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Atlas of vertebrate decay: a visual and taphonomic guide to fossil interpretation

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Abstract: Like many other important evolutionary transitions, our knowledge of the origin of vertebrates is limited to windows of exceptional preservation of soft-bodied fossils. Unfortunately, these fossils are rare and have been subjected to complex taphonomic filters including decay, collapse and distortion. In order to maximize our ability to utilize these crucial fossils to reconstruct the timing and sequence of evolutionary events, we are in need of a robust taphonomic framework with which to interpret them. Here we report the results of a series of experiments designed to examine patterns of transformation and loss during decay of important anatomical characters of chordates and primitive vertebrates (ammocoete, adult lamprey, hagfish, juvenile chondrichthyans and a non-vertebrate chordate, *Branchiostoma*). Complex and repeated patterns of transformation during decay are identified and figured for informative character complexes including eyes, feeding apparatus, skull and brain, muscles, branchial apparatus, axial structures, viscera, heart and fins. The resulting data regarding character decay and relative loss serve as a guide to recognition and interpretation of the anatomy of non-biomineralized fossil vertebrates. The methods and techniques outlined are eminently applicable to other soft-bodied groups and present a new way to interpret the exceptionally preserved fossil record.

Key words: chordate, experimental decay, comparative taphonomy, anatomy.

THE origin of vertebrates represents a landmark event in the history of life on Earth. The evolution of vertebrates from non-vertebrate relatives is associated with huge increases in morphological complexity, developmental changes (neural crest) and genome duplication events (Aburomia *et al*. 2003; Donoghue and Purnell 2005; Holland and Chen 2001). The fossil record should bring light to bear on this episode by providing answers to important

questions such as: is the apparent morphological jump between extant invertebrate chordates and vertebrates real, or do fossils fill this gap, indicating more gradual evolution? When, and over what period of time did these events take place? In what sequence was the vertebrate body plan assembled? Thus it is the fossils from this part of the tree of life that have the potential to shed light on the nature of macro-evolutionary processes as well as the nature of our own origins.

Despite its great potential, however, the fossil record of these events is hard to read, and interpretations remain equivocal. Of the several fossil taxa that have been discussed in the context of vertebrate origins and early evolution, many have proved to be highly contentious in terms of interpretation of their anatomy (Donoghue and Purnell 2005; Sansom *et al.* 2010*a*). Yunnanozoans from the Cambrian of China are a salient example having been variously interpreted as stem-vertebrates (Mallatt and Chen 2003), stem-deuterostomes (Shu *et al*. 2001), early hemichordates (Shu *et al*. 1996) or even non-deuterostomes (Donoghue and Purnell 2009 for review). Equally, *Jamoytius* from the Silurian of Scotland has been interpreted as a primitive amphioxus-like vertebrate or as a 'naked anaspid' (i.e. stem-gnathostome; Sansom *et al*. 2010*b*), implying radically different interpretations of vertebrate evolution.

What is the cause of such conflicting interpretations? The first vertebrates and their chordate relatives were entirely soft-bodied, pre-dating the evolution of readily fossilizable biomineralized hard tissues such as bone and dentine. As such, fossil remains of these organisms are preserved only under those rare circumstances that allow exceptional preservation of soft tissues. The morphology of these crucial fossils has, therefore, inevitably been altered by the complex taphonomic processes of decay, collapse and distortion, both before, during, and even after, preservation. It is these very processes that have adversely affected our ability as palaeontologists to recognize fossil morphology, make homology statements via comparison with homologous structures in a range of modern anatomical comparators, and assess the evolutionary significance of these fossils (Donoghue and Purnell 2009; Janvier 1998; Sansom *et al.* 2010*a,* 2010*b*, 2011).

A solution to the problem of lack of clarity of interpretation of non-biomineralized organisms lies in the choice of morphological comparator. All too often, direct comparisons are made between the morphology of fossils and the pristine anatomy of living relatives. Pristine anatomy is not, however, a suitable comparison. Better data is needed about how the anatomy of living representatives is affected by decay (Briggs 1995, 2003). By experimentally unlocking the sequences of morphological change and loss during decay in

living anatomical comparators, it is possible to identify collapsed and partially decayed anatomy in fossils and to make the crucial distinction between taphonomic loss and phylogenetic absence of particular characters i.e. whether a morphological character has been lost through decay, or was never present in the organism in the first place. Only by making these identifications and distinctions will it be possible to constrain interpretations of the anatomy and phylogenetic affinity of early vertebrate and chordate fossils, and ultimately the circumstances surrounding the origin of vertebrates.

To provide the framework for the interpretations of the anatomy of non-biomineralized vertebrates, we performed a series of experiments to investigate the sequences of change and loss during decay of key anatomical features that characterize chordate and vertebrate clades in a range of extant comparators: modern jawless vertebrates (hagfish and lamprey – both larval and adult), a jawed vertebrate (the chondrichthyan dogfish – both embryonic and juvenile) and an invertebrate chordate (the cephalochordate, amphioxus). None of these organisms on their own makes an appropriate proxy for the early chordate or early vertebrate condition. Instead we focus on the decay of informative characters and character complexes observed in the range of conditions exhibited in modern comparators. Investigating in this way allows us to reconstruct patterns of decay that are common amongst, or distinguish between, the extant phylogenetic bracket i.e. making taphonomy comparative in the same sense of comparative anatomy. It is the combined knowledge of decay of chordate and vertebrate characters, not organisms, that enables us to reconstruct decay patterns and shed light on the anatomy and taphonomy of stem-chordate and early vertebrate fossils (*contra* Conway Morris and Caron 2012).

The sequence of change and loss of morphological complexes during decay are described and illustrated below i.e. feeding apparatuses, branchial apparatuses, eyes, muscles, axial structures, viscera and hearts, and fins. These data, summarized in Table 1, serve as a unique source for interpretation of putative fossil vertebrates by allowing robust comparative anatomy in light of taphonomy.

MATERIALS AND METHODS

In order to best capture the morphological variation observed in the non-biomineralized characters of interest, a range of extant chordates were chosen for decay – the cephalochordate Amphioxus (*Branchiostoma lanceolatum*), the Atlantic hagfish (*Myxine glutinosa*), the brook and river lampreys (*Lampetra planeri* and *Lampetra fluviatilis*), the

spiny dogfish (*Squalus acanthias*) and lesser-spotted catshark (*Scyliorhinus canicula*). Both the adult and larval lamprey (ammocoete hereafter) were investigated because they have very different anatomies, pre- and post-metamorphosis. Different stages (embryonic and pre-hatchling) were also used for the chondrichyans because of their different anatomies (e.g. external vs internal gills respectively). Whilst the Urochordata (tunicates) are widely seen as more closely related to the vertebrates than to the cephalochordata (Delsuc *et al*. 2006; Jeffries 1986), they remain an unsuitable anatomical comparator for non-biomineralized chordate characters due to their morphological and developmental specialization (Swalla and Smith 2008).

Experimental taphonomy is often performed at the scale of whole body changes which can be very informative for reconstructing the taphonomic history of particular fossils or the taphonomic thresholds of particular fossil yielding strata (Briggs 1995, 2003; Briggs and Kear 1994). In order to shed light on the evolutionary significance of an exceptionally-preserved soft-tissue fossil, it is necessary, however, to understand the taphonomic history of individual anatomical characters; it is these anatomical characters that are the unit of phylogenetic investigation and allow identification of the affinity of a fossil organism (Sansom *et al*. 2010*a*, 2011). Here, anatomical characters are grouped into eight categories: feeding apparatus (mouth, tentacles, teeth, associated cartilages and muscles), branchial apparatus (gill slits, openings, lamellae and support structures), eyes, muscles (principally trunk myomeres), axial structures (including notochord, dorsal nerve cord and vertebral elements), viscera (including heart, liver and gut) and fins (both paired and unpaired). The terminology for anatomical features follows that of Marinelli and Strenger (1954, 1956, 1959), Janvier (1996), Robson *et al*. (2000) and De Beer (1931).

The experimental methodology follows that of Sansom *et al*. (2010) for amphioxus and ammocoete and Sansom *et al.* (2011) for adult lamprey and hagfish. A minimum of 34 specimens of each (not three as stated by Turner *et al*. (2010) and Conway Morris and Caron (2012)) were incubated post-mortem at 25 degrees Celsius for a 60, 200 or 300 days. Specimens were terminally sampled by photography and dissection at approximately logarithmic intervals. Terminology of decay stages follows those defined by Sansom *et al.* (2010*a*, 2011). For chondrichthyans, the appropriate developmental stages were not readily available. Fertilized eggs were provided by University Marine Biological Station, Millport, Scotland (dogfish) and by Station Biologique de Roscoff, France (catshark). They were incubated in aerated artificial seawater at 18 degrees Celsius with weekly water changes. The dogfish were incubated for 60 days until they reached developmental stages 31 or 32 of

Ballard *et al*. (1993; approximately 35–50mm in length) whilst the catsharks were incubated for 120 days until they reached stages 32–34 (around 80–100mm in length). After incubation, the experimental methodology follows that of Sansom *et al.* (2010*a*, 2011). Four embryonic dogfish specimens (*Squalus*) were photographed only, with no dissection, over a period of 330 days of decay. Seventeen pre-hatchling stage catshark specimens (*Scyliorhinus*) were terminally sampled involving observation and photography of specimens whilst still inside their decay-containers, and, following specimen extraction, dissection for internal information. Illustrations of decayed anatomy are based upon these photographs in the same manner as would be appropriate for illustrations of fossil anatomy. Results are summarized in Table 1 and described in detail below.

TAPHONOMIC CHANGES OF CHARACTERS

Eyes

Amphioxus. Branchiostoma does not have eyes, but possesses an anterior pigment spot. In the very early stages of decay, this concentrated area of pigment ('eye spot'), becomes more diffuse (Fig. 1A, day 1). After becoming fainter, it is completely lost after 11 days of decay.

Lamprey. The simple paired eye spots of the ammocoete are difficult to observe externally, but during the early stages of decay, discolouration and bulging make them more visually apparent (Fig. 1D, day 1) before later loss.

In the adult lamprey, the paired, complex eyes become clouded within the first few days including the initially glassy lens (Fig. 1E). As decay progresses, the eyes remain as intact sacks of black pigment (presumably from the pigment layer at the back of the eye), which can become disarticulated but still sealed, with the lens inside (Fig. 1E, day 135). In the later stages of decay, the eye capsule ruptures and, if unconfined, the pigment can disperse. The only recognizable remains of the eyes are the cloudy, hard lenses which persists beyond 300 days of decay (Fig. 1E, day 296).

Hagfish. The eyes of *Myxine glutinosa*, unlike those of *Eptatretus*, are beneath a layer of skin and muscle and are only 500 microns in size, and as such, it has not been possible to make observations of hagfish eye decay in our experiments. Nevertheless, decay of comparable paired subcutaneous eye spots has been observed in the ammocoete.

Shark. Embryonic stage eyes are initially blood red in colour, but rapidly lose this colouration leaving a robust, black pigmented, open cup (Fig. 1B). Similarly, the pre-hatchling stage rapidly loses emerald pigmentation from the iris, but the black pigmentation associated with the cartilaginous sclera is retained well into the later stages of decay (Fig. 1C). The eye lens of the hatchling stage, like that of adult lamprey, becomes initially cloudy and persists into the later stages, but unlike lampreys, it does not retain its firmness and spherical shape.

Mouth and Feeding apparatus

Amphioxus. The mouth and feeding apparatus is composed of several components which differ in their comparative decay profiles. The velum apparatus is more decay prone than the other feeding structures, being lost before the oral tentacles (buccal cirri). Within the tentacles, the distal curved ends are the most decay prone, and disappear before the more robust connective base uniting them (Fig. 2A, day 15). The oral hood, between the velum and the buccal cirri, is simple in structure and undergoes little change, other than thinning, before eventual loss.

Lamprey. The ammocoete and adult lamprey have very different feeding apparatuses. The buccal tentacles of the ammocoete (not thought to be homologous to the buccal cirri of amphioxus (Yasui and Kaji 2008)) are extremely decay prone, being completely lost within three days (the tentacles are on the interior of the oral hood and therefore not shown in Figure 2). The small lower lip and velum are similarly decay prone. The velum's association with the branchial region means that it decays and loses detail at a similar timescale to the branchial region. The anterior oral hood is more decay resistant, often remaining articulated with the head – despite decay of the mouth and branchial region – although it loses its distinctiveness (Fig. 2B, day 90).

The oral tentacles (papillae) surrounding the oral disc of the adult lamprey are more decay resistant than the buccal tentacles and oral hood of the ammocoete (Fig. 2C, day 135). Of the cartilages associated with feeding, the annular cartilage encircling the mouth is the most robust and decay resistant (Fig. 2C day 135); the attached lingual spinosa cartilages are also decay resistant, and commonly remain attached to the annular cartilage until they decay away. The only other lingual cartilage that survives into the relatively later stages of decay is the piston cartilage (Fig. 2C, day 135). Prior to their loss, there is little anatomical

transformation of these cartilages other than disarticulation. The lingual musculature is only slightly more decay prone than the lingual cartilages. As in the ammocoete, the velum decays at the same time as the branchial region. Of the keratinous teeth, the lateral lingual teeth on the oral disc are disarticulated and lost relatively early, but the teeth associated with the annular cartilage persist well into the late stages of decay, even once the annular cartilage is lost (Fig. 2C, day 296; Sansom *et al.* 2010*a*).

Hagfish. The flesh surrounding the oral tentacles is lost very quickly, leaving exposed tentacle cartilages. Similarly, the velum cartilages are more decay resistant than the associated softer tissue, explaining the relative robustness of this character (Sansom *et al*. 2011, fig. 2). The difference between the rate of decay of the 'soft' and 'hard' cartilages causes consistent patterns of decay (Sansom *et al.* 2011). The majority of the dentigerous cartilage, which bears the teeth, is soft and lost to decay before the hard 'handlebar moustache shaped' posterior component (Fig. 2D, day 15). Similarly, the soft cartilage connecting the lingual cartilages is lost early leaving a consistent pattern of five disarticulated subrectagular cartilages, one with a notch (Fig. 2D, day 200). The lingual musculature is more decay resistant than other kinds of musculature. Its associated rod-like perpendicular cartilage is also very decay resistant. The white pulp of the keratinous teeth decays relatively early leaving a translucent sheath.

Shark. Anatomically, the jaws and teeth of Chondrichthyes are very different from the feeding apparatus of the other chordates analysed, but we used embryonic and hatchling stages which lack teeth of any kind; the pre-hatchling stage possesses Meckel's cartilage (lower jaw) and a pterygoquadrate (upper jaw). In early stages of decay, the cartilages of the upper and lower jaw become easily disarticulated but retain their characteristic shape (Fig. 2E, day 14), but later retain only a rough curved shape (Fig. 2E, day 36).

Skull and brain

Amphioxus. Cephalochordates do not possess any skull or brain tissues.

Lamprey. The trabeculae and otic capsules of the ammocoete skull are relatively more resistant than most other head tissues, including the mucocartilage tissues (a tissue type unique to ammocoetes) (Fig. 3A). The adult lamprey skull is more complex. The cartilaginous skull elements, including the basicranium, tectal cartilages and otic capsules, have similar

decay profiles undergoing softening, disarticulation and loss on a comparable timescale (Fig. 3B, day 135). Decay causes the otic capsules to appear as open cups. The otic capsules retain their three-dimensional shape throughout decay, unlike the eyes (see above) which collapse as sacks of pigment. The brain of the ammocoete and adult lamprey undergo the same sequence of liquefaction and loss of tissue leaving an empty brain cavity (Fig. 2B, day 8); this happens over different time scales in different ontogenetic stages.

Hagfish. The hagfish skull has an unique configuration which undergoes a complex sequence of transformation during decay. Decay begins at the anterior with the loss of the nasal tube, followed by nasal capsule, tentacle cartilages and more gracile cartilages to the posterior (e.g. velar cartilages) (Fig. 4A). The most decay resistant elements of the skull are the palatine and otic capsules (Fig. 3C, day 48). The connection between the notochord and skull and brain is fairly robust; they retain articulation beyond the point at which surrounding tissues have been lost (Fig. 3C, day 11). The brain of the hagfish is also surprisingly robust maintaining its shape, including lobes of the telencephalon and diencephalon, after the loss of surrounding soft tissues such as the skin and nasal apparatus (Fig. 3C, day 35, Fig. 4A). Although the overall structure of the skull changes during decay, as described above, the individual hard cartilage components retain their shape until loss.

Shark. The pre-hatchling stage dogfish has a skull quite unlike that of the adult stage (De Beer 1931, figs 21–22). In the early stages of decay, the articulated skull and brain are easily extracted (Fig. 3D, day 14). In later stages, the anterior portion of the skull (rostral process and nasal capsule) is lost, leaving paired otic capsules articulated with the vertebral column and trabecular cartilages extending anteriorly to form a fan-shape (Fig. 3D, day 22, Fig. 4B). Following this stage, the skull loses any recognisable features and becomes an undifferentiated mass of cartilages of limited structural integrity which nevertheless remains articulated with the vertebral column (Fig. 3D, days 56 and 91). The skull and brain have barely developed in the embryonic stage, which is therefore not an appropriate model for comparative decay of this anatomical complex.

Muscles

Amphioxus. During early decay, the V or chevron-shaped myomeres of *Branchiostoma* maintain their shape but shrink, with gaps opening between adjacent myomeres. The width of

the gap varies along the trunk, and as decay proceeds their width increases as myomeres shrink further (Fig. 3E, day 6; Fig. 4B; see also Briggs and Kear 1994). The orientation of the myomeres becomes more irregular as they lose their chevron shape (Fig. 3E, day 60; Fig. 4B). Complete loss of serially repeating myomere structure was not observed within the 60 day period over which *Branchiostoma* was decayed.

Lamprey. The ammocoete and adult lamprey myomeres undergo a similar pattern of decay. Initially, biofilms form around the myomeres with gaps in-between, thus replicating external topography (Fig. 3F–G, Fig. 4B). The strips of biofilm can become slightly offset from the corresponding myomeres underneath. Patches of fibrous muscle tissue are evident at the surface of the body, in gaps in the biofilm and skin (Fig. 3G, day 21). The ventral portion of the myomeres is weakened and lost to decay first, which can have the effect of causing the originally W-shaped myomeres to become, Z and then V-shaped. The W-shapes are relatively more decay resistant in the post-anal portion of the tail. The most decay resistant muscles are those flanking the notochord (see axial structures). In the ammocoete, the tissues of the myomeres can be lost almost completely, but their former disposition can still be observed because their boundaries are replicated through the pattern of pigment retained in the skin (Fig. 3F, day 200).

Hagfish. The ventral surface of the body of the hagfish deteriorates rapidly during decay. This transforms the W-shaped myomeres, first to Z-shaped and then V-shaped. The degradation of the ventral body surface and loss of ventral myomeres affects the trunk and posterior portion of the body first and can lead to coiling of this region. In the later stages of decay, only the dorsal portion of the myomeres towards the head are retained before complete loss.

Shark. The pattern of vascularization that reveals the W-shape of the myomeres of embryos is lost rapidly following death (within 9 days). Similarly, the myomeres of the pre-hatchling stage do not retain their shape well during decay, unlike those of its non-gnathostome relatives. Trunk myomeres surrounding the vertebral column are generally more robust than others (Fig. 3I, day 22). In addition, antero-posterior gaps can occasionally form between myomeres on the trunk exposing the vertebrae.

Branchial apparatus

Amphioxus. During the first few days of decay, the numerous postero-ventrally oriented gill slits become less oblique. The gill bars are relatively decay resistant, but some lose their shape leading to the appearance of 'gaps' in the series of gill slits (Fig. 5A, day 21, Fig. 6A). As decay progresses, the left and right gill bars can become separated leading to splaying of the branchial region (see also Sansom *et al*. 2010*a*, supplementary information).

Lamprey. The ammocoete and adult lamprey have significant differences in the arrangement of their branchial region but follow broadly similar patterns of decay. The branchial region softens and collapses relative to the more rigid head and trunk regions. Externally, decay results in the appearance of a postero-ventral 'line' connecting the seven circular gill openings (Fig. 5B–C, days 3, 15). In the adult lamprey, decay can cause the trematic rings which surround each gill opening to become more visually pronounced (Fig. 5C, day 50). The lateral walls of the branchial region, including the branchial cartilage, decay before the ventral or dorsal regions (Fig. 5C, day 50). Internally, the gill arrangement (pouches in the adult or pharyngeal cavity in the ammocoete, gill lamellae in both stages) is maintained even once the lateral branchial walls are lost.

Hagfish. The single external gill opening (oesophagocutaneous duct) and fatty connective tissue surrounding the gills of the hagfish are lost early. The numerous gill pouches, associated afferent ducts, and ventral aorta are more decay resistant and can persist even when surrounding supporting tissues (e.g. body wall) have been lost (Fig. 5D, day 4, Fig. 6A). Later, the soft branchial tissues are all lost to decay, but the cartilaginous branchial arches of the head persist, often articulated with the lingual cartilage (Fig. 6A).

Shark. The fine filamentous external gills of the embryonic stage are extremely labile and heavily degraded within 24 hours (Fig. 5E). Only the most proximal parts of the external gills persist, but are lost eventually. The pre-hatchling stage has a very different branchial apparatus consisting of 5 internal gill arches with cartilaginous supports. The gill lamellae are very labile (lost within three days), but less so than the external filaments of the embryonic stage. The cartilaginous gill arches are more resistant (Fig. 6A). In later decay stages (days 22–36) they lose their characteristic shape and become fibrous and harder to recognise (Fig. 5F, day 56).

Axial structures

The structure of the notochord varies between amphioxus, where the collagenous sheath is filled with discoidal muscle cells resembling stacked poker chips (Briggs and Kear 1994), and jawless vertebrates, where it is filled with large acellular vacuoles (Richardson and Wright 2003) or densely packed vacuolated epithelial cells (Welsch *et al.* 1998).

Amphioxus. The notochord of cephalochordates is unique in its extension to the anterior most point of the organism. It is, however, this rostral extension that decays earliest (Fig. 7A, day 15). The notochord along the trunk of the body, however, is relatively decay resistant, maintaining its structure beyond that of surrounding tissues (Fig. 7A, day 35, 60). Given the relatively small size and internal location of the dorsal nerve cord of *Branchiostoma*, it was very difficult to make observations of its decay. Nevertheless, sections of nerve cord were observed to persist in at least one specimen at day 60 (Fig. 7A).

Lamprey. The notochord of the ammocoete and adult lampreys is extremely decay resistant, persisting when all other tissues have decayed, especially toward the anterior. As it changes during decay, the initially clear filling of the notochord condenses into irregular serially repeating units (Fig. 6B, Fig. 7C, day 63). The notochord loses its rigidity, and collapses following loss of the liquefied contents, eventually leaving only the notochord sheath (Fig. 7B, day 130, Fig. 7C, day 93). The dorsal nerve cord is also decay resistant, but not as robust as the notochord. When exposed due to loss of surrounding tissues, it can become disarticulated (Fig. 7C, day 296). The muscles surrounding the notochord are also relatively decay resistant compared to other muscles, and remain firmer than other muscles for longer. In more advanced stages of decay, the trunk can appear to consist of three axial bands of equal height – a central lumen for the notochord flanked by two blocks of muscles which exhibit myomere 'banding' (Fig. 6B, Fig. 7B, day 28, Fig. 7C day 93).

Hagfish. The transparent contents of the notochord of hagfish also condenses to form a broken white line in the interior, akin to that of lampreys (Fig. 6B, Fig. 7D). The sheath is also the most resistant part of the notochord, surviving after the surrounding tissues have decayed and the notochord has collapsed. The dorsal nerve cord can lose its association with the notochord during decay and also develop a pattern of a broken white line (Fig. 6B, Fig. 7D, day 15). The dorsal trunk myomeres surrounding the notochord are relatively more decay resistant than other muscles, but not to the same extent as in lampreys.

Shark. The axial elements of the pre-hatchling stage consist of a notochord, dorsal nerve chord, dorsal aorta and vertebral elements. The dorsal aorta is lost within the first few days of decay. The dorsal nerve chord and neural arches surrounding it become softer and are lost to decay during later stages (Fig. 6B, Fig. 7E). The notochord sheath and centra surrounding the notochord, however, are extremely decay resistant, often being the only features of the decayed organism to survive extraction from their decay container. This leaves the appearance of a chain of cylinders with concave constrictions and a collapsed sheath in their centre uniting them (Fig. 6B, Fig. 7E, day 91). No observations were made on the decay of axial structures in the embryonic stage because it was not possible to collect internal data (due to size and limited specimen numbers).

Viscera

Amphioxus. The atriopore is rather short-lived and is lost during early decay stages (15 days; Fig. 8). The midgut-caecum (a potential homolog to the vertebrate liver) becomes more visible after the early stages of decay as the myomeres shrink. Latterly it breaks up (Fig. 2F, day 11) and is lost, but not before the gut. The gut also fragments and is often best visualised in the tail region. It was not possible to make observations about the decay of the subendostylar vessel, the only potential homolog of the vertebrate heart.

Lamprey. The liver of the ammocoete and adult lamprey undergo a similar sequence of decay. At the early stages, the liver softens and becomes oily. It can persist in this condition well into the later stages of decay (Fig. 8), maintaining its characteristic triangular shape and orange colour (Fig. 2G–H). The liver also becomes buoyant which can cause it to disarticulate from the body and float (Fig. 7G, day 11). The gut of the ammocoete follows a similar temporal pattern of change and loss as the liver. The gut of the adult lamprey is atriophied (the adult brook lamprey does not feed) making observations during decay less informative. The distinction between the atrium and ventricle of the heart is lost very quickly in both the ammocoete and the adult lamprey (3 and 11 days respectively) and the heart is lost altogether in early decay of the ammocoete. The adult lamprey heart is, however, relatively decay resistant remaining as a distinct unit, sometimes disarticulated, well into the late stages of decay. It is characterized by its size, shape, position and central ‘hole’ (Fig. 2H, days 35, 207). Unexpectedly, the heart of the adult lamprey is actually more decay resistant than the

pericardiac cartilage that surrounds it.

Hagfish. The large gut of the hagfish decays very quickly, being completely lost within two days. This is associated with loss of the ventral surface of the body, and thus the slime glands (Sansom *et al.* 2011). The different subunits of the liver decay at different rates. The gall bladder liquefies and is lost early, after which the anterior lobe of the liver is lost; the posterior lobe persists, however, into relatively later decay stages when it can become disarticulated (Fig. 2I, day 15). As in the lamprey, the liver becomes oily and buoyant, causing the body to float with the hepatic region as a centre of buoyancy. The main cardiac heart quickly loses structure and is lost completely within 6 days. The accessory hearts, near the liver, head and tail, are lost even earlier.

Shark. During development, pre-hatchlings transfer the contents of their external egg sac to an internal egg sac. For some specimens, this process was not complete so the external egg sac was manually removed before decay. In the early stages of decay, the ventral surface of the body ruptures exposing the viscera. Subsequently, the intestine with its spiral fold and the internal yolk sac are lost rapidly (Fig. 2J, days 0, 56). Unlike other parts of the viscera, parts of the liver can persist and become disarticulated (Fig. 2J, days 156). Often only one lobe of the liver persists, although it is not clear which. The heart of the pre-hatchling stage rapidly loses structure within the first few days and persists until day 56 in at least one specimen.

Fins

Amphioxus. The smaller, dorsal part of the simple caudal fin of *Branchiostoma* is the first to lose its shape during decay. The fin web is only lost completely following shrinkage of myomeres in the tail region (Fig. 1F, Fig. 9).

Lamprey. The fins of the ammocoete and adult lamprey undergo a similar sequence of decay. The smaller anterior dorsal fin is the first to collapse and decay, followed by the larger posterior dorsal fin. The posterior dorsal fin survives relatively longer in the adult lamprey stage, presumably because of the presence of extensive cartilaginous fin rays (Fig. 1H–I, Fig. 9). The caudal fin does not collapse in the same way as the dorsal fins, but undergoes shrinkage instead (Fig. 1H–I, Fig. 9) and may become patchy. Commonly, the characteristic tail shape of lampreys (directed ventrally *in vivo*) varied during death and decay, both

between specimens and over time for some specimens.

Hagfish. The skin surrounding the caudal fin is degraded within 24 hours of decay, but the fin retains its shape. After the skin has been lost, the cartilaginous fin rays are retained, and can persist into the later stages of decay (Fig. 1G, day 35, Fig. 9). Only the posterior-most fin rays, united at their proximal ends, remain articulated with the tail (Fig. 1G day 63, Fig. 9).

Shark. The developing median fin fold of the embryonic stage is altered within the first few days of decay leading to warping and loss of shape (Fig. 1J). The developing dorsal, caudal and paired fins remain visible until later stages. In the pre-hatchling stage, the collapse of the ventral surface of the body during decay can cause the paired fins (both pelvic and pectoral) to become disarticulated from the body (Fig. 1K, day 156). Patchy and cracked biofilms formed around the paired and unpaired fins. The caudal fin warps during decay becoming wavy, rather than remaining flat. Complete loss of the fins was not observed over the time scale of the experiments.

DISCUSSION AND CONCLUSIONS

The experiments reveal repeated and identifiable sequences of change, transformation, and loss of anatomical characters during decay. Conducting these trials on a range of different proxies allows identification of common patterns of change during decay through comparative taphonomy, comparative in the same sense as comparative anatomy i.e. identifying sequences of transformation shared in different groups of organism that likely represent the ancestral condition that would be observed in the stem-lineage of those groups. In addition to the detailed descriptions above, the results are summarized in Table 1 and Figure 8. Many characters exhibit complex taphonomic transformation series, with significant aspects of their shape, structural integrity, or arrangement changing over time as decay proceeds. For example, the notochord is initially stiff and three-dimensional; the infilling tissue initially condenses and breaks up (lamprey and hagfish) but eventually liquefies and disperses, leaving the notochord sheath as a hollow tube which collapses to form a flat structure with raised (apparently thickened) margins. Additionally, the cartilages of the vertebrate skull are observed to undergo repeated patterns of change whereby some connecting ‘soft’ elements are lost early whilst others retain their shape and can become disarticulated. A few characters

approximate to a binary condition of presence or absence whereby little change was observed in structure or shape before loss, or charactesr were lost relatively rapidly (e.g. branchial cartilage, heart symmetry, sensory lines). These data regarding character transformation and relative loss will serve as an invaluable tool for the interpretation of fossil non-biomineralized chordates and vertebrates. By observing and recording the sequence of decay-induced change in each character, partially decayed anatomy in fossils may now be more confidently recognised. Furthermore, it is now possible to understand how fossil anatomy might have been transformed allowing reconstruction of not only the original anatomy of the organism, but also the circumstances of the formation of that fossil and the amount of decay that fossil may have been exposed to prior to preservation.

For example, the acute, chevron-shaped segmental blocks observed in *Metaspriggina* (from the Cambrian of Canada) have been interpreted as myomeres (Conway Morris 2008), yet wide gaps are exhibited between them; wide gaps are not observed between the myomeres of any extant chordate, and they necessitate a different interpretation of myomere function and/or action. Reference to decay data indicates, however, that decay induced shrinkage of myomeres in amphioxus causes these structures to have the same appearance in terms of proportions and disposition as those in *Metaspriggina* (Figs 1F, 3E; see also Briggs and Kear 1994). From decay data we can therefore infer that *Metaspriggina* has not only been subjected to a certain amount of decay before, or during, preservation, but also that its phylogenetic affinities may be closer to that of cephalochordates rather than vertebrates because vertebrates do no exhibit the same pattern of myomere shrinkage during decay (Fig. 3F-I). The rest of the anatomy of *Metaspriggina* can therefore be interpreted in this light. A further example is *Myllokunmingia* from the Cambrian of China (also known as *Haikouichthys*) which exhibits well preserved gill pouches with gill filaments and cartilaginous elements of the branchial basket and arcualia (Shu *et al*. 1999, 2003; Hou *et al*. 2002). Decay experiments reveal that these vertebrate characters are relatively labile and lost early (Figs 5, 6, 8). We can therefore infer that some *Myllokunmingia* specimens have been preserved before major loss of characters to decay. Furthermore, given that the vertebrate braincase is relatively decay resistant, its absence in *Myllokunmingia*, which preserves other more labile cartilaginous tissues, can be interpreted as a true phylogenetic absence rather than taphonomic loss (Sansom *et al.* 2011). Decay data therefore indicate that based on the combination of presence and absence of vertebrate synapomorphies, *Myllokunmingia* is a stem-vertebrate in an evolutionary meaningful sense.

Ultimately, application of the new decay data in this way will allow constraints to be applied to anatomical interpretation and to phylogenetic affinity of crucial soft-bodied fossils and therefore better elucidation of the origin and early evolution of the vertebrates. The methods and techniques outlined are eminently applicable to other soft-bodied groups and demonstrate a new way to interpret the exceptionally preserved fossil record.

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EXPLANATIONS OF FIGURES AND TABLES

FIG. 1. Decay of eyes (A-E) and fins (F-K). A, amphioxus (white arrow for eye spot). B, embryonic dogfish eyes. C, pre-hatchling catshark. D, ammocoete (white arrow for eye spot). E, adult lamprey eyes with insets for extracted eye lenses and dissected pigmented eye sac. F, amphioxus caudal fin. G, hagfish caudal fin (extracted rather than *in situ*). H ammocoete dorsal and caudal fins. I, adult lamprey dorsal and caudal fins. J, embryonic dogfish dorsal, caudal, ventral and paired fins. K, pre-hatchling catshark dorsal, caudal, ventral and paired fins (disarticulated arrowed).
For this and all other photograph figures, numbers represent number of days of decay. Colour levels of images have been adjusted to improve balance without removing any data and some have been compiled from smaller images to improve resolution. For scale, green mesh apertures are 2mm x 2mm and background white grid is 10mm x 10mm.

FIG. 2. Decay of the feeding apparatus (A-E) and viscera (F-J). A, amphioxus. B, ammocoete. C, adult lamprey (lateral and ventral perspectives for day 0, otherwise lateral) with extracted annular and lingual cartilages (day 135) and keratinous teeth (days 135 and 296). D, hagfish in ventral perspective, external and dissected (day 0) with extracted cartilaginous and keratinous elements (day 15 and 200). E, catshark with extracted cartilaginous elements including Meckel's cartilage and palatoquadrate (day 14 and potential example at day 36). F, amphioxus. G, ammocoete. H, adult lamprey. I, hagfish. J, pre-hatchling catshark. White arrows indicate heart and black arrows indicate liver (midgut caecum in the case of amphioxus).

FIG. 3. Decay of the skull and brain (A-D) and myomeres (E-I). A, ammocoete with extracted trabecular cartilages (day 5) and dissection showing otic capsule (arrowed, day 8). B, adult lamprey from ventral perspective (sagittal dissection for day 8, extracted for day 35) with extracted head cartilages and teeth (day 135). C, hagfish (extracted, labelled in Fig. 4A). D, pre-hatchling catshark (extracted, labelled in Fig. 4A). E, amphioxus trunk. F, ammocoete trunk. G, adult lamprey trunk. H, hagfish trunk (external and dissected for day 0, otherwise extracted). I, pre-hatchling catshark (extracted, except day 36 *in situ*). Myomere figures are orientated with anterior to left, and where discernable, dorsal to top.

FIG. 4. A, Illustrations of decayed anatomy for skulls of hagfish and pre-hatchling catshark (relating to Fig. 3C-D). B, Illustrations of decayed anatomy for myomeres (relating to Fig. 3E-I).

FIG. 5. Decay of the branchial apparatus. A, amphioxus. B, ammocoete (upper row *in situ*, lower row dissected and extracted). C, adult lamprey (upper row external, lower row internal). D, hagfish (extracted and dissected). E, embryonic dogfish. F, pre-hatchling catshark (extracted and dissected). Structures are labelled in Fig. 6.

FIG. 6. A Illustrations of decayed anatomy of the branchial apparatus (relating to Fig. 5). B, Illustrations of decayed anatomy of axial structures (relating to Fig. 7).

FIG. 7. Decay of axial structures including notochord, dorsal nerve chord and vertebrae. A, amphioxus (rostral region for day 0 and day 15, trunk region for days 35 and 60). B, ammocoete (sagittal dissection day 0, otherwise extracted and dissected) labelled in Fig. 6B. C, adult lamprey (transverse dissection for day 0 and 28, lateral perspective otherwise, extracted days 63 and 93). D, hagfish (dissected and extracted). E, pre-hatchling catshark (dissected and extracted). Black arrows and white arrows indicate the notochord and dorsal nerve cord respectively; other structures are labelled on Fig. 6B.

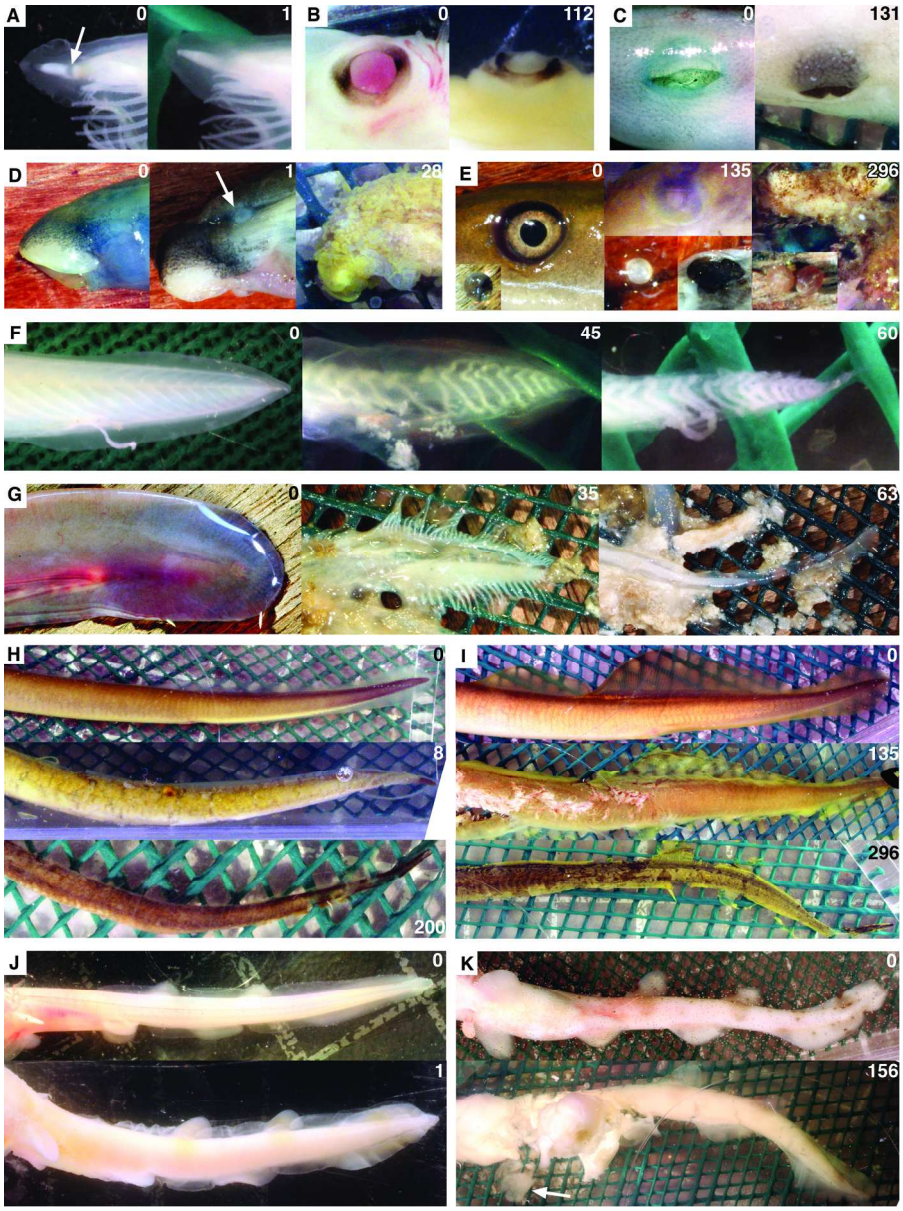
FIG. 8. Summary of decay sequences for amphioxus (A), adult lamprey (B), ammocoete (C) and hagfish (D). Observations of the decay of each character (yellow, pristine; orange, decaying; red, onset of loss; terminal point, complete loss) are used to rank them according to last occurrences. Blue indicates stages. Adapted from Sansom *et al*. (2010*a*, 2011).

FIG. 9. Illustrations of stages of decay of fins including caudal fins and median dorsal fins (relating to Fig 1F-K).

TABLE 1. Summarized results of the experimental decay for each character.

Eyes	Eye spots are more decay prone than complex eyes.
	Lenses, and especially pigment, persist after collapse and disarticulation.
Feeding apparatus	Sheath of keratinous teeth are very decay resistant, persisting into final stages.
	Harder cartilages (annular, lingual and potentially Meckel's) persist relative to other feeding apparatus.

Skull and Brain	The skull of lamprey, hagfish and dogfish undergo repeated patterns of transformation during decay. These taphonomic changes completely alter the appearance of the skull relative to the pre-decay condition.
Branchial apparatus	Amphioxus gill bars are more recalcitrant than cyclostome gill pouches which are more recalcitrant than external filaments. The branchial region of cyclostomes softens and laterally collapses during decay but pouch structure and gill lamellae (if present) can persist. External gills are more decay prone than internal gills, being lost within days.
Muscles	The ventral portion of myomeres are lost first which can result in transformation from W, to Z, to V-shaped myomeres. Relative recalcitrance of the muscles flanking the notochord can change apparent dimensions of myomeres. Decay can cause myomeres to shrink, and to appear irregular and offset.
Axial structures	Despite different histologies, the notochord undergoes the common pattern of liquifaction and loss of the central filling leaving just a sheath The notochord filling of adult lampreys and hagfish condenses to form an internal broken white line. The notochord, and to a lesser extent, the dorsal nerve cord are amongst the most recalcitrant features of chordates Of the vertebral elements, centra are much more robust than neural arches or arcualia.
Viscera	Ventral body surfaces rupture, exposing viscera. The heart quickly loses details of its arrangement and structure, but can persist as a feature until late in decay (in lamprey at least). The liver exudes oil and becomes buoyant in early decay stages with specific lobes persisting into late decay stages.
Fins	Presence of cartilaginous fin rays retards decay of fins. For lampreys and amphioxus, the caudal (and dorsal) fins can be lost before the adjacent body margin.



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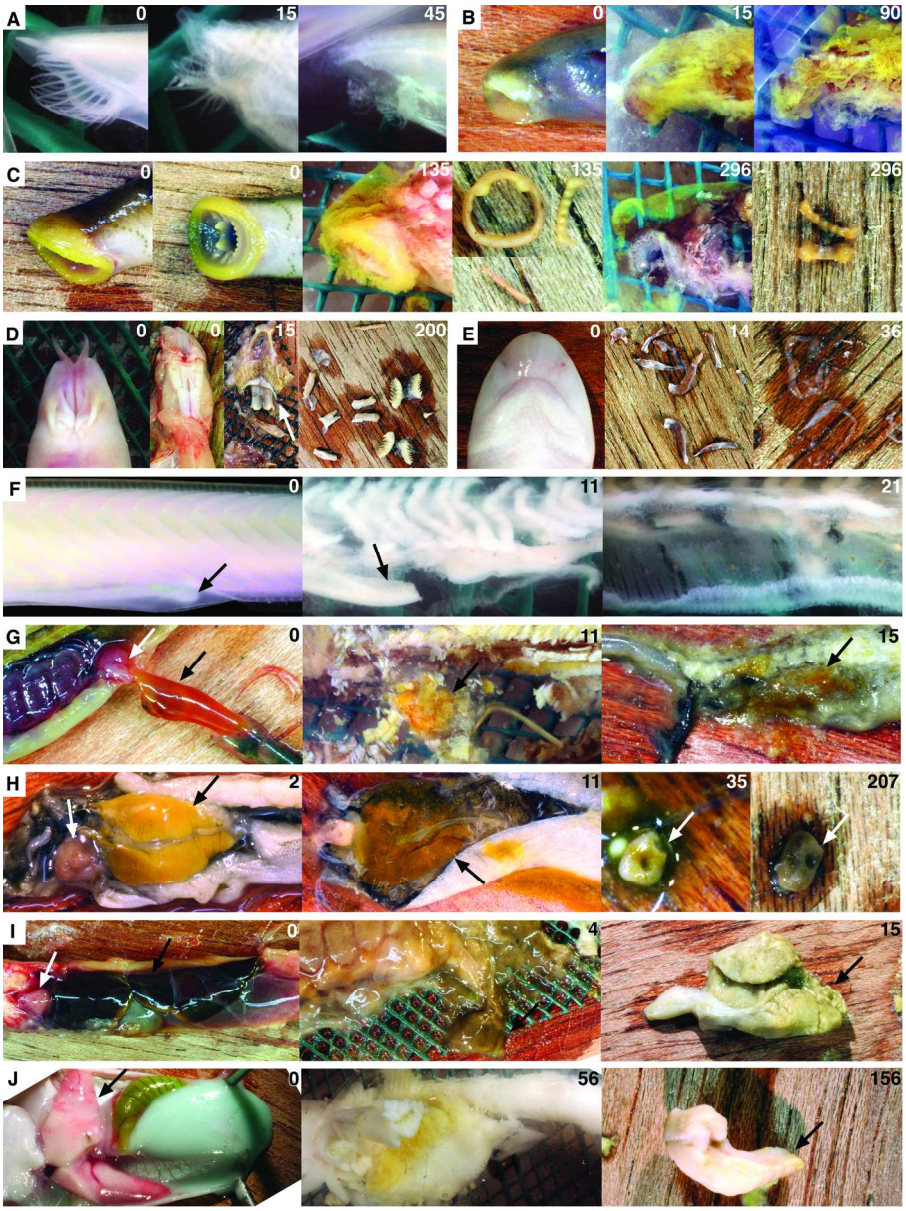

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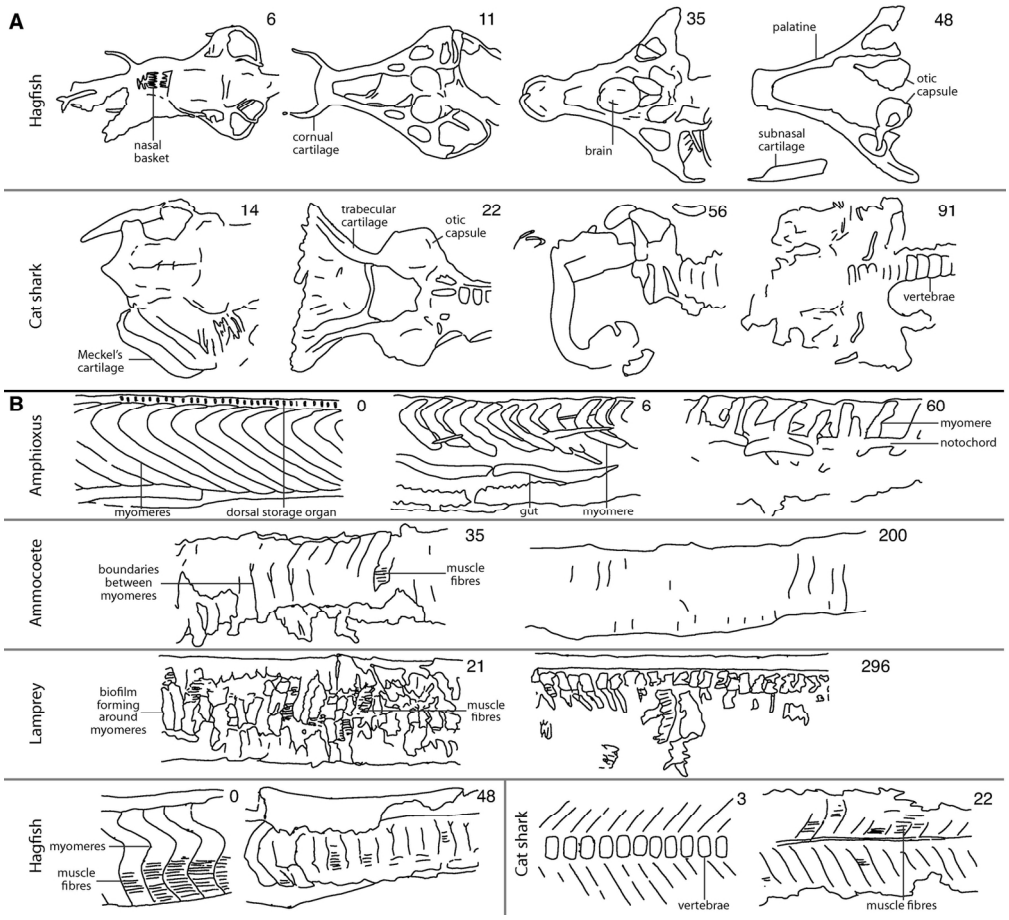

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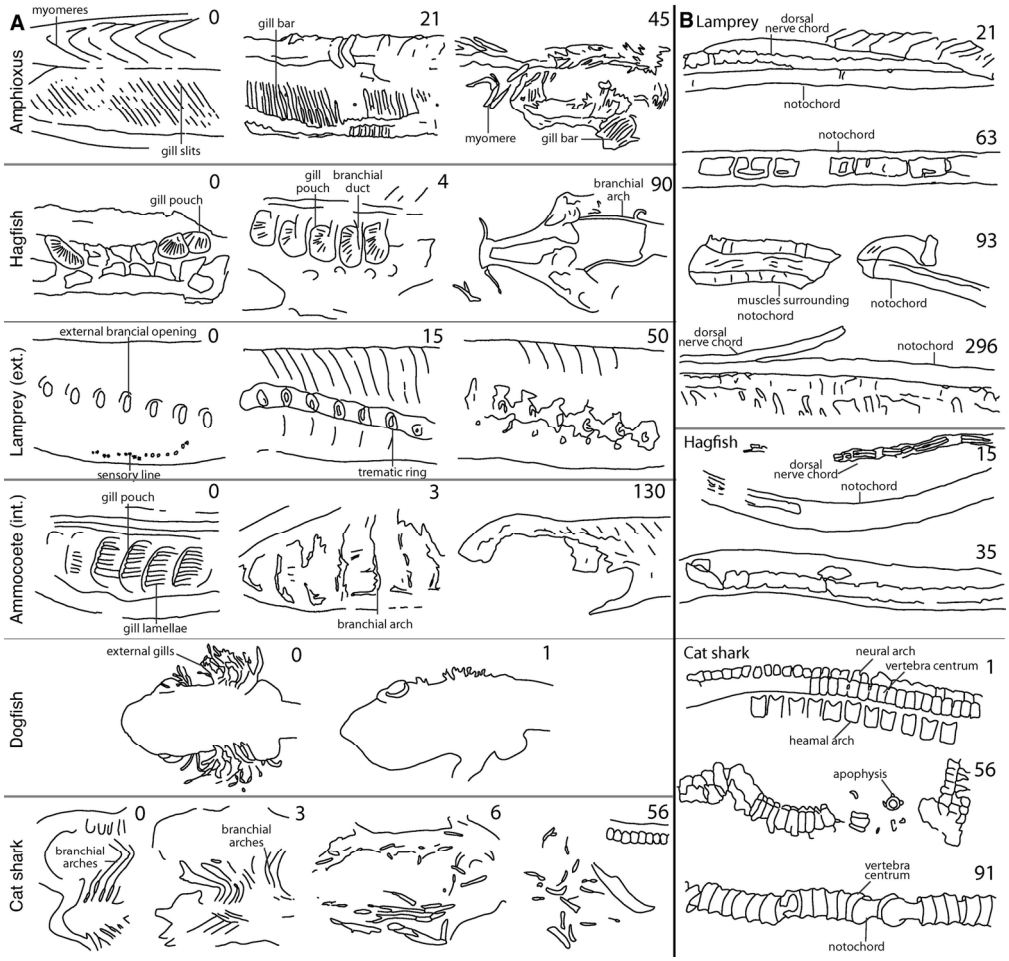

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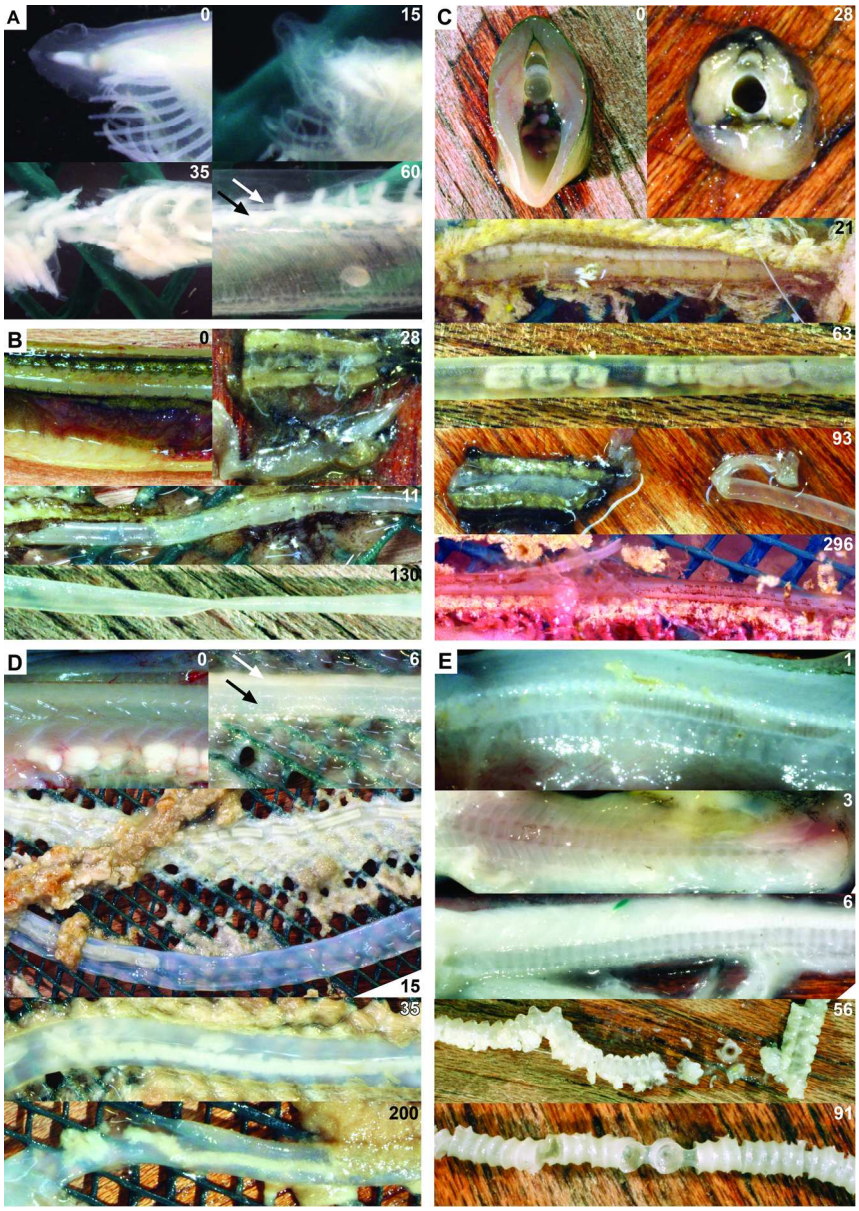

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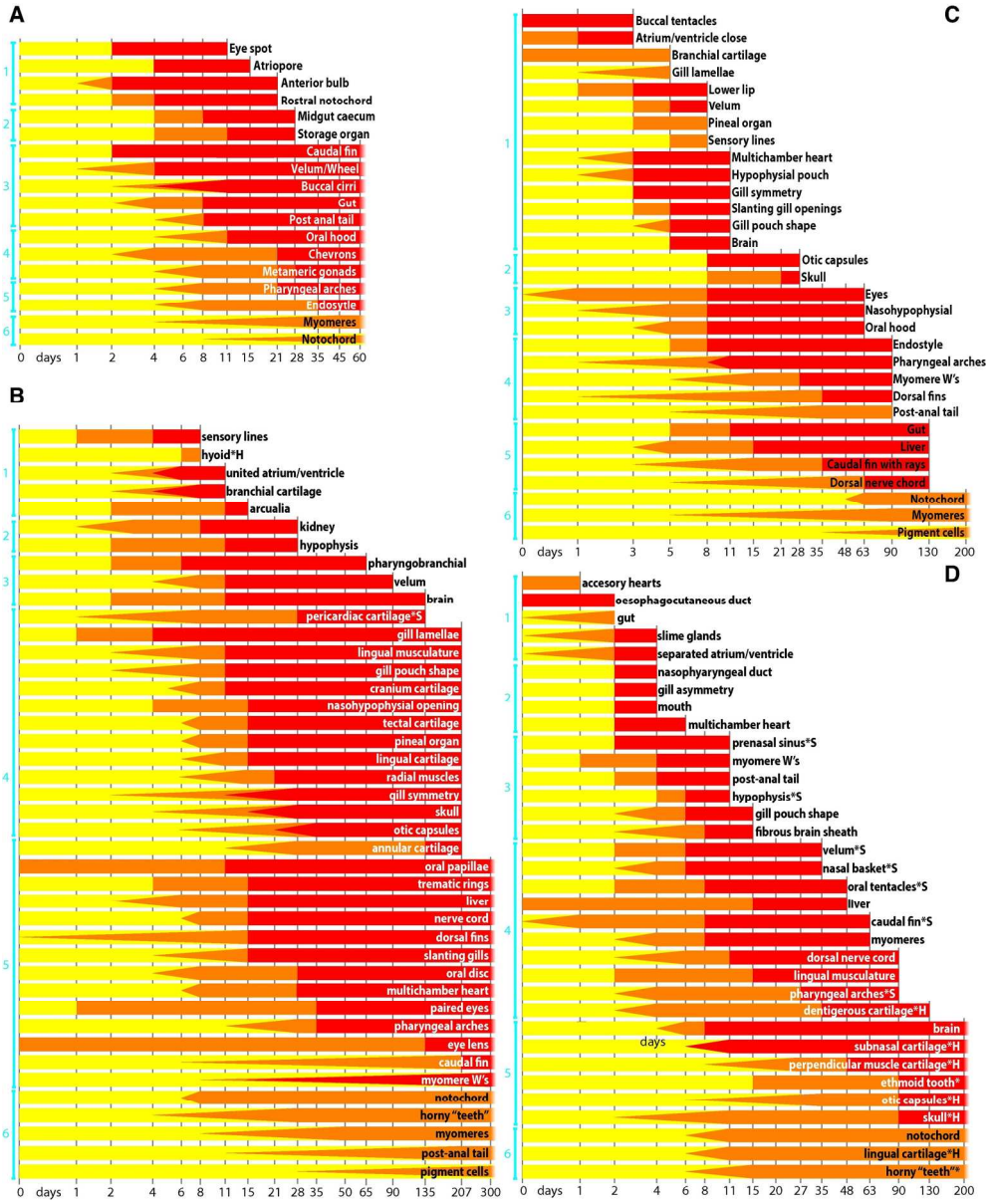

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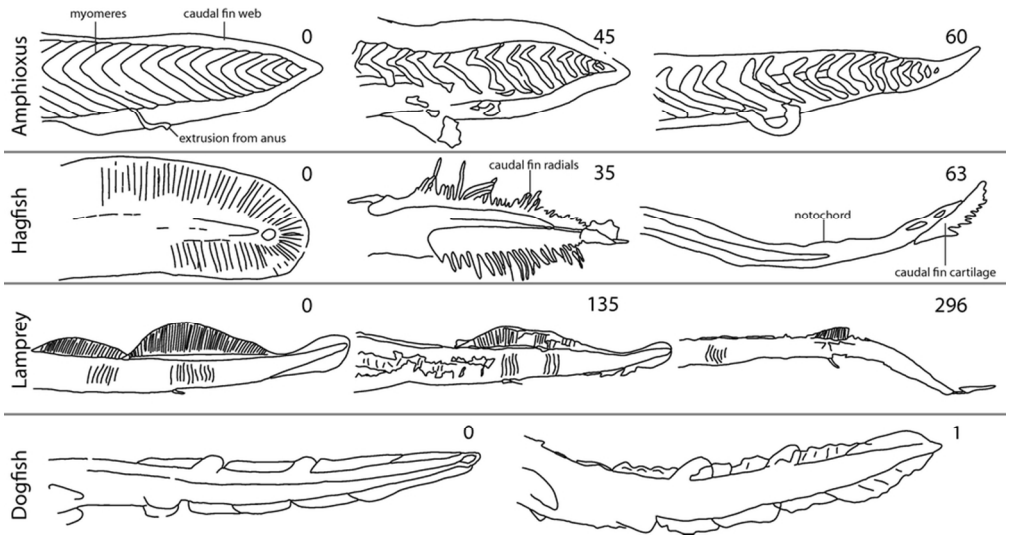

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