

LETTERS

Devonian arthrodire embryos and the origin of internal fertilization in vertebrates

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Evidence of reproductive biology is extremely rare in the fossil record. Recently the first known embryos were discovered within the Placodermi¹, an extinct class of armoured fish, indicating a viviparous mode of reproduction in a vertebrate group outside the crown-group Gnathostomata (Chondrichthyes and Osteichthyes). These embryos were found in ptyctodontids, a small group of placoderms phylogenetically basal to the largest group, the Arthrodira^{2,3}. Here we report the discovery of embryos in the Arthrodira inside specimens of *Incisoscutum ritchiei* from the Upper Devonian Gogo Formation of Western Australia⁴ (approximately 380 million years ago), providing the first evidence, to our knowledge, for reproduction using internal fertilization in this diverse group. We show that *Incisoscutum* and some phyllolepid arthrodires possessed pelvic girdles with long basipterygia that articulated distally with an additional cartilaginous element or series, as in chondrichthyes, indicating that the pelvic fin was used in copulation. As homology between similar pelvic girdle skeletal structures in ptyctodontids, arthrodires and chondrichthyes is difficult to reconcile in the light of current phylogenies of lower gnathostomes^{2,3,5}, we explain these similarities as being most likely due to convergence (homoplasy). These new finds confirm that reproduction by internal fertilization and viviparity was much more widespread in the earliest gnathostomes than had been previously appreciated.

The first appearance of viviparity was considered to have evolved within the chondrichthyes, where today it is the dominant form of reproduction, occurring far less commonly among the osteichthyes^{6,7}. However, discovery of viviparity within ptyctodontids, a group belonging in the extinct class Placodermi¹, shifted the evolutionary origin of this reproductive mode back to the most phylogenetically basal gnathostome (jawed vertebrate) group^{2,3}. All extant chondrichthyan taxa are sexually dimorphic, with males possessing distinct erectile claspers that are elongate modifications of the pelvic fins used for sperm transfer. Approximately 55% of all chondrichthyes are viviparous⁷ and molecular phylogenies indicate that this mode of reproduction arose many times within both chondrichthyes^{7,8} and actinopterygians^{7,9,10}. Once viviparity develops, the complex physiological requirements that accompany live-bearing generally prohibit reversal back to external spawning⁷, although there are rare cases of reversal from live-bearing to egg-laying in squamate reptiles¹¹. The arthrodires are the most diverse group of placoderms (>200 species) and they show no signs of sexual dimorphism. Bone morphology and ossification patterns in ptyctodont embryos from *Materpiscis* and *Austroptyctodus*, preserved in three-dimensional uncrushed form from the Upper Devonian Gogo Formation of Western Australia¹ (see Supplementary Information), now allow us to reinterpret small arthrodires discovered within the body cavities of two adult arthrodires from Gogo, *Incisoscutum ritchiei*, as representing embryos (Fig. 1). The small arthrodires were previously presumed to have been

eaten by the larger adult fish⁴. However, the excellent preservation of the small arthrodires does not match poorly preserved stomach contents observed in other placoderms^{12–14}. The small *Incisoscutum* specimens were described as ‘gut contents’ on the basis of the disorganized arrangement of one specimen (P50934); their posterior position behind the trunk shield; presumed gastric etching of some of the trunk plates; and one embryo (P57640) facing posteriorly in relation to the adult⁴. However, the overall position of these embryos (Fig. 1a, b) is exactly the same as the position of embryo 2 within the ptyctodont *Austroptyctodus*¹ (WAM 86.9.662, see Supplementary Information). The disorganized arrangement of dermal plates in P50934 is a result of scattering following the opening of the abdominal cavity. Embryo 3 within *Austroptyctodus* shows a similar disorganized pattern of dermal plates, with some plates outside the abdominal cavity (see Supplementary Information). Worm casts scattered throughout the body and surrounding matrix indicate that the *Incisoscutum* carcass was open to the environment for some time after death (Fig. 1c, d). The posterior orientation of the small arthrodire inside P57640 was considered to be evidence that it was a prey item swallowed head first⁴, but this is consistent with the orientation of all four known embryonic ptyctodonts¹. The stomach contents of *Coccoosteus cuspidatus* (Middle Devonian period, Scotland) were described as being an ovoid phosphatic mass, with prey items including the scales of acanthodians and possible dipnoan scales¹². In addition, detrital grains interpreted as gizzard stones occur inside *Coccoosteus cuspidatus*¹⁴. The complete preservation of the dermal plates and the absence of other taxa associated with the embryonic plates suggest that the arthrodires within adult *I. ritchiei* specimens can now be excluded as stomach contents.

The presumed gastric ‘acid etching’ on the dermal plates of the arthrodire embryos, forming an ‘open weave’ appearance on the bone surface⁴, can now be attributed to an early stage of ossification. Differential ossification is also seen in the juveniles of ichthyosaurs¹⁵, pterosaurs¹⁶, the placoderms *Bothriolepis*¹⁷ and *Asterolepis*¹⁸, and the ptyctodont embryos¹. Juveniles (13–14 mm) of the antiarch *Asterolepis ornata* show lamellar bone with an irregular network of bony trabeculae on the external surface of the lamina. The second stage of development is the formation of the spongy middle layer¹⁸. Larger individuals (longer than 30 mm) have fine meshed reticular ornament, which sometimes bears tubercles. The ptyctodont and *Incisoscutum* embryos appear to have reached this final developmental stage, although, unlike *Asterolepis*, they remained *in utero*.

Further new evidence of arthrodire reproductive biology has come to light with our re-examination of the pelvic girdle structure in *Incisoscutum* and in the Phyllolepidia, a dorsoventrally flattened group of basal arthrodires (Fig. 2). The pelvic girdle is well known in *Incisoscutum* and the perichondrally ossified pelvic radials are well preserved⁴. The posterior large facet for the ‘metapterygium’ identified previously⁴ is here reinterpreted as being for a long, posteriorly

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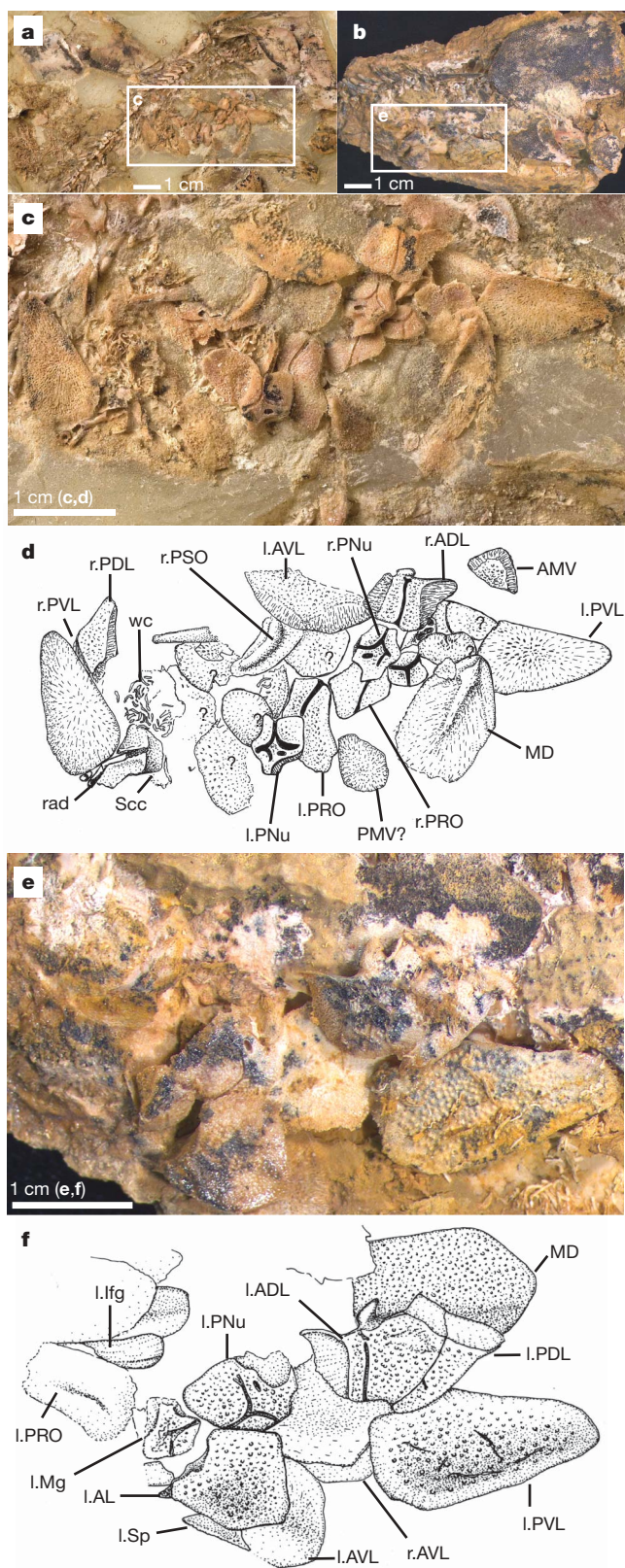


Figure 1 | *Incisoscutum ritchiei* specimens showing embryo fossils. **a, c, d**, P50934; **b, e, f**, P57640. AMV, anterior median ventral; I.AL, left anterolateral; I.AVL/r.AVL, left and right anterior ventrolaterals; I.lfg, left inferognathal; I.Mg, left marginal; I.PDL, left posterior dorsolateral; I.PVL/r.PVL, left and right posterior ventrolaterals; I.Sp, left spinal; MD, median dorsal; PMV?, posterior median ventral?; r.ADL, right anterior dorsolateral; I.PNu/r.PNu, left and right paranuchals; r.PDL, right posterior dorsolateral; I.PRO/r.PRO, left and right preorbitals; r.PSO, right postsuborbital; rad, radialia; Scc, scapulocoracoid; wc, worm casts.

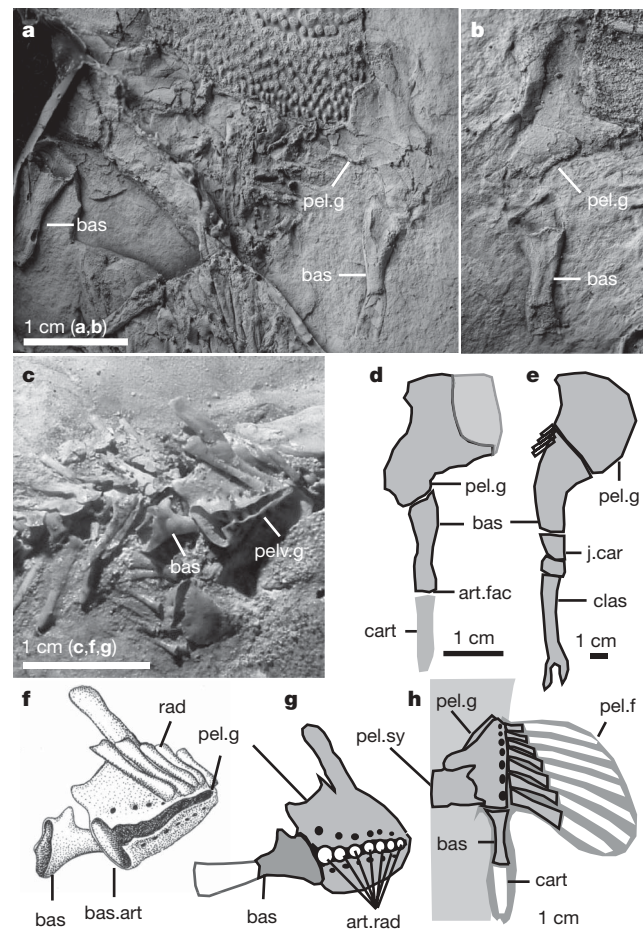


Figure 2 | Placoderm and shark pelvic structures. **a, b, d**, The phyllolepid *Austrophyllolepis ritchiei* (MV P160746) showing pelvic girdle in ventral (**a**) and dorsal (**b**) aspects, and compared, after being restored (**d**), with the shark *Cobelodus* (**e**). **c, f–h**, *Incisoscutum ritchiei*. **c, f, g**, WAM 04.10.02. **g**, Pelvic girdle restored (full extent of basipterygium unknown). **h**, Pelvic fin structures restored in dorsal view showing restored fin cartilages in white. art.fac, articular facet for additional pelvic elements; art.rad, radial articulations; bas, basipterygium; bas.art, basipterygial articulation facet; cart, cartilage distal to basipterygium; clas, clasper; j.car, junction cartilages; pel.f, pelvic fin; pel.g, pelvic girdle; pel.sy, pelvic symphysis; rad, radialia.

facing basipterygium (Fig. 2c, f) by comparison with the pelvic girdle in selachians (Fig. 2e). The basipterygium is seen in WAM 04.10.2 (Fig. 2c, f) and is here reconstructed as in life (Fig. 2g, h).

The pelvic girdle in phyllolepid was first described from *Austrophyllolepis* from the Middle Devonian strata of Victoria, Australia¹⁹. It was compared with claspers in the stem chondrichthyan *Cobelodus*, but dismissed owing to lack of demonstrable sexual dimorphism. We now identify a broad-based pelvic plate articulating to a long, posteriorly directed basipterygium in these phyllolepid (Fig. 2a, b, d). The distal end of the basipterygium has an articulation surface for an additional cartilaginous segment or series. Further complete pelvic girdles of similar gross morphology occur in another phyllolepid, *Cowralepis*, from New South Wales, Australia²⁰. The long basipterygia of *Cowralepis*, *Austrophyllolepis* and *Incisoscutum* all show a remarkably chondrichthyan-like morphology (Fig. 2e). A comparable structure is unknown in early osteichthyan pelvic girdles, although juvenile sturgeons do show a series of pelvic basals in similar topological position to the basipterygium linking the last radial with the metapterygium²¹. In view of the presence of embryos within *Incisoscutum*, in association with a long basipterygium, this structure probably had a role in copulation as it does in chondrichthyans. It must have articulated distally with at least one other unossified cartilage, possibly for a clasper in males and/or a non-clasping cartilage

in females. The proximal part of the pelvic girdle (pelvic basal plate and basipterygium) appears to be the same shape and size in both male and female phyllolepid, comparable to modern chondrichthyan pelvic girdles. In *Squalus* the pectoral girdle and basipterygium are of the same shape and size in both sexes, so only the distal elements differ in morphology. This loss of ossification in the distal pelvic fin skeleton most probably corroborates with the use of the pelvic fin in erectile copulation, as occurs in chondrichthyans.

Viviparity has been reported in several fossil fish groups including the Middle Triassic actinopterygian *Peltopleurus lissocephalus* Brough 1939²² the coelacanth *Holophagus* Watson 1927²³ and the holocephalan *Delphyodontos dacriformes* Lund 1980²⁴. This indicates that viviparity developed within Osteichthyes without claspers, and often with no signs of external sexual dimorphism. The presence of embryos in the arthrodire *Incisoscutum* indicate that internal fertilization was the main reproductive mode for this species, and that viviparity could have evolved in other eubranchyothoracid arthrodires.

Ptyctodontid claspers are dermal bones²⁵ having a groove for cartilaginous internal support that has been suggested as being homologous with the cartilaginous basipterygium of elasmobranch claspers²⁶. We have no evidence, however, of sexual dimorphism shown in the pelvic fins of arthrodires, but this is not a requirement for internal fertilization, as shown by the various families of extant viviparous teleosts and the sarcopterygian *Latimeria*²³.

Mapping of viviparity and the presence of claspers onto recent hypotheses of gnathostome interrelationships shows that there is no parsimonious reconciliation of these features with current phylogenetic schemes (Fig. 3a, b). Claspers in ptyctodontids and chondrichthyans are thus here interpreted as being non-homologous, as to accept homology based on these phylogenies^{2,3} implies either a loss of claspers in osteichthyans (and/or some acanthodians³), or a monophyletic grouping of placoderms and chondrichthyans, which assumes claspers might have been present in other placoderm

groups²⁷. We leave the problem unresolved, and accept that the most likely explanation for this similarity in pelvic fin morphology in ptyctodontids and chondrichthyans is homoplasy, or convergence.

Our discovery of the first embryos in arthrodire fishes thus demonstrates that internal fertilization was a more widespread reproductive strategy in the earliest jawed vertebrates. Furthermore, our observation that certain arthrodires (including phyllolepid) had elongated pelvic basipterygia that articulated distally with additional cartilage elements as in chondrichthyans supports the view that a copulatory mode of reproduction involving manipulation of the pelvic fin for sperm delivery had evolved at this early stage of vertebrate evolution. Although homology between such structures in placoderms (ptyctodontids) and chondrichthyans is not yet resolved, such observations provide informative new data to contribute to the ongoing debate concerning the origins and interrelationships of the first jawed vertebrates.

METHODS SUMMARY

The specimens of *Incisoscutum* were prepared at the Natural History Museum, London, using the standard acetic acid technique. Reference 1 provides details of this method as applied to preparing out embryonic ptyctodontid placoderms. The specimens of *Austrophyllolepis* were prepared using negative preparation by immersing the specimens in 10% HCl overnight to dissolve away the weathered bone. They were then washed in water for 24 h, dried and hardened using 3% paraloid in acetone. A latex peel (darkened with Indian ink) of the cleaned mould was then made, and whitened with ammonium chloride sublimate for high-contrast photography.

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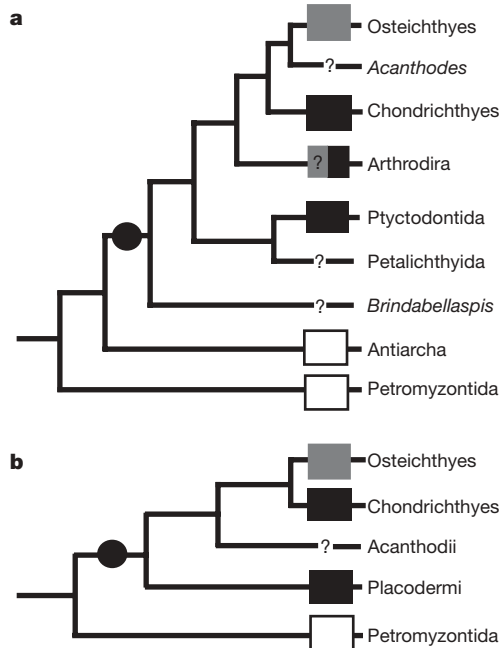


Figure 3 | Lower gnathostome phylogenies showing reproductive strategies. Viviparity has arisen many times in vertebrate evolution using different morphologies for internal fertilization. **a**, After Brazeau³. **b**, After Young²⁶ and Goujet and Young² (based on two broad assumptions: (1), placoderm monophyly; (2), all placoderms reproduced by internal fertilization). Black circle, presence of pelvic fins; black rectangle, viviparity with claspers present; grey rectangle, spawning and/or internal fertilization without claspers; white rectangle, spawning mode of reproduction; ?, pelvic region too poorly known to infer reproductive mode.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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