Donoghue, P. (2017). Evolution: divining the nature of the ancestral vertebrate. *Current Biology*, *27*(7), R277-R279. https://doi.org/10.1016/j.cub.2017.02.029

DISPATCH

Evolution: Divining the Nature of the Ancestral Vertebrate (au: is this correct? It was originally spelled 'Diving')

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Inferences of the ancestral vertebrate are increasingly complex because the previously understudied cyclostomes have been revealed as simplified and specialised. New research uncovers another ancestral vertebrate character, resolving a century of debate over whether the ancestral vertebrate bore gills.

We are all vertebrates, distinguished from our spineless invertebrate kin by the possession of boney vertebrae, but we still possess (**au:ok?)** their cartilaginous evolutionary rudiments — among a vast swathe of other anatomical characters, including paired sense organs and a differentiated brain. Divining **(au: ok?)** the origin of vertebrates and, therefore, the nature of the ancestral vertebrate, has been one of the most popular games in evolutionary biology. However, the rules of the game have changed over time, along with changing perspectives on the most primitive living vertebrates and our nearest living invertebrate relative.

For ever such a long time, cephalochordates were identified as the nearest invertebrate relative of vertebrates, and the vertebrate bodyplan was considered metaphorically (if not literally), to be an elaboration of this simple chassis, principally through the addition of a new head. This view was confused when tunicates were resolved as the closest relatives of vertebrates (Figure 1). Similarly, the most primitive of living vertebrates have been identified among the living cyclostomes, the hagfishes and lampreys. Both possess a bilaterally acting keratinous feeding apparatus that is taken by some to betray their kinship to the exclusion of jawed vertebrates — the living 'gnathostome' group, which includes sharks, boney fishes and ourselves (cyclostome monophyly). However, both hagfish and lampreys share mutually exclusive features with jawed vertebrates, suggesting a close relationship with jawed vertebrates to the exclusion of the other cyclostome lineage (cyclostome paraphyly).

Classically, anatomical evidence has been interpreted to support cyclostome paraphyly, uniting lampreys and jawed vertebrates to the exclusion of hagfish, which are identified as the most primitive vertebrate lineage. Indeed, some argued that hagfish should be cast from the vertebrates altogether, and they have long served as a model representing a half-way stage to vertebrate supremacy. Hagfish eyes, for instance, are simple, lacking a lens, cornea, intra- and extra-ocular muscles, and they have been used as a model for understanding the evolutionary assembly of the eye from invertebrate chordate pigment spots to the vertebrate camera eye [1]. However, others view the hagfish eye, which in some species is less sensitive to light than the animal's cloaca [2], as vestigial [3].

Support for hagfish degeneracy is found in molecular phylogenetics, which invariably recovers cyclostome monophyly. Indeed, cyclostome monophyly

versus paraphyly is one of the iconic examples of phylogenetic conflict between molecular and morphological evidence [4]. However, morphological support for cyclostome paraphyly has waned recently. This has occurred principally because most of the morphological evidence has been recycled by phylogeneticists long past its use-by date [5]. For instance, hagfish were perceived to lack the adaptive immune system of lampreys and jawed vertebrates, but it has since (**au:ok?)** been shown that cyclostomes share an adaptive immune system that is distinct from the immunoglobulin-based system of jawed vertebrates [6].

In other ways, morphological support for cyclostome paraphyly has diminished because of new insights into cyclostome biology. Lampreys were long recognised to possess dorsal cartilaginous vertebrae-like rudiments, suggesting a closer relationship to gnathostomes. However, hagfish embryology has revealed that these organisms also possess cartilaginous structures similar to (**au:ok?)** vertebrae, though they are ventral in position [7]. Together, this evidence suggests that the two lineages of cyclostomes are mutually degenerate, and the ancestral vertebrate was more akin to living gnathostomes in possessing both dorsal and ventral vertebral rudiments that have been lost in a mutually exclusive manner in the hagfish and lamprey lineages [7]. These and other insights have emerged as a consequence of the return to fashion of cyclostomes as evolutionary models for early vertebrates, after a century of neglect [8]. It comes as something of a surprise to discover then, that some of our misconceptions of the ancestral vertebrate are rooted in misconceptions of the biology of jawed vertebrates, on which most research has been lavished.

Published recently in *Current Biology*, Gillis and Tidswell [9] report on their fate-mapping analysis of gill development in the little skate, *Leucoraja erinacea*. This study is significant because of a long-standing controversy concerning the distinct endodermal and ectodermal embryonic origin of gills in cyclostomes and gnathostomes, respectively. Indeed, this distinction has been marshalled as one of the few anatomical characters that support the monophyly of cyclostomes [10], considered sufficiently significant that cyclostomes and gnathostomes have been perceived to have evolved from an ancestral vertebrate lacking gills [10]. The development of cyclostome gills, from out-pocketing of foregut endoderm, has been well characterised classically, but the development of gnathostome gills is less clear. Gills have been described as developing from pharyngeal ectoderm in sturgeon [11] and lungfish [12], but zebrafish gills develop from endoderm [13], and various authors have argued for at least some endodermal contribution [10,14–18].

In an attempt to resolve the primitive gnathostome condition, Gillis and Tidswell [9] studied gill development in the little skate, a chondrichthyan and, therefore, an outgroup to the boney vertebrates in which gnathostome gill development has previously been investigated. In comparing gill development in chondrichthyans and osteichthyans, similarities are likely inherited characteristics from the crown ancestor of all gnathostomes. Using a lipophilic dye to track the fate of early pharyngeal endoderm, Gillis and Tidswell [9] injected the dye into to the pharyngeal cavity prior to gill slit perforation, precluding contamination with pharyngeal ectoderm. The internal and external

gill filaments that subsequently developed were labelled with the dye, indicating that they are derived from pharyngeal endoderm, as in cyclostomes.

So, after decades of attempts to explain away the apparently fundamental embryological distinction between the gills of cyclostomes and gnathostomes, it appears that at least primitively, they have a common endodermal origin after all. Thus, misgivings concerning their common evolutionary origin in the ancestral vertebrate can be dismissed. Like so many differences between hagfishes, lampreys, and jawed vertebrates, these characters have to be reinterpreted to have evolved in the vertebrate stem-lineage, broadening the already wide gulf in bodyplan complexity between living vertebrates and their invertebrate chordate relatives [5]. Ultimately, this makes attempts to explain the assembly of the vertebrate bodyplan all the more challenging.

Does the origin of vertebrates reflect a major evolutionary leap? Or does it betray as vestigial the bodyplans of invertebrate chordates, since they are surely simpler than those of the last common ancestor shared with tunicates (clade Olfactores) and cephalochordates (phylum Chordata). To be sure, the tunicate bodyplan is much reduced, mirroring the wholesale reduction in its genome [19]. The fossil record provides little help since the characters that might discriminate stem-vertebrates from stem-Olfactores, are embryological and, therefore, have little chance of preservation. And the picture is further confused by the peculiar manner in which chordate organisms decay, with derived characters capitulating to autolytic and microbial processes before more primitive characters, making the phylogenetic interpretation of fossil remains challenging [20]. Thus, attempts to elucidate the assembly of the vertebrate bodyplan must rest with experiments like these from Gillis and Tisdwell [9], attempting to find cryptic 'vertebrate' characters among our spineless kin.

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Figure 1: Current understanding of the interrelationships of deuterostomes, including chordates, olfactores, vertebrates, cyclostomes and gnathostomes.

Gill slits (pharyngeal pores) evolved first in the deuterostome stem-lineage. Gillis and Tisdwell [9] resolve debate over the homology of cyclostome and gnathostome gills, adding yet another character to the long inventory that distinguishes vertebrates from their invertebrate relatives.

In Brief
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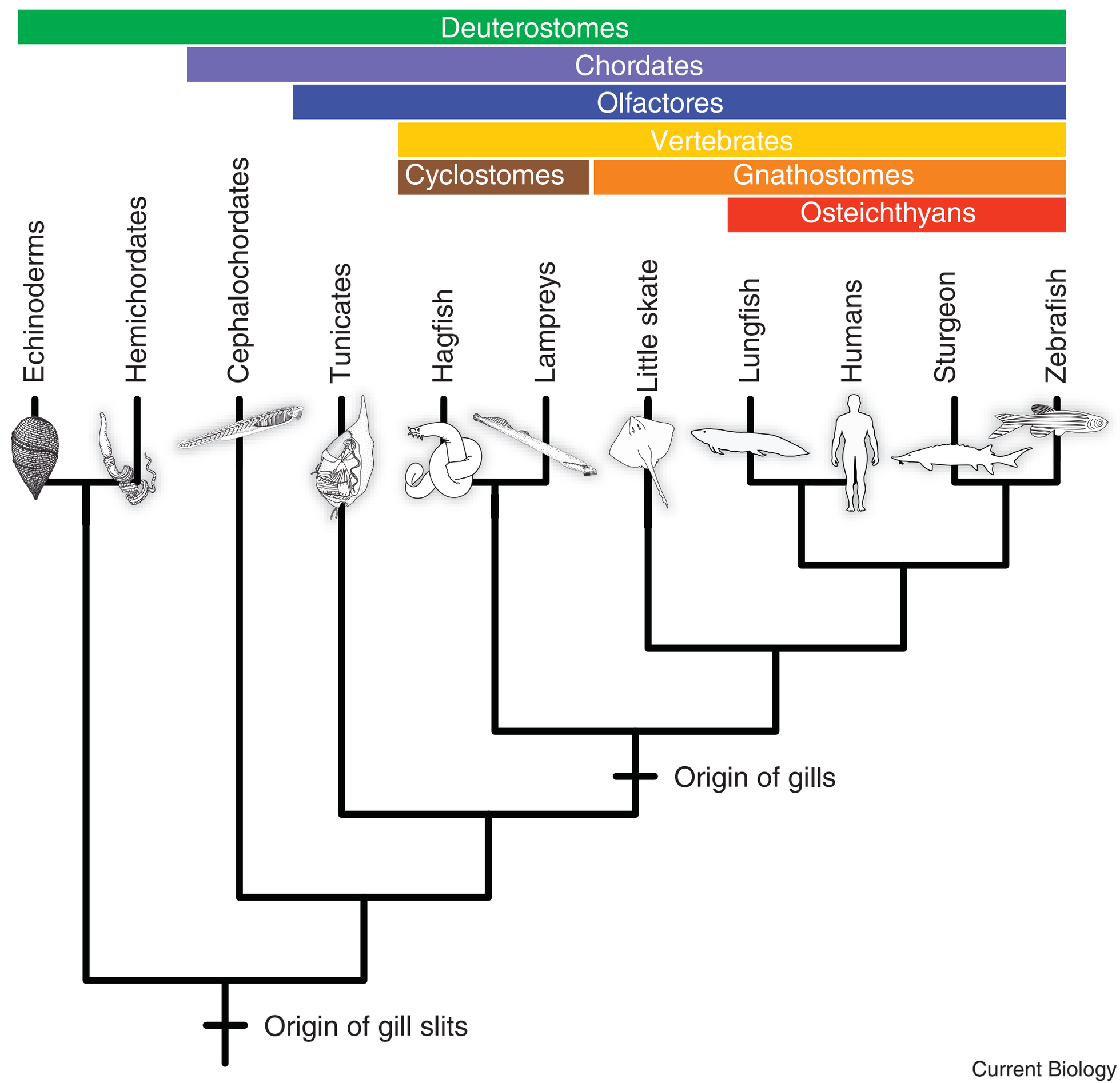
Deuterostomes
Chordates
Olfactores
Vertebrates
Cyclostomes
Gnathostomes
Osteichthyans
Echinoderms
Hemichordates
Cephalochordates
Tunicates
Hagfish
Lampreys
Little skate
Lungfish
Humans
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Origin of gills
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