Unusual anal fin in a Devonian jawless vertebrate reveals complex origins of paired appendages

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Jawed vertebrates (gnathostomes) have undergone radical anatomical and developmental changes in comparison with their jawless cousins (cyclostomes). Key among these is paired appendages (fins, legs and wings), which first evolved at some point on the gnathostome stem. The anatomy of fossil stem gnathostomes is, therefore, fundamental to our understanding of the nature and timing of the origin of this complex innovation. Here, we show that Euphanerops, a fossil jawless fish from the Devonian, possessed paired anal-fin radials, but no pectoral or pelvic fins. This unique condition occurs at an early stage on the stem-gnathostome lineage. This condition, and comparison with the varied condition of paired fins in other ostracoderms, indicates that there was a large amount of developmental plasticity during this episode—rather than a gradual evolution of this complex feature. Apparently, a number of different clades were exploring morphospace or undergoing multiple losses.

1. Introduction

The evolution of jawed vertebrates from their jawless ancestors represents a landmark event in the evolutionary history of vertebrates. Radical genetic, developmental and morphological changes took place during this episode, and the body plan was largely overhauled [1,2]. This includes the origin and early evolution of novel and fundamental structures such as teeth, bone and importantly, paired appendages. The evolution of paired appendages in the stem gnathostomes enabled more sophisticated control of movement and, subsequently, the development of a diverse array of complex fins and tetrapod limbs. Genetic and developmental investigations of paired fins have suggested evolutionary scenarios for the initial origin of these fundamental features [3,4], but cannot provide a time scale of the events or the sequence of intermediate stages. The wide morphological gulf between extant jawless vertebrates (lampreys and hagfish; together the cyclostomes) and extant jawed vertebrates (chondrichthyans, osteichthyans and tetrapods) offers us few insights into the actual timing and sequence of evolutionary events.

Reprieve comes from the ostracoderms, a diverse array of extinct Palaeozoic jawless fish. Reconstructing the anatomy and phylogenetic relationships of these important fossil taxa allows us to bridge the morphological gap between cyclostomes and jawed vertebrates, to reconstruct the acquisition of characters along the gnathostome stem, and thus the circumstances of the origin of jawed vertebrates from jawless ancestors [1,2,5]. Fossil data are invaluable in this context as they can reveal unexpected or unknown anatomical conditions at an early stage of a clade. This is especially true for the evolution of vertebrate paired appendages for which fossils have yielded a variety of conditions ranging from complete absence, through simple flaps, to complex paired fins with skeletal support [5–7]. Although these fossil data allow us to build...
evolutionary scenarios [6,7], ambiguity remains over the true timing and sequence of events. There are two reasons for this ambiguity: the variety of paired appendage structures within and between stem-gnathostome clades, and the unknown condition of paired appendages in more primitive taxa. Here, we describe new data from *Euphanerops*, a key taxon from the Devonian, phylogenetically placed right at the split of cyclostomes and jawed vertebrates [8,9]. *Euphanerops* is interpreted here as possessing a paired anal fin—a unique condition in vertebrates with far reaching ramifications for our understanding of the evolution of paired appendages.

2. Material and methods

*Euphanerops longaevus* is known from the Upper Devonian Escuminac Formation of Miguasha, Quebec, Canada [9,10]. The fossil material analysed here (36 specimens from the type horizon, and

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Figure 1. *Euphanerops* fins. (a) Whole body (MHNM 01–02; length 86 mm); (b)(i) anal fin region (MHNM 01–98), (ii) three-dimensional surface height map (where green is higher and purple is lower) and (iii) cross section with interpretation; (c) fin radials crossing and at angles in part and counter-part (MHNM 01–02); (d) anal fin (MHNM 01–123) showing paired series of radials (red and blue in interpretation, below); (e) extent of digestive system (MHNM 01–163).
many more specimens from the overlying VI unit, previously identified as *Endeiolepis* (junior synonym) is housed at the Museum d’Histoire Naturelle, Miguasha (MHNM) and the Natural History Museum, London (NHM). Additional material of *Hardistiella montanensis* was from the University of Montana Paleontological Centre (UMPC) and the Carnegie Museum of Natural History, Pittsburgh (CMNH). Specimens have been analysed using photography with polarized light, alcohol immersion and colour balancing, camera lucida drawings, SEM of uncoated specimens under partial vacuum (Hitachi 3600) with energy dispersive X-ray spectroscopy (EDX) for analysis of elemental composition and focus variation microscopy to assess three-dimensional configuration of fossils.

3. Results

In addition to the very clearly hypocercal, lamprey-like caudal fin, *Euphanerops* possesses a median fin towards the posterior, just anterior to the constriction between the torso and tail (figure 1a). Using the relative positions of the mouth and gut in multiple specimens, the median fin is clearly on the ventral surface. The preservation exhibited in *Euphanerops* specimens from unit VI, which includes gut-infills, provides unambiguous evidence of the extent of the digestive system. It extends to the ventral body margin at a point immediately anterior to the ventral fin (figure 1c), making it an anal fin rather than a pelvic fin. The anal fin is approximately as tall as it is long, in the region of 5–10 mm in size, except in MHNM 01–123, which, although incomplete, is a substantially larger specimen (figure 1d). The fin contains distinct skeletal radial elements. EDX analysis reveals a composition dominated by calcium and phosphorous; the radials are most likely apatite, but whether this reflects biomineralization or diagenetic phosphatization is unclear [9]. The radial elements generally extend out from the body margin in a fan-like fashion, but there are important deviations from this pattern: in some specimens, radial elements are observed to cross and form distinct angles (MHNM 01–02, 01–98, 01–123, 01–181). Two distinct series of radials are observed in MHNM 01–123, one perpendicular to the body margin, overlain by another at a more acute angle (figure 1d), whereas comparison of part and counter-part of MHNM 01–02 reveals a similar pattern (figure 1c). Furthermore, the region of the ventral fin of specimen MHNM 01–98 preserves two sets of radials on distinct and separate sedimentary laminae, with 100 µm of matrix between (figure 1b), although diagenetic compaction of sediment means that the gap would have been larger in *vivo*. The orientation of the radial elements differs slightly between the layers (figure 1b). A pattern of two series of overlapping and crossing radial elements in the ventral fin is, therefore, repeated across different specimens (15–22 pairs of radials). It is not, however, observed in all specimens of *Euphanerops* that preserve this region, but this is what would be expected as a consequence of preservation and fossil exposure—paired ventral fins originating near the midline will often overlie each other, separated by a thin layer of rock, but with only one visible on the exposed surface.

*Euphanerops* has also been observed to possess a long series of short ‘fin radials’ forming a narrow ribbon from the mouth to the anus [9]. This structure is potentially paired, diverging laterally towards the head. The preservation of part and counter-part of MHNM 01–98 reveals a similar pattern (figure 1c). Furthermore, the region of the ventral fin of specimen MHNM 01–98 preserves two sets of radials on distinct and separate sedimentary laminae, with 100 µm of matrix between (figure 1b), although diagenetic compaction of sediment means that the gap would have been larger in *vivo*. The orientation of the radial elements differs slightly between the layers (figure 1b). A pattern of two series of overlapping and crossing radial elements in the ventral fin is, therefore, repeated across different specimens (15–22 pairs of radials). It is not, however, observed in all specimens of *Euphanerops* that preserve this region, but this is what would be expected as a consequence of preservation and fossil exposure—paired ventral fins originating near the midline will often overlie each other, separated by a thin layer of rock, but with only one visible on the exposed surface.

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4. Discussion

Given the unambiguous position of the gut and its ventral location, the fin is clearly an anal fin. Rather than a result of post-mortem displacement of posterior elements [9, p. 187], the repeated pattern of crossing and overlapping series of radial elements strongly indicates that there was a laterally paired series of elements, inserted into the body wall close to...
the ventral midline of the body (figure 2). A paired disposition of the anal fin is most unusual and potentially unique for vertebrates. In-depth analysis of function is beyond the scope of this manuscript, but comparison could be made with the ventrally directed, paired pelvic fins of various modern gnathostomes that insert close to the ventral midline (e.g. Atlantic cod (Gadus morhua), Nassau grouper (Epinephalus striatus), Moorish idol (Zanclus cornutus) or claspers of some chondrichthyes). This unusual paired post-anal condition is all the more striking, considering the early stage of vertebrate evolution at which it occurs.

Paired pectoral fins are known throughout jawed vertebrates and are homologous with those of the jawless osteostracans [11]. Paired fin ‘precursors’ of unclear homology are observed in some anaspids and some thelodonts [1,7,12,13]. Paired pelvic fins are known in ‘higher’ placoderms and crown-group gnathostomes [5,13], but it seems a pelvic girdle may have been present in antiarchs [14]. Observations of pelvic fins in jawless vertebrates have either been revised [8,15] or could equally be interpreted as representing pectoral fins [7], or, in the case of the thelodont Shehia, are based on a single taphonomically ambiguous specimen (fig. 24 in [16]). Anal fins are observed in jawed vertebrates (with the potential exception of antiarchs), some anaspids and at least one thelodont [5,15,17]. Lampreys do not possess an anal fin, although isolated examples have been reported [18]. We find no evidence for anal fins in fossil lampreys, including Hardistiella, and patterns of character decay provide no evidence that fins might be absent owing to taphonomic loss [19–21]. Some osteostracans possess a horizontal caudal lobe, which is reconstructed as plesiomorphic, but its homology with anal fins is unclear [5,9,11]. Many stem gnathostomes, including representatives of each group, lack any kind of paired fins or anal fin.

Placing this diversity of appendage-like structures in a phylogenetic context reveals that the paired anal fin of Euphanerops cannot be securely homologized with either the paired fins or anal fin of jawed vertebrates or other stem gnathostomes (figure 2). Phylogenetic schemes exhibit some differences with regard to the relative placement of anaspids, thelodonts and pteraspidimorphs [2,5,8,11], but the ambiguity over the homology of the Euphanerops fin, and paired fins more broadly, remains, whichever scheme characters are mapped onto. This is in no small part owing to the variety of structures observed within these supposedly monophyletic groups of stem-gnathostome and ambiguity over their interrelationships and ancestral conditions. There are two possible ways to view the patterns that emerge: different appendage structures have appeared independently in different groups, or, conversely, there has been a single origin of paired and anal fins followed by multiple losses in a variety of clades. The unusual paired anal fin of Euphanerops lends support to the idea that there was some degree of developmental and evolutionary plasticity in some stem gnathostomes. After the Devonian and the extinction of most stem-taxa, the gnathostome body plan exhibits fewer deviations from the formula of paired pectoral, paired pelvic, unpaired dorsal and unpaired anal appendages. The discovery of new anatomical conditions in new taxa or existing taxa will hopefully bring light to bear on the timing and sequence of events underlying the origin and diversification of vertebrate appendages. Furthermore, it is clear that we are in need of better phylogenetic frameworks for relationships within stem-gnathostome clades, particularly the thelodonts and anaspids, in order to be able to better understand character polarity, make statements about homology and ultimately, to reconstruct the sequence of acquisition of complex characters along the gnathostome stem. The evolution of paired appendages in vertebrates has been speculated to have occurred via co-option of the molecular circuitry regulating medial fins, which are present even in the most basal chordates [3,22]. Our evidence of paired anal fins in Euphanerops suggests that this co-option might have occurred more than once, and even earlier in post-cyclostome vertebrate evolution than previously thought.

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