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The Locomotory System of Pearlfish *Carapus acus*: What Morphological Features are Characteristic for Highly Flexible Fishes?

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ABSTRACT The body curvature displayed by fishes differs remarkably between species. Some nonmuscular features (e.g., number of vertebrae) are known to influence axial flexibility, but we have poor knowledge of the influence of the musculotendinous system (myosepta and muscles). Whereas this system has been described in stiff-bodied fishes, we have little data on flexible fishes. In this study, we present new data on the musculotendinous system of a highly flexible fish and compare them to existing data on rigid fishes. We use microdissections with polarized light microscopy to study the three-dimensional anatomy of myoseptal tendons, histology and immunohistology to study the insertion of muscle fiber types into tendons, and μ-CT scans to study skeletal anatomy. Results are compared with published data from stiff-bodied fishes. We identify four important morphological differences between stiff-bodied fishes and *Carapus acus*: (1) *Carapus* bears short tendons in the horizontal septum, whereas rigid fishes have elongated tendons. (2) *Carapus* bears short lateral tendons in its myosepta, whereas stiff-bodied fishes bear elongated tendons. Because of its short myoseptal tendons, *Carapus* retains high axial flexibility. In contrast, elongated tendons restrict axial flexibility in rigid fishes but are able to transmit anteriorly generated muscle forces through long tendons down to the tail. (3) *Carapus* bears distinct epineural and epipleural tendons in its myosepta, whereas these tendons are weak or absent in rigid fishes. As these tendons firmly connect vertebral axis and skin in *Carapus*, we consider them to constrain lateral displacement of the vertebral axis during extreme body flexures. (4) Ossifications of myoseptal tendons are only present in *C. acus* and other more flexible fishes but are absent in rigid fishes. The functional reasons for this remain unexplained. J. Morphol. 273:519–529, 2012. © 2011 Wiley Periodicals, Inc.

KEY WORDS: myoseptal tendons; body flexibility; fish locomotion; *Carapus acus*

INTRODUCTION

The body curvature displayed by fishes is extremely diverse among species. On one hand, fast swimming open ocean predators like tunas or lamnid sharks are known for their relatively stiff bodies that display only slight body curvatures. On the other hand, some species like eels show extreme body curvatures during routine turns (review in: Domenici, 2003). One reason for this difference in body curvatures could be a simple correlation between axial flexibility and number of vertebrae (Lindsey, 1978; Long and Nipper, 1996; Brainerd and Patek, 1998). However, exceptions to this simple correlation have been found in sharks (Kajiura et al., 2003). Furthermore, Porter et al. (2009) identified a set of 11 nonmuscular features that correspond to the observed body curvatures during routing turns in five species of sharks. Thus, the correlation between axial morphology and axial flexibility seems to be more complex than previously thought.

Besides nonmuscular features that influence body flexibility, the musculotendinous system might be another complex that significantly influences axial flexibility. Although this idea has never been explored, body curvature is not a result of nonmuscular body mechanics but is under active control of the musculotendinous system of the body, i.e., actively produced by trunk muscles.

In fishes, these muscles are organized in segmented blocks of specific three-dimensional (3D) folding, the myomeres. Adjacent myomeres are separated by thin sheets of connective tissue, the myosepta. Muscles and myosepta form the musculotendinous system of the trunk. Within this system, muscle fibers insert into myosepta, and myosepta are connected to the fibrous dermis and

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the vertebral column (Gemballa and Röder, 2004; Danos et al., 2008). Some features of the musculotendinous system of the trunk have been identified to be highly conservative among gnathostome fishes. For example, the 3D-folding of myosepta is similar in all investigated species. Furthermore, myosepta bear tendinous tracks of collagen fibers oriented in a specific way and referred to as myoseptal tendons. As this condition occurs in many widely separated gnathostome taxa (from sharks to derived teleostean groups), it represents the gnathostome groundplan condition (Gemballa et al., 2003a).

Recent studies revealed some musculotendinous features in rigid fishes (tunas, lamnid sharks) that clearly differ from the groundplan condition (Donley et al., 2004; Gemballa et al., 2006; Shadwick and Gemballa, 2006). Most striking features in these species are elongated myoseptal tendons (lateral tendons) that reach up to 20% of body length and might hamper body flexibility and the lack of another set of tendons (epineural tendon [ENT] and epipleural tendon [EPT]) that is usually present in fishes. In contrast to rigid fishes, our knowledge of the musculotendinous system of extremely elongate and flexible species is scarce. So far, only *Anguilla rostrata* has been investigated in sufficient detail (Danos et al., 2008). Its system of myoseptal tendons differs in several respects from that of more rigid fishes. Myomeres are numerous and the lateral tendons, being elongated in rigid fishes, are short. Moreover, the ENT and EPT that are absent in rigid fishes are very pronounced.

The aim of this study is to examine the musculotendinous system of another highly flexible but non-eel species and to test whether the contrasting morphology between rigid fishes and flexible fishes holds true. For this purpose, we considered pearlfish, *Carapus acus* (family Carapidae, tribe Carapini) to be an excellent candidate. Species of the Carapini (Carapidae, Ophidiiformes) live in different invertebrate hosts such as sea stars, sea cucumbers, or bivalves (Markle and Olney, 1990; Parmentier et al., 2000a). The adults of *C. acus* (Emery, 1880) are inquilinists, live inside the respiratory tree or body cavity of sea cucumbers and use them as a shelter (Trott, 1970; Shen and Yeh, 1987; Van Den Spiegel and Jangoux, 1989; Kloss and Pfeiffer, 2000; Parmentier and Vandewalle, 2005). The behavior of penetration has been closely observed (Fig. 1; Arnold, 1953, 1956; Gustato et al., 1979; Kloss and Pfeiffer, 2000; Parmentier and Vandewalle, 2003, 2005). Having detected a sea cucumber, the fish swims, almost vertically, head facing downwards alongside the integument until the cloaca of the sea cucumber is found. Two strategies are observed for entering (Arnold, 1953; Gustato et al., 1979; Kloss and Pfeiffer, 2000; Parmentier and Vandewalle, 2005): (1) head first by propelling itself with violent strokes of the tail; (2) tail first by placing the head at the cloaca of the sea cucumber (Fig. 1A) and moving the thin tail forward alongside its own body at the level of the lateral line until his caudal extremity is inside the holothurian (Fig. 1B–E). After this initial penetration, the carapid redresses itself (Fig. 1F–H) and enters the holothurian with backward movements (Kloss and Pfeiffer, 2000; Parmentier and Vandewalle, 2003, 2005).

Obviously, during penetration the fish displays extreme body curvatures (e.g., Fig. 1B–E). Given the data from previous studies listed above, we predict that *C. acus* will bear short myosepta with short lateral tendons and distinct ENT and EPT. We expect other features of the musculotendinous system in *C. acus*, for example, connections of myoseptal tendons and red muscles and attachment of myosepta to the vertebral column, being clearly different from those in rigid fishes of the open ocean. Thus, we consider this study to be a step towards a characterization of the locomotor morphology of extremely flexible fishes that will stay in contrast to the well-described morphology of rigid fishes (e.g., Shadwick and Gemballa, 2006).

MATERIALS AND METHODS

Material Examined

We studied two species of *C. acus* (pers. collection C. Schwarz). One specimen (159 mm total length [TL]) was used for clearing and staining, the other (137 mm TL) for µCT-scans and sagittal sectioning.

The distribution of muscle fiber types in *C. acus* was recorded from cross serial sections of four specimens with a TL of 80–150 mm from the collection of the Laboratoire de Morphologie Fonctionnelle et Evolutive of the University of Liège, Belgium.

For living observations of the axial flexibility and extreme turns, we collected 21 living specimens of *C. acus* from the dissection of 434 sea cucumbers (mainly *Holothuria tubulosa*), which were collected in the Mediterranean Sea (Calvi, Corsica) in June 2008. Specimens were kept in tanks with fresh sea water at ambient water temperature. The penetrations into sea cucumbers were filmed simultaneously from a lateral and dorsal position (Sony VX 1000 Standard digital camera; Panasonic HC5 High definition digital camera at 25 frames per second). Video frames were exclusively used for qualitative analysis.

Myosepta and Horizontal Septum

We carried out microdissections on a cleared and stained specimen of *C. acus* to analyze the morphology of the myosepta and their collagen fiber tracks. After fixation in 6% formaldehyde for several weeks, we skinned and then cleared and double stained (Alcian Blue 8GX for cartilage, Alizarin Red for bone) the specimen according to the procedure of Dingerkus and Uhler (1977), modified by Prochel (2006). For a better visualization of the connective tissue and the examination of the collagen tracks and tendons within myosepta, the specimen was transferred stepwise into pure ethanol.

Prior to excision of a myoseptum, we recorded its axial position, which we defined as the anteriormost position of its insertion line on the vertebral column (axial position $0.0L$ is the tip of the snout; $1.0L$ is the tip of the caudal fin). The overall myoseptal length (i.e., distance between tip of the anteriorly pointing cone to the posteriorly pointing cone) was measured with a stereo microscope (Zeiss Stemi 2000C). Three-dimensional drawings of the myosepta were prepared every 10% of TL. The length was calculated in millimeter from the drawings by using a 2 mm

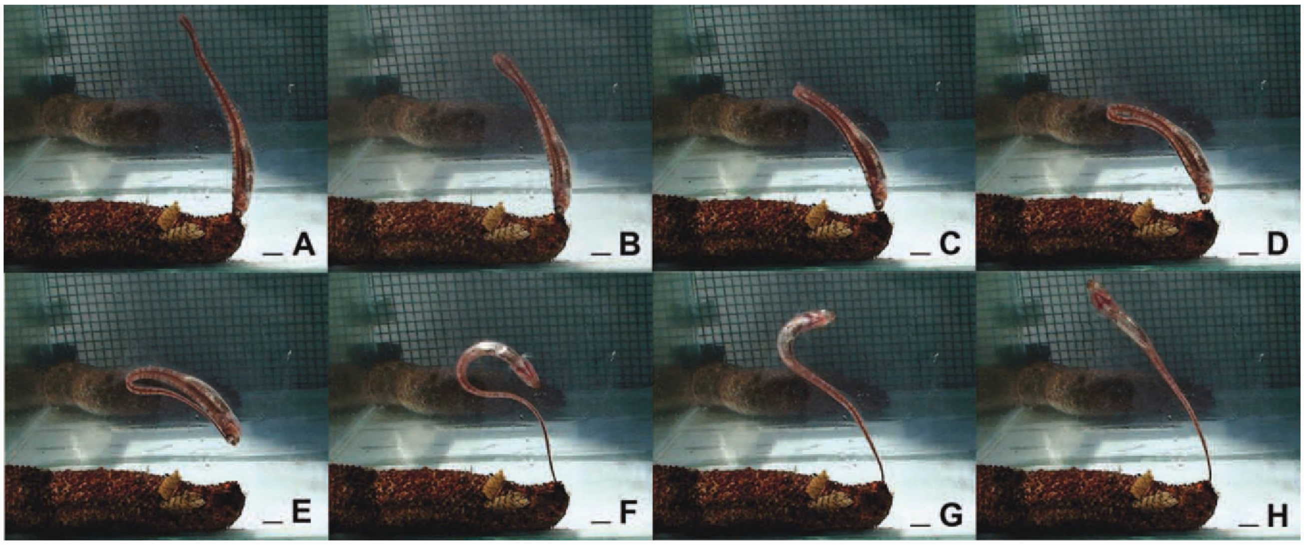

Fig. 1. *C. acus* penetrating the sea cucumber tail first. Note the extreme body flexure in the middle of the trunk (**A–F**). Time interval between two pictures 0.27 ms. Scale bar 1 cm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

standard scale bar slide with 200 subdivisions. The 3D-drawings of myosepta and records of the attachment line were later combined with the 3D view of the vertebrae obtained from the μCT scan (see below). The time-consuming microdissections did not allow addressing intraspecific variation of myoseptal length. We are, however, confident that our results are representative for the species because vertebral anatomy in *C. acus* shows little variation (Parmentier et al., 2004) and myoseptal anatomy has poor intraspecific variation in other species (Gemballa and Treiber, 2003; Gemballa and Röder, 2004).

Each myoseptum was dissected out close to its insertion line along the vertebral column and the horizontal septum by using fine iris spring scissors (FST Vannas Mini; for details of the method see Gemballa et al., 2003a). For further investigations of collagen fiber tracts, excised myosepta were kept separately in ethanol. The different myosepta were spread on microscopic slides and photographed under polarized lights (Zeiss Polarized S and analyzer A53 adapted to stereomicroscope; for details of the method see Gemballa and Hagen, 2004). Under these conditions, collagenous fibers and tendons of a myoseptum appeared bright white, whereas remaining parts of a myoseptum appeared black (see Gemballa et al., 2003b).

Having removed all myosepta of one side of the body, we dissected the remaining horizontal septum (i.e., from 0.36% TL to 0.40% TL). The collagen fiber tracts in the horizontal septum were visualized according to the procedure described for the myosepta.

Distribution of Muscle Types and Insertions of Myoseptal Tendons into Red Muscles

Specific associations of red muscles with myosepta and the horizontal septum were studied on sagittal sections of parts of the trunk. Sections were prepared from decalcified parts, embedded in paraffin and serially sectioned at 10 or 15 μm. The sections were stained with Azan (Domagk, 1933) and belong to the collection of the Zoologische Schausammlung Tübingen, Germany. In these sections, red muscles were identified according to their slender appearance in contrast to white fibers.

The distribution of red and white muscles was analyzed from serial cross-sections of *C. acus*, which belong to the collection of the Laboratoire de Morphologie Fonctionnelle et Evolutive, Belgium. In these sections, we distinguished different muscle fiber types according to their adenosine triphosphatase (ATPase) activity (see Meyer-Rochow et al., 1994 and Parmentier et al., 2003 for details). Moreover, cross-sections of 6–7 μm were stained for glycogen using Schiff's periodic acid method (PAS; Hotchkiss, 1948).

Computer Tomography of the Vertebral Column

The segmentation of the vertebral axis in Actinopterygii was discussed during the last decades and resulted in many different views (Gadow, 1894; Schauinsland, 1906; Remane, 1936; Asano, 1977; Grande and Bemis, 1998; Meunier and Ramzu, 2006; Ward and Brainerd, 2007). In this study, we use the terminology introduced by Remane (1936) with a division of the vertebral column in caudal and precaudal centra. Because we only investigated postanal myosepta (i.e., posterior to 0.2% TL), we confined the investigation of the vertebral column to the same body region.

The morphology of the vertebral centra was obtained from μCT scans, which were carried out at the Laboratory for Preclinical Imaging and Imaging Technology of the Werner Siemens-Foundation, University of Tübingen, using the micro-CAT-II high resolution animal X-ray computer tomography (CT; Siemens Preclinical Solutions, Knoxville, TN). For the scans, an X-ray voltage of 70 kVp and an anode current of 500 μA were used with an exposure time of 250 ms per projection. A total of 360 projections over 360° of rotation were acquired. Projection data were rebinned by 2 and reconstructed using a Shepp-Logan filter, into a 512 × 512 × 768 matrix having an isotropic voxel size of 19 μm. Three-dimensional-images of vertebrae, obtained from μCT scans, were combined with 3D-drawings of myosepta, obtained from camera lucida drawings, by comparing many landmarks (e.g., insertion of myosepta to vertebrae, position of myoseptal cones in relation to vertebrae). Data were finally combined in a single figure generated with Adobe Photoshop.

The lengths of the vertebral centra were obtained by importing images of the vertebral axis and a 2 mm standard scale bar slide with 200 subdivisions in a measurement tool (Zeiss Axio Vision). These images were made with a stereo microscope (Zeiss Stemi 2000C) and a camera lucida.

RESULTS

Morphology and Spatial Arrangement of Myosepta

A single myoseptum is not a plain sheet but a complex 3D arrangement of specific anteriorly and

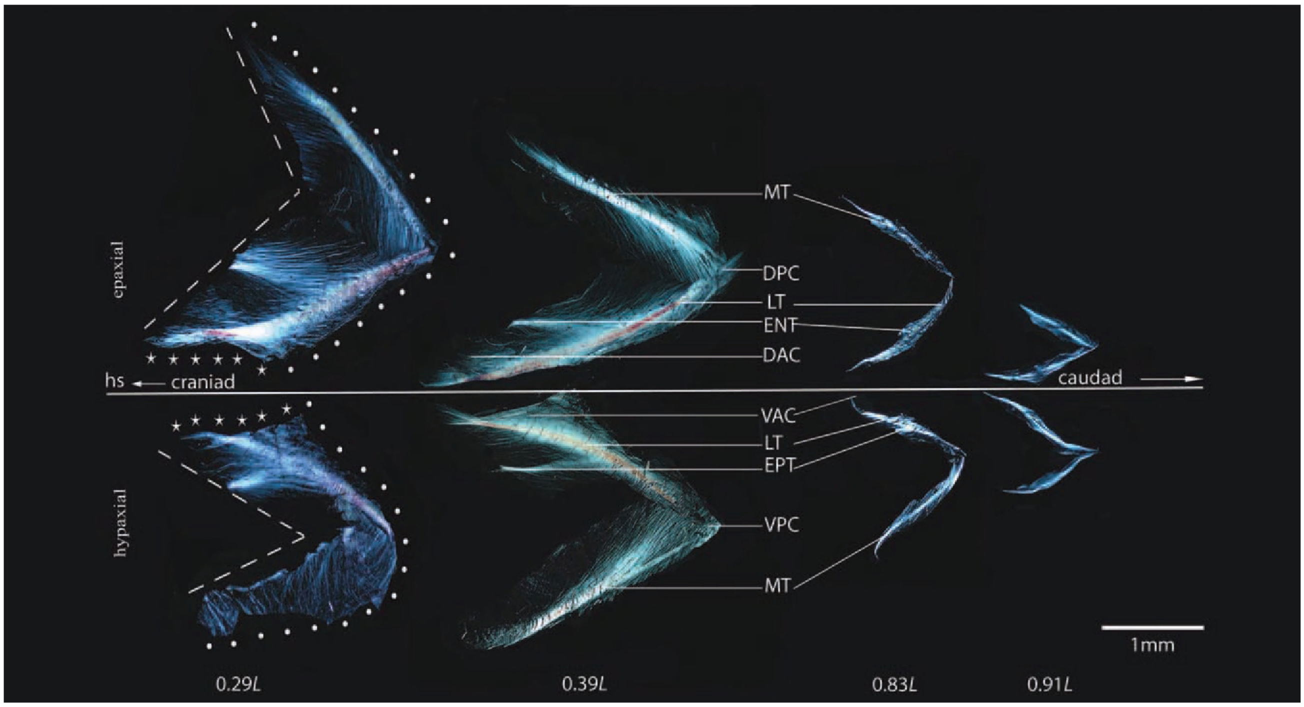

Fig. 2. Polarized light micrographs of four selective myosepta in *C. acus* (left body side at axial positions 0.29*L*, 0.39*L*, 0.83*L*, and 0.91*L*). Tendinous fiber tracts are visible as white strands; mineralizations are red. In the anterior body region (myosepta at 0.29*L* and 0.39*L*), the LTs of the epaxial and hypaxial body region show mineralizations, which gradually disappear in subsequent myosepta and are absent in posterior myosepta (0.83*L* and 0.91*L*). Insertion lines of myosepta to horizontal septum (series of asterisks), to median septum (dashed line), and to the integument (dotted line) are indicated for the myoseptum at 0.29*L*. DAC, dorsal anterior cone; DPC, dorsal posterior cone; EPT, epipleural tendon; ENT, epineural tendon; hs, horizontal septum; LT, lateral tendon; MT, myorhabdoid tendon; VAC, ventral anterior cone; VPC, ventral posterior cone.

posteriorly pointing myoseptal cones that project into the musculature and separate subsequent myomeres. The following description of the morphology of myosepta is based on the terminology introduced by Alexander (1969) and modified by Gemballa et al. (2003a). Viewed laterally, a myoseptum shows a "W"-shape, which is bisected into epaxial and hypaxial halves by the horizontal septum at the midhorizontal level.

Epaxially as well as hypaxially, each myoseptum bears two cones, one pointing anteriorly and one pointing posteriorly (see Figs. 2 and 7 for an overview of the morphology of a myoseptum). The anteriorly pointing epaxial cone is termed "dorsal anterior cone" (DAC) and the posteriorly pointing epaxial cone is termed "dorsal posterior cone" (DPC). These two cones are connected by myoseptal tissue termed as "epaxial sloping part" (ESP). The DPC is linked to the dorsal most part (i.e., dorsal midline of the trunk) by the "epaxial flanking part." This epaxial arrangement is almost mirror imaged in the hypaxial part, which bears a ventral anterior cone (VAC) and a "ventral posterior cone" (VPC). VAC and VPC are connected by the "hypaxial sloping part" (HSP). The "hypaxial flanking part" extends from VPC cranially to the ventral midline.

The W-shape of the myosepta is also reflected in a W-shaped line of attachment to lateral and median structures. Laterally, each myoseptum attaches to the skin (Fig. 2 dotted line). Midsagittally, it attaches to the vertebral column and the median septum (Fig. 2 dashed line; for details see Fig. 7 and further descriptions below). This shape of a myoseptum with four cones remains similar along the trunk (Fig. 2).

The Myoseptal Tendons

A myoseptum is not a homogenous sheet of connective tissue but bears distinct collagen fiber tracts referred to as the myoseptal tendons. Each myoseptum contains a set of six tendons, three of them located epaxially and three located hypaxially. Lateral tendons connect the anterior and posterior cone of a myoseptum (Fig. 2). The lateral tendons are the only tendons in *Carapus* that bear mineralizations (red areas in Fig. 2). The degree of mineralization changes markedly along the series of myosepta. Lateral tendons of anterior myosepta are clearly mineralized (Fig. 2, see 0.29*L* and 0.39*L*), whereas posterior myosepta show no mineralizations (Fig. 2, see 0.83*L* and 0.91*L*). We observed that the mineralization of the myosepta

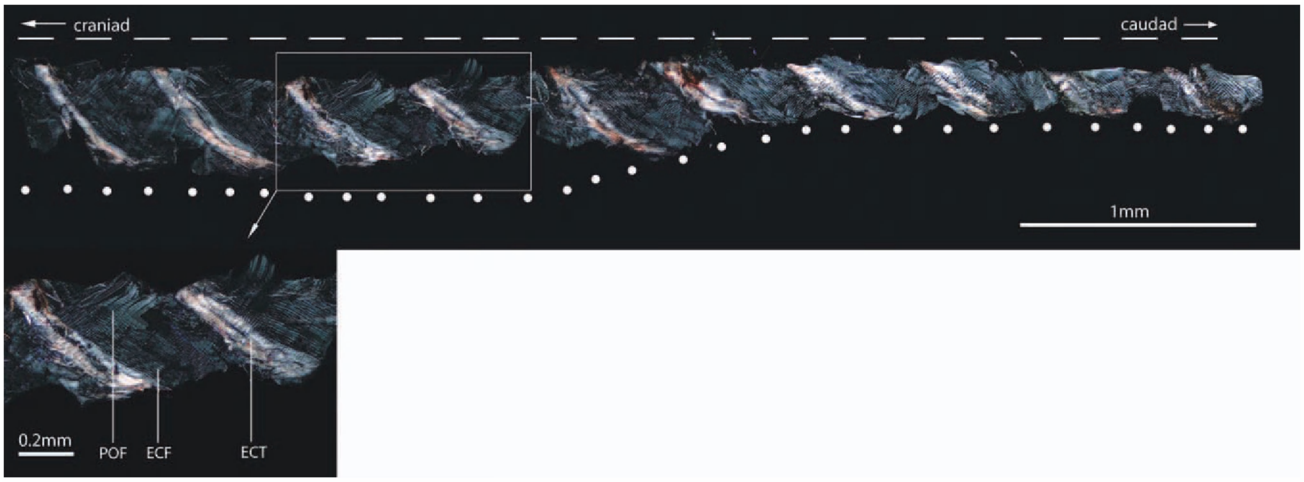

Fig. 3. Polarized light micrograph of the horizontal septum. Top view of left body side of *C. acus*. ECTs, into which the myosepta insert, are mineralized. Between adjacent ECTs fine epicentral fibers (ECF) are visible. ECFs run parallel to ECTs. Posterior oblique fibers (POF) run perpendicular to the ECT and ECF direction.

gradually disappeared from 20% TL toward the posterior body region.

In addition to the longitudinally oriented lateral tendon, each sloping part bears a caudolaterally oriented tendon termed ENT in the ESP and a corresponding EPT in the HSP. These tendons are anchored in the vertebral column, run caudolaterally to the skin and show no mineralizations. A third set of tendons, the myorhabdoid tendons, are present in the flanking parts of the myosepta. Epaxially and hypaxially (starting at 0.32*L*), each flanking part bears a myorhabdoid tendon that runs caudocranially from the posterior cone to the dorsal or ventral midline of the body (Fig. 2). The myoseptal length (i.e., the rostrocaudal extension of the lateral tendon between the tip of the anterior cone to the tip of the posterior cone) decreases alongside the trunk from 5.1% (anterior myosepta) to 0.081% of body length (posterior myosepta; Fig. 4).

The Horizontal Septum

The horizontal septum consists of a cross fiber array of collagen fibers in two different directions (Fig. 3). The nomenclatures of the different parts in the horizontal septum that is used in this study was first established by Kafuku (1950) and modified by Gemballa et al. (2003b).

Most prominent in *C. acus* is the caudolateral fiber direction. It is formed by distinct tendon like structures, the epicentral tendons (ECTs). They insert into the anterior margin of the centra and run in caudolateral direction to the skin. These ECTs form the line of attachment of the epaxial and hypaxial myoseptum on the horizontal septum. The remaining fibers of the horizontal septum are extremely thin and hardly detectable compared with the prominent ECTs (Fig. 3). Parallel to and between ECTs, thin epicentral fibers (ECFs) run caudolaterally in the horizontal septum. These ECFs are crossed by craniolaterally oriented posterior oblique fibers (POFs). ECTS, ECFs and POFs are present in the horizontal septum throughout the trunk (Fig. 3).

Distribution of Muscle Fiber Types and Insertion of Muscles into Myosepta

Myomeres consist of packages of red, pink, and white muscle fibers (Fig. 5A,B). White muscle packages are surrounded by a layer of red muscles in which pink muscles are nested inside (Fig. 5A). The superficial layer of red muscles is present along the whole trunk. At a given axial position, it is thickest at the midhorizontal position and extends dorsoventrally (Fig. 5A). A thin layer of deeper red muscle extends around the horizontal septum from

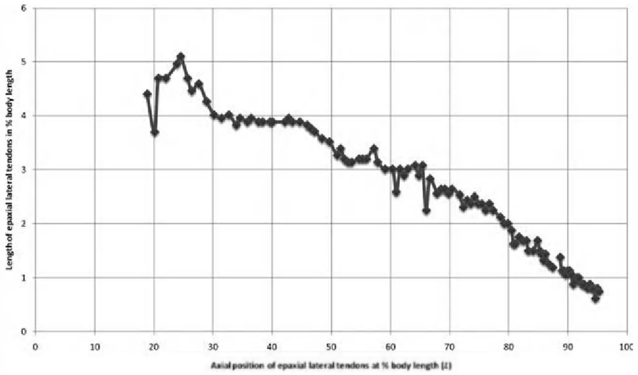

Fig. 4. Length of epaxial lateral tendons at different axial positions. Data were obtained from 104 individual myosepta.

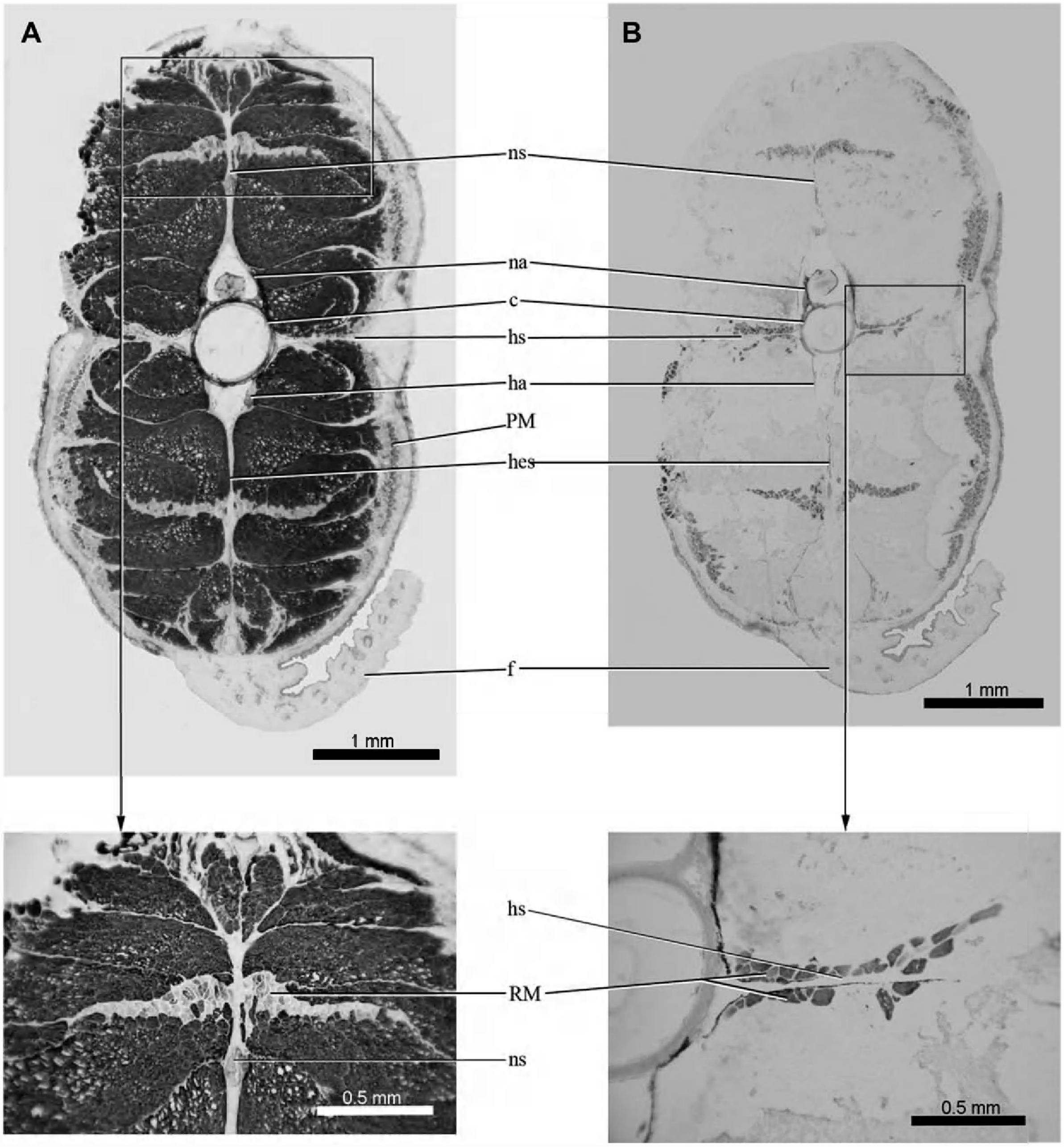

Fig. 5. Histological cross-sections of the trunk of *C. acus* stained with PAS, for illustrating the distribution of red and white muscle fibers. (**A**) Transverse section of the body. Red muscles (bright areas) represent a superficial layer on white muscle packages (dark area). (**B**) Red muscle fibers (dark area) run alongside the horizontal septum to the centra. c, centra; f, fin; ha, hemal arch; hes, hemal spine; hs, horizontal septum; na, neural arch; ns, neural spine; PM, pink muscles; RM, red muscles.

the surface medially to the vertebral centra (Fig. 5B). Another thin package of red muscles is present between white muscle packages at the level of the neural and hemal spines (Fig. 5A).

Sagittal sections gave us some information about the association of muscle fiber types and myoseptal tendons. White muscle fibers insert into all kinds of myoseptal tendons (lateral tendons, ENT/EPT and myorhabdoid tendons) as they occupy most of the space in a myomere (Figs. 5 and 6). However, red muscles form only thin superficial layers or layers around the horizontal septum (Fig. 5). We found no evidence for red muscle fibers inserting into the lateral or ENT. However, in the dorsal- and ventralmost parts of our sections, we identified red muscles that insert into myorhabdoid tendons (Fig. 6A,B). Around the horizontal septum, some red muscle fibers appeared to be anchored in the connective tissue of the horizontal septum (Fig. 6C; hypaxially).

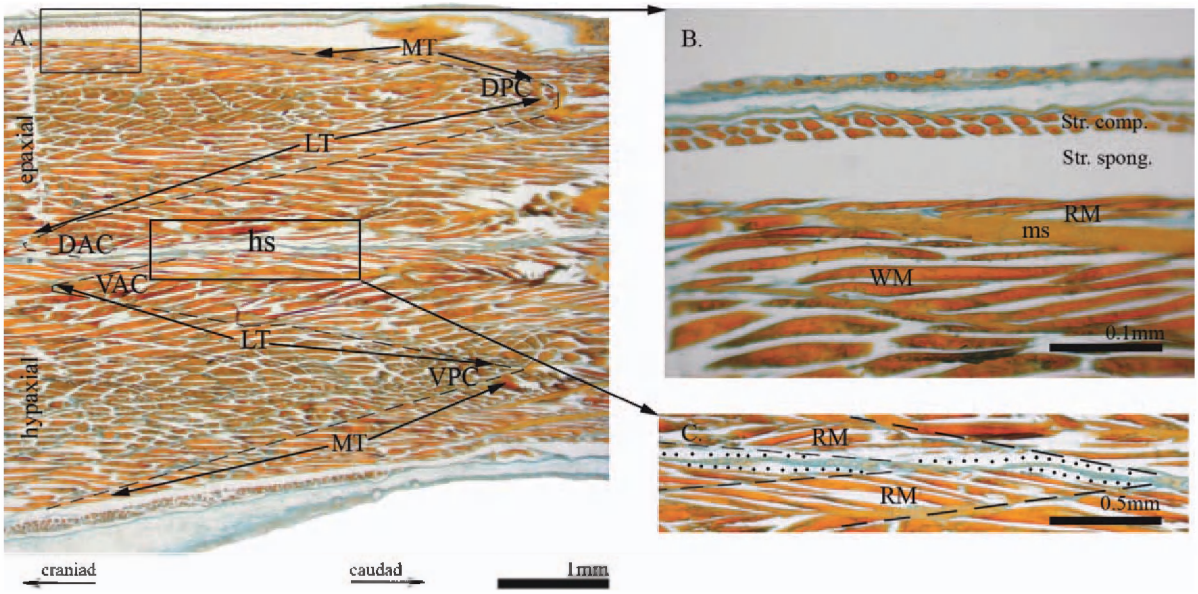

Fig. 6. Sagittal sections of *C. acus*. (**A**) Overview of the anterior body region (0.27*L*). (**B**) Epaxial insertion of red muscles into myorhabdoid tendons of myosepta. (**C**) Red muscle fibers connect adjacent myosepta (dashed lines). Epaxially, no insertion of red muscles into the horizontal septum (dotted line) can be found, whereas hypaxially some red muscles fibers insert into the horizontal septum. DAC, dorsal anterior cone; DPC, dorsal posterior cone; hs, horizontal septum; LT, lateral tendon; MT, myorhabdoid tendon; RM, red muscles; Str. comp, stratum compactum; Str. spong, stratum spongiosum; VAC, ventral anterior cone; VPC, ventral posterior cone; WM, white muscles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The Insertion of Myosepta on the Vertebral Axis

The vertebral axis of *C. acus*, in which the myosepta insert, consists of amphicoelous vertebrae with characteristic processes (Fig. 7). The insertion of a myoseptum into the vertebral axis changes gradually along the series of myosepta. Figure 7 depicts the condition found in the anterior and midbody region. In this condition, the medial part of an epaxial myoseptum (i.e., the medial attachment line) runs along the anterior margin of a vertebral centrum *N* and turns caudally at the base of the neural arches (Fig. 7: dotted line). It proceeds horizontally across the neural arch of the subsequent vertebra (*N* + 1) and follows the neural spine of this vertebra to its distal end. At the tip

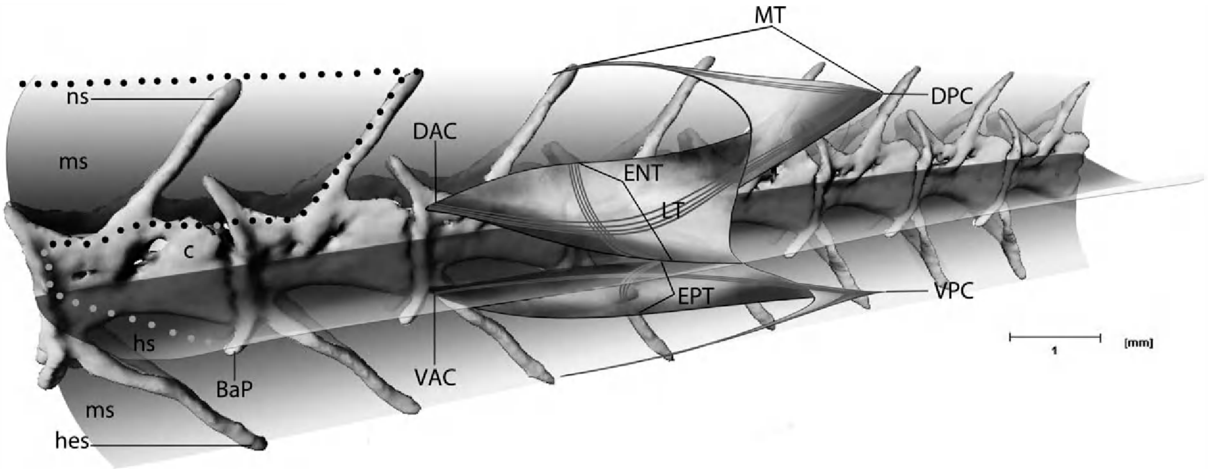

Fig. 7. Three-dimensional view of a part of the vertebral column of *C. acus* with one representative myoseptum at 0.5*L* (left body side). Data were combined from μ-CT scans (vertebral column) and from camera lucida drawings (myosepta and insertion lines). The dotted line indicates the insertion line of a myoseptum on the vertebral column. BaP, basapophysis; c, vertebral centrum; DAC, dorsal anterior cone; DPC, dorsal posterior cone; ENT, epineural tendon; EPT, epipleural tendon; hes, hemalspine; hs, horizontal septum; ms, median septum; MT, myorhabdoid tendon; ns, neural spine; VAC, ventral anterior cone; VPC, ventral posterior cone.

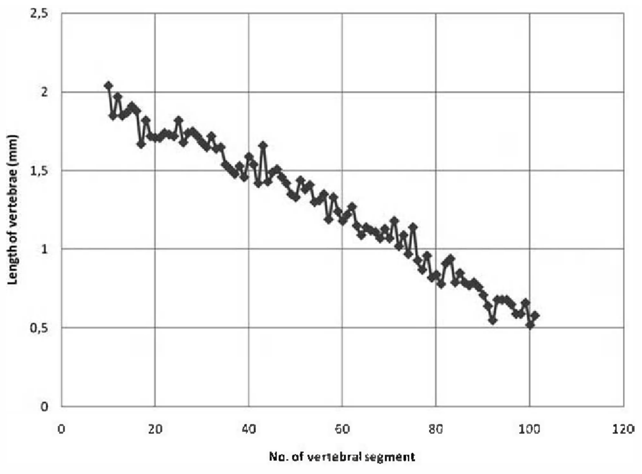

Fig. 8. Length of successive vertebrae from cranial to caudal. Data were obtained from 102 individual centra.

of this neural spine, it turns into a craniodorsal direction and ends at the dorsal midline. In summary, the medial attachment line extends over two vertebral segments. This extension decreases gradually toward the posterior trunk but clearly remains more than one segment.

Hypaxially, myosepta insert into the anterior margin of the basapophysis. At the tip of the basapophysis, the medial attachment line runs posteriorly in a horizontal direction until it reaches the hemal spine. It proceeds horizontally across the basapophysis of another vertebra ($N + 1$) to the next hemal spine. Here, it turns ventrocaudally along the spine. At the distal tip of the hemal spine, the attachment line turns in cranioventral direction and ends at the ventral midline (not shown in Fig. 7). We found a constant rostrocaudal reduction in the overall length of vertebrae (Fig. 8) from 2 mm anteriorly to 0.5 mm posteriorly. Thus, not only the myoseptal length (Fig. 4) decreases (see above) but also the length of the vertebrae.

DISCUSSION

Morphological Features That Might Influence Axial Flexibility

The body curvature displayed by fishes differs remarkably between species. In this study, we investigated pearlfish (*C. acus*), a species that displays extreme body curvatures during penetration of its invertebrate host (Fig. 1). The morphological correlates for a high axial flexibility in fishes are currently under debate. It is likely that a high number of vertebrae are crucial for a high axial flexibility (Lindsey, 1978; Long and Nipper, 1996; Brainerd and Patek, 1998). However, other nonmuscular features of the trunk have been identified to play a role, such as second moment of area of centra, centrum length or transverse height (Porter et al., 2009). So far, features of the musculotendinous system of the trunk have only been little explored with respect to their impact on flexibility (Danos et al., 2008).

Tendonlike Structures of Flexible Fishes Compared With Rigid Fishes

Comparative studies revealed that the gnathostome ancestor already had W-shaped myosepta equipped with six tendons in each myoseptum (myorhabdoid, lateral, and ENT/EPT). This set of six tendons is still observed in many living species (Gemballa et al., 2003a). However, several modifications have been observed in rigid fishes with respect to relative length and robustness of tendons.

In these fishes, the myoseptal cones are elongated and, thus, the lateral tendons are of a considerable relative length. This modification must have occurred independently several times as it is observed in many distantly related groups, such as sharks (Lamnidae), jacks (Carangidae), swordfish (Xiphiidae), mackerels and tunas (Scombridae; Gemballa and Treiber, 2003; Donley et al., 2004; Shadwick and Gemballa, 2006; Gemballa et al., 2006, 2007). Typically, the length of lateral tendons in these fishes increases from 6–10% of body length anteriorly to 15–25% in the posterior body region, although the length of an individual segment decreases. Such an elongation of tendons is not observed in *C. acus*. In contrast, the length of lateral tendons in this species decreases from 5% in the anterior body region to less than 1% of body length in the posterior body region (Fig. 4). This decrease in tendon length is even sharper than the decrease in length of vertebrae (Fig. 8). In eels, lateral tendons are also very short. Along the body, the length of the tendons remains almost constant around 5% of body length (Danos et al., 2008). Few published data on other relatively flexible species from various systematic groups (e.g., small sharks, polypterids, sturgeons, blenniids, snakeheads; Gemballa and Treiber, 2003; Gemballa and Röder, 2004; Gemballa et al., 2006; Shadwick and Gemballa, 2006) indicate that the eel condition is matched. Thus, a short lateral tendon almost constant in length is significant for flexible fishes, whereas an elongated lateral tendon is significant for rigid fishes. A decrease in length, however, as observed here for *C. acus*, has never been observed in any other species and might be a special feature allowing for the extreme body flexibility.

In terms of the ENT and EPT, the condition found in *Carapus acus* matches the condition in *Anguilla anguilla* (Danos et al., 2008). These tendons are prominent in all body regions and firmly connect the vertebral axis and skin. Besides these detailed data sets on whole myoseptal series in *Anguilla* and *Carapus*, we have data from individual myosepta of other species that are in accordance with this obser-

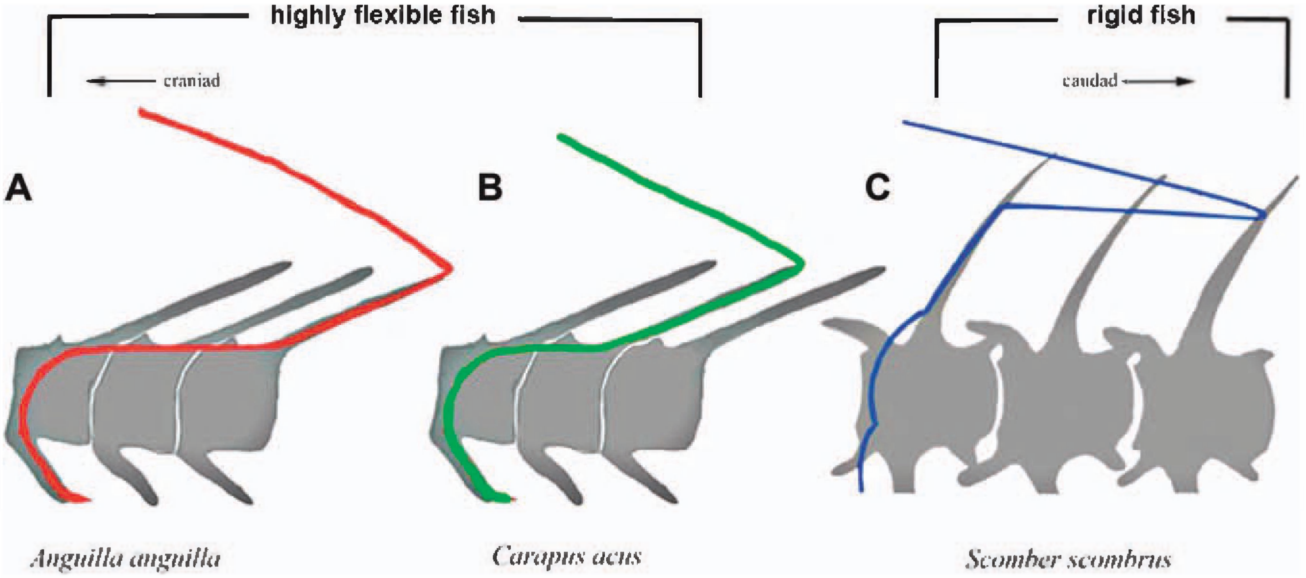

Fig. 9. Myoseptal attachment line on the vertebral column in two flexible fishes compared with a rigid fish (*Scomber scombrus*). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

vation. In contrast, ENT and EPT are weakly developed or even absent in rigid fishes (for summary of data see Shadwick and Gemballa, 2006).

Besides tendon length, the attachment of myosepta on the vertebral axis differs between flexible and rigid fishes. In flexible fishes, the attachment line crosses two or three vertebral segments along the neural arch and then joins up with the neural spine (Fig. 9A,B). In contrast, the attachment line in rigid fishes remains within one vertebral centrum and follows the neural spine of the same segment. It turns caudally at a more dorsal position (Fig. 9C).

The horizontal septum of rigid fishes consists of prominent and elongated ECT and posterior oblique tendons (POTs), especially, in the posterior body region (Westneat et al., 1993; Westneat and Wainwright, 2001; Gemballa et al., 2003b). In *C. acus* (Fig. 3), these tendons remain short and partially indistinct along the whole body. Data from a comparative study on the horizontal septum suggest that this is another widespread difference between rigid and flexible fishes (Gemballa et al., 2003b; Danos et al., 2008).

Ossifications in myoseptal tendons as found here in *C. acus* are widespread among teleosts but never occur in sharks, basal actinopterygians, and derived acanthopterygians (Patterson and Johnson, 1995; Gemballa et al., 2003a). Thus, the fact that rigid sharks and rigid tunas and mackerels (Acanthopterygii) lack ossifications in their myoseptal tendons might have either a phylogenetic or a functional reason. So far, we have no data to explain specific functional roles of myoseptal ossifications.

Functional Implications of Differences Between Flexible and Rigid Fishes

Given the structural differences between flexible and rigid fishes described above, the question remains whether these differences are likely to contribute to the observed differences in body flexibility. Several skeletal features that influence body flexibility have been identified in quantitative studies (Porter et al., 2009). However, although not quantified yet, it had been suggested that part of the body flexibility is also influenced by the tendinous system of the trunk (Porter et al., 2009). In rigid fishes, the extremely elongated lateral tendons are the best candidates to reduce body flexibility and, thus, cause the measured great turning radii (Domenici, 2003). In contrast, flexible fishes with shorter tendons, such as *C. acus* and others (e.g., *Polypterus*, *Anguilla*; Gemballa and Bartsch, 2002; Gemballa and Röder, 2004; Shadwick and Gemballa, 2006; Danos et al., 2008) show very flexible bodies (e.g., during penetration of *Carapus* into host; Fig. 1). Among others (e.g., reduced vertebral length and enhanced number of vertebrae as in *Carapus*), one reason for their enhanced body flexibility might be the short myoseptal tendons (see Fig. 4 for *Carapus*) and the reduced rostrocaudal extension of the medial attachment of myosepta to the vertebral axis (see Fig. 7 for *Carapus*).

The function of the ENT and EPT is much less clear than that of the lateral tendons. Thus, the following explanation of differences we found between rigid fishes (indistinct or absent ENT/EPT) and flexible fishes (distinct ENT/EPT; Fig. 2) remains speculative. As extreme body curvatures inevitably cause a lateral displacement of the vertebral column to the convex side, structures that constrain this displacement maybe needed. The pronounced ENT and EPT observed in flexible fishes might serve as such. They provide a firm connection between vertebral axis and skin, which likely hampers the lateral displacement of the vertebral axis during extreme body flexures. It might also be responsible for the generation of intramuscular pressure that has been measured during extreme flexures in fast start responses of some fishes (Westneat et al., 1998).

The differences in myoseptal tendons in rigid and flexible fishes are part of a musculotendinous system that produces characteristic axial curvatures during swimming by acting on the axial skeleton. If rigid and flexible fishes bear at least two partly different systems, it might well be that the mechanisms by which they produce such curvatures also differ. Indeed, studies on swimming mechanics and swimming physiology support this view.

In rigid fishes (tunas, lamnid sharks), the elongated lateral tendons are likely to be part of a long distance force transmission system. For continuous swimming, muscle forces are transmitted from the anterior and midbody toward the caudal fin by insertions of either superficially placed or deep red muscles into the lateral tendons (tunas: Knower et al., 1999; Shadwick et al., 1999; Altringham and Shadwick, 2001; Katz et al., 2001; sharks: Donley et al., 2004, 2005; Gemballa et al., 2006; swordfish: Gemballa et al., 2007). In these fishes, activity of anterior muscles causes bending at a more posterior position. In contrast, muscle activity in other fishes causes local bending (Coughlin et al., 1996; Katz et al., 1999; Donley and Shadwick, 2003). Therefore, there is no functional need for elongated tendons connected to red muscles in such fishes. Accordingly, in *C. acus*, we neither found elongated lateral tendons nor insertion of red muscles into these tendons.

Data on tunas suggest that long distance force transmission may also be achieved by elongated tendons of the horizontal septum (i.e., ECT and POTs; Westneat et al., 1993; Westneat and Wainwright, 2001). In *C. acus* as well as in other flexible fishes, these tendons are short. Our histological investigations gave some indications of an insertion of red muscles into these tendons. However, if this is part of a direct force transmission from red muscles into the horizontal septum, it will definitely not serve for force transmission over longer distance as such short ECT and posterior oblique tendons as in *Carapus* are likely to cause local bending.

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