

Research



Cite this article: Charest F, Johanson Z, Cloutier R. 2018 Loss in the making: absence of pelvic fins and presence of paedomorphic pelvic girdles in a Late Devonian antiarch placoderm (jawed stem-gnathostome). *Biol. Lett.* **14**: 20180199.
<http://dx.doi.org/10.1098/rsbl.2018.0199>

Received: 21 March 2018

Accepted: 21 May 2018

Subject Areas:

developmental biology, evolution, palaeontology

Keywords:

placoderm, fossilized ontogeny, atavism, allometric growth, perichondral bone, endochondral bone

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Electronic supplementary material is available online at <https://dx.doi.org/10.6084/m9.figshare.c.4116899>.

Palaeontology

Loss in the making: absence of pelvic fins and presence of paedomorphic pelvic girdles in a Late Devonian antiarch placoderm (jawed stem-gnathostome)

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Within jawed vertebrates, pelvic appendages have been modified or lost repeatedly, including in the most phylogenetically basal, extinct, antiarch placoderms. One Early Devonian basal antiarch, *Parayunnanolepis*, possessed pelvic girdles, suggesting the presence of pelvic appendages at the origin of jawed vertebrates; their absence in more derived antiarchs implies a secondary loss. Recently, paired female genital plates were identified in the Late Devonian antiarch, *Bothriolepis canadensis*, in the position of pelvic girdles in other placoderms. We studied these putative genital plates along an ontogenetic series of *B. canadensis*; ontogenetic changes in their morphology, histology and elemental composition suggest they represent endoskeletal pelvic girdles composed of perichondral and endochondral bone. We suggest that pelvic fins of derived antiarchs were lost, while pelvic girdles were retained, but reduced, relative to *Parayunnanolepis*. This indicates developmental plasticity and evolutionary lability in pelvic appendages, shortly after these elements evolved at the origin of jawed vertebrates.

1. Introduction

Jawed vertebrates (Gnathostomata) share short-based pectoral and pelvic appendages supported by endoskeletal elements [1]. Pectoral girdle-supported fins first evolved in fossil jawless osteostracans [2], whereas pelvic fins originated in the most basal gnathostomes, the antiarch placoderms [3,4], based on dermal and endoskeletal pelvic girdles in a specimen of the Early Devonian *Parayunnanolepis xitunensis* [3]. Beyond this, pelvic fins were reported in the Late Devonian (*ca* 380 million years ago) derived antiarch *Bothriolepis canadensis* [5,6], but this has been questioned, and pelvic appendage absence in derived antiarchs is interpreted as a secondary loss [3,7,8]. Furthermore, other skeletal elements, dorsal to the posterior ventrolateral (PVL) plates (pelvic girdle position in other placoderms [9]) of the *Bothriolepis* thoracic shield, were interpreted as an anal plate [5,10] or female dermal genital plates [11]. Here, bony pelvic plates in 32 *Bothriolepis* specimens ranging from 28 to 186 mm total dorsal armour length (TALd) are described. Based on their morphology, histology and ontogenetic changes, we suggest that these represent the endoskeletal pelvic girdles of *Bothriolepis*, as do the aforementioned anal and genital plates.

The *Bothriolepis* pelvic girdle is relatively smaller than in *Parayunnanolepis*, and fin radials are lost (but with occasional atavistic reappearance), suggesting

reduction of the pelvic girdle in *Bothriolepis* as part of the loss of pelvic appendages in antiarchs. We also document the presence of perichondral bone, previously problematic in antiarchs [12], and endochondral bone in *Bothriolepis* (electronic supplementary material, table S1).

2. Material and methods

A size series of 340 specimens (4–205 mm TALd) of *B. canadensis* (Upper Devonian Escuminac Formation, Canada) was examined (Leica MZ9.5). Specimens preserving a pelvic girdle (32) were photographed (Nikon D80/Leica IC80) and drawn (camera lucida). Specimens are housed in the Musée d'Histoire Naturelle de Miguasha (MHNM) and the Natural History Museum (UK; NHMUK).

Pelvic girdle growth was studied, measuring length (Lpelg) and width (Wpelg) of left and right (L, R) girdles. Analyses were performed using R (v. 3.3.2; libraries PSYCH, lmodel2, car) to estimate: (i) girdle bilateral symmetry by comparing the mean of 'L minus R' with a mean of zero (*t*-test), (ii) Spearman rank correlation of the number of concentric lines on the girdle surface relative to Lpelg, Wpelg and TALd, and (iii) allometric growth with major axis and two ordinary least square regressions on log₁₀ transformed data (electronic supplementary material).

Girdles were removed from three specimens and embedded in EpoFix Resin (Struers). Thin sections were prepared (Secotom-50), ground, polished (LaboPol-35, Struers) and sealed (Araldite 2020 epoxy, coverslips). Slides were examined (Leica DMLB microscope) and photographed (AmScope: MUIOOO camera, Toupview, v. 3.7 2013).

Elemental composition analyses were performed with a scanning electron microscope (SEM; JEOL 6460LV) equipped with an energy dispersive spectrometer (Cu calibrated; 100 s; EDS, INCA X-sight, Oxford Instruments).

3. Results

Pelvic girdles were observed within the trunkshield above the posterior margin of the PVL plates (figure 1*a–d*) in 32 specimens larger than 28 mm TALd (electronic supplementary material, table S2). In 73 smaller specimens (4–28 mm TALd), girdles were not observed (unmineralized/not yet developed); thus TALd at the onset of girdle development is unknown. The pelvic girdle includes two symmetrical semicircular bony plates (half-girdles; electronic supplementary material, table S3). Half-girdles join at a median suture in 25 specimens, including the smallest (figure 2*a–c*), being separated in seven specimens. Left and right half-girdles show contiguous ossification centres (foci) surrounded by hemi-concentric lines (figure 1*b,d,e,g,j,s*), interpreted as growth lines, increasing significantly in number with TALd and girdle width (electronic supplementary material, table S4). In two specimens (greater than 100 mm), the median suture is imperceptible, but foci and a few concentric lines appear as bulges on the surface (figure 1*g*; electronic supplementary material, figure S1), making the girdle appear unpaired.

The *Bothriolepis* pelvic girdle lacks articular surfaces for fin radials. Ossified elements, showing similar relative positions and orientations to the girdle, are present in two small specimens (figure 2*a–g*; electronic supplementary material, table S2). These elements, whether flat

with peripheral lamellar bone or tubular, are interpreted as fin radials.

In most specimens, including the smallest, the girdle elemental composition shows a low carbon content (4–20%) and a calcium:phosphorus ratio (2.06–2.71) similar to *Bothriolepis* dermal bones (1.66–2.31; electronic supplementary material, table S5). High carbon content is measured in one specimen (60%, MHNM 02-2426, 48.69 mm), suggesting the presence of cartilage, while calcium (6.90%) and phosphorus (0.61%) suggest mineralization was initiated, but incomplete. Despite these chemical differences, morphology is similar between carbon-rich (figure 2*e–g*) and ossified girdles (figure 1*s*). Mineralization in the pelvic girdle suggests the presence of bone; histologically, dorsal and ventral lamellar sheaths (perichondral) cover intermediate trabecular (endochondral) bone (figure 1*j–r*), and bone resorption and remodelling is observable in this region (re.z, re.l, figure 1*m,n,r*; electronic supplementary material). The perichondral sheaths show lines of arrested growth, suggesting, as for the surface concentric lines, cyclical bone deposition around the girdle (figure 1*n*).

The girdle appears wider than long in small and medium-sized (80–100-mm) specimens, while nearly as long as wide in large specimens (figure 1*j* versus *e*). Pelvic girdle length and width show positive allometric growth relative to TALd; this tendency is stronger for girdle length (electronic supplementary material, table S6). The pelvic girdle of *Parayunnanolepis* is proportionally larger than in juvenile *Bothriolepis* (figure 2*h*; electronic supplementary material). One small juvenile of the antiarch *Asterolepis ornata* (14–16 mm TALd; figure 4.35 in [13]) shows a 0.45 mm pelvic element with similar position and symmetrical appearance to *Bothriolepis*. This element shows slight overlap with the 99% equiprobability ellipse of *Bothriolepis* girdle length relative to TALd (figure 2*h*), but is smaller than in the smallest *Bothriolepis* specimen. No dermal pelvic girdle, intromittent organs or genital plates were observed in *B. canadensis*.

4. Discussion

(a) *Bothriolepis* pelvic girdle and fins among placoderms

The so-called 'median anal plate' of *Bothriolepis*, with its circular shape and concentric ridges [5] resembles the pelvic girdle described above, but this resemblance is less obvious in Stensiö's 'anal plate' (fig. 54 in [10]). Additionally, the anal plate in other placoderms is more posterior relative to the pelvic girdles [14], the latter located above the posterior margin of the PVL plates in all placoderms and in the same relative position when PVL plates are absent [9]. The putative *Bothriolepis* 'genital plates' (fig. 2*h–j* in [11]) closely resemble separated half-girdles in terms of position, presence of medial focal points and small size relative to the trunkshield length, with separation resulting from fracture at the weaker medial suture postmortem (e.g. figure 1*o*). Considering the inferred mating behaviour in antiarchs, with male claspers gripping the internal ornament of the female genital plates [11], it is unlikely that the *Bothriolepis* pelvic girdle had any reproductive function.

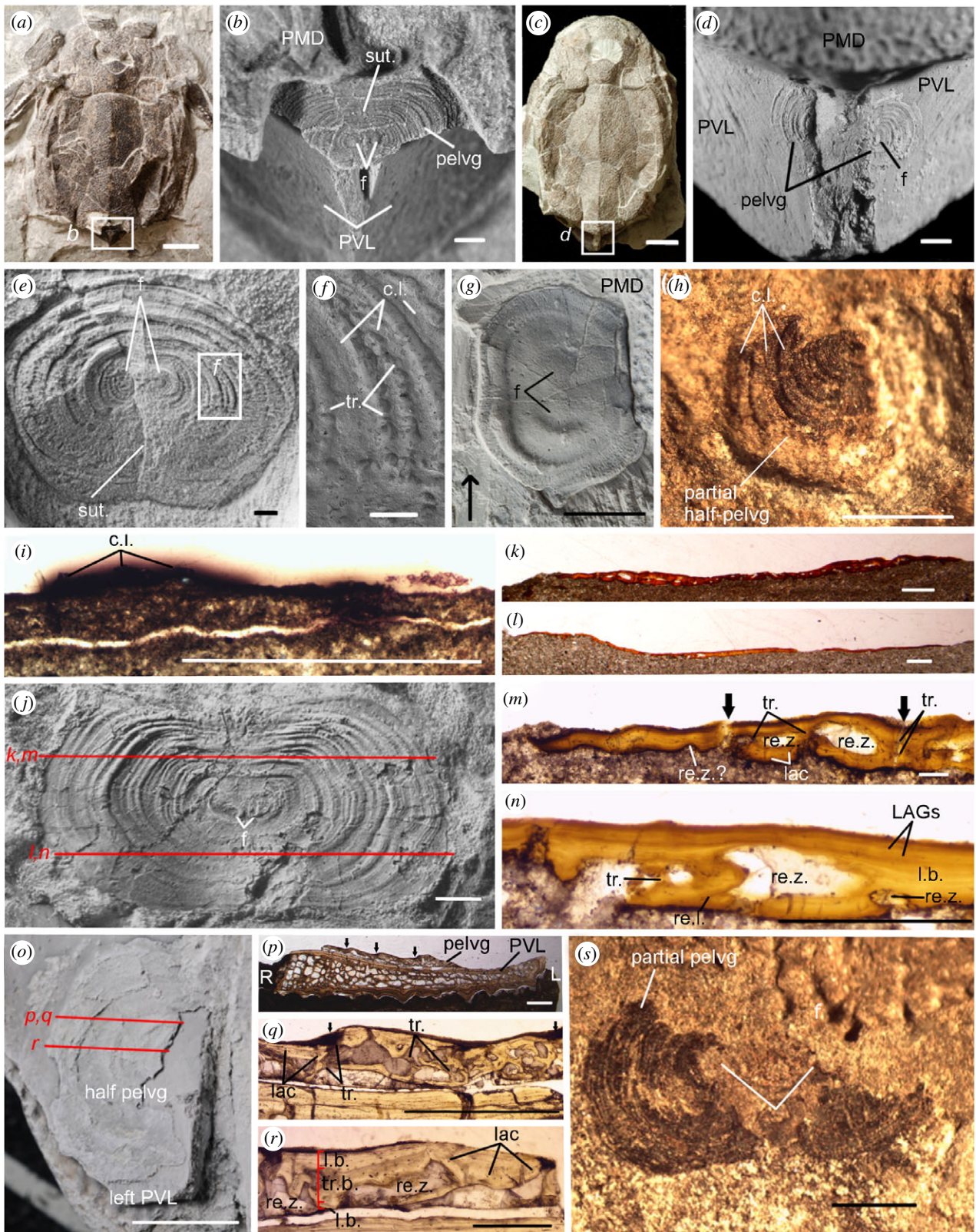


Figure 1. *Bothriolepis canadensis* pelvic girdles. Immature specimens (a,b) MHNM 02-3333, (c,d) MHNM 02-3397. (e,f) Girdle with concentric lines (ventral view, SEM), medium-sized specimen (MHNM 02-3668). (g) Large specimen (MHNM 02-3461; arrow towards head). (h,i) Partial pelvic girdle, immature specimen (MHNM 02-1421). (j–n) Medium-sized specimen (MHNM 02-988). (o–r) Medium-sized specimen (MHNM 02-2119) with partial left girdle. (s) Immature specimen (MHNM 02-3170). c.l., concentric line; f, focus; L, left; lac, lacunae; LAGs, lines of arrested growth; l.b., lamellar bone; pelvg, pelvic girdle; PMD, posterior median dorsal plate; PVL, posterior ventrolateral plates; R, right; re.l., reversal (cement) line; re.z., resorption zone; sut., suture; tr., trabeculae; tr.b., trabecular bone. Black arrows, limits between concentric lines. (b,d,e,g,j,o) Specimens dusted with ammonium chloride. Scale bars: (a,c,g) 10 mm, (b,d,e,h–j,p,q,s) 1 mm, (f,k,l) 0.5 mm, (m) 0.01 mm, (n,r) 0.005 mm and (o) 5 mm. (Online version in colour.)

The ‘pelvicbone’ figured by Stensiö (figs 59 and 60 in [6]) differs from the pelvic girdle described earlier, and ‘fins’ [6] are too anterolaterally positioned and have not been observed in any of the 340 specimens examined here. *Bothriolepis* pelvic half-girdles

are small and ovoid, as in rhenanid and ptyctodontid placoderms [9,15,16], but are fused, rather than independent. The presence of a suture between half-girdles, as well as proximity of their foci, indicates early ontogenetic fusion.

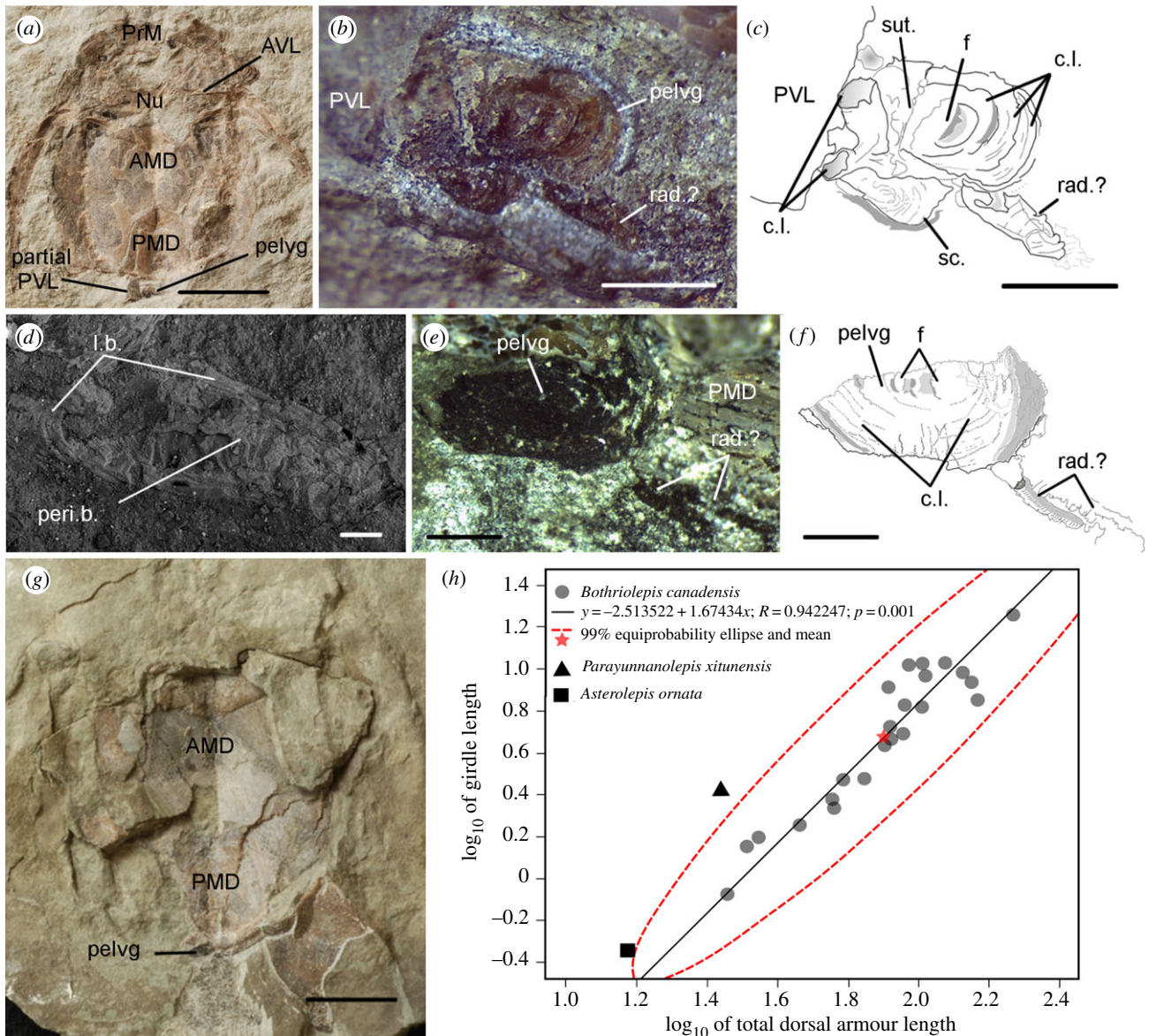


Figure 2. *Bothriolepis canadensis* pelvic girdles. (a–d) Immature specimen (MHNM 02-1454), pelvic girdle and radial. (e–g) Immature specimen (MHNM 02-2426), pelvic girdle and radials. (h) Scatterplot of girdle length relative to TALD with MA regression plus 99% equiprobability ellipse. AMD, anterior median dorsal plate; AVL, anterior ventrolateral plate; c.l., concentric lines; f, focus; l.b., lamellar bone; Nu, nuchal plate; pelvg, pelvic girdle; Peri.b., perichondral bone; PMD, posterior median dorsal plate; PrM, premedian plate; PVL, posterior ventrolateral plate; rad., radial; sc, scale; sut., suture. Scale bars, (a,g) 10 mm; (b,c,e,f) 1 mm and (d) 0.1 mm. (Online version in colour.)

(b) Paedomorphosis and the phylogenetic reduction of the pelvic girdle

Paired appendage losses are frequent among gnathostomes, notably in teleosts, where pelvic fins are often modified, reduced or lost [1,17]. The sequence of gnathostome appendage development is proximo-distal, while loss of elements is distal to proximal, i.e. addition or truncation are more frequent distally [17,18].

The presence of ossified fin radials in only a few immature *Bothriolepis* individuals of our series suggests the absence of pelvic fins in this derived antiarch, with the occasional reappearance of radials, i.e. an atavism [19]. The positive allometry of the pelvic girdle relative to TALD in *Bothriolepis* suggests the pelvic girdle had a great potential for variation (i.e. developmental plasticity, evolutionary lability) [20]. Heterochronic patterns such as paedomorphosis are recognized to have played a role in antiarch evolution [20]. The *Bothriolepis* pelvic girdle seems significantly reduced in size (paedomorphic) relative to the basal *Parayunnanolepis* (figure 2h), but without a comparative

ontogenetic trajectory, this inference remains tentative (electronic supplementary material). However, if the pelvic element in the 14–16 mm *Asterolepis* specimen [13] is a girdle, the absence of preserved pelvic girdle in *Bothriolepis* smaller than 28 mm suggests paedomorphosis as well, with either belated onset of development (girdle absent) or belated mineralization relative to *Asterolepis*. Consequently, a paedomorphic evolutionary trend in antiarch pelvic appendages, proceeding from the loss of the distal fin to the size reduction of the proximal girdle, with adult morphologies resembling early ontogenetic stages (i.e. no radials/fin absence, small girdles) is suggested. The lack of the dermal pelvic girdle, present in other placoderms [9], and rarity of scales ([21]; figure 2b,c; electronic supplementary material, figure S1) constitute a reduction of the post-armour dermal skeleton, which also suggests paedomorphosis in *Bothriolepis*. However, the absence of pelvic fins in *Bothriolepis* species showing a heavily scale-covered tail [22] excludes a direct link between the reduction of the pelvic appendages and the reduction of the post-armour dermal skeleton in *B. canadensis*.

The ontogenetic series of *B. canadensis* presented here is of great importance in the interpretation of the pelvic skeletal elements, previously documented as an anal plate [5,10] or female genital plates [11], but here identified as the pelvic girdle. Moreover, taking into account the ontogenetic stages of the specimens of *Parayunnanolepis* and *Asterolepis*, within the *Bothriolepis* pelvic girdle ontogenetic trajectory, allows us to infer that paedomorphosis may underlie the secondary loss of pelvic appendages in antiarchs. Integration of data from fossil early vertebrate ontogenies, relatively rare although increasingly recovered from nursery assemblages like those at Miguasha, adds crucial information to long-standing questions involving not only the origin of the gnathostome appendages, but also their evolutionary development (e.g. sequence of appendage loss).

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Data accessibility. Data are available as electronic supplementary material.

Authors' contributions. R.C. conceived the project, all authors examined fossil specimens; F.C. performed data analyses, constructed figures and wrote the electronic supplementary material with input from R.C. and Z.J. F.C. wrote the first version; all authors contributed to the final version. All authors approved the final version of the manuscript and agree to be held accountable for the content therein.

Competing interests. We have no competing interests.

Funding. F.C. was supported by Parc national de Miguasha, R.C. by NSERC (238612, 2017-06200) and the Research Chair in Paleontology and Evolutionary Biology – UQAR.

Acknowledgements. We thank C. Belzile (ISMER-UQAR; SEM-EDS), A. Caron (UQAR; statistical advice), M. Chevrinai (UQAR; thin sections), I. Upeniece (University of Latvia; *A. ornata* data), J. Willett (MHNM; preparation) and three anonymous referees for their constructive comments.