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DOI:
10.1098/rspb.2017.2418

PROCEEDINGS OF
THE ROYAL SOCIETY B
BIOLOGICAL SCIENCES

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Journal:	*Proceedings B*
Manuscript ID	RSPB-2017-2418.R1
Article Type:	Research
Date Submitted by the Author:	n/a
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Subject:	Palaeontology < BIOLOGY, Taxonomy and Systematics < BIOLOGY, Evolution < BIOLOGY
Keywords:	Chondrichthyes, gill skeleton, scales, Middle Devonian, gnathostome phylogeny, computed tomography
Proceedings B category:	Palaeobiology

An early chondrichthyan and the evolutionary assembly of a shark body plan

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Abstract

Although relationships among the major groups of living gnathostomes are well established, the relatedness of early jawed vertebrates to modern clades is intensely debated. Here, we provide a new description of *Gladbachus*, a Middle Devonian (Givetian ~385-million-year-old) stem chondrichthyan from Germany, and one of the very few early chondrichthyans in which substantial portions of the endoskeleton are preserved. Tomographic and histological techniques reveal new details of the gill skeleton, hyoid arch and jaws, neurocranium, cartilage, scales and teeth. Despite many features resembling placoderm or osteichthyan conditions, phylogenetic analysis confirms *Gladbachus* as a stem chondrichthyan and corroborates hypotheses that all acanthodians are stem chondrichthyans. The unfamiliar character combination displayed by *Gladbachus*, alongside conditions observed in acanthodians, implies that pre-Devonian stem-chondrichthyans are severely under-sampled and strongly supports indications from isolated scales that the gnathostome crown group originated at the latest by the early Silurian (~440 mya). Moreover, phylogenetic results highlight the likely convergent evolution of conventional chondrichthyan conditions among earliest members of this primary gnathostome division, while skeletal morphology points towards the likely suspension feeding habits of *Gladbachus*, suggesting a functional origin of the gill slit condition characteristic of the vast majority of living and fossil chondrichthyans.

Keywords:

Chondrichthyes, gnathostomes, gill skeleton, scales, Middle Devonian, jaws

1. Introduction

The early evolution of the Chondrichthyes (cartilaginous fishes) has long been obscured by an impoverished fossil record [1-3]. This has only recently been improved through discoveries of partly articulated bodies [4-7] and braincases from the Lower and Middle Devonian [8-10], coupled with computed tomography (CT scanning) [11-16]. The Lower and Middle Devonian record of chondrichthyans remains sparse, but the influx of high quality data from slightly younger material, especially from the Upper Devonian and Carboniferous [14-17], combined with insights from earlier studies [18-21] has transformed our understanding of the early evolution of the crown group (Holocephali and Elasmobranchii). In a complementary manner, the origin of total group Chondrichthyes has been amended by serial large-scale analyses of early gnathostome phylogeny [22-27], which consistently recover acanthodians [28] as stem-chondrichthyans. However, because the acanthodian and chondrichthyan taxa included in these data matrices are largely unaltered from Brazeau [29] and Davis *et al*. [30], these results are not truly independent.

Here, we present a CT analysis and re-description of *Gladbachus adentatus* [31] from the Middle Devonian (Givetian ~385-million-year-old) of Germany, one of the earliest chondrichthyans known from articulated remains. Unlike *Doliodus* [1,4-7], the earliest and most completely described 'unambiguous' stem chondrichthyan [32], *Gladbachus* has never been included among acanthodians, but, like *Doliodus*, recent analyses and discussion [16,32] suggest that it might illuminate conditions bridging the acanthodian-chondrichthyan transition. Thus, a primary aim of the present work is to test the assumed chondrichthyan affinity of *Gladbachus* in light of the current acanthodians-as-stem-chondrichthyans hypothesis. Here, we have constructed a new early gnathostome data base, with an augmented chondrichthyan component with taxa and characters from analyses by Pradel *et al*. [14], Coates *et al*. [16] and Coates & Tietjen [17]. This analysis presents the most detailed context, thus far, to reconstruct the evolutionary assembly of the chondrichthyan morphotype: a body-plan that has persisted, more-or-less conservatively, for at least 370 million years.

2. Materials and methods

(a) Specimens

The subject of this study, *Gladbachus adentatus* [31] is known from a single, dorsoventrally compressed individual, UMZC (University Museum of Zoology, Cambridge, UK) 2000.32 [31,33], collected from the Lower Plattenkalk, Upper Givetian, Upper Middle Devonian, of Unterthal, Bergisch Gladbach (Germany). The specimen (electronic supplementary material, figure S1) consists of three pieces embedded within a rectangular slab of resin, with only the dorsal surface visible for direct inspection.

(b) Computed tomography, anatomical reconstruction and histological thin sections.

Whole specimen scans: large scale scans were completed by the High Resolution X-ray Computed Tomography facility at the University of Texas at Austin (UTCT, www.digimorph.org).

Scales, denticles, teeth, and calcified cartilage were examined using synchrotron μCT: all data were collected at beamline 13-BM-D at the Advanced Photon Source at Argonne National Laboratory. Image reconstruction used GSECARS tomography processing software (http://cars9.uchicago.edu/software/idl/tomography.html), which dark-current corrects and white-field normalizes acquired data prior to performing gridding-based image reconstruction. Further details are provided in the electronic supplementary material.

Histological thin sections: doubly polished thin sections were studied using a Zeiss Axioskop Pol microscope equipped with Nomarski DIC optics.

Anatomical reconstruction: Mimics v. 17 (biomedical.materialise.com/mimics; Materialise, Leuven, Belgium) was used for the three-dimensional modeling, including segmentation, three-dimensional object rendering, STL polygon creation and kinematics. 3D Studio Max (Autodesk.com/products/3ds-max; Autodesk, San Rafael, USA) was used for further editing of the STLs (color, texture, lighting), kinematics, and mirroring for the final restoration.

(c) Phylogenetic and phenetic analysis

The phylogenetic data matrix is developed from sources including iterations of the early gnathostome data matrix by Brazeau [29], Davis *et al*. [30] and Zhu *et al*. [22]; most recently updated by Lu *et al*. [23], Qiao *et al*. [26], Zhu *et al*. [27] and Burrow *et al*. [34]. Chondrichthyan content includes substantial additions of new data drawn from Pradel *et al*. [14,15], Coates *et al*. [16], Coates & Tietjen [17], and observation of original specimens (electronic supplementary material).

Phylogenetic Methods: The primary character matrix consists of 84 ingroup taxa and 2 outgroup taxa (Galeaspida and Osteostraci) coded for 262 characters. Character and taxon sampling sources and discussion are provided in electronic supplementary material. Phylogenetic analyses used maximum parsimony implemented in PAUP*4.0.152 [35]. Nodal support was assessed via bootstrapping [36] and Bremer Decay Indices [37], carried out using AutoDecay [38] and PAUP*. Details of phylogenetic methods are provided in the electronic supplementary material. Character state transitions by node for the strict consensus cladogram of the MPTs were reconstructed in PAUP* assuming hard polytomies with DELTRAN [39] optimization (see Davis et al. [30], Coates et al. [16]).

A Principle Coordinate analysis (PCO) [40,41] was performed on the Hamming distance matrix [42] of the character data. Computed dissimilarity was restricted to characters coded for both taxa, and distances were normalized to the number of characters coded for both members in each taxon pair. For the PCO, all characters were treated as equally weighted and unordered.

3. Results

(a) Specimen description, including (b) results of computed tomography, anatomical reconstruction and thin section histology

Head length including the gill skeleton (figure 1*a*,*b*) is ~21cm, and head plus trunk length as preserved with the caudal region mostly absent is ~60cm (electronic supplementary material, figure S1), implying a total body length of approximately 80cm.

Although considered one of the few 'unambiguous sharks' of the Lower and Middle Devonian [32], details of *Gladbachus* anatomy do not conform in a straightforward manner with contemporary models of early chondrichthyan anatomy. The internal skeleton consists of calcified cartilage with no perichondral bone, yet the cartilage surface lacks the tightly connected tesserae that is a hallmark of chondrichthyan skeletal anatomy [15,43]. Rather, most cartilage surfaces bear a mesh of continuously calcified ridges (electronic supplementary material, figure S2), broadly resembling the ‘wood-like’ [44] texture observed in some Mesozoic elasmobranchs. Discrete tesserae are visible only in the walls of the semicircular canals, but these are irregularly sized and shaped, with broad intertesseral spaces. Thin section histology and synchrotron microtomography show that poorly delineated tesserae are distributed elsewhere in the skeleton, but concealed beneath the continuously mineralized cartilage surface.

The dermal skeleton includes no large plates. Head scales (figure 1*d*) are mostly larger than trunk scales (figure 1*f*) and the lateral line runs between scales. However, scale shape, composition and histology are remarkable, as they resemble conditions observed in 'placoderms' [45,33], and differ markedly from polyodontode scales like those of *Doliodus* [4,46] and mongolepids [47,48]. In *Gladbachus*, scale and branchial denticle crowns consist of overlapping, mono-layered, cellular dentine tubercles (electronic supplementary material, figure S3), lacking neck and basal canals. The standard, total-group chondrichthyan scale growth pattern of areally apposed odontodes [32] is absent, as are growing monodontode scales [49], and the non-growing placoid scales characteristic of modern chondrichthyans. Instead, the reconstructed growth pattern of *Gladbachus* scales is linear and bidirectional. Most unusually for a chondrichthyan, the cranial scales are asymmetric, with irregular and inconsistent shapes. Fin spines, and spines associated with girdles and the flank region, are completely absent.

The anterior section of the braincase is not preserved (figure 1*a*,*b*), thus evidence of a precerebral fontanelle is unknown, *contra* Heidtke and Kratschmer [31]. The right postorbital process includes traces of a jugular canal, and a groove on the posterior surface, likely for articulation with the upper jaw (electronic supplementary material, figure S4). The basicranium is compressed against the subjacent visceral arches, and too poorly preserved to demonstrate presence or absence of a ventral cranial fissure, or canals for all or part of the dorsal aorta network. Reconstructions of the vestibular, semicircular canals and ampullary spaces (electronic supplementary material, figure S4*c*) demonstrate that the otic capsules were large and widely separated across the midline. In extant gnathostomes, this degree of lateral separation is manifest only in embryonic forms, and resemble adult conditions observed in 'placoderms' [50-53]. A pair of ring-shaped structures flanking the dorsal ridge, next to the anterior lip of the persistent otico-occipital fissure probably represents endolymphatic duct openings. This location for the endolymphatic ducts is consistent with the absence of an endolymphatic fossa or single, median endolymphatic foramen, which characterizes all Recent and fossil conventional chondrichthyans.

The jaws, hyoid arch, and gill skeleton are exceptionally complete (figures 1*a*,*b* and 2, electronic supplementary material, figure S5), providing ready comparison with recently described in-group [15,54] and out-group [55] examples. Mandibular arch morphology (electronic supplementary material, figure S6) is more conventional than previously understood. *Contra* previous descriptions [31,56], there is no palatal symphysis. The difference in length between the upper and lower jaws is considerable (figure 2*e*), and comparable to conditions in *Acanthodes* [57,30], implying that a significant portion of the upper dentition was born on the underside of the neurocranium; presumably on the internasal plate (cf. *Ptomacanthus* [29]; *Doliodus* [5]). Notably, the scales and teeth (mixed) bordering the gape are preserved as continuous, subparallel bands spanning the inter-orbital space (figures 1*a*,*b*). The palatoquadrate is generally comparable to that of an early, conventional chondrichthyan (e.g. *Orthacanthus* [58]). The well-developed otic process bears a broad posterodorsal rim; the palatine process is broad and short, but there is no evidence of a flange or process contributing to a palatobasal articulation (figure 2*a*). Preserved most completely on the left side of the specimen, the anterior of the palate is thrust beneath the postorbital process (figure 1*a*). The section of jaw visible in front of the preserved portion of the braincase is the anterior extremity of Meckel's cartilage. The posterior portion of Meckel's cartilage is also exposed on the dorsal surface of the specimen, but rotated through 90 degrees, such that the dorsal surface is compressed against the mesial surface of the palatoquadrate.

The hyoid arch (figures 1*a*,*b* and 2*c*,*d*) is morphologically distinct from the gill arches. There is no interhyal, and both the large and well-mineralized ceratohyal and slender first ceratobranchial articulate with a broad basihyal. The five gill arches (electronic supplementary material, figure S7) are positioned caudal to the braincase, as in non-holocephalan chondrichthyans. Epibranchials are present on the first four arches, with anteriorly-directed simple pharyngobranchials (with no suprapharyngobranchials) present in the first three gill arches (figures 1*a* and 2*c*) as in osteichthyans and *Ozarcus* [15]. A pair of short, laterally directed cartilages medial to the base of the second gill provide the only evidence of hypobranchials. The fifth arch ceratobranchials are unusually broad, nearly rectangular, and keeled along the anterior margin. Remarkably, and uniquely, these resemble the posteriormost ceratobranchials of *Paraplesiobatis*, a Lower Devonian 'placoderm' [55] (electronic supplementary material, figure S8). The ceratobranchials of *Gladbachus* fourth and fifth gill arches articulate with a large basibranchial copula, which is separated from the basihyal by a large gap. This revised description provides the first accurate association of dorsal to ventral parts of each arch.

Although reported and named as toothless, *Gladbachus* possesses a dentition of small, mono-, bi-, and tri-cuspid teeth lining the jaws, with branchial denticles lining gill arches I-IV (figure 1*a*-*c*, electronic supplementary material, figure S3). The teeth are individually separate, and despite a suggestion of lingual to labial alignment, there is no trace of whorl-like families as in conventional, non-holocephalan, chondrichthyans.

(c) Phylogenetic analysis and principal coordinates analysis.

Phylogenetic analysis of the data set returned 249,600 most parsimonious trees (TL = 691, CI = 0.396, RI = 0.785, RCI = 0.311). The strict consensus cladogram of MPTs (figure 3*a*, electronic supplementary material, figure S11) strongly corroborates recent phylogenetic hypotheses of early gnathostomes [23-27], reconstructing all taxa usually referred to as acanthodians in a paraphyletic assemblage branching from the chondrichthyan stem. Here, *Gladbachus* is also recovered as a stem-chondrichthyan, as the sister taxon to a poorly resolved set of climatiid acanthodians and conventional chondrichthyans (including crown clade Chondrichthyes). A monophyletic group uniting diplacanthid, ischnacanthid and acanthodid acanthodians forms a clade that is the sister group of all other total-group chondrichthyans, including *Gladbachus*.

The widely discussed Lower and Middle Devonian sharks *Doliodus* and *Pucapampella* branch from close to the apex of the chondrichthyan stem. *Pucapampella* is recovered in an uncertain position relative to several 'acanthodian' genera and the clade of conventional chondrichthyans. *Doliodus* is recovered as a sister taxon to conventional chondrichthyans.

Contra Qiao *et al*. [26] and Zhu *et al.* [27], *Ramirosuarezia* is not recovered among 'acanthodian' stem chondrichthyans, but rather, is nested among stem-gnathostomes, with other taxa, such as *Qilinyu*, *Entelognathus* and *Janusiscus* branching crownward of the paraphyletic placoderms.

Within the chondrichthyan crown-group, xenacanths and ctenacanths (*sensu lato*) form a clade branching from the elasmobranch stem. A further, poorly resolved cluster, including *Homalodontus*, *Tristychius*, *Acronemus* and hybodontids branches from more crownward nodes, suggesting successive sister groups to the elasmobranch crown and close relatives. Holocephalans include the symmoriids, corroborating the arrangement found in Coates *et al*. [16].

A phylogenetic analysis on a reduced sample of chondrichthyans, focusing on the relationships among stem members (figure 3*b*), recovered 24 MPTS, with a mostly resolved set of relationships among acanthodians along the chondrichthyan stem. In this reduced analysis, *Gladbachus* again branches from within the 'acanthodians', suggesting that the position of *Gladbachus* within this paraphyletic assemblage is not the result of noise introduced into the data set by the large chondrichthyan sample.

Principal coordinates (PCO) analysis of the character data recovers all four of the traditional gnathostome divisions ('placoderms', 'acanthodians', osteichthyans and chondrichthyans) as discrete clusters in the space defined by the first three PCO axes (figure 3*c*,*d*). Notably, *Gladbachus* clusters with chondrichthyans in the PCO, despite its phylogenetic position among 'acanthodians'. *Gladbachus*, *Doliodus* and *Pucapampella* each occupy positions in PCO space between conventionally defined chondrichthyans and 'acanthodians', however, these three genera are all significantly closer in PCO space to chondrichthyan taxa than to 'acanthodians' (t-tests of the intertaxon distances in PCO space for *Gladbachus, Doliodus,* and *Pucapampella* yield p-values of $1.39*10^{-6}$, 0.01, and $8.65*10^{-8}$, respectively).

4. Discussion

(a) Tree shapes and implications for evolutionary timescale.

Due principally to its plesiomorphic scale conditions and absence of a dentition consisting of toothwhorls, *Gladbachus* is reconstructed close to the base of chondrichthyan total-group (figure 3*a*,*b*), removed from *Doliodus* and *Pucapampella*, which have traditionally been ascribed to the Chondrichthyes, but interleaved among taxa normally referred to as 'acanthodians'. Accordingly, despite a phenetic similarity to conventionally defined chondrichthyans, phylogenetically, *Gladbachus* is an acanthodian-grade stem-chondrichthyan.

Support for the acanthodian branching pattern is weak (electronic supplementary material, figure S11), but consistent with recent analyses [23-27]. Recent reconstructions have recovered traditional acanthodian family-level sets: acanthodids, ischnacanthids, diplacanthids and climatiids (electronic supplementary material, figure S9). In all of these trees, the climatiids group with conventional chondrichthyans, echoing results of Brazeau [29] and Davis *et al*. [30]. Diplacanthids, ischnacanthids and acanthodids fall into one of two arrangements, 1) as successive sister groups to more crownward taxa [25,27], or 2) as in the present analysis, a monophyletic clade [23,24]. Here, we propose resurrecting the term Acanthodii to define the diplacanthid-ischnacanthid-acanthodid clade.

Support for the chondrichthyan crown clade is strong (figure 3*a*), introducing new data for the elasmobranch branch and corroborating the topology found in Coates *et al*. [16]. A time-calibrated phylogeny using the strict consensus tree (figure 4) places the origin of the crown group at least as early as the end-Middle Devonian. The initial evolutionary radiation of crown chondrichthyans is primarily post-Devonian, forming a significant component of the vertebrate recovery after the end-Devonian

Hangenberg extinction [59,60], which is evident from faunas recorded at Lower Carboniferous localities such as Glencartholm [61], Bearsden [61], and Bear Gulch [62,63].

Conventional chondrichthyan conditions, exemplified by *Doliodus* [4], are present by the middle-Lower Devonian (Pragian: ~410mya), and a minimum date for the origin of the chondrichthyan total-group is currently tethered to the late Silurian (Ludlow: ~423 mya) by the earliest well-preserved osteichthyan (*Guiyu*) [64]. However, the earliest 'acanthodian' stem-chondrichthyan body fossils (*Nerepisacanthus*) are only slightly younger (Pridoli: ~419 mya) [65], and a wide variety of 'acanthodians' are known from the Lower Devonian (e.g. *Ptomacanthus*, *Brochoadmones*, *Cassidiceps* and *Promesacanthus* [29,66-69]). Such diversity, first apparent in the Lochkovian (figure 4) supported by a taphonomically biased record of articulated specimens, implies either a sudden radiation in the early Lower Devonian or a severely under-sampled history of Silurian stem-sharks. The latter hypothesis is supported by isolated scales scattered through the Middle Ordovician to Silurian [48,49], including strikingly characteristic, classically defined 'acanthodian' scales from the Rhuddanian (Llandovery ~440mya) of the Siberian Platform [70]. In the present phylogenetic context, we prefer to combine the micro- and macro-/articulated fossil records, which strongly suggest that the chondrichthyan total group, and, therefore, the gnathostome crown node, dates to at least the earliest Silurian, ~440 million-years-ago. Thus, the early history of chondrichthyans consists of two phases (figure 4): a Silurian-Devonian evolutionary radiation of micromeric, acanthodian-like taxa, and a subsequent Carboniferous radiation of the crown clade, initially dominated by holocephalans [16,63].

(b) Palaeobiological inferences

Gladbachus adds to an increasingly populated chondrichthyan stem lineage that also includes *Acanthodes* [24,30], *Ptomacanthus* [29,66], *Pucapampella* [8-10] and *Doliodus* [4-7]. The resultant data on early chondrichthyan morphological diversity captures endoskeletal detail comparable to the content of early osteichthyans, contributing to a more balanced interpretation of the initial gnathostome radiation. However, there is no straightforward emerging sequence of character acquisition for the chondrichthyan crown group (crown clade apomorphies are listed in electronic supplementary material, figure S10). The current analysis highlights conflicting patterns of character-state distributions, implying repeated and convergent evolution of chondrichthyan-like specializations among the earliest total-group members. For *Gladbachus*, PCO analysis clearly identifies the chondrichthyan-like nature of its body plan (figure 3*c*), but this stands in marked contrast to its reconstruction as representative of a previously unrecognized 'acanthodian' lineage (figures 3*a*,*b* and 4). *Gladbachus* approaches a quantifiably defined shark space, but does so from a phylogenetically distinct origin (electronic supplementary material, figure S10).

The character combination observed in *Gladbachus*, alongside the array of contrasting conditions observed in Early Devonian acanthodians (figure 4), defies conventional hypotheses of morphologically segregated acanthodian and chondrichthyan morphotypes, reinforcing the hypothesis that pre-Devonian stem-chondrichthyan diversity is fundamentally under-sampled. Reasons for the absence of substantial Silurian remains of crown gnathostomes are unclear, although restricted environmental specificity has been conjectured [71]. *Gladbachus* is a morphotypic outlier, in the sense that although phylogenetically placed within the acanthodian grade, it lacks fin spines, its scales lack synapomorphies shared with any acanthodian subgroup, and its estimated body length (electronic supplementary material, figure S1) is two to three times greater than contemporary or earlier 'acanthodians', with the notable exception of gyracanthids [60]. Furthermore, several features of the skeletal morphology suggest that *Gladbachus* was a continuous ram suspension feeder [72], somewhat like modern basking sharks (*Cetorhinus*). The head, including the gill skeleton, accounts for ~25% of estimated total body length, the reconstructed oral aperture is likely to have been in a near-perpendicular plane to the direction of forward movement, the dentition is minimal, and the lower jaw is long and slender (figure 2*b*,*c*). To the best of our knowledge, this is the earliest combination of such features known in any jawed vertebrate, adding to an emerging picture of total-group chondrichthyans as early, nektonic specialists, in contrast to the reconstructed demersal habits of their heavily skeletonized osteichthyan and 'placoderm' contemporaries [73]. Aspects of this character-suite occur repeatedly among stem chondrichthyans, suggesting that the familiar gill slit condition of sharks might originate from such early, and apparently multiple, natural experiments in suspension feeding.

5. Conclusion

Gladbachus offers a glimpse of early chondrichthyan diversity yet to be discovered. Significantly, *Gladbachus* scales, if discovered as isolated specimens, would be unrecognizable as chondrichthyan in the new, total-group sense, unlike an increasing variety of Silurian and Ordovician [47-49,74] scale-based taxa assigned with increasing confidence to the chondrichthyan total-group. Insights offered by *Gladbachus* and other early chondrichthyans suggest that the morphological disparity in the early members of the chondrichthyan total group was likely substantially greater than that which is observed in the more-or-less stable shark-morphotype which has persisted from the Middle Devonian through to the present. Accordingly, the importance of *Gladbachus* lies in its apparent morphological incongruence with its phylogenetic position, hinting at multiple paths leading to the modern shark-like body plan.

Data accessibility. Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.84mh3

Authors' contributions. M.I.C. and J.A.F. conceived the idea and designed the research; K.E.C. provided additional input. I.J.S. and P.S.A. provided all thin section histological analyses, related figure preparations and comparative scale data. K.E.C. and M.I.C. completed initial CT renderings; M.K.T. generated present CT renderings and produced the figures. K.E.C. provided comparative developmental data. M.L.R. and P.J. La R. conducted the synchrotron CT scanning. J.A.F. and M.I.C. conducted phylogenetic analyses; J.A.F. conducted PCO analysis. The manuscript was drafted by M.I.C. with significant input from J.A.F., I.J.S. and K.E.C.

Competing interests. The authors declare no competing interests.

Funding. This work was supported by grants DEB-0917922 and DEB 1541491 from the National Science Foundation (USA) (to MIC). GeoSoilEnviroCARS is supported by the National Science Foundation - Earth Sciences (EAR-1128799) and Department of Energy- GeoSciences (DE-FG02-94ER14466). The Advanced Photon Source, a U.S. Department of Energy (DOE) Office of Science User Facility, is operated for the DOE Office of Science by Argonne National Laboratory under Contract No. DE-AC02-06CH11357.

Acknowledgements. We thank J.A. Clack and M. Lowe for specimen loan, U. Heidtke for provision of materials recording early preparation of specimen, and K. A. Hardy for assistance with computed tomography renderings. We thank the GeoSoilEnviroCARS (Sector 13), Advanced Photon Source (APS), Argonne National Laboratory for access and support to complete significant portions of this work.

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FIGURES

Figure 1. *Gladbachus adentatus* Heidtke and Krätschmer [31]. (*a*) Rendering of cranial and pectoral girdle remains in dorsal view and (*b*) ventral view. (c) mandibular tooth; (d) cranial roof scale; (e) branchial denticle; (f) trunk scale. All denticles and scales rendered semitransparent from micro-computed tomography scans. Abbreviations: bhy, basihyal; chy, ceratohyal; hb, hypobranchial; mc, Meckel's cartilage; na, neural arches; nc, neurocranium; or, orbital ring; pop, postorbital process; pq, palatoquadrate; sco, scapulocoracoid.

Figure 2. *Gladbachus adentatus* Heidtke and Krätschmer [31]. (*a*) Palatoquadrate; (*b*) Meckel's cartilage; (*c*) jaws, hyoid arch and gill arches restored, dorsal view; (*d*) gills reconstructed ventral view; (*e*) reconstruction of articulated mandibular arch, left lateral view. Abbreviations: adf, adductor fossa; bhy, basihyal; cbr, ceratobranchial; chy, ceratohyal; cop, copula; ebr, epibranchial; end, endolymphatic duct; fm/oc, foramen magnum/occipital cotylus; gl, glenoid; hb, hypobranchial; hy, hyomandibula; mp, mesial process; opr, otic process; pbr, pharyngobranchial; pop, postorbital process; ppr, palatine process; pq, palatoquadrate; q, quadrate condyle, unmineralized site of; ssc, semicircular canal network; ocf, otico-occipital fissure.

Figure 3. Phylogenetic placement of *Gladbachus adentatus* Heidtke and Krätschmer [31]. **(***a*) parts *i* and *ii* join at arrow heads. Strict consensus, complete tree. (*b*) Strict consensus of chondrichthyan total group obtained from reduced taxon set; arrow head joins arrow head of (a) part *i*. Branch colours: black, stem group gnathostomes; green, Osteichthyes; magenta, acanthodid stem Chondrichthyes; red, non-acanthodid stem Chondrichthyes; purple, Holocephali (crown Chondrichthyes); blue, Elasmobranchii (crown Chondrichthyes). Circles mark nodes with bootstrap support greater than 50% and/or decay values greater than 1; filled circles mark nodes with bootstrap support greater than 75% and/or decay values greater than

3. (*c*) Phenetic results: PCO 1 (18.1% explained variance) is plotted on the vertical axis and PCO 2 (9.6%) is plotted on the horizontal axis. (*d*) PCO 1(vertical) vs. PCO 3 on the horizontal axis (8.1%). Details of the PCO analysis are presented in the Supplementary Notes. The four traditionally named groups (placoderms in purple, acanthodians in green, chondrichthyans in blue, osteichthyans in red) cluster in distinct non-overlapping regions on the first three PCO dimensions. Relevant stem chondrichthyan taxa are indicated in each plot.

Figure 4. Early chondrichthyan phylogeny: simplified strict consensus of phylogenetic analysis results calibrated against Ordovician-Carboniferous chronostratigraphic chart. Consensus computed from matrix with 86 taxa and 262 characters. Taxon bar colour: black, non-chondrichthyan; magenta, acanthodid stem chondrichthyan; red, non-acanthodid stem chondrichthyan; purple, holocephalan crown chondrichthyan; blue, elasmobranch crown chondrichthyan. Bar length corresponds to earliest occurrence stage-duration. Brown bar signifies total range of chondrichthyan-like scale-based taxon. Timescale (Ma) from Cohen *et al*. [75]. Complete cladogram shown in figure 3*a*.

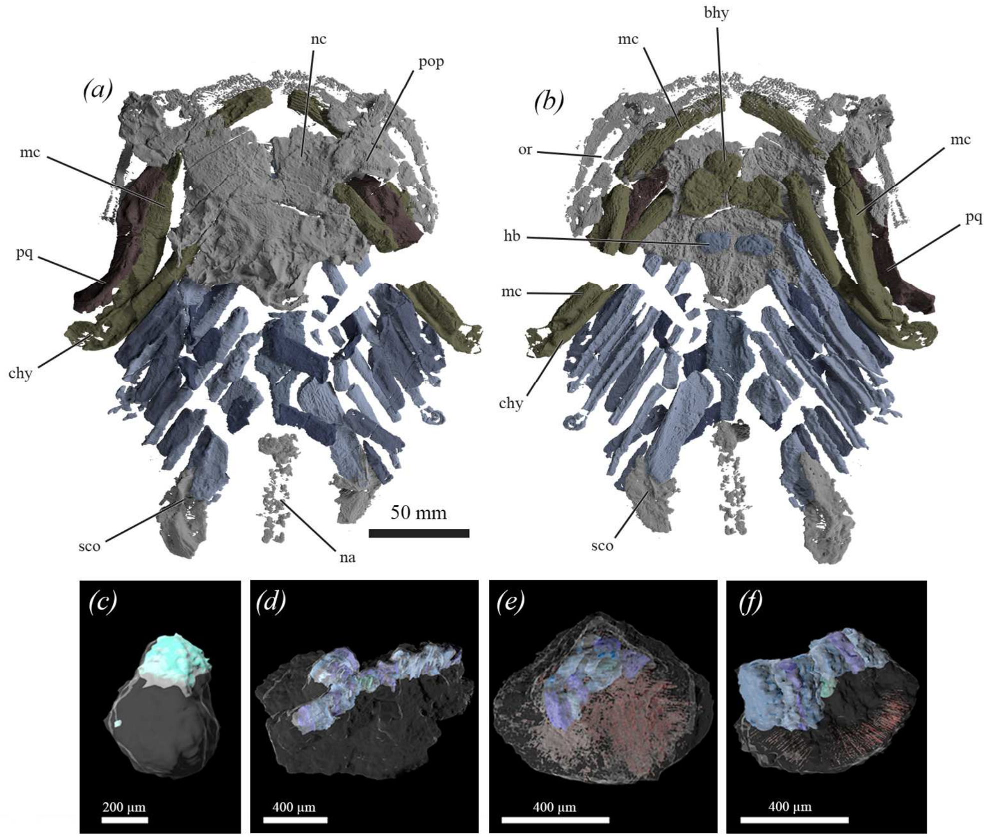

Figure 1

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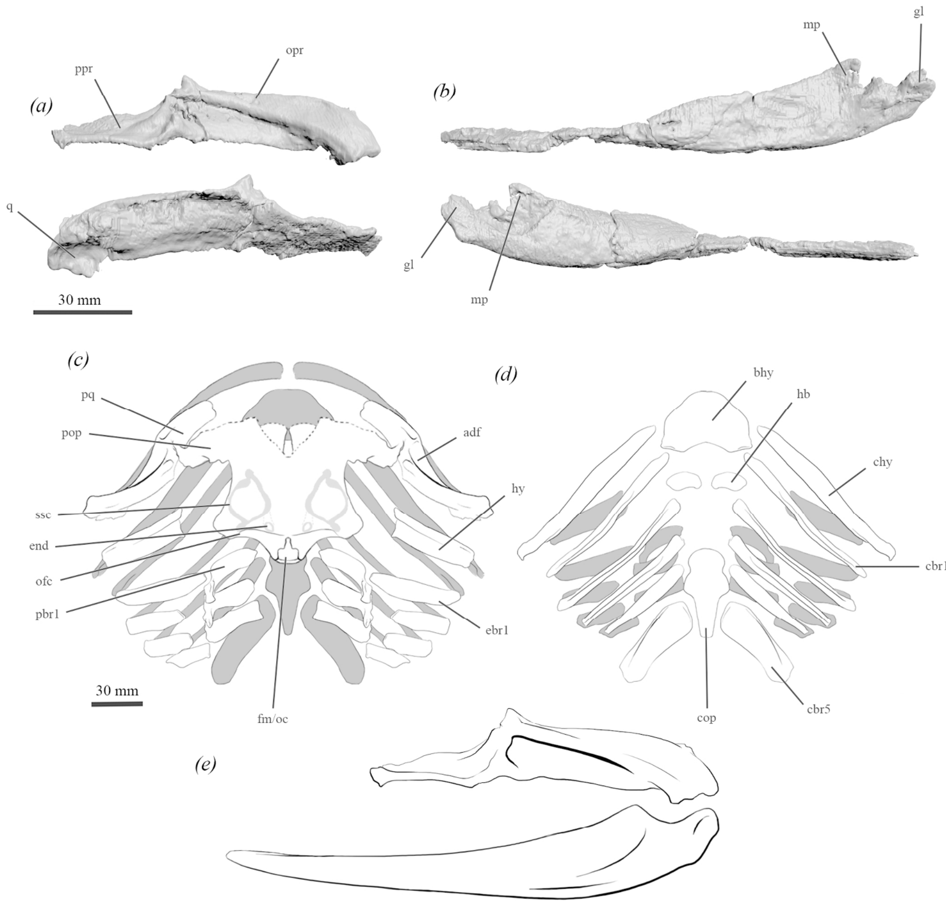

Figure 2

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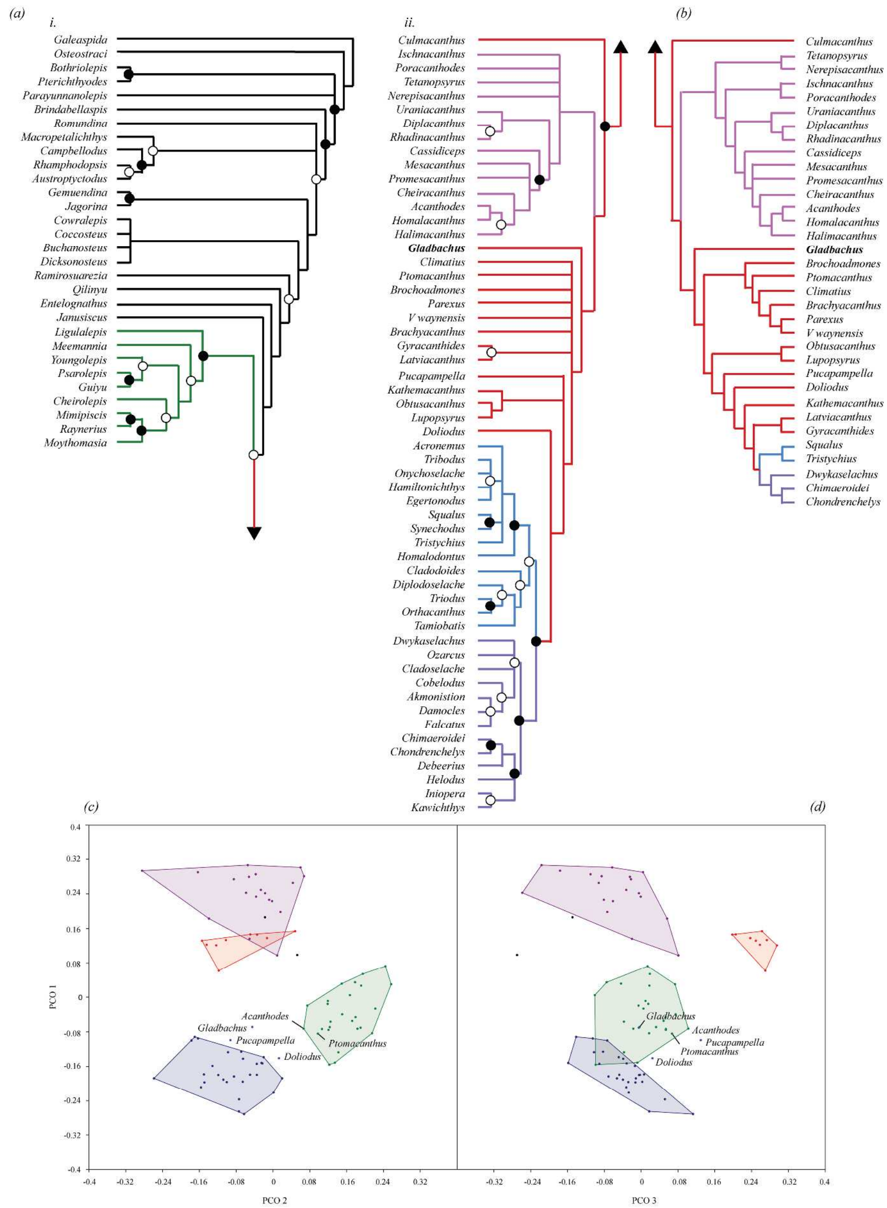

Figure 3

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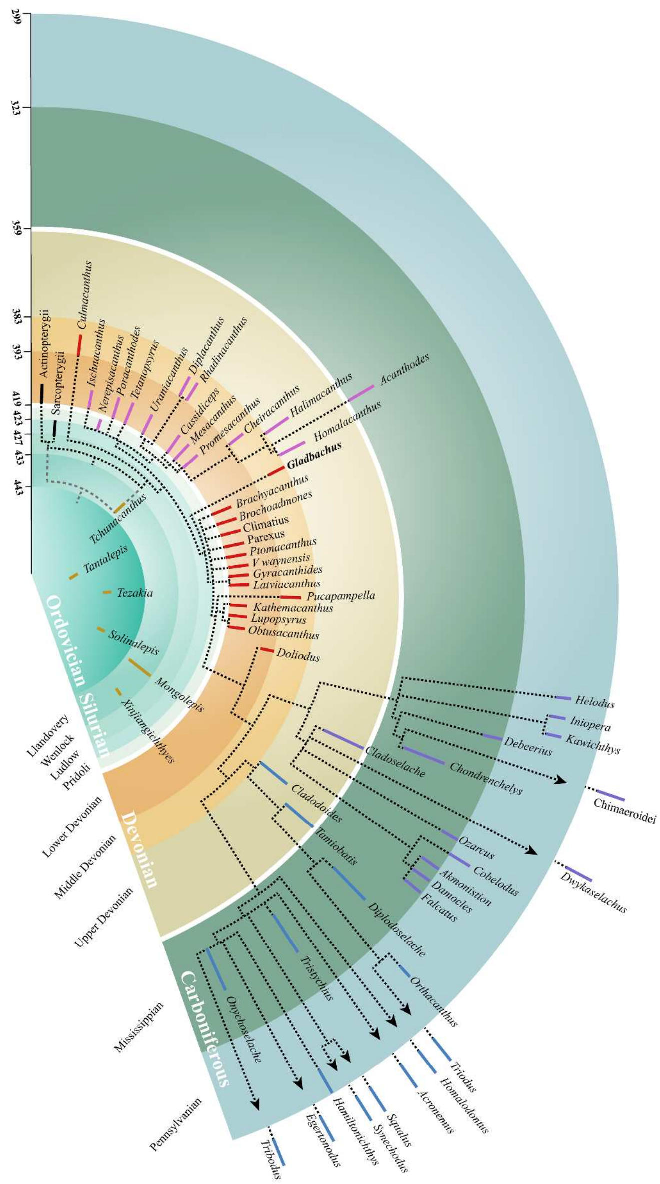

Figure 4

293x426mm (300 x 300 DPI)