LETTERS

Non-random decay of chordate characters causes bias in fossil interpretation

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Exceptional preservation of soft-bodied Cambrian chordates provides our only direct information on the origin of vertebrates[1,2]. Fossil chordates from this interval offer crucial insights into how the distinctive body plan of vertebrates evolved, but reading this pre-biomineralization fossil record is fraught with difficulties, leading to controversial and contradictory interpretations[3,4]. The cause of these difficulties is taphonomic: we lack data on when and how important characters change as they decompose, resulting in a lack of constraint on anatomical interpretation and a failure to distinguish phylogenetic absence of characters from loss through decay[3]. Here we show, from experimental decay of amphioxus and ammocoetes, that loss of chordate characters during decay is non-random: the more phylogenetically informative are the most labile, whereas plesiomorphic characters are decay resistant. The taphonomic loss of synapomorphies and relatively higher preservation potential of chordate plesiomorphies will thus result in bias towards wrongly placing fossils on the chordate stem. Application of these data to *Cathaymyrus* (Cambrian period of China) and *Metaspriggina* (Cambrian period of Canada) highlights the difficulties: these fossils cannot be placed reliably in the chordate or vertebrate stem because they could represent the decayed remains of any non-biomineralized, total-group chordate. Preliminary data suggest that this decay filter also affects other groups of organisms and that 'stem-ward slippage' may be a widespread but currently unrecognized bias in our understanding of the early evolution of a number of phyla.

The fossil record offers unique and crucial insight into many important episodes in the evolution of life, and allows reconstruction of the extinct relatives that comprise the stem lineages of major extant clades and phyla (crown groups). Our view of the fossil record, however, is obscured by two persistent problems. First, most organisms are never preserved as fossils, and the patchy record provides only limited data from deep time. Second, even in cases in which exceptional preservation provides a more complete picture, fossils themselves are never anatomically complete or intact: they have been subject to the complex taphonomic processes and filters of decay and preservation. This degradation creates difficulties when comparing the morphology of fossils with the anatomy of extant relatives to identify the shared characters (synapomorphies) on which evolutionary analysis ultimately rests. Further complications arise because placement of fossils in the stems of extant clades reflects the absence of certain features; our failure to find a particular character in a fossil, however, may reflect post-mortem degradation of the original anatomical signal (taphonomic loss) rather than true evolutionary absence (where the fossil precedes the origin of the character). Failure to distinguish between the underlying causes of character absence will lead to erroneous evolutionary conclusions[3].

The main cause of these phylogenetic problems is not the patchiness of the fossil record, but our lack of taphonomic data to constrain anatomical interpretation and character coding in the fossils[3,4]. It is also implicitly assumed that anatomical loss through decay is random with respect to the phylogenetic informativeness of a character and affects characters independently. We have lacked the data required to test the validity of this assumption, but deviation from random and independent loss will clearly bias and distort our interpretation and understanding of the fossil record. Furthermore, identification of partially decayed remains requires knowledge not only of which characters decay rapidly and which persist, but also of how they decay.

Current understanding of the early evolution of chordates and the origin of vertebrates provides one of the most notable examples of the problems caused by taphonomic ambiguity. Fossil evidence from this interval has the potential to transform our understanding of the nature and timing of the fundamental episodes of initial body-plan development, increasing complexity and genome duplications, as it has done for the jawed vertebrates[2,5]. Continuing disagreement over interpretations of the fossils, however, means that no clear patterns or processes are currently discernable. Only a few fossil taxa have been discussed in the context of early chordate evolution, largely because they pre-date the advent of biomineralized hard tissues; the early chordate fossil record is consequently limited to rare instances of soft-tissue preservation. These soft-bodied fossils of purported chordates, such as *Metaspriggina*, *Pikaia*, *Cathaymyrus* and yunnanozoans from the Cambrian deposits of Canada and China (see, for example, refs 1, 6, 7), have proved to be highly contentious in terms of interpreting their anatomy. A broad range of phylogenetic affinities has been proposed for each taxon, and little consensus has been reached (Fig. 1a and Supplementary Fig. 1).

To constrain interpretation of the anatomies and affinities of soft-bodied chordates, we undertook a series of decay experiments using larval *Lampetra* (ammocoetes) and *Branchiostoma* (amphioxus). Of the extant chordates, urochordates and jawed vertebrates have undergone considerable genetic and morphological specialization[2,8] and the amphioxus (Cephalochordata) and the ammocoete (Vertebrata), with their relatively simple and comparable morphologies[9], are widely thought to be the best anatomical proxies for early chordates[8,10]. Both organisms have been used to make direct comparisons with purported fossil chordates[11,12].

Previous work in the field of experimental taphonomy (see, for example, refs 13–15), including some on *Branchiostoma*[16], has generally focused on the transformation of organisms as a whole rather than on individual character change, but we have developed a new approach based on analysis of the rate at which and sequence in which individual anatomical characters are transformed and lost during decay. The apomorphies of *Branchiostoma* and larval *Lampetra* were identified and categorised a priori in terms of the synapomorphies of the nested clades to which each belongs (for example Chordata, Cephalochordata or Vertebrata, Fig. 1b). We recorded how, and when, each of the

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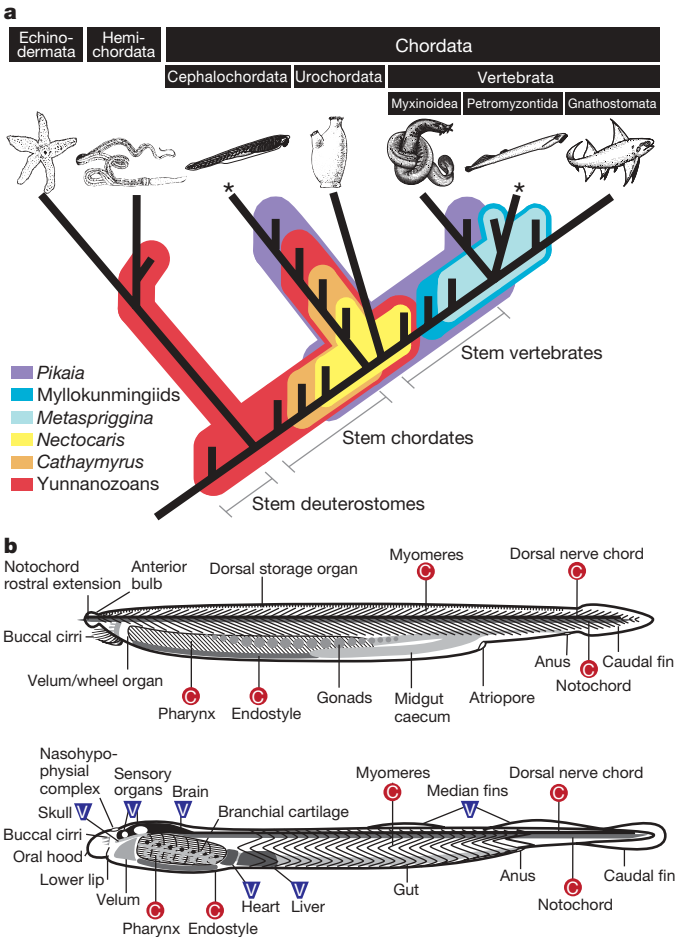

Figure 1 | Phylogeny and anatomy of chordates. **a**, Deuterostome phylogeny based on data from extant forms[7,29,30]. Asterisks mark the extant proxies used in the experiments. Each fossil taxon (coloured) has been proposed to have a wide range of affinities, with little consensus existing. References for each placement are available in Supplementary Information. **b**, Cephalochordate anatomy (top) and ammocoete anatomy (bottom). C, chordate synapomorphy; V, vertebrate synapomorphy.

synapomorphies decayed, allowing recognition of partially decayed characters, distinction between phylogenetic absence and taphonomic loss, and identification of patterns of loss.

For *Branchiostoma*, the general changes in pH, mass and state of organismal integrity through time (Supplementary Fig. 2), compare well with those documented in ref. 16, thus validating experimental taphonomy as replicable. Our quantitative data show that the rate of cumulative morphological character decay in both *Branchiostoma* and *Lampetra* follows the same sigmoidal pattern (Fig. 2c). Figure 2a, b shows the chronological sequences of character decay. Analysis of these sequences reveals a striking pattern: in both taxa, the first characters to decay are synapomorphies of relatively more derived clades (that is, cephalochordate synapomorphies for *Branchiostoma* and petromyzontid, cyclostome and [petromyzontid + gnathostome] synapomorphies for *Lampetra*). The more plesiomorphic characters (for example chordate notochord and myomeres) are the last to decay. Although there are some exceptions to this pattern, there is a very strong positive correlation between the rank of a character in the decay sequence and the phylogenetic rank at which a character is synapomorphic ($R_S = 0.75$ (Spearman's rank correlation coefficient) and $P = 0.0014$ for *Branchiostoma* (15 d.f.); $R_S = 0.71$ and $P = 0.000019$ for *Lampetra* (28 d.f.)).

Our results reveal two important patterns. First, homologous chordate characters in different taxa have comparable decay resistances, despite some fundamental structural differences (for example cephalochordate and vertebrate notochord and pharyngeal-arch histologies[1,16,17]). Second, they demonstrate that the loss of anatomical

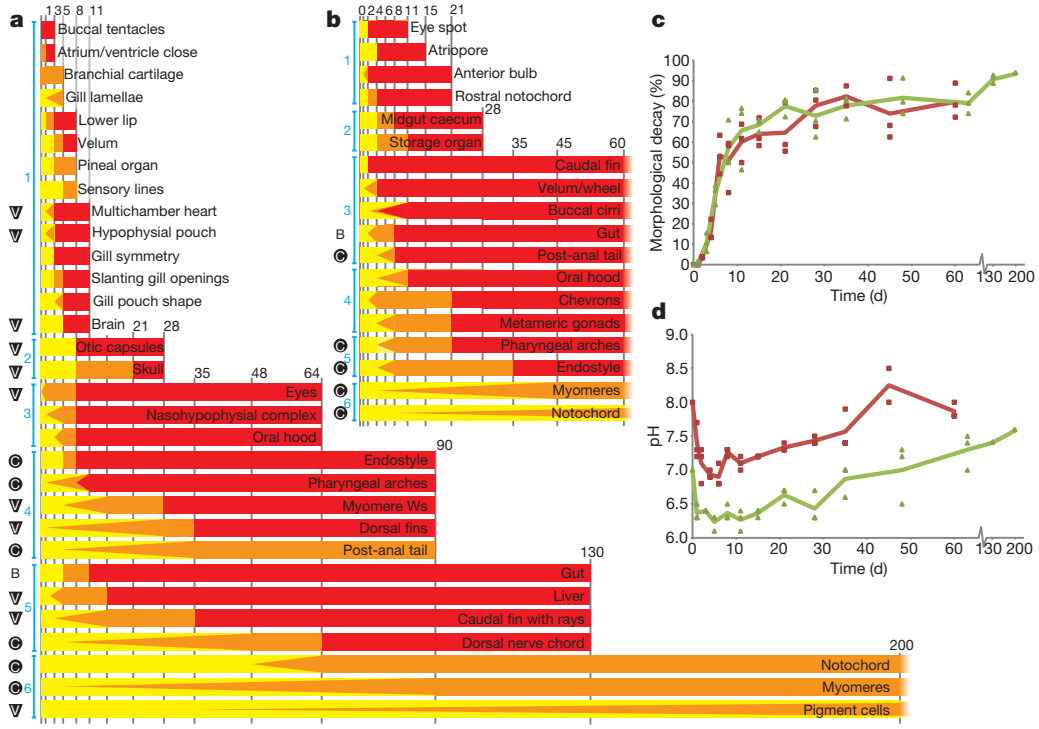

Figure 2 | Results of experimental decay. **a**, Anatomical characters of larval *Lampetra* ranked according to last occurrences in decay sequence (horizontal scale shows time in days). Observations are scored as pristine (yellow), decaying (orange), onset of loss (red) or complete loss (terminal point) (Methods Summary). Plesiomorphic characters are labelled for Chordata (C), Vertebrata (V) and Bilateria (B). Decay stages are indicated in blue. **b**, Character decay for adult *Branchiostoma* (as in **a**). 'Storage organ' has been historically termed 'fin rays'. **c**, Quantitative morphological decay through time for *Lampetra* (green) and *Branchiostoma* (red) (Methods Summary). **d**, pH changes through time for *Lampetra* (green) and *Branchiostoma* (red); initial pH differences reflect salinity. Lines connect means in **c** and **d**; d, days.

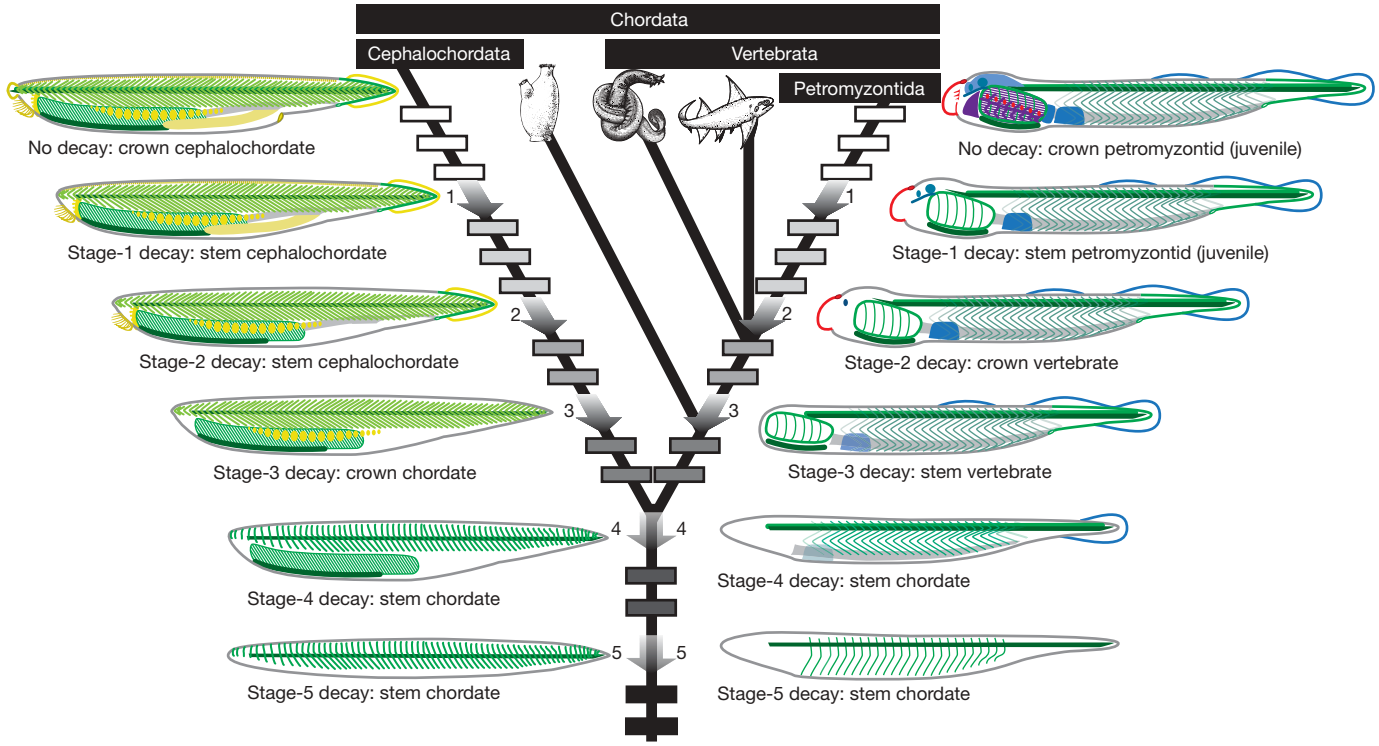

Figure 3 | Morphological decay stages of *Branchiostoma* (left) and larval *Lampetra* (right) and the phylogenetic position of each stage if interpreted as a fossil. Rectangles on branches of the phylogeny are morphological characters, their shade indicating order of loss (white, early; dark, late). As each organism decays, its phylogenetic position moves down the tree; thus, taphonomic bias exists in identification of fossil chordates. Characters are colour coded according to the hierarchical level for which they are informative (green, chordate; yellow, cephalochordate; blue, vertebrate; purple, cyclostome and vertebrate (*sensu* ref. 7); red, petromyzontid).

information during decay of chordates is non-random, which will affect the preservation potential of characters in the fossil record. The more phylogenetically informative anatomical features decay before the more plesiomorphic characters (that is, chordate synapomorphies). Significantly, this means that the fossil record will be biased towards preservation of symplesiomorphies because characters that decay very rapidly are generally less likely to be preserved, as the window of time during which preservation processes can act is of shorter duration. This is especially true for purported non-vertebrate fossil chordates, all of which are known from Burgess Shale-type deposits, in which the dominant mode of preservation of non-biomineralized anatomy is through stabilization of recalcitrant tissues as organic films[18–20]. These circumstances will strongly favour preservation of decay-resistant symplesiomorphies, and analysis of chordate remains will suggest phylogenetic placement of fossils in a more basal position than is correct (Fig. 3), potentially in the stem of the crown group to which they belong. Thus, our decay data strongly suggest that the fossil record of non-vertebrate chordates is skewed by a systematic bias of stem-ward slippage and should be reviewed in light of this.

Two examples illustrate our point. The fossil *Cathaymyrus* has characters interpreted as a notochord, pharyngeal 'striations', myomeres and few other informative anatomical features[21]. Parsimony therefore constrains its phylogenetic position as a stem chordate. However, when viewed in light of the decay bias we have identified, *Cathaymyrus* is comparable to *Branchiostoma* at an advanced stage of decay (Supplementary Fig. 3) and, as a result, the absence of more synapomorphic characters could be the result of non-preservation. A 'cone of phylogenetic uncertainty' therefore extends the range of potential placements of *Cathaymyrus* to encompass the crown groups of the non-biomineralized chordates (that is, cephalochordates, urochordates and juvenile cyclostomes; Fig. 1). *Metaspriggina* similarly exhibits stem-chordate characters only (chevron-shaped myomeres; the homology of apparently metameric anterior structures is uncertain)[7], but decay biases indicate that its true phylogenetic position could be much more derived (Supplementary Fig. 3). Our results therefore highlight the dangers inherent in assuming that placement of a non-biomineralized fossil in the stem of a major extant clade necessarily reveals significant information about the time of origin and patterns of character acquisition of that group. Putative fossil chordates that are placed in stem positions (stem chordate or stem vertebrate) because they possess only decay-resistant chordate characters (that is, they lack crown-group synapomorphies) must be treated with caution.

The prevalence of this decay bias of stem-ward slippage among clades that include soft-bodied fossils is unknown. Experimental decay data for the polychaete *Nereis*, for example, reveal that the most decay-resistant features are jaws and chaetae[15]. Previously these characters were considered to be phylogenetically informative, but questions have subsequently been raised about the utility of jaws and chaetae in polychaete and scolecodont (fossil polychaete) phylogeny and taxonomy as both are strongly tied to function[22,23]. More recent polychaete phylogenies indicate that it is the labile, soft-tissue characters that are more phylogenetically informative[24,25]; it is these characters that are the first to decay[15].

If this decay bias is widespread, the many important evolutionary episodes that are understood from the fossil record of exceptionally preserved soft-tissue remains will need careful reconsideration. More character-based decay analyses of more taxa are needed before we can develop widely applicable taphonomic models, but this new approach will help to constrain anatomical interpretations of what are currently the most controversial yet potentially critical fossils known. Every fossil represents the result of interactions between the processes of decay and the processes of preservation. Decay data show clear associations of characters that have similar decay resistances and are consequently lost at about the same time. Fossils that seem to preserve an ensemble of anatomical characters that are lost at very different stages of decay, and that lack associated characters of comparable decay resistance, are therefore worth closer scrutiny:

either their anatomical interpretation requires revision or each fossil reflects multiple phases of exceptional preservation of greater complexity than current models suggest. Determining which of these possibilities is the more parsimonious is contingent on our understanding of patterns of character decay.

METHODS SUMMARY

Adult *Branchiostoma lanceolatum* specimens (0.10–0.40 g) were collected from coastal waters at Argelès-sur-Mer, France[26]; we collected juvenile *Lampetra fluviatilis* specimens (0.38–2.39 g) from the River Ure, North Yorkshire, UK. All were killed by overdose of tricaine methanesulphonate (MS222; 2 mg ml^{-1} with buffer), which does not adversely affect bacteria[27]. Specimens were placed in individual 48-cm^3 or 182-cm^3 clear, polystyrene containers that were filled with standard artificial sea water (filtered, deionised water only for *Lampetra*) and had plastic mesh floor to facilitate extraction. The box lids were sealed closed with silicon grease (Ambersil M494). Oxygen saturation, irrespective of its initial value, converges rapidly upon anoxia[15,28]. In the case of *Branchiostoma*, decay follows the same stages under anoxic diffusion conditions as under free diffusion conditions[16]. We incubated the boxes at 25 ± 1.0 °C in cooled incubators for up to 200 days. Trials at 15 °C and 25 °C for *Branchiostoma* showed that temperature affected only the rate of decay, not the sequence or pattern of character loss.

We destructively sampled three individuals of each species at intervals that were varied to capture rapid early decay and later, slower stages (Fig. 2). The decay of each anatomical character (Fig. 2) for each individual animal was observed visually and through dissection, and scored according to three defined categories of morphological decay: pristine (same condition as at death; score 0), decaying (morphology altered from that of original; score 0.5) or lost (no longer observable or recognizable as the morphological feature; score 1). We calculated the percentage morphological decay (Fig. 2c) from these cumulative character scorings.

The correlation between the hierarchical level at which an anatomical character is synapomorphic and the rank of that character in the sequence of loss through decay was assessed through Spearman's correlation coefficient of decay ranks (Fig. 2a, b) and synapomorphy ranks (for *Branchiostoma*, synapomorphies of Cephalochordata rank 0, Chordata rank 1; for *Lampetra*, synapomorphies of Petromyzontida rank 0, Cyclostomata rank 1, Vertebrata (*sensu* ref. 7) rank 1, Craniata rank 2, Chordata rank 3; each rescaled for ties).

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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