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

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26 **Endochondral bone is the main internal skeletal tissue of nearly all osteichthyans^{1,2} —**
 27 **the group comprising more than 60,000 living species of bony fishes and tetrapods.**
 28 **Chondrichthyans (sharks and their kin) are the living sister group of osteichthyans and**
 29 **have cartilaginous endoskeletons, long considered the ancestral condition for all jawed**
 30 **vertebrates (gnathostomes)^{3,4}. The absence of bone in modern jawless fishes and the**
 31 **absence of endochondral ossification in early fossil gnathostomes appears to lend**
 32 **support to this conclusion. Here we report the discovery of extensive endochondral bone**
 33 **in a new genus of ‘placoderm’-like fish from the Early Devonian (Pragian) of western**
 34 **Mongolia described using x-ray computed microtomography (XR-μCT). The fossil**
 35 **consists of a partial skull roof and braincase with anatomical details providing strong**
 36 **evidence of placement in the gnathostome stem group. However, its endochondral space**
 37 **is filled with an extensive network of fine trabeculae resembling the endochondral bone**
 38 **of osteichthyans. Phylogenetic analyses place this new taxon as a proximate sister group**
 39 **of the gnathostome crown. These results provide direct support for theories of**
 40 **generalised bone loss in chondrichthyans^{5,6}. Furthermore, they revive theories of a**
 41 **phylogenetically deeper origin of endochondral bone and its absence in**
 42 **chondrichthyans as a secondary condition^{7,8}.**

44 **Systematic palaeontology**

45 Placodermi M’Coy 1848
 46 *Minjinia turgenensis* gen. et sp. nov.

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

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

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

59 **Formation and age.** Upper part of Tsagaansalaat Formation, Pragian (Early Devonian)^{9,10}.

60

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

65

66 **Description**

67 MPC-FH100/9.1 consists of a partial braincase and skull roof (Fig. 1). The skull roof is
68 ornamented with sparsely distributed stellate tubercles resembling those of the Siberian
69 ‘placoderm’ *Dolganosteus*¹¹. Towards the midline of the skull roof, the tubercles are larger
70 and more pointed, and are more broadly separated from each other by unornamented fields.
71 The specimen shows signs of extensive post-mortem transport, with angles of the braincase
72 worn off and much of the skull roof and some of the braincase preserved as a mould.
73 Individual skull roof ossifications cannot be identified, although this may be due to the
74 dominantly mouldic preservation. There appears to have been a prominent nuchal plate
75 eminence comparable to acanthothoracids¹²⁻¹⁴.

76

77 **Endoskeletal tissue.** The braincase of MPC-FH100/9.1 is well ossified, comprising an
78 external bony sheath filled with an extensive matrix of spongy tissue (Fig. 2a-b; Extended
79 Data Fig. 1; Supplementary Videos 1 & 2). The trabecles forming this tissue are irregular and
80 branching, less than 1mm thick and often curved, and resemble most closely the
81 endochondral tissue of osteichthyans (Fig. 2c-d). As such, we interpret this as endochondral
82 bone. Notably, this is found in all preserved regions of the braincase, in contrast to the
83 isolated trabeculae identified as endochondral bone in *Boreaspis*¹⁵ and *Bothriolepis*¹⁶. The
84 margins of the braincase, the endocranial walls, and the boundaries of nerve and blood
85 canals, are formed from a thicker tissue which we interpret as perichondral bone. This
86 suggests that the endoskeleton of *Minjinia* comprises osteichthyan-like endochondral bone,
87 with an ossified perichondrium. To address the possible alternative explanation that it is an
88 aberrant instance of calcified cartilage, we compared the structure of this tissue with rarely-
89 preserved mineralized cartilage in the stem chondrichthyan *Diplacanthus crassismus*
90 (National Museums of Scotland specimen NMS 1891.92.334; Fig. 2e-f) observed using
91 synchrotron tomography. The cancellae within the endochondral tissue of *Minjinia* are
92 irregular, with a diameter of approximately 1-2mm. This tissue is distinctly unlike the

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

Neurocranium. The braincase is preserved from the level of the right posterior orbital wall to the posterior end of the occipital ridge. Occipital glenoid condyles are not preserved, but broad, flat parachordal plates are present, separated by a midline groove that accommodated a relatively narrow notochordal tunnel. A transverse fissure spans the basicranial surface at about mid-length of the preserved portion. It clearly demarcates the anterior margin of the parachordal plates and may correspond to the ventral cranial fissure of crown-group gnathostomes. However, unlike in crown gnathostomes, it is traversed by a substantial anterior extension of the cranial notochord. The courses of the lateral dorsal aortae are marked by a pair of sulci on the lateral margins of the parachordal plates. A narrow, shallow sulcus for the efferent hyoid artery is present on the preserved right side of the specimen, immediately behind the level of the orbit (Fig. 1a).

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

In posterior view, a tall, narrow median otic ridge is evident and resembles the morphology of *Romundina*¹⁷ and *Arabosteus*¹⁸. Similar to these taxa, the median otic ridge is flanked by two large occipital fossae for the epaxial musculature. The notochordal tunnel is approximately the same size as or smaller than the foramen magnum, as in ‘placoderms’ and in contrast with crown-group gnathostomes. A metotic fissure is absent.

Endocast. A partial cranial endocast is preserved, consisting of the hindbrain cavity, partial midbrain cavity, labyrinth cavities, and posteromedial corner of the orbital region. The two primary trunk canals of the trigeminal nerve (N.V₁ and N.V_{2,3}) are preserved (Fig. 1; Extended Data Fig. 2). The acoustic (N.VIII) and facial nerve (N.VII) canals share a common trunk canal behind the trigeminal nerves, as in many other ‘placoderms’^{17,19-21}. The supraorbital branch opens into the rear wall of the orbit and part of its supraorbital course

is preserved (Extended Data Figs. 2, 3). A slender branch extends below the labyrinth and divides into palatine and hyomandibular branches (Extended Data Figs. 2, 3). As in other ‘placoderm’-grade taxa, the vagus nerve (N. X) trunk canal is very large in diameter and exits from immediately behind the labyrinth cavity (Fig. 1; Extended Data Fig. 2). The spino-occipital region resembles other ‘placoderms’ in being extended. At least four spino-occipital nerve canals are present in a linear series, and the penultimate canal is largest in diameter (Fig. 1; Extended Data Fig. 2). Intercalating these is a network of occipital artery canals branching from the dorsal aortae.

The skeletal labyrinth is not complete on either side of the specimen, but can mostly be reconstructed according to the assumption of bilateral symmetry. The most significant feature is that the labyrinth and endolymphatic cavity are joined to the main endocavity chamber (Fig. 1). This is a striking contrast to other ‘placoderms’ and closely resembles crown-group gnathostomes²². The endolymphatic canals are elongate and tubular, extending posterolaterally to reach the skull roof, though external openings cannot be clearly identified. The anterior semi-circular canal follows the saccular cavity closely as in petalichthyids²³ (Fig. 1; Extended Data Fig. 2). However, the horizontal and posterior canals appear to extend well away from the saccular chamber (Fig. 1, Extended Data Fig. 2). The dorsal junctions of the anterior and posterior canals are joined in a crus commune, as in *Romundina*¹⁷ and *Jagorina*¹⁹. A sinus superior is absent.

Phylogenetic analyses

We conducted phylogenetic analyses under four different protocols: equal weights parsimony, implied weights parsimony, an unpartitioned Bayesian analysis, and a Bayesian analysis with characters partitioned by fit determined under implied weights parsimony²⁴ (see Extended Data Figs. 4-7). All phylogenetic analyses consistently place *Minjinia* as a stem-group gnathostome, proximate to the gnathostome crown (Fig. 3, Extended Data Figs 4,5). Equal weights parsimony recovers *Minjinia* in a position crownward of arthrodires but outside of a grade consisting of *Entelognathus*, *Ramiroswarezia*, and *Janusiscus*. Under implied weights, these three taxa move onto the osteichthyan stem and *Minjinia* is placed as the immediate sister taxon of the gnathostome crown. Under Bayesian analyses, arthrodires are resolved as more crownward than *Minjinia*. However, the latter analyses fail to recover arthrodires as a clade and the node uniting them with the crown to the exclusion of *Minjinia* is extremely weakly supported (posterior probability: 0.52-0.55). Under parsimony, the crownward position of *Minjinia* is unambiguously supported by the skeletal labyrinth and

endolymphatic duct being confluent with the main cranial cavity²² (Fig. 3). In common with arthrodires and the gnathostome crown, *Minjinia* possesses a division of the facial nerve deep to the transverse otic process (Fig. 1; Extended Data Fig. 2). However, *Minjinia* is excluded from the gnathostome crown group due to the absence of a metotic fissure and a posterior dorsal fontanelle, and presence of broad, flat parachordal plates expanded behind the saccular cavity (Fig. 3, Supplementary Information).

We undertook ancestral states reconstructions to assess the evolutionary history of endochondral bone (Fig. 3, Extended Data Figs. 6 & 7). Interestingly, parsimony analysis fails to recover secondary homology of this trait between *Minjinia* and osteichthyans. The crownward placement of *Minjinia* is, in fact, based on independent evidence relating to anatomical features of the braincase and endocast. However, the strict precision of parsimony reconstructions makes it insensitive to underlying uncertainty. To explore this, we used likelihood reconstructions and compared the ancestral state reconstructions under equal rates (ER) and all rates different (ARD) variants of the Mk model on branch-length-rescaled parsimony trees and Bayesian trees. On the parsimony trees, both models show substantial non-zero probabilities (0.23 for ER; 0.39 for ARD; Extended Data Table 1) for the presence of endochondral bone in the common node of *Minjinia* and Osteichthyes (Extended Data Fig. 6) in the parsimony trees. The ARD model shows the best likelihood score and a better AIC fit for endochondral bone (Extended Data Table 1), favouring repeated losses of this tissue over multiple gains (see Discussion). The values are substantially lower in the Bayesian trees (Extended Data Fig. 7, Extended Data Table 1), but this results from the relative positions of *Minjinia* and arthrodires, which is not well supported in those trees.

Discussion

Minjinia presents an unusual discovery of extensive endochondral bone in a ‘placoderm’-grade fish, with repercussions for the phylogenetic origin of this tissue and the problem of early gnathostome relationships more generally. The vertebrate skeleton is split into two systems: the exoskeleton (external achondral dermal bones) and endoskeleton (internal chondral bones)¹. Dermal bone evolved at least 450 million years ago in jawless stem gnathostomes, but the endoskeleton in these taxa is not endochondrally ossified (but see below). More crownward stem gnathostomes (osteostracans and ‘placoderms’) surround their cartilaginous endoskeleton in a sheath of perichondral bone. Extant chondrichthyans lack both dermal and perichondral bone, possessing a cartilaginous endoskeleton enveloped by prismatic calcified cartilage. Endochondral bone, in which the cartilaginous endoskeletal

precursor is invaded by and eventually replaced by bone, is widely considered an osteichthyan apomorphy based on clear prior polarity^{3,7,25,26}. However, recent work has cast doubt on this assertion. The recognition that dermal bone is secondarily lost in chondrichthyans^{27,28} is consonant with prior knowledge of the loss of perichondral bone in this same lineage²⁹. Taken together, this has revived uncertainty about the true phylogenetic timing of the origin of endochondral ossification⁸.

Minjinia does not represent the first report of endochondral bone outside of Osteichthyes. However, it is by far the most extensive and unequivocal example, and raises explicit questions in light of the proximity of *Minjinia* to the gnathostome crown. Isolated examples of trabecular bone, typically restricted to a small region of the neurocranium, have historically been reported in boreaspid osteostracans^{15,30}, buchanoosteid arthrodiroids³¹ and petalichthyids³². However, these reports have all been dismissed as misidentifications²⁶, possibly representing the retreat of perichondral bone deposited during cartilage growth³³. Most recently, trabeculae in supposed endoskeletal bones of *Bothriolepis* have been termed endochondral bone¹⁶, although the small scale of these is in line with ‘superficial’ perichondral trabeculae seen elsewhere. In line with ref. 26, we found no evidence of endochondral bone in material of *Buchanosteus* held in the Natural History Museum, London, or indeed in any other ‘placoderms’ we have examined. The *Epipetalichthys* holotype (Museum für Naturkunde, Berlin specimen MB.f.132.1-3) shows an apparently spongiöse infilling in the anterior region of the braincase, but the identity of this structure, or even whether it is biological, cannot be determined.

Does endochondral bone have a deep origin within the gnathostome stem group? This would imply repeated losses of this tissue. We do find some statistical support for this hypothesis (Fig. 3, Extended Data Figs. 6, 7; Extended Data Table 1), and the model is well justified on prior phylogenetic and biological grounds. Endochondral bone has long been known to be inconsistently developed across ‘primitive’ bony fishes: incomplete or entirely absent ossification of the endoskeleton is known in both Palaeozoic actinopterygians³⁴ and sarcopterygians³⁵, as well as more recent taxa³⁶. The frequent absence of endochondral bone is considered secondary, and other controlling factors such as body size, maturity, mechanical stress, and buoyancy can determine its degree of development¹. Our findings are also in agreement with studies establishing a genetic basis for secondary loss of all bone types within chondrichthyans^{5,37,38}, with the failure to produce endochondral bone likely representing arrested development of chondrocytes as opposed to a primary lack of ability⁶.

Another confounding factor in this question is the problem of ‘placoderm’ relationships. Although currently resolved as a deeply pectinate grade along the gnathostome stem, the backbone of this arrangement has poor statistical support and there is a lack of consistency in the arrangement of plesia. *Minjinia* itself highlights this uncertainty, given its highly unexpected character combinations. Notwithstanding its endochondral bone and crown-gnathostome-like inner ear structure, it strongly resembles ‘acanthothoracids’—the ‘placoderms’ widely considered among the most removed from the gnathostome crown (i.e. most ‘primitive’). This apparent character conflict could perhaps be more easily reconciled with a more coherent (though not necessarily monophyletic) ‘placoderm’ assemblage. Indeed, the highly pectinate structure of the ‘placoderm’ grade seems symptomatic of an overemphasis on characters and taxa resembling the crown group, thereby undersampling characters that could stabilise a clear picture of ‘placoderm’ interrelationships.

Minjinia reveals new data on ‘placoderm’ endoskeleton and tissue diversity from Mongolia—an otherwise extremely poorly known biogeographic realm for early gnathostomes. The phylogenetic placement of this ‘acanthothoracid’-like taxon crownward of all non-maxillate ‘placoderms’, in conjunction with possession of extensive endochondral bone, highlights the importance of material from traditionally undersampled geographic areas. The presence of endochondral bone renews the hypothesis that this tissue is evolutionarily ancient and was lost secondarily in chondrichthyans^{7,8}. This view is overall consistent with evidence of generalised bone loss in chondrichthyans, potentially as a result of the suppression of bone-generating molecular genetic pathways^{6,38}. Continued work in Mongolia and re-evaluation of phylogenetic datasets will be necessary to address this, with the results likely to lead to substantial re-evaluation of gnathostome phylogeny.

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

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Methods

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

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

Phylogenetic analysis. We conducted a parsimony analysis using TNT 1.5³⁹ and Bayesian analysis using MrBayes v 3.2.7⁴⁰. The dataset consisted of 95 taxa and 284 discrete characters based on a pre-existing dataset⁴¹. We employed Osteostraci and Galeaspida as composite outgroups. We conducted parsimony analysis using both equal weights and implied weights methods. Global settings were 1000 search replicates and a hold of up to 1 million trees. Equal weights parsimony analyses were conducted using the ratchet with default settings. Implied weights parsimony used a concavity parameter of 3 and the search was without the ratchet. Command lists are included in Supplementary Information. We conducted Bayesian analysis using both a partitioned and unpartitioned dataset. We used the Mkv model⁴² and gamma rate distribution. We ran the analyses for 5 million generations with a relative burn-in fraction of 0.25. Runs were checked for convergence using Tracer⁴³. We partitioned the dataset using a newly proposed method²⁴ that partitions the data according to homoplasy levels. Using the results of implied weights parsimony conducted in TNT, we created a text table of character fit values. We wrote an R⁴⁴ script to generate a list of partition commands for MrBayes. To reduce the number of partitions with small numbers of characters, we concatenated the partitions by rounding the fitness scores to 2 significant figures, yielding 10 individual partitions.

We assessed parsimony ancestral states visually using Mesquite⁴⁵. Likelihood and Bayesian ancestral states were estimated in R using the castor package⁴⁶. Prior to calculating likelihood ancestral states on parsimony trees, we scaled branch lengths using PAUP*⁴⁷ and calculated the likelihood scores for all of the trees under the Mkv model. The trees were then exported with branch lengths. To account for overall uncertainty in tree estimates, we estimated ancestral states on 100 trees randomly selected from the fundamental set of most parsimonious trees and two times 50 trees selected from the 75% last trees of each posterior tree distribution from the Bayesian analysis. We then run an ancestral states estimation Mk model (using the castor R package) using both the Equal Rates (ER) and All Rates Different (ARD) models. This resulted in 400 ancestral states estimations. For each estimation we extracted the overlap log likelihood, the AIC (counting one parameter for the ER model and

two for the ARD model) and the scaled log likelihood (probability) for the presence and absence of the endochondral bone character (character 4) for the last common node of *Minjina* and crown-group gnathostomes. We present the median value of these distributions of the estimations overall log likelihoods, AICs and presence or absence of endochondral bone in Extended Data Table 1.

Data availability

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

Code availability

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

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350 **Fig. 1 | MPC-FH100/9.1 a 'placoderm' skull roof and braincase from the Early**
351 **Devonian of Mongolia. a, Ventral view. b, Dorsal view. c, Left lateral view. d, Posterior**
352 **view. e, Braincase endocavity in dorsal view. Taupe: endoskeleton; grey: mould; pink:**
353 **endocavity; blue: exoskeleton. a.scc., anterior semicircular canal; cav.end., endolymphatic**
354 **cavity; crsp.ri, craniospinal ridge; d.end., endolymphatic duct; e.hy.a., sulcus for the efferent**
355 **hyoid artery; f.m.ep., epaxial muscle fossa; fo.mag., foramen magnum; h.scc., horizontal**
356 **semicircular canal; l.d.ao, sulcus for the lateral dorsal aorta; N.V, trigeminal nerve canal;**
357 **N.VII, facial nerve canal; N.VIII, acoustic nerve canal; N.X, vagus nerve canal; nch.,**
358 **notochordal canal; occ.ri, occipital ridge; orb., orbit; p.scc, posterior semicircular canal;**
359 **pr.pv., paravagal process; sac., saccus; soc., spino-occipital nerve canals. Scale bar, 20 mm.**

Fig. 2 | Endoskeletal mineralisation in fossil gnathostomes. **a**, Transverse tomographic slice through MPC-FH100/9.1. **b**, Three-dimensional rendering of trabecular bone structure. **c**, Transverse tomographic section through the braincase of the osteichthyan *Ligulalepis*. **d**, Three-dimensional rendering of the trabecular bone in *Ligulalepis* (**c** and **d** use data from⁴¹). **e**, Synchrotron tomography image of the calcified cartilage of the stem-group chondrichthyan *Diplacanthus crassissimus* specimen NMS 1891.92.334. **f**, Semi-transparent three-dimensional structure of calcified cartilage of NMS 1891.92.334. Scale bars, **a** and **b**, 10 mm; **c** and **d**, 1 mm ; **e** and **f**, 150 μ m.

Fig. 3 | Summary phylogenetic relations of early gnathostomes showing distribution of endochondral bone and exoskeletal armour. Squares at nodes indicate parsimony reconstruction for endochondral bone. Pie charts at nodes show likelihood reconstructions for the same character under the all-rates-different model (see Extended Data Figs 6 & 7 for competing reconstructions). Grey box indicates uncertainty. Loss of endochondral bone maps closely with generalised loss of bone in chondrichthyans where exoskeletal armour and perichondral bone are also absent.

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.

Extended Data Fig. 2 | Braincase endocavity of *Minjina*. **a**, Semi-transparent rendering of skull roof and braincase (grey and blue) showing extent of endocavity (pink). **b**, Ventral view. **c**, Dorsal view. a.scc., anterior semicircular canal; cav.end., endolymphatic cavity; d.end., endolymphatic duct; h.scc., horizontal semicircular canal; l.d.a., sulcus for the lateral dorsal aorta; N.V, trigeminal nerve canal; N.VIIhm, hyomandibular branch of facial nerve canal; N.VIIpal, palatine branch of facial nerve canal; N.VIII, acoustic nerve canal; N.X, vagus nerve canal, N.Xa, anterior branch of vagus nerve canal; N.Xp, posterior branch of vagus nerve canal; occ.a, occipital artery canals; p.scc, posterior semicircular canal; sac., sacculus; soc., spino-occipital nerve canals; sup.opht, canal for supra-ophtalmic nerve. Scale bars, 20 mm (upper scale bar associates with **a**, lower scale bar associates with **b** and **c**).

Extended Data Fig. 3 | Right orbital wall and innervation pattern of *Minjia*. **a**, orbit in anterolateral view showing disposition of nerve openings (pink infill). **b**, endocast in the same perspective showing the relationship between nerve canals and endocast.

Extended Data Fig. 5 | Results of Bayesian phylogenetic analysis using both partitioned and unpartitioned data. Majority-rules consensus trees with posterior probabilities shown along branches. Blue shading: osteichthyan total group (dark blue: crown group); orange shading: chondrichthyan total group (dark orange: crown group).

Endochondral bone estimation (bayesian – ARD)

Endochondral bone estimation (bayesian – ER)

Extended Data Fig. 7 | Likelihood ancestral state mapping of endochondral bone on unpartitioned Bayesian analysis results. ARD, all rates different model; ER, equal rates model.

Extended Data Table 1 | Tree distribution (100) ancestral states estimation results. ER = Equal rates model; ARD = All Rates Different model. The columns AIC and log.lik represent the median AIC and log.lik across the 100 parsimony and bayesian trees (for both models). The columns Absent and Present represent the median scaled likelihood for the endochondral bone state.

Node	Tree	Model	log.lik	AIC	Absent	Present
<i>Minjinia</i> :crown gnathostomes	Parsimony	ER	-27.60	57.20	0.94	0.06
		ARD	-25.47	54.93	0.61	0.39
	Bayesian	ER	-29.94	61.89	0.98	0.02
		ARD	-27.69	59.38	0.82	0.18

References

- Hall, B. K. *Bones and Cartilage*. (Academic Press, 2005).
- Janvier, P. *Early Vertebrates*. (Oxford University Press, 1996).
- Friedman, M. & Brazeau, M. A Reappraisal of the Origin and Basal Radiation of the Osteichthyes. *J Vertebr Paleontol* **30**, 36–56 (2010).
- Brazeau, M. D. & Friedman, M. The characters of Palaeozoic jawed vertebrates. *Zool J Linn Soc* **170**, 779–821 (2014).
- Eames, B. F. *et al.* Skeletogenesis in the swell shark *Cephaloscyllium ventriosum*. *Journal of Anatomy* **210**, 542–554 (2007).
- Marconi, A., Hancock-Ronemus, A. & Gillis, J. A. Adult chondrogenesis and spontaneous cartilage repair in the skate, *Leucoraja erinacea*. *eLife Sciences* **9**, 2813 (2020).
- Maisey, J. G. Heads and tails: a chordate phylogeny. *Cladistics* **2**, 201–256 (1986).
- Zhu, M. Bone gain and loss: insights from genomes and fossils. *Natl Sci Rev* **1**, 490–492 (2014).
- Alekseeva, R. E., Mendbayar, B. & Erlanger, O. A. *Brachiopods and Biostratigraphy of the Lower Devonian of Mongolia*. **16**, (Nauka, 1981).
- Alekseeva, R. E. *Devonian Biostratigraphy of Mongolia*. (Nauka, 1993).
- Mark-Kurik, E. in *Morphology, Phylogeny and Paleobiogeography of Fossil Fishes* (eds. Elliott, D. K., Maisey, J., Yu, X. & Miao, D.) 101–106 (2010).
- Stensiö, E. A. Contributions to the knowledge of the vertebrate fauna of the Silurian and Devonian of western Podolia. II. Notes on two arthrodires from the Downtonian of Podolia. **35A**, 1–83 (1944).
- Ørvig, T. Description, with special reference to the dermal skeleton, of a new radotinid arthrodire from the Gedinnian of Arctic Canada. *Colloque international C.N.R.S. no. 218* 43–71 (1975).
- Vaškaninová, V. & Ahlberg, P. E. Unique diversity of acanthothoracid placoderms (basal jawed vertebrates) in the Early Devonian of the Prague Basin, Czech Republic: A new look at Radotina and Holopetalichthys. *PLoS ONE* **12**, e0174794 (2017).

- 462 15. Wängsjö, G. The Downtonian and Devonian vertebrates of Spitsbergen. IX. *Norsk*
463 *Polarinstitutt Skrifter* **97**, 1–611 (1952).
- 464 16. Charest, F., Johanson, Z. & Cloutier, R. Loss in the making: absence of pelvic fins and
465 presence of pedomorphic pelvic girdles in a Late Devonian antiarch placoderm
466 (jawed stem-gnathostome). *Biology Letters* **14**, 20180199 (2018).
- 467 17. Dupret, V., Sanchez, S., Goujet, D. & Ahlberg, P. E. The internal cranial anatomy of
468 *Romundina stellina* Ørvig, 1975 (Vertebrata, Placodermi, Acanthothoraci) and the
469 origin of jawed vertebrates—Anatomical atlas of a primitive gnathostome. *PLoS ONE*
470 **12**, e0171241 (2017).
- 471 18. Olive, S., Goujet, D., Lelièvre, H. & Janjou, D. A new Placoderm fish
472 (Acanthothoraci) from the Early Devonian Jauf Formation (Saudi Arabia).
473 *Geodiversitas* **33**, 393–409 (2011).
- 474 19. Stensiö, E. A. La cavité labyrinthique, l'ossification sclérotique et l'orbite de *Jagorina*.
475 *Colloques internationaux du Centre national de la Recherche scientifique* **21**, 9–43
476 (1950).
- 477 20. Stensiö, E. A. Anatomical studies on the arthrodiran head. *K Sven Vetenskapsakad*
478 *Handl* **9**, 1–419 (1963).
- 479 21. Goujet, D. *Les poissons placodermes du Spitsberg*. (Cahiers de Paléontologie, Editions
480 du CNRS, 1984).
- 481 22. Davis, S. P., Finarelli, J. A. & Coates, M. I. *Acanthodes* and shark-like conditions in
482 the last common ancestor of modern gnathostomes. *Nature* **486**, 247–250 (2012).
- 483 23. Castiello, M. & Brazeau, M. D. Neurocranial anatomy of the petalichthyid placoderm
484 *Shearsbyaspis oepiki* Young revealed by X-ray computed microtomography.
485 *Palaeontology* **21**, 754 (2018).
- 486 24. Rosa, B. B., Melo, G. A. R. & Barbeitos, M. S. Homoplasy-Based Partitioning
487 Outperforms Alternatives in Bayesian Analysis of Discrete Morphological Data.
488 *Systematic Biology* **54**, 373 (2019).
- 489 25. Rosen, D. E., Forey, P. L., Gardiner, B. G. & Patterson, C. Lungfishes, tetrapods,
490 paleontology, and plesiomorphy. *Bull Am Mus Nat Hist* **167**, 159–276 (1981).
- 491 26. Gardiner, B. G. The relationships of the palaeoniscid fishes, a review based on new
492 specimens of *Mimia* and *Moythomasia* from Upper Devonian of Western Australia.
493 *Bull Br Mus nat Hist (Geol)* **37**, 173–428 (1984).
- 494 27. Zhu, M. *et al.* A Silurian placoderm with osteichthyan-like marginal jaw bones. *Nature*
495 **502**, 188–193 (2013).
- 496 28. Giles, S., Friedman, M. & Brazeau, M. D. Osteichthyan-like cranial conditions in an
497 Early Devonian stem gnathostome. *Nature* **520**, 82–85 (2015).
- 498 29. Miles, R. S. in *Interrelationships of Fishes* (eds. Greenwood, P. H., Miles, R. S. &
499 Patterson, C.) 63–103 (Academic Press London, 1973).
- 500 30. Janvier, P. *Les céphalaspides du Spitsberg*. (Éditions du Centre National de la
501 Recherche Scientifique, 1985).
- 502 31. Young, G. C. New information on the structure and relationships of *Buchanosteus*
503 (Placodermi: Euarthrodira) from the Early Devonian of New South Wales. *Zool J Linn*
504 *Soc* **66**, 309–352 (1979).
- 505 32. Stensiö, E. A. On the head of the macropetalichthyids. *Field Museum of Natural*
506 *History Publication Geological Series* **4**, 87–197 (1925).
- 507 33. Ørvig, T. Notes on some Paleozoic lower vertebrates from Spitsbergen and North
508 America. *Norsk Geologisk Tidsskrift* **37**, 285–353 (1957).
- 509 34. Schaeffer, B. The braincase of the holostean fish *Macrepistius*, with comments on
510 neurocranial ossification in the Actinopterygii. *American Museum Novitates* **2459**, 1–
511 34 (1971).

512 35. Cloutier, R. in *Devonian Fishes and Plants* (eds. Schultze, H.-P. & Cloutier, R.) 227–
513 247 (Verlag Dr. Friedrich Pfeil, 1996).

514 36. Grande, L. & Bemis, W. E. Osteology and Phylogenetic Relationships of Fossil and
515 Recent Paddlefishes (Polyodontidae) with Comments on the Interrelationships of
516 Acipenseriformes. *Memoir (Society of Vertebrate Paleontology)* **11** (Supplement to
517 **Number 1**), 1–121 (1991).

518 37. Venkatesh, B. *et al.* Elephant shark genome provides unique insights into gnathostome
519 evolution. *Nature* **505**, 174–179 (2014).

520 38. Ryll, B., Sanchez, S., Haitina, T., Tafforeau, P. & Ahlberg, P. E. The genome of
521 *Callorhynchus* and the fossil record: a new perspective on SCPP gene evolution in
522 gnathostomes. *Evolution & Development* **16**, 123–124 (2014).

523 39. Goloboff, P. A. & Catalano, S. A. TNT version 1.5, including a full implementation of
524 phylogenetic morphometrics. *Cladistics* **32**, 221–238 (2016).

525 40. Ronquist, F. & Huelsenbeck, J. P. MrBayes 3: Bayesian phylogenetic inference under
526 mixed models. *Bioinformatics* **19**, 1572–1574 (2003).

527 41. Clement, A. M. *et al.* Neurocranial anatomy of an enigmatic Early Devonian fish sheds
528 light on early osteichthyan evolution. *eLife Sciences* **7**, e34349 (2018).

529 42. Lewis, P. O. A likelihood approach to estimating phylogeny from discrete
530 morphological character data. *Systematic Biology* **50**, 913 (2001).

531 43. Rambaut, A., Drummond, A. J., Xie, D., Baele, G. & Suchard, M. A. Posterior
532 Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Systematic Biology* **67**,
533 901–904 (2018).

534 44. R Core Team. *R: A language and environment for statistical computing*. (R
535 Foundation for Statistical Computing, 2019).

536 45. Maddison, D. R. & Maddison, W. P. *Mesquite*. (2019).

537 46. Louca, S. & Doebeli, M. Efficient comparative phylogenetics on large trees.
538 *Bioinformatics* **34**, 1053–1055 (2017).

539 47. Swofford, D. L. *PAUP**. 4.0a166, (2019).

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