Fin ray patterns at the fin-to-limb transition

Thomas A. Stewart,a,b Justin B. Lemberga, Natalia K. Taftb, Ihna Yooa, Edward B. Daeschlerc, and Neil H. Shubin1,a

The fin-to-limb transition was marked by the origin of digits and the loss of dermal fin rays. Paleontological research into this transformation has focused on the evolution of the endoskeleton, with little attention paid to fin ray structure and function. To address this knowledge gap, we study the dermal rays of the pectoral fins of 3 key tetrapodomorph taxa—Sauripterus taylori (Rhizodontida), Eusthenopteronfoordi (Tristichopteridae), and Tiktaalkoroseae (Eipistostegalia)—using computed tomography. These data show several trends in the lineage leading to digitized forms, including the consolidation of fin rays (e.g., reduced segmentation and branching), reduction of the fin web, and, unexpectedly, the evolution of asymmetry between dorsal and ventral hemitrichia. In Eusthenopteron, dorsal rays cover the preaxial endoskeleton slightly more than ventral rays. In Tiktaalk, dorsal rays fully cover the third and fourth mesomeres, while ventral rays are restricted distal to these elements, suggesting the presence of ventralized musculature at the fin tip analogous to a fleshy “palm.” Asymmetry is also observed in cross-sectional areas of dorsal and ventral rays. Eusthenopteron dorsal rays are slightly larger than ventral rays; by contrast, Tiktaalk dorsal rays can be several times larger than ventral rays, and degree of asymmetry appears to be greater at larger sizes. Analysis of extant osteichthyans suggests that cross-sectional asymmetry in the dermal rays of paired fins is plesiomorphic to crown group osteichthyans. The evolution of dermal rays in crownward stem tetrapods reflects adaptation for a fin-supported elevated posture and resistance to substrate-based loading prior to the origin of digits.

dermal skeleton | evolution | fin-to-limb transition | paleontology

The fin-to-limb transition is characterized by the loss of fin rays (dermal skeletal rods that support the fin web) and the evolutionary origin of digits (1–3). Traditionally, efforts to explain how limbs evolved from fins have focused on transformation and novelty in the endoskeleton (4–18), with little emphasis placed on fin ray structure and function. Detailed anatomical description of dermal rays is lacking, in part, due to taphonomic bias. Individual rays are small, positioned superficially on the fin, and likely to be displaced during preservation. Additionally, when fossils are prepared, it is common practice for the dermal skeleton to be removed to allow for visualization of the endoskeleton beneath (19–21). This deficit in our knowledge is unfortunate: given the functional (22) and developmental (23, 24) integration of these skeletal systems in fins, comparative analyses of the dermal skeleton of tetrapodomorph paired fins can help elucidate how one of the greatest transitions in vertebrate history occurred.

General features of tetrapodomorph paired fins include an endoskeleton that is overlapped significantly by dermal rays that are tightly packed (i.e., with little fin membrane between them) and covered proximally by scales (25–27). The fins can be divided into a lobed region, which contains endoskeleton and fin musculature, and the fin web, which contains rays extending beyond the endoskeleton and interray webbing (28). The rays are ossified and diagnosed as lepidotrichia [sensu Goodrich (25)] because dorsal and ventral rays (hemitrichia) run apposed to one another for extended distances in the fin web. Rays are usually segmented, branching, and more robust on the anterior, or leading edge, of the fin (25, 26). In some tetrapodomorph clades, fin ray morphology is a diagnostic character. For example, rhizodontids have a long unsegmented proximal region that spans most of the ray’s length (29, 30), and rays of the osteolepoid Gogonatasandrewesae have distinctive perforations and cross-sectional geometry (31). Broadly, the distribution and polarity of fin ray characters within tetrapodomorphs are undescribed, although it is hypothesized that fin rays were reduced and ultimately lost as fin function shifted from resisting hydrodynamic forces to substrate-based loading (32).

To understand how dermal rays evolved in the paired fins of tetrapodomorph fishes, we analyzed pectoral fins of 3 key taxa, which are listed here by increasing proximity to the tetrapod crown group. First, we studied the rhizodontid Sauripterus taylori (Hall, 1843), both juvenile (33) and adult specimens (20, 34). Second, we studied the tristichopterid Eusthenopteron foordi (Whiteaves, 1881), which is known from a complete postlarval growth series (35–37). Both Eusthenopteron and Sauripterus are hypothesized to have been fully aquatic and to have used their pectoral fins primarily for swimming (20, 33, 37, 38) and perhaps, also to prop the animal up while resting on benthic substrate. Third, we studied the elpistostegid Tiktaalkoroseae (Daeschler, Shubin & Jenkins, 2006), the most crownward finned tetrapodomorph (39–41) known from at least 20 individuals that are estimated to range from 1.25 to 2.75 m in length (12, 42). Based on study of the endoskeleton and girdle, the pectoral fin of Tiktaalk is hypothesized to have been capable of holding the body up in an elevated posture on the substrate, and the animal is predicted to have occupied a benthic aquatic niche in littoral zones (12, 42, 43). The microcomputed

Significance

To explain how limbs evolved from fins, paleontologists have traditionally studied the endoskeleton. Here, we provide a comparative analysis of the other skeletal system of fins, the dermal skeleton. We describe dermal ray anatomy for 3 species of tetrapodomorph fishes. These data show that, prior to the origin of digits, dermal rays were simplified, the fin web became reduced in size, and the top and bottom of the fin became more asymmetric. These changes reveal how fins became adapted for interacting with the substrate prior to the fin-to-limb transition and that dorsoventral asymmetry is an important, understudied axis of diversification in paired fins.
tomography (μCT) data presented here show several trends in the evolution of dermal rays along the tetrapod stem including, unexpectedly, the evolution of dorsoventral asymmetry.

**Results**

**Pectoral Fin Rays of Sauripterus.** To visualize the pectoral fin rays of *S. taylori*, the specimen ANSP20581 was μCT scanned (*SI Appendix*, Table S1). The material includes the right pectoral fin of an adult individual (*SI Appendix*, Fig. S1). Dorsal hemitrichia are preserved partially embedded in matrix. Ventral hemitrichia were manually removed from the specimen during fossil preparation, and data on their distribution and morphology are unavailable. The fin was slightly deformed during preservation. Repositioning the fin to a more natural and articulated position requires only moderate translation of endoskeletal elements (*SI Appendix*, Fig. S2 and Movie S1). Hypotheses regarding the distribution of pectoral fin rays, discussed below, are not affected by this reconstruction.

At least 60 hemitrichia are present on the dorsal surface of the pectoral fin of *Sauripterus* (Fig. 1A). This is consistent with the ~65 rays observed in a cf. *Sauripterus* juvenile, ANSP20980 (Fig. 1B) (33), and indicates that nearly the full complement of dorsal hemitrichia is preserved. In ANSP20581, 20 rays are associated with the radius, 12 rays are distal to the intermedium, and at least 28 are distal to the ulnare (Fig. 1A). Dorsal rays extend proximally to fully cover the radius, cover the distal half of the intermedium, and fully cover distal radials 5 through 8. The fin rays are unsegmented and presumed to represent only the proximal portion of a large fin web, as is observed in juveniles (Fig. 1B) (20, 33). At their distal tips, rays can be segmented (33) and were likely branching, similar to other rhizodontids (29).

Rays vary in their morphology along the anteroposterior axis of the pectoral fin. In ANSP20581, along the anterior edge of the radius are 2 or 3 robust rays with geometry that is quite distinct from the more posterior rays. These anterior-most rays are dorsoventrally flattened and have broad bases, while the more posterior rays have tapering bases and are circular in their cross-section with a hollow core (20). Expanded anterior fin rays are also observed in the juvenile specimens (Fig. 1B) (33).

**Pectoral Fin Rays of Eusthenopteron.** To characterize dermal rays of the pectoral fin of *Eusthenopteron*, 2 specimens were μCT scanned (*SI Appendix*, Table S1). CMNH 8153 includes a pair of pectoral fins. Dorsal rays are differentially positioned and embedded in matrix (*SI Appendix*, Fig. S3). The orientation of these fins was determined by the curvature of the proximal articular surface of the radius (27). Neither fin was preserved with a humerus, and the dermal fin skeleton is disturbed for much of the left pectoral fin. CMNH 10926 comprises 2 pieces and includes an entire fish preserved in lateral aspect with the left side exposed. The anterior piece contains the skull, trunk, and pectoral fins (*SI Appendix*, Fig. S4). Portions of both left and right pectoral fins are preserved. The right fin, which is embedded in matrix, is more complete and missing the proximal and posterior portions. Consistent with previous descriptions, pectoral fin rays have a proximal unsegmented region, which constitutes a small proportion of total fin ray length, and a distal segmented region, which is branching in rays posterior to the radius (Fig. 2A and B) (27, 44).

The left pectoral fin of CMNH 8153 includes at least 30 rays (Fig. 2A and B). Although the fin web appears generally undisturbed, it is difficult to diagnose which endoskeletal element the rays might have been most closely associated with. This is because the endoskeleton is slightly repositioned as compared with other *Eusthenopteron* specimens (e.g., here the radius exhibits a greater angle with respect to the ulna) (3, 27), and rays are sometimes damaged and missing from one of the sides (Fig. 2A and B), making it challenging to diagnose the precise proximal extent of rays throughout the fin web. Additionally, the posterior flange of the ulnare is not preserved, and given that the pectoral fin of another specimen is described with ~40 rays (27), postaxial rays are likely missing. The left pectoral fin of CMNH 8153 includes 7 rays closely associated with the radius (Fig. 2C). The right pectoral fin of CMNH 10926 includes 5 rays associated with the intermedium and 4 with the third radial (Fig. 2D). These counts are consistent with another specimen showing 8 rays associated with the radius, 5 with the intermedium, 5 with the third radial, 7 with the third mesomere, and 10 with the posterior flange of the third radial (27).

In *Eusthenopteron*, dorsal and ventral hemitrichia differ slightly in their spatial distributions. On the right radius of CMNH 8153, dorsal hemitrichia extend further proximally than ventral hemitrichia (Fig. 2C), and on the intermedium and third radial of the right fin of CMNH 10526, dorsal hemitrichia extend further proximally than ventral hemitrichia (Fig. 2D). Concordant patterns between these 2 specimens indicate that in *Eusthenopteron* preaxial fin rays on the dorsal surface covered more of the endoskeleton than ventral rays.

**Fig. 1.** Dermal rays of the pectoral fin of *S. taylori*. (A) Right pectoral fin of ANSP20581 in dorsal perspective. Scanning reveals several robust and flattened rays on the leading edge of the radius. Posterior rays are thinner and round in cross-section. (B) Photograph of ANSP20980, juvenile of cf. *Sauripterus*, showing the full extent of the fin web. d1, first distal radial; d8, eighth distal radial; h, humerus; ir, intermedium; r, radius; u, ulna; ul, ulnare.
Pectoral Fin Rays of Tiktaalik. To analyze the anatomy of dermal rays of the pectoral fin of Tiktaalik, 3 specimens were μCT scanned (SI Appendix, Table S1). Pectoral fin materials are known from several Tiktaalik specimens; however, only 3 are preserved with dermal fin rays in close proximity with the endoskeleton. NUFV110 includes a nearly complete right pectoral fin. NUFV109 includes a mostly complete right fin with proximal elements removed so that they could be studied in isolation; the distal articulated portion was scanned. NUFV108 includes a disarticulated left pectoral fin.

In NUFV110, at least 33 lepidotrichia are preserved along the anterior and distal fin margin (Fig. 3 A and B). At least 10 rays are associated with the radius, 7 with the intermedium, 3 with each of the third and fourth radials, and 10 with the fourth mesomere. Postaxial radials and fin rays are not preserved in NUFV110. Several rays preserved on the ventral fin surface, adjacent to the ulnare, were not included in the reconstruction (Movie S2). We interpret these as having been displaced postmortem because of substantial separation between rays and the endoskeleton and because the ulnare was also preserved in a nonarticulated position. In NUFV109, 13 hemitrichia are preserved on the dorsal fin surface and 22 on the ventral fin surface (Fig. 3 C and D). These rays would have been associated with the fourth preaxial radial or fourth mesomere. It is challenging to demarcate precisely to which element they correspond due to slight anterior displacement of the rays during preservation. In NUFV108, 57 rays are preserved (SI Appendix, Fig. S5). Nine of these are dorsal to the radius, and the remainder is separated into 2 groups of 28 and 20 rays and not associated with particular endoskeletal elements.

The fin rays of Tiktaalik do not extend significantly beyond the distal terminus of the endoskeleton. Most rays are unbranching and unsegmented. In NUFV109, 1 cm

Fig. 2. Dermal rays of the pectoral fin of E. foordi. Left pectoral fin of CMNH 8153 in (A) dorsal and (B) ventral perspectives. (C) Radius of the right pectoral fin of CMNH 8153 in dorsal and ventral perspectives. (D) Distal and anterior portion of the right pectoral fin of CMNH 10926 in dorsal and ventral perspectives. In all images, dorsal hemitrichia are shown in yellow–orange, and ventral hemitrichia are cyan. ir, intermedium; m3, third mesomere; r, radius; r3, third radial; r4, fourth radial; u, ulna; ul, ulnare.

Dorsal and ventral hemitrichia differ in their distribution and morphology. At the distal portion of the fin, dorsal hemitrichia overlap the endoskeleton to a greater extent than do ventral hemitrichia. In NUFV110, dorsal hemitrichia cover from the distal-most region of the fin to the proximal side of the third mesomere (Fig. 3A), while ventral hemitrichia are restricted terminal to fourth mesomere (Fig. 3B). NUFV109 shows a similar pattern of preservation is observed in the pectoral fin of Howittichthys, a proposed elpistostegid (28).
pattern: dorsal hemitrichia cover the fifth mesomere, the anterior edge of fourth mesomere, and its anterior radial (Fig. 3C), while ventral hemitrichia are positioned distal to the fourth mesomere (Fig. 3D). Thus, we infer that the fifth mesomere was sandwiched between a pair of hemitrichia, while the third and fourth mesomers were covered only on the dorsal surface. Additionally, dorsal and ventral hemitrichia differ in their robustness, with dorsal hemitrichia being robust and ventral fin rays more gracile by comparison. This is most readily observed in the larger specimen, NUFV109 (Fig. 3 C and D, SI Appendix, Fig. S6, and Movie S3), and discussed in detail below.

Consistent patterns of asymmetry in endoskeletal coverage by hemitrichia between specimens in both Eusthenopteron and Tiktaalik suggest that the pattern is not the result of postmortem displacement of fin elements but rather, is a general feature of these fins. To test whether dorsoventral asymmetry in the coverage of fin rays relative to endoskeleton can reflect biological patterning and not simply taphonomy, we analyzed pectoral fin anatomy of...
Comparisons of Dorsal and Ventral Hemitrichia. As noted above, the dorsal and ventral hemitrichia of Tiktaalik specimen NUFV109 differ dramatically from one another in their size and geometry (Fig. 3 C and D, SI Appendix, Fig. S6, and Movie S3). To understand how this pattern evolved and might have developed, we quantified cross-sectional area (CSA) and second moment of area (SMA) of dorsal and ventral hemitrichia in Tiktaalik and Eusthenopteron. For each taxon, we compared rays in approximately the same position within the fin web between 2 individuals of different sizes. In Tiktaalik, the only region of the fin where such a comparison was possible is the fin tip. In Eusthenopteron, we compared preaxial lepidotrichia because they are the least disturbed and most complete between the 2 specimens. To estimate the relative sizes of Tiktaalik specimens, we compared humeral length, measuring from the entepicondyle to the middle of the proximal articular surface. In NUFV110, the humerus measures 5.07 cm, and in NUFV109, it measures 6.80 cm. Therefore, assuming that humeral length scales isometrically with standard length, we estimate that NUFV109 was 1.34 times the size of NUFV110. To estimate the relative sizes of the 2 Eusthenopteron specimens, we measured the approximate total length of CMNH 10926 (18.8 cm) and estimated the total length of CMNH 8153 by measuring the right radius (1.99 cm) and scaling this to a previous reconstruction (27). The estimated length of CMNH 8153 is 76.9 cm, near to the upper range of described specimens (35) and approximately 4 times the size of CMNH 10926.

For Tiktaalik, lepidotrichia on the preaxial side of the fourth mesomere were compared. In the smaller specimen, NUFV110, dorsal hemitrichia on average have a 1.93 times-larger CSA than the ventral rays (Fig. 5A). In the larger specimen, NUFV109, dorsal hemitrichia on average have a 2.67 times-larger CSA than ventral rays (Fig. 5B). For Eusthenopteron, lepidotrichia distal to the third radial were compared. Similar to Tiktaalik, dorsal hemitrichia have greater CSA as compared with corresponding ventral hemitrichia; however, the magnitude of asymmetry is less in Eusthenopteron and consistent between individuals of different sizes (Fig. 5 C and D). Dorsal rays are 1.10 and 1.09 times greater than ventral rays in CMNH 10926 and CMNH 8153, the smaller and larger specimens, respectively. All pairwise comparisons between the dorsal and ventral hemitrichia of a particular lepidotrichium show differences in CSA (Mann–Whitney U test, P value < 0.05) (SI Appendix, Table S2).

To assess whether dorsoventral asymmetry might be a general feature of the hemitrichia of paired fins in crown group osteichthians, we measured the CSAs of dorsal rays from the pectoral fins of Neoceratodus and 2 nonteleost actinopterygians, Polypterus (acipenseriformes) (Lucépède 1803) and Acipenser (leuciurus) (Lesueur 1818), the shortnose sturgeon (SI Appendix, Fig. S7). In Neoceratodus, 6 pairs of rays were sampled across the fin web. Fin ray morphology is heterogeneous along the anteroposterior axis: anteriorly, the rays are asymmetric, with dorsal campotrichia being 1.63 times larger than ventral campotrichia; more posteriorly (pairs 4, 5), the rays are not different from one another in CSA to a threshold of P > 0.05, and in the most posterior pair (6), the dorsal campotrichia are 0.84 times the size of the ventral campotrichia. Pairwise comparisons between dorsal and ventral rays of campotrichia pairs 1, 2, 3, and 6 show differences in CSA (Mann–Whitney U test, P value < 0.05) (SI Appendix, Table S2). For Polypterus and Acipenser, rays were sampled from the middle of the fin web. In Polypterus, dorsal hemitrichia on average have a CSA that is 0.83 times the size of the ventral rays. In Acipenser, dorsal hemitrichia on average have a CSA that is 1.05 times larger than the ventral rays. All rays of Polypterus and Acipenser were analyzed, and pairwise comparisons of hemitrichia of a lepidotrichium show differences in CSA (Mann–Whitney U test, P value < 0.05) (SI Appendix, Table S2).

Comparisons of dorsal and ventral hemitrichia were also conducted using the SMA, a descriptor of resistance to bending. We compared the bending for each hemitrichia with a neutral axis that corresponds with the dorsoventral axis of the fin (SI Appendix, Fig. S8). Broadly, these results are consistent with those presented above for CSA (SI Appendix, Fig. S8 and Table S2). Asymmetry in SMA is greater in Tiktaalik than Eusthenopteron. In Tiktaalik, asymmetry in SMA is greater in the larger specimen, whereas in Eusthenopteron, asymmetry in SMA is consistent between sizes. These results are robust to possible alternative orientations of the ventral hemitrichia in Tiktaalik. Furthermore, Polypterus and Acipenser also show asymmetry in SMA, suggesting that dorsoventral asymmetries in morphology and mechanics characterize the lepidotrichia of crown group osteichthians and that the sign and magnitude of such asymmetries differ between lineages.

Discussion

Tetrapodomorph pectoral fins vary in a number of fin ray properties, including branching, segmentation, length, and surprisingly, dorsoventral asymmetry. We analyze this variation in a phylogenetic context, taking into account what is known of the functional morphology of benthic actinopterygians and evolution of the pectoral fin endoskeleton. We argue that pectoral fins of crownward tetrapodomorphs were adapted to substrate-based
loading prior to the origin of digits, that the evolution of dorsoventral patterning is an important axis of morphological diversification in paired fins, and that analyses of fin function in Tetrapodomorpha should consider both dermal and endoskeletal systems.

Convergence with Benthic Actinopterygians. In tetrapodomorph pectoral fins, rays extend further proximally on the preaxial side than on the postaxial side. *Sauripterus* and *Eusthenopteron* have rays that contact the base of the radius on the fin’s leading edge and are terminal to the ulnare at the fin’s trailing edge. In *Tiktaalik*, rays show a similar pattern; they reach to the base of the radius anteriorly and become more distal posteriorly. Although postaxial rays are not preserved in close association with endoskeletal elements in *Tiktaalik*, these rays would likely have been positioned distal to the ulnare and associated with the radials posterior to the third and fourth mesomeres. The pattern of preaxial rays being more proximal is likely plesiomorphic to sarcopterygians (45, 46), although it can vary [e.g., an inverse pattern in *Neoceratodus* (Fig. 4) and a symmetrical pattern in *Glyptolepis* (46) and *Latimeria* (47)].

The pectoral fin rays of tetrapodomorphs are patterned along the anteroposterior axis. Uniformly, anterior rays are more robust, and species differ in the degree and spatial patterns of heterogeneity. For example, in *Sauripterus*, rays vary in thickness and in the geometry of hemitrichia proximally (Fig. 1A). In *Eusthenopteron*, the anterior rays are nonbranching and slightly thicker than more posterior rays (Fig. 2A and B). In *Tiktaalik*, the majority of the rays (anterior two-thirds) are unsegmented and nonbranching, and anterior rays are thicker than posterior rays (*SI Appendix*, Fig. S5). Among tetrapodomorphs, only the proposed elpistostegid *Howittichthys* (28) has been described similarly, with anterior rays of the pectoral fin that are unsegmented and unbranching (28).

Fig. 5. Comparisons of dorsal and ventral hemitrichia *Tiktaalik* and *Eusthenopteron*. CSA was calculated for 3 pairs of hemitrichia in the pectoral fins of *Tiktaalik* and *Eusthenopteron*. In all fins, the rays that were analyzed are numbered 1 through 3 from anterior to posterior. Volumetric renderings of the fin in *Left* indicate the position of the rays that were measured and also, the position of the cross-section (denoted by red lines) figured to *Right*. For both volumetric renderings and box plots, the color orange–yellow designates dorsal hemitrichia, and the color cyan designates ventral hemitrichia. *T. roseae* is shown in (A) NUFV110 and (B) NUFV109. *E. foordi* is shown in (C) CMNH 10926 and (D) CMNH 8153. The mean ratios of dorsal to ventral hemitrichial CSAs for the 3 lepidotrichia of each fin are reported as the variable “d/v.” Box plots show median value, the first and third quartiles, and maximum and minimum values; outlying points are denoted as gray dots.
The tetrapodomorph pectoral fin web was plesiomorphically of equal or greater size than the lobed region. This is observed in both Saaripeterus and Eusthenopteron (Figs. 1 and 2) and in many other species [e.g., Osteolepis (Osteolepididae) (48), Goolooongongia (Rhizodontida) (31), Cladaroysbлемma (Megalichthiidae) (31), and Panderichthys (Elpistostegida) (49)]. Only 2 tetrapodo
tomorph taxa deviate from this pattern: Tiktaalik and Howittichthys (28). In both cases, the fin web is substantially smaller than the lobed component of the fin. It is unlikely that the reduced lep
diotrichia length in these species is due to distal breakage. Tiktaalik
rays are of consistent length at the distal tip in specimens NUFIN010 and NUFIN110, and in both species, specimens are
preserved with a posterior distal fringe, where fragile and seg
mented components of the rays are preserved, although dis
articulated (SI Appendix, Fig. 55) (28).

Given the crownward phylogenetic position of Tiktaalik, we predict that dermal fin rays of pectoral fins become consolidated, reducing segmentation and branching, and that the length of the fin web was reduced prior to the fin-to-limb transition. Given the limited materials preserved of Howittichthys, it is difficult to
confidently assign its phylogenetic position (28) and to determine whether similarities with Tiktaalik in pectoral fin ray morphology reflect homoplasy or a plesiomorphic pattern among crownward elpistostegids.

The pectoral fin rays of Tiktaalik have converged on morphologies observed in benthic actinopterygians. Ray-finned fishes
have repeatedly evolved to occupy benthic niches (50). In these
phologies observed in benthic actinopterygians. Ray-finned fishes
have repeatedly evolved to occupy benthic niches (50). In these
lineages, the fin web often becomes regionalized, and lepidotrichia
that are in frequent contact with the substrate become un
branched, shorter, and thicker (50–55). These morphological
changes correlate with functional changes, specifically increased
flexural stiffness (i.e., resistance to bending) of fin rays (56). The
consolidated rays and reduced fin length in Tiktaalik are, there
therefore, consistent with previous predictions that its paired fins were adapted for interaction with the substrate (12) and suggest selec
tion for a stiffened fin web.

Asymmetry in Hemitrichia Coverage. The pectoral fins of sarcopterygians exhibit asymmetry in the extent to which dorsal and ventral rays cover the endoskeleton. This pattern is observed in Noecratodus, Eusthenopteron, and Tiktaalik. Species differ in the region of the fin that this asymmetry occurs and in the degree of this asymmetry. Asymmetries in the distribution of hemitrichia have implications for the distribution of fin ray musculature. In gnathostomes, pectoral fins plesiomorphically had dorsal and ventral muscles (i.e., fin ray extensors and flexors, respectively) that originate from the pectoral girdle and insert on the bases of the dermal rays (5). This is seen in Latimeria, for example, where muscles attach distally to an aponeurotic fascia that spans the proximal tips of the dermal rays (47, 57). Thus, dorsoventral asymmetry in the proximal extent of hemitrichia should cor
relate with the distal extent of fin ray extensors and flexors. In Eusthenopteron, pectoral fin flexors are predicted to have extended slightly more distally than extensors on the preaxial side. Similarly, in Tiktaalik, the ventral surface of the fin would have been signi
ficantly more muscular than the dorsal surface distally. Flexors would have covered the ventral surfaces of the third and fourth mesomers, analogous to a muscular “palm,” a pattern that had not been documented previously in tetrapodomorph fins.

Geological and anatomical evidence is strongly indicative that Tiktaalik was an animal interacting with the substrate in an aquatic context (12, 42, 43). However, changes in the distribution of paired fin musculature would have had implications for ter
restriality in future tetrapodomorphs. Dorsal extensor muscles, which were adapted for fin protraction and resisting hydrodynamic

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Methods

Quantifying Hemitrichial Asymmetry. For each specimen analyzed, we selected a set of 3 adjacent lepidotrichia that were straight and unbroken. Individual lepidotrichia were segmented using Amira and exported as 2-dimensional tiff stacks. These data were then imported to Fiji (version 2.0.0-c0-691.2Sn) (65) as “Raw Sequences” and converted to binary using the Huang method. Specimens were scaled by setting pixel size to the voxel dimensions from the CT scan. Using the package BoneJ (version 1.4.3) (66), we applied the “Moments of Inertia” function using default settings for all slices. This resulted in an image stack with orientation that corresponded to the long axis of the ray. Next, we applied the function “Slice Geometry” on the aligned data to recover CSAs and SMAs of slices orthogonal to the long axis of the ray. Finally, we trimmed the terminal few cross-sections, which are artificially small due to the fact that rays were first clipped precisely orthogonal to their long axis during extraction in Amira.

We compared the CSA and SMA of the dorsal and ventral hemitrichia of each lepidotrichium. A Shapiro test was used to assess whether the distributions of CSA and SMA were normal. Tests generally recovered $P$ values < 0.05, implying that the distribution of the data is significantly different from normal. Therefore, we next conducted Mann–Whitney $U$ test, which allows testing of whether population distributions are identical without assuming them to be normal. All statistical analyses were performed using R (version 3.3.1). Data were plotted using ggplot2 (version 3.1.0) (67).

When comparing SMAs, we analyzed SMAs around neutral axes that corresponded with the pectoral fin’s dorsoventral axis (SI Appendix, Fig. S8). For most hemitrichia, this inference is straightforward, with rays displaying distinct morphological polarity and reliable orientation within the fin web. However, the ventral hemitrichia of Tiktaalik pectoral fins do not show clear anatomical markers, and it is possible that they were displaced or rotated during preservation (e.g., Fig. 3D shows ventral rays slightly displaced and oblique to the dorsal rays). Therefore, to assess whether the results described are robust to alternative ventral ray orientations, we also considered alternative neutral axis of bending: the SMA around the minor axis. Results presented above are consistent between the different axes of bending: the magnitude of asymmetry in SMA is greater in Tiktaalik than other species, and it is greater in the larger Tiktaalik individual.

Data Availability. μCT data for Sauripterus (ANSP20581), Eusthenopteron (CMNH 10926, CMNH 8153), Tiktaalik (NUFV109, NUFV110), Neoceratodus (MCZ157440), Polypterus (FMNH1217440), and Acipenser (FMNH144017) are available on MorphoSource (Project P853) (68). Three-dimensional surface models of the endoskeletal and dermal skeletal elements of Tiktaalik (NUFV110) and Sauripterus (ANSP20581) pectoral fins are also available in the project. CSA and SMA values of each hemitrichia are available on the Dryad Digital Repository (https://doi.org/10.5061/dryad.zfg6s12kd) (69). The R code for analysis and plotting is available on GitHub (https://github.com/ThomasAStewart/tetrapodomorph_dermal_project) (70).

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