Axial regionalization in *Tiktaalik roseae* and the origin of quadrupedal locomotion

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Summary:

The origin of quadrupedal locomotion in tetrapods entailed the evolution of a regionalized axial skeleton with sacral ribs. These ribs provide linkage between the pelvis and vertebral column and contribute to body support and propulsion by the hind limb. The closest relatives of limbed vertebrates are not known to possess such a connection and, therefore, have typically been described as primarily supporting their bodies against the substrate with pectoral fins. However, data on the axial skeletons of stem tetrapods are sparce, with key features of specimens potentially concealed by matrix. Here we provide micro-computed tomography data of the axial skeleton of *Tiktaalik roseae* and show that its vertebrae and ribs are regionalized along the craniocaudal axis, including expanded and ventrally curved ribs in the sacral region. The sacral ribs would have contacted the expanded iliac blade of the pelvis in a soft tissue connection. No atlas-axis complex is observed, however the basioccipital-exoccipital complex is deconsolidated from the rest of the neurocranium, suggesting increased mobility at occipital-vertebral junction. Thus, axial regionalization that allowed for innovations in head mobility, body support and buttressing the pelvic fin evolved prior to the origin of limbs.

The earliest limbed vertebrates are characterized by a regionalized axial skeleton with cervical, thoracic, sacral, and caudal domains in the vertebral column and ribs[1-4]. These modules correspond to locomotor specializations, including providing support for load-bearing hind limbs[5-7]. *Acanthostega* and *Ichthyostega* have specialized ribs that connected to the ilium, either in a soft-tissue or bony articulation, providing mechanical linkage between the axial column and the pelvic girdle[1,2,4]. The structures that make this connection possible are not present in tetrapodomorph outgroups where the complete axial column has been described[8-10]. For example, the tristichopterid *Eusthenopteron* has vertebrae and ribs that are short and generally similar across their cranio-caudal distribution, lacks a sacral rib, and has a pelvis that is small as compared to the pectoral girdle[8]. Moreover, unlike *Acanthostega* and *Ichthyostega*, *Eusthenopteron* possessed a bony linkage between the shoulder girdle and cranium that would have limited head mobility[1,2,4,8].

Little is known of the axial columns of the closest relatives of limbed vertebrates. The vertebrae of *Panderichthys* are described from a brief series that evince no indication of regionalization[11]. The vertebrae of *Elpistostege* are known from a series of approximately 16 that, likewise, show no heterogeneity in their length or shape[12]. The axial skeleton of *Tiktaalik* has been largely obscured by matrix. The rostral ribs are broad and laterally expanded as compared to early tetrapodomorph conditions, and vertebral column has not been described[13]. However, the pelvis and pelvic fin of *Tiktaalik* are nearly the size of the pectoral appendage[14], differentiating its overall proportions from less crownward taxa, like *Panderichthys*[11,15]. The size and depth of the acetabulum, the general robusticity of the pubis, and the dorsally expanded iliac blades of *Tiktaalik* are further similarities shared with digited forms that are notably absent in other finned tetrapodomorphs[14].

Here, we present high-resolution micro-computed tomography scans of the type specimen of *Tiktaalik,* NUFV 108, that expose for the first time the vertebral skeleton and posterior ribs of *Tiktaalik* (Fig. 1, Movies S1,2). These data, and the new reconstruction they allow, reveal unexpected intermediate conditions as well as apomorphies that provide new insight on changes involved with the origin of limbed vertebrates and the functional context in which they arose.

Results

Vertebrae

The vertebrae of *Tiktaalik* are rhachitomous and surround an unconstricted notochord that was persistent into adulthood (Fig. 2). In specimen NUFV 108, elements of 40 vertebrae are preserved. These include ossified intercentra and neural arches, while pleurocentra are not identified. The size, shape, and spacing of intercentra and neural arches of *Tiktaalik* are similar to *Eusthenopteron*[1], suggesting that pleurocentra might have been present but, because of their small size, are difficult to identify within the field of preserved scales. However, their absence in *Tiktaalik* cannot be excluded; pleurocentra have likewise not been identified in *Panderichthys*[11] or *Elpistostege*[12], suggesting a possible 'reverse' rhachitomous pattern in elpistostegalians which is known in *Acanthostega, Ichthyostega,* and *Pederpes*[3].

Vertebrae are not preserved in association with the four most anterior ribs (Fig. 2 A,B). It is possible that these vertebrae were present but are not preserved in specimen NUFV 108. However, a similar condition is observed *Ichthyostega*, where preserved intercentra and neural arches also initiate at rib number five[3]. This well-defined gap in multiple taxa suggests that these vertebrae in the cervical domain were cartilaginous into adult stages in *Tiktaalik* and *Ichthyostega* and that the observed pattern is not an artifact of preservation or variation across ontogeny.

Intercentra are paired and have minor graded differences in their morphology across the series (Fig. 2 C). Proceeding caudally, intercentra become longer in the rostro-caudal direction, shorter dorsoventrally, and bear a larger articular facet for the ribs (Fig. 2 D-G). Similar rostro-caudal variation is observed in the presacral intercentra of *Eusthenopteron*[8]. *Tiktaalik* is distinguished from closely related taxa in having paired intercentra along the full series. In *Eusthenopteron,* the anterior five intercentra and the intercentra above the pelvis, at approximately position 32, are bilaterally fused[8]; *Acanthostega* has fused atlantal and sacral intercentra[1]; and in *Ichthyostega* most intercentra are fused, with only the anterior-most ones being paired[3].

Neural arches are inclined posteriorly and vary craniocaudally in their morphology. Frequently, they are laterally compressed in preservation, with the left and right halves occasionally

88   separating, as has been described in *Eusthenopteron*[8], *Panderichthys*[11], *Elpistostege*[12], and

89   *Acanthostega*[1]. Zygapophyses are not observed, unlike the condition of limbed vertebrates[2,3,16,17].

90   Cranially, neural arches have a simple saddle shape (Fig. 2 D,E). The rostral 30 arches show

91   subtle variation in their geometry, with more caudal neural arches having slightly more vertical

92   inclination relative to the notochord. By position 32, the neural arch pattern shifts abruptly, and

93   neural arches extend further dorsally and have a dorsal foramen. Neural arch 31 is broken

94   dorsally, and so it is unclear whether the transition in neural arch morphology occurs at position

95   31 or 32. Regardless, this shift in morphology is inferred to mark the trunk-tail boundary, a

96   change also observed in the ribs as described below. Further caudally, four vertebrae are

97   preserved. One of these is substantially more robust than all others (Fig. 2 F,G. Movie S1),

98   similar to neural arches preserved in the caudal domain of *Acanthostega*[1].

Ribs

100   Specimen NUFV 108 was physically prepared in 2004 and 2005 to expose rostral ribs[13]. μCT

101   imaging reveals additional ribs preserved beyond those previously identified, making for a total

102   of 56, including an uninterrupted series of 32 on the left side. Across the series, ribs have a

103   curved articular head that would have contacted the pleurapophyses of the intercentra. Ribs bear

104   a flange posteriorly on their proximal portion that varies in its mediolateral span across the

105   series, and they lack imbricating uncinate processes (Fig. 2 A,B). The rostral-most ribs extend

106   straight to a tapered, narrow tip. More caudally, at approximately rib number 5, the ribs become

107   longer and have a gentle ventral curvature. At approximately rib number 20, the ribs shorten in

108   their mediolateral span and have a broader base, gaining a more triangular shape. Ribs 31 and 32

109   are markedly distinct in their morphology from others in the series. Rib 31 is broad in dorsal

110   perspective and has unfinished distal surface that is rounded, while rib 32 shows substantial

111   ventral curvature as compared more cranial ribs (Fig. 2 A,B). An isolated post-sacral rib is

112   preserved to the left of the other axial elements (Fig. 1 A,B, Movie S2). Its morphology, narrow,

113   slightly recurved and posteriorly directed, is similar to the post-sacral ribs of *Acanthostega*[1] and

114   *Ichthyostega*[2]. No evidence of sternal structures is found.

Reconstruction of the pelvic region

The morphology of the pelvis of *Tiktaalik* was described previously[14], but, importantly, its position and relation to the axial column has remained unknown. The right pelvis of specimen NUFV 108 was preserved adjacent to the axial column, not in articulation[14]. Abrupt transitions in the morphology of the vertebrae and ribs at position 31 and 32 in *Tiktaalik* denote the trunk-to-tail transition and likely position of the pelvic girdle. In both *Eusthenopteron* and *Acanthostega*, transitions in vertebral and rib anatomy at this general position denote the trunk-to-tail transition and pelvic position. In *Eusthenopteron*, ribs are only present rostral to vertebrae 30, between vertebrae 30 and 32 the haemal arches enclosed the haemal canal and the become intercentra fuse bilaterally, and the pelvis is approximately ventral to vertebrae 32[8]. In *Acanthostega,* vertebrae 31 differs from those immediately rostral in having fused intercentra and bearing a distinctive and elongate rib with a ventral expansion that would have allowed for attachment to the girdle, likely *via* soft tissue[1].

New data on the on vertebrae and ribs of *Tiktaalik* allow for assessment of pelvic orientation and position (Fig. 3, Movie S3). The dorsal extent of the iliac blade of the pelvis would have approached ribs 31 and 32 of the series. While there is no articular facet on the internal surface of the girdle for a sacral rib[14], the proximity inferred from their anatomy, along with a comparison to other taxa, suggest a soft-tissue linkage. In *Acanthostega*, there is likewise no distinct articular facet or marked perimeter for the attachment of the sacral rib in the ilium[1]. Nevertheless, a soft-tissue connection between rib and ilium has been inferred based on rib morphology and the size and position of the two elements[1]. Similar patterns of connectivity have been proposed in other early tetrapods, such as *Eryops*[1,18]. A bony articulation between ribs and pelvis is only definitively present in more crownward tetrapods such as *Whatcheeria*[1,18,19]. The positioning of the pelvis of *Tiktaalik* suggested by the shape of the pubis and width of the body (Fig. S1, Supplementary Text) would entail a more posteroventral-facing acetabulum than previously proposed[14], more similar to the orientation of the pelvic fins of *Eusthenopteron*[8] than the laterally positioned limbs of Devonian limbed vertebrates[1,2,20].

Pelvic fin

Mechanical preparation of specimen NUFV 108 in 2005-2006 exposed parts of the pelvic fin[14]. µCT data reveal new details, including the full extent of the pelvic fin web and additional endoskeletal elements (Fig. 1, Fig. 4 A). Pelvic fin rays are unbranching and unsegmented.

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146 Similar to the pectoral fins of tetrapodomorphs, the pelvic fin rays are more robust on the leading
147 edge and more gracile on the posterior side[21]. Hemitrichia have accentuated asymmetry. Dorsal
148 hemitrichia are larger in cross section than ventral hemitrichia, as in the pectoral fin of
149 *Tiktaalik*[21] (Fig. 4 B). Two new pelvic endoskeletal elements are identified (Fig. 4 A,C). One,
150 inferred to be a tibia, has a robust proximal articular surface, and its distal margin appears
151 broken, making it unclear whether a more distal element might have articulated with it. The other
152 element is small with a posteriorly oriented ventral curving process, a feature not previously
153 observed in tetrapodomorph pelvic fins[8-10,15,22].

154 **Occipital-vertebral junction**

155 In specimen NUFV 108, the basioccipital-exoccipital complex is preserved apart from the rest of
156 the skull, medial to the pectoral girdles, and it comprises a bilateral pair of elements (Fig. 1, Fig.
157 S2 A-D). Examination of μCT data of specimen NUFV 110[23] confirms that the basioccipital-
158 exoccipital complex is deconsolidated from the rest of the skull in *Tiktaalik*. (Fig. S1 E-I). The
159 pattern of *Tiktaalik* differs from the general pattern among tetrapodomorphs, where the
160 basioccipital-exoccipital complex is fused both across the midline and to anterior neurocranial
161 elements[24,25]. In the tristichopterid *Mandageria fairfaxi*, the basioccipital-exoccipital complex is
162 also separated from more anterior elements, and this feature has been inferred to allow for
163 increased notochordal flexion at the occipital-vertebral junction[26]. Deconsolidation of skeletal
164 elements at the back of the skull in *Tiktaalik*, therefore, provides further evidence for increased
165 mobility at the head-trunk boundary, which was previously hypothesized based on the absence of
166 an operculum and extrascapular series[13].

167

168 **Discussion**

169 *Tiktaalik* exhibits a unique constellation of primitive and derived characters in the axial skeleton
170 that suggest it had a locomotor capacity intermediate to currently known finned elpistostegalians
171 and limbed vertebrates. These data, and the reconstruction they imply (Fig. 5), allow for new
172 hypotheses on the evolution of axial regionalization and the origin of quadrupedal locomotion in
173 early tetrapods.

6

174   The vertebrae of *Tiktaalik* adhere closely to plesiomorphic tetrapodomorph conditions. Most of

175   the preserved vertebrae are from the trunk, and they are similar to the trunk vertebrae of

176   *Eusthenopteron* both in degree of differentiation across the series and in overall construction,

177   except for slight differences in intercentral fusion and the potential lack of pleurocentra[8]. The

178   number of trunk vertebrae in *Tiktaalik* is similar to other tetrapodomorphs; *Eusthenopteron*,

179   *Acanthostega*, and *Ichthyostega* are also characterized by approximately 30 pre-sacral

180   vertebrae[1,2,8].

181   In contrast to the vertebral column, the ribs of *Tiktaalik* show numerous derived features that are

182   previously known only from limbed taxa (Fig. 6). As in *Acanthostega*[1] and *Ichthyostega*[2], the

183   ribs of *Tiktaalik* extend caudal to the trunk-tail boundary and are regionalized with a sacral

184   module. This is a departure from plesiomorphic tetrapodomorph pattern, seen in *Eusthenopteron*,

185   where ribs do not extend caudal to the trunk-tail boundary and those near to the pelvis are not

186   morphologically differentiated[8].

187   The rib anatomy of *Eusthenopteron*, coupled with a small ilium positioned ventrally to the

188   vertebral column, indicate the absence of any linkage between axial column and pelvic fin[8,24].

189   The sacral ribs of *Tiktaalik*, on the other hand, would have overlapped the pelvic girdle in lateral

190   perspective, with ribs lying medial to a large, plate-like ilium. Although there is no evidence of a

191   bony articulation, the nature of the expansion of both ribs and ilium, the degree of overlap

192   between the elements, and the unfinished distal margin of one sacral rib, indicates that a soft-

193   tissue connection was likely in *Tiktaalik*. Such a connection, also proposed to be present in early

194   limbed forms including *Acanthostega*[1], likely allowed for a degree of structural support and for a

195   restricted range of motion between the elements. A soft-tissue linkage between girdle and axial

196   column would have provided a less robust a connection than direct bony articulations

197   hypothesized for *Ichthyostega*[4] and observed more clearly in more crownward forms, like

198   *Whatcheeria*[19]. However, mobility of the pelvic girdle could have allowed for slight changes in

199   the orientation of the acetabulum during locomotor behaviors. The post-cranial skeleton of

200   *Tiktaalik*, therefore, reveals that sacro-iliac specializations arose in the ribs and pelvis prior to

201   modifications to the vertebral column. Subsequent modifications to the axial column observed in

202   limbed vertebrates include expansion of the dorsal extent of neural arches, either squared as in

203   *Acanthostega* or rounded as in *Ichthyostega,* and the origin of zygapophyses[1,2].

204   The presence of sacral ribs, robust pelvis, deep acetabulum, and large pelvic fin in *Tiktaalik*

205   indicate that the rear appendage was generating greater forces in locomotion than in other finned

206   elpistostegalians, such as *Panderichthys*. In addition, these features suggest that *Tiktaalik* was

207   capable of more axial support for the trunk when the pelvic fins were loaded against the substrate

208   than less crownward elpistostegalians. Despite these apomorphic features, *Tiktaalik* retains

209   numerous plesiomorphic characteristics in its pelvic anatomy, such a posteriorly facing

210   acetabulum, left and right pubes unfused along the midline, and lack of an ischium[14], which

211   imply that the pelvic fin was not able to retract as extensively as limbed forms such as

212   *Acanthostega* and *Ichthyostega*. The posterior orientation of the acetabulum of *Tiktaalik* and

213   concomitant inability to use retraction for limb propulsion suggests that the pelvic fin was unable

214   to play a significant role in terrestrial walking.

215   With a pelvis and pelvic fin subequal in size to the shoulder girdle and pectoral fin, the overall

216   proportions of the trunk and paired appendages of *Tiktaalik* hew closer to those of *Acanthostega*[1]

217   and *Ichthyostega*[2,20] than to *Eusthenopteron*[8,24] and *Panderichthyes*[11,15]. Pelvic and sacral

218   anatomy implies that *Tiktaalik* represents an intermediate condition in which a large pelvic

219   appendage was stabilized by the axial skeleton and capable of being used in diverse paddling,

220   walking, and propping behaviors on aquatic substrates. These functions of the pelvic fin were

221   antecedents to the terrestrial walking behaviors that were possible in later forms.

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323

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325

326 **Data and materials availability:** All data analyzed in the paper will be freely available.
327 Computed tomography data sets and STL files will be available for download from
328 MorphoSource prior to publication.
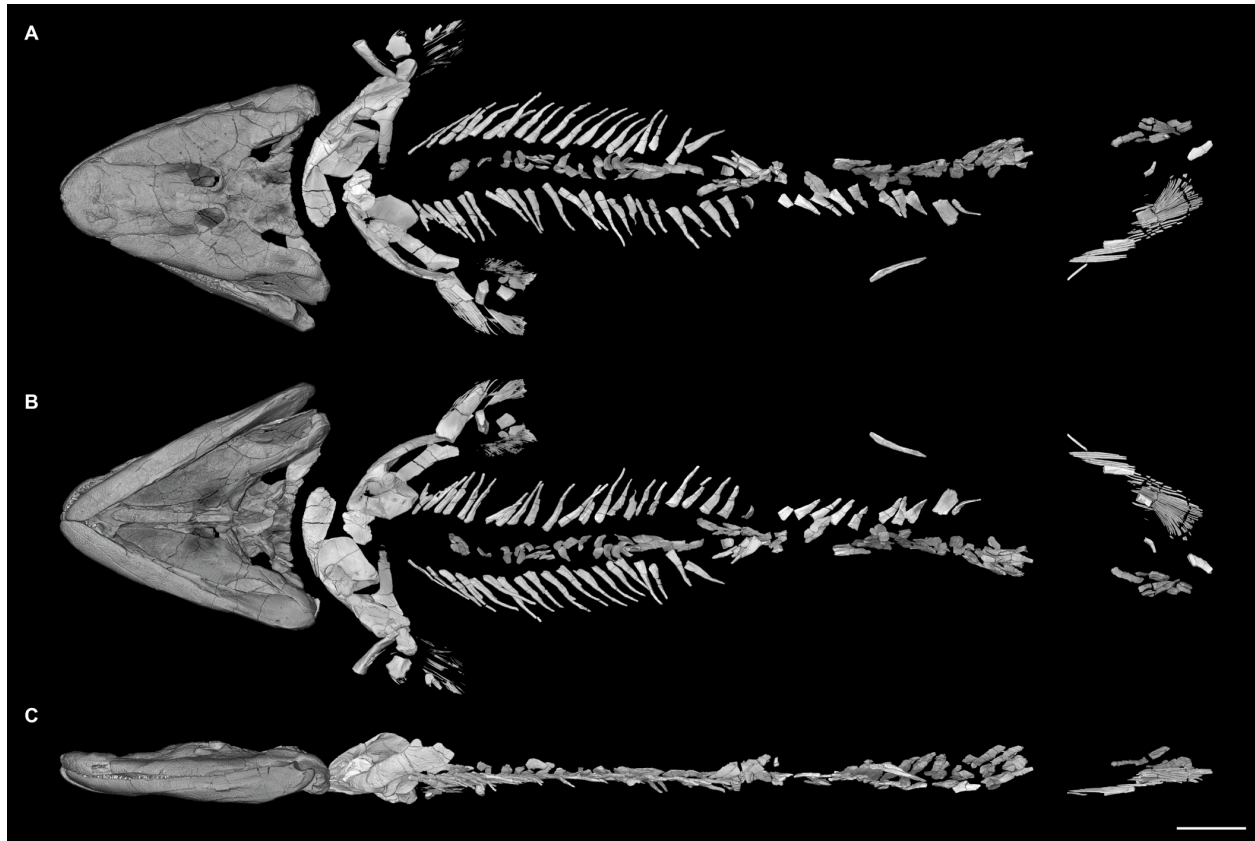
Fig. 1. Volumetric rendering of μCT scans of *Tiktaalik roseae*. Specimen NUFV 108 in (**A**) dorsal, (**B**) ventral, and (**C**) left lateral perspectives. μCT data reveal new detail on the ribs, vertebrae, and pelvic fin. The head, which was mechanically prepared and scanned separately[27], is positioned slightly anterior to its preserved position. Scale bar, 5 cm.
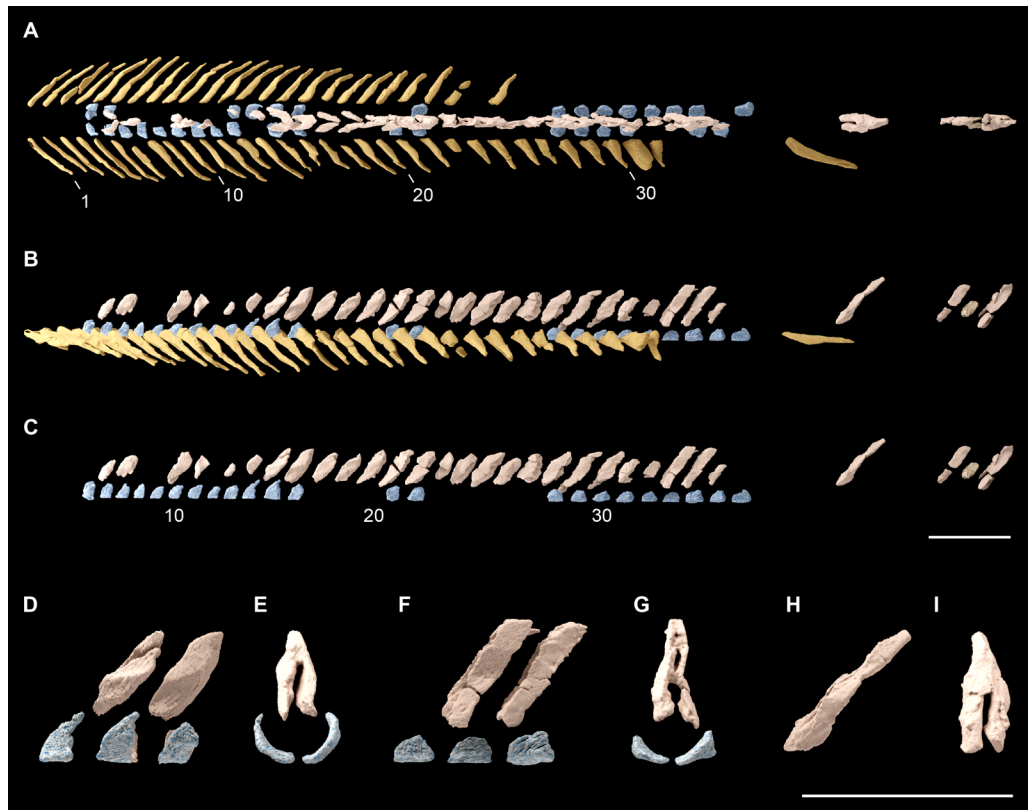
**Fig. 2. Vertebrae and ribs of *Tiktaalik roseae*.** Vertebrae and ribs in (**A**) dorsal and (**B**) lateral perspective. (**C**) Intercentra and neural arches in lateral perspective. (**D,E**) Intercentra and neural arches beginning at position 14 in left lateral and anterior perspective (**F,G**) Intercentra and neural arches beginning at position 32 in left lateral and anterior perspective. (**H,I**) Neural arch from the caudal region in left lateral and anterior perspective. Ribs are depicted in yellow, neural arches in tan, and intercentra in blue. Scale bars, 5 cm.
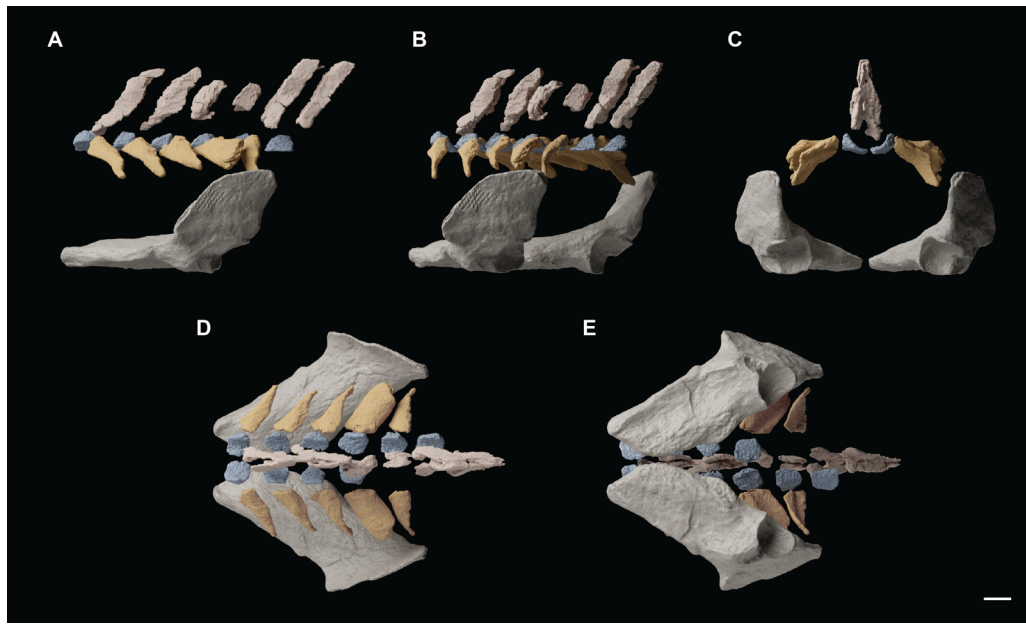
Fig. 3. Reconstructed sacral domain and pelvic girdle of *Tiktaalik roseae*. Reconstruction of the axial column and pelvis in (**A**) left lateral, (**B**) posterior-oblique, (**C**) posterior, (**D**) dorsal, (**E**) ventral perspectives. Ribs and pelvic girdle have been mirrored to produce the reconstruction. Ribs 31 and 32 show modified shape as compared to the more anterior elements and are inferred to have supported the pelvic girdle by a soft-tissue connection. Scale bar, 1 cm.
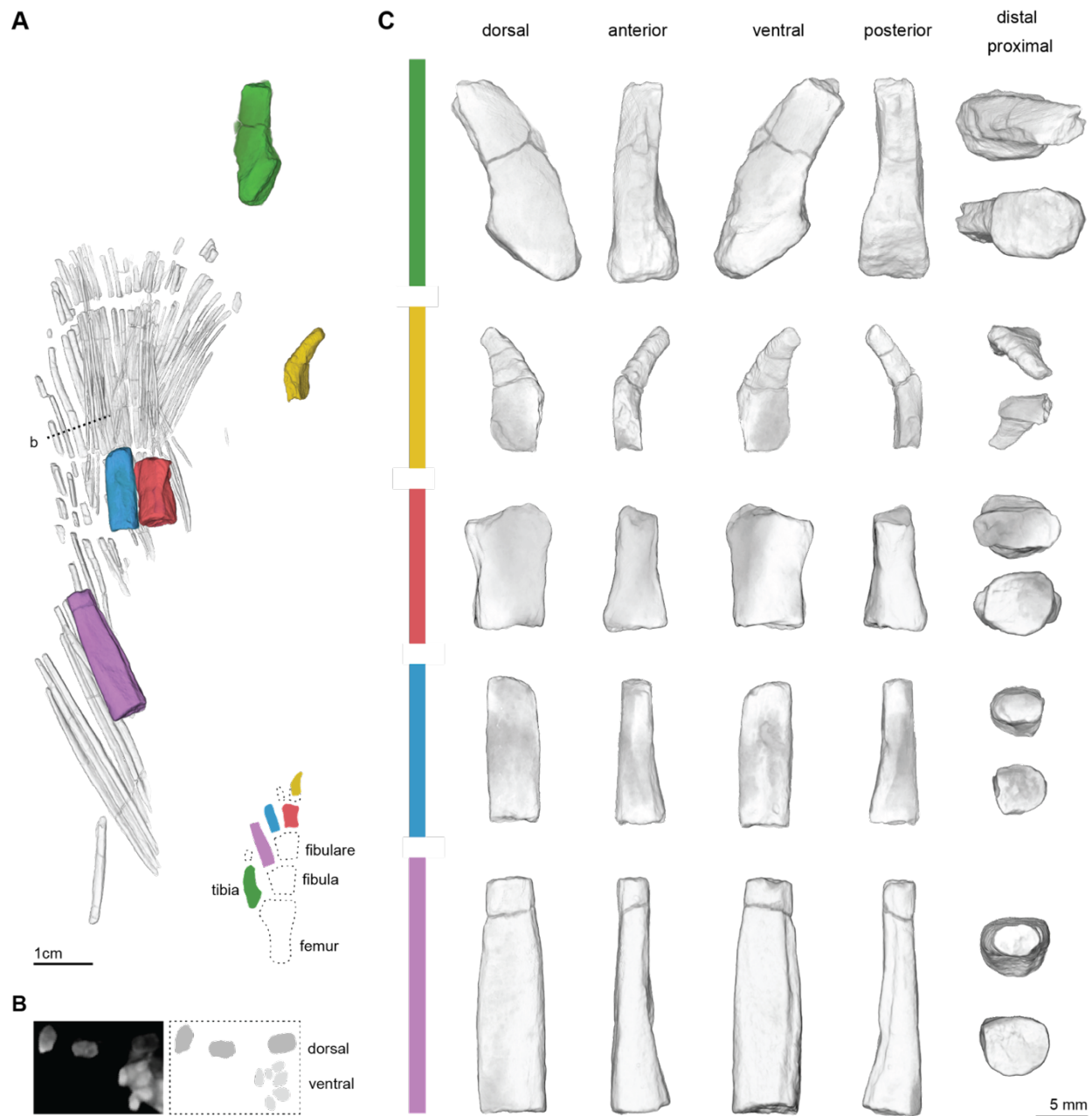
Fig. 4. Pelvic fin of *Tiktaalik roseae*. (**A**) Volumetric rendering of μCT data of the left pelvic fin of NUFV 108 and a reconstruction of the fin in ventral perspective. (**B**) Hemitrichia show dorso-ventral asymmetry. The digital cross section, left, and illustration, right, were taken at the position of the dashed line labeled 'b' in panel A. The cross section is oriented orthogonal to the plane of the fin web. (**C**) Endoskeletal elements of the pelvic fin in various orientations.
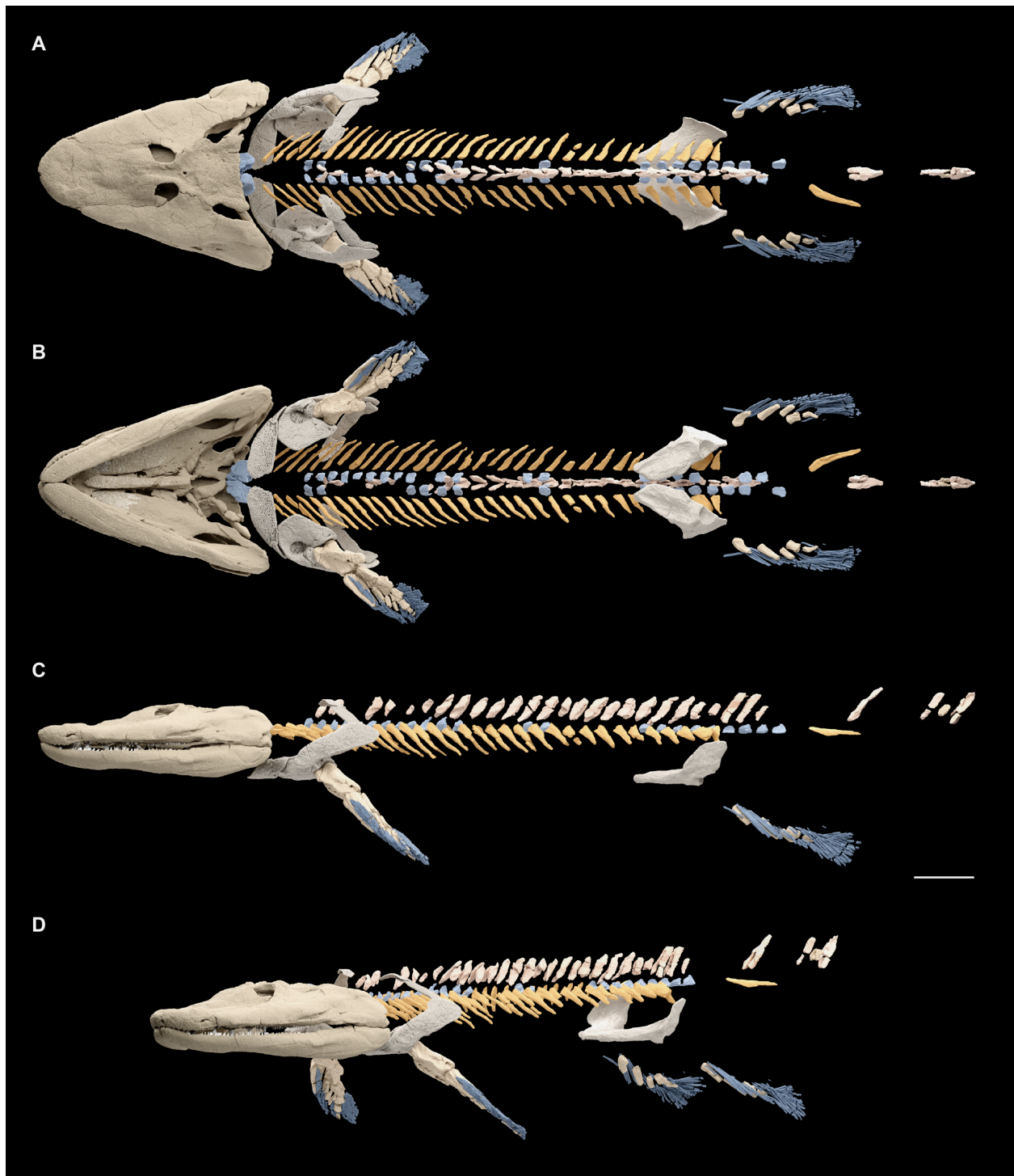
Fig. 5. Reconstruction of *Tiktaalik roseae*. Reconstruction in (**A**) dorsal, (**B**) ventral, (**C**) left lateral, and (**D**) oblique views. Cranial materials are repositioned according to Lemberg *et al*[23] to account for settling during preservation. Select elements that are preserved from only one side of NUFV 108 (*i.e.,* pre-sacral ribs, pelvic girdle, and fin) are reflected for symmetry. The pectoral fin is from specimen NUFV 110[21] and scaled to the length of the right humerus of NUFV 108. Additional skeletal elements are known for *Tiktaalik*, including branchial skeleton[13] and interclavicle[28], but have not been rendered here. Scale bar, 5 cm.

16

Eusthenopteron foordi

Tiktaalik roseae

Acanthostega gunnari

Ichthyostega sp.
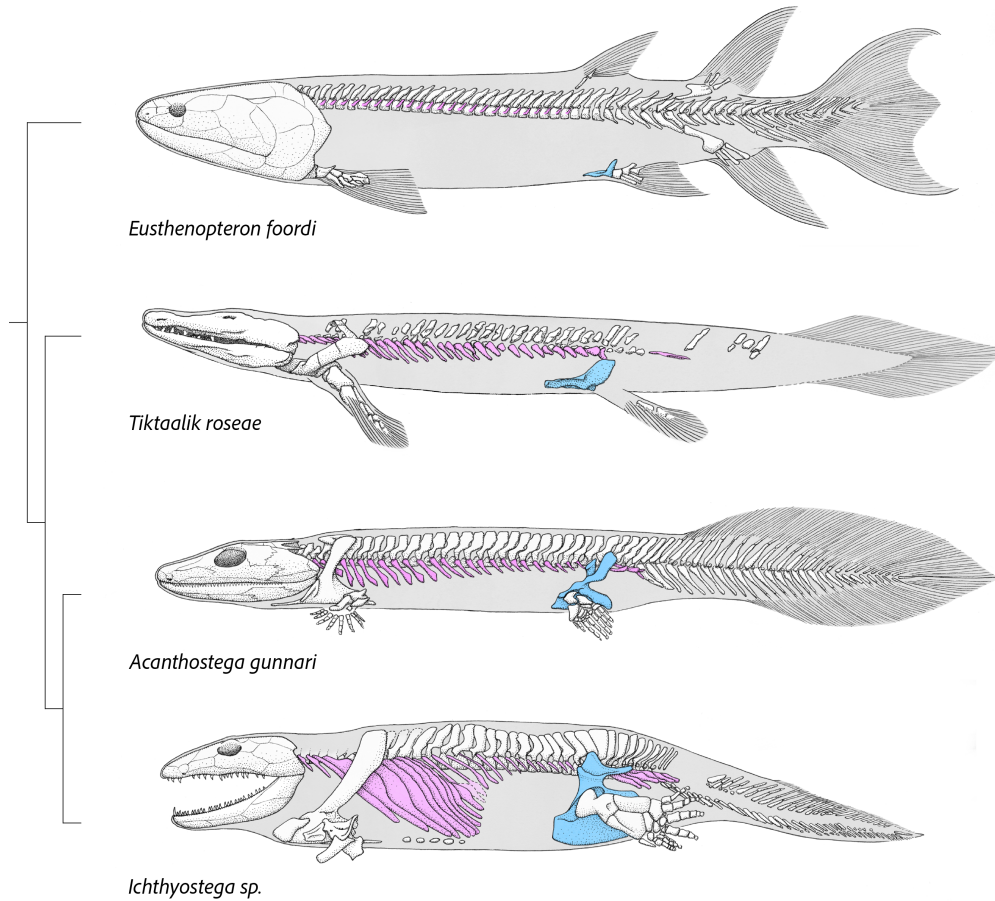
Fig. 6. Reconstructions of Devonian tetrapodomorphs. The anatomy of *Tiktaalik roseae* shows that specializations in the axial column for head mobility, body support and pelvic fin buttressing had evolved in elpistostegalians, prior to the origin of limbs. Illustration of *Tiktaalik roseae* based on specimen NUFV 108. Illustrations of other taxa are based on previously published descriptions: *Eusthenopteron foordi*[8,24], *Acanthostega gunnari*[1,2], *Ichthyostega sp.*[2,20]. Ribs are depicted in purple. Pelvic girdles are shown in blue.

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376
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Supplementary Materials for

378 **Axial regionalization in *Tiktaalik roseae* and the origin of quadrupedal**
379 **locomotion**

380

381 T.A. Stewart, J.B. Lemberg, E.J. Hillan, I. Magallanes, E.B. Daeschler, N.H. Shubin

382
383
384 **This PDF file includes:**
385
386   Materials and Methods
387   Supplementary Text
388   Figs. S1 to S3
389   Tables S1
390   Captions for Movies S1 to S4
391
392 **Other Supplementary Materials for this manuscript include the following:**
393
394   Movies S1 to S4
395

Materials and Methods

The material of *Tiktaalik roseae* was recovered during paleontological excavations near Bird Fiord on southern Ellesmere Island in 2004, 2006, 2008, and 2013. All specimens were recovered from a single locality (NV2K17; N77°09.895′ W86°16.157′) within the Fram Formation (Frasnian Stage, Late Devonian). The fossil material is curated in the Nunavut Fossil Vertebrate Collection (NUFV) at the Canadian Museum of Nature.

Computed tomography scanning

CT scans were collected at The University of Chicago's PaleoCT scanning facility with a GE Phoenix v|tome|x 240 kv/180 kv scanner. The post-cranial skeleton of NUFV 108 is contained in two blocks (Movie S1). Each of these blocks are too large for single multiscan. Therefore, each block was scanned each twice: first oriented vertically with the anterior edge down, and then rotated 180 degrees and scanned again with the posterior edge down. Scanning parameters for these four scans are provided in Table S1. CT data were reconstructed with Phoenix Datos|x 2 (version 2.3.3) and imported to VGStudio Max (version 2.2) to be cropped and exported as a tiff stack. For each block, the two multi-scans were manually stitched together and then manually segmented in Amira (version 20.2) (Thermo Fisher Scientific).

Surface scanning

The pelvis of NUFV 108 was previously physically prepared from the specimen and, therefore, is not included in the μCT scans. A 3D model of the pelvis was generated by surface scanning a cast of the pelvis using a FARO Design ScanArm 1.0 at a resolution of 40-75 μm.

Images and Animations

Volumetric images of the segmented μCT data were generated using Amira (Fig. 1, Fig. 4, Fig. S1). All other renderings of skeletal elements are surface models, which were generated by exporting segmentation label fields from Amira as surface files, or directly by surface scanning, and visualized in Blender (version 3.3.1). Movies were created by first exporting animations as tiff stacks from Amira or Blender and then using Adobe Premier (version 13.12) to combine and edit the images into movies.

19

427   *Supplementary Text*

428

429   In specimen NUFV 108, individual skeletal elements are three-dimensionally preserved with

430   minimal deformation, and the specimen has settled during preservation. Previous studies of

431   *Tiktaalik* have presented reconstructions of the cranial and pectoral fin skeleton based on μCT

432   data [21,23]. In this study, we describe the axial skeleton of *Tiktaalik* and use μCT data to produce a

433   three-dimensional model of specimen NUFV 108 that contains nearly all skeletal elements

434   known for this taxon. Additional elements known from *Tiktaalik* that are not included in the

435   model include components of the hyoid skeleton and the interclavicle. In generating our model,

436   numerous decisions were made on how to place the pieces. These decisions are based upon

437   comparisons between anatomical systems of *Tiktaalik* (*e.g.*, comparing intercentral and neural

438   arch anatomy, or comparing the pectoral and pelvic girdle), as well as comparisons to other

439   tetrapodomorphs and extant fishes. The new reconstruction of *Tiktaalik*, thus, represents a

440   hypothesis based on multiple lines of evidence.

441

442   Reconstruction of the vertebrae

443   Intercentra and neural arches are positioned according to their preserved rostrocaudal order.

444   Intercentra are assigned to either the left or right sides based curvature of the internal surface and

445   position of the articular facet. Left and right intercentra that were preserved near to one another

446   are reconstructed as paired. However, because intercentra are unfused can have shifted during

447   preservation, it is possible that some elements reconstructed as paired are slightly out of register

448   from their original position. Intercentra are reconstructed as associated with individual neural

449   arches. Likewise, it is possible that intercentra could be reconstructed modestly out of register

450   from their original neural arch. This uncertainty does not impact results presented in the

451   manuscript.

452   Intercentra are positioned so that they bound the lower portion of the notochord and wrap

453   dorsally. When both left and right sides are preserved for a vertebra, they are positioned so that

454   their internal curvature symmetrically fits around a notochord that is circular in cross section.

455   Intercentra are positioned under the assumption that the notochord is of a uniform cross section

456   between the head and pelvis, a feature observed in various taxa, including *Eusthenopteron* [8,24]

457   and *Latimeria* [29]. If only one intercentra was preserved for a vertebra, the element is positioned

458   so its internal curvature matched elements anterior or posterior it in the series.

459   Neural arches are occasionally broken, and whenever possible the pieces are re-

460   assembled. Neural arches from vertebrae 5-34 can be associated with ribs or intercentra.

461   However, four neural arches are preserved more caudally and without clear association to other

462   axial elements. The anterior-most of these four neural arches is preserved caudal and slightly

463   ventral to neural arch 32 (Movie S1). Its morphology is significantly more robust than those

464   immediately anterior (Fig. 2F-I), and it is identified as belonging to the caudal region based of

465   comparison to *Acanthostega*[1]. Although the neural arch could have been associated with

466   intercentra 34 or 35, it is depicted in the reconstruction with a gap between it and other elements

467   to denote uncertainty in position (Fig. 2). The three most-caudal neural arches are preserved in

468   close association with one another and separated by a substantial gap to other axial elements,

469   near to the pelvic fin (Fig. 1A,B). These neural arches, too, are depicted in the reconstructed with

470   gaps between them and other axial elements to denote ambiguity in their position (Fig. 2).

471   To reconstruct the dorsal position of neural arches, we first focused on the most complete

472   neural arches in the series (*e.g.*, Fig. 2F,G). Despite some lateral compression, these allowed us

473   to estimate the extent to which the arch would have wrapped around the notochord. When neural

474   arches were broken, if possible, they are reconstructed so that the apex of their internal curvature

475   aligns with the apex of other more complete neural arches in the series.

476   To constrain spacing of axial elements in the rostrocaudal direction, we considered the

477   preserved distance between ribs 1 and 32 in NUFV 108 to approximate the distance between ribs

478   1 and 32 in life. Across this distance, ribs and vertebrae are placed so that gaps between the

479   vertebrae were uniform, except when their position was uncertain (see discussion above of the

480   caudal-most 4 vertebrae and discussion below of the sacral rib). Vertebrae 32-36 are spaced at

481   distances similar to those of positions 1-32.

482   In the reconstruction of *Tiktaalik*, intercentra are positioned slightly anterior to their

483   corresponding neural arch. This positioning is based on several features. First, the positioning of

484   intercentra reveals the size of the notochord, and comparison of intercentral and neural arch

485   morphologies suggest that they are unlikely to have been aligned strictly dorsally, because this

486   would have produced a lateral overlap of the elements. Second, pleurocentra were not identified

487   for specimen NUFV 108. If large pleurocentra had been observed in the specimen, then the

488   vertebrae are likely to have been organized such that neural arches were positioned dorsal to

489   their corresponding intercentra, as in *Osteolepis* [9]. Therefore, the absence of pleurocentra

21

490 indicates they were either small or fused to the intercentra; both conditions predict that neural

491 arches and intercentra were not vertically aligned, but slightly out of register [3,8].

492 The neural arches of *Tiktaalik* lack zygopophyses. This suggests space between adjacent

493 neural arches. Therefore, they are situated with angles of inclination that maintain a slight gap

494 between adjacent elements. The caudal four neural arches are positioned with similar angles of

495 inclination as those in the trunk series.

496

497 Reconstruction of the ribs

498 The anterior-most rib on the left side is broken in two pieces, which were preserved in contact

499 with one another with a sharp angle between them (Fig. 1). These pieces are placed end-to-end to

500 reconstruct the original element (Fig. 2). Other ribs that are broken have pieces preserved in

501 close proximity with one another, and they are approximately aligned (*e.g.*, rib 23 on the right

502 side). In the reconstruction, the pieces of these other broken ribs are kept in their preserved

503 positions and have not been moved closer to one together in the reconstruction. This presentation

504 was done to preserve information on which features are broken and not to imply that any gaps in

505 individual ribs represent their original length and missing portions of the rib.

506 Two ribs on the left side (ribs seven and twelve) and one on the right side (rib six) were

507 displaced during preservation such that the distal portion of the rib was posteriorly oriented and

508 ventral to the rib that followed. Additionally, four ribs on the right side (ribs 20-23) are preserved

509 such their articular surfaces point posteriorly. In each of these cases, the individual ribs were

510 rotated and repositioned preserving the order of their proximal articular surfaces.

511 One rib is preserved to the left of the rest of the axial series, and it is identified as a post-

512 sacral rib. It is possible that it might have articulated upon intercentra 33-36, as approximately 5

513 post-sacral ribs are preserved in *Acanthostega* [1] and *Ichthyostega* [2,20]. However, the rib is

514 depicted in the reconstruction with a gap between it and other axial elements to denote ambiguity

515 in position.

516 Ribs were positioned relative to the vertebral column based on curvature of the proximal

517 articular surface. In many ribs, this portion is broken or incomplete. Therefore, across the series,

518 ribs are placed by first reconstructing the positions of those ribs with complete articular heads.

519 These ribs were placed so that their heads aligned with the curvature of the posterior margin of

520 the intercentra, which bear an articular facet. Ribs with damaged heads were then positioned to

521 maximize similarity in their orientation to those with complete heads.

22

522

523   Reconstruction of the pelvis

524   Rostrocaudal positioning of the pelvis of *Tiktaalik* is based upon transitions in rib and neural

525   arch anatomy. Specifically, the girdle is placed so that the dorsal extent of the ilium is

526   rostrocaudally aligned with the sacral ribs (ribs 31 and 32). This positioning is similar to what

527   has is proposed for the pelvic girdles of *Acanthostega* and *Ichthyostega*, which each have

528   approximately 30 pre-sacral vertebrae [1,2].

529   Dorsoventral positioning of the pelvic girdle of *Tiktaalik* is based on comparisons to

530   other tetrapodomorphs. Uniformly, tetrapodomorphs are reconstructed with the ventral portion of

531   the pelvic girdle approximately in line with the ventral portion of the pectoral girdle (*e.g.*,

532   *Eusthenopteron* [8], *Acanthostega* [1], *Ichthyostega* [2]). In *Tiktaalik*, thus, the pelvic girdle is placed

533   with a position that comports with the body thickness observed in the articulated pectoral region.

534   To reconstruct the medio-lateral splay of the pelvic girdle of *Tiktaalik*, first the

535   anteromedial portion is positioned near to the midline, as in *Eusthenopteron* [8]. Next, the girdle

536   was positioned to produce a taper in the body outline when viewed from the dorsal perspective.

537   Specimen MHNM 06-2067 of *Elpistostege* shows approximately 30% reduction in the width of

538   the trunk between the pectoral and pelvic fins [30]. The pelvic girdle of *Tiktaalik* is reconstructed

539   similarly, resulting in a narrow distance between the ilium and sacral ribs (Fig. S1 A-E). This

540   reconstruction predicts a more posterior orientation of the acetabulum than previously

541   hypothesized [14], one approximately similar to *Eusthenopteron* [8]. We regard this hypothesis of

542   pelvic positioning as more likely than one where the dorsal extent of the ilium is parallel to the

543   axial column (Fig. S1 F-I). Such a wide splay would result in an unusually ovate shape of the

544   trunk in cross section at the position of the pelvis (Fig. S1 H), which is not known among

545   tetrapodomorphs. Additionally, if a lateral orientation is constrained, but the angle between left

546   and right halves is increased to produce a more rounded cross-section, this increases the height of

547   the girdle in lateral perspective and yields a reconstruction where body thickness is greater at the

548   pelvis than the pectoral girdle (Fig. S1 H). As noted above, such an increase in body thickness is

549   not seen in other closely related taxa and regarded as unlikely.

550   Thus, positioning of the pelvis is constrained by both features of other anatomical

551   systems (*i.e.*, vertebrae, ribs, and pectoral girdle) and by comparisons to other tetrapodomorphs.

552   Although there is uncertainty in some features of the reconstruction, alternative hypotheses of

553   pelvic girdle positioning for *Tiktaalik* robustly predict that the dorsal extent of the ilium

554   approached the sacral ribs and that they overlapped in lateral perspective. Further, alternative

555   predictions also recover a pelvic fin of *Tiktaalik* that is more posteriorly oriented than in

556   *Acanthostega* and *Ichthyostega*[1,2].

557

558   <u>Reconstruction of the pelvic fin</u>

559   A line drawing of the pelvic fin is presented in Fig. 4 A that shows the estimated positions of the

560   preserved endoskeleton elements as well as estimates of the geometry of missing elements.

561   Along the proximodistal axis, fins generally taper dorsoventrally. Accordingly, proximal skeletal

562   elements have articular surfaces that are deeper in the dorsoventral direction than those more

563   distally positioned. As previously noted, element shown in purple in Fig. 4 has a similar

564   morphology to the intermedium of the pectoral fin of *Tiktaalik*[14]; it is, thus, reconstructed as

565   articulating with the fibula. This positioning contributed to the identification of the tibia. The

566   element identified as the tibia has an articular surface deeper dorsoventrally than any other

567   preserved pelvic endoskeletal elements and, therefore, would likely have been more proximally

568   positioned than the element shown in purple. The general pattern of tetrapodomorph pelvic fins

569   is such that one would predict only three possible more proximal elements: the femur, fibula, and

570   tibia. The geometry of this most robust element is inconsistent with either a femur or fibula, both

571   of which likely would have had two distal articular facets, and it is therefore identified as the

572   tibia.

573   In the drawing, the tibia is illustrated with a dashed component distal to it. The distal

574   geometry of the tibia is rough and uneven as compared to the distal surfaces of other pelvic

575   elements, like the intermedium, third mesomere, and third anterior radial. Therefore, this texture

576   is taken to indicate that the distal portion of the tibia might have broken off or was poorly

577   ossified. It is possible that a small element articulated distally with the tibia. We regard this

578   condition as unlikely, because neither *Eusthenopteron*[8] nor *Panderichthys*[15] have pelvic fins

579   showing an element articulating distally with the tibia.

580   Several endoskeletal elements of the pelvic fin are not preserved. Their approximate

581   geometries are estimated in the illustration. Mesomeres are typically not longer proximodistally

582   than those more proximal to them. Therefore, we estimated the relative lengths of the fibulare,

583   fibula based on the third mesomere (shown in red in Fig. 3). The approximate geometry of the

584   femur is based on the assumptions that it would be at least as long as the tibia and distally wide

585   enough to accommodate the tibia and fibula.

586   In the pectoral fin, fin rays overlap with the radius. The tibia, the homologous element in

587 the pelvic fin, is therefore expected to similarly have been covered by lepidotrichia. Accordingly,

588 it is positioned it in the 3D reconstruction so that dorsal hemitrichia would have reached

589 approximately to the base of the femur. Individual fin rays within the fin web are not

590 repositioned. The pelvic fin is placed relative to the girdle such that a femur, if present, would be

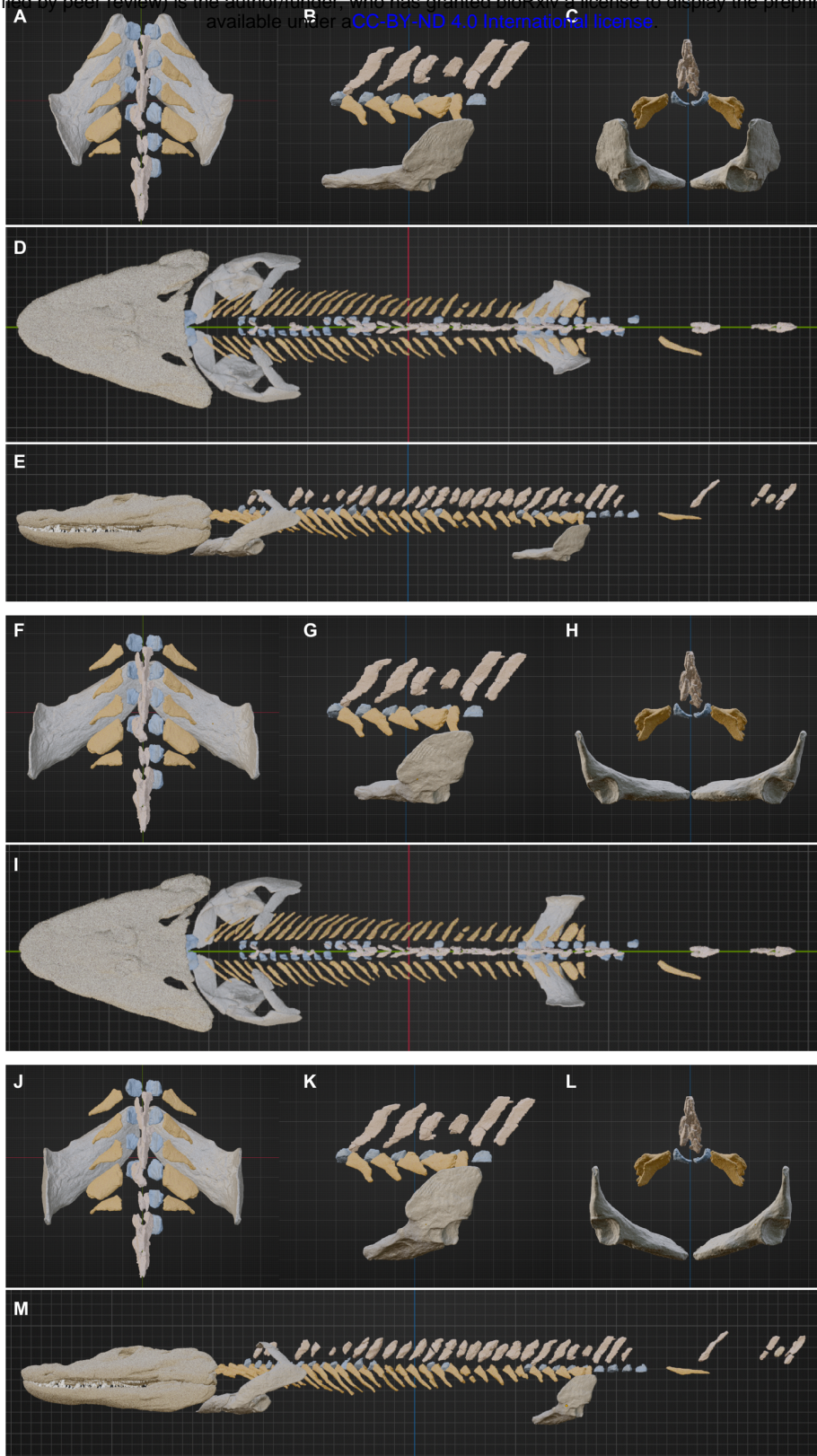591 extending straight from the acetabulum.

Fig. S1.
Alternative reconstructions of pelvic girdle of *Tiktaalik roseae*. Alternative hypotheses for the positioning of the pelvic girdle were considered when building the reconstruction, as reviewed in the Supplementary Discussion. 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

26

623   pelvic girdle is aligned with the ventral aspect of the pectoral girdle. This position, with a broad
624   body in the pelvic region, corresponds to previous reconstruction of the pelvic girdle[14]. Panels J-
625   M show a third reconstruction, where the left and right halves of the pelvis are rotated.
626
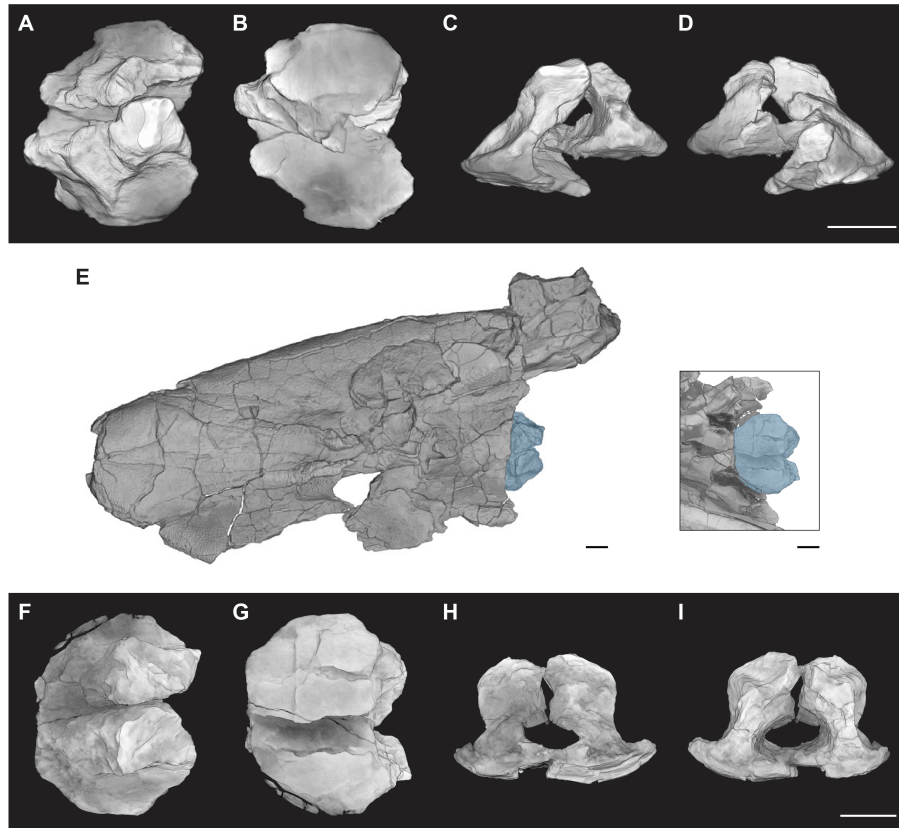
627
628
629
630 **Fig. S2.**
631 **Basioccipital-exoccipital complex of *Tiktaalik roseae*.** The basioccipital-exoccipital complex of
632 *Tiktaalik* is preserved in specimens NUFV 108 and NUFV 110 as paired elements that are
633 unfused to the rest of the braincase. In NUFV 108, the elements are preserved medial to the
634 pectoral girdle, as depicted in Fig. 1. The basioccipital-exoccipital elements of NUFV 108 shown
635 in preserved positions from (A) dorsal, (B) ventral, (C) anterior, and (D) posterior perspectives.
636 (E) In NUFV 110, the basioccipital-exoccipital complex is still contacting the rest of the skull.
637 The basioccipital-exoccipital complex of NUFV 110 from (F) dorsal, (G) ventral, (H) anterior,
638 and (I) posterior perspectives. Scale bars, 1 cm.
639
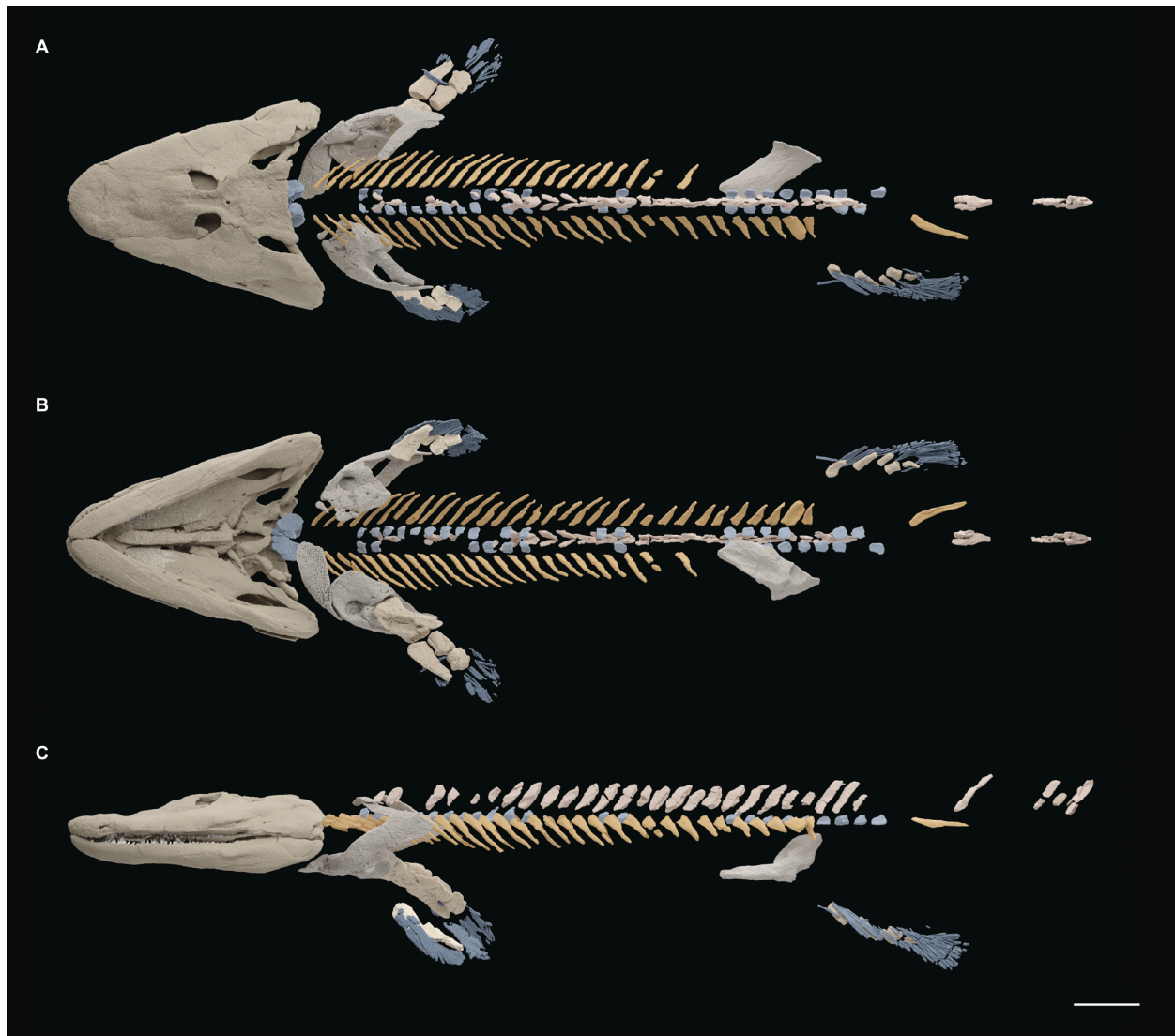
Fig. S3.

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

647   **Table S1.**
648

scan ID	Voltage (kV)	Current (µA)	Voxel Size (µm)	Filter (mm)	Scan Duration
pectoral block anterior	100	570	122.441	0.24 Cu	1hr44min
pectoral block posterior	100	570	88.038	0.24 Cu	5hr06min
pelvic block anterior	110	450	73.23	0.24 Cu	6hr48min
pelvic block posterior	110	450	73.23	0.24 Cu	6hr48min

649
650   Parameters for µCT scanning of specimen NUFV108
651

652 **Movie S1.**
653 Volumetric rendering of the two blocks containing the post-cranial skeleton of NUFV 108
654 including matrix
655
656 **Movie S2.**
657 Volumetric rendering of NUFV 108 with all segmented elements in their preserved position
658
659 **Movie S3.**
660 Rotation of the reconstructed sacral domain of *Tiktaalik roseae*
661
662 **Movie S4.**
663 Rotation of the reconstruction of *Tiktaalik roseae*