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# Exceptional preservation of nerve and muscle tissues in Late Devonian placoderm fish and their evolutionary implications

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**In this paper, we show exceptional three-dimensionally preserved fossilized muscle tissues in 380–384 Myr old placoderm fish (Late Devonian), offering new morphological evidence supporting the hypothesis that placoderms are the sister group to all other gnathostomes. We describe the oldest soft tissue discovered in gnathostomes, which includes striated muscle fibres, circulatory and nerve tissues, preserved as phosphatized structures precipitated by microbial infilling of small, protected areas under the headshield of the arthrodire, *Eastmanosteus calliaspis*. Muscle impressions have also been found in the ptyctodontid, *Austroptyctodus gardineri*. The specimens display primitive vertebrate muscle structures; in particular, shallow W-shaped muscle blocks such as those observed in lampreys. New information from fossilized soft tissues thus elucidates the affinities of the placoderms and provides new insights into the evolution and radiation of gnathostomes.**

**Keywords:** placoderms; phosphatized muscle; gnathostome phylogeny

## 1. INTRODUCTION

The placoderms were the most successful and diverse group of jawed fishes (gnathostomes) in the Devonian Period (416–358 Myr ago). Here, we describe the oldest soft tissue, ever discovered in gnathostomes, from placoderms endemic to the Gogo Formation (Frasnian), Canning Basin, Western Australia. It includes striated muscle fibres, circulatory structures and nerve fibres preserved in small protected areas under the headshield of *Eastmanosteus calliaspis* (Dennis-Bryan 1987). The processes of soft-tissue preservation in *E. calliaspis* are extraordinary because the preservation appears to have occurred in an oxygenated environment. In *Austroptyctodus gardineri* (Long 1997), early bacterial-mediated phosphatization, in addition to anoxia and rapid burial, provided favourable conditions for soft-tissue preservation within the body cavity (Hirschler *et al.* 1990).

The relationships of placoderms remain poorly understood despite their excellent fossil record (Young 1986). Recently, various cladograms have been proposed, which show the placoderms closely related to: sharks and rays (Elasmobranchii; Young 1986); bony fish (Osteichthyes; Forey 1980; Gardiner 1984); or, as the primitive sister group to all other gnathostomes (Goujet 2001; Goujet & Young 2004). There is also much debate concerning the interrelationships within the Notochordata. In *A. gardineri*, myomeres and myosepta, displaying the primitive vertebrate structure of a shallow W-shape similar to lampreys, have been identified. This discovery provides direct evidence for these particular character states within the Placodermi, and are, therefore, critical to our understanding of early evolution in these phyla.

## 2. TISSUE STRUCTURE AND MICROSTRUCTURE IN ARTHRODIRES

### (a) Eastmanosteus calliaspis

Muscle microstructure is best preserved in the arthrodire, *E. calliaspis* (WAM 00.3.1; WAM 06.2.1), where the phosphatized tissue consists of striated muscle fibres and nerve endings (figure 1*a*,*b*) with the possible associated blood capillaries (figure 1*c*). The external surface of the muscle block (figure 1*e*) demonstrates that each myomere is composed of fibril bundles (figure 1*f*,*g*) together with sarcolemmic membranes, which enclose the fibres (figure 1*f*). In some cases, where the internal fibre has decayed, only the sarcolemmic membrane that surrounded the muscle fibres is preserved. Similar preservation of the sarcolemmic membrane has been observed in fossils from the Cretaceous Santana Formation of Brazil (Martill 1988, 1990).

The phosphatized muscle fibres are composed of myofibrils that are approximately 20 μm in diameter and vary in length. The sarcomeres have been preserved in the striated fibres. Parallel rows of paired hydroxyapatite crystallite aggregates highlight the fibril banding, where the fibres ruptured along M bands during the decay (figure 1*a*). A similar pattern of fibril banding is reported from the horseshoe crab, *Mesolimulus* (Briggs *et al.* 2005), and the ray, *Squatina* (Wilby & Briggs 1997), both from Solnhofen. The high fidelity with which the subcellular detail has been replicated by individual crystalline aggregates of calcium phosphate is most probably bacterially induced, through bacterial decay, rather than bacterially controlled (Briggs & Kear 1993).

The nerve fibres, approximately 75 μm in length and 1.3 μm in diameter, stretch between muscle fibres with more than one muscle fibre innervated by the same axon. This results in all the muscle fibres contracting together as a motor unit. The neurons attached to the muscle fibres at motor end plates, indicated by branching structures located midway along the length of the fibres (figure 1*b*). Their multipolar shape is consistent with the shape of vertebrate neurons and differs from invertebrate neurons, which have a unipolar shape and lack myelin sheaths. The presence of myelin sheaths has not been demonstrated in basal gnathostomes, and cannot be identified here, despite their presence being considered a vertebrate characteristic. This absence may be due to the lack of preservation rather than myelin being absent in placoderms.

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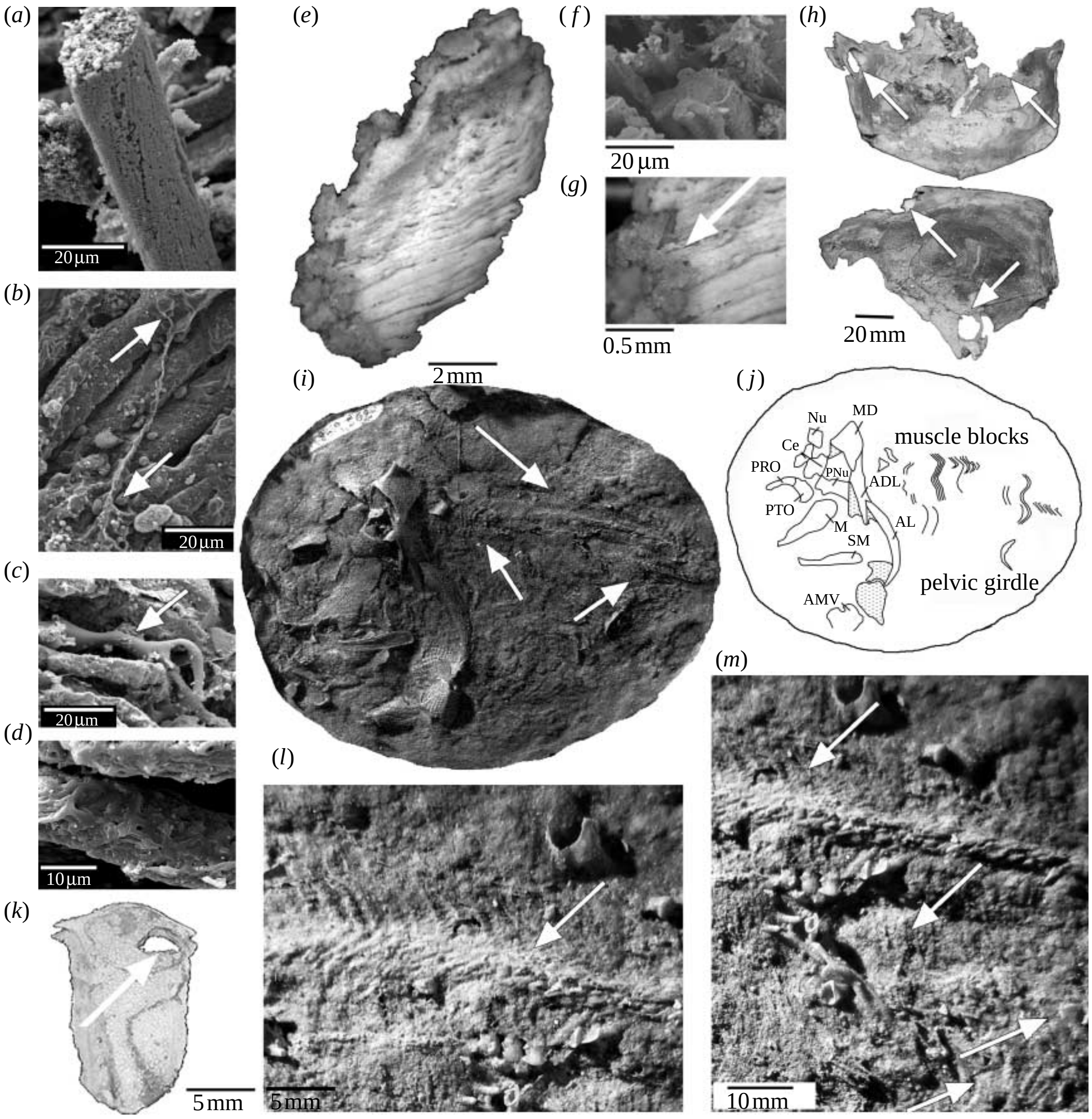

Figure 1. Preserved soft-tissue recovered from under the infragnathal plate from the placoderm, *Eastmanosteus calliaspis* (WAM 00.3.1). (*a*) A striated muscle fibre showing banding replaced by individual calcium phosphate spherulites arranged in pairs, preserving the H and Z bands. (*b*) A neuron with motor end plates (indicated by arrow) attaching to muscle fibres. (*c*) A capillary running between muscle fibres. (*d*) Preservation style made up of coarse massive crystalline replacement by calcium phosphate. (*e*) A section of phosphatized muscle tissue made up of muscle bundles. (*f*) Individual muscle fibres enclosed by sarcolemmic membrane. (*g*) Fibre bundles (indicated by arrow). (*h*) Scavenger holes (indicated by arrow) in the headshield of *E. calliaspis* (visceral view and lateral view). (*i*) Muscle fibres in the ptyctodontid, *Australolepis gardineri* (WAM 86.9.662). (*j*) Line drawing of *Australolepis gardineri* (WAM 86.9.662) indicating the position and extent of muscle blocks. (*k*) Scavenger holes (indicated by arrow) in anterior median ventral plate. (*l*) Dorsal muscle fibres running parallel to the long axis of the body attached to myomeres (indicated by an arrow). (*m*) 'W'-shaped muscle architecture in the ptyctodontid *Australolepis gardineri* (arrows indicate the points of flexure that give the W-shape).

Preserved circulatory structures (figure 1*c*) extend between muscle fibres and may wrap around them. They are of varying length and typically approximately 3 µm in diameter. Both the nervous tissue and circulatory structures appear to be smoother in texture than the muscle fibres. Their delicate structure means they are less likely to be preserved than muscle fibres, so we suggest that they were preserved early in the decay process.

### (b) *Preservation of soft tissue in* Eastmanosteus calliaspis

In contrast to the more common preservation conditions recorded by the Gogo Formation (i.e. rapid burial and dysaerobic bottom conditions), the skeleton of *E. calliaspis* (WAM 00.3.1) was open to the environment for some time after death, as indicated by disarticulation of many of the skeletal elements, the presence of scavenger holes in the dermal plates (figure 1*h*,*k*) and sponge spicules scattered throughout the matrix and the muscle fibres. The preserved soft tissue is restricted to two areas of the body, i.e. under the anterior median ventral plate and attached to the left inferognathal bone (figure 1*h*,*k*). In both the cases, the soft tissue was pressed against the dermal plates of the body, creating a locally protected environment favourable to rapid phosphatization (Briggs *et al.* 1993; Wilby 1993). These localized compartments would have created an anoxic milieu, which did not extend to the rest of the carcass. This anoxic effect,

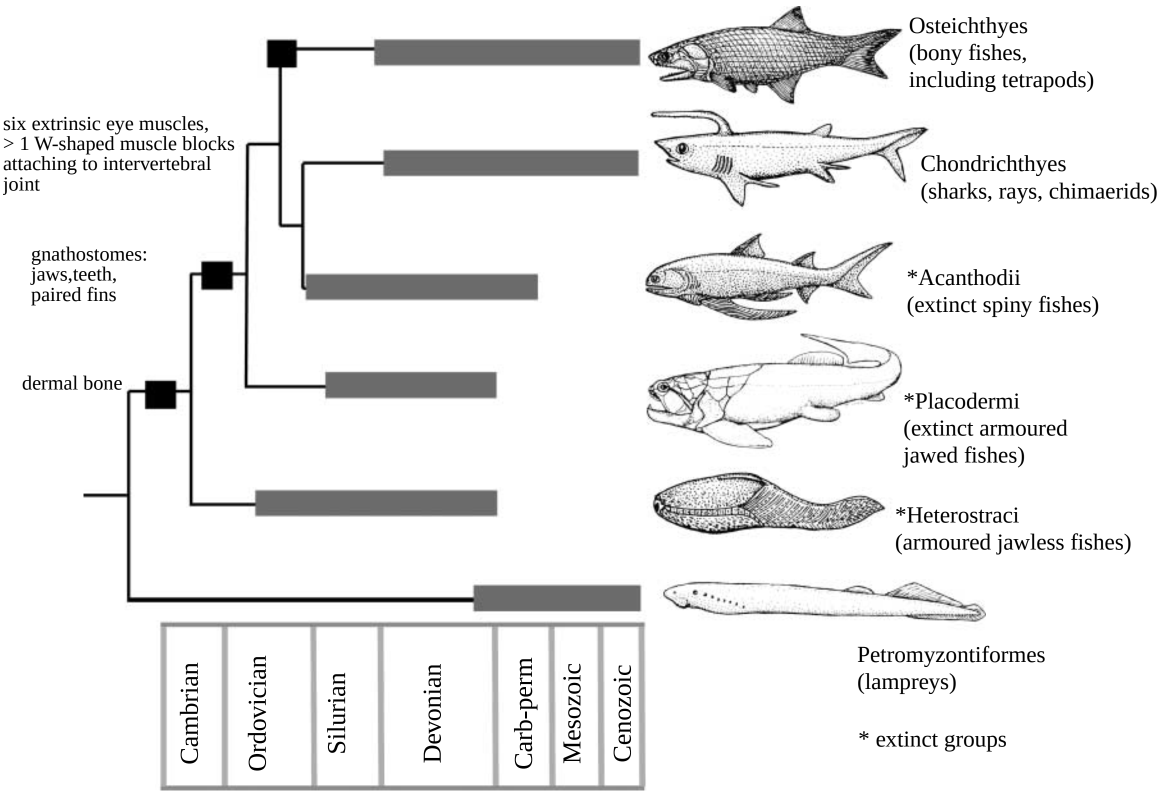

Figure 2. Phylogenetic tree supporting the hypothesis that placoderms are the sister group to all other gnathostomes. See table 1 for list of characters.

as well as the build up of CO$_2$ and volatile fatty acids with a resultant fall in pH, would have shifted the equilibrium of precipitation in favour of calcium phosphate rather than calcium carbonate (Briggs *et al.* 1993; Wilby 1993). If a drop in pH was sustained, due to closure caused by the overlying bone or sediment preventing diffusion, precipitation of calcium carbonate continues to be inhibited (Briggs & Kear 1993). There is discoloration of the upper portions of the calcium phosphate replacement indicating that a microbial film may have been present. This microbial film may have acted to concentrate and protect the phosphorus needed for the calcium phosphate aggregation and soft-tissue mineralization (Wilby *et al.* 1996).

## 3. MUSCLE ARCHITECTURE IN *AUSTROPTYCTODUS GARDINERI*

The ptyctodontid, *A. gardineri* (WAM 86.9.662; Long 1997), has a band of muscle preserved along the dorsal margin of the body above the vertebral column that retains approximate *in situ* position (figure 1*i*,*j*,*l*,*m*). Several myomeres are distinguished ventral to the notochord (figure 1*i*,*j*,*m*). Within each myomere, muscle fibres run parallel to the long axis of the body and are attached at either end to the myosepta (figure 1*l*). Their discrete nature probably resulted from internal fibre shrinkage and decay, which has been shown in decomposition experiments on *Branchiostoma* (Briggs & Kear 1993).

The architecture of the muscle blocks is 'W'-shaped, with one anteriorly and two posteriorly directed flexures, which is common to lampreys, hagfish, sharks and bony fish (figure 1*j*,*m*). The myomere curves posteriorly and then, just above the notochord, reverts back to an anterior direction with the centre of the W being low and rounded. The angle of the muscle flexure is more acute towards the tail. The dorsal portion of the myomere is anteriorly directed (figure 1*l*). The anteromedial extension connects each myoseptum to the next anterior neural arch and each spans one full vertebral segment. This differs from Osteichthyes, where each myomere extends across four to five vertebrae. No distinct horizontal septum is visible; however, the myosepta extended anteriorly at the boundary of the hypaxial and epaxial regions.

## 4. EVOLUTIONARY IMPLICATIONS

Character states of extant gnathostomes are defined by skeletal form and soft tissue characteristics. To date, lack of soft tissue data in placoderms has prevented their use, or their presence has been assumed in phylogenetic analyses. Comparing muscle structure and pattern of the Gogo placoderms with extant taxa indicates that the closest extant relative to the placoderm is the lamprey (figure 2, table 1). Both taxa have weak W-shaped myosepta; however, they lack the extensive development of the dorsal and the ventral limbs of the muscle blocks found in higher vertebrates. In addition, the absence of myelinated nerve fibre further supports a close relationship between placoderms and lampreys. These discoveries complement the recently described pattern of seven extrinsic eye muscle insertions for placoderms, a plesiomorphic condition also found in lampreys, although reduced to six eye muscle insertions in all

Table 1. List of characters: 1, mineralized endoskeleton; 2, jaws; 3, paired fins; 4, eyestalk; 5, trochlearis-innervated oblique eye muscle anterodorsal in position; 6, internal gill arch; 7, paired nasal sacs; 8, otoliths; 9, braincase; 10, ring of armour; 11, dermal fin spines; 12, fin rays; 13, prismatic cartilage; 14, W-shaped muscle; 15, anterior facing dorsal muscle limb; 16. hypaxial division of muscles and 17, chevron-shaped muscle blocks. (All character states coded as: 0, absent and 1, present.)

| taxa/character | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hyperoartia | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| Ostracoderm | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? |
| Placodermi | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| Chondrichthyes | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 |
| Acanthodii | 0 | 1 | 1 | ? | ? | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | ? | 1 | 1 |
| Osteichthyes | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 |

extant gnathostomes (Goujet & Young 2004). Other characters previously considered to be present only in placoderms and chondricthyans, such as the presence of a cartilaginous eye-stalk, are now known to be generalized gnathostome characters (Basden *et al.* 2000; Zhu *et al.* 2001).

Recent phylogenetic analysis suggests that conodonts are more derived than lampreys (Donoghue *et al.* 2000); however, as the architecture of conodont muscle blocks is V-shaped and no horizontal septum has been identified in any conodont, two reversals must have occurred within the conodonts to support their placement within a grouping of hagfish, lampreys and jawed fishes. Although, no horizontal septum has been identified in any of the Gogo Formation placoderms, the division of the myosepta at the boundary of the hypaxial and epaxial regions strongly suggests its presence. All of these developments herald further unforeseen discoveries concerning the soft-tissue anatomy of stem-gnathostomes and lungfish.

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