (i.e. the living radiation only), summarised in Fig. 3. All living ray-finned fishes display an everted telencephalon, representing the principal neuroanatomical synapomorphy of actinopterygians. However, the telencephalon of †*Coccocephalichthys* shows the contrasting evaginated condition, as seen in non-actinopterygian fishes. This indicates an everted forebrain originated in more crownward portions of the actinopterygian stem. Presence of an evaginated telencephalon in a Carboniferous actinopterygian also challenges the hypothesis that forebrain eversion arose due to developmental constraints associated with small body size in Devonian members of the group[45,46], as the forebrain region of the endocast of †*Coccocephalichthys* is small.

Partially developed inferior lobes of the hypothalamus in †*Coccocephalichthys* challenge the current assumption that the absence of this diencephalic outgrowth in *Polypterus* (Figs 2-3) represents a primitive condition for crown actinopterygians[1,47]. Presence of this feature in a stem

actinopterygian suggests an alternative scenario where it arose deep on the ray-fin stem, was lost in cladistians and retained by actinopterans, before developing fully in neopterygians[47]. †*Coccocephalichthys* also provides evidence that the myelencephalic gland of holosteans and chondrosteans traces its origins to a feature present in stem actinopterygians. The myelencephalic gland, a hematopoietic (blood-generating) structure enclosed within the endocranial cavity of non-teleost actinopterans, either overlies (lepisosteids) or embraces (*Amia*, chondrosteans) the myelencephalon[39,48]. In *Polypterus*, meningeal tissue occupying the same region as the myelencephalic gland of other taxa is differentiated and highly vascularised, and is called the cisterna spinobulbaris[20,38]. †*Coccocephalichthys* bears a similar membranous structure overlying the rhombencephalon at the level of the vagal nerves, considered to be homologous to the cisterna spinobulbaris of *Polypterus*. On this basis, we argue that modified rhombencephalic meningeal tissues are a general feature of ray-finned fishes, with subsequent modifications in holosteans and chondrosteans as a well-developed myelencephalic gland.

The brain of †*Coccocephalichthys* clarifies polarities of neuroanatomical features of deeply-branching crown lineages, with implications for brain evolution in more nested clades (Fig. 3). These data provide corroboration that features of *Polypterus* such as absence of intraventricular projections and the presence of a poorly differentiated corpus cerebelli represent primitive actinopterygian conditions. However, †*Coccocephalichthys* suggests that a conspicuous external aspect of neuroanatomy in *Polypterus* might be apomorphic. Like lungfishes and tetrapods, *Polypterus* has a telencephalon that is much larger than the midbrain[1], in contrast to the small structure in actinopterans[1] and *Latimeria*[49]. This distribution among extant bony fishes has been used to argue that a large telencephalon may be a generalised

osteichthyan feature[20] lost in actinopterans. However, the small telencephalon of †*Coccocephalichthys* (Fig. 1D) instead suggests the convergent origin of enlarged structures in *Polypterus* and a subset of sarcopterygians. This inference is supported by outgroup comparison to chondrichthyans, many of which have a small telencephalon[50]. At the same time, †*Coccocephalichthys* indicate that an apparent specialisation of *Polypterus* might in fact be a more general feature of actinopterygians. *Polypterus* is unique among extant jawed vertebrates in having an invaginated corpus cerebelli, a condition generally interpreted as a specialisation of that lineage[1,9]. However, the corpus cerebelli of †*Coccocephalichthys* also seems to be formed as an invagination (Supplemental Fig. 7) of the dorsal surface of the rhombencephalic region, matching the arrangement of *Polypterus*. Independent gains within both lineages, or a single gain at the base of actinopterygians followed by a loss in actinopterans, represent equally parsimonious scenarios. It is not possible to select between these alternatives in the absence of additional information on brain structure in other early actinopterygians.

The utility of fossil brains. †*Coccocephalichthys* reinforces studies of neural structures in fossil arthropods[10–12] that highlight the importance of fossil brains for patterns of neuroanatomical change in groups with deep evolutionary divergences. Beyond representing preservational curiosities, fossilised brains provide otherwise inaccessible trait data with implications for patterns of phylogenetic relationships and character polarity. We anticipate that preservation of neural tissue in fossil fishes is likely to be more common than widely thought[24], with assumptions of non-preservation leading to potentially valuable information on the evolution of brain structural diversity being overlooked. A careful survey of fish material from

taphonomically promising horizons has potential to yield novel anatomical information bearing on the evolution of brain structural diversity within the principal clade of aquatic vertebrates.

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Figure Legends

Fig. 1: Neurocranium, endocast, otoliths and preserved brain of †*Coccocephalichthys wildi* (MANCH: W.12451). a, Neurocranium in left lateral view. **b**, cutaways of neurocranium in dorsal (top) and left lateral (bottom) views showing brain and otoliths *in situ*. **c**, endocast in left lateral view. **d**, the brain and associated preserved soft tissues in dorsal (top) and lateral (bottom) views, with left otolith removed in the latter for clarity. cce, corpus cerebelli; hyp, hypophysis; mes, mesencephalon; ms, myelencephalic sheet; tel, telencephalon; I, olfactory nerve; II, optic nerve; III, oculomotor nerve; V, trigeminal nerve; VI, abducens nerve; IX, glossopharyngeal nerve; X, vagus nerve. Scale bars = 5 mm. Arrow indicates anterior for all panels.

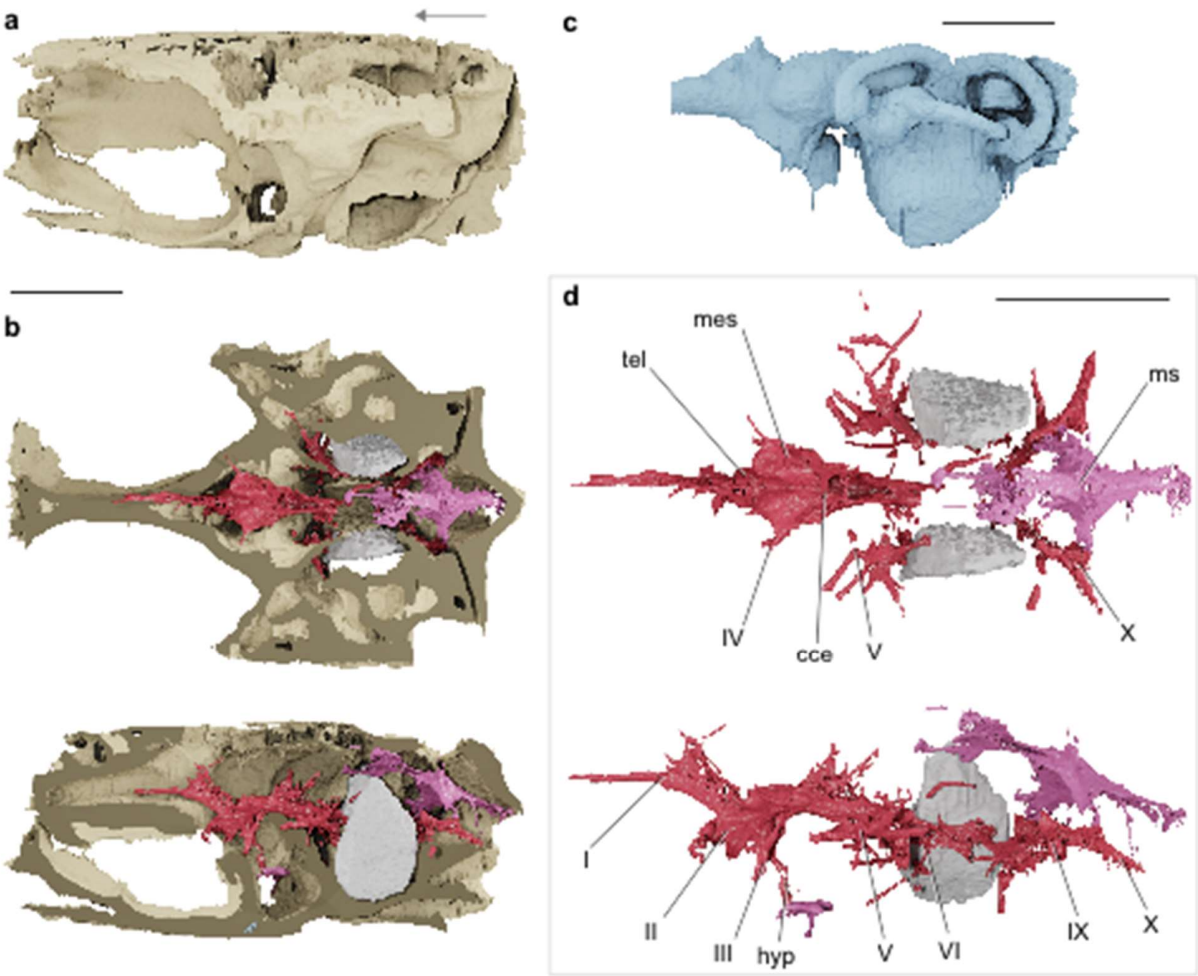


Fig. 2: Anatomical correspondence between preserved brain of †*Coccocephalichthys wildi* and those of extant fishes. a, three-dimensional rendering of the brain of †*Coccocephalichthys* in left lateral view. Scale bar = 10 mm. **b,** transverse sections through the brains of †*Coccocephalichthys* and selected jawed fishes from diceCT data. cce, corpus cerebelli; mes, mesencephalon; mye, myelencephalon; tel, telencephalon; tri (V), trigeminal nerve; ?, unidentified midbrain feature. Data for extant taxa are original scans from specimens in University of Michigan Museum of Zoology (see Methods and Supplementary Table 1) with the exception of *Lepidosiren* (UF:FISH:129826; Morphosource ark:/87602/m4/M167969). Silhouettes of extant taxa modified from phylopic2 (*Squalus*: Ignacio Contreras; *Lepidosiren*:

Roberto Diaz Sibaja; *Acipenser*, *Amia*, *Polypterus*: no copyright; *Squalus*, *Lepidosiren* are available under Creative Commons 3.0 license https://creativecommons.org/licenses/by/3.0/). Hypothetical †*Coccocephalichthys* silhouette original based on illustration in the public domain[51]. Scale bar = 1 mm.

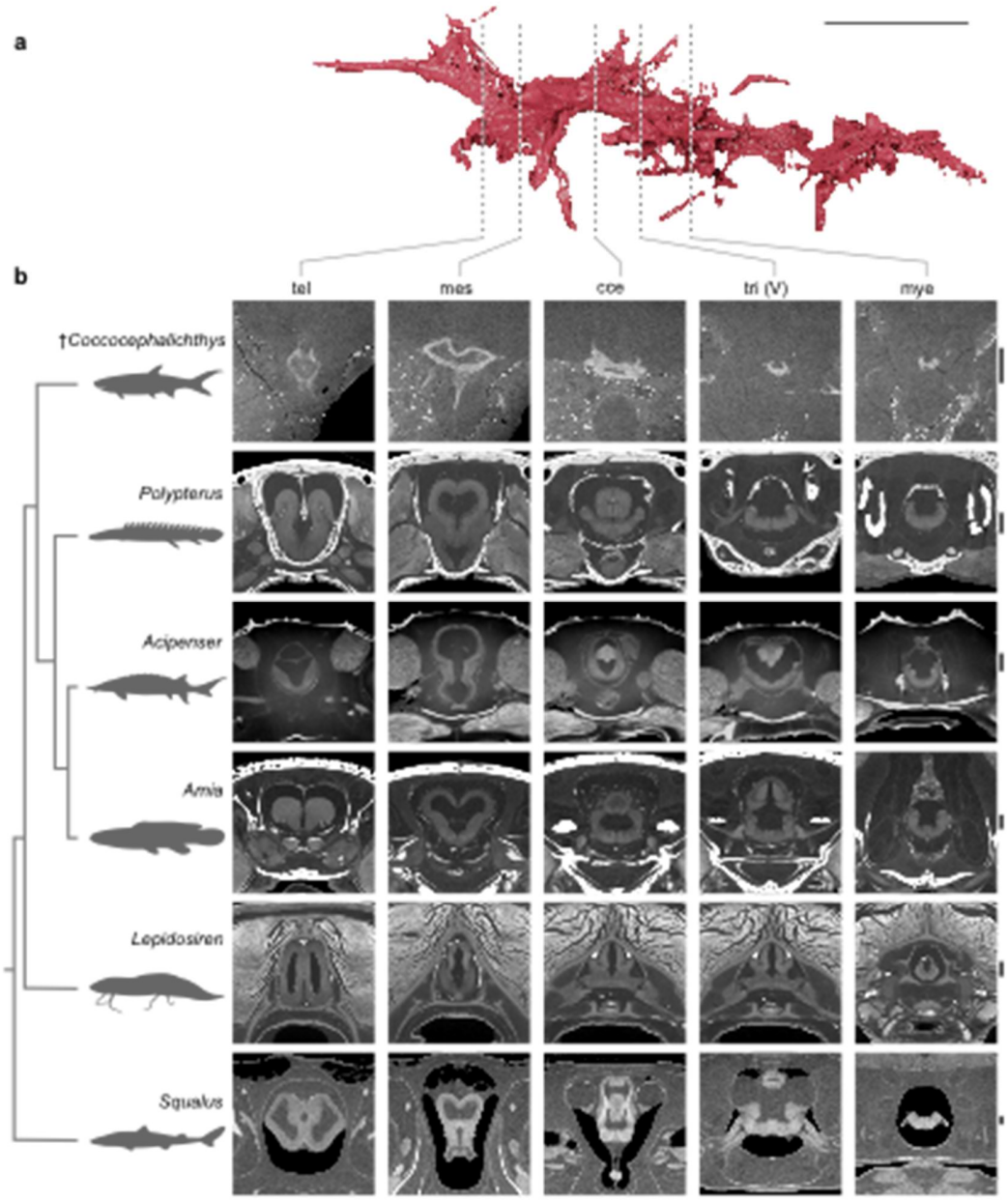
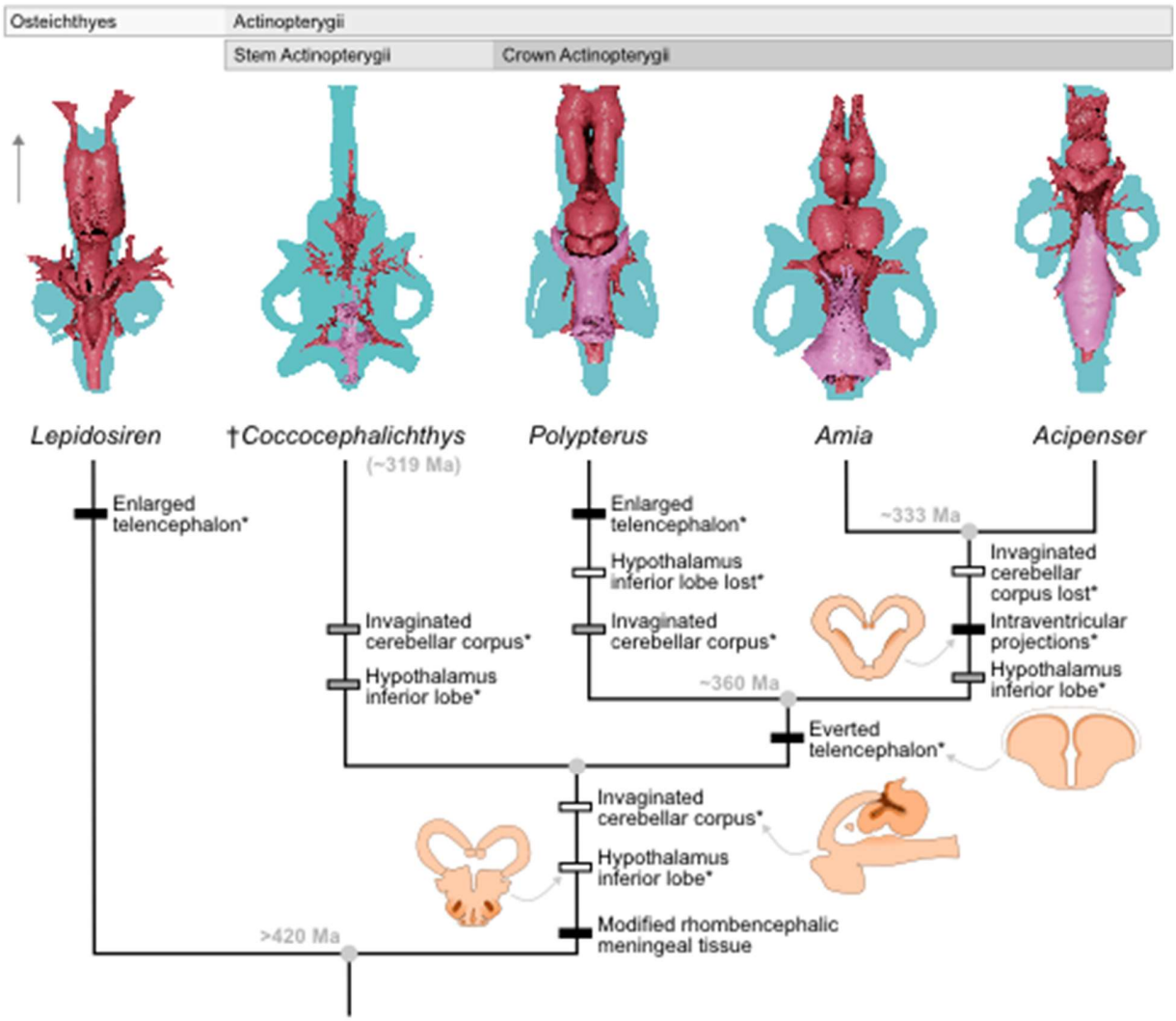


Fig. 3: Major anatomical transformations in actinopterygian brain structure illuminated by †*Coccocephalichthys.* Branch labels represent character modifications. Asterisk (*) indicates shift in position of character in cladogram due to anatomical information from †*Coccocephalichthys*. Black bars: unambiguous changes; grey bars: ACCTRAN optimisations; white bars: DELTRAN optimisations. Blue: endocast; red: brain and cranial nerves; pink: myelencephalic sheet. Arrow indicates anterior direction for 3D renders. Insets show transverse or sagittal sections through the relevant portions of the brain, with darker orange shading indicating specific regions of interest. Images not to scale. Node ages from Giles et al.[3].



Methods

Material examined

†*Coccocephalichthys wildi* is known from a single specimen (Manchester Museum, Wild Collection, MANCH: W.12451) from the roof of the Mountain Fourfoot Mine, Carre Heys, Trawden, Lancashire, UK. Accounts of its anatomy are given by Watson[52], Poplin[25], and Poplin & Veran[53]. Other three-dimensionally preserved actinopterygians hosted in nodules from this area include †*Trawdenia planti*[24,54] and †*Mesonichthys aitkeni*; these are all thought to derive from the so-called "Soapstone Bed." This horizon lies within the Pennine Lower Coal Measures above the Bullion Coal (= Upper Foot Coal) and the Mountain 1.2 m Coal (= Lower Mountain Coal), but below the Ardley Seam (=Arley Coal)[24,54,55]. This is within the Langsettian regional substage, which correlates with the upper part of the Bashkirian stage of the international timescale[56].

Preservation of brain tissues. The brain of †*Coccocephalichthys* appears to be preserved in a manner comparable to the younger (ca. 300 Ma) chondrichthyan brain reported by Pradel[13,31]. In both examples, the brain is preserved in three dimensions within an enclosed skeletal space (the braincase), potentially allowing development of a micro-environment favourable for the preservation of certain soft-tissue structures. The failure of cranial nerves to extend beyond the outer wall of the braincase in both examples provides support for this localised model of preservation. Exposed regions of soft tissue in the iniopterygian example show that its brain was preserved as calcium phosphate[13,31]. However, the unique specimen of †*Coccocephalichthys* lacks any clear external exposures of the brain or associated nerves, and the composition of the radiodense material capturing their structure remains unclear. The preservation of brains in these

Carboniferous fishes shows coarse similarities to concretion-hosted Devonian placoderms[15] and Jurassic invertebrates[57,58] that likewise preserve three-dimensional organs within enclosed bony, shelly, or chitinous carapaces. Collectively, these examples differ from the flattened preservation of neural and other soft tissues in arthropods from Cambrian shales[10,12]. For a more detailed discussion of modes of preservation for brain tissues see the Supplemental Material.

Pathways to preservation of brain tissues are poorly understood, especially due to the lack of experimental focus on neuroanatomical decay[59]. Available evidence indicates a relatively rapid deterioration of brain tissues, based on controlled decay of cephalochordates and agnathans[59–61]. However, data are lacking for taxa in which the brain is enclosed and protected by a robust endochondral ossification, as is the case in †*Coccocephalichthys*. As such, the timing and patterns of brain decomposition in bony fishes requires further investigation.

Diffusible Iodine-based contrast enhancement (diceCT)

Comparative specimens of *Squalus acanthias* (University of Michigan Museum of Zoology [UMMZ] 253084), *Polypterus senegalus* (UMMZ 195008), *Amia calva* (UMMZ 235291) and *Acipenser fulvicens* (UMMZ 219456) were prepared for diceCT by submerging specimens in 1.25% Lugol's solution (25g I_2 + 50g KI for every 2L of water) for roughly 14 days prior to scanning. DiceCT data for a specimen of *Lepidosiren paradoxa* (UF:FISH:129826) from the Florida Museum of Natural History Ichthyology Collection was obtained from Morphosource (ark:/87602/m4/M167969).

X-ray computed tomography

†*Coccocephalichthys wildi* and extant comparative material were scanned at the CTEES facility of the Department of Earth and Environmental Sciences, University of Michigan, using a Nikon XT H 225ST µCT scanner. The scan for †*Coccocephalichthys wildi* was set with 120 kV energy, 125 µA current and using a 0.5 mm copper filter. Eight frames were acquired for each projection, with an exposure time of 2.83 seconds, and the option for minimising ring artefacts was selected. Effective pixel size was 15.35 µm and geometric magnification of 13.031. Parameters for new scans of extant comparative material (*Squalus acanthias*, *Polypterus senegalus*, *Acipenser brevirostrum*, and *Amia calva*) are given in SI Table 1. Processing of data was conducted in Mimics v.21. A threshold encompassing the preserved neural soft tissue was determined and the resultant mask cleared, with the threshold values retained. The soft tissue was manually segmented using the circle and livewire tools. Segmentation was performed by three authors (RTF, MF and SG) and results compared for consistency. The hard tissues were segmented using the same method. Rendering was performed in Blender 2.91 (blender.org) using cycles rendering with addition of custom shading and reflection attributes to the material properties (principled BSDF material), coupled with ambient occlusion for better lighting of minute structures and realistic shading of internal cavities. An annotated interactive surface file of the brain of †*Coccocephalichthys* is also available through Zenodo (10.5281/zenodo.6560305) as an html file.

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Author contributions The project was conceived by M.F. and S.G. CT scanning was carried out by M.F. and R.F., with staining of extant material by R.F. and M.K. Segmentation of CT data was performed by M.F., S.G., D.G., and R.F. M.F, S.G., and R.F wrote the manuscript, with comments from all authors.

Competing interests The authors declare no competing interests.

Additional information

Supplementary information is available for this paper.

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Data availability The fossil described in this study is deposited in the collections of the Manchester Museum and the extant specimens in the University of Michigan Museum of Zoology. The reconstructed .TIFF stack, segmented Mimics file and .PLY files for †*Coccocephalichthys wildi* are available on Zenodo (10.5281/zenodo.6560305).

Extended Data figure legends

Extended Data Fig. 1. The brain (red) and myelencephalic sheet/gland (pink) of †*Coccocephalichthys wildi* and selected extant ray-finned fishes. a, †*Coccocephalichthys wildi*. **b,** *Acipenser brevirostrum*. **c,** *Amia calva*. **d,** *Polypterus senegalus*. Gray and white delimitations show margins between forebrain, midbrain and hindbrain across all taxa. Brains are aligned at the anterior– and posteriormost points of the forebrain (olfactory bulbs, telencephalon and diencephalon) and the posteriormost point of the fourth ventricle. Scale bar = 10 mm.

Extended Data Fig. 2. Sections through the brain of †*Coccocephalichthys wildi*. **a**, transverse section through the anterior portion of the telencephalon. **b**, axial section through the ventral portion of the telencephalon. **c**, transverse section through the posterior portion of the telencephalon. **d**, axial section through the dorsal portion of the telencephalon. **e**, transverse section through the anterior portion of the hypothalamus inferior lobes. **f**, transverse section through the posterior portion of the hypothalamus inferior lobes. Inset shows where each of sections (a)-(e) intersect the brain. h.inf, inferior lobe of the hypothalamus; l.hyp.re, lateral hypothalamic recess; tel, telencephalon; tel.sept, telencephalic septum. Scale bar = 2 mm.

Extended Data Fig. 3. Transverse sections and renders of the brain of †*Coccocephalichthys wildi*. **a**, **b**, the telencephalon. **c**, **d**, the mesencephalon and hypophysis. cce, corpus cerebellum; h.inf, inferior lobe of the hypothalamus; hyp, hypophysis; tel, telencephalon; mes, mesencephalon; ms, mesencephalic sheet; v. tr, velum transversum; 4th v, fourth ventricle; II, optic nerve; III, oculomotor nerve; IV, trochlear nerve, V, trigeminal nerve; VII, facial nerve.

Dorsal portion of forebrain and velum transversum digitally removed. Scale bar in a, c = 2.5 mm; scale bar in b, d = 5 mm.

Extended Data Fig. 4. Sections through the brain of †*Coccocephalichthys wildi* and *Amia calva*. **a**, transverse section through the diencephalon and mesencephalon of *Coccocephalichthys wildi*. **b**, transverse section through the diencephalon and mesencephalon of *Amia calva*. l.hyp.re, lateral hypothalamic recess. Scale bar = 2 mm.

Extended Data Fig. 5. Sagittal sections through the neurocranium of †*Coccocephalichthys wildi* showing the brain and associated structures. cce, corpus cerebelli, cr.c; crista cerebellaris, h.inf, hypothalamus inferior lobes; hyp, hypophysis; mes, mesencephalon; ms, myelencephalic sheet; rho, rhombencephalon; sc, spinal cord; tel, telencephalon; v.tr, velum transversum; 2nd v, second ventricle; 4th v, fourth ventricle; I, olfactory nerve; II, optic nerve. Scale bar = 10 mm.

Extended Data Fig. 6. The brain of †*Coccocephalichthys wildi* (red) rendered partially transparent to show brain ventricle configuration (white). a, dorsal view. **b,** left lateral view. die. v, diencephalic ventricle; 2nd v, second ventricle; 4th v, fourth ventricle. Scale bar = 5 mm.

Extended Data Fig. 7. Sections through the brain of †*Coccocephalichthys wildi* showing the rhombencephalic region. a, sagittal section through the brain. **b**, transversal section through the anterior portion of the rhombencephalon. **c**, axial section through the mesencephalic and rhombencephalic regions of the brain. cce, corpus cerebelli, crc, crista cerebellaris, inv, invagination of the cerebellum, 4th v, fourth ventricle. Scale bar = 1 mm.

Extended Data Fig. 8. The brain of †*Coccocephalichthys wildi* within the braincase. a, dorsal view, **b**, left lateral view. d.lat, dorsal lateral line nerve, hyo.VII, hyomandibular branch of the facial nerve, hyp, hypophysis, ms, mesencephalic sheet, I, olfactory nerve, II, optic nerve, IV, trochlear nerve, V, trigeminal nerve, VI, abducens nerve, VII, facial nerve, IX, glossopharyngeal nerve, X, vagus nerve. Scale bar = 1 mm.