

Supporting Information for

The axial skeleton of *Tiktaalik roseae*

Thomas A. Stewart, Justin B. Lemberg, Emily J. Hillan, Isaac Magallanes, Edward B. Daeschler,
Neil H. Shubin

Corresponding Authors: T.A. Stewart and N.H. Shubin
Email: tomstewart@psu.edu and nshubin@uchicago.edu

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Supporting Information Text

In NUFV 108, individual skeletal elements are three-dimensionally preserved with minimal breakage and deformation. Previous work reconstructed the cranium and pectoral fin of *Tiktaalik* from μ CT data (1, 2). In this study, we describe the axial skeleton and produce a three-dimensional model that contains nearly all skeletal elements known for the taxon. Additional elements that are not presented in the model include components of the pharyngeal skeleton (3), the interclavicle (4), and the posttemporal (5). In generating this 3D model, numerous decisions were made on how to place the elements. These decisions are based upon comparisons between anatomical systems of *Tiktaalik* (e.g., comparing intercentral and neural arch anatomy, or comparing the pectoral and pelvic girdle), as well as comparisons to other tetrapodomorphs and extant fishes. The new reconstruction of *Tiktaalik*, thus, represents a hypothesis based on multiple lines of evidence.

Positioning of the head

Lemberg *et al* (1) analyzed the several *Tiktaalik* specimens and produced a reconstruction of the head of NUFV 108 by placing individual elements of the cranium and lower jaws in life-like positions. We follow their placement in the present reconstruction.

The head of NUFV 108 is postulated to have moved postmortem, shifting rostrally 5.3 cm from the trunk (*i.e.*, distance between the basioccipital-exoccipital complex and the rest of the head plus the distance between the basioccipital-exoccipital complex and the vertebra associated with the first rib) (Fig. S1). This is consistent with the measured displacement between the left and right sides of the pectoral girdle, where the right side is preserved 4.1 cm rostral to the left side. We regard the hypothesis of rostral post-mortem movement of the head as more likely than the alternative hypothesis: that the preserved position of the head of NUFV 108 reflects its position in life, which would imply that *Tiktaalik* possessed a series of cartilaginous, non-rib bearing vertebrae immediately caudal to the head.

In the reconstruction of *Tiktaalik*, the head is moved caudal from its preserved position so that the most-rostral ribs are immediately caudal to the head. Ribs are observed on the first vertebra of *Eusthenopteron* (6), *Acanthostega* (7), and *Ichthyostega* (8, 9). Among tetrapodomorphs, *Panderichthys* is unique in possessing rostral vertebrae without ribs (10). Thus, this positioning of the head in *Tiktaalik* is consistent with the general tetrapodomorph pattern. As further evidence against the alternative hypothesis (of a series of non-rib vertebrae immediately caudal to the head), the present reconstruction recovers the trunk-to-tail transition and position of the pelvis at approximately vertebrae 31. This is also consistent with the general tetrapodomorph pattern of pelvic positioning, seen in *Eusthenopteron* (6), *Acanthostega* (7), and *Ichthyostega* (8, 9) discussed below. If a series of non-rib vertebrae had been immediately caudal to the head of *Tiktaalik*, then the trunk-to-tail transition and position of the pelvis would occur approximately at vertebra 36; such a pattern is unknown among early tetrapods and therefore regarded as unlikely. Elements in the axial column of *Tiktaalik* are enumerated under the assumption that the first rib belongs to the first axial segment.

Reconstruction of the vertebrae

Intercentra and neural arches are positioned according to their preserved rostrocaudal order. Intercentra are assigned to either the left or right sides according to the curvature of the internal

surface and position of the articular facet. Left and right intercentra that were preserved near to one another are reconstructed as paired. However, because intercentra are unfused and can have shifted during preservation, it is possible that some elements reconstructed as paired are slightly out of register from their original position. Intercentra are reconstructed as associated with individual neural arches. Likewise, it is possible that intercentra could be reconstructed modestly out of register from their original neural arch. This uncertainty does not impact results presented in the manuscript.

Intercentra are positioned so that they bound the lower portion of the notochord and wrap dorsally. When both left and right sides are preserved for a vertebra, they are positioned so that their internal curvature symmetrically fits around a notochord that is circular in cross section. Intercentra are positioned under the assumption that the notochord is of a uniform cross section between the head and pelvis, a feature observed in various taxa, including *Eusthenopteron* (6, 11) and *Latimeria* (12). If only one intercentra was preserved for a vertebra, the element is positioned so that its internal curvature matches elements anterior or posterior to it in the series.

Neural arches are occasionally broken, and whenever possible the pieces are re-assembled. Most neural arches can be associated with ribs or intercentra. However, caudal to neural arch 34, four neural arches are preserved without clear association to other axial elements. The rostral-most of these four neural arches is preserved ventral to neural arch 32 (Fig. 1, Movie S2). Its morphology is markedly more robust than those immediately anterior (Fig. 2 F-I), and it is identified as belonging to the caudal region based of comparison to the vertebrae of *Acanthostega* (7). While this neural arch could have been associated with intercentra 34 or 35, it is depicted in the reconstruction with a gap between it and other elements to denote uncertainty in position (Fig. 3). The three caudal-most neural arches are preserved near to the pelvic fin in close association with one another and separated by a substantial gap to other axial elements (Fig. 1 A,B). These neural arches, too, are depicted in the reconstruction with gaps between them and other axial elements to denote uncertainty in their position (Fig. 3).

To reconstruct the dorsal position of neural arches, we first focused on the most complete neural arches in the series (e.g., Fig. 2 F,G). Despite some lateral compression, these allowed us to estimate the extent to which the arch would have wrapped around the notochord. When neural arches were broken, if possible, they are reconstructed so that the apex of their internal curvature aligns with the apex of other more complete neural arches in the series.

To constrain spacing of axial elements in the rostrocaudal direction, we considered the preserved distance between ribs 1 and 32 in NUFV 108 as representative of the distance between ribs 1 and 32 in life. Across this distance, ribs and vertebrae are placed so that gaps between the vertebrae were uniform, except when their position was uncertain (see discussion above of the caudal-most four vertebrae and discussion below of the caudal rib). Vertebrae 33-36 are spaced at distances similar to those of positions 1-32.

In the reconstruction of *Tiktaalik*, intercentra are positioned slightly anterior to their corresponding neural arch. This positioning is based on several features. First, the positioning of intercentra reveals the size of the notochord, and comparison of intercentral and neural arch morphologies suggest that they are unlikely to have been aligned strictly dorsally, because this would have produced a lateral overlap of the elements. Second, pleurocentra were not identified for NUFV 108. If large pleurocentra had been observed, then the vertebrae are likely to have been organized such that neural arches were positioned dorsal to their corresponding intercentra, as in *Osteolepis* (13). Thus, the absence of identifiable pleurocentra suggests that they were small and unossified and that neural arches and intercentra were not vertically aligned, but slightly out of register (6, 14).

The neural arches of *Tiktaalik* lack zygapophyses. This suggests space between adjacent neural arches. Therefore, they are situated with angles of inclination that maintain a slight gap between

adjacent elements. The caudal four neural arches are positioned with similar angles of inclination as those in the trunk series.

Reconstruction of the ribs

The anterior-most rib on the left side is broken in two pieces, which were preserved in contact with one another with a sharp angle between them (Fig. 1). These pieces are placed end-to-end to reconstruct the original element (Fig. 2). The other ribs that are broken have pieces preserved in proximity with one another, and they are approximately aligned (e.g., rib 23 on the right side). In the reconstruction, the pieces of these other broken ribs are kept in their preserved positions and have not been moved closer to one together. This presentation is meant to preserve information on which features are broken and not to imply that any gaps in individual ribs represent their original length and missing portions of the rib.

Two ribs on the left side (ribs seven and twelve) and one on the right side (rib six) were displaced during preservation such that the distal portion of the rib was posteriorly oriented and ventral to the rib that followed. Additionally, four ribs on the right side (ribs 20-23) are preserved such their articular surfaces point posteriorly. In each of these cases, the individual ribs were rotated and repositioned preserving the order of their proximal articular surfaces.

One rib is preserved to the left of the rest of the axial skeleton, and it is identified as a post-sacral rib. It is possible that it might have articulated upon intercentra 33-36, as *Acanthostega* had five post-sacral ribs (7) and *Ichthyostega* had six post-sacral ribs (8, 9). However, the rib is depicted in the reconstruction with a gap between it and other axial elements to denote ambiguity in position.

Ribs were positioned relative to the vertebral column according to the curvature of the proximal articular surface. In many ribs, this portion is broken or incomplete. Therefore, across the series, ribs are placed by first reconstructing the positions of those ribs with complete articular heads; these ribs were placed so that their heads aligned with the curvature of the posterior margin of the intercentra, which bears an articular facet. Ribs with damaged heads were then positioned to maximize similarity in their orientation to those with complete heads.

Positioning of the pectoral girdle

The pectoral girdle was positioned along the rostro-caudal axis according to the position of the cranium and with reference to the anatomy of closely related taxa. Specifically, it is positioned sufficiently caudal to the head so as to account for the hyoid skeleton, which is known for NUFV 108 (3) but is not included in the μ CT data. This produces a positioning where the glenoid fossa is in register with the fifth rib, similar to *Acanthostega* and *Ichthyostega* (Fig. 6). The pectoral girdle was positioned in the dorso-ventral direction so that its bottom lies slightly below the ventral boundary of the lower jaws. The clavicle was positioned so that it approaches the ventral midline and allows space for the interclavicle, which is known from other specimens and not depicted here (4). The cleithrum was positioned such that its height approximated the height of the head. The anocleithrum and supracleithrum were positioned to be oriented towards the postparietal and to allow for space for the posttemporal, which is known from NUFV 110. A bony lateral

extrascapular is not known for any specimen of *Tiktaalik* and, therefore, hypothesized to have been absent in this taxon (5).

Positioning the pelvic girdle

Both the pelvis and pelvic fin have been displaced relative to the rest of the body during preservation, and cues from the axial skeleton have been used to infer their life positions. The position of the pelvis of *Tiktaalik* in the rostrocaudal axis is based upon transitions in vertebral and rib anatomy. Specifically, the girdle is placed so that the dorsal extent of the ilium is rostrocaudally aligned with ribs 31 and 32. The resultant positioning is consistent with *Eusthenopteron*, *Acanthostega*, and *Ichthyostega*, where approximately 30 ribs are rostral to the pelvic girdle.

Dorsoventral positioning of the pelvic girdle of *Tiktaalik* is based on comparisons to other tetrapodomorphs. Uniformly, tetrapodomorphs are reconstructed with the ventral portion of the pelvic girdle approximately in line with the ventral portion of the pectoral girdle (e.g., *Eusthenopteron* (6), *Acanthostega* (7), *Ichthyostega* (8)). Thus, in *Tiktaalik* the pelvic girdle is placed with a position that comports with the body thickness in the dorso-ventral direction that is predicted from the reconstructed head and pectoral region.

To reconstruct the medio-lateral splay of the pelvic girdle of *Tiktaalik*, first the anteromedial portion is positioned near to the midline, as in *Eusthenopteron* (6). Next, the girdle was positioned to produce a taper in the body outline when viewed from the dorsal perspective. Such a tapering is typical of tetrapodomorphs. In *Elpistostege*, the pelvic girdle is not yet described; however, the width of the trunk at the position of pelvic fin insertion approximately 66% as wide as the trunk at the position of pectoral fin insertion (based on Fig. 1 A,B of Cloutier et al. (15)). In *Acanthostega* the pelvic girdle is approximately 48% narrower than the pectoral girdle at the widest extent of each element (based on Figs. 18 and 20 of Coates (7)). In *Ichthyostega* the pelvic girdle is approximately 51% as wide as the pectoral girdle at the widest extent of each element (based on Fig. 1 B of Pierce et al. (9)). In *Tiktaalik*, the pelvic girdle is reconstructed to be 63% narrower than the pectoral girdle. In this position, the femur would extend approximately posteriorly. If the pelvic girdle were reconstructed with a narrower mediolateral splay, it would result in the femur pointing towards the midline, which we regard as unlikely. The reconstructed positioning results in a narrow distance between the ilium and ribs 31 and 32, consistent with the prediction that these axial elements were functionally associated with the hind fin (Fig. S3 A-E).

This reconstruction predicts a more posterior orientation of the acetabulum than previously hypothesized (16), one approximately similar to *Eusthenopteron* (6). We regard this hypothesis of pelvic positioning as more likely than one where the dorsal extent of the ilium is parallel to the axial column (Fig. S3 F-I). Such a wide splay of the pelvic girdle would result in an unusually ovate shape of the trunk in cross section at the position of the pelvis (Fig. S3 H), which is not known in tetrapodomorphs. Additionally, if a lateral orientation of the iliac blade is constrained but the angle between left and right halves is increased to produce a more rounded cross-section, the height of the girdle in lateral perspective would be increased and yield a reconstruction where body thickness is deeper at the pelvis than the pectoral girdle (Fig. S3 H). As noted above, such an increase in body thickness rostrocaudally is not seen in other closely related taxa and is regarded as unlikely.

Thus, positioning of the pelvic girdle is constrained both by features of other anatomical systems of *Tiktaalik* (i.e., vertebrae, ribs, head, and pectoral girdle) and by comparisons to other tetrapodomorphs. Although there uncertainty inherent in a reconstruction like this, alternative hypotheses of pelvic girdle positioning for *Tiktaalik* robustly predict that the dorsal extent of the

ilium approached the sacral ribs (those in positions 31 and 32) and that the pelvic fin of *Tiktaalik* that is more posteriorly oriented than in *Acanthostega* and *Ichthyostega* (7, 8).

Reconstruction of the pelvic fin

A line drawing of the pelvic fin is presented in Fig. 6 A that shows the estimated positions of the preserved endoskeleton elements as well as estimates of the geometry of missing elements.

Along the proximodistal axis, fins generally taper dorsoventrally. Accordingly, proximal skeletal elements have articular surfaces that are deeper in the dorsoventral direction than those more distally positioned. As previously noted, element shown in purple in Fig. 6 has a similar morphology to the intermedium of the pectoral fin of *Tiktaalik* (16); it is, thus, reconstructed as articulating with the fibula. This positioning contributed to the identification of the tibia. The element identified as the tibia has an articular surface deeper dorsoventrally than any other preserved pelvic endoskeletal elements and, therefore, would likely have been more proximally positioned than the element shown in purple. The general pattern of tetrapodomorph pelvic fins is such that one would predict only three possible more proximal elements: the femur, fibula, and tibia. The geometry of this most robust element is inconsistent with either a femur or fibula, both of which likely would have had two distal articular facets, and it is therefore identified as the tibia.

In the drawing, the tibia is illustrated with a dashed component distal to it. The distal geometry of the tibia is rough and uneven as compared to the distal surfaces of other pelvic elements, like the intermedium, third mesomere, and third anterior radial. Therefore, this texture is taken to indicate that the distal portion of the tibia might have broken off or was poorly ossified. It is possible that a small element articulated distally with the tibia. We regard this condition as unlikely, because neither *Eusthenopteron* (6) nor *Panderichthys* (17) have pelvic fins showing an endoskeletal element articulating distally with the tibia.

Several endoskeletal elements of the pelvic fin are not preserved. Their approximate geometries are estimated in the illustration. Mesomeres are typically not longer proximodistally than those more proximal to them. Therefore, we estimated the relative lengths of the fibulare, fibula based on the third mesomere (shown in red in Fig. 6). The approximate geometry of the femur is based on the assumptions that it would be at least as long as the tibia and distally wide enough to accommodate the tibia and fibula.

Positioning the pelvic fin

In the 3D reconstruction of the pelvic fin, individual endoskeletal elements were repositioned according to their hypothesized positions, described above. Individual fin rays within the fin web are not repositioned. Endoskeletal elements preserved in contact with the fin web were rotated and slightly translated. In the pectoral fin, fin rays overlap the radius. Therefore, the tibia, the homologous element in the pelvic fin, is expected to similarly have been covered by lepidotrichia and is positioned so that dorsal hemitrichia reach its base. The full fin was placed according to the position of the pelvic girdle such that the femur, if present, would have extended straight from the acetabulum.

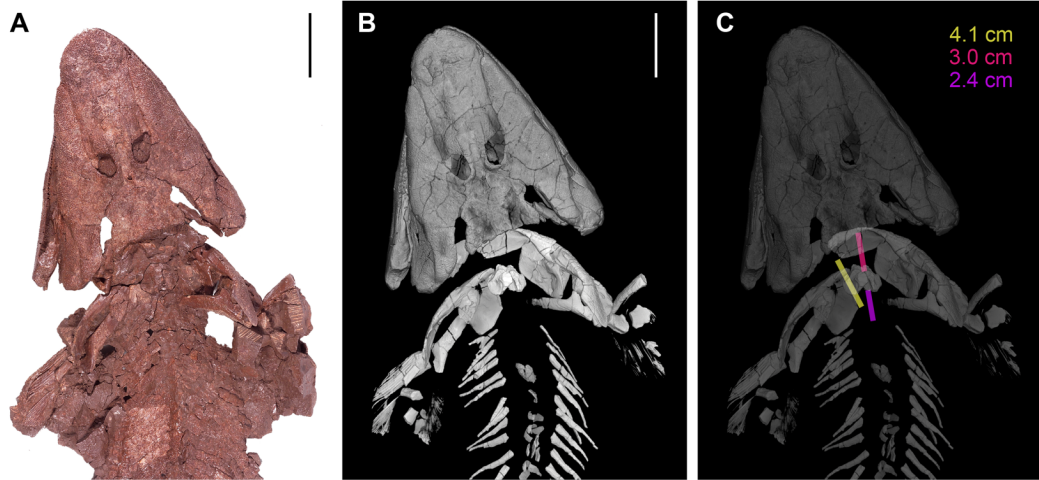


Fig. S1. Rostral skeletal elements of NUFV 108 were displaced post-mortem. (A) Photograph of NUFV 108 before the head was separated from the rest of the pectoral block (Photo credit: E.B. Daeschler). (B) Volumetric rendering of μ CT data with the head, which was separated by mechanical preparation and scanned separately from the more caudal materials (1), repositioned to match the preserved position. (C) Volumetric rendering with reduced opacity to show measured distances between skeletal elements used to infer degree of post-mortem skeletal movement. Yellow corresponds to the distance between the posterior medial margin of the left and right shoulder girdles. Pink corresponds to the distance between the rostral margin of the basioccipital-exoccipital complex and its original position on the cranium. Purple corresponds to the distance between the caudal margin of the basioccipital-exoccipital complex and vertebra associated with the first rib. Scale bars, 5 cm.

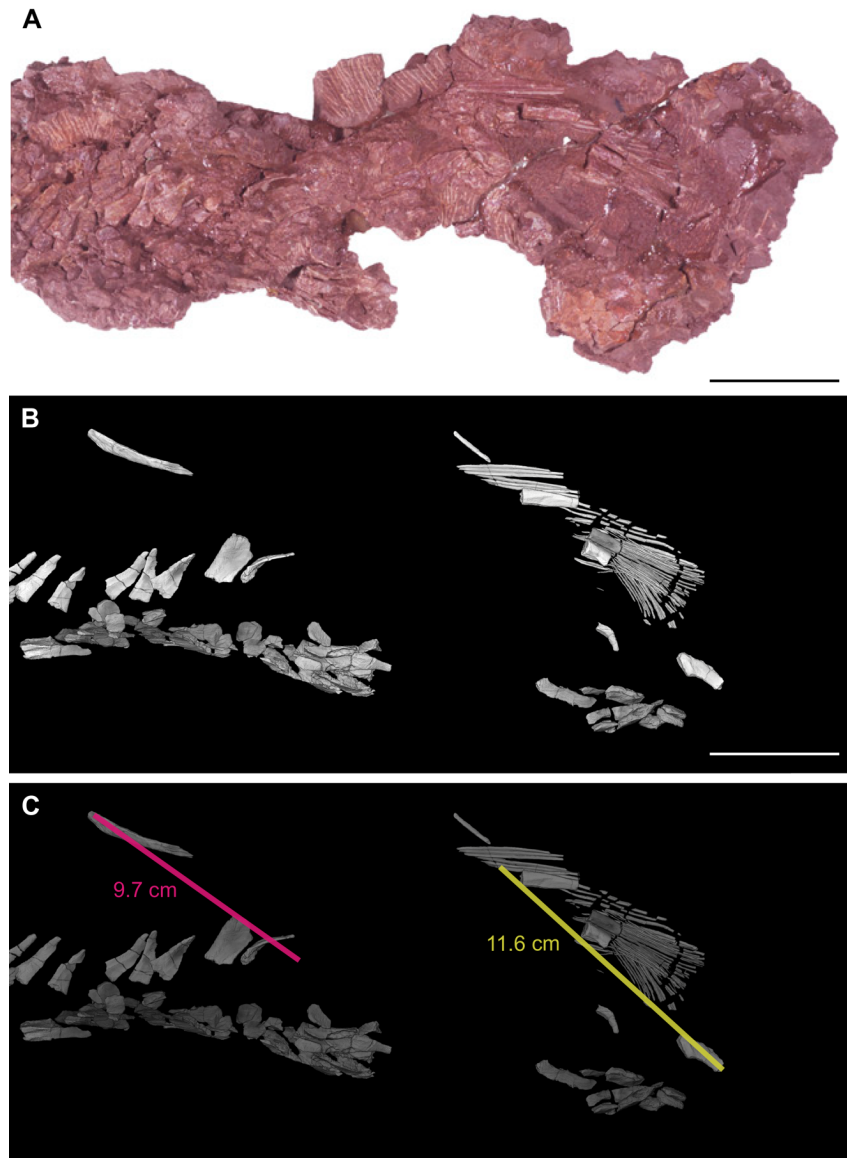


Fig. S2. The pelvic fin of NUFV 108 has been displaced post-mortem. (A) Photograph of the caudal portion of NUFV 108 (Photo credit: E.B. Daeschler). (B) Volumetric rendering of μ CT data of the block showing preserved positions of the axial skeleton and pelvic fin. (C) Various skeletal elements caudal to vertebra 32 have been displaced post-mortem. For example, a caudal rib has moved at least 9.7 cm from its position relative to more rostral ribs (distance measured shown in pink), the tibia of the left pelvic fin has moved at least 11.6 cm from its position relative to the rest of the fin (distance measured shown in yellow), and the fin web has been rotated mediolaterally such that its proximal portion is directed away from the axial column. Scale bars, 5 cm.

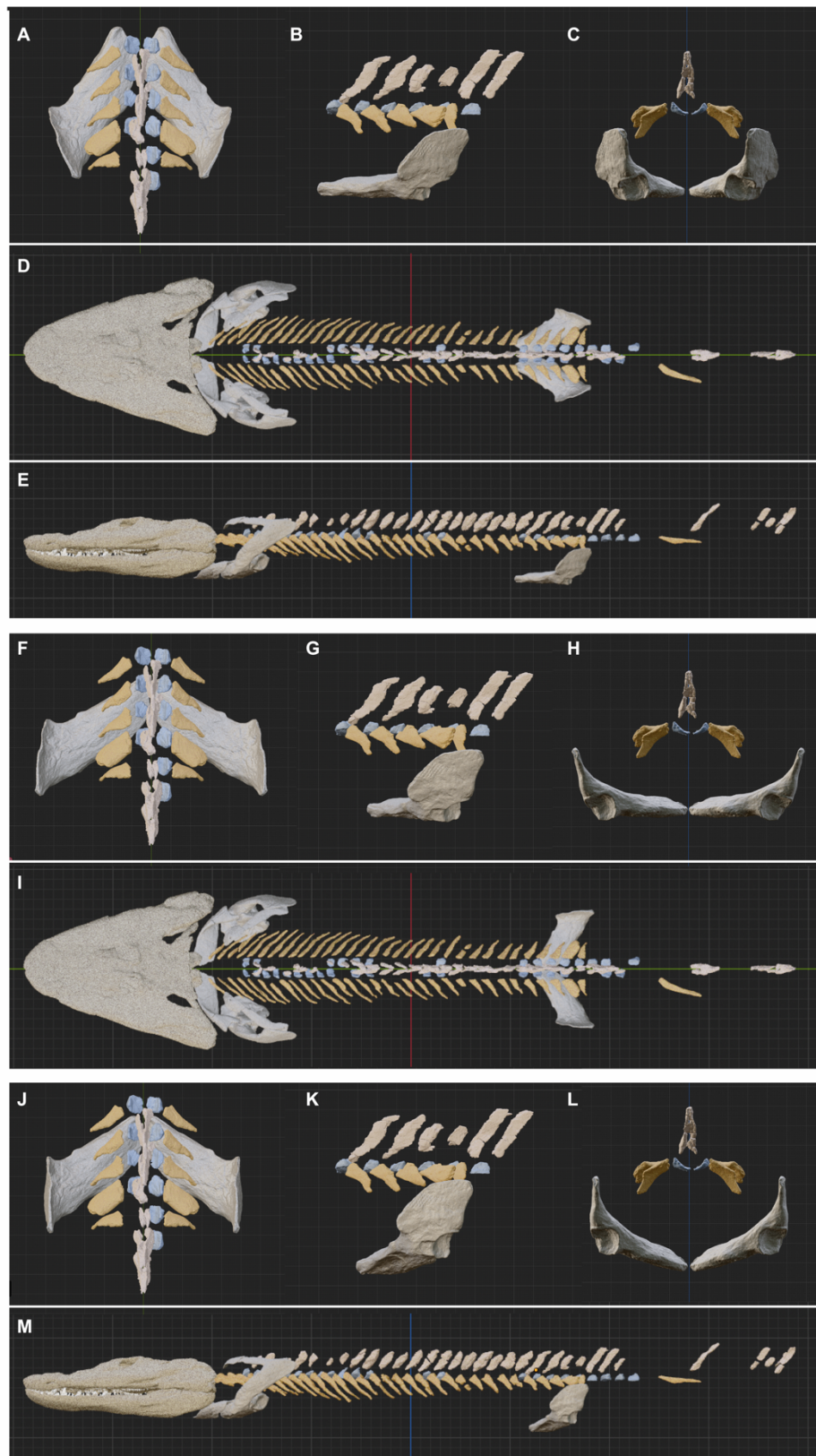


Fig. S3. Alternative reconstructions of pelvic girdle of *Tiktaalik roseae*. Alternative hypotheses for the positioning of the pelvic girdle were considered when building the reconstruction, as discussed in the Supplementary Text. Panels A-E show the reconstruction of the pelvic girdle presented in the main manuscript. Panels F-I compare that condition with an alternative positioning, where the dorsal extent of the ilium is parallel to the rostro-caudal axis and the ventral aspect of the pelvic girdle is aligned with the ventral aspect of the pectoral girdle. This

position, with a broad body in the pelvic region, corresponds to previous reconstruction of the pelvic girdle (16). Panels J-M show a third reconstruction, where the left and right halves of the pelvis are further rotated, resulting in a deeper body.

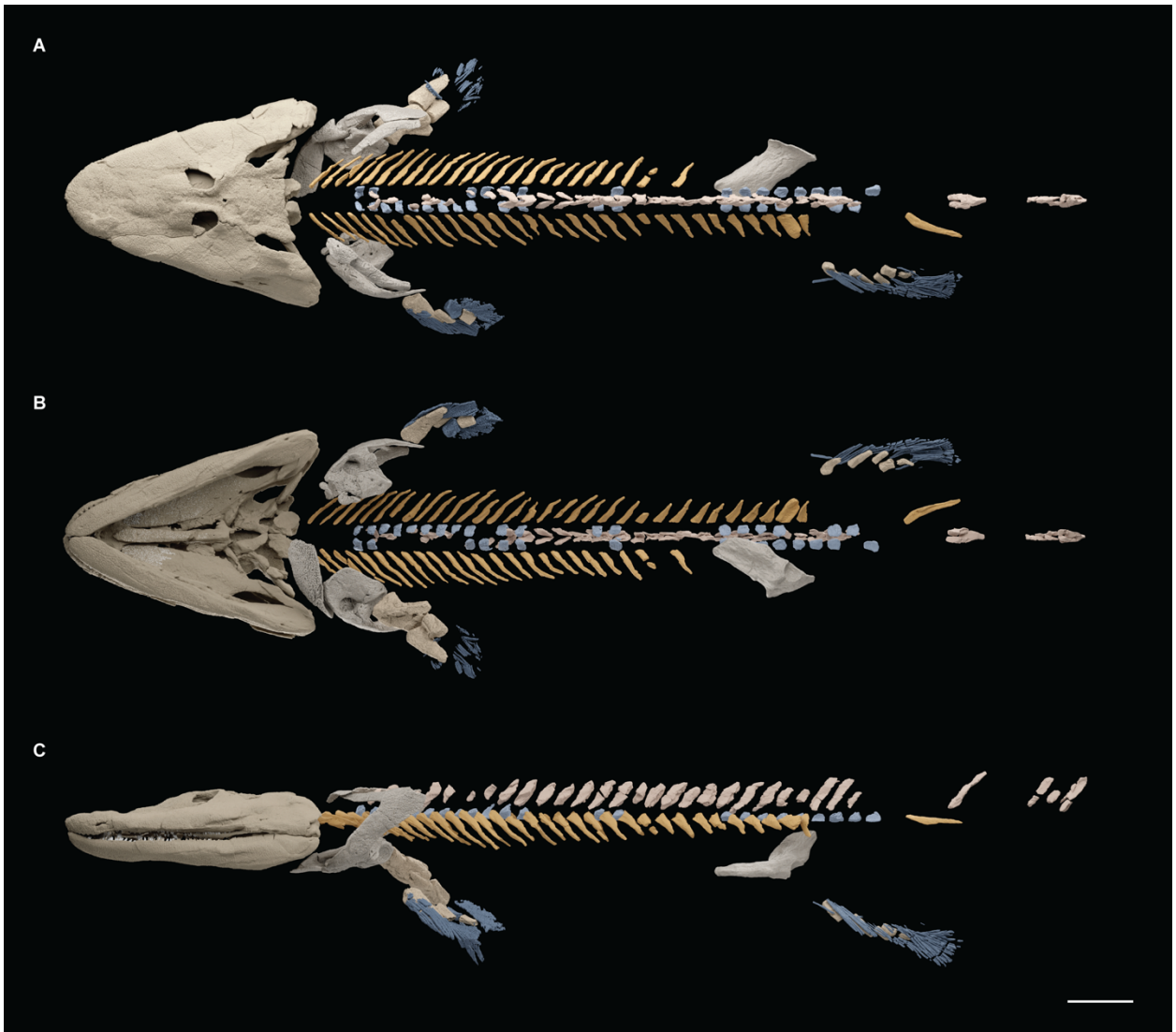


Fig. S4. NUFV 108 with elements repositioned. Rendering of all skeletal elements of NUFV 108 that have been μ CT scanned are shown here in their reconstructed positions. These images differ from the reconstruction in Fig. 7, which shows several elements duplicated for left-right symmetry and coupled with the more complete pectoral fin of another specimen. Scale bar, 5 cm.

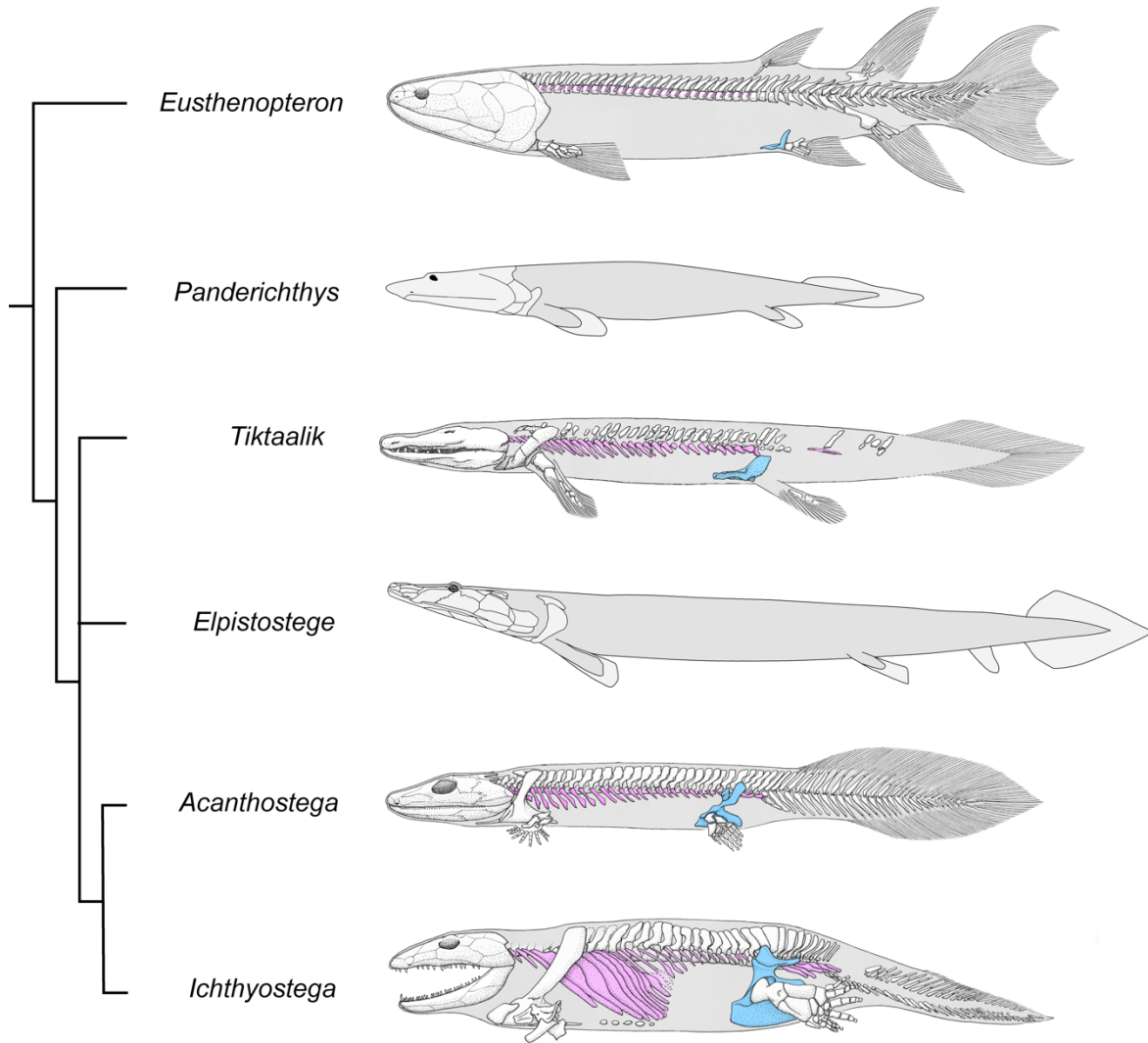


Fig. S5. Overall body proportions of Devonian tetrapodomorphs. Illustration of *Tiktaalik* is based on NUFV 108. Illustrations of other taxa are based on previously published descriptions: *Eusthenopteron* (6, 11), *Panderichthys* (17), *Elpistostege* (15), *Acanthostega* (7, 8), *Ichthyostega* (8, 9). Taxa are approximately scaled to the length of the lower jaw. The phylogeny is from Stewart *et al* (18) and is the strict consensus tree of their maximum parsimony analysis.

Table S1. Parameters for μ CT scanning of NUFV 108.

scan ID	Power (W)	Voltage (kV)	Current (μ A)	Voxel Size (μ m)	Filter (mm)	Scan Duration
pectoral block anterior	57	100	570	122.441	0.24 Cu	1hr44min
pectoral block posterior	57	100	570	88.038	0.24 Cu	5hr06min
pelvic block anterior	49.5	110	450	73.23	0.24 Cu	6hr48min
pelvic block posterior	49.5	110	450	73.23	0.24 Cu	6hr48min

Movie S1 (separate file). Volumetric rendering of the two blocks containing the post-cranial skeleton of NUFV 108 including matrix

Movie S2 (separate file). Volumetric rendering of NUFV 108 with all segmented elements in their preserved position

Movie S3 (separate file). Rotation of the reconstructed sacral domain of *Tiktaalik roseae*

Movie S4 (separate file). Rotation of the reconstruction of *Tiktaalik roseae*

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