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1 **Endocast and bony labyrinth of a Devonian ‘placoderm’ challenges stem gnathostome**
2 **phylogeny**

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25 **Summary:**

26 Our understanding of the earliest evolution of jawed vertebrates depends on a credible
27 phylogenetic framework for the jawed stem gnathostomes collectively known as ‘placoderms’.
28 However, their relationships, and even whether ‘placoderms’ represent a single radiation or a
29 paraphyletic array, remain contentious. Here we describe the endocranial cavity and bony
30 labyrinth of *Brindabellaspis stensioi*, commonly recovered as a taxon of uncertain affinity
31 branching near the base of ‘placoderms’. While some features of its braincase and endocast
32 resemble those of jawless vertebrates, its inner ear displays a repertoire of crown gnathostome
33 characters. Both parsimony and Bayesian analyses suggest that prevailing hypotheses of
34 ‘placoderm’ relationships are unstable, with newly-revealed anatomy pointing to a radical
35 revision of early gnathostome evolution. Our results call into question the appropriateness of
36 arthrodire-like ‘placoderms’ as models of primitive gnathostome anatomy and raise questions of
37 homology relating to key cranial features.

38 **Results**

39 One of the major transitions in vertebrate history was the evolution of gnathostomes, or jawed
40 vertebrates, from jawless ancestors. The major morphological gap apparent when considering
41 only living vertebrate diversity—extant jawless fishes comprise just hagfish and lamprey—is
42 largely filled in by the fossil record [1]. ‘Placoderms’, the most crownward assemblage on the
43 gnathostome stem, occupy a pivotal place in this discussion. Traditional hypotheses of
44 relationships posit a monophyletic Placodermi [2–4], whereas most recent analyses recover
45 ‘placoderms’ as a paraphyletic array from which crown gnathostomes arose over 420 million
46 years ago [5–12]; but see ref [13]. In either scenario, antiarchs are typically recovered near the
47 base of the ‘placoderm’ assemblage, sharing some anatomical similarities with fossil agnathan
48 outgroups such as galeaspids and osteostracans, with arthrodires recovered proximate to the
49 gnathostome crown [1, 5–7, 9–11]; but see ref [12]. Uncertainty surrounding the relationships

between different ‘placoderm’ groups, as well as their broader taxonomic status, are compounded by an uneven understanding of anatomy across the radiation, particularly of the phylogenetically informative braincase and brain cavity—endocast (see Supplementary Materials).

Brindabellaspis stensioi [14] is a ‘placoderm’-grade stem gnathostome from the Early Devonian of New South Wales, Australia. Although almost exclusively recovered among the earliest diverging ‘placoderms’ [5–9, 11, 12, 15–17], it has variably been allied with rhenanids [13] acanthothoracids [4] and antiarchs [13], some of which are of dubious monophyly. Comparisons with fossil jawless fishes have frequently been drawn on the basis of gross external and braincase anatomy [14] and general proportions of the endocast [1, 18]. Other distinctive features, such as a large endolymphatic cavity, have been interpreted as autapomorphies [14, 19]. Here, we provide high-resolution CT data of two more recently discovered specimens (Figure 1), detailing unexplored parts of the endocast and offering a reinterpretation of previously described regions of the braincase and skull roof.

Tomographic data reveals the position of dermal bone sutures, clarifying the structure of the skull roof. Unlike in previous interpretations [14, 20], we identify an independent median pineal plate sitting posterior to the rostral plate, rather than a single rostopineal (Figures 1, 2). We also confirm the presence of four bones contributing to the lateral margin of the skull roof, contra refs [14, 20]. *Brindabellaspis* possesses an elongate ossification (postmarginal) flanking the serial lateral line-bearing bones, resembling maxillate ‘placoderms’ [6, 11] and early osteichthyans [21]; in most other ‘placoderms, the postmarginal is either lost or present only as the tip of the lateral corner of the skull roof [3].

Broadly speaking, our results affirm past descriptions of the endocavity [14], although with key additions and clarifications. The extremely short telencephalic region (see comparison to other taxa in Figure S3) of the endocast has a flat anterior face with no bulge anterior to the olfactory and terminal nerves, contra ref [14] (Figure 1c and e;). We also confirm that the hypophysis is oriented anteroventrally [14] (Figure 1e). Although an anteriorly or anteroventrally

directed hypophysis has sometimes been considered restricted to fossil agnathans (e.g. *Shuyu* [22], *Mimetaspis* [23]: Figure S3) and ‘placoderms’ [19], it is also reported in crown gnathostomes [17, 24–26]. *Brindabellaspis* has a laterally expansive cerebellum, which is wider than both the forebrain and hindbrain, and an anteroposteriorly elongate vagus complex, which is several times wider than the spino-occipital nerve foramina posterior to it (Figures 1d and f, and S2). These were previously suggested as being shared between extinct jawless fishes and *Brindabellaspis*, but are now known to be widespread in other stem and crown gnathostomes [17, 19, 25] (Figures 3 and S2), and are presumably plesiomorphic for the gnathostome crown. As described by Young [14], the olfactory tracts are elongate, and diverge anteriorly towards the widely separated and laterally positioned nasal capsules (Figures 1c, 3, and S2). Divergent olfactory tracts are otherwise only known in crown gnathostomes [17, 25–27]; in other ‘placoderms’ and the galeaspid *Shuyu* [22] the olfactory tracts are parallel and typically short (Figure 3), although it is possible that this arrangement is a reflection of the orbital position of the nostrils in *Brindabellaspis*. The myelencephalic region of the endocast anterior to the vagus nerve in *Brindabellaspis* accounts for some 16% of the total length of the brain cavity, which is inconsistent with the proportions in most ‘placoderms’ (20–30%, Figures 3 and S3) but within the range of extinct agnathans and crown gnathostomes (between 8–20%, Figures 3 and S3).

Our CT data reveal important new anatomical details of the bony labyrinth and endolymphatic complex. In addition to features identified in the endocast, the bony labyrinth of *Brindabellaspis* (Figures 1c–f, 3, and S2) bears important similarities to those of crown gnathostomes, with considerable difference to those of other ‘placoderms’. The labyrinth is anteroposteriorly short, and all three semicircular canals have large diameters. The anterior semicircular canal is much shorter than its posterior counterpart, and in dorsal view the two diverge at a much smaller angle than in other ‘placoderms’ such as the rhenanid *Jagorina* and arthrodire *Kujdanowiaspis* (Figures 1c–f and 4, and S2). Most strikingly, CT data demonstrate that the anterior and posterior semicircular canals of *Brindabellaspis* join in a crus commune,

with a dorsoventrally long sinus superior developed ventrally. This configuration is typical of crown gnathostomes [17, 25–27], and the combination is unknown in other ‘placoderms’ (Figure 4). There is no significant preampullary portion of the posterior semicircular canal, and the utricle does not separate the anterior and external semicircular canals (both contra the condition in all known ‘placoderms’ except *Romundina* [19]). While incomplete ventrally, the curvature of the saccus suggests that it is significantly smaller than in other ‘placoderms’, barely protruding laterally (Figures 3 and 4). It is also restricted ventral to the plane of the external semicircular canal, a condition seen elsewhere only in crown gnathostomes (Figures 3 and 4). Despite the lack of an external semicircular canal or utricular chamber in agnathans [22], a number of labyrinth characters can be polarised across the jawless-jawed vertebrate transition. Osteostracans possess small angles between anterior and posterior semicircular canals, and a crus commune but no developed sinus superior (Figure 4). Quantitative analyses of the endocast and bony labyrinth in stem and early gnathostomes is challenging: these features have been described using computed tomography for a single jawless fish [22] and just two ‘placoderms’ [19, 28]; other comparisons are known only from historical interpretive drawings or wax models [23, 29–32]. As such, it is difficult to determine the extent to which morphological features of the bony labyrinth reflect the external geometry of the skull. Detailed three-dimensional data for a range of extinct jawless and jawed fishes will be crucial to probing these relationships in the future.

Although previously considered autapomorphic [14], our data allow similarities to be drawn between the endolymphatic systems of *Brindabellaspis* and crown gnathostomes. The complex in *Brindabellaspis* can be divided into three distinct sections: a large, well-developed endolymphatic sac; an ascending duct connecting the vestibular chamber to the endolymphatic sac; and a distal duct extending from the sac, through the dermal bone, and opening externally (Figures 1d-f, 3, 4, and S2). There is no “second sac”, contra ref [19]. In both jawless and jawed stem gnathostomes, the endolymphatic complex is a simple tube-like structure that extends unidirectionally, and is positioned close to the labyrinth [18–19, 22–23]. In contrast, the crown gnathostome system is

more complex and divided into three distinct regions [33], much as in *Brindabellaspis*, and located mesially, closer to the brain cavity than the labyrinth [34–35] (Figures 3 and 4). Although incompletely known, the endocavities of the antiarchs *Minicrania* [36] and *Phymolepis* [37] hint at the presence of a mesially-directed endolymphatic duct and an endolymphatic sac, as well as a relatively short hindbrain.

Discussion

A revised and expanded morphological matrix, analysed under both parsimony and Bayesian frameworks, provides novel—and conflicting—insights into early gnathostome evolution. Under parsimony analysis, jaw-bearing gnathostomes fall into one of two monophyletic groups (Figures 4 and S4). The more stemward of these contains the bulk of traditionally-recognised ‘placoderms’, albeit with arthodires representing a nested radiation within this clade. *Brindabellaspis* is recovered as the earliest diverging member of a clade comprising, successively: antiarchs, maxillate ‘placoderms’, and crown gnathostomes. The position of *Brindabellaspis* and antiarchs as proximate to the gnathostome crown, with arthodires representing a nested radiation within a clade of ‘placoderms’, is unexpected [1, 5–7, 9–11]; (but see ref [13]) and indicates the importance of endocranial data and previous biases towards external morphology. The placement of *Brindabellaspis* is supported by features of the bony labyrinth and endolymphatic complex, and of antiarchs by characters including absence of opercular suspension on the braincase, a posteriorly open cuccularis fossa, and a long obstantic margin. However, results under Bayesian analyses differ from our parsimony analysis and recall more common hypotheses of placoderm paraphyly [5–7, 9–11, 17], with antiarchs recovered as the earliest-diverging ‘placoderm’ clade and arthodires as sister taxa to maxillate ‘placoderms’ and the gnathostome crown. Outside of these nodes, however, other ‘placoderms’—including *Brindabellaspis*—fall in a polytomy, and arthodires are recovered as paraphyletic. Support values for early gnathostome relationships across our parsimony tree are low, and the proximity of antiarchs to the gnathostome crown node

raises several questions of homology. The transition from posteriorly-positioned to anteriorly-positioned nasal capsules, as well as changes to jaw suspension, are now optimised as evolving twice: once within the clade comprising *Romundina*, rhenanids, ptyctodonts, petalichthyids and arthrodiros; and once within the clade comprising maxillate ‘placoderms’ and crown gnathostomes. The recovery of arthrodiros as removed from maxillate ‘placoderms’ plus crown gnathostomes also requires a number of homoplasies in the skull roof and trunk armour. Resolving these joint conflicts of phylogenetic relationships and anatomical evolution represents a fundamental challenge of early gnathostome evolution, and is one that cannot be resolved without detailed CT-based reassessment of the anatomy of key ‘placoderm’ taxa.

Our work adds considerably to knowledge of labyrinth and endocast variation across stem gnathostomes, highlighting the major impact that CT-based descriptions and re-examination of key taxa can have on both phylogenetic resolution and schemes of morphological evolution. The unexpected character combination in *Brindabellaspis* suggests that endocranial characters previously considered exclusive to crown gnathostome are likely widely distributed amongst a diversity of stem jawed vertebrates. However, outstanding questions remain about the homology of features common to both arthrodiros and crown gnathostomes, notably in the skull roof and nasal capsules. The conflicting phylogenetic hypotheses of relationships presented here highlight major uncertainties on the gnathostome stem, calling into question long-standing assumptions about patterns of character evolution. Recent work on the diversity of ‘acanthothoracid’ dentitions, also revealed by CT data, suggests a more complex picture of dental character evolution and provides independent evidence that at least some ‘acanthothoracids’ may branch closer to the gnathostome crown node than previously thought [12]. Notably, the position of arthrodiros as removed from maxillate ‘placoderms’ plus crown gnathostomes challenges previous understanding of arthrodire-like taxa as representative of the primitive gnathostome condition [11, 28, 38].

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Author contributions: G.Y., J.L. and Y.Z. designed the research project; J.L., S.G., Y.Z. and Y.H. carried out the reconstruction of datasets and digital segmentation; Y.Z., S.G. and J. L. performed the phylogenetic analysis and constructed figures; M.B. and S.G. performed principal component analyses; Y.Z., J.L., S.G., and M.B. wrote the manuscript; and all authors reviewed and revised the manuscript. Y.Z. and S.G. contributed equally.

Declaration of Interests: The authors declare no competing interests.

Figure Legends

Figure 1 The skull of the ‘placoderm’ *Brindabellaspis stensioi*, based on high-resolution CT.

- (A) Ventral view of endocranium (ANU 49493)
- (B) Dorsal view of skull roof (AM F81911)
- (C) Dorsal view of endocranial cavity (ANU 49493)
- (D) Left lateral view of endocranium (ANU 49493)
- (E) Right lateral view of endocranial cavity (ANU 49493)
- (F) Enlarged bony labyrinth and endolymphatic sac region
- (G) Mesial view of the skeletal labyrinth (ANU 49493)

(H) Ventral view of the skeletal labyrinth (ANU 49493)

Abbreviations: a.ps, efferent pseudobranchial artery; asc, anterior semicircular canal; asc.amp, ampullae of anterior semicircular canal; c.c, crus commune; conj.asc+esc, conjunction of anterior and external semicircular canals; die, diencephalon; e.endd, exit of endolymphatic duct; ends, endolymphatic sac; esc, external semicircular canal; esc.amp, ampullae of external semicircular canal; fo.hy, hypophysial fossa; hy.v, hypophysial vein; mes, mesencephalon; met, metencephalon; mye, myelencephalon; nc, nasal capsule; olf.t, olfactory tract; pit.v, pituitary vein; psc, posterior semicircular canal; psc.amp, ampullae of posterior semicircular canal; rec.p, pineal recess; rec.pap, parapineal recess; rec.utr, utricular recess; sac, sacculus; spio, spino-occipital nerves; s.su, sinus superior; tel, telencephalon; 0, terminal nerve; II, optic nerve, III, oculomotor nerve; IV, trochlear nerves; Vpro, profundus branch of trigeminal nerve; Vtg, maxillary and mandibular branches of trigeminal nerve; VI, abducens nerve; VII, facial nerve; VIII, otic nerve; IX, glossopharyngeal nerve; X, vagus nerve; Xa, anterior branch of vagus nerve. Scale bars, 1 cm.

Figure 2. Braincase of *Brindabellaspis stensioi* (AM F81911), based on high-resolution CT.

(A) Dorsal view, showing the dermal skull roof.

(B) Ventral view, showing the segmented skull roof and neurocranium.

(C) Left lateral view of whole skull.

(D) Left lateral view of neurocranium only. Segmenting could not be completed in the otic region of the braincase due to very low contrast between the braincase and surrounded limestone matrix, which was only partially acid-etched. Digital transverse sections showing the dermal bone sutures.

Abbreviations: Ce, central; Mg, marginal; Nu, nuchal; Pi, pineal; Pm, postmarginal; Pn, postnasal; Pna, anterior paranuchal; Pnu, paranuchal; Prm, premedian; Pro, preorbital; Pto, postorbital; Ro, rostral.

Scale bar, 1 cm.

Figure 3. Comparative morphology of crania in selected early vertebrates, aligned and scaled to match skeletal labyrinth dimensions (grey bar). Cranial outlines (light grey) and endocast outlines (yellow) in dorsal (left) and ventral (right) views. Data sources for each genus are provided in Supplementary Information.

Figure 4. Phylogeny showing the evolution of the inner ear and endolymphatic complex, and endocasts in early vertebrates.

(A) The inner ear and endolymphatic complex of selected crown gnathostomes in lateral and dorsal view, showing the shared characters between those in *Brindabellaspis* and the crown gnathostomes (boxed area). The phylogeny is a simplified version of the parsimony strict consensus tree (Figure S4)

(B) Brain cavity of selected gnathostomes in lateral view, mapped in the full version of the parsimony strict consensus tree (Figure S4)

STAR Methods

KEY RESOURCES TABLE

Note for the specimens listed in the Key Resources Table

Young assigned six specimens to *Brindabellaspis stensioi*, three of which are skulls [14]. Five additional specimens, which also provide information on skull morphology, were described in 2018 [20]. The similar size, proportion, condition of the nasal opening inside the dermal orbit, distinctive dermal ornament of flat-topped tubercles, and the proximity of all specimens supports their referral to *Brindabellaspis stensioi*. The endocranial morphology of *Brindabellaspis* redescribed here is based mainly on X-ray computed microtomography of two specimens, AM F81911 and ANU 49493, from ref [20]. We list in the key resources table six specimens from the original paper and the five additional specimens. Additional ‘CPC’ numbers, used by Young [14] for some ANU specimens, are given in parentheses for completeness. All but

the last two specimens are held in the Department of Applied Mathematics, Research School of Physics, ANU.

Anatomical notes on new skull roof interpretation

The original identification of skull roof bones in *Brindabellaspis* [14] has been revised twice [3, 20] on the basis of external examination. Tomographic data reveals newly recognized sutures, allowing a revised interpretation of the skull roof pattern. This differs significantly from previous reconstructions in the following details:

Firstly, previous interpretations have reconstructed a large rostopineal plate with a posterior convex margin, resembling the plate in the acanthothoracid *Romundina* and arthrodiros such as *Buchanosteus*. In our new interpretation, however, this posterior extension is in fact a separate pineal plate. The rostral has a sinuous posterior margin, which interdigitates with the narrow, rectangular pineal and the large, quadrate preorbital (Figure 2a).

Secondly, previous interpretations do not agree on the bones around the posterolateral boundary of the orbit and the lateral edge of the skull roof. Our new interpretation resembles that of Young [3], with a series of four bones (contra the five in refs. 14,20) forming the lateral edge of the skull roof: a postnasal; a bar-shaped bone forming the ventral margin of the orbit and tentatively identified as a postorbital plate; a postmarginal; and a paranuchal (Figure 2a). The postmarginal canal runs through the postmarginal, rather than through the presumed postorbital plate (as reconstructed by refs. 14,20).

The skull roof of *Brindabellaspis* is highly apomorphic, particularly with regard to the anteriorly elongated bill-like premedian and accompanying lateral postnasals. Interestingly, its skull roof pattern shows some resemblance to the recently discovered Silurian maxillate placoderms, *Entelognathus* and *Qilinyu* [6, 11]. In both *Brindabellaspis* and maxillate placoderms, a large postmarginal is present and forms a substantial portion of the lateral margin of the skull roof, outflanking the serial bones bearing the lateral line canal (carried by the postorbital, marginal and anterior and posterior paranuchals). In most other placoderms, the postmarginal is

either much reduced, forming only the tip of the lateral corner of the skull roof, as in many arthrodires, or completely lost, as in *Romundina*, petalichthyids and ptyctodonts. Notably, some of the earliest-diverging osteichthyans such as *Guiyu* primitively have large ossifications flanking the bones bearing the lateral line canals. These bones form a considerable part of posterolateral margin of the skull roof [21], although the exact homology of these two bones (named extratemporal and accessory extratemporal in *Guiyu*) to the postmarginal of placoderms is difficult to establish. Finally, in *Brindabellaspis* the lateral-line-bearing marginal and anterior paranuchals are a series of rounded bones like in *Entelognathus* and *Qilinyu*, rather than the large polygon or irregularly shaped bones in most other placoderms.

Notes on endocast and labyrinth morphology in stem gnathostomes

The endocranial cavity morphology seen in ‘placoderms’ highlights the extreme diversity present across the assemblage, and with reference to outgroups allows us to reconstruct character polarity.

The two groups of jawless stem gnathostomes (or “ostracoderms”) that have well-preserved endocranial cavities, namely osteostracans and galeaspids, display considerable variation in morphology. The close affinity of osteostracans to jawed vertebrates is supported by features of the inner ear, in particular its drum-shaped vestibular cavity (sacculus), and anteroposterior asymmetry [23,30,32]. In contrast, the galeaspid *Shuyu*, despite possessing separated nasal sacs and olfactory tracts as in jawed vertebrates, lacks vestibular structures; the ventral part of the labyrinth, or *pars inferior*, is simply a continuation of the anterior and posterior semicircular canals as in living cyclostomes [22]. The whole profile of the inner ear in *Shuyu* also resembles living cyclostomes in being anteroposteriorly symmetrical. Both *Shuyu* and osteostracans possess a distinct crus commune, but the crus commune of osteostracans does not appear to extend into a developed sinus superior. The ventral extension of the crus commune seems variable in the osteostracans with inner ear morphology known. It is somewhat more pronounced in *Kiaeraspis*

auchenaspidoidea (Figure 4; ref.30:text-Figure 19) than in *Mimetaspis hoeli* (ref. 23:text-Figure 18) and *Norselaspis glacialis* (ref. 32: Figure 30), but is still more similar to the condition in *Romundina* than the distinct sinus superior in *Brindabellaspis* and crown gnathostomes. A better understanding of the crus commune in osteostracan inner ears is hampered by the lack of updated digital data, the availability of which would allow dissection of individual structures in strictly directional views.

Arthrodiroidea represent some of the first described and “stereotypical” placoderms [2,29,38-40]. In recent phylogenetic analyses, arthrodiroidea are typically resolved close to the crown gnathostome node [5,6,11,15,16], but in our parsimony analysis are nested within a monophyletic group containing many conventional ‘placoderms’. The endocranial cavity, especially the labyrinth morphology, is well-known in *Kujdanowiaspis* [29, 41, 42] and *Buchanosteus* [31], both of which display derived (or apomorphic) features when compared to agnathan outgroups. These features include a very long hindbrain; elongated general profile of the labyrinth; absence of crus commune, and laterally directed endolymphatic duct (Figure 4). In agnathans and most ‘placoderms’ the endolymphatic duct runs parallel in dorsal view (Figs. 2 and 3). The telencephalic and diencephalic regions of the arthrodiro endocranium resemble *Romundina* in having a bulged dorsal “forehead” and a ventral pre-hypophysial “step” anterior to the hypophysial fossa (Figure S3). The olfactory tracts proximally join the robust and long optic nerve canals that are perpendicular to the axis of the endocranium. The condition in which the olfactory tracts proximally join the robust and long optic nerve canal is shared in *Shuyu*, other ‘placoderms’ such as *Jagorina* [43], and *Brindabellaspis* (Figures. 1d, 2, 3, and S2), suggesting it is the primitive gnathostome condition. Of special interest is the incompletely known endocranium in the derived arthrodiro *Tapinosteus* [29]. *Tapinosteus* belongs to the arthrodiro subgroup Eubranchyothoraci [44] and was supposedly adapted to free-swimming lifestyle with a fusiform body, resembling crown gnathostomes. However, the preserved part of the inner ear resembles that of other arthrodiro in the low general profile and the absence of crus commune. The olfactory tracts are also parallel.

Although data are limited, this indicated that endocast and inner ear morphology are unlikely to broadly reflect ecological mode.

Together with arthrodires, antiarchs were among the first ‘placoderms’ to be described [39]. Antiarchs are typically resolved at the base of the ‘placoderm’ position close to *Brindabellaspis* in recent phylogenetic analyses. Notably, antiarchs possess various features difficult to compare to other ‘placoderm’ subgroups, such as a unique skull roofing pattern and “lateral plate”, a cheek bone bearing the dental and occlusal surface, a pectoral appendage clad in macromeric dermal plates, and a long, box-like trunk armour with multiple median dorsal plates. It is unclear whether these features represent derived or primitive gnathostome conditions—or indeed apomorphies—partly due to the lack of neurocranial evidence in the group. Endocavity anatomy is known in most detail for *Minicrania* [36], a primitive antiarch from the Early Devonian of Yunnan, China. External observation of the specimen suggests the presence of a bulbous endolymphatic sac not unlike the one in *Brindabellaspis*. The putative endocavity in *Minicrania* shows a short hindbrain section, and, based on the imprint of the braincase on the visceral surface of the skull roof, it is likely that most antiarchs display this condition. The distal part of the endolymphatic duct in the yunnanolepiform antiarch *Phymolepis* [37] is mesially directed as in *Brindabellaspis*, providing potential further support for a close relationship between antiarchs, *Brindabellaspis* and the gnathostome crown. In addition, antiarchs appear to have an anteroposteriorly compact labyrinth, with the anterior and posterior semicircular canals in dorsal view forming an angle close to that in *Brindabellaspis* and most crown gnathostomes, and smaller than that in petalichthyids and arthrodires. It is worth noting that these observations are not included in the current data matrix due to the limited data available for *Minicrania* and *Phymolepis*. Detailed descriptions based on tomographic data of this and other taxa is likely to yield more support for the systematic position of antiarchs, providing more insights into the primitive gnathostome characters in the future.

Petalichthyids include morphologically diverse taxa such as *Diandongpetalichthys*, *Eurycaraspis* and macropetalichthyids, but are traditionally united in one group. To date, the

endocranial cavity is only reported in the macropetalichthyids *Macropetalichthys* and *Shearsbyaspis*. The former is more completely preserved [42]. *Shearsbyaspis* is digitally reconstructed based on tomographic data, but the posterior part of cranium in the examined specimen is lost [45]. The olfactory tracts in both taxa are unusually long, but are anteriorly directed and parallel, as in most other ‘placoderms’, and the endocast in *Macropetalichthys* also displays an elongate hindbrain region contributed by both the otic and the cranio-spinal sections. In both taxa, the large and bulbous sacculus, thin semicircular canals and pre-ampullary portions of the anterior and external semicircular canals resemble those of arthrodires and rhenanids. *Macropetalichthys* is unique in having the distal part of the endolymphatic duct vertically ascends and flares. This cavity was labelled “fosse endolymphatique” by Stensiö [42], following chondrichthyan terminology. However, unlike the chondrichthyan condition, the endolymphatic duct first extends posteriorly for most of its length, and this flared section is far more distal than the endolymphatic sac in *Brindabellaspis*, resulting in unclear homology. However, the three-dimensional morphology of endolymphatic complex in *Macropetalichthys* is poorly known, and more information is needed to determine whether the flared condition is independent from those in *Brindabellaspis* and crown gnathostomes or not.

Rhenanids are ‘placoderm fishes’ with flat, disc-shaped bodies like those of stingray, and the dermal skeleton deviates considerably from the typical ‘placoderm’ bauplan. As in *Macropetalichthys*, the endocranial cavity of the rhenanid *Jagorina* lacks an updated three-dimensional description, despite good preservation of the neurocranium [42, 43, 46]. The inner ear of *Jagorina* resembles arthrodires in displaying a bulbous utricular and sacculus chamber, anterior and external semicircular canals separated by the utricular recess, and a simple tube-like endolymphatic duct. The olfactory tracts are coded as “diverged” in current matrix based on drawings by Stensiö [43], the otic section of the hindbrain is long as in most other ‘placoderms’, and the craniospinal section is very short as in crown gnathostomes.

Brindabellaspis was previously assigned into Acanthothoraci, a poorly known and loosely defined placoderm group showing considerable morphological disparity, and which is probably paraphyletic. The labyrinth of the acanthothoracid *Romundina* displays an interesting combination of inner ear and endolymphatic features [19], representing a possible intermediate between *Brindabellaspis* and arthrodire / rhenanid conditions. The general profile is not elongated, but is not as longitudinally compressed as in *Brindabellaspis* and the crown group. The angle between the anterior and posterior semicircular canals in dorsal view is sharper than the condition in rhenanids and arthodires, but is larger than that in agnathans, crown-group, and *Brindabellaspis*. The crus commune is present but the sinus superior is not developed, more-or-less resembling the condition in osteostracans. The posterior semicircular canal does not have an extensive preampullary section; the sacculus is large, dorsally positioned in relation to the external semicircular plane, and is flat inclined in anterior view; and the auditory nerve bifurcates before entering the labyrinth cavity, all as in other placoderms. However, the sacculus is irregularly shaped, and the utricular does not separate the ampullary ends of anterior and external semicircular canals, which joins before entering the utricular chamber, as in *Brindabellaspis* and crown gnathostomes (Figs. 2 and 3).

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents may be directed to and will be fulfilled by the lead contact, Jing Lu (lujing@ivpp.ac.cn).

METHOD DETAILS

X-ray computed microtomography Both specimens were scanned with the HeliScan CT Scanner (ANU1) in the CT Lab, Department of Applied Mathematics, Research School of Physics, Australian National University. ANU 49493 was scanned in 2017 with a 3 mm aluminium filter (resolution: 30 microns). The specimen was placed 161 mm from the source, with detector position 625 mm from the source, and was probed separately with a polychromatic X-ray beam (Bremsstrahlung radiation) with accelerating voltage of 120kV and a current of

120 μ A. AM F81911 was first scanned in 2017 with a 3 mm aluminium filter (resolution: 34 microns), with the specimen placed 180 mm from the 120kv accelerating voltage and 120 μ A current polychromatic X-ray beam source; the detector was placed 786 mm from the source. AM F81911 was re-scanned in 2018 to obtain a more focused view of the inner ear area, using the same filter as the 2017 scan (resolution: 35 microns). The specimen was positioned 187 mm from the source, and detector position positioned 599 mm from the source; it was probed separately with 100kv accelerating voltage and 150 μ A current polychromatic X-ray beam (Bremsstrahlung radiation). All reconstructions of these scans were based on 3600 radiographic projections on a 2872 $\times$ 2840 Pixium Flat Panel camera. Tomograms were segmented in Mimics (biomedical.materialise.com/mimics; Materialise, Leuven, Belgium) and the completed models exported as .ply files to Blender (blender.org) for rendering.

Phylogenetic analysis Parsimony analysis was performed in PAUP v. 4.0a158 [47] with the following settings: 500 random addition sequences, five trees held at each step, maxtrees set to automatically increase, nchuck =10 000, chuckscore =1, tree bisection and reconstruction strategy enabled. The data matrix is based on refs 9,11, 17 and 34. We added or amended 14 characters (see character list for full details), revised a number of codings and removed redundant characters to give a total of 118 taxa and 360 characters. We also added two taxa: *Minicrania* and *Phymolepis*. The four taxa in Vařkaninová et al. [12] were not included due to the low proportion of characters that can be coded on the basis of published data; all four act as wildcard taxa. Osteostraci and Galeaspida were set as the outgroup. All characters are unweighted, and six characters (c.62, c.123, c.160, c.256, c.258, c.262) were ordered. The full character list is given below. Bremer support values were calculated in TNT, with only Bremer Indices higher than one retained in the strict consensus tree nodes. An optimization tree, showing all ambiguous character changes, was generated in MacClade [49] (see Supplementary Data 5). The Bayesian analysis was run in MrBayes v.3.2.6 [50] under the Mkv model, with Galeaspida set as the outgroup. The

analysis was run until the standard deviation of split frequencies reached less than 0.01, indicating convergence had been reached, and this was confirmed in Tracer [51]. The first half of each run was discarded as burn-in.

The results of both the parsimony and Bayesian analyses are summarized in Supplementary Figure 6. The parsimony analysis resulted in 818628 most parsimonious trees of 1106 steps; the large number of trees is mostly due to very poor resolution within total group chondrichthyans. The complete strict consensus tree with node support is summarised in Supplementary Figure 4a. RI = 0.793; CI = 0.35; HI=0.649; RC=0.278. The Bayesian analysis is summarized in Supplemental Figure 4b.

Notes on homoplasies invoked by our parsimony analysis

Although supported by our parsimony analysis, the phylogenetic distance between arthrodires and maxillate ‘placoderms’ plus the gnathostome crown demands a number of homoplasies. These broadly fall into three categories: jaw suspension (c.92, c.93, c.328), pectoral fin articulation (c.194), and the posterior nose – anterior nose transition. In previous hypotheses of relationships, the posterior nose to anterior nose transition (an anatomical complex of several characters: c.112, c.115, c.127, c.134 c.157) happened only once: at the node subtending arthrodires, maxillate ‘placoderms’ and crown gnathostomes. In the scenario of relationships hypothesised in our parsimony analysis, early members of each of the two jawed vertebrate clades are ‘posterior nosed’, with the transition to an anterior nose occurring independently within them (once in maxillate ‘placoderm’ and crown gnathostomes, and in a stepwise manner in the clade comprising *Romundina*, rhenanids, ptyctodonts, petalichthyids and arthrodires). There are also three homoplasies between the skull roof and trunk armour of arthrodires and maxillate ‘placoderms’ (c.92, c.313, c.323, c.328).

DATA AND SOFTWARE AVAILABILITY

Data availability: The CT data that support the findings of this study, as well as 3D surface files of described material, are deposited on figshare: <https://figshare.com/s/d040d38b2e0ae3501f65>.

All other data files are included in the Supplementary Materials.

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