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Copulation in antiarch placoderms and the origin of gnathostome internal fertilisation

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Reproduction in jawed vertebrates (gnathostomes) involves either external or internal fertilization¹. It is commonly argued that internal fertilization can evolve from external, but not the reverse. Male copulatory claspers are present in certain placoderms²⁻⁴, fossil jawed vertebrates retrieved as a paraphyletic segment of the gnathostome stem group in recent studies⁵⁻⁸. This suggests that internal fertilization could be primitive for gnathostomes, but such a conclusion depends on demonstrating that copulation was not just a specialized feature of certain placoderm subgroups. The reproductive biology of antiarchs, consistently identified as the least crownward placoderms⁵⁻⁸ and thus of great interest in this context, has until now remained unknown. Here we show that certain antiarchs possessed dermal claspers in the males, while females bore paired dermal plates inferred to have facilitated copulation. These structures are not associated with pelvic fins. The clasper morphology resembles that of ptyctodonts, a more crownward placoderm group⁷⁻⁸, suggesting that all placoderm claspers are homologous and that internal fertilization characterized all placoderms. This implies that external fertilisation and spawning, which characterizes most extant aquatic gnathostomes, must

(g) views; e, large hooked dermal element in ventral view; f, reversed image; h-m, male *Microbrachius dicki* specimens showing claspers with close-up views of some specimens. h-i, NHMUK PV P73397; j, NHMUK PV P77402; k, NHMUK PV P77401; l-m, NHMUK PV P77405. Abbreviations: cls, dermal clasping elements; clsd, distal clasping element; clst, terminal hooked clasping element; gr, groove; mls, midline suture; orn, ornament; PVL, posterior ventrolateral plate; ri, ridge; sp, spines; sut, suture between clasper and PVL; tvr, transverse lateral ridge; vl, ventral lamina; vlr, ventrolateral ridge.

Microbrachius, a small bothriolepidoid antiarch, is known from complete articulated specimens from the Middle Devonian (Givetian) of Scotland¹⁴, the Early-Middle Devonian of China^{15,16}, and by isolated plates from the Essi Farm site in Estonia. Numerous new articulated specimens from the Eday Flags, Orkney Islands, Scotland, show either male dermal claspers or female genital plates in life position (Figs. 1, 2; Extended Data Fig.3), and one specimen from Estonia shows an isolated right posterior ventrolateral plate (PVL) with an attached dermal clasper (Fig. 1a-c), (Supplementary Information: A1,2, Extended Data Figs 1,2).

The clasper of *Microbrachius* is a deeply grooved dermal bone (Fig. 1a-c, h-m) that curves laterally, similar to the main hooked dermal clasper element of ptyctodontid placoderms^{2,4}. The groove (gr, Fig. 1 l,m) may have served to transfer sperm, or encased a structure that carried the sperm canal. The articulated specimens show some of the claspers in mesial contact, sutured in the midline (Fig. 1i-j) indicating they were not mobile. The extended wing of the clasper extends laterally as far as the width of the trunkshield (Fig. 1h,j,k), potentially enabling a male *Microbrachius* clasper to reach the cloaca of a female if the two individuals were side by side (Fig. 3). Ventrally the claspers have well defined dermal ornamentation of small posteriorly directed spines, and have a series of larger spines along the distal margin (Fig. 1l, m). Variation in clasper size likely reflects individual sexual maturity (Extended Data Fig.3).

Female *Microbrachius dicki* (Fig. 2 f-I, 3c) show paired blade-like structures in the same region corresponding to the male clasper (Fig. 2g-h). These blades carry a distinctive ornament of curving ridges and marginal tubercles on their dorsal (i.e. internal) surfaces: the only internally facing ornament in their dermal skeleton. Identical internal ornament is seen in *Pterichthyodes*¹⁴, where it occurs on separate dermal plates preserved atop the dorsal (internal) side of the subanal lamina of the PVL plates (Fig. 2a-d) in similar position as the male claspers. Within other placoderms, where separate male and female dermal elements are known, they are similarly positioned^{2,4}. These dermal plates are flat and taper to meet the lateral ends of the transverse ventral ridge inside the lateral lamina of the PVL plate (Fig. 2f-h). We interpret these structures in *Microbrachius* and *Pterichthyodes* as female genital plates, similar to the post-pelvic plates found in some female ptyctodonts⁴, and suggest that the claspers attached to them during mating by gripping the internal ornament which faced into the cloacal chamber.

Two specimens of the Late Devonian antiarch *Bothriolepis* also show small semicircular plates sitting on the distal area of the subanal lamina of the PVLs (Fig. 2j- m). They are only visible in dorsal view, and show a slight thickening anteriorly with a line of roughened pits for ligamentous or muscle attachment near the anterior margin. We interpret

these as female genital plates as they do not resemble male claspers in *Microbrachius*, and occupy the same topology as the paired genital plates in female *Pterichthyodes*. We also identify new features on the subanal lamina of the PVL plates in other antiarchs which likely relates to their reproductive anatomy (Extended Data Fig. 4, Supplementary Information:B4).

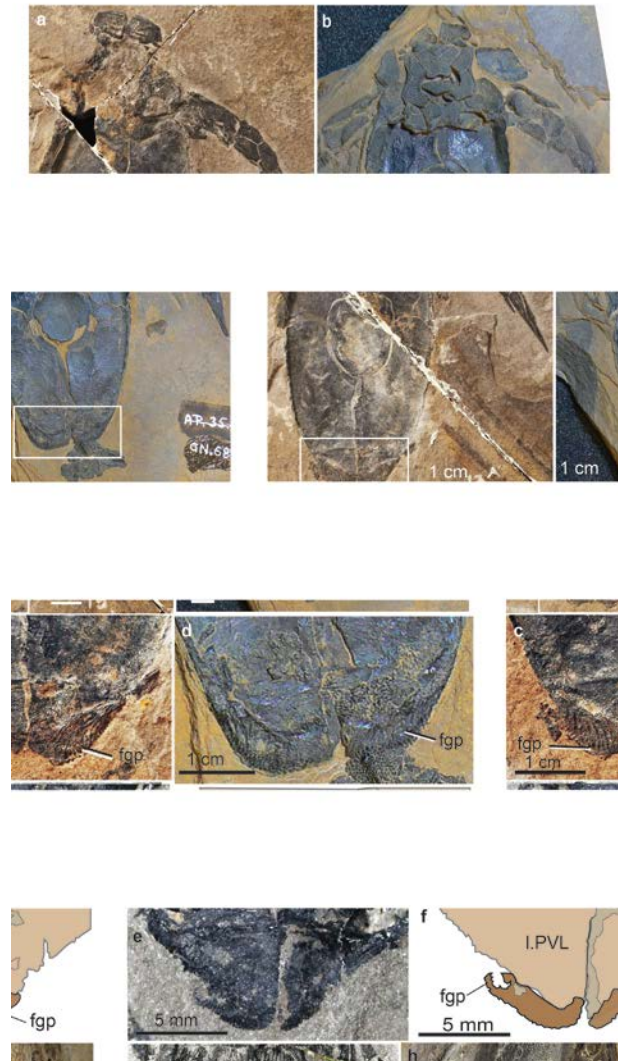


Figure 2. Female reproductive structures in Middle-Late Devonian antiarchs. a,c, *Pterichthyodes milleri*; NHMUK PV P32544; b,d, UMZC 687. e-g, *Microbrachius dicki*; e-f, NHMUK PV P73398; g, NHMUK PV P73399; h,i, *Bothriolepis canadensis*, V11127 (Smithsonian), showing armour (h) and female genital plates (j). i, *Bothriolepis* sp. ANU V1040, close up of paired female genital plates. Abbreviations: fgp, female genital plates; l.PVL, left posterior ventrolateral plate.

We thus have compelling evidence for a clasper-based system of internal fertilization in *Microbrachius*, strong circumstantial evidence for the same system in *Pterichthyodes*, and plausible evidence for the same in *Bothriolepis*. Although male claspers have not been described in antiarchs other than *Microbrachius*, even from taxa such as *Bothriolepis*^{17, 18}, *Asterolepis*¹⁹ and *Remigolepis*²⁰ known from hundreds of articulated specimens with tails

preserved, we suggest that internal fertilization is general for the Antiarchi. As the dermal skeleton is quite reduced in advanced antiarchs we propose that the claspers in some forms might have also been cartilaginous and thus not well preserved. Further evidence for internal fertilisation in antiarchs comes from their large hatchlings¹³ (Supplementary Information: B4).

The clasper in *Microbrachius* is clearly different from the pelvic girdle and fin, known only in one antiarch: *Parayunnanolepis* from the Early Devonian of China²¹. More derived asterolepidoid and bothriolepidoid antiarchs lack pelvic girdles and fins, which are

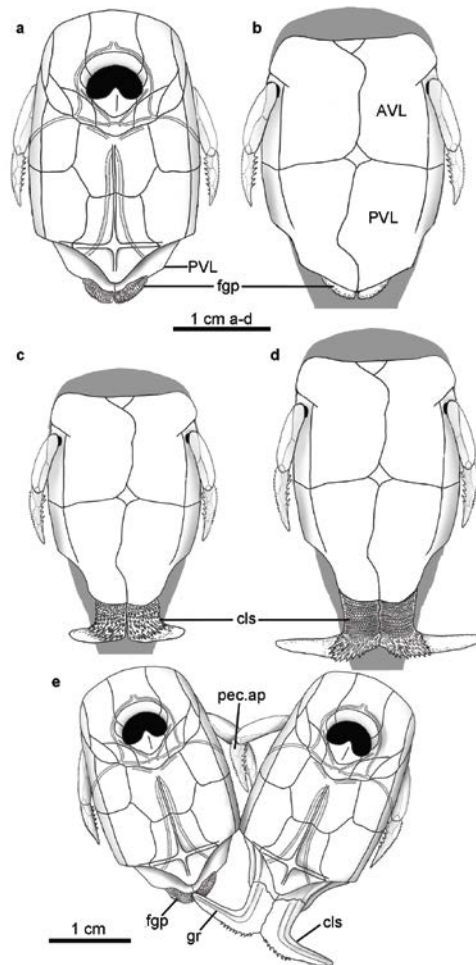


Figure 3. Sexual dimorphism in *Microbrachius dicki*. Reconstruction of female *Microbrachius dicki* in (a) dorsal and (b) ventral views; c,d, male *Microbrachius dicki* ventral views showing variations in clasper development; e, hypothetical mating position for *Microbrachius*. Abbreviations as for Figs 1, 2 plus pec.ap, pectoral appendage.

thus assumed to be lost (secondarily absent) in these groups¹⁹. In chondrichthyans, the clasper is attached to the posterior extremity of the pelvic fin metapterygium^{9,22,23}. In ptyctodont and arthrodire placoderms, the clasper is immediately posterior to the pelvic fin⁴, and has previously been interpreted as an elaboration of the pelvic fin skeleton^{2,3}. However, new evidence shows that the clasper of arthrodires does not articulate directly with the pelvic

girdle or fin⁴. In ptyctodonts the endoskeleton of the clasper (if present) was unossified¹², making it more difficult to determine its precise relationship to neighbouring structures, but the dermal components of the claspers are consistently preserved some distance posterior to the pelvis^{2,4,12}. All known placoderm claspers thus differ from chondrichthyan claspers in being independent from the pelvis and pelvic fin. The principle dermal element of the ptyctodont clasper is a large curved, grooved plate (Fig. 1e, f) that distinctly resemble and is thus likely homologous to the dermal clasper of *Microbrachius*, whereas arthrodire claspers have a much smaller but plausibly homologous dermal bone tip^{3,4}. Based on similarities in position (behind and independent of pelvic region), and materials (dermal bone), the claspers of antiarchs, ptyctodonts and arthrodires are most probably homologous with each other, but not homologous with the claspers of chondrichthyans.

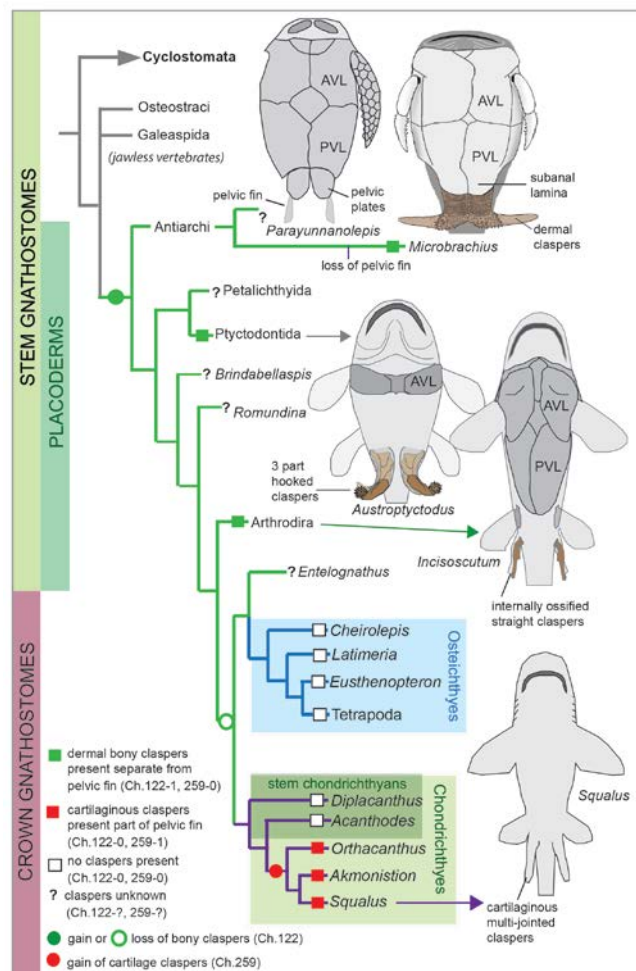


Figure 4. Phylogeny of major lineages of gnathostomes, based on analysis of an expanded version of the dataset from ref 8. Distribution and morphology of two kinds of claspers are mapped on the phylogeny. Claspers are most parsimoniously inferred to have evolved in the most recent common ancestor of all gnathostomes, then lost before or at the node of crown group gnathostomes. Claspers developed as a modified part of the pelvic fin skeleton appears as a synapomorphy of all chondrichthyans. Full tree with branch supports and states for all terminal taxa in Extended Data Figures 5 and 6. See SI for further details of the analysis.

To evaluate the evolution of claspers and reproductive biology across gnathostomes, we expanded upon a recently published phylogenetic analysis⁸ with the addition of 14 placoderm taxa, three new characters (256-258), and one character (122) split into two (122, 159) (Supplementary Information: C7,8). Our analyses of both expanded and original data sets yielded very similar trees, which supported placoderm paraphyly and placed antiarchs as the sister group to all other gnathostomes (Extended Data, Figs. 5,6; Supplementary Information 6.1). Our analyses found shorter trees for the original dataset⁸ supporting a more orthodox position for ptyctodonts lower on the gnathostome stem (Extended Data Figs. 7, 8; Supplementary Information C7,8). The shortest tree indicates that bony claspers separate from the pelvic fin arose in the most recent common ancestor (MRCA) of jawed vertebrates and were lost in the MRCA of crown gnathostomes (Fig. 3; Extended Data, Figs 5,6).

These results have intriguing biological implications. The implied loss of bony claspers and implied reversion to external fertilisation in crown gnathostomes appears heterodox: loss of internal fertilization and acquisition of external fertilization is not widely accepted, at least in vertebrates^{1,11} although it could have happened multiple times in invertebrates²⁴. The shared, unique morphology and post-pelvic position of claspers in all placoderms is more consistent with a single origin, and thus represents a potential synapomorphy supporting placoderm monophyly²⁵.

If all placoderm claspers are homologous, as we suggest, this gives rise to alternative implications with equally startling significance for early vertebrate evolution. If placoderm paraphyly is accepted, based on the optimal trees here (and consistent with the majority of recent analyses^{5-8,23}), then external fertilization and spawning employed by the majority of recent bony fishes and many lissamphibians must have evolved from clasper-mediated internal fertilization. If claspers are accepted as *prima facie* evidence of placoderm monophyly²⁵, the transformation of cranial architecture within the Placodermi documented by several recent analyses⁵⁻⁸ must be entirely convergent on crown gnathostomes, as the antiarchs belong to the cluster of primitive placoderms with posteriorly placed rostronasal capsules and a trabecular "upper lip"⁸. Placoderm monophyly is also inconsistent with the co-occurrence of placoderm and osteichthyan characteristics in Silurian taxa such as *Entelognathus*⁷. Resolution of the status of placoderms, which will require data from both fossil anatomy and the reproductive physiology of extant fishes, is one of the most pressing tasks currently facing early gnathostome palaeontology.

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Supplementary Information is attached.

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Author Contributions. The project was designed by JAL, with material examined and described by JAL, EMK, ZJ, KT, BC and PEA. MSYL performed phylogenetic analyses with input from JAL, GCY and BC. MN, JDB and RJ collected and prepared material, provided site information, and input to the discussion. Illustrations were made by JAL and BC with photography supplied for some specimens by the Natural History Museum, London, and by the Institute of Geology at Tallinn University of Technology. All authors contributed to data interpretation, figures and writing of the MS.

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Extended Data Figure 1. Top, map showing locality of the Essi Farm site, Estonia, and stratigraphic section where the fossils were found. Modified from Mark-Kurik (1999). Below, *Microbrachius* sp. plates from Essi Farm, Estonia. A, GIT 628-37, sample showing several small plates and fragments; B, GIT 628-9, right lateral plate, visceral view; C, 628-3, posterior median dorsal plate, dorsal view; D, GIT 628-25, right posterior ventrolateral plate,

visceral view; E, GIT 628-18, anterior section of anterior ventrolateral plate, lateral view showing brachial process. All specimens held within the Institute of Geology, Tallinn University of Technology, Estonia, Collection GIT 628.

Extended Data Figure 2. Top, map of the Orkney Islands with an asterisk marking the location where the specimens of *Microbrachius dicki* described in this paper were collected. Below, stratigraphical column of the upper part of the Middle Devonian in the Orkney Islands with the position of the Eday Flagstone Formation fish beds marked by a dotted line.

Extended Data Figure 3. Growth of claspers in *Microbrachius dicki* males. a, b, NHMUKVP P 77400, claspers only weakly developed, no lateral wing; close up of claspers in b; c,d, NHMUK VP P 77403 showing further caudally-directed growth of claspers, d, close up of claspers showing fusion in midline. Scale bars are 1 cm.

Extended Data Figure 4. Top, *Yunnanolepis porifera*, Xitun Formation, Yunnan, China. Specimen IVPP V19359) in a, dorsal view; b, ventral view and c, showing posterior region of trunkshield prepared to show internal side of the PVL plates. p.ri, strong ridge on the dorsal surface of the posterior region of the PVL plates. Below, a-b, *Bothriolepis* sp., Gogo Formation, Western Australia (P223045, Museum Victoria, Melbourne). c, *Bothriolepis canadensis*, Escuminac Formation, Quebec, Canada. (UF 252, Field Museum, Chicago). Abbreviations: m.att? muscle attachment area; ri.i, internal ridge, ri.o, outer ridge; pl, platform; sb.l, subanal lamina; tvr, transverse ridge (= *crista transversalis interna posterior*, Stensiö 1948).

Extended Data Figure 5. Strict consensus tree from 7039 trees (L=640) from analysis of the expanded dataset (85 taxa, 259 chars). Numbers on branches denote Bremer and bootstrap support. Green squares denote presence of bony claspers (character 122), red squares denote presence of cartilaginous claspers (character 259), and white squares absence of both types of claspers. Circles denote gain/loss of the two types of claspers under the most-parsimonious optimisation.

Extended Data Figure 6. Majority-rule consensus tree, and also one of the most-parsimonious trees (length 640) from analysis of the expanded dataset (85 taxa, 259 chars). Numbers on branches indicate % of MPTs which contain a particular clade (100% unless otherwise indicated). Green squares denote presence of bony claspers (character 122), red squares denote presence of cartilaginous claspers (character 259), and white squares absence of both types of claspers. Circles denote gain/loss of the two types of claspers under the most-parsimonious optimisation.

Extended data Figure 7. Strict consensus tree from 808 trees (L=611) from reanalysis of the dataset in Dupret et al.⁸ Numbers on branches denote Bremer and bootstrap support.

Extended data Figure 8. Majority-rule consensus tree, and also one of the most-parsimonious trees (length 611) from analysis of the data in Dupret et al.⁸ Numbers on branches indicate % of MPTs which contain a particular clade (100% unless otherwise indicated).

Extended data Figure 9. Reconstruction showing hypothetical mating *Microbrachius*, with male to the right, female on left. Artwork by Brian Choo.