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1 **Endochondral bone in an Early Devonian ‘placoderm’ from Mongolia**

2

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28 **Endochondral bone is the main internal skeletal tissue of nearly all osteichthyans—the**
29 **group comprising more than 60,000 living species of bony fishes and tetrapods.**
30 **Chondrichthyans (sharks and their kin) are the living sister group of osteichthyans and**
31 **have cartilaginous endoskeletons, long considered the ancestral condition for all jawed**
32 **vertebrates (gnathostomes). The absence of bone in modern jawless fishes and the**
33 **absence of endochondral ossification in early fossil gnathostomes appears to lend**
34 **support to this conclusion. Here we report the discovery of extensive endochondral bone**
35 **in *Minjinia turgenensis*, a new genus and species of ‘placoderm’-like fish from the Early**
36 **Devonian (Pragian) of western Mongolia described using x-ray computed**
37 **microtomography (XR- μ CT). The fossil consists of a partial skull roof and braincase**
38 **with anatomical details providing strong evidence of placement in the gnathostome stem**
39 **group. However, its endochondral space is filled with an extensive network of fine**
40 **trabeculae resembling the endochondral bone of osteichthyans. Phylogenetic analyses**
41 **place this new taxon as a proximate sister group of the gnathostome crown. These**
42 **results provide direct support for theories of generalised bone loss in chondrichthyans.**
43 **Furthermore, they revive theories of a phylogenetically deeper origin of endochondral**
44 **bone and its absence in chondrichthyans as a secondary condition.**

45

46 The vertebrate skeleton comprises two main systems: the exoskeleton (external achondral
47 dermal bones) and endoskeleton (internal chondral bones and cartilages, as well as some
48 intramembranous bones)¹. An ossified exoskeleton evolved at least 450 million years ago in
49 jawless stem gnathostomes^{2,3}, but the endoskeleton in these taxa is not endochondrally
50 ossified. Endochondral bone, in which the cartilaginous endoskeletal precursor is invaded by
51 and eventually replaced by bone, is widely considered an osteichthyan apomorphy³⁻⁷ and
52 such a reliable identifying character gives the group its name. Extant chondrichthyans lack

53 dermal bone and possess a mainly cartilaginous endoskeleton enveloped by a structurally
54 diverse range of tessellate calcified cartilage⁸. Outgroups of the gnathostome crown also lack
55 endochondral ossification. Galeaspid surround their cartilaginous skeleton in globular
56 calcified cartilage{NianZhong:2005tj}, while osteostracan and ‘placoderm’ endoskeletons
57 were sheathed in perichondral bone³. Consequently, the last common ancestor of jawed
58 vertebrates was long thought to have been perichondrally ossified, but lacking endochondral
59 ossification³.

60 In this paper, we describe a new genus and species of ‘placoderm’ from the Early
61 Devonian of western Mongolia. Although Mongolia is known for some of the geologically
62 oldest putative gnathostome fossils (isolated chondrichthyan-like scales⁹⁻¹²), it remains a
63 poorly sampled region of the world with respect to early vertebrates. ‘Placoderms’ were until
64 now known from only a single fragmentary occurrence¹³ in the early Middle Devonian
65 (Eifelian). Our new data highlight the importance of Mongolia as a key region for studies of
66 early gnathostome evolution. We describe a braincase and partial skull roof representing the
67 first substantial body fossil of an early gnathostome from Mongolia and displaying an
68 unexpected occurrence of endochondral bone analysed using XR- μ CT. We conducted
69 phylogenetic analyses to reconstruct the evolutionary relationships of this new taxon. To
70 explore the evolutionary history of endochondral bone in light of this new discovery, we used
71 parsimony and maximum likelihood ancestral states reconstruction. Finally, we discuss these
72 results in the context of earlier statements about endochondral bone in non-osteichthyans,
73 new developments in understanding the complexity and diversity of chondrichthyan
74 endoskeletal tissues, and current uncertainties about early gnathostome phylogenetic
75 relationships.

76

77 **Systematic palaeontology**

78 Gnathostomata Gegenbaur, 1874¹⁴

79 *Minjinia turgenensis* gen. et sp. nov.

80

81 **Etymology.** Generic name honours the memory of Chuluun Minjin for his extensive
82 contributions to the Palaeozoic stratigraphy of Mongolia, his enthusiastic support of this
83 work, and introducing us to the Yamaat River locality. Specific name recognises the
84 provenance of the fossil from the Turgen region, Uvs aimag of western Mongolia.

85

86 **Holotype.** Institute of Paleontology, Mongolian Academy of Sciences MPC-FH100/9.1, a
87 partial braincase and skull roof.

88

89 **Type locality.** Turgen Strictly Protected Area, Uvs province, western Mongolia; near the top
90 of the stratigraphic sequence that occurs between the Tsagaan-Salaat and Yamaat Rivers.

91

92 **Formation and age.** Upper part of Tsagaansalaat Formation, Pragian (Early Devonian) ^{15,16}.

93

94 **Diagnosis.** ‘Placoderm’-grade stem gnathostome with endochondral bone, deep epaxial
95 muscle cavities flanking a slender occipital ridge, and the following possible autapomorphies:
96 dermal bones covered in sparsely placed tubercles, penultimate spino-occipital nerve canal
97 substantially larger in diameter than others.

98

99 **Description**

100 MPC-FH100/9.1 consists of a partial braincase and skull roof (Fig. 1). The skull roof is
101 ornamented with sparsely distributed finely ridged tubercles resembling those of the Siberian
102 ‘placoderm’ *Dolganosteus*¹⁷; the tubercles become more broadly separated towards the

103 midline of the skull. They are distinct from those of *Dolganosteus* in that towards the midline
104 of the skull roof, the tubercles are larger and more pointed. The specimen shows signs of
105 extensive post-mortem transport, with angles of the braincase worn off and much of the skull
106 roof and some of the braincase preserved as a mould. Individual skull roof ossifications
107 cannot be identified, although this may be due to the dominantly mouldic preservation. There
108 appears to have been a prominent nuchal plate eminence comparable to certain
109 acanthothoracids such as *Romundina*¹⁸ and *Arabosteus*¹⁹.

110

111 **Endoskeletal tissue.** The braincase of MPC-FH100/9.1 is well ossified, comprising an
112 external bony sheath filled with an extensive matrix of spongy tissue (Fig. 2a-b; Extended
113 Data Fig. 1; Supplementary Video 1). The trabecles forming this tissue are irregular and
114 branching, less than 1 mm thick and often curved, and resemble most closely the
115 endochondral tissue of osteichthyans (Fig. 2c-d; Supplementary Video 2). As such, we
116 interpret this as endochondral bone. Notably, this is found in all preserved regions of the
117 braincase, in contrast to the isolated trabeculae previously identified as endochondral bone in
118 *Boreaspis*²⁰ and *Bothriolepis*²¹. The margins of the braincase, the endocranial walls, and the
119 boundaries of nerve and blood canals, are formed from a thicker tissue which we interpret as
120 perichondral bone. This suggests that the endoskeleton of *Minjinia* comprises osteichthyan-
121 like endochondral bone, with an ossified perichondrium. To address the possible alternative
122 explanation that it is an aberrant instance of calcified cartilage, we compared the structure of
123 this tissue with rarely-preserved mineralized cartilage in the stem chondrichthyan
124 *Diplacanthus crassismus* (National Museums of Scotland specimen NMS 1891.92.334; Fig.
125 2e-f) observed using synchrotron tomography. The cancellae within the endochondral tissue
126 of *Minjinia* are irregular, with a diameter of approximately 1-2 mm. This tissue is distinctly

127 unlike the calcified cartilage of *Diplacanthus* in appearance, which consists of a densely
128 packed matrix of irregularly stacked chondrons between 20-60 µm in diameter.

129

130 **Neurocranium.** The braincase is preserved from the level of the right posterior orbital wall
131 to the posterior end of the occipital ridge. Occipital glenoid condyles are not preserved, but
132 much of the rest of the broad, flat parachordal region is present, separated by a midline
133 groove that accommodated a relatively narrow notochordal tunnel. An asymmetric transverse
134 fissure spans the basicranial surface at about mid-length of the preserved portion. It appears
135 to demarcate the anterior margin of the parachordal plates and may correspond to the ventral
136 cranial fissure of crown-group gnathostomes. However, unlike in crown gnathostomes, it is
137 traversed by a substantial anterior extension of the cranial notochord. The courses of the
138 lateral dorsal aortae are marked by a pair of sulci on the lateral margins of the parachordal
139 plates, though only a short part of the canal is preserved on the right side of the specimen. A
140 narrow, shallow sulcus for the efferent hyoid artery is present on the preserved right side of
141 the specimen, immediately behind the level of the orbit (Fig. 1a).

142 The lateral surface of the braincase is preserved on the right side as a mouldic
143 impression in the matrix (Fig. 1). A sharply demarcated hyoid fossa is present on the lateral
144 wall of the otic region (Fig. 1). Posterior to this, a stout but pronounced vagal process with a
145 pair of rounded eminences likely corresponds to the branchial arch articulations. There is no
146 evidence for a pair of anterior and posterior divisions to the vagal process, which are
147 typically seen in other ‘placoderms’. A well-developed ‘placoderm’-like craniospinal
148 process is absent; its homologous position is instead covered in perichondral bone and
149 marked by a low ridge (Fig. 1).

150 In posterior view, a tall, narrow median occipital ridge is evident and resembles the
151 morphology of *Romundina*²² and *Arabosteus*¹⁹. Similar to these taxa, the median otic ridge is

152 flanked by two large occipital fossae for the epaxial musculature. The notochordal tunnel is
153 approximately the same size as or smaller than the foramen magnum, as in ‘placoderms’ and
154 in contrast with crown-group gnathostomes. A metotic fissure is absent.

155

156 **Endocast.** A partial cranial endocast is preserved, consisting of the hindbrain cavity, partial
157 midbrain cavity, labyrinth cavities, and posteromedial corner of the orbital region. The two
158 primary trunk canals of the trigeminal nerve (N.V₁ and N.V_{2,3}) are preserved (Fig. 3). The
159 acoustic (N.VIII) and facial nerve (N.VII) canals share a common trunk canal behind the
160 trigeminal nerves, as in many other ‘placoderms’²²⁻²⁵. The facial nerve canal branches into
161 palatal and hyomandibular branches between the saccular chamber and rear orbit wall (Fig. 3;
162 Extended Data Fig. 2), indicating this division was internal (deep) to the otic process. The
163 supraorbital branch opens into the rear wall of the orbit and part of its supraorbital
164 course is preserved (Fig. 3; Extended Data Fig. 2). A slender branch extends below the
165 labyrinth and divides into palatine and hyomandibular branches (Fig 3; Extended Data Fig.
166 2). As in other ‘placoderm’-grade taxa, the vagus nerve (N. X) trunk canal is very large in
167 diameter and exits from immediately behind the labyrinth cavity (Fig. 3). The spino-occipital
168 region resembles other ‘placoderms’ in being extended. At least four spino-occipital nerve
169 canals are present in a linear series, and the penultimate canal is largest in diameter (Fig. 3).
170 Intercalating these is a network of occipital artery canals branching from the dorsal aortae.

171 The skeletal labyrinth is not complete on either side of the specimen, but can mostly
172 be reconstructed according to the assumption of bilateral symmetry. The most significant
173 feature is that the labyrinth and endolymphatic cavity are joined to the main endocavity
174 chamber (Fig. 3). This is a striking contrast to other ‘placoderms’ and closely resembles
175 crown-group gnathostomes²⁶. The endolymphatic canals are elongate and tubular, extending
176 posterolaterally to reach the skull roof, though external openings cannot be clearly identified.

177 The anterior semi-circular canal follows the saccular cavity closely as in petalichthyids²⁷(Fig.
178 3). However, the horizontal and posterior canals appear to extend well away from the
179 saccular chamber (Fig. 3). The dorsal junctions of the anterior and posterior canals are joined
180 in a crus commune, as in *Romundina*²² and *Jagorina*²³. A sinus superior is absent.

181

182 **Phylogenetic analyses**

183 We conducted phylogenetic analyses under four different protocols: equal weights
184 parsimony, implied weights parsimony, an unpartitioned Bayesian analysis, and a Bayesian
185 analysis with characters partitioned by fit determined under implied weights parsimony²⁸ (see
186 Extended Data Figs. 3-6). All phylogenetic analyses consistently place *Minjinia* as a stem-
187 group gnathostome, proximate to the gnathostome crown (Fig. 4, Extended Data Figs 3, 4).
188 *Minjinia* is recovered in a position crownward of arthrodires but outside of a grade consisting
189 of *Entelognathus*, *Ramirosuarezia*, and *Janusiscus*. Under implied weights parsimony, these
190 three taxa move onto the osteichthyan stem and *Minjinia* is placed as the immediate sister
191 taxon of the gnathostome crown. Under parsimony, the crownward position of *Minjinia* is
192 unambiguously supported by the skeletal labyrinth and endolymphatic duct being confluent
193 with the main cranial cavity²⁶ (Supplementary Information). In common with arthrodires and
194 the gnathostome crown²⁹, *Minjinia* possesses a division of the facial nerve(Fig. 3; Extended
195 Data Fig. 2) deep to the transverse otic process. However, *Minjinia* is excluded from the
196 gnathostome crown group due to the absences of a metotic fissure and a posterior dorsal
197 fontanelle, and presence of broad, flat parachordal plates expanded behind the saccular cavity
198 (Fig. 3, Supplementary Information).

199 We undertook ancestral states reconstructions to assess the evolutionary history of
200 endochondral bone (Fig. 4; Extended Data Figs. 5 & 6; Supplementary Information).

201 Interestingly, parsimony analysis fails to recover secondary homology of this trait between

202 *Minjinia* and osteichthyans. The crownward placement of *Minjinia* is, in fact, based on
203 independent evidence relating to anatomical features of the braincase and endocast. However,
204 the resolution becomes ambiguous if missing data in either *Entelognathus* or *Ramirosuarezia*
205 are resolved as having endochondral bone. The reconstruction becomes similarly ambiguous
206 if *Janusiscus* is moved a single branch (requiring only two additional steps) onto the
207 chondrichthyan stem. The strict precision of parsimony reconstructions makes it insensitive
208 to this underlying uncertainty. To explore this, we used likelihood reconstructions and
209 compared the ancestral state reconstructions under equal rates (ER) and all rates different
210 (ARD) variants of the Mkv model on branch-length-rescaled parsimony trees and Bayesian
211 trees. Both models show substantial non-zero marginal likelihoods if endochondral bone is
212 assumed present in the common node of *Minjinia* and Osteichthyes, with ARD strongly
213 favouring its presence (0.33 for ER; 0.81 for ARD; Fig. 3, Table 1, Extended Data Figs. 5, 6,
214 Supplementary Table 1). Under the ARD model, there is nearly equivocal support for
215 presence or absence of endochondral bone at the gnathostome crown node (Table 1). The
216 ARD model shows the best fit for endochondral bone (likelihood ratios 4.75 for parsimony [p
217 = 0.029] and 5.26 for Bayesian, [p = 0.022]) (Table 1, Supplementary Table 1), favouring
218 repeated losses of this tissue over multiple gains (see Discussion).

219

220 **Discussion**

221 *Minjinia turgenensis* presents an unexpected discovery of extensive endochondral bone in a
222 ‘placoderm’-grade fish, with repercussions for the phylogenetic origin of this tissue and the
223 problem of early gnathostome relationships more generally. The prevailing hypothesis has
224 been that endochondral bone is an osteichthyan apomorphy^{3,7,29}. However, recent discoveries
225 have cast doubt on this assertion. The recognition that dermal bone is secondarily lost in
226 chondrichthyans^{30,31} (Fig. 4) is consonant with prior knowledge of the loss of perichondral

227 bone in this same lineage³². Taken together, this has revived uncertainty about the true
228 phylogenetic timing of the origin of endochondral ossification³³. *Minjinia* provides direct
229 corroboration for a more ancient origin.

230 *Minjinia* does not represent the first report of endochondral bone outside of
231 Osteichthyes. However, it is by far the most extensive and unequivocal example and raises
232 explicit questions in light of the proximity of *Minjinia* to the gnathostome crown (Fig. 4;
233 Extended Data Figs. 3, 4). Isolated examples of trabecular endoskeletal bone have historically
234 been reported in boreaspid osteostracans^{20,34}, a rhenanid³⁵, arthrodires³⁶, a ptyctodont³⁷, and a
235 petalichthyid^{38,39}. However, these reports are nearly all unillustrated statements; they have all
236 been considered tenuous³ or dismissed as misidentifications⁵. In line with these assessments,
237 we found no evidence of endochondral bone in material of *Buchanosteus* held in the Natural
238 History Museum, London, or indeed in any other ‘placoderms’ we have examined. The
239 *Epipetalichthys* holotype (Museum für Naturkunde, Berlin specimen MB.f.132.1-3) shows an
240 apparently spongiose infilling in the anterior region of the braincase, but the identity of this
241 structure, or even whether it is biological, cannot be determined. The *Epipetalichthys* tissue
242 figured by Stensiö³⁸ was very superficial, and possibly represents the retreat of perichondral
243 bone deposited during cartilage growth³⁹. Most recently, trabeculae in supposed endoskeletal
244 pelvic bones of *Bothriolepis* have been termed endochondral bone²¹, although the small scale
245 of these is in line with ‘superficial’ perichondral trabeculae seen elsewhere³⁸. The reported
246 examples in boreaspid osteostracans have also been dismissed by later authors^{3,5}. Although
247 they warrant further study, their tissue structures are unlikely to be homologous to
248 osteichthyans owing to their phylogenetic remoteness and nested position in the
249 Osteostraci⁴⁰.

250 Among chondrichthyans, endochondral bone has been mentioned in ‘acanthodians’^{3,41}
251 and superficial bone-like tissues have been reported in the skeletons of extant

252 chondrichthyans. We are unable to substantiate statements about acanthodians: no authors
253 have cited primary sources or specimens. One possible source is Watson's⁴² description of
254 "massive ossification" of the endoskeleton of *Diplacanthus*. However, our synchrotron data
255 of this same specimen (Fig. 2) shows that this tissue is undoubtedly calcified cartilage. Some
256 authors have speculated that the superficial mineralised tissue in the jaws of acanthodians or
257 chondrichthyans may have developed in an endochondral position³⁹. Histological studies
258 show that endoskeletal mineralization in the jaws of acanthodians is globular calcification
259 and occasionally 'sub-tessellate'^{8,43}. Recent comparative histology and development in extant
260 chondrichthyans has shown the presence of an extensive canalicular network in the tesserae⁴⁴
261 and a trabecular tesseral network in some vertebral elements⁴⁵, both resembling bone.
262 Whether these represent homologues of osteichthyan examples remains open to debate;
263 future works could employ synchrotron microtomography of stem-chondrichthyan cartilages
264 to address these questions.

265 Does endochondral bone have a deep origin within the gnathostome stem group? This
266 would imply repeated losses of this tissue. We do find statistical support for this hypothesis
267 (Fig. 4, Table 1, Extended Data Figs. 5, 6, Supplementary Table 1), and the model is well
268 justified on prior phylogenetic and biological grounds. Endochondral bone has long been
269 known to be inconsistently developed across 'primitive' bony fishes: incomplete,
270 polymorphic, or entirely absent ossification of the endoskeleton is known in both Palaeozoic
271 actinopterygians^{41,46,47} and sarcopterygians {Cloutier:wm}, as well as more recent taxa⁴⁸. The
272 frequent absence of endochondral bone in osteichthyans is considered secondary, and other
273 controlling factors such as body size, maturity, mechanical stress, and buoyancy can
274 determine its degree of development¹. Our findings are also in agreement with studies
275 establishing a genetic basis for secondary loss of all bone types within chondrichthyans⁴⁹⁻⁵¹,

276 with the failure to produce endochondral bone likely representing arrested development of
277 chondrocytes as opposed to a primary lack of ability⁵².

278 Another confounding factor in this question is the problem of ‘placoderm’
279 relationships. Although currently resolved in most analyses as a deeply pectinate grade along
280 the gnathostome stem (Fig. 4), the backbone of this arrangement has poor statistical support,
281 even in the present analysis (Extended Data Figs. 3). There is a lack of consistency in the
282 arrangement of plesia and Bayesian tip-dating methods have even recovered a monophyletic
283 Placodermi⁵³. *Minjinia* itself highlights this uncertainty, given its highly unexpected character
284 combinations. Notwithstanding its endochondral bone and crown-gnathostome-like inner ear
285 structure, it resembles ‘acanthothoracids’—the ‘placoderms’ widely considered among the
286 most removed from the gnathostome crown (i.e. most ‘primitive’): it possesses deep epaxial
287 fossae either side of a prominent occipital ridge and a nuchal eminence otherwise seen only
288 in acanthothoracids such as *Romundina*¹⁸ and *Arabosteus*¹⁹. This apparent character conflict
289 could perhaps be more easily reconciled with a more coherent (though not necessarily
290 monophyletic) ‘placoderm’ assemblage. Indeed, the highly pectinate structure of the
291 ‘placoderm’ grade seems symptomatic of an overemphasis on characters and taxa resembling
292 the crown group, thereby undersampling characters that could stabilise a clear picture of
293 ‘placoderm’ interrelationships.

294 *Minjinia turgenensis* reveals new data on ‘placoderm’ endoskeleton and tissue
295 diversity recorded from Mongolia—an otherwise extremely poorly known biogeographic
296 realm for early gnathostomes. The phylogenetic placement of this ‘acanthothoracid’-like
297 taxon crownward of all non-maxillate ‘placoderms’, in conjunction with possession of
298 extensive endochondral bone, highlights the importance of material from traditionally
299 undersampled geographic areas. The presence of endochondral bone renews the hypothesis
300 that this tissue is evolutionarily ancient and was lost secondarily in chondrichthyans^{6,33}. This

301 view is overall consistent with evidence of generalised bone loss in chondrichthyans,
302 potentially as a result of the suppression of bone-generating molecular genetic pathways^{51,52}.
303 Continued work in Mongolia and re-evaluation of phylogenetic datasets will be necessary to
304 address this, with the results likely to lead to substantial re-evaluation of gnathostome
305 phylogeny.

306

307

308 **Methods**

309

310 **X-ray computed microtomography.** We scanned MPC-FH100/9.1 using the Nikon XT
311 225s at the Museum of Paleontology, University of Michigan with the following parameters:
312 200kV, 140 μ A, over 3123 projections and a voxel size of 32.92 μ m. We conducted
313 segmentation using Mimics 19.0 (<http://biomedical.materialise.com/mimics>; Materialise,
314 Leuven, Belgium) and we imaged models for publication using Blender
315 (<https://www.blender.org>).

316 **Synchrotron light propagation phase contrast tomography.** We imaged *Diplacanthus*
317 *crassismus* specimen NMS 1891.92.334 on Beamline 19 of the European Synchrotron
318 Radiation Facility, using propagation phase-contrast synchrotron microtomography. We
319 performed a spot scan with an energy of 116keV, achieving a voxel size of 0.55 μ m. We
320 processed the resulting tomograms using VG StudioMax 2.2 (Volume Graphics, Germany),
321 and prepared images in Blender.

322 **Phylogenetic analysis.** We conducted a parsimony analysis using TNT 1.5⁵⁴ and Bayesian
323 analysis using MrBayes v 3.2.7⁵⁵. The dataset consisted of 95 taxa and 284 discrete
324 characters based on a pre-existing dataset⁵⁶. We employed Osteostraci and Galeaspida as
325 composite outgroups. We conducted parsimony analysis using both equal weights and

326 implied weights methods. Global settings were 1000 search replicates and a hold of up to 1
327 million trees. Equal weights parsimony analyses were conducted using the ratchet with
328 default settings. Implied weights parsimony used a concavity parameter of 3 and the search
329 was without the ratchet. Command lists are included in Supplementary Information. We
330 conducted Bayesian analysis using both a partitioned and unpartitioned dataset. We used the
331 Mkv model⁵⁷ and gamma rate distribution. We ran the analyses for 5 million generations with
332 a relative burn-in fraction of 0.25. Runs were checked for convergence using Tracer⁵⁸. We
333 partitioned the dataset using a newly proposed method²⁸ that partitions the data according to
334 homoplasy levels. Using the results of implied weights parsimony conducted in TNT, we
335 created a text table of character fit values. We wrote an R⁵⁹ script to generate a list of
336 partition commands for MrBayes.

337 We assessed parsimony ancestral states visually using Mesquite⁶⁰. Likelihood and
338 Bayesian ancestral states were estimated in R using the castor package⁶¹ version 1.5.7. Prior
339 to calculating likelihood ancestral states on parsimony trees, we scaled branch lengths using
340 PAUP*⁶² and calculated the likelihood scores for all of the trees under the Mkv model with
341 gamma rate parameter. The trees were then exported with branch lengths. To account for
342 overall uncertainty in tree estimates, we estimated ancestral states on 100 trees randomly
343 selected from the fundamental set of most parsimonious trees and two times 50 trees selected
344 from the 75% last trees of each posterior tree distribution from the Bayesian analysis. We
345 then run an ancestral states estimation Mk model (using the castor R package) using both the
346 Equal Rates (ER) and All Rates Different (ARD) models. This resulted in 400 ancestral states
347 estimations. For each estimation we extracted the overlap log likelihood, the AIC (counting
348 one parameter for the ER model and two for the ARD model) and the scaled log likelihood
349 (probability) for the presence and absence of the endochondral bone character (character 4)
350 for the last common node of *Minjinia* and crown-group gnathostomes. We present the median

351 value of these distributions of the estimations overall log likelihoods, AICs and presence or
352 absence of endochondral bone in Table 1.

353

354 **Data availability**

355 The holotype specimen of *Minjinia turgenensis* will be permanently deposited in the
356 collections of the Institute of Paleontology, Mongolian Academy of Sciences. Original
357 tomograms are available at (doi:10.6084/m9.figshare.12301229) and rendered models are
358 available at (doi:10.6084/m9.figshare.12301223). The phylogenetic character list and dataset
359 are available as Supplementary Information S1 and S2. The LifeScience Identifier for
360 *Minjinia turgenensis* is urn:lsid:zoobank.org:act:82A1CEEC-B990-47FF-927A-
361 D2F0B59AEA87

362

363 **Code availability**

364 R code for generating partitions based on character fits and code for likelihood ancestral
365 states reconstructions and plots are available in the Supplementary Information.

366

367 **References**

- 368 1. Hall, B. K. *Bones and Cartilage*. (Academic Press, 2005).
- 369 2. Janvier, P. *Early Vertebrates*. (Oxford University Press, 1996).
- 370 3. Donoghue, P. C. J., Sansom, I. & Downs, J. P. Early evolution of vertebrate skeletal
371 tissues and cellular interactions, and the canalization of skeletal development. *Journal*
372 *of Experimental Zoology* **306B**, 278–294 (2006).
- 373 4. Rosen, D. E., Forey, P. L., Gardiner, B. G. & Patterson, C. Lungfishes, tetrapods,
374 paleontology, and plesiomorphy. *Bull Am Mus Nat Hist* **167**, 159–276 (1981).

- 375 5. Gardiner, B. G. The relationships of the palaeoniscid fishes, a review based on new
376 specimens of *Mimia* and *Moythomasia* from Upper Devonian of Western Australia.
377 *Bull Br Mus nat Hist (Geol)* **37**, 173–428 (1984).
- 378 6. Maisey, J. G. Heads and tails: a chordate phylogeny. *Cladistics* **2**, 201–256 (1986).
- 379 7. Friedman, M. & Brazeau, M. A Reappraisal of the origin and basal radiation of the
380 Osteichthyes. *J Vertebr Paleontol* **30**, 36–56 (2010).
- 381 8. Maisey, J. G., Denton, J. S. S., Burrow, C. & Pradel, A. Architectural and
382 ultrastructural features of tessellated calcified cartilage in modern and extinct
383 chondrichthyan fishes. *Journal of Fish Biology* **15**, 37–23 (2020).
- 384 9. Karatajūtė-Talimaa, V. & Novitskaya, L. *Sodolepis*—a new representative of
385 Mongolepidida (Chondrichthyes?) from the lower Silurian of Mongolia.
386 *Paleontologicheskii Zhurnal* **5**, 96–103 (1997).
- 387 10. Karatajūtė-Talimaa, V. & Novitskaya, L. *Teslepis*—a new representative of
388 mongolepid elasmobranchs from the Lower Silurian of Mongolia. *Paleontologicheskii*
389 *Zhurnal* **4**, 36–43 (1992).
- 390 11. Karatajūtė-Talimaa, V., Novitskaya, L. & Rozman, K. S. *Mongolepis*—a new lower
391 Silurian genus of elasmobranchs from Mongolia. *Paleontologicheskii Zhurnal* **1**, 76–
392 86 (1990).
- 393 12. Andreev, P. *et al.* The systematics of the Mongolepidida (Chondrichthyes) and the
394 Ordovician origins of the clade. *PeerJ* **4**, e1850–e1850 (2016).
- 395 13. Vorobjeva, E. I. & Janvier, P. Observations to the note by E Vorobjeva: ‘First
396 discovery of Palaeozoic fishes on the territory of the Mongolian Republic’.
397 *Palaeontology and Biostratigraphy of Mongolia*, Moscow, 3, 134–136 (in Russian).
398 *Geobios* **17**, 379–380 (1984).

- 399 14. Gegenbaur, C. *Grundriss der vergleichenden Anatomie*. 1–680 (Wilhelm Engelmann,
400 1874).
- 401 15. Alekseeva, R. E., Mendbayar, B. & Erlanger, O. A. *Brachiopods and Biostratigraphy*
402 *of the Lower Devonian of Mongolia*. **16**, (Nauka, 1981).
- 403 16. Alekseeva, R. E. *Devonian Biostratigraphy of Mongolia*. (Nauka, 1993).
- 404 17. Mark-Kurik, E. in *Morphology, Phylogeny and Paleobiogeography of Fossil Fishes*
405 (eds. Elliott, D. K., Maisey, J., Yu, X. & Miao, D.) 101–106 (2010).
- 406 18. Ørvig, T. Description, with special reference to the dermal skeleton, of a new radotinid
407 arthrodire from the Gedinnian of Arctic Canada. *Colloque international C.N.R.S. no.*
408 *218* 43–71 (1975).
- 409 19. Olive, S., Goujet, D., Lelièvre, H. & Janjou, D. A new Placoderm fish
410 (Acanthothoraci) from the Early Devonian Jauf Formation (Saudi Arabia).
411 *Geodiversitas* **33**, 393–409 (2011).
- 412 20. Wängsjö, G. The Downtonian and Devonian vertebrates of Spitsbergen. IX. *Norsk*
413 *Polarinstitut Skrifter* **97**, 1–611 (1952).
- 414 21. Charest, F., Johanson, Z. & Cloutier, R. Loss in the making: absence of pelvic fins and
415 presence of paedomorphic pelvic girdles in a Late Devonian antiarch placoderm
416 (jawed stem-gnathostome). *Biology Letters* **14**, 20180199 (2018).
- 417 22. Dupret, V., Sanchez, S., Goujet, D. & Ahlberg, P. E. The internal cranial anatomy of
418 *Romundina stellina* Ørvig, 1975 (Vertebrata, Placodermi, Acanthothoraci) and the
419 origin of jawed vertebrates—Anatomical atlas of a primitive gnathostome. *PLoS ONE*
420 **12**, e0171241 (2017).
- 421 23. Stensiö, E. A. La cavité labyrinthique, l'ossification sclérotique et l'orbite de *Jagorina*.
422 *Colloques internationaux du Centre national de la Recherche scientifique* **21**, 9–43
423 (1950).

- 424 24. Stensiö, E. A. Anatomical studies on the arthrodiran head. *K Sven Vetenskapsakad*
425 *Handl* **9**, 1–419 (1963).
- 426 25. Goujet, D. *Les poissons placodermes du Spitsberg*. (Cahiers de Paléontologie, Editions
427 du CNRS, 1984).
- 428 26. Davis, S. P., Finarelli, J. A. & Coates, M. I. *Acanthodes* and shark-like conditions in
429 the last common ancestor of modern gnathostomes. *Nature* **486**, 247–250 (2012).
- 430 27. Castiello, M. & Brazeau, M. D. Neurocranial anatomy of the petalichthyid placoderm
431 *Shearsbyaspis oepiki* Young revealed by X-ray computed microtomography.
432 *Palaeontology* **61**, 369–389 (2018).
- 433 28. Rosa, B. B., Melo, G. A. R. & Barbeitos, M. S. Homoplasy-based partitioning
434 outperforms alternatives in Bayesian analysis of discrete morphological data.
435 *Systematic Biology* **54**, 373 (2019).
- 436 29. Brazeau, M. D. & Friedman, M. The characters of Palaeozoic jawed vertebrates. *Zool*
437 *J Linn Soc* **170**, 779–821 (2014).
- 438 30. Zhu, M. *et al.* A Silurian placoderm with osteichthyan-like marginal jaw bones. *Nature*
439 **502**, 188–193 (2013).
- 440 31. Giles, S., Friedman, M. & Brazeau, M. D. Osteichthyan-like cranial conditions in an
441 Early Devonian stem gnathostome. *Nature* **520**, 82–85 (2015).
- 442 32. Miles, R. S. in *Interrelationships of Fishes* (eds. Greenwood, P. H., Miles, R. S. &
443 Patterson, C.) 63–103 (Academic Press London, 1973).
- 444 33. Zhu, M. Bone gain and loss: insights from genomes and fossils. *Natl Sci Rev* **1**, 490–
445 492 (2014).
- 446 34. Janvier, P. *Les céphalaspides du Spitsberg*. (Éditions du Centre National de la
447 Recherche Scientifique, 1985).

- 448 35. Ørvig, T. Histologic studies of placoderms and fossil elasmobranchs. *Arkiv för Zoologi*
449 **2**, 321–454 (1951).
- 450 36. Young, G. C. New information on the structure and relationships of *Buchanosteus*
451 (Placodermi: Euarthrodira) from the Early Devonian of New South Wales. *Zool J Linn*
452 *Soc* **66**, 309–352 (1979).
- 453 37. Miles, R. S. & Young, G. C. in *Problems in Vertebrate Evolution* (eds. Andrews, S.
454 M., Miles, R. S. & Walker, A. D.) 123–198 (Academic Press, 1977).
- 455 38. Stensiö, E. A. On the head of the macropetalichthyids. *Field Museum of Natural*
456 *History Publication Geological Series* **4**, 87–197 (1925).
- 457 39. Ørvig, T. Notes on some Paleozoic lower vertebrates from Spitsbergen and North
458 America. *Norsk Geologisk Tidsskrift* **37**, 285–353 (1957).
- 459 40. Sansom, R. Phylogeny, classification and character polarity of the Osteostraci
460 (Vertebrata). *Journal of Systematic Palaeontology* **7**, 95–115 (2009).
- 461 41. Schaeffer, B. The braincase of the holostean fish *Macrepistius*, with comments on
462 neurocranial ossification in the Actinopterygii. *American Museum Novitates* **2459**, 1–
463 34 (1971).
- 464 42. Watson, D. M. S. The acanthodian fishes. *Philosophical Transactions of the Royal*
465 *Society of London. Series B, Biological Sciences* **228**, 49–146 (1937).
- 466 43. Burrow, C. J., Davidson, R. G., Blaauwen, den, J. L. & Newman, M. J. Revision of
467 *Climatius reticulatus* Agassiz, 1844 (Acanthodii, Climatidae), from the Lower
468 Devonian of Scotland, based on new histological and morphological data. *J Vertebr*
469 *Paleontol* **35**, e913421 (2015).
- 470 44. Dean, M. N., Socha, J. J., Hall, B. K. & Summers, A. P. Canaliculi in the tessellated
471 skeleton of cartilaginous fishes. *Journal of Applied Ichthyology* **26**, 263–267 (2010).

- 472 45. Atake, O. J., Cooper, D. M. L. & Eames, B. F. Bone-like features in skate suggest a
473 novel elasmobranch synapomorphy and deep homology of trabecular mineralization
474 patterns. *Acta Biomaterialia* **84**, 424–436 (2019).
- 475 46. Pearson, D. M. & Westoll, T. S. The Devonian actinopterygian *Cheirolepis* Agassiz.
476 *Transactions of the Royal Society of Edinburgh: Earth Sciences* **70**, 337–399 (1979).
- 477 47. Giles, S. *et al.* Endoskeletal structure in *Cheirolepis* (Osteichthyes, Actinopterygii), An
478 early ray-finned fish. *Palaeontology* **58**, 849–870 (2015).
- 479 48. Grande, L. & Bemis, W. E. Osteology and Phylogenetic Relationships of Fossil and
480 Recent Paddlefishes (Polyodontidae) with Comments on the Interrelationships of
481 Acipenseriformes. *Memoir (Society of Vertebrate Paleontology)* **11 (Supplement to**
482 **Number 1)**, 1–121 (1991).
- 483 49. Eames, B. F. *et al.* Skeletogenesis in the swell shark *Cephaloscyllium ventriosum*.
484 *Journal of Anatomy* **210**, 542–554 (2007).
- 485 50. Venkatesh, B. *et al.* Elephant shark genome provides unique insights into gnathostome
486 evolution. *Nature* **505**, 174–179 (2014).
- 487 51. Ryll, B., Sanchez, S., Haitina, T., Tafforeau, P. & Ahlberg, P. E. The genome of
488 *Callorhinchus* and the fossil record: a new perspective on SSCP gene evolution in
489 gnathostomes. *Evolution & Development* **16**, 123–124 (2014).
- 490 52. Marconi, A., Hancock-Ronemus, A. & Gillis, J. A. Adult chondrogenesis and
491 spontaneous cartilage repair in the skate, *Leucoraja erinacea*. *eLife Sciences* **9**, 2813
492 (2020).
- 493 53. King, B., Qiao, T., Lee, M. S. Y., Zhu, M. & Long, J. A. Bayesian morphological
494 clock methods resurrect placoderm monophyly and reveal rapid early evolution in
495 jawed vertebrates. *Systematic Biology* **66**, 499–516 (2016).

- 496 54. Goloboff, P. A. & Catalano, S. A. TNT version 1.5, including a full implementation of
497 phylogenetic morphometrics. *Cladistics* **32**, 221–238 (2016).
- 498 55. Ronquist, F. & Huelsenbeck, J. P. MrBayes 3: Bayesian phylogenetic inference under
499 mixed models. *Bioinformatics* **19**, 1572–1574 (2003).
- 500 56. Clement, A. M. *et al.* Neurocranial anatomy of an enigmatic Early Devonian fish sheds
501 light on early osteichthyan evolution. *eLife Sciences* **7**, e34349 (2018).
- 502 57. Lewis, P. O. A likelihood approach to estimating phylogeny from discrete
503 morphological character data. *Systematic Biology* **50**, 913 (2001).
- 504 58. Rambaut, A., Drummond, A. J., Xie, D., Baele, G. & Suchard, M. A. Posterior
505 summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* **67**,
506 901–904 (2018).
- 507 59. R Core Team. *R: A language and environment for statistical computing*. (R
508 Foundation for Statistical Computing, 2019).
- 509 60. Maddison, D. R. & Maddison, W. P. *Mesquite*. (2019).
- 510 61. Louca, S. & Doebeli, M. Efficient comparative phylogenetics on large trees.
511 *Bioinformatics* **34**, 1053–1055 (2017).
- 512 62. Swofford, D. L. *PAUP**. 4.0a166, (2019).

513

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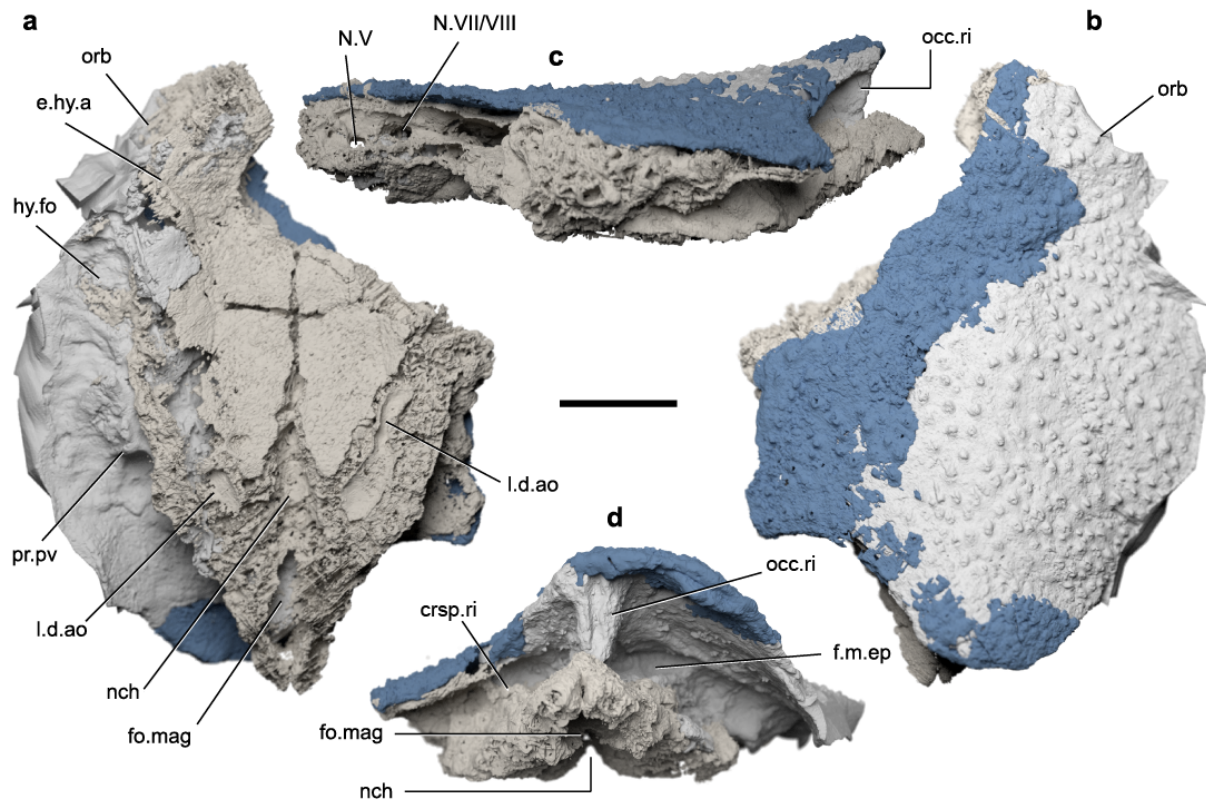
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531

532 **Author Contributions:** MDB conceived and designed the study. MDB, AJ, YAA, and EZ
533 participated in all field seasons. RPD and AJ undertook preliminary CT scanning and
534 segmentation that revealed the fossil was a 'placoderm' and had endochondral bone. RS
535 discovered the first vertebrate remains in the first field season at Yamaat Gol in 2010. SG
536 undertook the segmentation of *Minjinia* with input from MDB. AJ performed segmentation
537 of *Diplacanthus* tissue. MC provided input on occipital comparative morphology of
538 'placoderms'. RPD provided data and comparative analyses and data for endoskeletal tissue.
539 YAA provided background on the geology, palaeontology, and stratigraphy of the type
540 location; EZ and YAA organized field logistics and permitting. MDB, SG, MC, RPD, and AJ
541 undertook the anatomical interpretation and prepared the figures. MDB and SG conducted the
542 phylogenetic analyses. RS conducted the parsimony branch support analyses. TG wrote the
543 script for generating MrBayes partitions from TNT's character fits table and conducted the
544 likelihood and model-fitting analyses. The manuscript was written by MDB, RPD, and SG.
545

546 **Competing interests statement:**

547 The authors declare no competing interests.



548

549 **Fig. 1 | MPC-FH100/9.1 a 'placoderm' skull roof and braincase from the Early**

550 **Devonian of Mongolia. a, Ventral view. b, Dorsal view. c, Left lateral view. d, Posterior**

551 **view. e, Braincase endocavity in dorsal view. Taupe: endoskeleton; grey: mould; blue:**

552 **exoskeleton. crsp.ri, craniospinal ridge; e.hy.a, sulcus for the efferent hyoid artery; f.m.ep,**

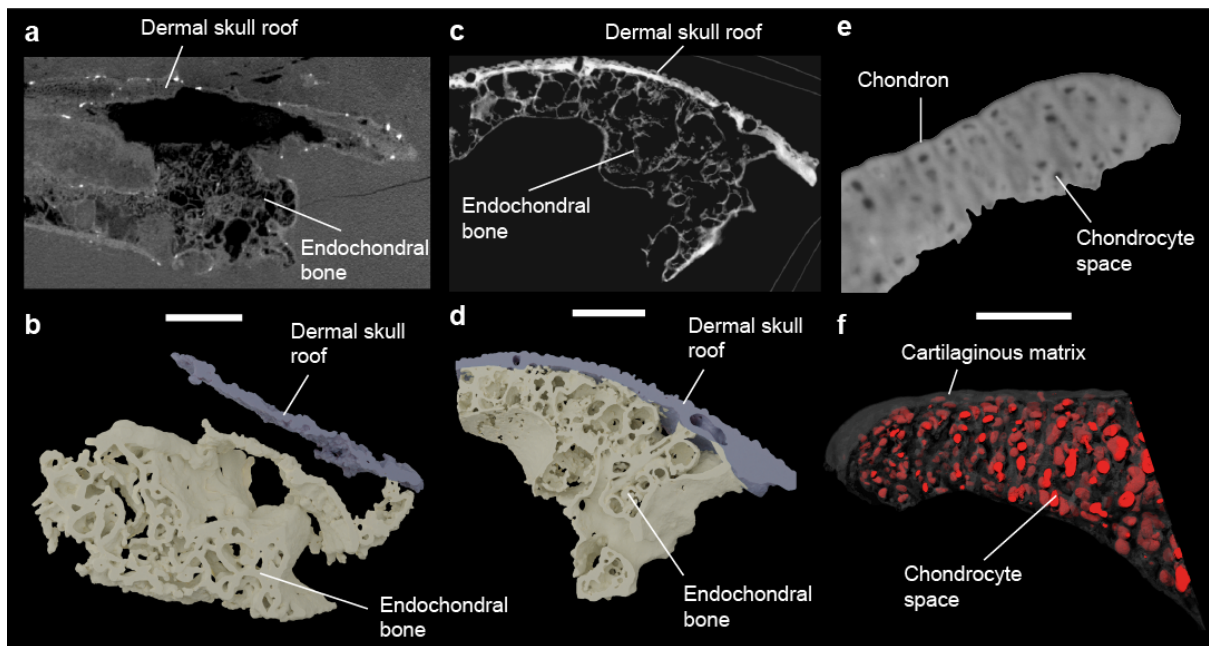
553 **epaxial muscle fossa; fo.mag., foramen magnum; hy.fo, hyodean fossa; l.d.ao, sulcus for the**

554 **lateral dorsal aorta; N.V, trigeminal nerve canal; N.VII, facial nerve canal; N.VIII, acoustic**

555 **nerve canal; nch, notochordal canal; occ.ri, occipital ridge; orb, orbit; pr.pv, paravagal**

556 **process. Scale bar, 10 mm.**

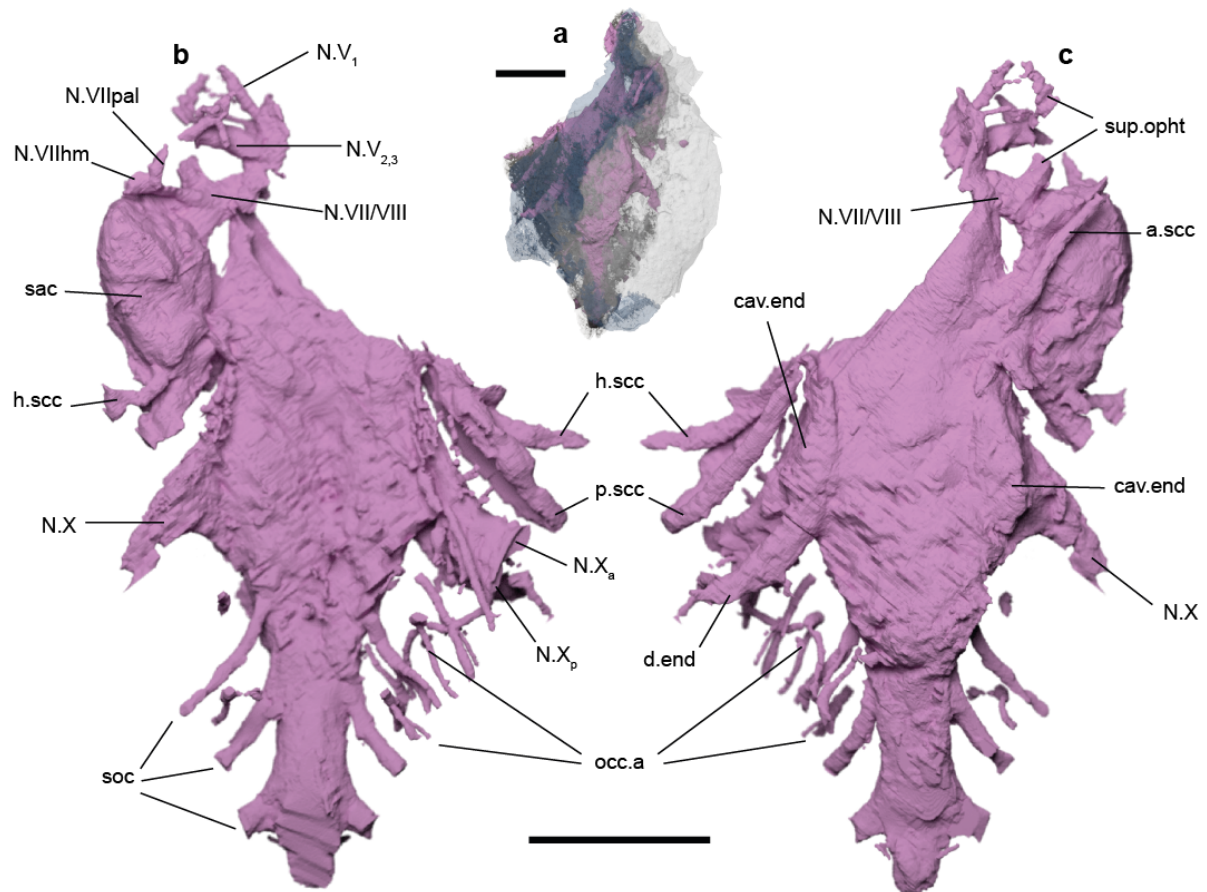
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558

559 **Fig. 2 | Endoskeletal mineralisation in fossil gnathostomes.** **a**, Transverse tomographic
 560 slice through MPC-FH100/9.1. **b**, Three-dimensional rendering of trabecular bone structure.
 561 **c**, Transverse tomographic section through the braincase of the osteichthyan *Ligulalepis*. **d**,
 562 Three-dimensional rendering of the trabecular bone in *Ligulalepis* (**c** and **d** use data from⁵⁶).
 563 **e**, Synchrotron tomography image of the calcified cartilage of the certatohyal of the stem-
 564 group chondrichthyan *Diplacanthus crassisimus* specimen NMS 1891.92.334. **f**, Semi-
 565 transparent three-dimensional structure of calcified cartilage of NMS 1891.92.334. Scale
 566 bars, **a** and **b**, 10 mm; **c** and **d**, 1 mm; **e** and **f**, 150 μ m.

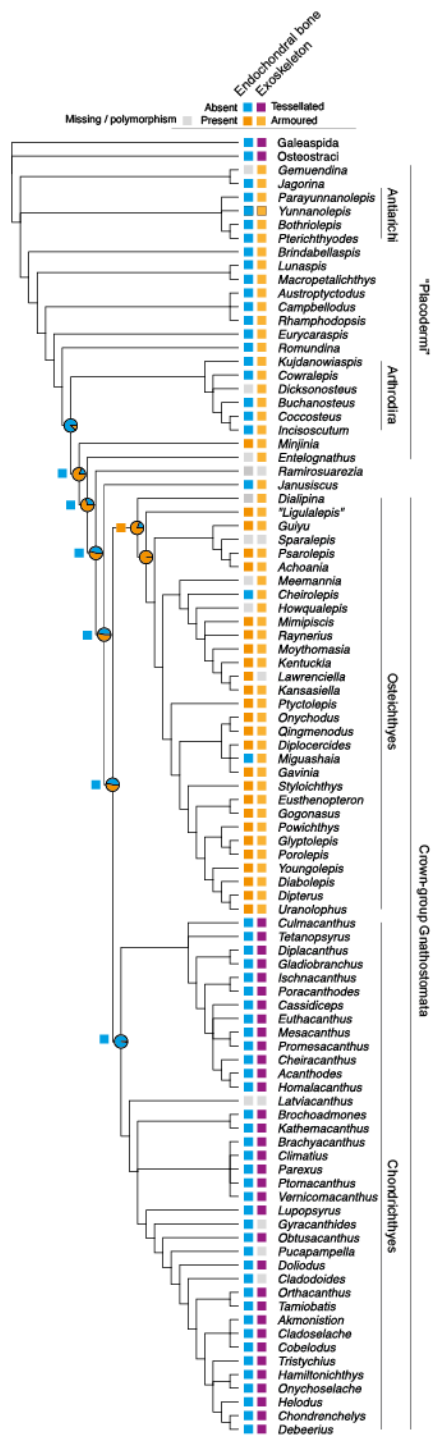
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568

569 **Fig. 3 | Braincase endocavity of *Minjinia*.** **a**, Semi-transparent rendering of skull roof and
 570 braincase (grey and blue) showing extent of endocavity (pink). **b**, Ventral view. **c**, Dorsal
 571 view. a.scc, anterior semicircular canal; cav.end, endolymphatic cavity; d.end, endolymphatic
 572 duct; h.scc, horizontal semicircular canal; N.V, trigeminal nerve canal; N.VIIhm,
 573 hyomandibular branch of facial nerve canal; N.VIIpal, palatine branch of facial nerve canal;
 574 N.VIII, acoustic nerve canal; N.X, vagus nerve canal, N.X_a, anterior branch of vagus nerve
 575 canal; N.X_p, posterior branch of vagus nerve canal; occ.a, occipital artery canals; p.scc,
 576 posterior semicircular canal; sac, sacculus; soc, spino-occipital nerve canals; sup.opht, canal
 577 for supra-ophthalmic nerve. Scale bars, 10 mm (upper scale bar associates with **a**, lower scale
 578 bar associates with **b** and **c**).

579



580

581 **Fig. 4 | Strict consensus tree from parsimony analysis of early gnathostomes showing**
 582 **distribution of endochondral bone and exoskeletal armour.** Squares at nodes indicate
 583 parsimony reconstruction for endochondral bone. Pie charts at nodes show likelihood
 584 reconstructions for the same character under the all-rates-different model (see Extended Data
 585 Figs 6 & 7 for competing reconstructions). Grey box indicates uncertainty. Loss of

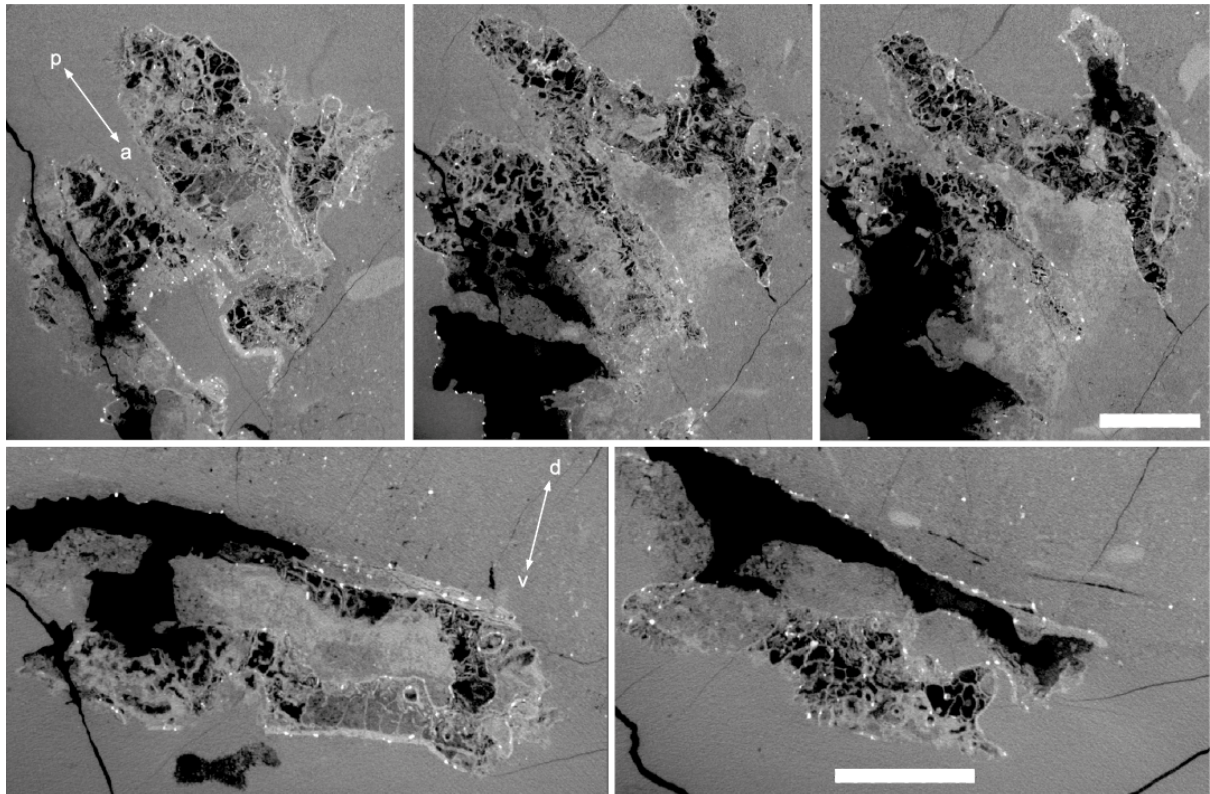
586 endochondral bone maps closely with generalised loss of bone in chondrichthyans where
 587 exoskeletal armour and perichondral bone are also absent.

588

589 **Table 1 | Tree distribution (n=100) ancestral states estimation results.** ER = Equal rates
 590 model; ARD = All Rates Different model. The columns AIC and log.like represent the
 591 median AIC and log.lik across the 100 parsimony and Bayesian trees (for both models). The
 592 like.ratio column is the likelihood ratio for the models compared on these trees. The columns
 593 Absent and Present represent the median scaled likelihood for the endochondral bone state.

trees	model	log.like.	like.ratio	AIC	node	Absent	Present
Parsimony (equal weights)	ER	-28.91	4.74	59.82	Minjinia:Gnathostomes	0.67	0.33
	ARD	-26.54		57.09		0.19	0.81
	ER				Crown Gnathostomes	0.91	0.09
	ARD					0.46	0.54
Bayesian (unpartitioned)	ER	-29.66	5.26	61.32	Minjinia:Gnathostomes	0.73	0.27
	ARD	-27.03		58.06		0.17	0.83
	ER				Crown Gnathostomes	0.79	0.21
	ARD					0.22	0.78

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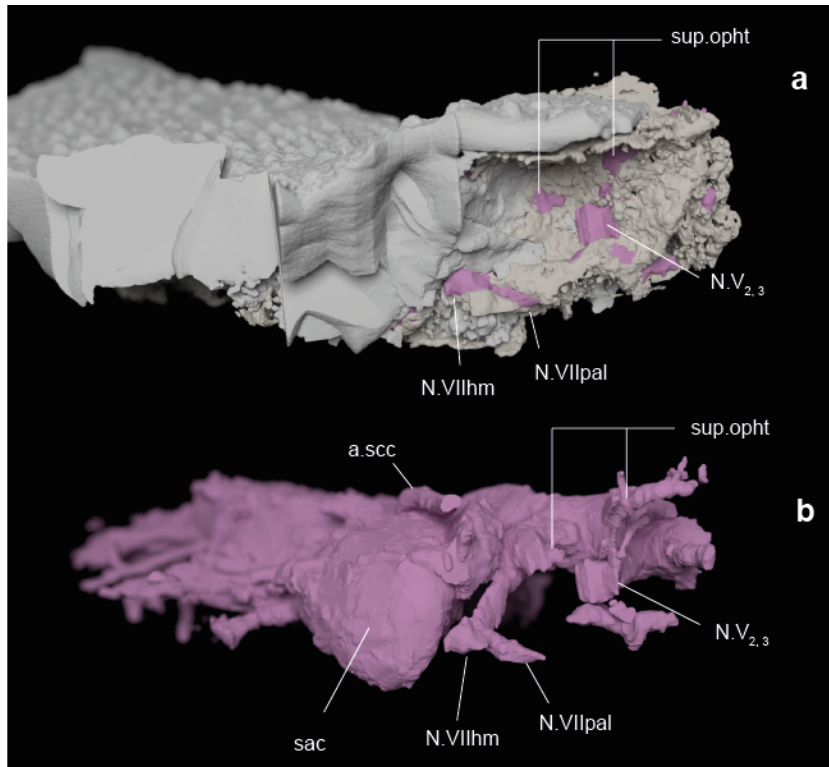
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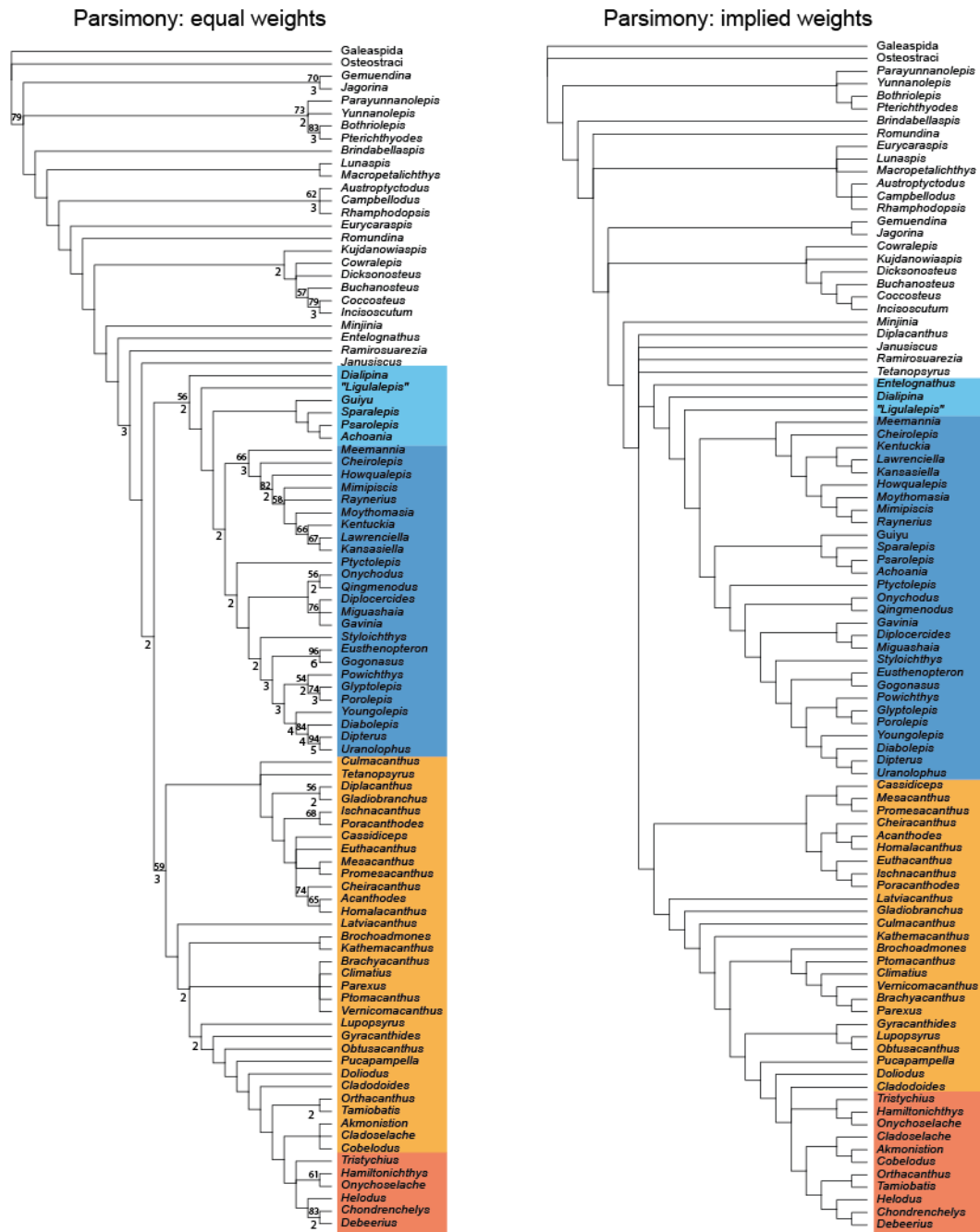
Extended Data Fig. 1 | Tomograms of endoskeletal ossification in *Minjinia*. Top row: semi-coronal sections through braincase. Double-headed arrows indicate anterior-posterior (a-p) dorsal-ventral (d-v) axes. Bottom row: semi-transverse sections through posterior part of endocranium. Voids of black space represent mouldic preservation. Scale bars, 10 mm and apply across each row of panels.



602

603 **Extended Data Fig. 2 | Right orbital wall and innervation pattern of *Minjinia*.** **a**, orbit in
 604 anterolateral view showing disposition of nerve openings (pink infill). **b**, endocast in the
 605 same perspective showing the relationship between nerve canals and endocast. a.scc., anterior
 606 semicircular canal; N.V_{2,3} trunk of the trigeminal nerve canal for branches 2 and 3; N.VII_{hm},
 607 hyomandibular branch of facial nerve canal; N.VII_{pal}, palatine branch of facial nerve canal;
 608 sac., sacculus; sup.opht, canal for supra-optalmic nerve. Scale bars, 20 mm (upper scale bar
 609 associates with **a**, lower scale bar associates with **b** and **c**).

610



611

612 **Extended Data Fig. 3 | Results of phylogenetic parsimony analysis.** Dataset consists of 95

613 taxa and 284 characters. Both trees are strict consensus topologies. Equal weights parsimony

614 analysis using the ratchet resulted in 240 trees with a length of 831 steps. Implied weights

615 parsimony analysis using random addition sequence + branch-swapping resulted in 8 optimal

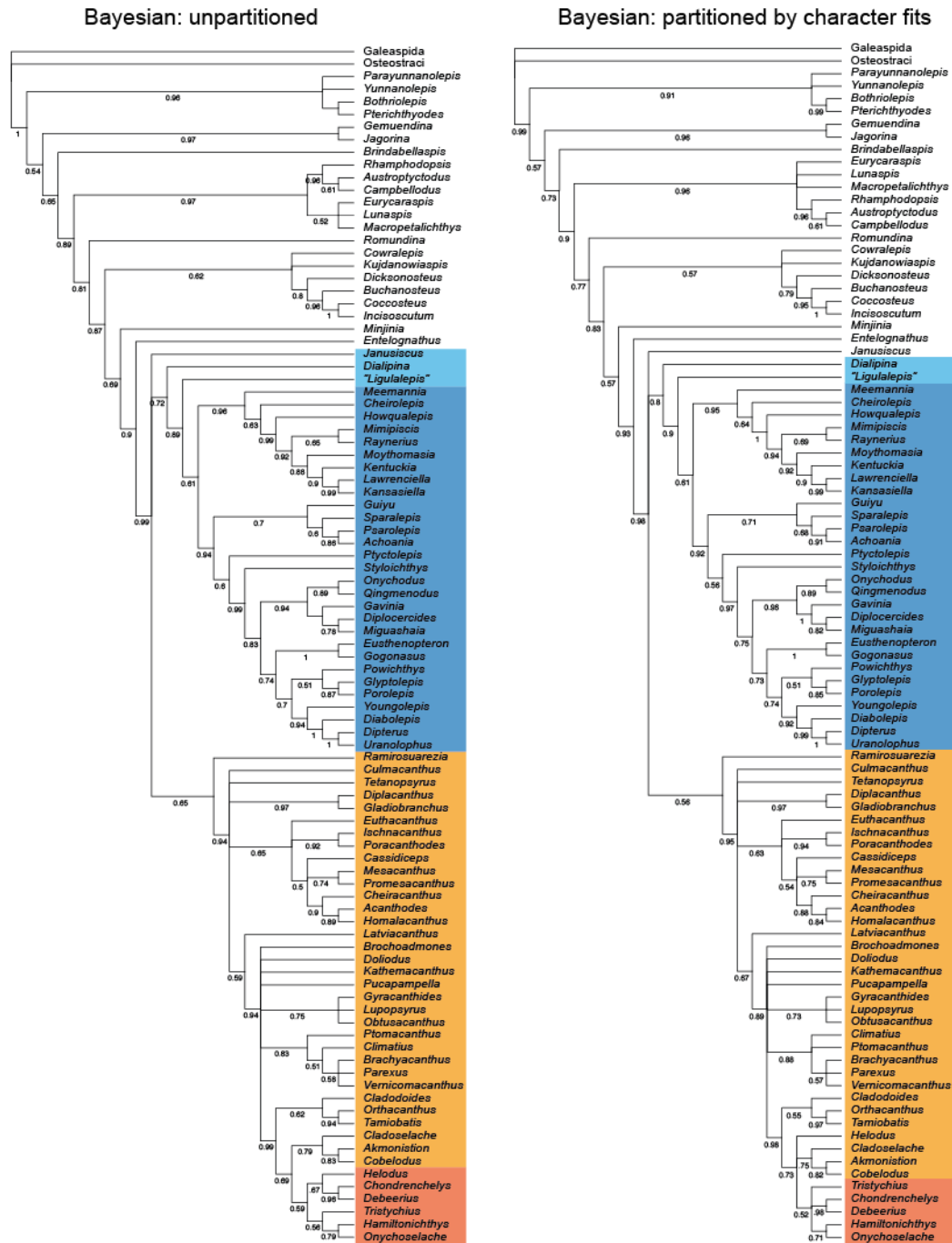
616 trees with score 85.20513. Double-digit figures above internal branches are bootstrap values

617 of 50% and over; single-digit figures below branches are Bremer decay index values. Blue

618 shading: osteichthyan total group (dark blue: crown group); orange shading: chondrichthyan

619 total group (dark orange: crown group).

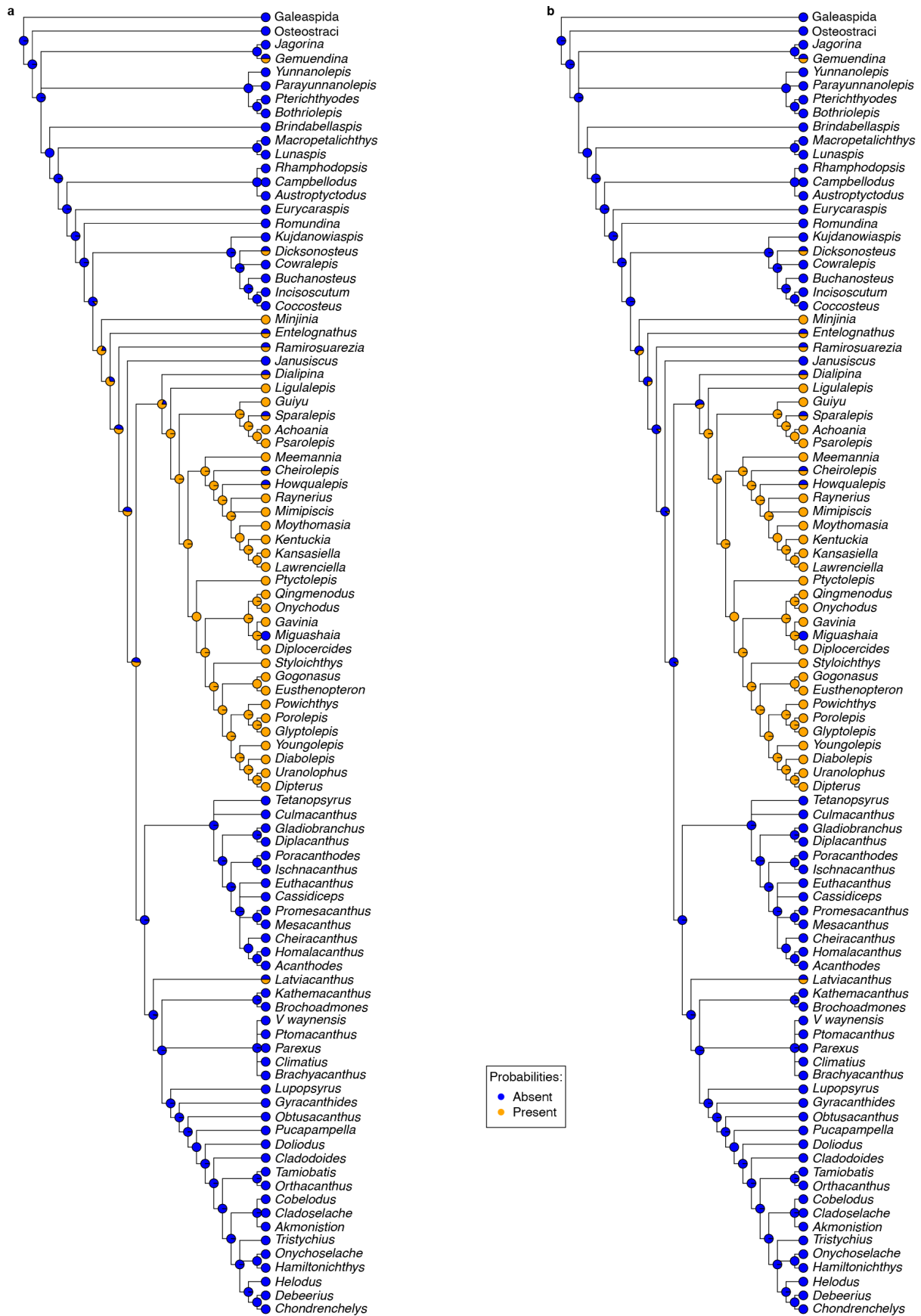
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624 along branches. Blue shading: osteichthyan total group (dark blue: crown group); orange

625 shading: chondrichthyan total group (dark orange: crown group).

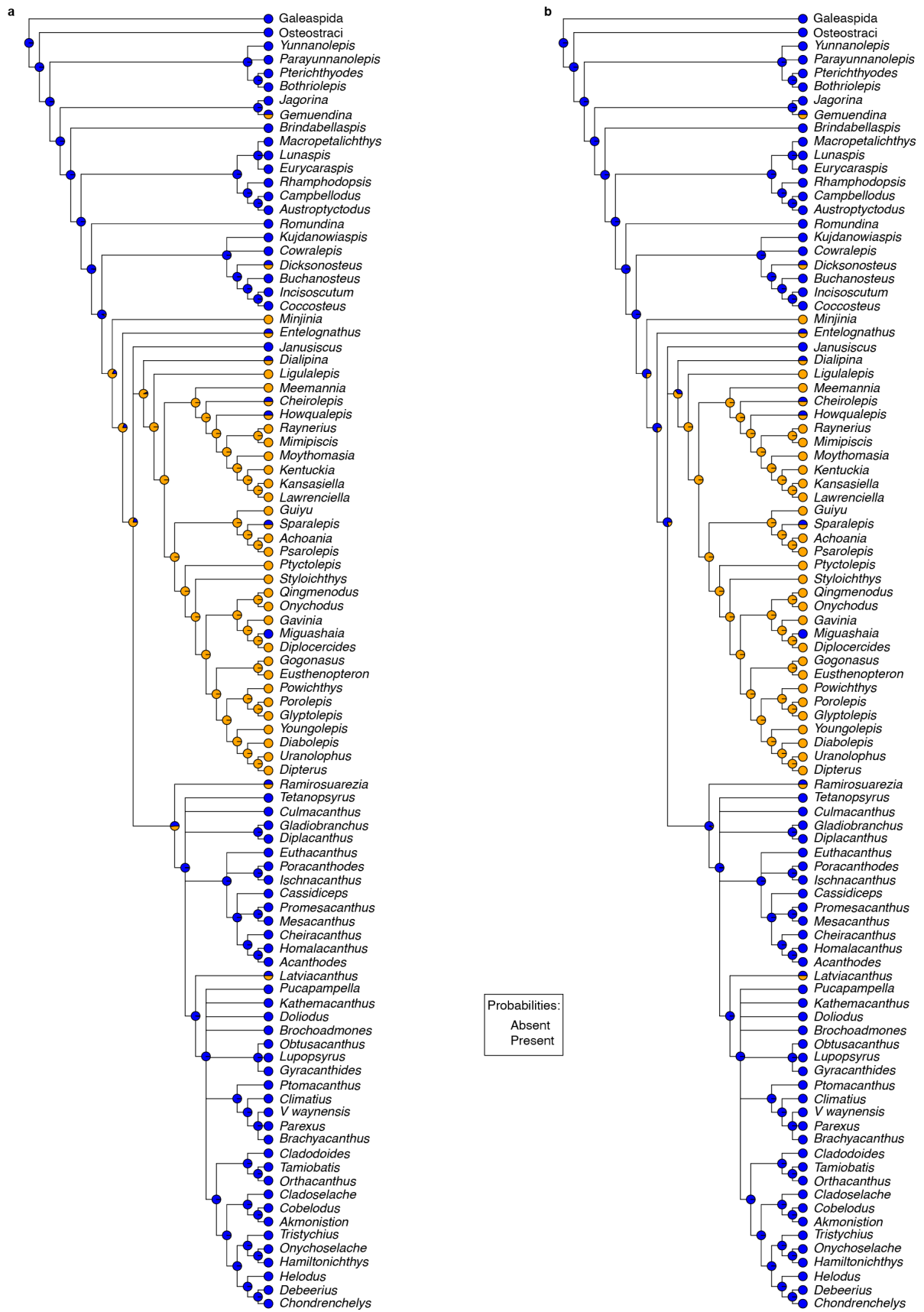
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629 **equal weights parsimony results.** **a**, ARD, all rates different model; **b**, ER, equal rates

630 model.

631



632

633 **Extended Data Fig. 6 | Likelihood ancestral state mapping of endochondral bone on**

634 **unpartitioned Bayesian analysis results. a**, ARD, all rates different model; **b**, ER, equal
635 rates model.