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

2   Rodrigo T. Figueroa[1,2]; Danielle Goodvin[2,3]; Matthew A. Kolmann[2]; Michael I. Coates[4]; Abigail M.

3   Caron[4]; Matt Friedman*[1,2,5]; Sam Giles*[5,6]

4   [1]Department of Earth and Environmental Sciences, University of Michigan, 1100 N University Ave, Ann

5   Arbor, MI 48109, USA

6   [2]Museum of Paleontology, University of Michigan, 1105 N University Ave, Ann Arbor, MI 48109, USA

7   [3]Department of Forestry, Wildlife, and Fisheries, University of Tennessee, 2505 E J. Chapman Dr,

8   Knoxville, TN 37996, USA

9   [4]Department of Organismal Biology and Anatomy and Committee on Evolutionary Biology, University

10   of Chicago, 1027 E 57th St, Chicago, IL 60637, USA

11   [5]Department of Earth Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK

12   [6]School of Geography Earth and Environmental Sciences, University of Birmingham, Birmingham B15

13   2TT, UK

14   *corresponding authors

15

16   **SUMMARY**

17   **Brain anatomy provides key evidence for ray-finned fish relationships[1], but two key**

18   **limitations obscure our understanding of neuroanatomical evolution in this major**

19   **vertebrate group. First, the deepest branching living lineages are separated from the**

20   **group's common ancestor by hundreds of millions of years, with indications that aspects of**

21 **their brain morphology–like other aspects of their anatomy[2,3]–are specialised relative to**

22 **primitive conditions. Second, there are no direct constraints on brain morphology in the**

23 **earliest ray-finned fishes beyond the coarse picture provided by cranial endocasts: natural**

24 **or virtual infillings of void spaces within the skull[4–8]. Here we report brain and cranial**

25 **nerve soft-tissue preservation in †*Coccocephalichthys wildi,* a ~319-million-year-old (Myr)**

26 **ray-finned fish. This oldest example of a well-preserved vertebrate brain provides a unique**

27 **window into neural anatomy deep within ray-finned fish phylogeny. †*Coccocephalichthys***

28 **indicates a more complicated pattern of brain evolution than suggested by living species**

29 **alone, highlighting cladistian apomorphies[9] and providing temporal constraints on the**

30 **origin of traits uniting all extant ray-finned fishes[9–11]. Our findings, along with a growing**

31 **set of studies in other animal groups[12–16], point to the significance of ancient soft tissue**

32 **preservation in understanding the deep evolutionary assembly of major anatomical systems**

33 **outside of the narrow subset of skeletal tissues[17–20].**

34

35 MAIN

36 Actinopterygian (ray-finned fish) brains display anatomical innovations not seen in other

37 vertebrates, most notably a forebrain that grows in a unique way: through eversion of the dorsal

38 walls of the telencephalon, rather than evagination of its lateral walls[21,22]. This results in a

39 forebrain formed of two solid hemispheres that do not enclose a ventricle[23]. Brain anatomy

40 therefore provides important evidence for the monophyly and interrelationships of ray-finned

41 fishes, a major radiation containing roughly half of all vertebrate species[24]. Brain anatomy in

42 non-teleost fishes is limited to a handful of examples, reflecting the low diversity of the deepest

43 extant branches of the ray-finned fish tree of life. Fossils provide limited constraints on brain

44   structure deep in actinopterygian phylogeny. In contrast to teleosts, where the inner walls of the

45   neurocranium are generally widely separated from most parts of the brain, the contours of the

46   cranial endocavity (endocast) in non-teleosts appear to capture some aspects of neural anatomy[25].

47   For over a century, rare natural endocasts[4,26] and a handful of models from physical

48   tomography[5,27,28] provided the only constraints on brain structure in early ray-finned fishes. The

49   recent widespread application of computed tomography yields a greater number of examples

50   spanning the very deepest branches of the actinopterygian tree[7] to the teleost and holostean

51   stems[25,29], and several groups in between[6,30,31]. These provide information on gross morphological

52   patterns of actinopterygian brain evolution and represent an important source of characters for

53   phylogenetic analysis[6,26]. However, there are significant disconnects between our understanding

54   of neural anatomy in fossil species, where information derives exclusively from the endocavity,

55   and living forms, where only brain anatomy is well documented. This stems from two practical

56   limitations: the low preservation potential of brain tissues in the fossil record combined with a

57   poor understanding of endocavity anatomy in living taxa. Consequently, key evolutionary steps

58   that preceded the origin of living actinopterygian brains remain unknown.

59

60   Although rare, there is a growing record of preserved neural tissue in fossils. Palaeozoic

61   arthropods provide the most examples[12–16], although there is a solitary report of a preserved brain

62   in a Carboniferous cartilaginous fish allied to extant ratfishes[19]. Here we report an exceptionally

63   preserved brain and associated cranial nerves in the Pennylvanian (Bashkirian; ~319 Myr) ray-

64   finned fish †*Coccocephalichthys wildi*, representing the first known fossil example for

65   actinopterygians. Current analyses place this taxon outside the group containing all living

66   species[29]. Details of brain structure in †*Coccocephalichthys* therefore bear on interpretations of

67   neural morphology during the early stages of evolution in a principal lineage of backboned

68   animals. Using µCT of fossil material in concert with diceCT imaging of extant species[32], we

69   provide a revised picture of brain evolution in bony fishes.

70

71   **Description**

72

73   **Endocast and otoliths.** The endocast of †*Coccocephalichthys*, like that of other Palaeozoic

74   actinopterygians, is clearly differentiated into areas that appear to correspond to regions of the

75   brain (Fig. 1a). It agrees most closely with that described for *Lawrenciella*[28,33]. Only a single pair

76   of otoliths, filling the saccular chamber, are preserved (Fig. 1b,d). These are large and teardrop

77   shaped in lateral view, similar to those reported in some other Palaeozoic and early Mesozoic

78   actinopterygians[34]. Their mesial and lateral surfaces are slightly convex and concave,

79   respectively.

80

81   **Overall preservation of the brain.** Within the cranial cavity lies a symmetrical object that is

82   denser than the surrounding matrix (Fig. 2, Extended Data Figs. 1–5). It extends from the level of

83   the orbit to the oticooccipital fissure. It comprises three principal structures: a central, hollow

84   body that lies on the midline; ramifications on either side of the central body that are in some

85   cases are clearly associated with endoskeletal nerve foramina; and a diamond-shaped sheet that

86   lies posterodorsal to the other elements. The central body includes three regions: a long, narrow

87   anterior extension; a swollen middle region comprising a horizontal plate with two dorsal

88   hemispheres and a ventral outgrowth; and flattened posterior tube with a slit-like opening on the

89   dorsal midline. Based on preservational style[19] and comparison with neural features in extant

90   jawed vertebrates (Fig. 2, Extended Data Fig. 3), we interpret this structure as a preserved brain.

91   The three regions described above roughly correspond to the forebrain, midbrain and hindbrain,

92   respectively, and collectively occupy around 5% of the endocranial volume.

93

94   **Forebrain.** The forebrain, comprising the olfactory bulbs, telencephalon and diencephalon, lies

95   anterior to, and is considerably smaller than, the midbrain (Fig. 1). An elongate, slender

96   extension anterior to the telencephalic body represents the olfactory nerve, but the olfactory

97   bulbs are difficult to identify. The olfactory nerve extends to the midpoint of the orbit as a single

98   tract before dividing anteriorly. A dorsal sheet extends into the pineal chamber posterior to the

99   divergence of the olfactory tract from the telencephalon. This structure may represent the

100   remnants of the velum transversum. Thin filaments connect the anterior and posterior margins of

101   this sheet to the inner walls of the endocranial chamber, and paired anterior cerebral veins exit

102   from its base. The body of the telencephalon is formed by two small, paired swellings separated

103   by a median septum that is visible most clearly anteriorly and posteriorly (Extended Data Fig. 5).

104   The swellings are moderately expanded laterally, giving the telencephalon an ellipsoidal profile

105   in axial section (Fig. 2; Extended Data Fig. 5). Each swelling is hollow and encloses a large

106   ventricular space, indicating that the forebrain is evaginated as in sarcopterygians and

107   chondrichthyans[35–37,21]. By contrast, all living ray-finned fishes possess an everted

108   telencephalon[9,21,38–40] (Fig. 2B). We interpret an additional tissue layer dorsal to the

109   telencephalon as part of the meningeal tissue of the forebrain.

110   There is no clear boundary dividing the telencephalon and diencephalon. A moderate

111   expansion posteroventral to the telencephalon corresponds with an ellipsoidal ventricle within

112   the main body of the brain, indicating the presence of partially developed hypothalamic inferior

113   lobes (Extended Data Fig. 4,5). The lobes are visible in cross-section as small ellipsoid structures

114   of a slightly denser material than the matrix, but less dense than the external brain wall. The right

115   lobe is apparent externally on the right side of the brain as a low swelling. A slender and

116   ventrally elongated hypophysis extends from behind the hypothalamus. It leads to a

117   differentiated distal portion in contact with the buccohypophysial canal, and a posterior

118   expansion associated with the saccus vasculosus. The ventricular space within each

119   hypothalamic inferior lobe is connected with that of the hypophysis (the diencephalic ventricle)

120   via a narrow canal, named the lateral hypothalamic recess[41]. The morphology of this structure in

121   †*Coccocephalichthys* is similar to that of *Amia* (Extended Data Fig. 6).

122

123   **Midbrain.** The mesencephalic lobes, the dorsal surfaces of which comprise the optic tectum, are

124   well-developed and oval in dorsal view (Fig. 1). The lobes are connected posteriorly, level with

125   the cerebellar region, and diverge anteriorly. Two nerves emerge from the surface of the

126   mesencephalon: a narrow, anterodorsally directed trochlear (IV) nerve; and a stout,

127   anteroventrally directed oculomotor (III) nerve, which dichotomises within the braincase wall

128   and enters the orbital cavity through two foramina. A third feature, which leaves the anterior

129   margin of the midbrain, is of unclear identity. The optic chiasma is preserved on the

130   anteroventral surface of the mesencephalon, along with the proximal portions of the optic (II)

131   nerves. These extend and diverge beyond the external margin of the midline optic foramen.

132

133   Ventricles are apparent in sections through the midbrain (Fig. 2, Extended Data Figs. 1,2). The

134   second (mesencephalic) ventricle mirrors the shape of the optic tectum, and is V-shaped in axial

135   section and U-shaped in horizontal section. There does not appear to be either a torus

136   longitudinalis or torus semicircularis within the second ventricle. Anteriorly, the mesencephalic

137   ventricles connect to a tube-like ventricle that opens at the roof of the diencephalic region of the

138   brain. Posteriorly the mesencephalic ventricles contact the fourth ventricle through a narrow

139   tube-shaped connection.

140

141   **Hindbrain.** Few features of the hindbrain are preserved. The anteriormost portion of the

142   hindbrain is developed as small rounded cerebellar auricular lobes, which are separated by the

143   posterior limits of the mesencephalic lobes (Fig. 1). Posterior to these lies the recessus lateralis

144   of the fourth ventricle, which is continuous with a thin, dorsally-extensive rhombencephalic tela

145   choroidea. The cerebellar corpus is barely developed. The fourth ventricle is open dorsally. It is

146   anteroposteriorly elongate and circular in axial section, and lies ventral to the mesencephalic

147   ventricle (Fig. 2, Extended Data Figs. 1,2). A cerebral aqueduct connecting the second and fourth

148   ventricles is not apparent. The internal walls of the fourth ventricle lack pronounced ridges, but it

149   is difficult to say whether this is original or a taphonomic artifact. Two thin, posteroventrally

150   directed branches of the abducens (VI) nerve leave the ventral surface of the brain level with

151   posterior margin of the fourth ventricle. More ventrally, an additional branch extends from the

152   saccular chamber in the direction of the posterior myodome. Due to the position and path of this

153   branch, we identify it as a distally diverging branch of the abducens nerve.

154

155   The trigeminofacial nucleus and associated nerves are separated from the body of the hindbrain,

156   and this is presumably a taphonomic artifact (Fig. 1). The trigeminofacial complex on the right

157   of the specimen appears to be associated with the alar wall of the rhombencephalon, which has

158   pulled away from the remainder of the hindbrain. Nerve branches located at the front of this

159   complex are enclosed within skeletal canals and can thus be identified most readily by

160   comparison with endocasts described for Palaeozoic actinopterygians, although we caution that

161   this nomenclature needs review in comparison to nerve patterns in extant non-teleost

162   actinopterygians. Two stout nerves emerge anterolaterally from the front of this complex, the

163   most anterior of which enters the canal identified as that for the trigeminal (V) nerve, and the

164   more posterior one the lateralis branch of the facial (VIIlat) nerve. A third nerve, which leaves

165   the complex anteroventrally, enters the canal for the main branch of the facial (VII) nerve. More

166   posteriorly, a series of nerves are associated with the inner ear and otolith, and most likely

167   correspond to branches of the octavolateralis (VIII) nerve. The anterior branch of the anterior

168   ramus of the octavolateralis extends some way dorsally into the anterior ampulla, with the

169   posterior branch of the anterior ramus entering the utriculus. A posteroventral branch contacts

170   the anterior margin of the otolith within the saccular cavity. Two to three additional rami attach

171   to the medial margin of the otolith, and further branches may be present posteriorly.

172

173   A diamond-shaped sheet lies posterodorsal to the preserved portion of the brain, in close

174   association with the roof of the endocranial cavity (Fig. 1, Extended Data Fig. 3). This structure

175   is in a similar position to the meninx primitiva, modified to a cisterna spinobulbularis in

176   *Polypterus*[42,43], and a myelencephalic gland in other early ray-finned fishes[44]. The dorsal surface

177   of the tissue sheet bears a medially located opening surrounded by a thin layer of tissue that

178   extends as a tube toward the posterodorsal fontanelle of the neurocranium. The vagus (X) nerve

179   lies ventral to this sheet, extending posterolaterally to exit from the braincase via the

180   oticooccipital fissure. Anterior to the vagus nerve root, the glossopharyngeal nerve extends

181   laterally towards the endocranial wall.

182

183 **Discussion and Conclusions**

184 **Correspondence between brains and endocasts.** It has been widely assumed that there is close

185 correspondence between brain and endocast shape in early ray-finned fishes[4,6,26,42]. However, the

186 brain as preserved in †*Coccocephalichthys* does not closely conform to the inner surface of the

187 endocavity (Fig. 1, Extended Data Fig. 1). It seems likely that the brain has contracted to some

188 degree during preservation, but the fact that many cranial nerves both connect with the brain

189 itself and extend out of their neurocranial foramina places a limit on the degree of shrinkage.

190 There is also a clear positional match between regions of the preserved brain and areas of the

191 endocavity hypothesised to accommodate them. Living ray-finned fishes show varying degrees

192 of correspondence between brain and endocast morphology[46,47] (Fig. 3), although in no case does

193 the brain completely fill the endocavity in a way comparable to lungfishes and some tetrapods[48–

194 51]. This does not invalidate endocasts as sources of characters or information about

195 neuroanatomy, but stresses that the anatomy of brains and endocavities should not be treated as

196 interchangeable.

197

198 **Patterns of brain evolution in bony fishes.** The principal lineages of ray-finned fishes show

199 substantial differences in both brain and endocavity structure (Fig. 3, Extended Data Fig. 3).

200 Living members of early-diverging groups like cladistians and chondrosteans provide important

201 clues about primitive brain anatomy in actinopterygians. However, both groups show extreme

202 morphological specialisations resulting from their long independent evolutionary histories. As a

203 stem actinopterygian separated from the common ancestor of all living species by tens—rather

204 than hundreds—of millions of years[3,29,52,53], †*Coccocephalichthys* provides unique information

205 bearing on primitive brain anatomy in ray-finned fishes and sequences of change within the

206 group. Most notably, the brain of †*Coccocephalichthys* allows us to clarify neurological

207 synapomorphies of the ray-finned fish total group (i.e. the living radiation and all closely related

208 fossil taxa) and crown group (i.e. the living radiation only), summarised in Fig. 3. An everted

209 forebrain, the principle neuroanatomical feature of ray-finned fishes, is absent in

210 †*Coccocephalichthys*, indicating that this feature originated in more crownward portions of the

211 actinopterygian stem. Absence of this feature also nullifies the hypothesis that forebrain eversion

212 in actinopterygians arose due to developmental constraints associated with small body size in

213 Devonian members of the group[54,55].

214

215   The presence of partially developed inferior lobes of the hypothalamus in

216 †*Coccocephalichthys* challenges the current assumption that the absence of this diencephalic

217 outgrowth in *Polypterus* represents a primitive condition for crown ray-finned fishes[1,56].

218 Presence of this feature in a stem actinopterygian suggests an alternative scenario where it arose

219 deep on the ray-finned fish stem, and was retained by actinopterans and lost in cladistians, before

220 developing fully in neopterygians[56]. †*Coccocephalichthys* also provides evidence that the

221 myelencephalic gland of holosteans and chondrosteans can trace its origins to a feature present in

222 stem actinopterygians. The myelencephalic gland is a hematopoietic (blood-generating) structure

223 enclosed within the endocranial cavity of non-teleost actinopterans, where it either overlies

224 (lepisosteids) or embraces (*Amia*, chondrosteans) the myelencephalon[44,57]. In *Polypterus*, the

225 meningeal tissue occupying the same region as the myelencephalic gland of other taxa is

226 differentiated and highly vascularised, and is identified as the cisterna spinobulbaris[42,43].

227 †*Coccocephalichthys* bears a similar membranous structure overlying the rhombencephalon at

228   the level of the vagal nerves, which we consider to be homologous to the cisterna spinobulbaris

229   of *Polypterus*. On this basis, we argue that modified rhombencephalic meningeal tissues are a

230   general feature of ray-finned fishes, with subsequent modifications in holosteans and

231   chondrosteans as a well-developed myelencephalic gland.

232

233   The brain of †*Coccocephalichthys* aids in discerning which neuroanatomical features of

234   deeply-branching crown lineages are primitive versus derived, with implications for patterns of

235   brain evolution in more nested clades (Fig. 3). These data provide remarkable corroboration that

236   features of *Polypterus* such as the absence of intraventricular projections and the presence of a

237   poorly differentiated corpus cerebelli represent primitive actinopterygian conditions. However,

238   †*Coccocephalichthys* suggests that perhaps the most conspicuous external aspect of

239   neuroanatomy in *Polypterus* might be apomorphic. Like sarcopterygians, *Polypterus* has an

240   enlarged telencephalon[9], in contrast to the small structure in actinopterans[38,39] and

241   chondrichthyans[35]. Distribution among extant taxa suggests the shared condition in *Polypterus*

242   and sarcopterygians may be an osteichthyan feature[42] lost in actinopterans. However, the absence

243   of enlarged telencephalon in †*Coccocephalichthys* makes it more parsimonious to infer the

244   convergent origin of similar geometries in *Polypterus* and sarcopterygians. At the same time,

245   †*Coccocephalichthys* suggests that an apparent specialisation of *Polypterus* might in fact be a

246   more general feature of ray-finned fishes. *Polypterus* is unique among extant jawed vertebrates

247   in having an invaginated corpus cerebelli, a condition most parsimoniously interpreted as a

248   specialisation of that lineage[9,11,58]. However, the corpus cerebelli of †*Coccocephalichthys* also

249   seems to be formed as an invagination of the dorsal surface of the rhombencephalic region of the

250   brain, matching the arrangement of *Polypterus*. Independent gains within both lineages, or a

251 single gain at the base of actinopterygians followed by a loss in actinopterans, represent equally

252 parsimonious scenarios. It is not possible to select between these alternatives in the absence of

253 additional information on brain structure in other early actinopterygians.

254

255 **The utility of fossil brains.** †*Coccocephalichthys* reinforces results from studies of neural

256 structures in fossil arthropods[12–16] that highlight the importance of fossil brains for patterns of

257 neuroanatomical evolution in groups with deep evolutionary divergences. Beyond representing

258 preservational curiosities, fossilised brains provide otherwise inaccessible trait data with

259 implications for patterns of phylogenetic relationships and character polarity. We anticipate that

260 preservation of neural tissue in fossil fishes is likely to be more common than widely thought,

261 with assumptions of non-preservation leading to potentially valuable information being

262 overlooked. A careful survey of fish material from taphonomically promising horizons has

263 potential to yield novel anatomical information bearing on the evolution of brain structural

264 diversity within the principal clade of aquatic vertebrates.

265

266 **References**

267 1.   Nieuwenhuys, R., ten Donkelaar, H. J. & Nicholson, C. The Meaning of It All. in *The Central*

268 *Nervous System of Vertebrates: Volume 1 / Volume 2 / Volume 3* (eds. Nieuwenhuys, R., ten

269 Donkelaar, H. J. & Nicholson, C.) 2135–2195 (Springer, 1998). doi:10.1007/978-3-642-18262-4_24.

270 2.   Friedman, M. The early evolution of ray-finned fishes. *Palaeontology* **58**, 213–228 (2015).

271 3.   Giles, S., Xu, G. H., Near, T. J. & Friedman, M. Early members of 'living fossil' lineage imply

272 later origin of modern ray-finned fishes. *Nature* **549**, 265–268 (2017).

273 4.   Moodie, R. L. A new fish brain from the coal measures of Kansas, with a review of other fossil

274 brains. *J. Comp. Neurol.* **25**, 135–181 (1915).

275   5.   Nielsen, E. *Studies on Triassic Fishes: Glaucolepis and Boreosomus. 1*. (Reitzel, 1942).

276   6.   Giles, S. & Friedman, M. Virtual reconstruction of endocast anatomy in early ray-finned fishes

277   (Osteichthyes, Actinopterygii). *J. Paleontol.* **88**, 636–651 (2014).

278   7.   Lu, J., Giles, S., Friedman, M., den Blaauwen, J. L. & Zhu, M. The oldest actinopterygian

279   highlights the cryptic early history of the hyperdiverse ray-finned fishes. *Curr. Biol.* **26**, 1602–1608

280   (2016).

281   8.   Edinger, T. Recent Advances in Paleoneurology. *Prog. Brain Res.* **6**, 147–160 (1964).

282   9.   Nieuwenhuys, R. Brachiopterygian Fishes. in *The Central Nervous System of Vertebrates:*

283   *Volume 1 / Volume 2 / Volume 3* (eds. Nieuwenhuys, R., ten Donkelaar, H. J. & Nicholson, C.) 655–

284   699 (Springer, 1998). doi:10.1007/978-3-642-18262-4_13.

285   10.   Anzeiger, A. & Page, P. Beitrag zur anatomie des Zentralnervensystems und des geruchsorgans

286   von *Polypterus bichir. Aus Dem Anat. Inst. Univ. Freibg.* 308–324 (1887).

287   11.   Ikenaga, T. *et al.* Morphological analysis of the cerebellum and its efferent system in a basal

288   actinopterygian fish, *Polypterus senegalus. J. Comp. Neurol.* **530**, 1231–1246 (2022).

289   12.   Ma, X., Hou, X., Edgecombe, G. D. & Strausfeld, N. J. Complex brain and optic lobes in an early

290   Cambrian arthropod. (2012) doi:10.1038/nature11495.

291   13.   Cong, P., Ma, X., Hou, X., Edgecombe, G. D. & Strausfeld, N. J. Brain structure resolves the

292   segmental affinity of anomalocaridid appendages. (2014) doi:10.1038/nature13486.

293   14.   Ma, X., Cong, P., Hou, X., Edgecombe, G. D. & Strausfeld, N. J. An exceptionally preserved

294   arthropod cardiovascular system from the early Cambrian. *Nat. Commun.* **5**, 3560 (2014).

295   15.   Edgecombe, G. D., Ma, X. & Strausfeld, N. J. Unlocking the early fossil record of the arthropod

296   central nervous system. *Philos. Trans. R. Soc. B Biol. Sci.* **370**, 20150038 (2015).

297   16.   Strausfeld, N. J., Ma, X. & Edgecombe, G. D. Fossils and the evolution of the arthropod brain.

298   *Curr. Biol.* **26**, R989–R1000 (2016).

299   17.   Johanson, Z. Placoderm branchial and hypobranchial muscles and origins in jawed vertebrates. *J.*

300   *Vertebr. Paleontol.* **23**, 735–749 (2003).

301   18.   Trinajstic, K., Marshall, C., Long, J. & Bifield, K. Exceptional preservation of nerve and muscle

302   tissues in Late Devonian placoderm fish and their evolutionary implications. *Biol. Lett.* **3**, 197–200

303   (2007).

304   19.   Pradel, A. *et al.* Skull and brain of a 300-million-year-old chimaeroid fish revealed by

305   synchrotron holotomography. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 5224–8 (2009).

306   20.   Maldanis, L. *et al.* Heart fossilization is possible and informs the evolution of cardiac outflow

307   tract in vertebrates. *eLife* **5**, (2016).

308   21.   Braford, M. R. Stalking the everted telencephalon: comparisons of forebrain organization in basal

309   ray-finned fishes and teleosts. *Brain. Behav. Evol.* **74**, 56–76 (2009).

310   22.   Briscoe, S. D. & Ragsdale, C. W. Evolution of the chordate telencephalon. *Curr. Biol.* **29**, R647–

311   R662 (2019).

312   23.   Nieuwenhuys, R. The development and general morphology of the telencephalon of

313   actinopterygian fishes: synopsis, documentation and commentary. *Brain Struct. Funct.* **215**, 141–157

314   (2011).

315   24.   Nelson, J. S., Grande, T. C. & Wilson, M. V. H. *Fishes of the World*. (John Wiley & Sons, 2016).

316   25.   Giles, S., Rogers, M. & Friedman, M. Bony labyrinth morphology in early neopterygian fishes

317   (Actinopterygii: Neopterygii). *J. Morphol.* **279**, 426–440 (2018).

318   26.   Coates, M. I. Endocranial preservation of a Carboniferous actinopterygian from Lancashire, UK,

319   and the interrelationships of primitive actinopterygians. *Philos. Trans. R. Soc. B Biol. Sci.* **354**, 435–

320   462 (1999).

321   27.   Poplin, C. M. Etude de quelques paleoniscides pennsylvaniens du Kansas. *Cah. Paléontol. Ed.*

322   *CNRS Paris* (1974).

323   28.   Hamel, M.-H. & Poplin, C. The braincase anatomy of *Lawrenciella schaefferi* , actinopterygian

324   from the Upper Carboniferous of Kansas (USA). *J. Vertebr. Paleontol.* **28**, 989–1006 (2008).

325   29.   Latimer, A. E. & Giles, S. A giant dapediid from the Late Triassic of Switzerland and insights

326   into neopterygian phylogeny. *R. Soc. Open Sci.* **5**, 180497 (2018).

327   30.   Argyriou, T. *et al.* Internal cranial anatomy of Early Triassic species of †*Saurichthys*

328   (Actinopterygii: †Saurichthyiformes): implications for the phylogenetic placement of

329   †saurichthyiforms. *BMC Evol. Biol.* **18**, 161 (2018).

330   31.   Choo, B., Lu, J., Giles, S., Trinajstic, K. & Long, J. A. A new actinopterygian from the Late

331   Devonian Gogo Formation, Western Australia. *Pap. Palaeontol.* **5**, 343–363 (2019).

332   32.   Gignac, P. M. *et al.* Diffusible iodine-based contrast-enhanced computed tomography (diceCT):

333   an emerging tool for rapid, high-resolution, 3-D imaging of metazoan soft tissues. *J. Anat.* **228**, 889–

334   909 (2016).

335   33.   Pradel, A., Maisey, J. G., Mapes, R. H. & Kruta, I. First evidence of an intercalar bone in the

336   braincase of "palaeonisciform" actinopterygians, with a virtual reconstruction of a new braincase of

337   *Lawrenciella* Poplin, 1984 from the Carboniferous of Oklahoma. *Geodiversitas* **38**, 489–504 (2016).

338   34.   Friedman, M. & Giles, S. Actinopterygians: the ray-finned fishes—an explosion of diversity. in

339   *Evolution of the vertebrate ear : evidence from the fossil record* (eds. Clack, J. A., Fay, R. R. &

340   Popper, A. N.) 17–49 (Springer International Publishing, 2016). doi:10.1007/978-3-319-46661-3_2.

341   35.   Smeets, W. J. A. J. Cartilaginous Fishes. in *The Central Nervous System of Vertebrates: Volume*

342   *1 / Volume 2 / Volume 3* (eds. Nieuwenhuys, R., ten Donkelaar, H. J. & Nicholson, C.) 551–654

343   (Springer, 1998). doi:10.1007/978-3-642-18262-4_12.

344   36.   Nieuwenhuys, R. Lungfishes. in *The Central Nervous System of Vertebrates: Volume 1 / Volume*

345   *2 / Volume 3* (eds. Nieuwenhuys, R., ten Donkelaar, H. J. & Nicholson, C.) 939–1006 (Springer,

346   1998). doi:10.1007/978-3-642-18262-4_16.

347   37.   Nieuwenhuys, R. The Coelacanth Latimeria chalumnae. in *The Central Nervous System of*

348   *Vertebrates: Volume 1 / Volume 2 / Volume 3* (eds. Nieuwenhuys, R., ten Donkelaar, H. J. &

349   Nicholson, C.) 1007–1043 (Springer, 1998). doi:10.1007/978-3-642-18262-4_17.

350   38.   Nieuwenhuys, R. Chondrostean Fishes. in *The Central Nervous System of Vertebrates: Volume 1*

351   */ Volume 2 / Volume 3* (eds. Nieuwenhuys, R., ten Donkelaar, H. J. & Nicholson, C.) 701–757

352   (Springer, 1998). doi:10.1007/978-3-642-18262-4_14.

353   39.   Meek, J. & Nieuwenhuys, R. Holosteans and Teleosts. in *The Central Nervous System of*

354   *Vertebrates: Volume 1 / Volume 2 / Volume 3* (eds. Nieuwenhuys, R., ten Donkelaar, H. J. &

355   Nicholson, C.) 759–937 (Springer, 1998). doi:10.1007/978-3-642-18262-4_15.

356   40.   Northcutt, R. G. Forebrain evolution in bony fishes. *Brain Res. Bull.* **75**, 191–205 (2008).

357   41.   Morona, R., López, J. M., Northcutt, R. G. & González, A. Comparative analysis of the

358   organization of the cholinergic system in the brains of two Holostean fishes, the Florida gar

359   *Lepisosteus platyrhincus* and the bowfin *Amia calva*. *Brain. Behav. Evol.* **81**, 109–142 (2013).

360   42.   Jarvik, E. *Basic structure and evolution of vertebrates*. (Academic Press, 1980).

361   43.   Bjerring, H. C. Facts and thoughts on piscine phylogeny. in *Evolutionary Biology of Primitive*

362   *Fishes* (eds. Foreman, R. E., Gorbman, A., Dodd, J. M. & Olsson, R.) 31–57 (Springer US, 1985).

363   doi:10.1007/978-1-4615-9453-6_3.

364   44.   Chandler, A. C. On a lymphoid structure lying over the myelencephalon of *Lepisosteus*. *Univ.*

365   *Calif. Publ. Zool.* **9**, 85–104 (1911).

366   45.   Coates, M. I. Actinopterygians from the Namurian of Bearsden, Scotland, with comments on

367   early actinopterygian neurocrania. *Zool. J. Linn. Soc.* **122**, 27–59 (1998).

368   46.   Fine, M. L., Horn, M. H. H. & Cox, B. *Acanthonus armatus* , a deep-sea teleost fish with a

369   minute brain and large ears. *Proc. R. Soc. Lond. B Biol. Sci.* **230**, 257–265 (1987).

370   47.   Herzog, H., Klein, B. & Ziegler, A. Form and function of the teleost lateral line revealed using

371   three-dimensional imaging and computational fluid dynamics. *J. R. Soc. Interface* **14**, 20160898

372   (2017).

373   48.   Rowe, T. B., Macrini, T. E. & Luo, Z.-X. Fossil evidence on origin of the mammalian brain.

374   *Science* **332**, 955–957 (2011).

375   49.   Neubauer, S. Endocasts: possibilities and limitations for the interpretation of human brain

376   evolution. *Brain. Behav. Evol.* **84**, 117–134 (2014).

377   50.   Clement, A. M., Nysjö, J., Strand, R. & Ahlberg, P. E. Brain - Endocast relationship in the

378   Australian lungfish, *Neoceratodus forsteri*, elucidated from tomographic data (Sarcopterygii: Dipnoi).

379   *PLoS ONE* **10**, (2015).

380   51.   Watanabe, A. *et al.* Are endocasts good proxies for brain size and shape in archosaurs throughout

381   ontogeny? *J. Anat.* **234**, 291–305 (2019).

382   52.   Figueroa, R. T., Friedman, M. & Gallo, V. Cranial anatomy of the predatory actinopterygian

383   *Brazilichthys macrognathus* from the Permian (Cisuralian) Pedra de Fogo Formation, Parnaíba Basin,

384   Brazil. *J. Vertebr. Paleontol.* **39**, e1639722 (2019).

385   53.   Stack, J. & Gottfried, M. D. A new, exceptionally well-preserved Permian actinopterygian fish

386   from the Minnekahta Limestone of South Dakota, USA. *J. Syst. Palaeontol.* **19**, 1271–1302 (2021).

387   54.   Striedter, G. F. & Northcutt, R. G. Head size constrains forebrain development and evolution in

388   ray-finned fishes. *Evol. Dev.* **8**, 215–222 (2006).

389   55.   Folgueira, M. *et al.* Morphogenesis underlying the development of the everted teleost

390   telencephalon. *Neural Develop.* **7**, 212 (2012).

391   56.   Schmidt, M. Evolution of the hypothalamus and inferior lobe in ray-finned fishes. *Brain. Behav.*

392   *Evol.* **95**, 302–316 (2020).

393   57.   van der Horst, C. J. The myelencephalic gland of *Polyodon*, *Acipenser* and *Amia*. *K. Akad. Van*

394   *Wet. Te Amst. Proc. Sect. Sci.* **28**, 432–442 (1925).

395   58.   Graña, P., Folgueira, M., Huesa, G., Anadón, R. & Yáñez, J. Immunohistochemical distribution

396   of calretinin and calbindin (D-28k) in the brain of the cladistian *Polypterus senegalus*. *J. Comp.*

397   *Neurol.* **521**, 2454–2485 (2013).

398

399 **Figure Legends**

400

401 **Fig. 1: Neurocranium, endocast, otoliths and preserved brain of †*Coccocephalichthys wildi* (MM**

402 **W.12451). a**, Neurocranium in left lateral view. **b**, cutaways of neurocranium in dorsal (top) and left

403 lateral (bottom) views showing brain and otoliths *in situ*. **c**, endocast in left lateral view. **d**, the brain and

404 associated preserved soft tissues in dorsal (top) and lateral (bottom) views, with left otolith removed in

405 the latter for clarity. cce, corpus cerebelli; hyp, hypophysis; mes, mesencephalon; ms, myelencephalic

406 sheet; tel, telencephalon; I, olfactory nerve; II, optic nerve; III, oculomotor nerve; V, trigeminal nerve; VI,

407 abducens nerve; IX, glossopharyngeal nerve; X, vagus nerve. Scale bars = 5 mm. Arrow indicates anterior

408 for all panels.
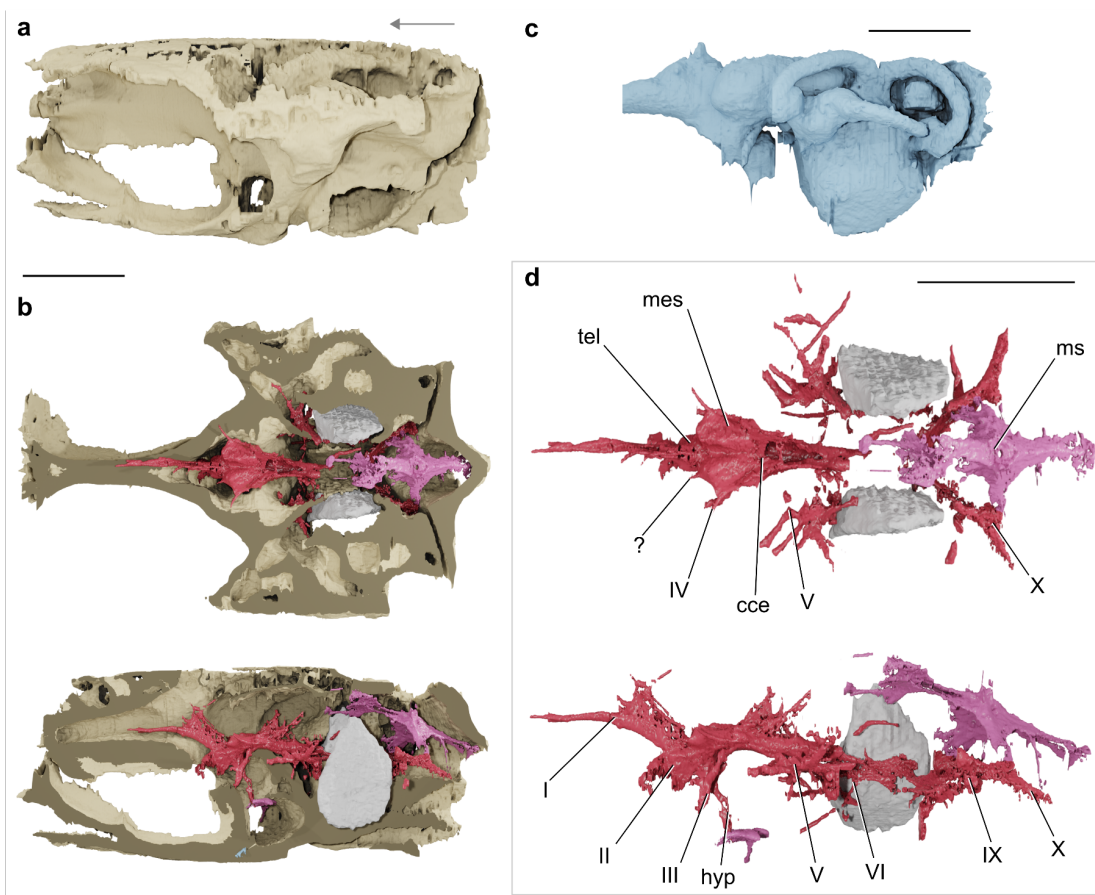
409

410

411   **Fig. 2: Anatomical correspondence between preserved brain of †*Coccocephalichthys wildi* and those**

412   **of extant fishes. a**, three-dimensional rendering of the brain of †*Coccocephalichthys* in left lateral view.

413   Scale bar = 10 mm. **b,** transverse sections through the brains of †*Coccocephalichthys* and selected jawed

414   fishes from diceCT data. cce, corpus cerebelli; mes, mesencephalon; mye, myelencephalon; tel,

415   telencephalon; tri (V), trigeminal nerve; ?, unidentified midbrain feature. Silhouettes of extant taxa from

416   phylopic2 (*Squalus*, Ignacio Contreras; *Lepidosiren*, Roberto Diaz Sibaja; *Acipenser*, Maija Karala, *Amia*,

417   no copyright; *Polypterus*, no copyright). †*Coccocephalichthys* silhouette original based on

418   '*Palaeoniscus*'.[59] Scale bar = 1 mm.
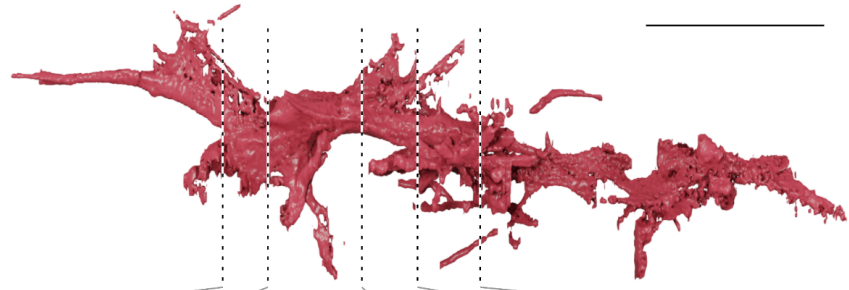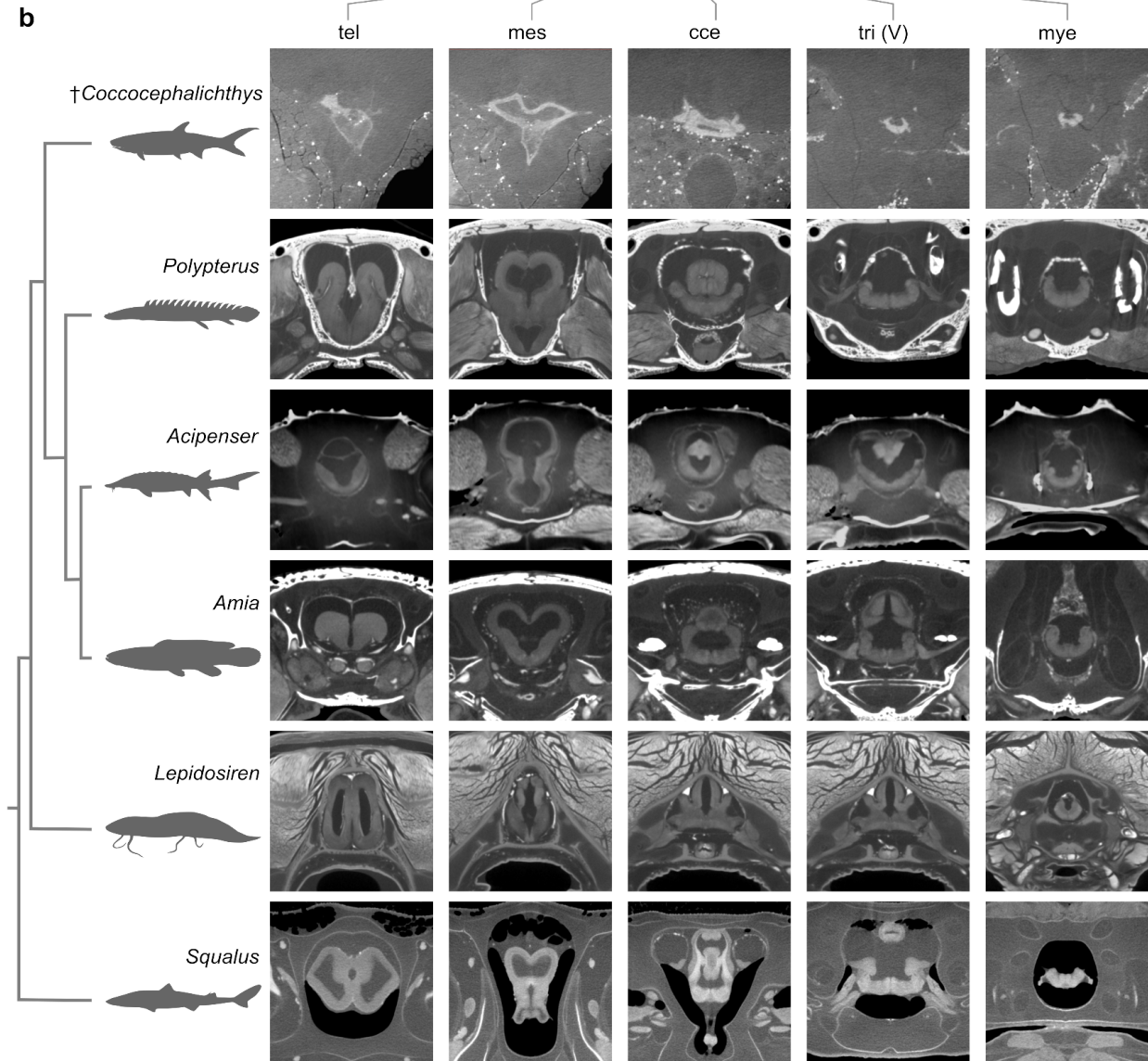
419

420

421

422

423 **Fig. 3: Major anatomical transformations in actinopterygian brain structure illuminated by**

424 **†*Coccocephalichthys.*** Branch labels represent character modifications. Asterisk (*) indicates shift in

425 position of character in cladogram due to anatomical information from †*Coccocephalichthys*. Black bars:

426 unambiguous changes; grey bars: ACCTRAN optimisations; white bars: DELTRAN optimisations. Blue:

427 endocast; red: brain and cranial nerves; pink: myelencephalic sheet. Arrow indicates anterior direction for

428 3D renders. Insets show transverse or sagittal sections through the relevant portions of the brain, with

429 darker orange shading indicating specific regions of interest. Images not to scale. Node ages from Giles et

430 al.[3].

431

432

433
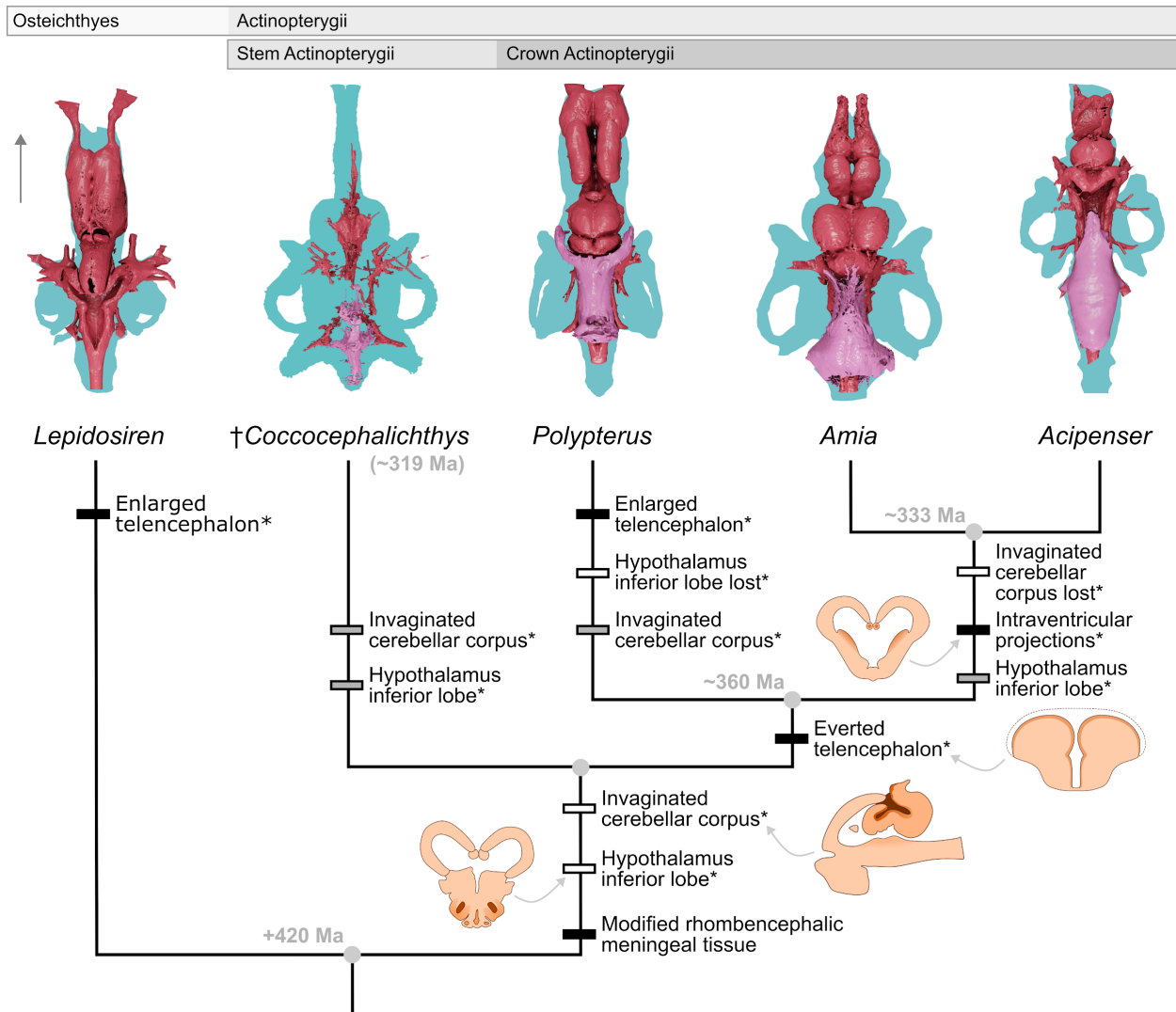
Methods

Material examined

436 †*Coccocephalichthys wildi* is known from a single specimen (Manchester Museum, Wild

437 Collection, 12451) from the roof of the Mountain Fourfoot Mine, Carre Heys, Trawden,

438 Lancashire, UK. Accounts of its anatomy are given by Watson[60], Poplin[27], and Poplin & Véran[61].

439 Other three-dimensionally preserved actinopterygians hosted in nodules from this area include

440 *Trawdenia planti* and *Mesonichthys aitkeni*; these are all thought to derive from the so-called

441 "Soapstone Bed." This horizon lies within the Pennine Lower Coal Measures above the Bullion

442 Coal (= Upper Foot Coal) and the Mountain 1.2 m Coal (= Lower Mountain Coal), but below the

443 Ardley Seam (=Arley Coal)[26,62,63]. This is within the Langsettian regional substage, which

444 correlates with the upper part of the Bashkirian stage of the international timescale[64].

445

Diffusible Iodine-based contrast enhancement (diceCT)

447 Comparative specimens of *Squalus acanthias* (University of Michigan Museum of Zoology

448 [UMMZ] uncatalogued), *Polypterus senegalus* (UMMZ 195008), *Amia calva* (UMMZ 235291)

449 and *Acipenser fulvicens* (UMMZ 219456) were prepared for diceCT by submerging specimens in

450 1.25% Lugol's solution (25g I_2 + 50g KI for every 2L of water) for roughly 14 days prior to

451 scanning. DiceCT data for a specimen of *Lepidosiren paradoxa* (UF:FISH:129826) from the

452 Florida Museum of Natural History Ichthyology Collection was obtained from Morphosource

453 (ark:/87602/m4/M167969).

454

X-ray computed tomography

456   †*Coccocephalichthys wildi* and extant comparative material were scanned at the CTEES facility

457   of the Department of Earth and Environmental Sciences, University of Michigan, using a Nikon

458   XT H 225ST µCT scanner. The scan for †*Coccocephalichthys wildi* was set with 120 kV energy,

459   125 µA current and using a 0.5 mm copper filter. Eight frames were acquired for each projection,

460   with an exposure time of 2.83 seconds, and the option for minimising ring artifacts was selected.

461   Effective pixel size was 15.35 µm and geometric magnification = 13.031. Parameters for extant

462   comparative material (*Squalus acanthias*, *Polypterus senegalus*, *Acipenser brevirostrum*, and

463   *Amia calva*) can be accessed through the Supplemental Material.

464

465   **Methods references**

466   59.   Lankester, E. R. & Ridewood, W. G. *Guide to the gallery of fishes*. (British Museum (Natural

467   History). Department of Zoology, 1908).

468   60.   Watson, D. M. S. The structure of certain palaeoniscids and the relationships of that group with

469   other bony fish. *Proc. Zool. Soc. Lond.* **95**, 815–870 (1925).

470   61.   Poplin, C. M. & Véran, M. A revision of the actinopterygian fish *Coccocephalus wildi* from the

471   Upper Carboniferous of Lancashire. *Spec. Pap. Palaeontol.* **52**, 7–29 (1996).

472   62.   Coates, M. I. & Tietjen, K. 'This strange little palaeoniscid': a new early actinopterygian genus,

473   and commentary on pectoral fin conditions and function. *Earth Environ. Sci. Trans. R. Soc. Edinb.*

474   **109**, 15–31 (2018).

475   63.   Hough, E. *Geology of the Burnley area (SD82NW and SD83SW)*. (2004).

476   64.   Waters, C. N. *et al. A revised correlation of carboniferous rocks in the British Isles*. (Geological

477   Society of London, 2011). doi:10.1144/SR26.

478

489 **Author contributions** The project was conceived by M.F. and S.G. CT scanning was carried out

490 by M.F. and R.F., with staining of extant material by R.F. and M.K. Segmentation of CT data

491 was performed by M.F., S.G., D.G., and R.F. M.F, S.G., and R.F wrote the manuscript, with

492 comments from all authors.

493

494 **Data availability** The fossil described in this study is deposited in the collections of the

495 Manchester Museum and the extant specimens in the University of Michigan Museum of

496 Zoology. The reconstructed .TIFF stack, segmented Mimics file and .PLY files for

497 †*Coccocephalichthys wildi* are available on Zenodo (10.5281/zenodo.6560305).

498

499

500   **Extended Data figure legends**

501   **Extended Data Fig. 1. Sagittal sections through the neurocranium of †*Coccocephalichthys***

502   ***wildi* showing the brain and associated structures.** cce, corpus cerebelli, cr.c; crista

503   cerebellaris, h.inf, hypothalamus inferior lobes; hyp, hypophysis; mes, mesencephalon; ms,

504   myelencephalic sheet; rho, rhombencephalon; sc, spinal cord; tel, telencephalon; v.tr, velum

505   transversum; 2nd v, second ventricle; 4th v, fourth ventricle; I, olfactory nerve; II, optic nerve.

506   Scale bar = 10 mm.
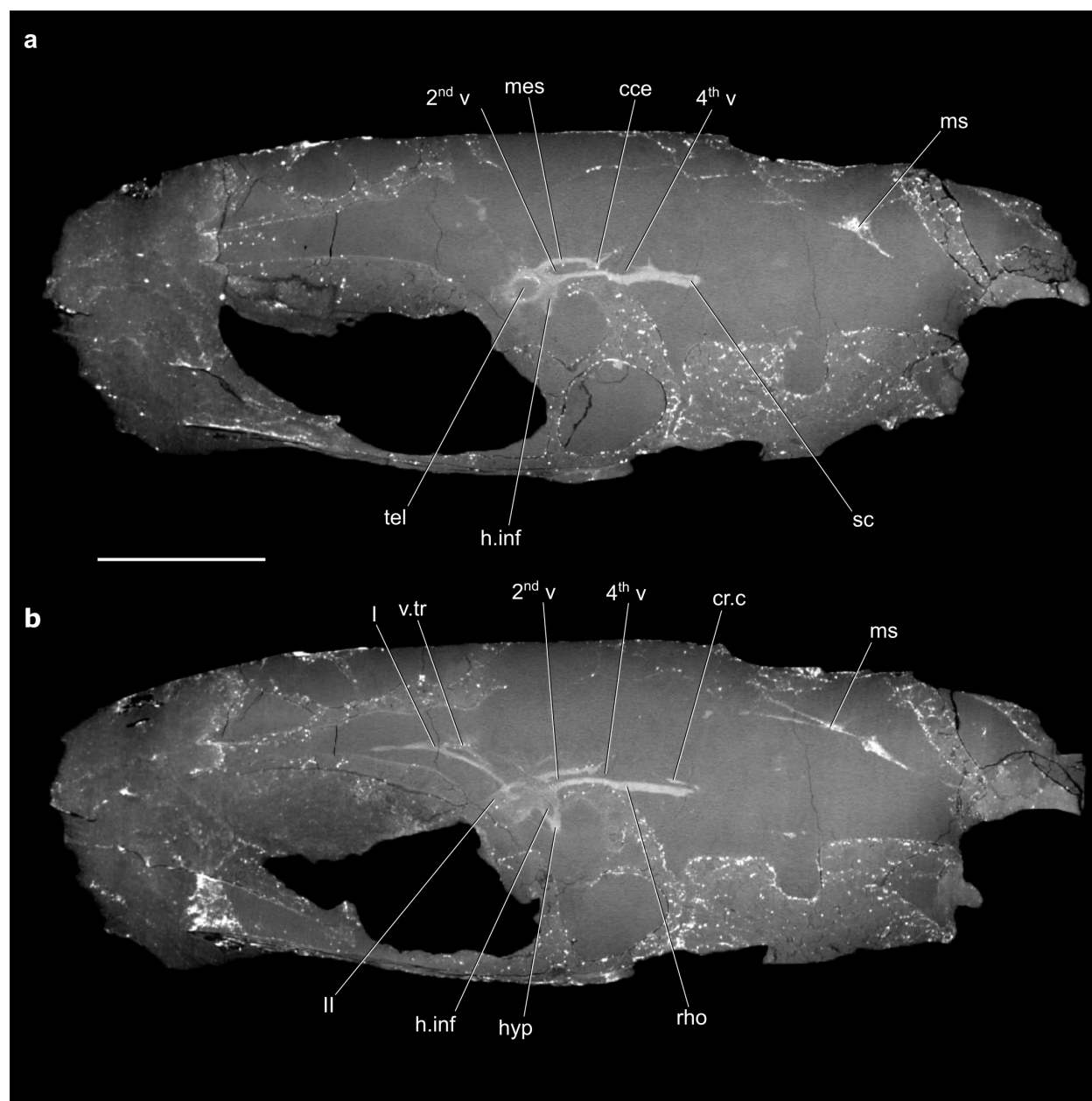
507

508

509

510 **Extended Data Fig. 2. The brain of †*Coccocephalichthys wildi* (red) rendered partially**

511 **transparent to show brain ventricle configuration (white). a,** dorsal view. **b,** left lateral view.

512 die. v, diencephalic ventricle; 2nd v, second ventricle; 4th v, fourth ventricle. Scale bar = 5 mm.
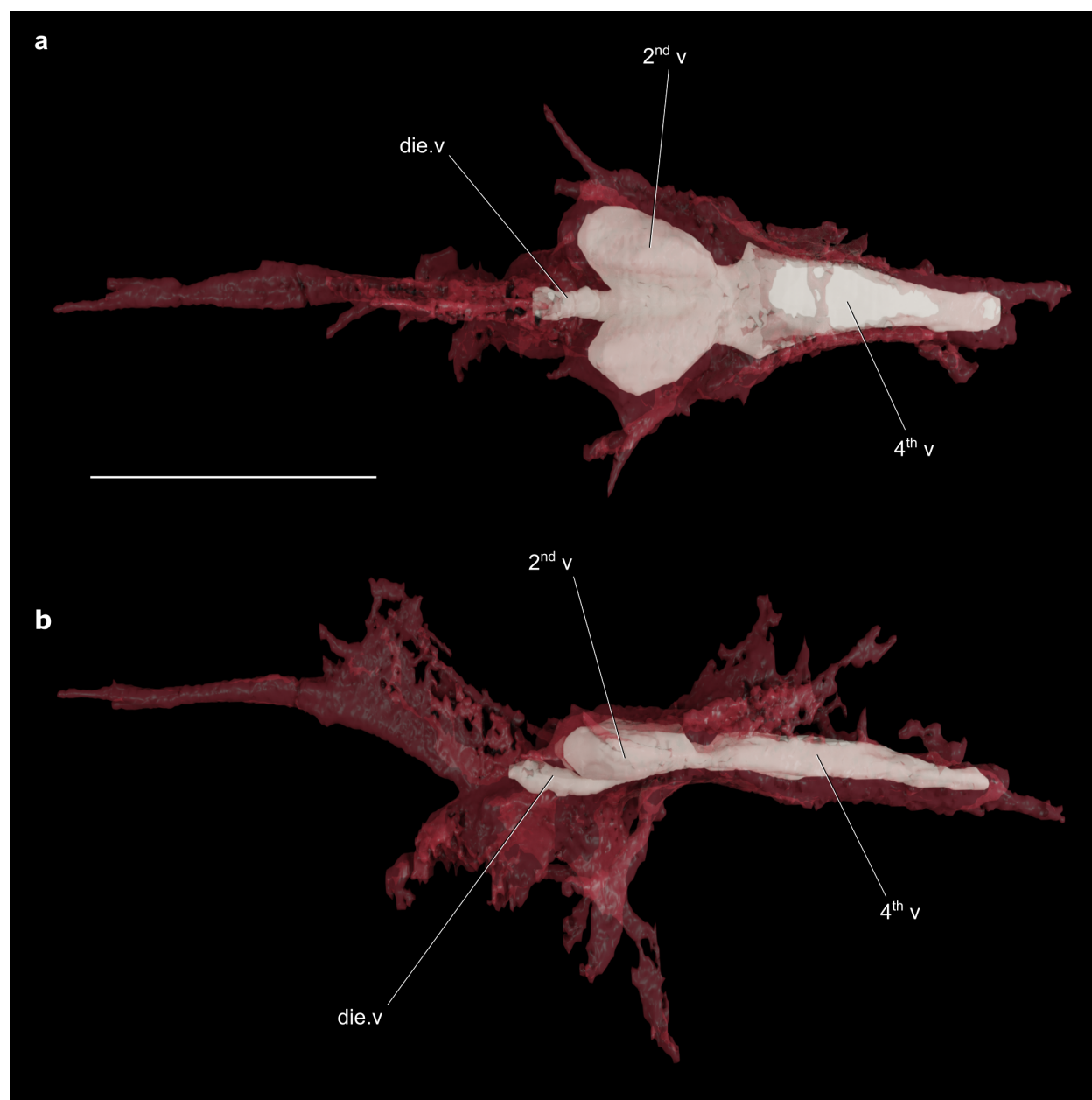
513

514

515

516 **Extended Data Fig. 3. The brain (red) and myelencephalic sheet/gland (pink) of**

517 **†*Coccocephalichthys wildi* and selected extant ray-finned fishes. a,** †*Coccocephalichthys*

518 *wildi*. **b,** *Acipenser brevirostrum*. **c,** *Amia calva*. **d,** *Polypterus senegalus*. Gray and white

519 delimitations show margins between forebrain, midbrain and hindbrain across all taxa. Scale bar

520 = 10 mm.
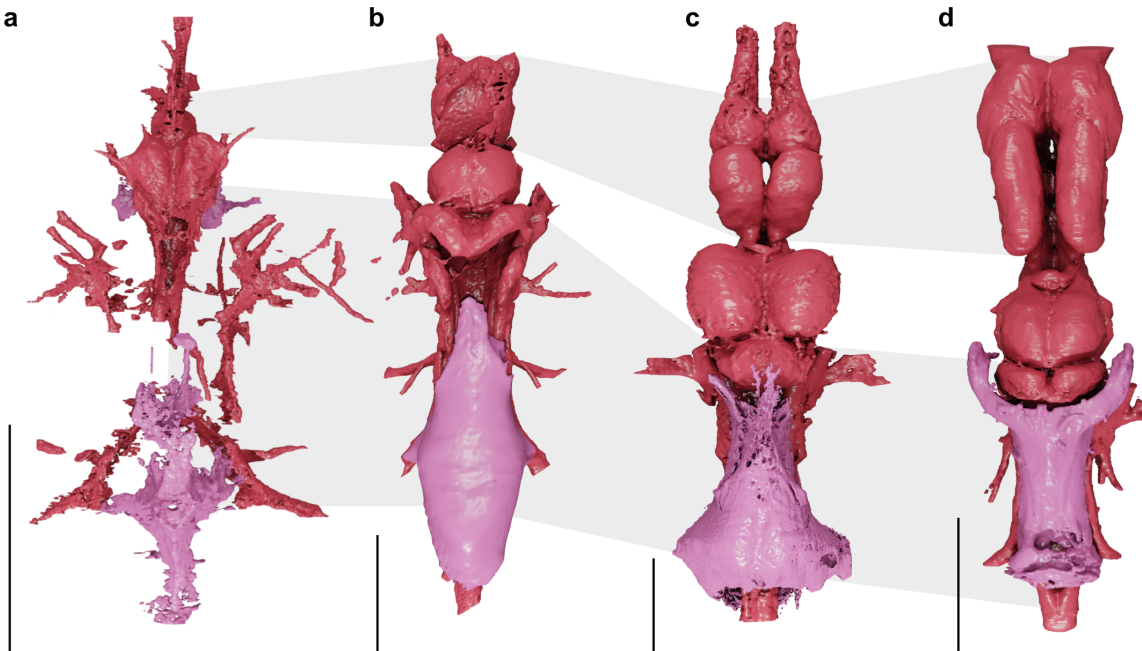
521

522
523
524

525　　**Extended Data Fig. 4. Transverse sections and renders of the brain of †*Coccocephalichthys***

526　　*wildi*. **a**, **b**, the telencephalon. **c**, **d**, the mesencephalon and hypophysis. cce, corpus cerebellum;

527　　h.inf, inferior lobe of the hypothalamus; hyp, hypophysis; tel, telencephalon; mes,

528　　mesencephalon; ms, mesencephalic sheet; v. tr, velum transversum; 4th v, fourth ventricle; II,

529　　optic nerve; III, oculomotor nerve; IV, trochlear nerve, V, trigeminal nerve; VII, facial nerve.

530　　Dorsal portion of forebrain and velum transversum digitally removed in renders. Scale bar in a, c
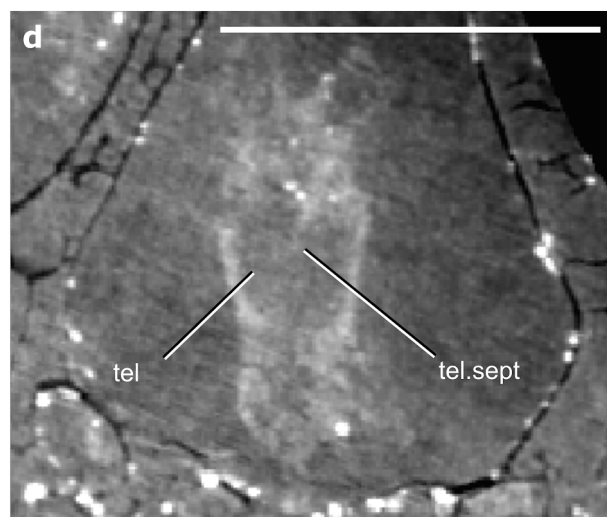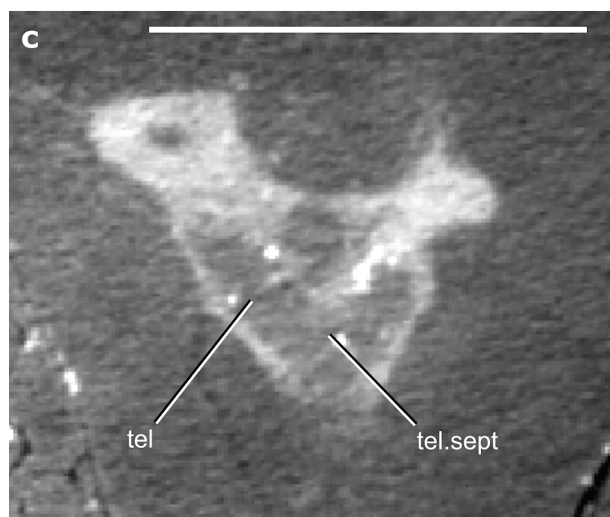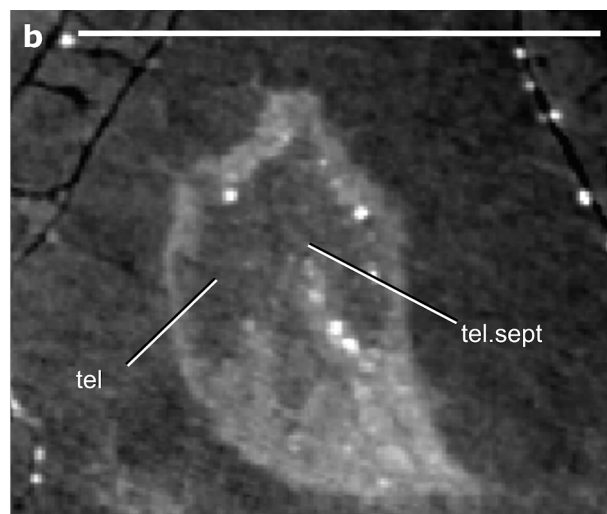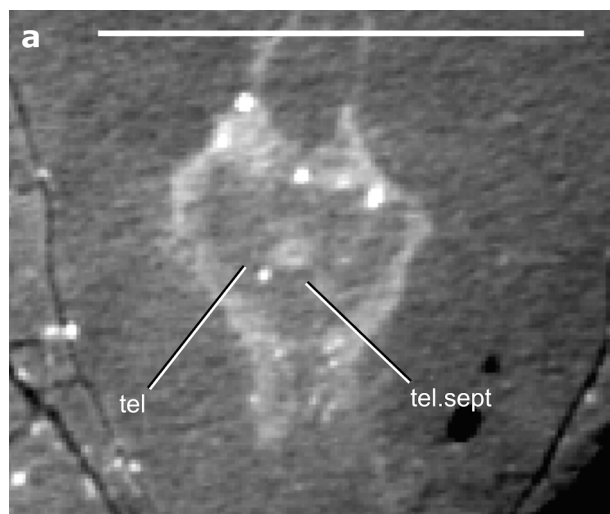
531　　= 2.5 mm; scale bar in b, d = 5 mm.
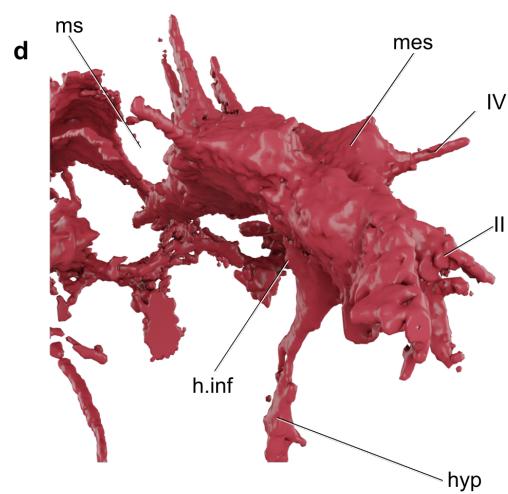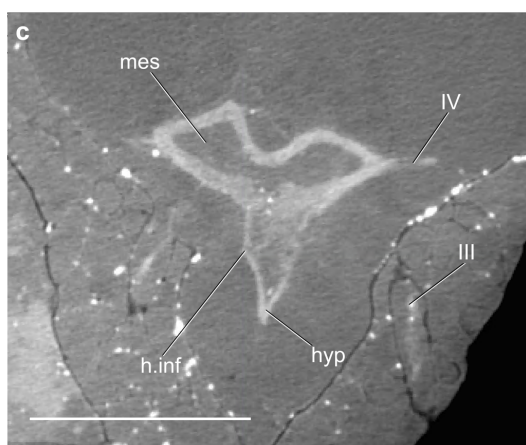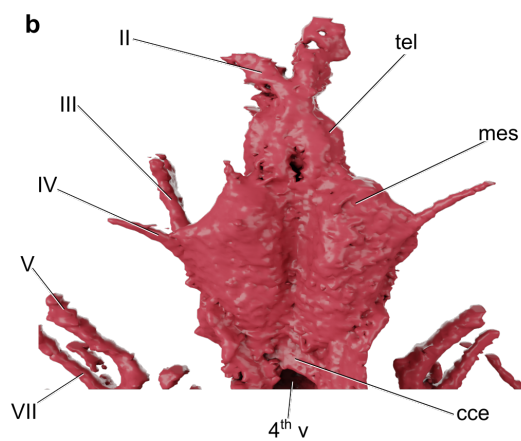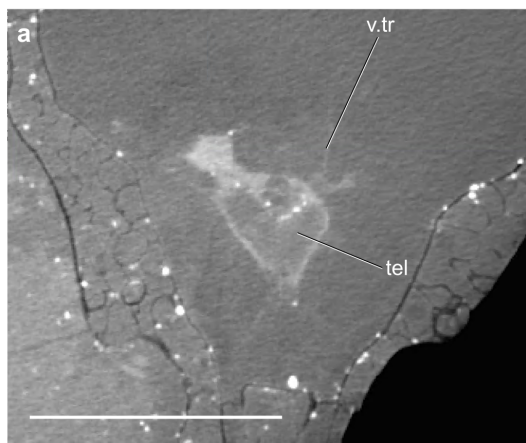
532

533

534

535   **Extended Data Fig. 5. Sections through the brain of †*Coccocephalichthys wildi*. a,** transverse

536   section through the anterior portion of the telencephalon. **b,** axial section through the ventral

537   portion of the telencephalon. **c,** transverse section through the posterior portion of the

538   telencephalon. **d,** axial section through the dorsal portion of the telencephalon. **e,** transverse

539   section through the anterior portion of the hypothalamus inferior lobes. **f,** transverse section

540   through the posterior portion of the hypothalamus inferior lobes. h.inf, inferior lobe of the

541   hypothalamus; l.hyp.re, lateral hypothalamic recess; tel, telencephalon; tel.sept, telencephalic

542   septum. Scale bar = 2 mm.
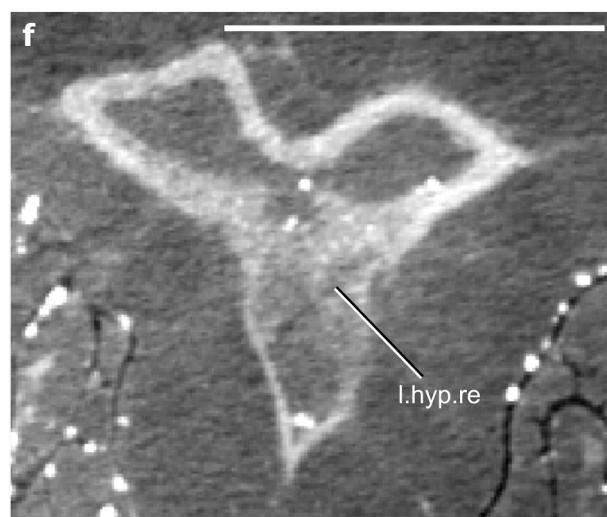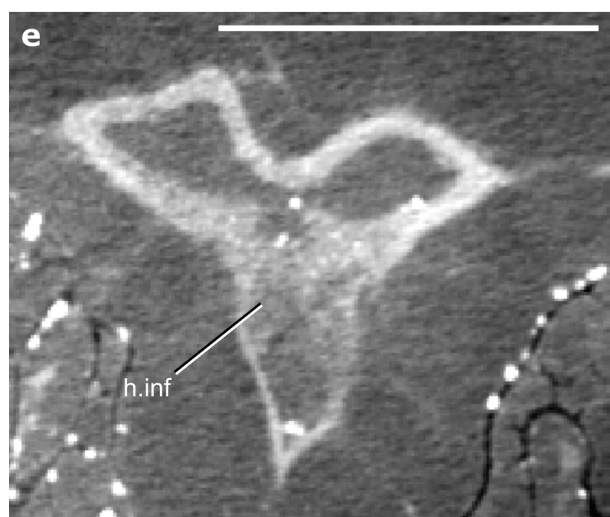
545 **Extended Data Fig. 6. Sections through the brain of †*Coccocephalichthys wildi* and *Amia***

546 *calva*. **a**, transverse section through the diencephalon and mesencephalon of *Coccocephalichthys*

547 *wildi*. **b**, transverse section through the diencephalon and mesencephalon of *Amia calva*. l.hyp.re,

548 lateral hypothalamic recess. Scale bar = 2 mm.

549

550