

Development of the Axial Skeleton and Median Fin in the Australian Lungfish, *Neoceratodus forsteri*

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Abstract: New observations on the axial skeleton of the extant lungfish *Neoceratodus forsteri* (Dipnoi; Sarcopterygii) indicate that neural and haemal arch elements develop more independently than previously believed. For example, while the cartilaginous neural arches/spines begin development anteriorly, just behind the skull, the distal supraneurals first form separately in the posterior region of the axial skeleton. Proximal supraneurals develop subsequently, but initially lack clear association with either neural arches/spines or distal supraneurals. This contradicts previous studies of *Neoceratodus* and other extant lungfish suggesting that the supraneurals and more distal radials develop from a single cartilage. This was suggested as a unique sarcopterygian pattern, but our new observations suggest a closer resemblance to the actinopterygian condition. With respect to the caudal fin skeleton, the heterocercal tail of Devonian lungfish is replaced in Carboniferous and younger taxa by a diphyccercal tail, comprising elongate and equal dorsal and ventral lobes. Whether these lobes resulted from fusion of dorsal/anal and caudal fins or expansion of dorsal and anal fin rays at the expense of the caudal fin is uncertain. However, comparing ontogenetic development of *Neoceratodus* with Devonian taxa indicates that the elements of the ventral diphyccercal lobe in *Neoceratodus* are homologous to haemal elements present in the caudal fin of Devonian forms, but loss of supporting basal plates in the dorsal fins of Devonian taxa allowed supraneural elements of the dorsal fin to expand to form the dorsal lobe of the *Neoceratodus* tail.

INTRODUCTION

Lungfish (Dipnoi) are represented by only three living genera (*Neoceratodus*, *Lepidosiren* and *Protopterus*) but have a rich fossil record, extending back to the early Devonian (approximately 400 million years ago). The postcranial skeleton has been described in detail for the living taxa [1], and is preserved to varying degrees in fossil taxa (*Uranolophus* [2]; *Griphognathus* [3, 4]; *Rhinodipterus* [5]; *Conchopoma* [6]; *Dipterus* [7]; *Fleurantia*, *Scaumenacia* [8]; *Barwickia*, *Howidipterus* (Long, pers. obs.); *Soederberghia*, [9]; see [1] for general review). Major changes to the dipnoan postcranial skeleton occurred during the Devonian, involving the loss of discrete dorsal and anal median fins and a change from a heterocercal caudal fin to the diphyccercal fin characterising extant taxa (dorsal and ventral lobes equal in size). Associated with this is the loss of supporting basal plates in the dorsal and anal fins (e.g., *Dipterus*) and at the anterior margin of the second dorsal fin in taxa such as *Barwickia*, *Howidipterus*, *Soederberghia*, *Rhynchodipterus* and *Pentlandia* (all Devonian in age [7, 9]). The diphyccercal tail fin developed by the early Carboniferous, the geological period following the Devonian [7, 9, 10], but Arratia *et al.* [1] questioned whether this represented a true caudal fin homologous to that of Devonian lungfish and to other fishes in general. Arratia *et al.* [1] noted that an unusual cartilage, the postcaudal cartilage,

formed at the posterior tip of the notochord, separating the dorsal and ventral lobes of the diphyccercal caudal fin. Thus, the dorsal and ventral lobes could be considered developmentally distinct from one another. Arratia *et al.* [1, pp. 134, 163] considered that in Carboniferous and younger fossil taxa, the dorsal lobe could have formed from the fusion of the dorsal fins and dorsal caudal fin lobe of Devonian taxa, while the ventral lobe formed from the fusion of the anal fin and ventral lobe of the Devonian caudal fin. However, they were less certain about the composition of the diphyccercal caudal fin in living taxa, where there was no indication of the dorsal and anal fins fusing to the caudal fin during ontogeny. Alternatively, following Abel [11], Arratia *et al.* [1, p. 164] suggested that the diphyccercal fin could have formed from loss of the caudal fin and expansion of the dorsal and anal fin rays posteriorly.

Again, one line of evidence supporting this hypothesis was the presence of postcaudal cartilages at the tip of the notochord, dividing the caudal fin into independent dorsal and ventral units. However, as described below, Devonian taxa such as *Dipterus*, *Scaumenacia* and *Barwickia* also possess an extensive mineralised posterior section of the notochord (the anterior part of the notochord is unmineralised and so not preserved), comparable to the postcaudal cartilage of living lungfish. Thus, the dorsal and ventral caudal lobes may have been independent for a substantial portion of lungfish evolutionary history. Further examination of the ontogenetic development of the lungfish axial skeleton in *Neoceratodus* may shed light on this problem.

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With respect to other regions of the lungfish axial skeleton, Arratia *et al.* [1] noted that the neural spines develop independently from the neural arches, while the supraneurals and dorsal radials develop subsequently from these neural spine cartilages (the ventral haemal elements also were said to develop from a single cartilage). However, we follow Friedman [9], who noted that classically, the neural spine is a distal outgrowth from each neural arch, whether these outgrowths fuse medially or not. The ‘neural spine’ of Arratia *et al.* is more properly termed a proximal supraneural [7, 9].

The observation that more distal supraneurals and radials develop from the proximal supraneural in living lungfish [1, 9] differs markedly from actinopterygians (ray-finned fishes), where the more distal axial skeletal elements develop independently from one another [1]. However, the smallest lungfish specimen available to Arratia *et al.* [1] was a 4.3cm individual of *Lepidosiren*; our observations on *Neoceratodus*, described below, suggest that much of the development of the axial skeleton has occurred by this size. Access to smaller and younger individuals indicates that there is a substantial degree of independent development of neural and haemal elements of the axial skeleton, with the more distal elements developing independently, to the extent of originating in different regions of the postcranial axial skeleton (proximal supraneurals anteriorly and distal supraneurals posteriorly) or being well separated from previously developed elements (infrahaemals from haemals). These features are more similar to the development of actinopterygian axial skeletons, suggesting an osteichthyan pattern of development rather than a uniquely lungfish or sarcopterygian pattern.

MATERIALS AND METHODS

Neoceratodus forsteri naturally inhabits the Burnett and Mary rivers in northeastern Australia and is listed as vulnerable under the Environmental Protection and Biodiversity Conservation Act 1999. Fortunately, an ongoing breeding program for *Neoceratodus* has been set up at Macquarie University, Sydney (with support from the

Australian Research Council and Macquarie University to J. Joss), providing all the animals used in this study. Fertilised eggs collected from dedicated lungfish spawning ponds were reared in standard aquaria according to the described protocols [12]. Forty-three specimens ranging in age from stage 40 to 53 were examined (5 specimens for stages 40 and 42/43 combined; 4 each for stages 46, 47; 2 for stage 49; 7 each for stages 50-53), staged using external morphology provided on the website: http://mac-0170.bio.mq.edu.au/~gjoss/lungfish_development/lungfishSQL.php. Individuals were anaesthetized in water containing 0.005% clove oil and killed by immersion in 10% neutral buffered formalin (Macquarie University Animal Ethics Committee approval # 2006/020). Each specimen was individually cleared and stained with Alcian blue for cartilage, and Alizarin red S for bone [13]. Specimens were photographed using an Olympus SZH stereo zoom microscope to visualise skeletal elements and captured with the BTVpro image software. To confirm independent development of these cartilaginous elements, one specimen was photographed with an Olympus inverted phase contrast microscope, in order to visualise cell condensation prior to cartilaginous matrix deposition. Fossil lungfish specimens were whitened with ammonium chloride sublimate or left uncoated, depending on the protocol of the institution (NMV, Museum Victoria, Australia; P., Palaeontology, Natural History Museum, London). Images were modified in Adobe Photoshop to improve brightness and contrast. Terminology employed to describe elements of the axial skeleton is taken from several sources [1, 7, 9]. With respect to the neural arch series, these elements include, from proximal to distal, the neural arch, neural spine (developing as noted above), proximal supraneural, distal supraneural and radials. In the haemal arch series, elements include the haemal arches, haemal spines, infrahaemals and radials. The radials support the fin rays (camptotrichia in extant forms and lepidotrichia in fossil taxa).

RESULTS

In the series of ontogenetic stages described below, we are concerned with the developmental timing of the cartilaginous elements of the *Neoceratodus forsteri* axial

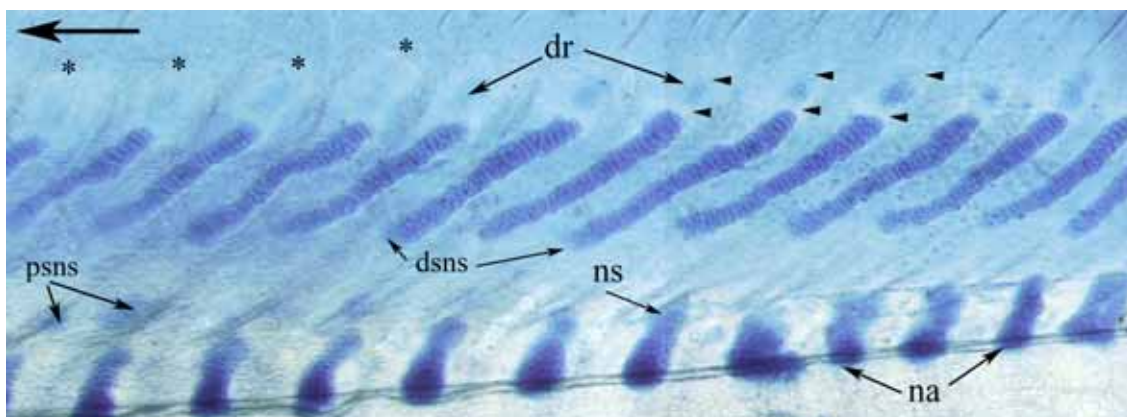


Fig. (1). *Neoceratodus forsteri*. Stage 53, closeup of independently developing supraneural spines; dorsal radials not developing independently. Abbreviations: na, neural arch; ns, neural spine; dr, dorsal radials; dsns, distal supraneural; psns, proximal supraneural. Stars/asterisks indicate regions where cartilaginous cells of the dorsal radials will condense. More posteriorly, the black arrowheads indicate cartilage cells that have formed but as yet unstained. Between the arrowheads, cartilage cells are stained by alcian blue. Cartilage cells are present between the distal supraneural and the developing dorsal radial, but neither these cells, nor regions of condensation, are present between proximal and distal supraneurals.

skeleton, as indicated by Alcian blue staining. Fig. (1) shows a sequence of developmental stages for the distal radials (dr) that we suggest applies to all cartilaginous elements of the axial skeleton. First, a condensation field forms (Fig. 1, asterisks). Cells develop within this field and then, as matrix develops around these cells, proteoglycans in the cartilaginous matrix are stained (Fig. 1, between the arrowheads). As a general rule, the appearance of alcian staining can be linked to the appearance and development of a cartilaginous element, and is applied to our observations below. Although staining can be incomplete or variable in and among specimens, our observations are consistent in individuals examined for each stage.

Stage 40 (Fig. 2)

In this early ontogenetic stage, the head, gut, and notochord (nc) can all be easily recognized (specimen not cleared and stained). Notably, an extensive unpaired dorsal fin (d.fin) develops that continues anteriorly to a point just behind the head. This fin is present through all stages examined herein (e.g., Figs. 3-5), and is marked by alcian blue staining although no cartilage deposition is involved (is non-specific).

Stage 42/43 (Fig. 3a)

In stage 42/43, the postcranial notochord and remainder of the yolk sac are visible (y.sac). The cranial skeleton is developing, including the otic capsules (ot). The pectoral fin (p.fin) is beginning to develop, including the scapulocoracoid and humerus. Branchial arches (br.a) are also beginning to develop, although cartilaginous elements of the axial skeleton have not.

Stage 46 (Fig. 3b, c)

This stage shows the earliest development of the cartilaginous axial skeletal elements. Neural arches (na) develop dorsal to the notochord, with the first or earliest elements positioned posterior to the cranium (Fig. 3b; see [14] for a more detailed description). These neural arches are small and extend posteriorly along the notochord. The neural arches have roughly triangular bases which develop dorsally (or distally) to form the neural spines (ns, Fig. 3c).

Stage 47 (Fig. 3d-f)

Development of the axial skeleton at this ontogenetic stage is very similar to the previous stage, with the neural arches continuing to develop posteriorly along the notochord

(Fig. 3d). Distal growth of the neural spines continues (Fig. 3e). A concentration of lightly stained cells, ventral to the notochord, is visible just posterior to the anus (an, Fig. 3d). The cells form clusters associated with each myotome (Fig. 3f) and are similar to comparable cell clusters along the dorsal notochord, representing the developing neural arches (na). We suggest these ventral condensations represent the first development of the haemal arches (ha). It is important to note that these cell clusters are larger further posterior relative to the anus, indicating that these were the first to begin developing. This is supported by later stages, where a developmental gap persists between these earlier forming elements and the anus (Fig. 4a).

Stage 50 (Fig. 3g-i)

The axial skeleton of stage 49 is similar to that of stage 47, with the neural arches/spines continuing to develop posteriorly along the notochord (specimen not illustrated). By stage 50 (Fig. 3g-i), substantially more development of the axial skeleton has occurred. The neural arches now extend posterior to the anus (Fig. 3g); the first (anterior) neural arch/spine becomes fused to the braincase (n1, Fig. 3h; [14]). Anterior neural arches/spines continue to lengthen, with the distal margins becoming squarer in shape, although more posterior neural arches/spines remain pointed (Fig. 3h).

Two additional series of axial skeletal elements have begun to develop, including the proximal and distal supraneurals. The proximal supraneurals (psns) develop distal to the neural arches/spines and are best developed approximately one-third of the way along the body, above neural arches/spines 6-13 (Fig. 3g, h). These represent the first-formed proximal supraneurals. From this point, the proximal supraneurals are developing anteriorly and posteriorly (Fig. 3g). Distal supraneurals (dsns) begin development more posteriorly, just behind the level of the anus (Fig. 3g). Comparable to the proximal supraneurals, the distal supraneurals are largest in the middle of the series of developing elements, which again indicates that the distal supraneurals are developing bidirectionally (Fig. 3i).

It is important to note that the two series of cartilaginous supraneurals begin their development in separate regions of the axial skeleton, and that the first proximal supraneurals develop above less well-developed neural arches rather than the elongate neural arches/spines anteriorly (Fig. 3g, h). The proximal supraneurals also appear to be offset from, or developing in between, the neural arches/spines (Fig. 3h). The distal supraneurals also begin to develop over more

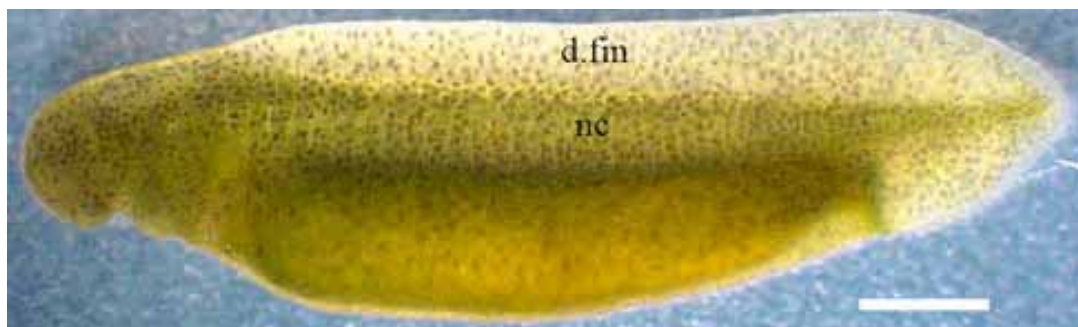


Fig. (2). *Neoceratodus forsteri*. Stage 40. From website http://mac-0170.bio.mq.edu.au/~gjoss/lungfish_development/lungfishSQL.php. Abbreviations: d.fin, elongate dorsal fin; nc, notochord. Scale bar=1.0 mm.

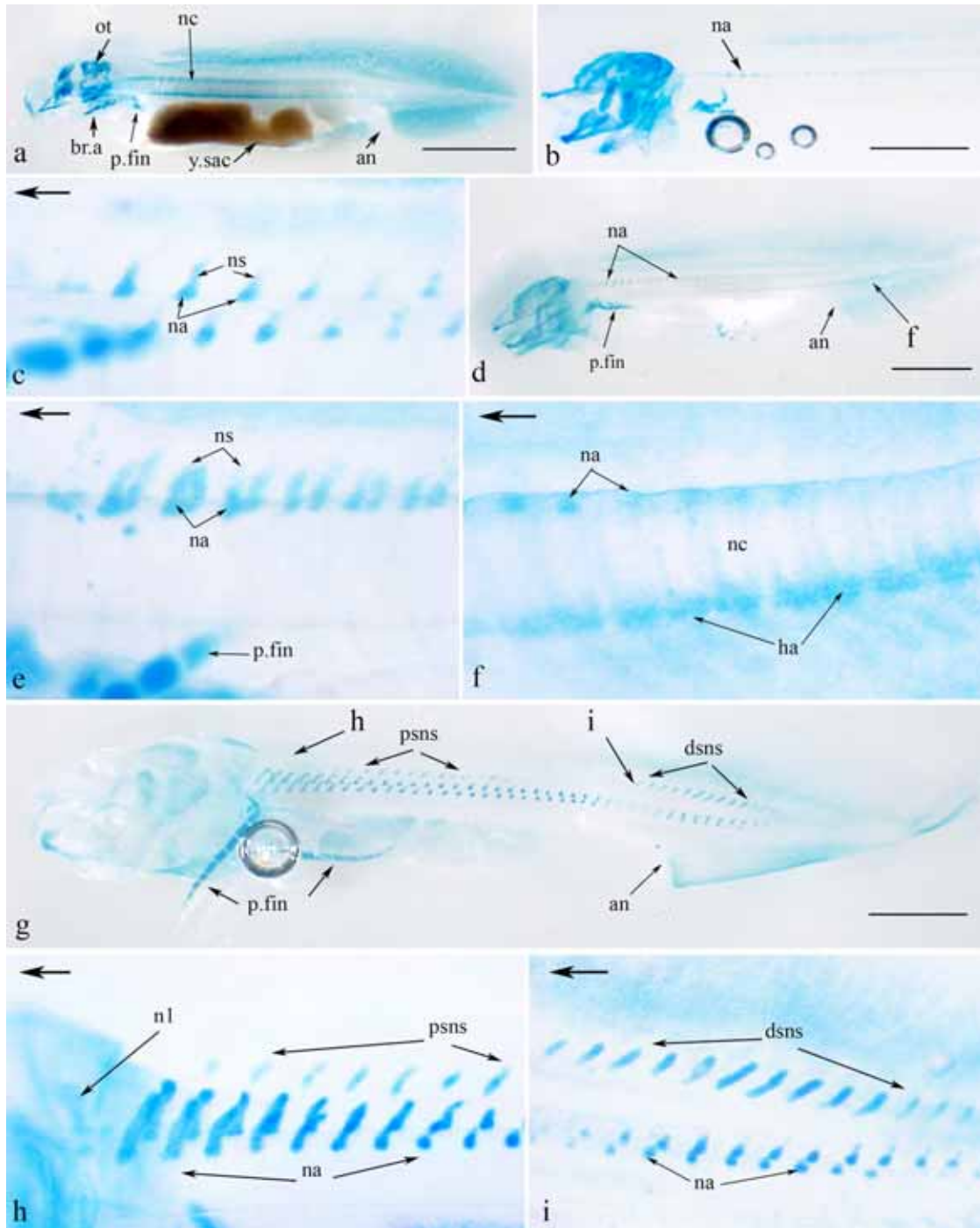


Fig. (3). *Neoceratodus forsteri*. **a**, Stage 42/43; **b, c**, Stage 46; **c**, closeup of neural arch and spine; **d-f**, Stage 47, 'f' in Fig. (3d) indicates region shown in Fig. (3f); **e**, closeup of neural arch and spine; **f**, closeup of notochord and developing neural and haemal bases; **g-i**, Stage 50, 'h, i' in Fig. (3g) indicate views shown in Fig. (3h) and (3i); **h**, closeup of developing proximal supraneural spines; **i**, closeup of distal supraneural spines. Abbreviations: as in Figs. (1, 2), also an, anus; br. a, branchial arch; ha, haemal arch; ha/h.sp, haemal arch/spine; n1, first neural arch, incorporated into braincase; ot, otic capsule; p.fin, pectoral fin; y.sac, yolk sac. Scale bar=0.25 cm.

poorly developed neural arches and are well-separated from these. Compared to the development of the neural spine from the neural arch, as a continuous unit, these observations suggest that the proximal and distal supraneurals develop independently.

To confirm independent development of supraneurals, a *Neoceratodus* specimen of stage 53 was examined under an inverted phase contrast microscope, focusing on a region of

the axial skeleton where the neural arch/spine and supraneurals are developing (Fig. 1). If the supraneurals are forming as segmentation events from the neural spine, condensing precartilaginous cells (cell outlines) should be present prior to any cartilage matrix deposition and so prior to stages visualised by Alcian blue staining of cartilage. If these cells connect proximal and distal supraneurals to each other or to the neural arches/spines, then it could be argued

that the former elements do not originate independently, only that cartilage formation occurs independently within a broader developmental field.

In Fig. (1), the neural arches and spines are visible above the notochord. Cartilaginous cells and their precursors can perhaps be best seen in conjunction with the most dorsal, or most distal, radials (dr), developing from the margin of the distal supraneurals. In Fig. (1), there is a posterior-anterior gradation in radial development (anterior indicated by the large black arrow in Fig. 1). More posteriorly, small condensations of cells can be seen; blue staining is visible in the middle of these condensations, indicating that cartilaginous matrix is being deposited. Unstained cells, represented by their outlines and more faintly visible (arrowheads), surround the middle cells and connect these to the distal supraneurals. More anteriorly, these distinct cells grade into a whitish or ‘fuzzy’ area, believed to represent regions where precartilaginous cells will condense (asterisks,

Fig. 1). Aggregations of these cells represent a stage in cartilage development prior to matrix deposition (e.g., [15, 16]). However, these aggregations are not seen between the proximal end of the distal supraneurals and the proximal supraneurals, nor between the neural spine and the proximal supraneurals. Thus, precartilaginous cells are absent and these cartilaginous elements are not developing within a field or region of these cells, but independently.

Stage 51 (Fig. 4a)

The cranial skeleton and pectoral girdle are well developed by Stage 51 and the pelvic girdle and fin are beginning to form (pel.fin, Fig. 4a). The postcaudal cartilage (pcc) is present near the tip of the notochord, with two centres of cartilage development visible. These cartilages develop well separated from any other elements of the axial skeleton. Development of the proximal and distal supraneurals continues in anterior and posterior directions. These supraneural series have begun to overlap, near a point

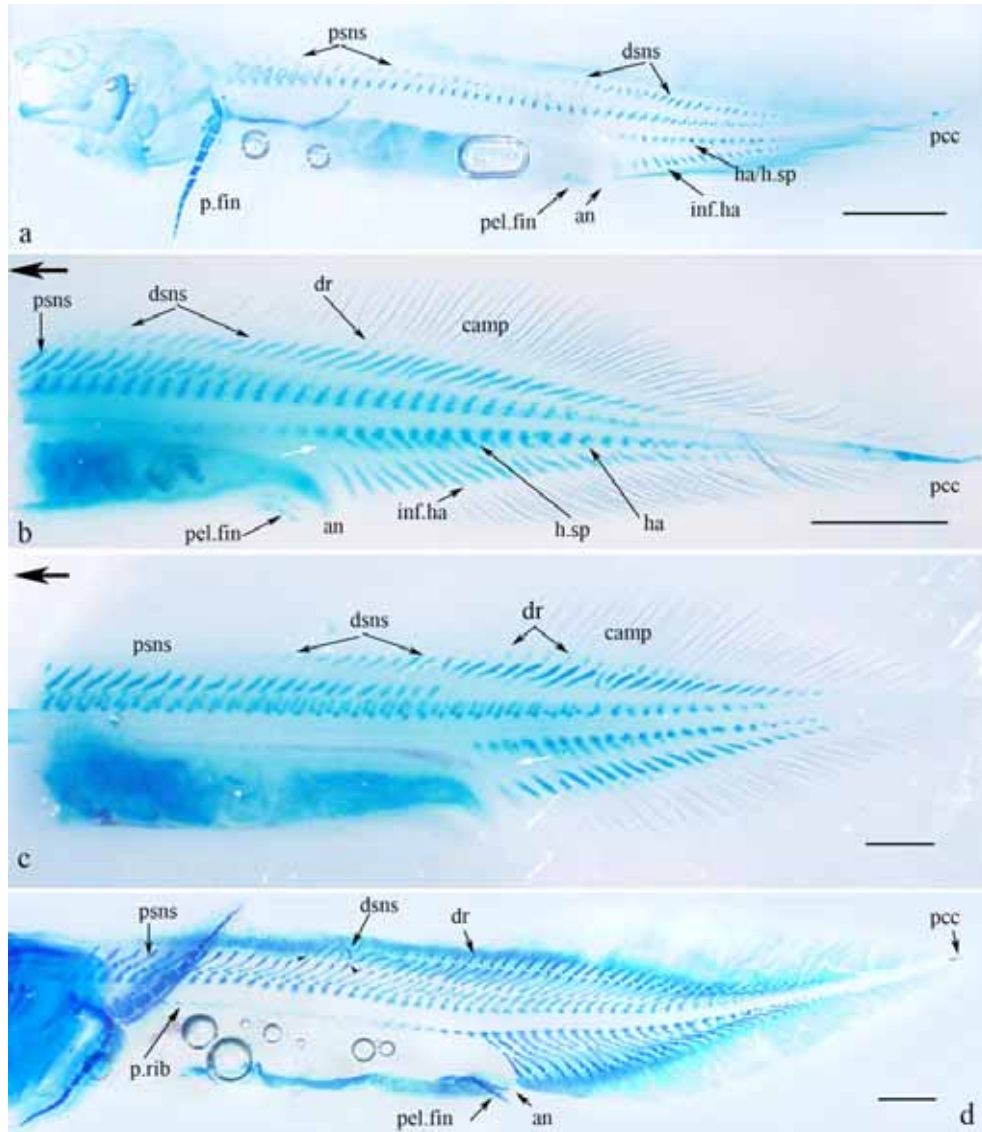


Fig. (4). *Neoceratodus forsteri*. **a**, Stage 51; **b**, Stage 52, view of diphycercal tail; **c**, Stage 52, view of diphycercal tail; **d**, Stage 53. Abbreviations: as in previous figures, also ha/h.sp, haemal arch/spine; inf.ha, infrahaemal arch; pel.fin, pelvic fin; p.rib, postcranial rib. Scale bars: **a**, **b**=0.25 cm; **c**, **d**=0.1 cm.

in line with the pelvic fin, but still remain separated (i.e., there is a noticeable gap between these elements in Fig. 4a). Ventral to the notochord, haemal arches and spines (ha/h.sp) are developing, as are the more distal infrahaemals (inf.ha). The larger, better-developed haemal elements show both arches and spines and represent the first formed elements (seen more clearly in Fig. 4b, st. 52). These are separated from the anus by 2-3 smaller, or later-forming haemal arches (Fig. 4a-c, small white arrow). Thus, as noted above, there is a developmental gap just posterior to the anus relative to more caudal haemal arches. As discussed below, this gap may represent the position of the anal fin, present in Devonian lungfish taxa, but absent from most Carboniferous and younger forms. As with the dorsal axial skeletal elements, it appears that the infrahaemals develop separately from the haemal arches/spines (Fig. 4a-c).

Stage 52 (Fig. 4b, c)

During this stage, the proximal supraneurals are developing to fill in the gap between the neural arches/spines and the distal supraneurals. The proximal supraneurals are still offset relative to the neural arches/spines (Fig. 4b), although they appear to be developing or growing towards the neural spines, resulting in a somewhat curved shape (more anteriorly, Fig. 4c). The postcaudal cartilages have increased in size/length (Fig. 4b). New elements of these cartilages can be seen just anteriorly, where they appear to be invading the notochord itself (indicated by the small patches of blue). The dorsal radials (dr) are beginning to develop, as are the camptotrichia (camp), or the fin rays supported by the

dorsal radials. The camptotrichia extend towards the tip of the notochord, developing much further posteriorly than the cartilaginous axial elements. Posteriorly, several camptotrichia have formed before any supporting supraneural or infrahaemal arches.

Stage 53 (Figs. 4d, 5)

Stage 53 shows continued development of all elements of the postcranial axial skeleton, including the pleural ribs (p.rib, Fig. 4d), which begin to develop anteriorly and progress posteriorly. Mineralisation of neural and haemal arch elements has begun, indicated by alizarin staining in the main part of the element, while the proximal and distal ends remain cartilaginous (Fig. 5). Notably, at a point slightly posterior to the anus, the proximal supraneurals, as a distinct element relative to the neural arch and spine, are no longer present. This marks the end of the posterior development of the proximal supraneurals, such that the distal supraneurals are positioned dorsal to the neural arch and spine. Development of new distal supraneurals anteriorly becomes somewhat irregular (dsns, Figs. 4d, 5), with these being crooked and misshapen and with two supraneurals associated with one proximal supraneural in some cases (Fig. 4d, smaller black arrowheads).

In a larger specimen, beyond the range of the standard stages, mineralisation has advanced in the axial skeleton and also in the skull and pectoral girdle (Fig. 5c, clth). The distal supraneurals no longer appear as irregularly shaped, but there is a size break in the distal supraneural series, indicated by the black arrowhead in Fig. (5c). Compared to the more

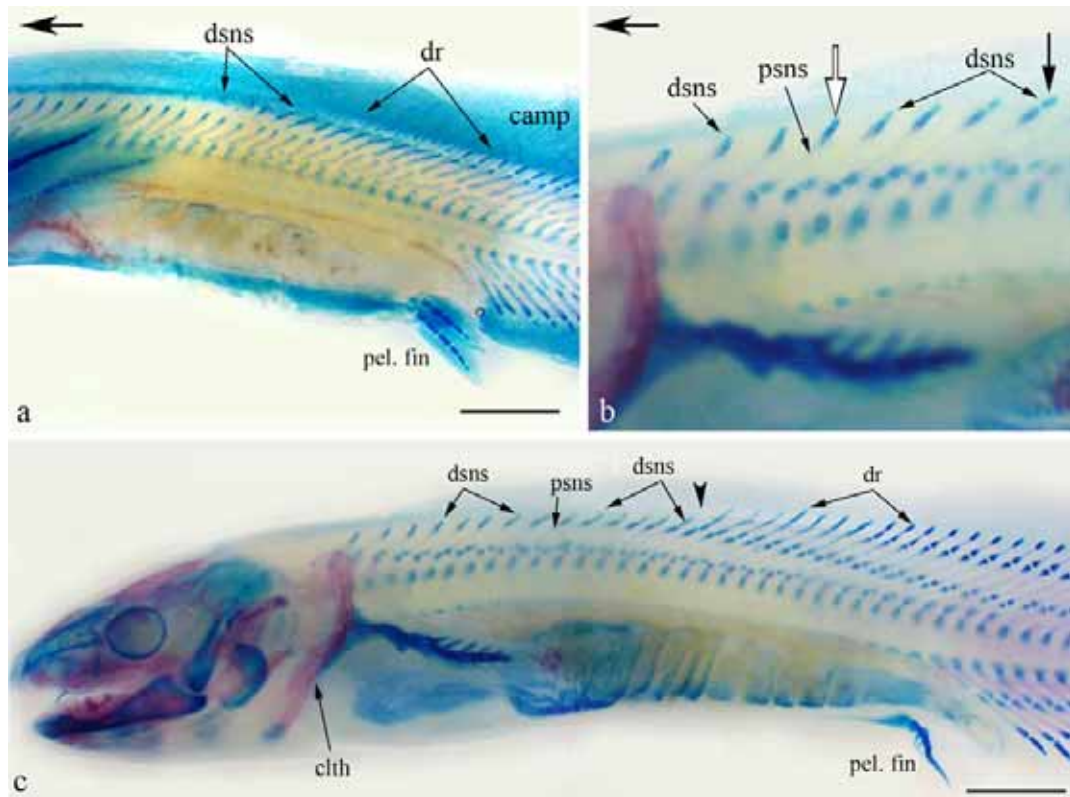


Fig. (5). *Neoceratodus forsteri*. **a**, Stage 53; **b**, **c**, specimen beyond range of standard stages. **b**, closeup of developing supraneurals anteriorly; **c**, right lateral view of specimen shown in **b**. Abbreviations: as in previous figures, also camp, camptotrichia; clth, cleithrum. Scale bar: **a**=0.1 cm; **c**=0.25 cm.

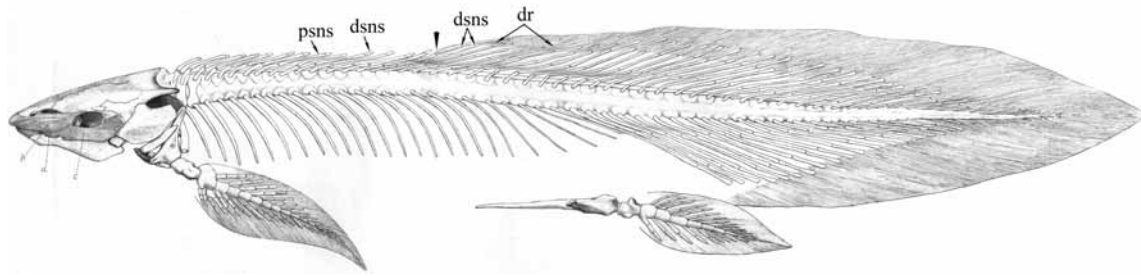


Fig. (6). Adult *Neoceratodus* skeleton [17]. Larger black arrow indicates developmental discontinuity between anterior and more posterior distal supraneurals. Abbreviations: as in previous figures. Specimen is 1.4 m long.

posterior distal supraneurals behind the size break, the more anterior distal supraneurals do not appear to develop as independent elements. This is best seen in Fig. (5b), where, posteriorly, a small distal supraneural is separated slightly from the proximal supraneural (black arrow). Anteriorly, the distal supraneurals become less distinct and appear continuous with the proximal supraneural (white arrow). This is particularly true when compared to the development of the distal supraneurals in earlier ontogenetic stages, where they were separated from other vertebral elements by a considerable gap (e.g., Fig. 4a). The developmental sequence in this anterior region of the postcranial skeleton involves formation of distal supraneurals from the distal end of the proximal supraneural and subsequent separation of these elements, perhaps by joint formation.

Fig. (6) shows an adult male skeleton (approximately 1.4m long [17]). Interestingly, the size distinction between the more anterior and posterior distal supraneurals remains (Fig. 6, larger black arrow), marking the beginning of the elongate and continuous dorsal fin and at this point, two distal supraneurals articulate with a single proximal supraneural. We suggest these correspond to the irregular supraneurals seen in the earlier ontogenetic stages described above. Unfortunately, adult *Neoceratodus* skeletons are not known in large numbers, so more individuals should be examined with respect to these features.

DISCUSSION

This study of early ontogenetic stages of the Australian lungfish, *Neoceratodus forsteri*, provides new information with respect to the development of the lungfish axial skeleton as well as the identity of the caudal fin. Although elements of the postcranial axial skeleton form a continuous functional unit later in ontogeny, several first develop as independent cartilaginous units in separate regions of the axial skeleton; by comparison, the neural/haemal arches and spines do not develop independently from one another [9].

Among the independently developing elements, the distal supraneurals first appear posteriorly, and the proximal supraneurals anteriorly. Both of these sets of elements develop bidirectionally, and in later ontogenetic stages, overlap one another, but still remain separated and independent (Fig. 4b-d). With respect to the haemal elements, the haemal arches begin development notably posterior to the anus (Fig. 4). During all ontogenetic stages examined, an elongate, unpaired dorsal fin fold extends to a point just behind the head. These observations can be used to interpret evolutionary change within the axial skeleton of

Devonian and Carboniferous lungfish. These changes have been well documented [e.g., 7], but primarily with respect to external morphology, where loss of the median unpaired dorsal and anal fins occurs by the early Carboniferous, resulting in the postcranial morphology characterising all living lungfish taxa [2, 7, 9, 18].

The dorsal and anal fins have been recognised as a correlated evolutionary module for actinopterygians [19], evolving in early actinopterygians as a positioning module, regulating the equivalent anteroposterior position of these fins along the axial skeleton. This module also included a component responsible for patterning the fin elements [19]. We suggest this module can also be recognised in lungfish, not only through the equivalent position of the unpaired dorsal fins and anal fin in fossil lungfish such as *Scaumenacia*, *Fleurantia*, *Uranolophus*, *Barwickia*, *Howidipterus* [2, 8, 9], but also their coordinated loss at the end of the Devonian. The exception to this is *Phaneropleuron*, which has lost the dorsal fins, but retains the anal fin [7, 9]. The dorsal-anal fin module can also be recognised in fossil lungfish via morphological similarities between these fins, particularly with respect to the oar-shaped fin support supporting more distal radials. This oar-shaped element occurs in the first dorsal and anal fins of several Devonian taxa such as *Griphognathus*, *Howidipterus*, *Barwickia* (oar, Fig. 7), *Soederberghia*, and *Pentlandia*, but importantly, also occurs at the anterior margin of the more elongate second dorsal fin in the latter four taxa [8, 9], Fig. (7). In the anal and first dorsal fin, the oar-shaped element forms the main supporting element of the fin, but in the second dorsal fin, there is a series of more posterior neural elements behind it.

The axial skeletons of the Devonian taxa *Fleurantia* and *Scaumenacia* [8] indicate that the oar-shaped element of the second dorsal fin is the first of these elements to be lost, with the element associated with the first dorsal fin still present in *Fleurantia*. As noted [9], the distal supraneurals at the anterior margin of the second dorsal fins in these taxa lack a one-to-one relationship with the proximal supraneurals; as well, more distal dorsal radials (supporting lepidotrichia) are absent. In *Scaumenacia*, two proximal supraneurals articulate with 4 distal supraneurals, while in *Fleurantia*, 3 distal supraneurals are supported. This represents the number of distal supraneurals supported by the oar-shaped element at the anterior margin of the second dorsal fin of *Howidipterus*, *Barwickia* and *Pentlandia* [9]. It appears that the oar-shaped element was lost, with the out-of-register distal supraneurals coming to contact the proximal supraneurals in its absence

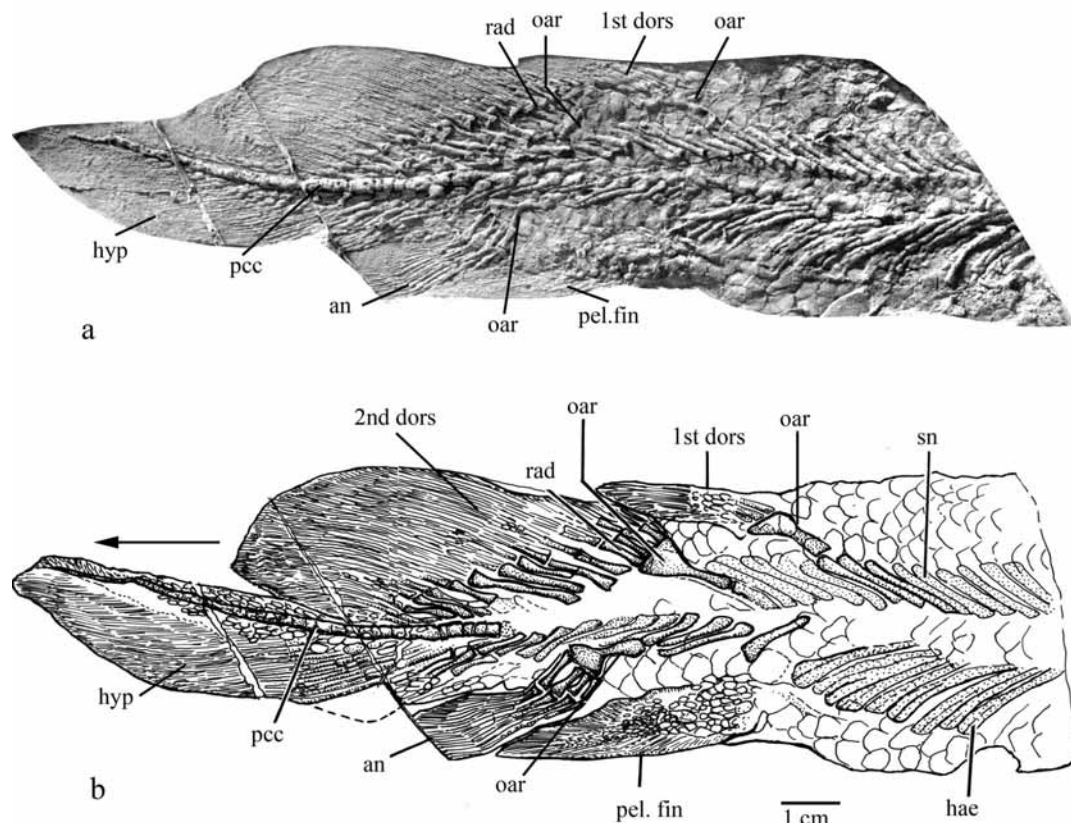


Fig. (7). *Barwickia downunda*. **a, b**, NMV181784, posterior fins and axial skeleton. **a**, photograph. **b**, line drawing. Abbreviations: as in previous figures, also rad, fin radial.

[9]. Interestingly, the oar-shaped fin support has also been lost from the first dorsal fin of *Scaumenacia*, which is now noticeably elongated [8]. We suggest that loss of these oar-shaped elements allowed the neural elements associated with the dorsal fins to expand along the axial skeleton, represented in ontogeny of the extant genus *Neoceratodus* by the bidirectional development of the proximal and distal supraneurals.

Regionalisation of the median dorsal fins in the derived teleost taxon Acanthopterygii has been described recently [19]. In this group, the lepidotrichia are modified into spines, effectively forming an anterior dorsal fin, separated to varying degrees from the normal lepidotrichia posteriorly, which form a posterior or second dorsal fin. Within the Acanthopterygii, loss of the anterior spines leads to a loss of regionalisation in the dorsal median fins, with one result being that the dorsal fin becomes elongated. An example of this occurs in Pleuronectiformes; in one pleuronectiform group, Psettodoidei (halibut), spines are retained, while in another, the Pleuronectoidei (flatfish, flounder), the spines are lost and the dorsal fin is continuous and expanded anteriorly. In another example [19], the Mahi Mahi (*Coryphaena hippurus*; Perciformes) also lacks spines (secondarily) and has an elongated dorsal fin.

A similar process can be envisaged for lungfishes, with regionalisation occurring with respect to the dorsal median fins and particularly the oar-shaped element at the front of the second dorsal fin. The fact that this element resembles the fin support for the first dorsal fin (e.g., *Soederberghia*

[9]) suggests that it developed from some type of duplication event associated with the median fins. Loss of the oar-shaped element occurs (in a taxon such as *Fleurantia*), which may be related to the loss of the fin support for the first dorsal fin (e.g., in *Scaumenacia*). Loss of both these elements results in the loss of the regionalisation in the dorsal median fins; as noted, in *Scaumenacia*, the first dorsal fin is notably elongate compared to taxa where a fin support is present, e.g., *Fleurantia* [8] or a variety of other Devonian taxa. By comparison to the Acanthopterygii, loss of regionalisation appears to result in fin expansion in *Neoceratodus*. One possibility is that the first and second dorsal fins both expand and coalesce to form a continuous dorsal fin.

This is supported by the ontogeny of *Neoceratodus* and the presence of the elongate unpaired dorsal fin. With respect to ontogeny, the cartilaginous distal supraneurals begin to condense and form posteriorly, with subsequent development both anteriorly and posteriorly, and independent of other vertebral elements. However, in the anteriormost part of the dorsal vertebral column, as illustrated in Fig. (5), the distal supraneurals develop as condensations from the proximal supraneurals. This development differs from that more posteriorly; the presence of misshapen distal supraneurals (Fig. 4d) and a notable size break (Fig. 5b) in this region suggests some type of developmental discontinuity. The misshapen distal supraneurals are lost in specimens larger than stage 53 (compare Fig. 5a with Fig. 5b, c), appearing above posterior supraneurals 12-17 in Fig. (4d), but absent here in Fig. (5c). We suggest this developmental discontinuity indicates the

presence of the second dorsal fin posteriorly and the region of the first dorsal fin anteriorly, in conjunction with the extensive unpaired fin fold running along the dorsal surface (Fig. 5c). Therefore, we hypothesise that the extensive unpaired dorsal fin of *Neoceratodus* (and by inference, all Carboniferous and younger taxa) is derived from the expansion and coalescence of the first and second dorsal unpaired fins present in Devonian taxa.

With respect to the ventral diphycercal lobe of *Neoceratodus*, the haemal/infrahaemal elements begin development posterior to the anus, leaving a space or gap that is filled by radials later in ontogeny (Fig. 4a, b). This gap is in a position comparable to the location of the narrow-based anal fin in Devonian taxa (Fig. 7, an), suggesting that the anal fin was lost during lungfish evolution and that the ventral lobe of the diphycercal fin is not formed by an expansion of the anal fin posteriorly. The alternative is that the radials comprising the ventral lobe of the diphycercal fin are homologous to the caudal fin radials in the ventral or hypochordal lobe of the heterocercal caudal fin of Devonian taxa (hyp, Fig. 8).

This is not an unrealistic scenario, given the morphology of the heterocercal caudal fin in taxa such as *Barwickia* (Figs. 7, 8a, c). Here, the ventral or hypaxial lobe is large and dominates the caudal fin, while the dorsal or epicercal lobe is almost absent. Once the discrete nature of the second dorsal fin was lost (loss of oar-shaped radial, as described above), the dorsal fin and its radials could not only extend anteriorly, but also posteriorly, continuing along the dorsal surface of the notochord (black arrow, Fig. 7b). Although the oar-shaped radial supporting the anal fin was also lost from Devonian taxa, expansion or extension was restricted by the presence of the anus anteriorly and the well-developed

hypaxial lobe posteriorly. Thus, the diphycercal fin of Carboniferous and living lungfish combines expanded dorsal fin radials in the dorsal lobe, but caudal fin radials in the ventral.

With respect to the question of the homology of the caudal fin of post-Devonian lungfish [1], it was observed that the tip of the notochord in living lungfishes was formed by a series of cartilaginous segments called postcaudal cartilages [1, 20]. Early developmental stages of these cartilages can be seen in *Neoceratodus*, although there appears to be individual variation (e.g., an older, larger specimen has a less well-developed postcaudal cartilage; pcc, compare Fig. 4d and 4b). The postcaudal cartilages effectively divided the diphycercal fin into separate dorsal and ventral halves, such that the development of radials in each of these halves did not extend to the tip of the notochord [1]. Rather than the caudal fin being a single development unit, the dorsal and ventral lobes were said to develop independently [1]. In fact, the development of the arches and radials becomes increasingly irregular towards the tip of the notochord on some specimens [1]; Fig. (7). Two evolutionary scenarios were described; either the median dorsal and anal fins merged with the caudal fin, or the caudal fin was lost, with expansion of the dorsal and anal fins posteriorly [1]. It was inferred that postcaudal cartilages were present in Carboniferous and younger fossil taxa possessing caudal fin morphologies comparable to living lungfish. However, several Devonian taxa also possess an elongate mineralised section of the notochord, extending from the tip of the notochord to the region of the second dorsal fin, including *Howidipterus*, *Barwickia*, *Dipterus* and *Scaumenacia*, all taxa with heterocercal caudal fins (Figs. 7, 8). If the postcaudal cartilage separates the caudal fin into

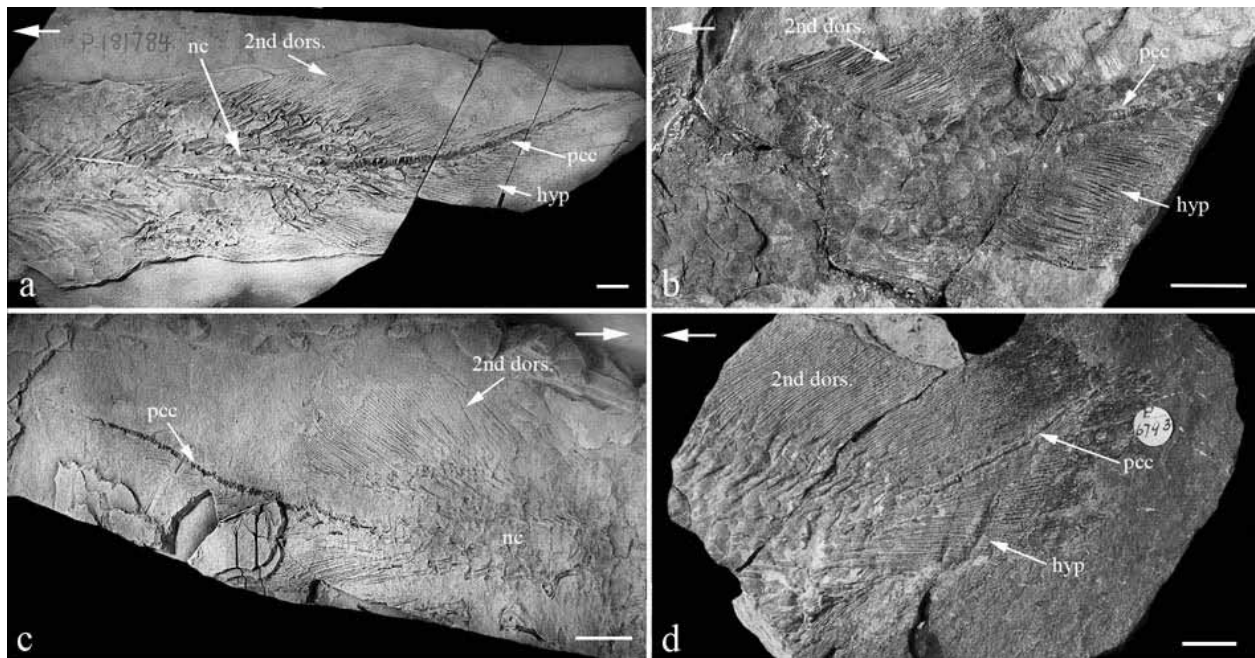


Fig. (8). Heterocercal caudal fins of Devonian lungfish, with postcaudal cartilages. **a**, *Barwickia downnunda*, NMV181784, right lateral view; **b**, *Dipterus valenciennesi*, P.15312; **c**, *Barwickia downnunda*, NMV59812, left lateral view; **d**, *Scaumenacia curta* P.6793. Larger white arrows indicate anterior. Abbreviations: as in previous figures, also hyp, hypochordal lobe of the caudal fin; 1st dors, unpaired first dorsal fin; 2nd dors, unpaired second dorsal fin. Scale bar=1 cm.

independent dorsal and ventral units in extant lungfish, we suggest the cartilage also did so in Devonian fish. This is supported by our observations discussed above, for example, that Devonian taxa such as *Barwickia* (Figs. 7, 8) possess a ventral caudal lobe but virtually no dorsal lobe. Thus, independence of the dorsal and ventral caudal fin lobes may have a deeper evolutionary history than previously appreciated.

CONCLUSIONS

Evidence from the ontogenetic development of the extant Australian lungfish can be used to interpret the evolutionary history of the lungfish axial skeleton. Most elements of the axial skeleton develop independently, contrary to previous assertions [1, 9]. This pattern is more comparable to that seen in actinopterygians, and suggests that this is characteristic of the Osteichthyes as a whole. As well, changes in the median unpaired dorsal fins in a variety of Devonian lungfish taxa suggest that loss of certain elements within the fin (oar-shaped supports) resulted in an expansion to fin radials along the skeleton. This is also seen in certain modern actinopterygian groups. Also by comparison to Devonian taxa, this expansion of the dorsal fin posteriorly is suggested to have extended along the dorsal or epicercal lobe of the caudal fin. A comparable expansion did not occur in the ventral part of the fin; instead *Neoceratodus* ontogenetic development indicates that the anal fin was lost. We suggest that previous suggestions that the caudal fin of Carboniferous and younger lungfish evolved from the expansion of the dorsal and anal unpaired median fins is only partially correct; this expansion occurred dorsally, but not ventrally. The ventral lobe represents the ventral lobe of the typical heterocercal tail.

ABBREVIATIONS

an	=	Anus
br.a	=	Branchial arch
camp	=	Camptotrichia
clth	=	Cleithrum
d.fin	=	Elongate dorsal fin
dr	=	Dorsal radials
dsns	=	Distal supraneural
ha	=	Haemal arch
ha/h.sp	=	Haemal arch/spine
hyp	=	Hypochordal lobe of the caudal fin
inf.ha	=	Infrahaemal arch
n1	=	First neural arch, incorporated into braincase
na	=	Neural arch
nc	=	Notochord
ns	=	Neural spine
oar	=	Oar-shaped fin support
ot	=	Otic capsule
pcc	=	Postcaudal cartilage
pel.fin	=	Pelvic fin

p.fin	=	Pectoral fin
psns	=	Proximal supraneurals
rad	=	Fin radial
sn	=	Supraneural
y.sac	=	Yolk sac
1st dors	=	Unpaired first dorsal fin
2nd dors	=	Unpaired second dorsal fin

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