Structure and evolution of the horizontal septum in vertebrates

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Abstract

Although the horizontal septum (HS) has been identified as playing a role in fish biomechanics and in path finding of cells during zebrafish development, its morphology is poorly known. However, it is generally regarded as an evolutionarily conserved structure. To test this idea, we applied a novel combination of techniques to analyse the HS of 35 species from all major gnathostome clades in which is visualized its collagen fibre architecture. Results show that the HS is a conserved trait only with respect to the presence of caudolateral [= epicentral] and craniolateral [= posterior oblique] collagen fibre tracts, but differs remarkably with respect to the specifications of these tracts. Our data revealed several evolutionary changes within vertebrates. In the gnathostome ancestor, the two tracts are represented by evenly distributed epicentral fibres (ECFs) and posterior oblique fibres (POFs). ECFs are condensed to distinct epicentral tendons (ECTs) in the actinopteran ancestor. POFs independently evolved to distinct posterior oblique tendons (POTs) at least two times within teleosts. Within basal teleostomes, POFs as well as ECFs or ECTs were lost two times independently. POTs were lost at least three times independently within teleosts. This view of a homoplastic HS remains stable regardless of the competing phylogenies used for analysis. Our data make problematic any generalization of biomechanical models on fish swimming that include the HS. They indicate that the pathfinding role of the HS in zebrafish may be extended to gnathostome fishes, but not to agnathans, sarcopterygian fishes and tetrapods.

Introduction

The well-known ‘W’-shaped myosepta of fishes are divided into an epaxial and hypaxial part by a horizontal sheet of connective tissue, the horizontal septum (HS) which is generally regarded as standard part of their segmented musculature (e.g. Liem *et al.*, 2001). However, for the vast majority of vertebrates this structure has never been evaluated in an evolutionary context with the exception of two German notes (Harder, 1975; Hennig, 1983). Neither its structure nor its distribution among extant vertebrate clades has been surveyed. Indeed, in recent times the HS and associated axial structures have attracted interest in two fields of integrative biological research.

The first field is biomechanics. The collagen fibre architecture of the HS was described in specialized steady swimmers, tunas and mackerels (Westneat *et al.*, 1993; Westneat & Wainwright, 2001). It was hypothesized to play a role in swimming mechanics of these fishes because associated red muscles were thought to transfer their forces along tendon-like structures in the HS to the vertebral axis. Red muscles, however, are present in all vertebrates, including the most basal groups, the Myxini (hagfishes) and the Petromyzontiformes (lampreys, e.g. Bone, 1978; Videler, 1993; Vogel & Gemballa, 2000). Although the hypothesis of red muscles force transfer through the HS is widely cited in the literature on fish swimming, it remains unclear whether the condition described in scombrids (tunas and mackerels) is also true for other groups of vertebrates.

The second field is developmental genetics. Members of the hedgehog (Hh) family of signaling molecules are critical for patterning of many vertebrate tissues (Varga *et al.*, 2001). It was suggested that axial Hh signalling is required for proper formation of the HS in zebrafish

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(van Eeden *et al.*, 1996; Schauerte *et al.*, 1998; Karlstrom *et al.*, 1999).

Apart from these data on the HS in a few species, very little is known about this widespread vertebrate feature. Only Gemballa & Britz (1998) have reported on the architecture of the HS in some derived teleoteans, the acanthomorphs. The evolutionary history and architecture of the HS are largely unknown. Recent studies have shown that a single HS is absent in hagfishes and lampreys. Instead, these groups have multiple layers of horizontal lamellae that may functionally replace the HS (Vogel & Gemballa, 2000). Within gnathostomes, the HS was shown to be absent in sarcopterygians (Gemballa & Ebmeyer, 2003; Gemballa *et al.*, 2003).

The main objective of this paper is to trace the evolutionary transformations of the HS within vertebrates. In particular, we intend to evaluate whether the HS is a conserved structure with a certain biomechanical function or not. To achieve these aims we are in need of a data set on the taxonomic distribution and the collagen fibre structure of the HS that considers basal and derived representatives of all major vertebrate clades. By adopting techniques used for analysing the vertical myosepta (e.g. Gemballa & Britz, 1998) we are now able to reliably identify certain structural features [epicentral fibres (ECFs), epicentral tendons (ECTs), posterior oblique fibres (POFs) and posterior oblique tendons (POTs)] of the HS. For each of these features we analyse, whether they represent conserved, nonconserved or homoplastic features of the HS within vertebrates. The new information and perspective we present for the evolutionary history of the HS has an impact on the understanding of its biomechanical function.

Materials and methods

Morphological techniques

For preparation of specimens we followed the clearing and staining (c & s) procedure described by Dingerkus & Uhler (1977) and the modifications given in Gemballa & Britz (1998) and Gemballa *et al.* (2003). After clearing and staining whole specimens were preserved in ethanol until closer examination of the HS took place. We used small iris spring scissors to cut the epaxial and hypaxial myosepta along their insertion line on the HS. Having removed all myosepta we investigated the attachment of the HS to axial structures and finally excised the septum close to its line of insertion for closer investigation.

Excised horizontal septa were spread out on slides and investigated by DIC and polarized light microscopy. Owing to the birefringent property of collagen, polarized light was used to visualize the collagen fibre pathways as white strands within horizontal septa. Septa were photographed under a stereomicroscope with polarized light (Zeiss Polarizer S and analyzer A53 with stereomicroscope Stemi 2000C and camera Fujix DC HC-300Z; 1000 × 1500 pixel; ZEISS, Oberkochen, Germany) or under a polarized light microscope (Zeiss Axioplan with camera MC 100). Membranous ossifications within collagen fibre tracts were difficult to recognize under polarized light or in polarized light micrographs, but were easy to recognize in translucent light or incident light under a stereomicroscope due to the Alizarin Red staining of bone in the c & s specimens. They were not documented separately by photographs.

Species studied and systematic framework

We surveyed 35 species of the gnathostome clade. Among them were three species of chondrichthyans. One of them, *Chimaera monstrosa*, belongs to the holocephalan clade that is generally accepted as sister-group to all remaining chondrichthyans, the sharks and rays (Elasmobranchii). We also included representatives of the four basal groups of the actinopterygian radiation (Cladistia, Acipenseriformes, Ginglymodi and Halecomorphi). Among the derived actinopterygians, the Teleostei we also chose basal representatives belonging to the clades Elopomorpha, Osteoglossomorpha and Clupeomorpha (see Table 1).

In support of our results from the microdissections we additionally used histological sections of selected specimens. Blocks of the trunk were embedded in paraffin wax and sectioned either transversally or sagittally (10 or 20 μm thickness; Azan- Domagk staining; see Table 1).

The higher-level vertebrate phylogeny we use for our analysis is one that is widely accepted and used in current textbooks (Lauder & Liem, 1983; Nelson, 1994; Janvier, 1996; Helfman *et al.*, 1997; Liem *et al.*, 2001). It is mainly based on myological and osteological characters. However, the higher-level relationships in teleosts are still largely unresolved. We evaluated our results in the light of various phylogenies based either on morphological (Lauder & Liem, 1983; Rosen, 1985; Stiassny, 1986; Patterson & Rosen, 1989; Stiassny & Moore, 1992; Johnson & Patterson, 1993; Arratia, 1997; Johnson & Patterson, 1996), molecular (Lê *et al.*, 1993; Inoue *et al.*, 2001) or on total evidence (Wiley *et al.*, 1998, 2000).

Results

Our investigations revealed a conservative arrangement of two directions of collagen fibres in the HS of all gnathostomes investigated. The two directions form a cross fibre array of collagen fibres in the HS (Fig. 1). One direction, the caudolateral direction, is here referred to as epicentral fibres (ECFs), if it consists of evenly distributed fibres, or as epicentral tendons (ECTs), if the fibres are concentrated into distinct tendon-like structures. The ('vertical') myosepta attach to the horizontal septum (HS) along this direction (Fig. 1a). The other direction, the craniolateral direction, is here referred to as posterior oblique fibres (POFs), if it consists of evenly distributed

Table 1 List of investigated specimens. Total length [mm] of each specimen is listed.

Chondrichthyes	
Holocephali: *Chimaera monstrosa* (L., 1758)	423* (tr), 508, 523, 533
Elasmobranchii: *Galeus melastomus* (Rafinesque, 1810)	282, 315
Elasmobranchii: *Scyliorhinus canicula* (L., 1758)	306
Basal actinopterygii (nonteleosts)	
Cladistia: *Polypterus ornatipinnis* (Boulenger, 1902)	145, 229
Cladistia: *P. delhezi* (Boulenger, 1899)	179
Cladistia: *P. senegalus* (Cuvier, 1829)	175
Cladistia: *P. palmas* (Ayres, 1850)	94, 158, 186, 197
Chondrostei: *Acipenser ruthenus* (L., 1758)	86* (tr), 125* (tr, sag), 145
Chondrostei: *A. gueldenstaedti* (Brandt & Ratzeberg, 1833)	273, 288* (sag)
Ginglymodi: *Lepisosteus platyrhincus* (De Kay 1842)	122* (tr), 166, 173, 218
Halecomorphi: *Amia calva* (L., 1766)	422
Actinopterygii, basal Teleostei	
Osteoglossomorpha: *Arapaima gigas* (Cuvier, 1829)	214
Osteoglossomorpha: *Notopterus notopterus* (Pallas, 1769)	182, 191
Osteoglossomorpha: *Papyrocranus afer* (Günther, 1861)	181
Elopomorpha: *Megalops cyprinoides* (Broussonet, 1782)	185
Elopomorpha: *Conger conger* (L., 1758)	298
Ostariophysi: *Hoplias malabaricus* (Bloch, 1794)	151
Ostariophysi: *Hydrocynus forskålii* (Cuvier, 1819)	192
Ostariophysi: *Danio rerio* (Hamilton, 1822)	42, 45, 52
Clupeomorpha: *Engraulis encrasicholus* (L., 1758)	106
Actinopterygii, Teleostei, Euteleostei	
Protacanthopterygii: *Salmo trutta* (L., 1758)	130, 208
Protacanthopterygii: *Osmerus eperlanus* (L., 1758)	84
Scopelomorpha: *Lampanyctus crocodilus* (Risso, 1810)	116
Actinopterygii, Teleostei, Euteleostei, Acanthomorpha	
Polymixiomorpha: *Polymixia lowei* (Günther, 1859)	110 (AMNH 086102SW)
Paracanthopterygii: *Phycis blennoides* (Brünnich, 1768)	143, 151
Paracanthopterygii: *Molva dipterygia* (Pennant, 1784)	232
Beryciformes: *Beryx splendens* (Lowe, 1834)	323
Percomorpha: *Scomber scombrus* (L., 1758)	275, 385
Percomorpha: *Channa obscura* (Günther, 1861)	139, 143, 148, 157, 160* (sag)
Percomorpha: *Trachurus trachurus* (L., 1758)	216, 225
Percomorpha: *Lepomis gibbosus* (L., 1758)	138* (sag), 143, 149
Percomorpha: *Serranus hepatus* (L., 1766)	85
Percomorpha: *Scorpaena porcus* (L., 1758)	223
Percomorpha: *Blennius ocellaris* (L., 1758)	127
Percomorpha: *Trigla lyra* (L., 1758)	236

AMNH: American Museum of Natural History, New York, taxonomic nomenclature of taxa according to Helfman *et al.* (1997). *Part of the trunk of these specimens was studied histologically, tr = transverse sections, sag = sagittal sections).

fibres, or as posterior oblique tendons (POTs), if the fibres are concentrated to distinct tendon-like structures. A single HS is composed of a series of ECFs or a series of ECTs crossed by a series of POFs or a series POTs. In a single HS we found either ECFs crossed by POFs, or ECTs crossed by POFs, or ECTs crossed by POTs, but never POTs crossed by ECFs. ECTs may ossify to epicentral bones (ECB). If an epicentral series ossifies only partly, the posterior epicentral elements of the series will always remain tendinous (Fig. 1b). ECB can be combined with POFs or POTs in a single HS.

We demonstrate the features of the HS by the example of selected representatives of the major gnathostome clades. We separately describe the horizontal septa of (1) chondrichthyans, of (2) the basal actinopterygians *Polypterus*, *Lepisosteus*, *Acipenser* and *Amia*, of (3) basal teleosts, of (4) euteleosts, and of (5) acanthomorphs.

The HS of Chondrichthyes

In chondrichthyans the HS consists exclusively of evenly distributed ECFs that are crossed by evenly distributed POFs (Fig. 2a,b). Both fibre directions are interwoven and are sometimes difficult to differentiate. The septa are flexible in a rostrocaudal direction, but are resistant to strain in the direction of ECFs and POFs. Along the body axis we found the HS to be prominent in the anterior part of the body. It weakens posteriorly until it is absent in the posterior half. The absence of the HS was evident during microdissections and was further confirmed using standard histological techniques for *Chimaera*. The septum is attached to the vertebral centra in sharks and to the connective tissue sheet of the notochord in *Chimaera*.

The HS in basal actinopterygians

We found the most diverse architecture of the HS among basal actinopterygians. The basal polypterids are unique in having bones that are preformed as cartilage instead of ECFs or ECTs (Fig. 2c). These bones are referred to as dorsal ribs (e.g. Bartsch & Gemballa, 1992 ; Bartsch *et al.*, 1997) and they occur along the whole body axis from the first to the last but nine or ten vertebrae. They extend from the transverse process of a vertebra to the inner face of a bony ganoid scale (Gemballa & Bartsch, 2002). The dorsal ribs are crossed by evenly distributed POFs (Fig. 2c).

Bones are never present in the HS of the remaining basal actinopterygians (representatives of Chondrostei, Ginglymodi and Halecomorphi). In the chondrostean *Acipenser* (Fig. 2d) the caudolateral direction is represented by ECTs. They are crossed by posterior oblique fibres. Each vertebral segment bears one ECTs and, along each ECTs, a vertical epaxial myoseptum is attached on the upper face and a vertical hypaxial myoseptum is attached on the lower face. The condition in *Acipenser* strongly resembles that in the bowfin *Amia* (Fig. 2e). The

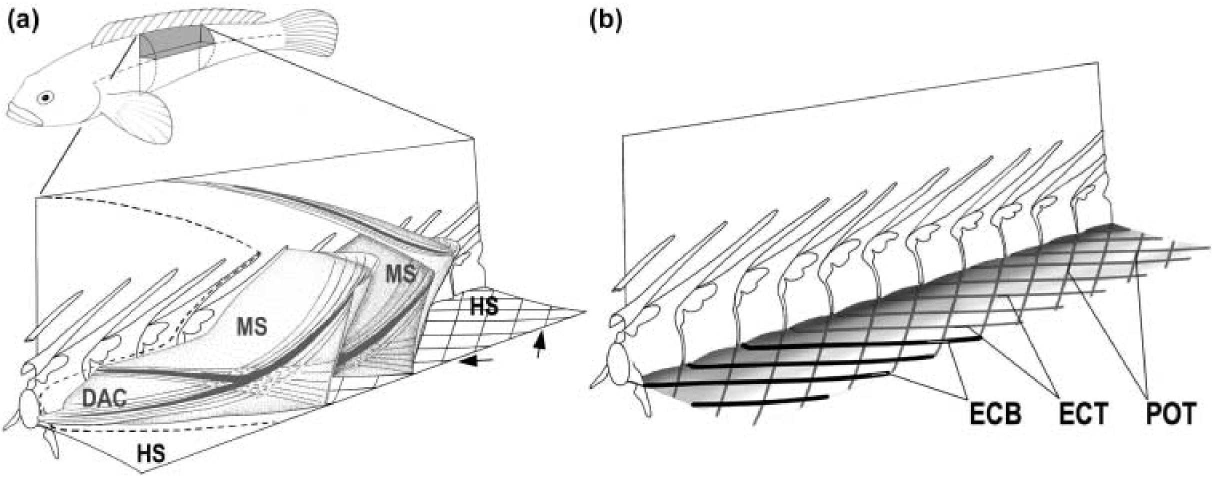

Fig. 1 Schematized representation of the mid trunk of a fish. Dorsal and oblique anterior view of left side. (a) Relationship between horizontal septum (HS) and myosepta (grey; only epaxial part shown). Myoseptum (MS) in front only partially shown, the one in the background represents a complete epaxial MS. Another MS between these two is omitted. Anteriorly, the MS is drawn out into a cone (DAC) that projects into the musculature. Dashed line represents line of attachment of MS to HS, vertebral column and median septum. Black lines in posterior part of HS represent the caudolateral and craniolateral directions of its collagen fibre tracts (arrows). Figure is redrawn from Gemballa *et al.* (2003). See there for descriptions of myoseptal fibre architecture. (b) Myosepta omitted from A. In the HS, a cross fibre array of collagen tracts is present. The caudolateral direction is represented by epicentral tendons (ECTs, grey) or their membranous ossifications (ECBs; black). The craniolateral direction is represented by posterior oblique tendons. Notice from A that a MS attaches to the HS along an ECB or ECT.

Ginglymodi (*Lepisosteus*) completely lack an HS. Neither the microdissections nor the histological sections provided any evidence for the existence of an HS in this group. We found one surprising similarity in the HS of *Acipenser* and the investigated chondrichthyans. Microdissections as well as histological sections revealed that in *Acipenser* the HS ended at an axial position of 65% of total length. This was not observed in any other of the fishes studied.

The HS of basal Teleostei

Osteoglossomorpha and Elopomorpha

In all osteoglossomorph and elopomorph genera considered in this study, the craniolateral direction in the HS was represented by POFs that never condensed to discrete tendons (Fig. 2f–h). We found differences in the caudolateral direction. A series of ECTs is present in the osteoglossomorphs *Arapaima* (Fig. 2f) and *Papyrocranus* and the elopomorph *Conger* (Fig. 2g). The series of ECTs is ossified in the osteoglossomorph *Notopterus* and the elopomorh *Megalops* (Fig. 2h).

Clupeomorpha and Ostariophysi

The series of ECTs was ossified in the clupeomorph *Engraulis*. The craniolateral direction was represented by very delicate and thin, almost unrecognizable posterior oblique fibers (POFs, Fig. 2i). Among ostariophysans we found a highly conservative condition that differs remarkably from that of *Engraulis*. In all three ostariophysan genera examined the craniolateral and caudolateral direction consisted of discrete ECTs or POTs (Fig. 2k,l). No collagenous fibres ocurred between these tendons.

The HS of basal Euteleostei (the 'protacanthopterygii') and Neoteleostei

The HS in the two 'protacanthopterygian' species *Salmo* and *Osmerus* (Fig. 3a,b) is rather uniform. Both species have distinct ECTs that are crossed by evenly distributed posterior oblique fibres. ECTs are also very distinct in the scopelomorph *Lampanyctus* (Fig. 3c). However, the condition of the craniolateral direction is difficult to classify. There are a small number of tendon-like elements. These are not as distinct as POTs in many other teleosts (compare Fig. 3c to Figs. 2k,l and 3f–h). Because their architecture resembles that of POFs, we consider them as those. However, they are not evenly distributed, in contrast to the usual situation in teleosts (compare Fig. 3c to Fig. 2f–h).

The HS of Acanthomorpha

Paracanthopterygii

The architecture of the HS is similar in the two investigated gadiforms *Phycis* and *Molva* (Fig. 3d,e). Both show ECTs that run in the myosepta marking the division into hypaxial and epaxial myosepta. Myoseptal sheets form acute cones closely dorsal and ventral to the ECTs. Hence, they are at acute angles to the ECTs and are impossible to remove without damaging the tendons. Therefore, the

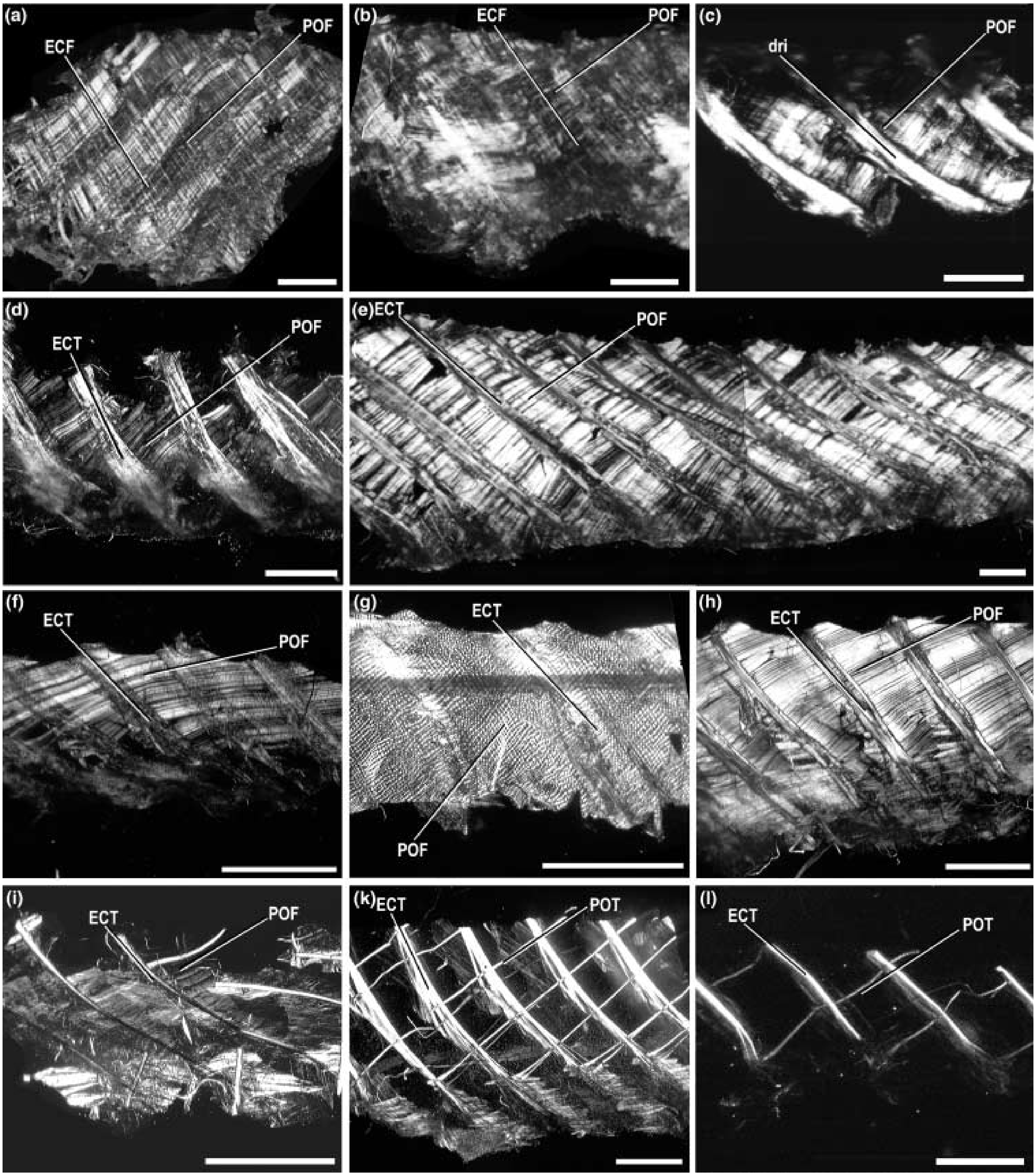

Fig. 2 Horizontal septa of selected chondrichthyans, basal actinopterygians and basal teleosts under polarized light. Left side of body, top view, anterior to left, top of micrographs represent medial attachment of septum to backbone. Collagen fibre tracts are visible as white strands. The caudolateral direction is represented by epicentral fibres (ECFs) or epicentral tendons (ECTs), the craniolateral direction by either POFs or posterior oblique tendon (POTs). Lines indicate fibre orientations. (a) *Chimaera monstrosa*. (b) *Scyliorhinus canicula*. (c) *Polypterus ornatipinnis*. Epicentral direction is substituted by dorsal ribs (dri). (d) *Acipenser gueldenstaedtii*. (e) *Amia calva*. (f) *Arapaima gigas*. (g) *Conger conger*. (h) *Megalops cyprinoides*. (i) *Engraulis encrasicholus*. (k) *Hoplias malabaricus*. (l) *Danio rerio*. Scale bar 2 mm (b-l) or 500 μm (M). Ossifications of ECTs occur in l and m but are camouflaged under polarized light by the white strands of ECTs.

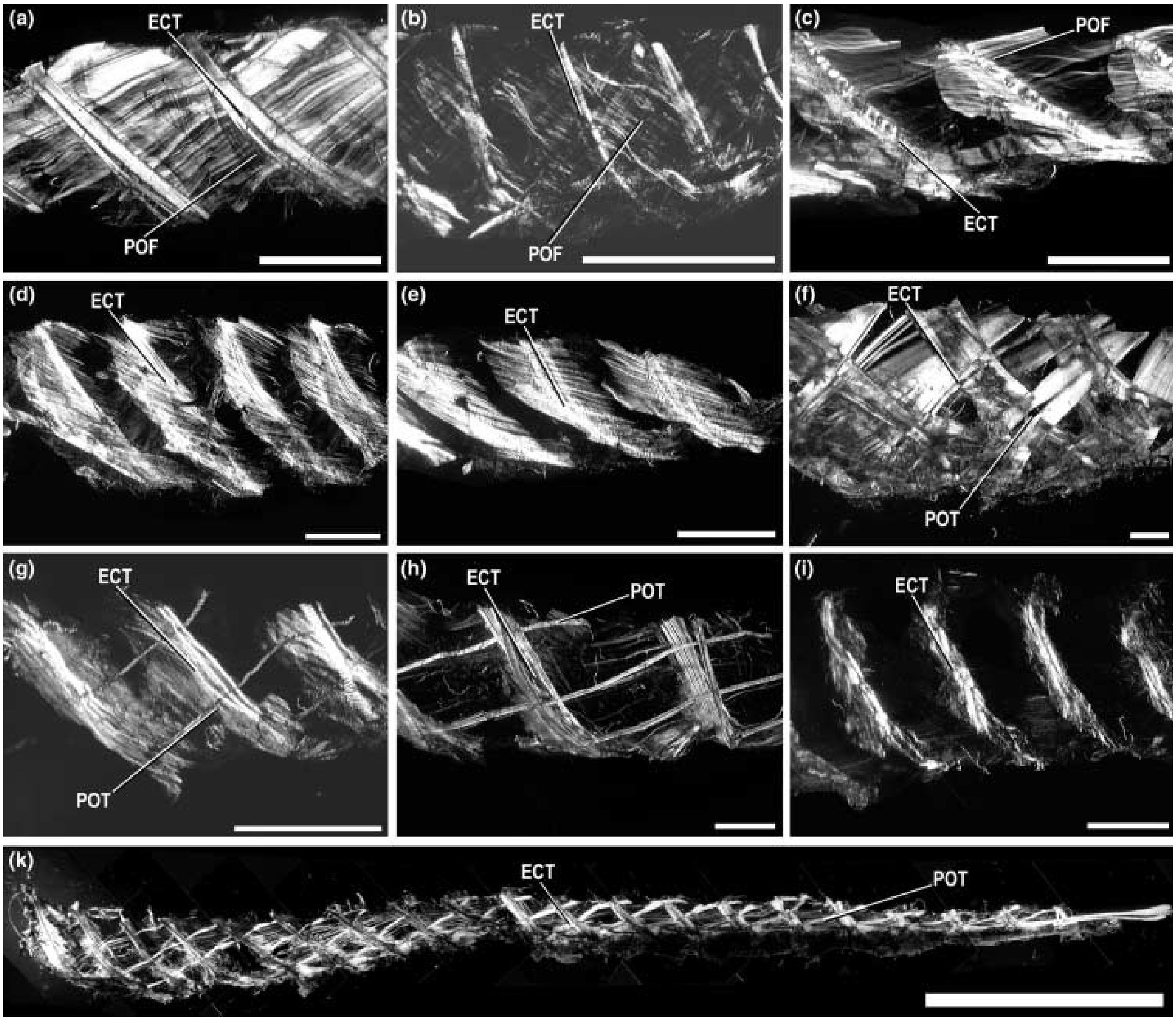

Fig. 3 Horizontal septa of selected euteleosts under polarized light. Left side of body, top view, anterior to left, top of micrographs represent medial attachment of septum to backbone. Collagen fibre tracts are visible as white strands. The caudolateral direction is represented by epicentral tendons (ECTs), the craniolateral direction by either posterior oblique fibres (POFs) or posterior oblique tendon (POTs) or absent. (a) *Salmo trutta*. (b) *Osmerus eperlanus*. (c) *Lampanyctus crocodilus*. (d) *Molva dipterygia*. POTs/POFs absent. (e) *Phycis blennoides*. POTs/POFs absent. (f) *Beryx splendens*. (g) *Serranus hepatus*. (h) *Trachurus mediterraneus*. (i) *Blennius ocellaris*. POTs/POFs absent. (k) *Scomber scombrus.* Scale bar 2 mm (a.-i) or 3 cm (k). Ossifications of ECTs occur in h-k, but are camouflaged under polarized light by the white strands of ECTs.

micrographs show some myoseptal fibres along the ECTs (Fig. 3d,e). However, between subsequent epicentrals there is always a region that is devoid of collagen fibres. The craniolateral direction of the POFs is clearly absent. After excision, subsequent epicentrals only keep their position with respect to each other because of the presence of endomysial fibres that fill the space between muscle fibres and are not digested during the clearing procedure. The only difference in the epicentral series of *Phycis* and *Molva* is the occurrence of membranous ossifications in the anterior part of the epicentral series in *Molva*, whereas epicentrals remain tendinous in *Phycis*.

Acanthopterygii

Among acanthopterygians, the situation found in paracanthopterygians was closely matched only in *Blennius* (Fig. 3i). In this genus we recorded a series of distinct ECTs, but no POFs or tendons. In the remaining eight acanthopterygian genera we found a series of ECTs that was crossed by distinct POTs (Fig. 3d–k). The latter appear to be different in terms of their robustness. The POTs of *Beryx* (Fig. 3f) are broader than the ECTs and tend to fan out distally, whereas those of *Scorpaena* and *Serranus* (Fig. 3g) are thin compared to the ECTs. *Trigla*, *Channa*, *Lepomis*, *Trachurus* (Fig. 2h), *Scomber* (Fig. 3k) have POTs of intermediate thickness compared to *Beryx* or *Serranus*.

Discussion

Biomechanics of the HS: does a unifying feature exist?

In a biomechanical model developed by Westneat *et al.* (1993) for scombrid fishes, the POTs were identified as tendons that transfer muscular forces and induce bending whereas the ECTs were thought to resist radial expansion of contracting muscle fibres and contribute to stiffening of the body. One of the concluding remarks (derived from preliminary comparative studies) from Westneat *et al.* (1993, p.203) was that this way of force transfer and body stiffening might be a unifying evolutionary feature enabling aquatic undulatory locomotion. One of their suggestions was to survey the design of the HS in order to integrate phylogeny and physiology.

In a recent study, Gemballa & Vogel (2002) have shown that white muscles attach to vertical myosepta, but do not attach to the HS. Red muscles are situated laterally around the HS. Thus, only these muscular fibres are able to transmit their forces via the POTs of the HS to the backbone.

In this study we present evidence that there is no unifying mechanism for the transfer of red muscular forces to the backbonbe by the POTs or the HS. Some genera lack POTs and have POFs instead (all chondrichthyans, basal actinopterygians except *Lepisosteus*, osteoglossomorphs, elopomorphs, *Salmo*). In these cases, red muscular forces can be transferred by POFs. This is impossible in those genera (*Lepisosteus*, *Phycis*, *Molva*, *Blennius*) that are shown to lack both POTs or POFs, and improbable in those that have fibres of very low density or robustness in the POT-direction (*Osmerus*, *Engraulis*, *Lampanyctus*).

Our conclusion from these findings is that pathways of red muscular forces should be reevaluated in fishes. That POTs and ECTs firmly interconnect backbone and dermis has not been considered in biomechanical models to date. The dermis consists of helically arranged fibres in vertebrates (Gemballa & Bartsch, 2002) that are put into tension during swimming (Wainwright *et al.*, 1978). Mechanically, dermis fibres and HS fibres form a continuum along which tension will be transferred to the backbone. From our own observations (mostly during the data acquisition for Gemballa & Vogel (2002), some during this study) on a wide variety of fishes it became clear that most of the red muscle fibres are attached to the myosepta instead of the POTs. Their forces might be transmitted to the skin via the myosepta. Westneat *et al.* (1993) revealed a specialized morphology (the AOT-ITL-POT loop) in scombrids that has never been found in any species outside this group. Thus, the functional implications of this study cannot be applied to taxa other than scombrids.

The HS: evolutionary novelties and patterns of homoplasy

In this study we have shown that the HS in principle consists of a cross-fibre array of collagen fibres or tendons. The caudolateral direction is represented as ECFs or ECTs (sometimes ossified as epicentral bones), the craniolateral direction as POFs or POTs. These results clearly show that any evolutionary treatment of the HS will have to consider five features instead of only one ('ECFs', 'ECTs', 'POFs', 'POTs', 'ossifications of epicentrals'). We discuss these findings in the light of phylogenetic hypotheses of vertebrate relationships (Fig. 4).

ECFs and ECTs

Evenly distributed ECFs are present in chondrichthyans, basal actinopterygians (excl. *Lepisosteus*), basal teleosts (osteoglossomorphs, elopomorphs, and clupeomorphs), the 'protacanthopterygians' *Salmo* and *Osmerus*, and *Lampanyctus*. From this character distribution we conclude that ECFs belong to the ground pattern of the last common ancestor of gnathostomes (Fig. 4a). The lack of ECFs in sarcopterygians (Gemballa & Ebmeyer, 2003) and in *Lepisosteus* (this study) has to be interpreted as secondary losses of this structure.

In all actinopterygians studied, ECFs are condensed to ECTs, except in the basal polypterids in which the epicentral direction is represented by dorsal ribs. We conclude that ECTs evolved from ECFs in the last common ancestor of actinopterans (actinopterygians minus polypterids).

ECTs ossify in a variety of teleosts. Patterson & Johnson (1995) give an extensive record of ossification patterns in teleosts and a literature review. From their overview it becomes clear that membranous ossifications of ECTs must have evolved several times independently within teleosts. The distribution of ossifications among teleosteans we recorded here (*Engraulis*, *Megalops*, *Notopterus*, *Molva*, *Blennius*) confirms this view.

POFs and POTs

Posterior oblique tendons are present in ostariophysans and in all investigated acanthomorphs except *Molva*, *Phycis*, and *Blennius*. Additional descriptions (Patterson & Johnson, 1995; Gemballa & Britz, 1998) report the presence of POTs among acanthomorphs and their absence in several nonacanthomorphs. However, the technique used by Patterson & Johnson (1995), which was different from that applied here and in Gemballa & Britz (1998), was not adequate to reliably identify intermuscular tendons (see Gemballa & Britz, 1998 for evaluation of techniques). Because it is probable that they have overlooked POTs, we do not integrate most of their data into our analysis of the evolution of the POTs

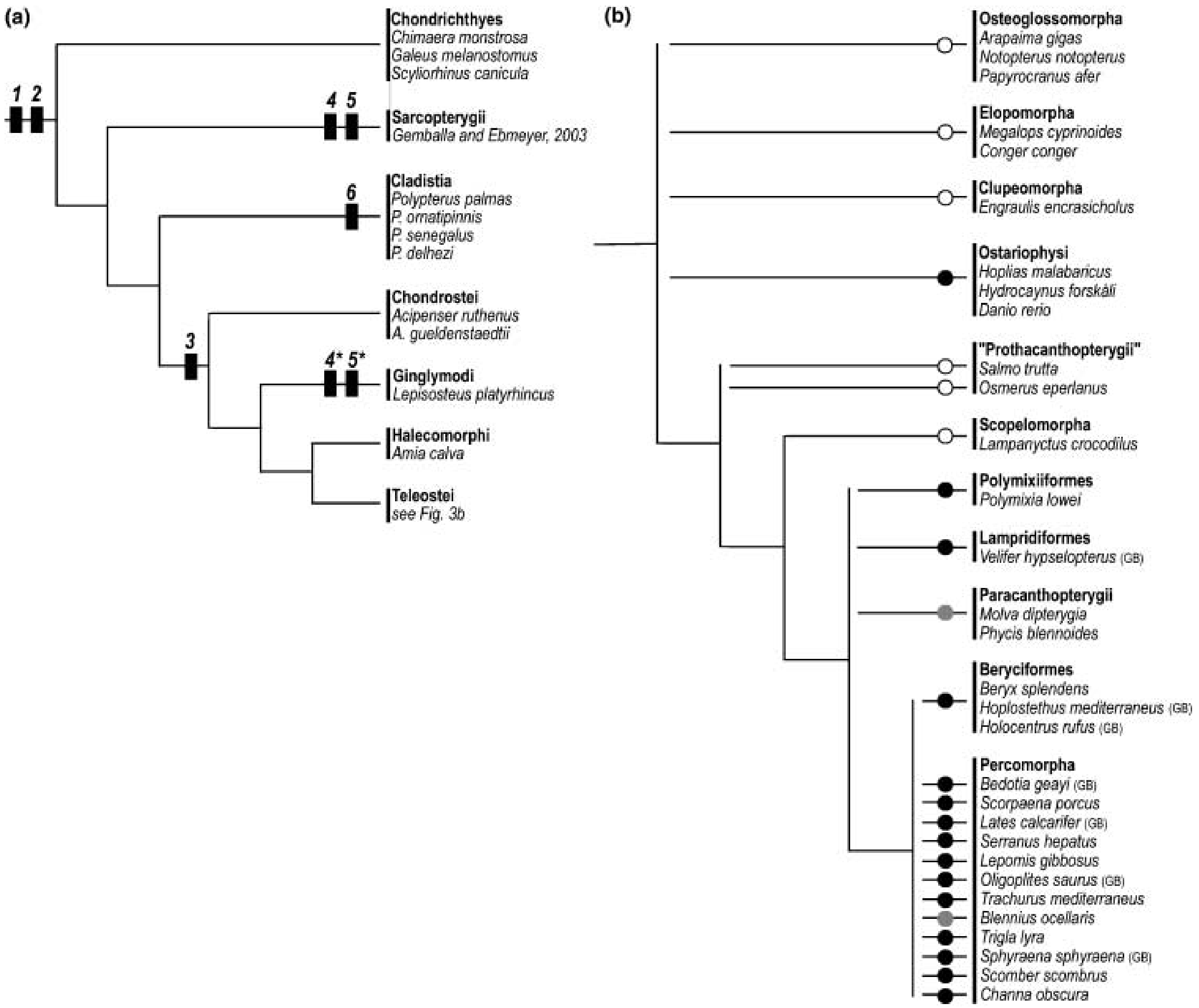

Fig. 4 Summary of the conclusions derived from comparative investigations of the horizontal septum (HS). Features are mapped on a phylogeny derived from various publications (see Materials and methods). We left dichotomies unresolved where competing phylogenetic hypotheses exist. Left cladogram: Transformations of HS during early gnathostome evolution. Boxes indicate synapomorphies. 1: evenly distributed epicentral fibres (ECFs), 2: evenly distributed posterior oblique fibres, 3: epicentral tendons evolve from ECFs, 4 and 4*: convergent loss of ECFs/ECTs, 5 and 5*: convergent loss of posterior oblique fibres, 6: no ECFs; dorsal ribs with cartilaginous precursor occupy epicentral direction. Right cladogram: Distribution of posterior oblique fibres (plesiomorphic within teleosts; open circles) and posterior oblique tendons (derived within teleosts; full black circles). Posterior oblique fibres/tendons are secondarily absent in three of the 30 listed teleost genera (grey circles). (GB) indicates data reported by Gemballa & Britz, 1998. See text for discussion.

within teleosts. Nevertheless, it becomes clear from the distribution of POFs and POTs in the teleosts studied by our technique (see Fig. 4b of this study and data from Gemballa & Britz, 1998), that this part of the HS is homoplastic instead of conservative: POTs evolved at least twice from POFs, namely in the ostariophysans and in acanthomorphs. The plesiomorphic POF condition was retained in the remaining nonacanthomorph teleosts. A secondary loss of POTs or POFs can also be deduced from the present data, namely within paracanthopterygians and *Blennius*. Our data also indicate that extensive studies of the horizontal septa in certain taxa may hold potential for the generation of new characters for phylogenetic analyses (e.g. Do all paracanthopterygians lack POTs? Do all ostariophysans possess POTs?). Also ctenosquamate and stenopterygian representatives have yet to be studied.

Horizontal septa are absent outside the gnathostomes (Vogel & Gemballa, 2000). However, myxinids and petromyzontids have multiple horizontal lamellae. Like the horizontal septa in gnathostomes, these have caudolateral and craniolateral fibre directions and are closely associated with red muscles (Vogel & Gemballa, 2000). Despite these similarities, it remains unclear whether the horizontal lamellae and horizontal septa evolved independently or are full or at least in part homologs. As long as this homology is in question, we consider the ECF and POF-fibre direction in a single HS as a gnathostome synapomorphy (Fig. 4a). Further arguments for a homology of lamellae and septum may come from developmental genetics if specific genes or developmental pathways can be identified that are directly involved in the formation of the HS. A homology of lamellae and HS is likely, if such genes exist in teleosts (e.g. zebrafish) and myxininds and petromyzontids, but are absent in outgroups.

Concluding remarks

The data on the structure of the HS of 35 species representing all major gnathostome taxa presented here have implications in three fields, biomechanics, phylogenetics, and developmental genetics. We demonstrate that the HS underwent considerable changes during gnathostome evolution. Evenly distributed fibres condensed to distinct tendons or were reduced independently several times. One consequence of this is that the hypothesized unifying system of a force-transmitting system including red muscles and POTs of the horizontal system does not exist in fishes. Future biomechanical models have to reevaluate the pathways of red muscular forces during steady swimming in fishes. Anatomical findings suggest that vertical myosepta instead of horizontal septa may be involved. In the zebrafish, *Danio rerio*, the HS plays an important role in path finding of cells and tissues during development. It is still questionable and remains to be tested experimentally whether this path finding developmental role of the HS in zebrafishes can be extended to vertebrates in general. We are not able to solve this, but at least, our data allow to comment on this question from the standpoint of evolutionary morphology. This role might be fulfilled by the HS in the vast majority of gnathostome fishes (excluding the few gnathostome fishes that reduced the HS). However, the path finding developmental role of the HS may not be extended to vertebrates in general, since the septum is absent in agnathans, sarcopterygian fishes, and terrestrial vertebrates (Tetrapoda).

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