Tetrapod-like axial regionalization in an early ray-finned fish

Lauren Cole Sallan*

Department of Organismal Biology and Anatomy, University of Chicago, 1027 E, 57th Street, Chicago, IL 60637, USA

Tetrapods possess up to five morphologically distinct vertebral series: cervical, thoracic, lumbar, sacral and caudal. The evolution of axial regionalization has been linked to derived Hox expression patterns during development and the demands of weight-bearing and walking on land. These evolutionary and functional explanations are supported by an absence of similar traits in fishes, living and extinct. Here, I show that, Tarrasius problematicus, a marine ray-finned fish from the Mississippian (Early Carboniferous; 359–318 Ma) of Scotland, is the first non-tetrapod known to possess tetrapod-like axial regionalization. Tarrasius exhibits five vertebral regions, including a seven-vertebrae ‘cervical’ series and a reinforced ‘sacrum’ over the pelvic area. Most vertebras possess processes for intervertebral contact similar to tetrapod zygaphyses. The fully aquatic Tarrasius evolved these morphologies alongside other traits convergent with early tetrapods, including a naked trunk, and a single median continuous fin. Regional modifications in Tarrasius probably facilitated pelagic swimming, rather than a terrestrial lifestyle or walking gait, presenting an alternative scenario for the evolution of such traits in tetrapods. Axial regionalization in Tarrasius could indicate tetrapod-like Hox expression patterns, possibly representing the primitive state for jawed vertebrates. Alternately, it could signal a weaker relationship, or even a complete disconnect, between Hox expression domains and vertebrate axial plans.

Keywords: axial patterning; neck; evolutionary development; biomechanics; sacrum; terrestriality

1. INTRODUCTION

Tetrapod vertebral columns are usually divided into five morphologically distinct regions—cervical, thoracic, lumbar, sacral and caudal [1,2]. Regions are deterministically patterned by expression of specific Hox genes during the development of all examined tetrapod [2–8]. This strong molecular–morphological relationship has been used to generate developmental hypotheses of body evolution and infer Hox expression from regional identity in fossil and living forms [2,4,9–13]. Even the origin of the tetrapod body plan has been linked to clade-specific elongation of the trunk and related changes in the placement of nested Hox expression domains [9,13,14]. The process was capped by the appearance of a flexible, distinct neck (cervical region) and the evolution of a distinct sacrum in early tetrapods and their relatives [13–17]. Regional distinctions, many involving reinforcement of the axial column, arose in multiple tetrapod lineages from the Devonian (416–359 Ma) onwards and are widely considered to be an adaptation to terrestrial lifestyles [14]. As such, these traits have been used to date a Devonian and/or Mississippian (359–318 Ma) transition to land [14].

The described scenario of tetrapod axial evolution and regulation has been supported by evidence, or a lack-there-of, from non-tetrapod fishes. The axial Hox domains of model ray-finned fishes (actinopterygians), such as the zebrafish Danio, the stickleback Gasterosteus and the medaka Oryzias, are anteriorized and highly overlapped relative to tetrapods [18–21]. This pattern is hypothesized by many researchers to be primitive for vertebrates and preclusive of tetrapod-like regionalization, particularly when combined with the lack of terrestrial selective regime [2,9,16,19–22]. However, recent examination of Hox patterning in the shark Scyliorhinus has revealed a more tetrapod- than teleost-like distribution of domains, opening new possibilities for the jawed vertebrate ancestral state [22].

No extant or extinct non-tetrapod has been reported to exhibit tetrapod-like vertebral regions in terms of number and morphology. This is an unfortunate situation, as convergent cases are required to tease apart initial selective pressures and developmental prerequisites from drift and ‘phylogenetic constraint’. Some non-tetrapod groups have apomorphic regions: teleosts have modified the caudal column, while ostariophysans (e.g. zebrafish), chondrichthyans, placoderms and lungfishes have specialized the anteriormost column [1,9,19,23,24]. However, the molecular controls on these are unknown. Despite such differentiation, all non-tetrapodomorph vertebrates are traditionally designated as ‘caudal’ or ‘pre-caudal’ or ‘monospondylos’ and ‘dispondylos’ based on the presence of haemal arches in the tail [1,13,17–20,22,25]. These practices might have obscured convergence with tetrapod axial morphology.

Tarrasius problematicus [26] is a fossil ray-finned fish (Actinopterygii) found in the Mississippian marine sediments of Scotland, phylogenetically branching off either the actinopterygian or actinopteran stem [26–35] (see...
electronic supplementary material). Tarrasius has been reconstructed multiple times, always with largely homogeneous vertebral arches (neural, ventral, haemal) surrounding an exposed notochord, a morphological state described for most Paleozoic actinopterygian axial columns [26–33]. Re-examination of all catalogued specimens of Tarrasius in the United Kingdom, including a type specimen (National Museums of Scotland (NMS) 1885.54.13), two previously undescribed individuals (NMS 1891.53.34 and 1996.32.5), and several which formed the basis for recent reconstructions (Natural History Museum, London (NHM) P18062–3, P18061, P18060, P11666–7 and P4704) revealed radically different vertebral morphologies. Here, I show that Tarrasius is the first non-tetrapod fish known to exhibit tetrapod-like axial regionalization, in terms of both distribution and vertebral functional morphology. Tarrasius exhibits five distinct axial series, four with fused arches and centra, two with pleural ribs and all marked by abrupt changes in morphology as described below (figure 1).

2. MATERIAL AND METHODS

All specimens of Tarrasius in the collections of the NMS, NHM and British Geological Survey, Edinburgh (GSE) were examined (see electronic supplementary material). High-resolution photographs were taken using Nikon D70 and NikonD70s cameras with a Sigma DG Macro lens or a Leica DFC490 digital camera mounted on a Zeiss Stemi SV-6 microscope. A new reconstruction of Tarrasius (figure 1ii) is based primarily on specimens NMS 1996.32.5, type NMS 1885.54.13, NMS 1891.53.34, NHM P11667, NHM P18060, NMS 1885.54.24, NMS 1891.53.13, NHM P11665, NHM P18061, NHM P18062 and syntype GSE5674 (see electronic supplementary material).

Specimens of the fusiform early actinopterygian Phanerosteon were examined for comparison of axial columns within Carboniferous taxa from the same locality (figure 2), and because of a potential relationship with Tarrasius (see electronic supplementary material). Reconstruction of the axial column in Phanerosteon (figure 2d) is based on specimens NMS 1885.54.24, NMS 1891.53.13, NHM P11665, NHM P20205, NHM P20206 and NHM P22966 (see electronic supplementary material). Other Devonian and Carboniferous actinopterygians in the collections of the aforementioned museum, Carnegie Museum of Natural History (CMNH) and Field Museum of Natural History (FMNH) were surveyed (see electronic supplementary material), including Tarrasius’ presumptive, but now doubtful (see electronic supplementary material), sister taxa Paratarrasius and ‘Apholidotos’ from the Serpukhovian Mississippian of Bear Gulch, Montana [33]. All other early actinopterygians either lacked axial material, retained a limited number of vertebral elements, had axial columns obscured by scales, and/or had unrestricted notochords with arches similar to Phanerosteon (common in taxa with naked trunks) [29]. Other relevant fish and tetrapod axial columns were surveyed via the literature and available reconstructions.

3. SYSTEMATIC PALEONTOLOGY

Osteichthyes, Huxley 1880
Actinopterygii, Cope 1887
Tarrasiidae, Woodward 1891
Tarrasius problematicus, Traquair 1881

(a) Types

Syntype GSE5674 (figure 1f) is a semi-articulated fish in part, displayed in left lateral view with a near-complete dermal skull and articulated posterior tail with continuous median fin and scale cover. The trunk has been lost during diagenesis [26–29]. Syntype GSE5675 is an articulated posterior tail in part and in left lateral view, with scale cover and fins [26–29]. Type NMS 1885.54.13 (figure 1c, o–s, u–z, f) is a nearly complete fish in part and counterpart, with only the first vertebral series and skull area missing from the slab. Vertebrae are preserved in off-centre lateral view.

(b) Horizon and locality

Mississippian (Middle Visean), Glencartholm Volcanic Beds, Carboniferous Sandstone, Dumfriesshire, Scotland [26,27,29,30].

(c) Amended diagnosis of genus and species

Tarrasius is an actinopterygian fish on the basis of the following characters: presence of acrodin caps on the marginal teeth, anterior portion of dorsal fin supported by radials, caudal radials that are shorter than the ventral/haemal arch and spine together, a single median rostral and consolidated, notched nasals in the snout, single preoperculum in the cheek, a t-shaped dermosphenotic above the orbit, two infraorbitals below the orbit (lacrimal and jugal) a dermohyal on the dorsal portion of the hyomandibular, and an ornamented postcleithrum in the shoulder girdle dermal series, and the absence of a jugal canal [28,29,34,36–40] (see figure 1 and electronic supplementary material).

Tarrasius is distinguished from other Paleozoic actinopterygians on the basis of the following traits (and combination thereof): an axial column regionalized into five series with distinct morphologies, with fused centra and arches in the first four (formula: 7:9:7:6:20+), highly elongate tail (more than 50% of total body length), ossified anterior trunk ribs, three supraneurals per neural spine, confluent median fins, pectoral fins superficially paddle-shaped with fine, densely packed rays, pectoral radials robust and in close contact, pelvic fins and girdle absent, unbranched median fin rays, trunk lacking squamation except for diamond-shaped lateral line scales, micromeric scales on the tail, thickened ridge bordering jaw margins on maxilla and dentary, teeth with blunt, square crowns and central concavities on the maxilla and dentary, premaxilla with prenasal and postnasal projections, robust fangs on the premaxilla, and a nearly vertical suspensorium (see figure 1 and electronic supplementary material).

4. DESCRIPTION OF THE AXIAL COLUMN

Two articulated specimens of Tarrasius exhibit complete axial regions (NMS 1996.32.5, NHM P18062), while several others possess more than one articulated vertebral series (NHM P11667, NHM P18061, NHM 1891.53.34, type NMS 1885.54.13) (see boxed material in figure 1). All specimens agree in the placement of regions and general morphology of vertebrae within them. Observed differences in the latter are attributable to quality of preservation and plane of view (figure 1). The totality of the material indicates that, like tetrapods, Tarrasius possessed
Figure 1. *Tarrasius problematicus*. Photographs are of the best individual examples for each row, while drawings represent all material used in reconstruction at bottom (ii). Boxes encompass material from a single individual. Some material has been inverted so that anterior is left for all specimens, simplifying comparison. Unmarked scale bars represent 1 mm. Cranial and fin material: (a) NHM P18062 skull and pectoral fin photograph (inverted), (b) NHM 1996.32.5 skull showing endocranial material, (c) type NMS 1885.54.13 shoulder girdle and pectoral fin, (d) NHM P18062 skull and pectoral fin (inverted), (e) NHM P18060 impression of skull (inverted), (f) syntype GSE5674 skull, (g) NMS 1996.32.5 premaxillary tooth showing distinct acrodin caps, (h) NHM P18062 premaxillary tooth with acrodin cap. First vertebral series: (i) NMS 1995.32.5 photograph, (j) NMS 1995.32.5 drawing showing separated centrum halves, (k) NHM P18062 (inverted), (l) NHM P11667 (inverted). Second series: (m) NMS 1996.32.5 photograph, (n) NMS 1996.32.5, (o) type NMS 1885.54.13 preserved off centre, (p) NHM P18062 (inverted), (q) NHM P18061 preserved off centre, (r) NHM P11667 with separated centrum halves (inverted). Third series: (s) type NMS 1885.54.13 photograph, (t) NMS 1996.32.5, (u) type NMS 1885.54.13, (v) NHM P18062 (inverted), (w) NHM P18061. Fourth series: (x) NMS 1891.53.34 photograph with robust, three dimensional preservation, (y) NHM 1996.32.5, (z) type NMS 1885.54.13, of-centre (inverted from counterpart), (aa) NHM P18062 (inverted), (bb) NHM P18061, (cc) NMS 1891.53.34 drawing. Fifth series: (dd) NMS 1996.32.5 photograph with arches in medial view, (ee) NMS 1996.32.5 drawing, (ff) type NMS 1885.54.13 (inverted from counterpart), (gg) NHM P18060 (inverted), (hh) NHM P18060 (inverted). Abbreviations: ac, acrodin cap; an, angular; ap, articular process; ba, branchial arch; br, branchiostegal rays; c, centrum; cf, caudal fin; cl, cleithrum; cv, clavicle; cn, tooth crown; cr, caudal fin radials; df, dorsal fin; dh, dermohyal; dl, dorsal ligament; dn, dentary; dp, dermopterotic; dr, dorsal fin radial; ds, dermosphenotic; ep, epiphyseal processes; ef, epiphyseal fossa; fr, frontal; ha, haemal arch; hs, haemal spine; ju, jugal; la, lachrymal; ll, lateral line; lr, lateral ridge; mx, maxilla; mn, mandible; na, neural arch; nl, nasal; nn, neural arch restriction (‘neck’); no, notochord exposure; ns, neural spine; op, operculum; pa, parietal; pc, postcleithrum; pf, pectoral fin; pm, premaxilla; po, preoperculum; pr, pectoral radials; ps, parapneumoid; pt, posttemporal; rb, rib; ro, rostral; sc, supracleithrum; sn, supranasal; so, suboperculum; soc, supraorbital canal; sq, squamation; va, ventral arch; vr, ventral ridge.
five morphologically distinct axial regions, four of which originate in the trunk (figure 1).

The first axial region, observed in NMS 1996.32.5, NHM specimens P18062-3 and P11666-67 and GSE 5674 (figure 1i–ii), runs to the origin of the dorsal fin. It contains seven rectangular, superficially amphicoelus vertebrae. These are formed by fusion of perichondral centra, neural and ventral arches. The vertebrae bear inclined articular processes at each corner (two pointed ventral units and two rectangular dorsal units), which provides for extensive intervertebral contact (figure 1j). The posterodorsal articular process, formed by the neural arch, is relatively large and articulates with a nearly horizontal neural spine (figure 1i–j). Each neural spine sits above posterior members of the series. Short, paired ribs articulate with each centrum; rib fossae are located on the vertebrae beneath diagonal lateral ridges (figure 1j).

The second axial region (NMS 1996.32.5, NMS 1885.54.13, NHM P11666-7 and NHM P18062-3) starts at the origin of the dorsal fin and continues to the midline of the body cavity (figure 1m–r,ii). This series contains nine bracket-shaped vertebrae, taller than those of the first region. The vertebrae are formed by the fusion of elongate centra, robust, laterally expanded neural arches and petal-shaped ventral arches (figure 1m–r). The neural arch forms a posteriorly inclined articular process that contacts both its posterior neighbour and a needle-like neural spine (figure 1m–r). Each neural spine articulates with three supraneurals, each supporting a short, sigmoidal dorsal fin radial (figure 1m–o,ii). Curved, presumably paired ribs are found in NMS1995.32.5, which probably articulated with each centrum (figure 1m,n). Probable rib fossae lie ventral to a tapered lateral ridge as in the first series (figure 1m,n,p).

The third axial region (NMS 1885.54.13, NMS 1996.32.5, NHM P18061 and NHM P18062-3) begins at the midline of the body cavity (figure 1r–w). This region holds seven vertebrae formed by elongated centra, neural arches and hypertrophied tear-dropped ventral arches (figure 1r–w). Ventral arches bear paired, pointed articular processes (epiventrals) and fossae for the same. These start out horizontal and become more ventrally directed along the series (figure 1s,ii). Neural arches are posteriorly inclined and exhibiting a restriction or ‘neck’ at their fusion with the centrum (figure 1s–w). This gives the dorsal portion of each vertebra an hourglass shape (figure 1s,ii). As in other series, neural arch elements articulate with the neural spines (figure 1s–w).
Each neural spine articulates with three dorsal supraneurals (figure 1i-u). The expanded dorsal process of each supraneurals which articulates with an elongated, sigmoidal dorsal fin radial (figure 1ii).

The fourth axial region in *Tarrasius* (NMS 1891.53.34, NMS 1885.54.13, NMS 1996.32.5, NHM 18061 and NHM P18062-3) extends across the posterior limit of the body cavity to the tail base and covers the beginning of the haemal arch series (figure 1x–cc,i,ii). Posteriorly inclined, hypertrophic neural arches, centra and ventral arches fuse at the midline, giving six vertebrae a horizontal v-shape in lateral view (figure 1x–cc). Centra are expanded ventrally and exhibit a prominent lateral ridge (figure 1xi–cc). The neural arches are curved dorsally and join to the centra at a slightly restricted, diagonally oriented neck (figure 1x–cc). They expand distally to form a thickened ball-in-socket joint with robust, sword-shaped neural spines (figure 1x–cc). Each neural spine is inclined diagonally, resting on its posterior neighbour, and articulates with three supraneurals. Ventral arches are Y-shaped and bear a sigmoidal lateral ridge (figure 1x–cc). Haemal arches appear distinct from the ventral arches in this series, suggesting fusion of these separate ossifications in other actinopterygians [1] (figure 2). The first two haemal arches are short and cover the ventral surface of the vertebrae, while other haemal arches are thick, elongate and curve posteriorly, cupping the distal-most ventral arches to form ball-in-socket joints (figure 1x–cc). Haemal arches after the first articulate distally with thin haemal spines (figure 1x,aa–cc).

The fifth posterior-most axial region in *Tarrasius* is present in nearly all specimens examined, but the exact morphology obscured by scales in many of them (figure 1dd–hh,ii). It contains 20 or more sets of vertebral arches with little intervertebral or dorsal–ventral contact between them. Hemicircular, thin centra incompletely surround the notochord and are fused to small neural arches bearing needle-like neural spines (figure 1dd–hh). The overall morphology of the vertebral elements in this series is identical to that of the arches of caudal peduncle (figure 2c). Each neural spine appears to be fused to one supraneural extending to articulate with a short, sigmoidal dorsal fin radial at the body margin (figure 1i). Two further supraneurals lie between each vertebral set (figure 1ii). The neural arches in NMS 1996.32.5 bear the scars of the dorsal ligament, marking the dorsal extent of the notochord (figure 1dd,ee). Each centrum is paired with a gracile y-shaped element formed by fusion of the haemal and ventral arches (figure 1dd–gg,ii). Haemal spines are thin and long, extending to the body margin where they appear to support fin rays (figure 1ii). Caudal radials are intercalated between the haemal spines.

The vertebral column in *Tarrasius* (figure 1ii) is significantly inclined posteroventrally, an arrangement primarily based on the articulated specimen NMS 1996.32.5 (figure 1s,ij,m,nt,3,ii). The angles of various neural spines, supraneurals, and dorsal radials would require the tail region to be largely above the dorsal skull in an animal with a perfectly straight spine (figure ii). In fact, localized curvature and inclination of the axis is normal for many living and fossil vertebrates [1,14] (figure 2).

5. DISCUSSION (a) Identity and homology of vertebrate axial regions

As described above, *Tarrasius* possesses axial regions analogous in position and number with the cervical, thoracic, lumbar, sacral and caudal series of tetrapods (figure 2). Whether they are homologous or convergent in identity requires discussion of regional definitions and developmental patterning. Evidence from *Tarrasius* and a survey of osteichthyans suggests that the extent of the ‘caudal’ region is inconsistently defined between tetrapods and fishes (figure 2) [1,25,41]. The distribution of ribs, long associated with the trunk, is variable, homoplasic and limited by the body cavity rather than identity [1,3,18,25,41] (figure 2). In contrast, haemal arch placement, normally linked to the tail, appears fixed. They begin near the end of the body cavity and within the homologous sacral series in the digitized tetrapod *Acanthostega* and the tetrapodomorph fish *Eusthenopteron* [13,17] (figure 2a–c) and between the anterior expression limits of *Hox11* (tetrapod sacral marker) and *Hox12* (tetrapod caudal marker) paralogues in both the shark *Scyliorhinus* and the zebrafish *Danio* [18,22] (figure 2f). In zebrafish, haemals can even be rib-bearing, a conjunction of elements also observed in the sacral and adjacent ‘caudal’ vertebrae of *Acanthostega* [17,18] (figure 2f).

If the anterior limit of haemal arches in vertebrates is specifically related to the expression of tetrapod sacral *Hox* domains, and there is tight relationship between vertebrate regional identity and *Hox* expression, the position of haemal arches in *Tarrasius* (figures 1x–cc and 2e) suggests that its fourth (‘sacral’) region is homologous with the sacral region of tetrapods [4,5,11–14]. In tetrapods, this region involves the first vertebra articulating with the pelvis and several subsequent elements morphologically and/or positionally distinct from the caudal series, sometimes referred to as postscapral or urostylc in amphibians (and early tetrapods) (figure 2) [3,6,14,17].

A generally strong relationship between the regional identity and specific *Hox* expression domains in vertebrates would mean that the axial *Hox* domain pattern for *Tarrasius* matched that shared by tetrapods and sharks [2–8,22] (figure 2c), but not the anteriorized ‘primitive’ state of model actinopterygians (e.g. zebrafish) [18–21] (figure 2e). As noted above, *Tarrasius* sits somewhere on the stem of living actinopterygii, actinopteri and/or neopterygii, nested clades that contain teleosts [28–35]. Under this scenario of shared regional identity, a tetrapod distribution of *Hox* domains would be ancestral for gnathostomes and osteichthyans by the rules of parsimony [11]. In contrast, axial patterning in model teleosts (figure 2f) would be derived: the axis of *Danio* has far fewer trunk vertebrae than *Tarrasius* and many other bony fishes (figure 2g) [1,18,25]. Indeed, experimental insertion of zebrafish *Hoxa11* regulatory genes into mice reduces trunk vertebral counts by anteriorizing the sacrum [42].

Evidence from *Tarrasius* and *Scyliorhinus* suggests a distinct cervical region is not a tetrapod-specific trait evolved through axial elongation [2,9,13,15]. In zebrafish, both the *HoxC3* marker for tetrapod forelimb position and *Hoxa* marker for tetrapod cervical–thoracic transition are expressed behind the pectoral fin origin [1,16,18]. If an elongated, *Hox*-defined cervical region is ancestral for vertebrates, then it would be likely that
other superficially similar morphologies arose under different circumstances in *Tarrasius* and tetrapods, and cannot be attributed to a single selective regime, lineage, niche or habitat (terrestrial or aquatic). In either case, the limited number of appearances of tetrapod-like morphological regionalization of the vertebrate axial column indicates either very specific sets of selective pressures are needed or that requisite variation is generated only rarely among bony fishes.

### 6. CONCLUSIONS

Convergent axial regionalization in *Tarrasius* complicates the conditions surrounding the appearance of this trait in tetrapods and scenarios for the transition to land. For the moment, it appears that axial regionalization proved a dead end in the aquatic *Tarrasius*, while the same trait in tetrapods, like so much else, was likely exapted for terrestriality [13,14,17]. Other convergent cases would help refine the evolutionary pathway(s) and environmental circumstances leading to vertebral regionalization. No other non-tetrapod instances are documented, but this is likely to change with informed surveys of the osteichthyan fossil record. Finally, studies of axial *Hox* expression patterns for additional vertebrates, particularly non-teleost actinopterygians and lungfishes [9], are needed to resolve the strength of the relationship between specific *Hox* genes and axial morphologies. The association in tetrapods may be accidental rather than deterministic, one of many possible methods of generating similar body plans.

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