

microRNAs revive old views about jawless vertebrate divergence and evolution

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Most living vertebrates are jawed vertebrates or gnathostomes, like sharks or mammals. However, two small fish groups, hagfishes (67 species) and lampreys (about 40 species), are devoid of jaws and are either ignored by the public or regarded as pests. Both are eel-shaped, cartilaginous, and scale-less, and their overall anatomy is roughly similar to that of other fishes, with a brain, spinal cord, and sensory capsule, although more simple than that of gnathostomes (Fig. 1). First regarded as intestinal worms or degenerate jawed vertebrates (1) and then gathered into a group, the cyclostomes (rounded mouth), on the basis of basic anatomical characteristics (horny teeth, pouch-shaped gills, single nostril, and lack of paired fins) (2), these fishes became finally considered in evolutionary times as a monophyletic offshoot of primitive vertebrates, sister group to jawed vertebrates (3), and this became the received view for most of the of the 20th century (4).

In the late 1970s and with the rise of the cladistic principles for assessing phylogenetic relationships on the basis of phenotypic characters, it was suggested that cyclostomes might be paraphyletic; that is, they are a basal grade in the vertebrate tree, with lampreys being more closely related to gnathostomes than to hagfishes (5). This phylogenetic pattern implied that cyclostomes could throw light on the early steps of the assembly of the vertebrate body plan and that hagfishes could document the most generalized condition for a number of vertebrate characters (6). However, it soon raised heated debates, because an increasingly large number of molecular sequence data provided increasingly strong support for the old theory of cyclostomes monophyly (7, 8); that is, hagfishes and lampreys were actually sister groups that had diverged in the early Paleozoic, up to 500 million years (Myr) ago. Morphologists who defended cyclostome paraphyly argued that molecular sequence-based trees were inconclusive because of the uncertainty as to the outgroups of the vertebrates (i.e., their closest relatives and either tunicates or amphioxus) (Fig. 1) or because of biases in the methods of molecular tree reconstruction that were at odds with the original standard parsimony methods

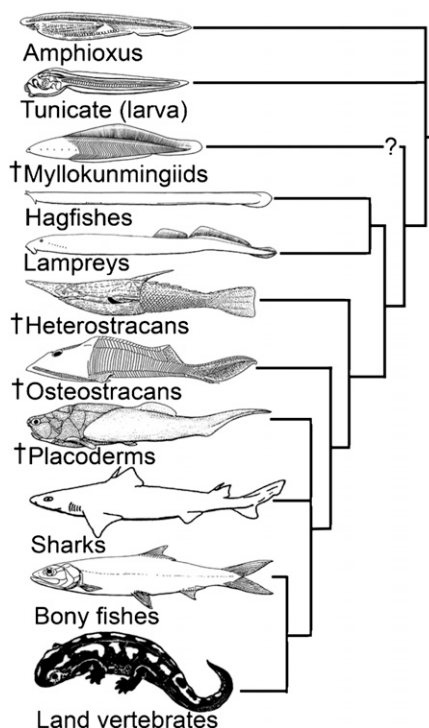


Fig. 1. Evidence for monophyly of the cyclostomes (hagfishes and lampreys) based on microRNAs distribution and expression profiles among vertebrates deprives phylogeneticists from the possibility to explore the stepwise assembly of the vertebrate body plan on the sole basis of phenotypic characters of these living jawless vertebrates. The pattern of the chordate tree now returns to what it was over a century ago, except for the relationships of extinct groups, such as the jawless ostracoderms (e.g., heterostracans and osteostracans) and the jawed placoderms. The investigations by Heimberg et al. (11) on microRNAs suggest that the cyclostomes, particularly hagfishes, underwent a spectacular degeneracy since their divergence from other vertebrates (that is, the loss of numerous phenotypic characters) and that the most recent common ancestor of the vertebrates was probably more complex than the living cyclostomes. This discovery may again question the status of the ostracoderms as jawless stem gnathostomes. †, Palaeozoic groups.

of cladistics (9). Clearly, a third independent source of characters was needed to resolve this conflict, and much hope was put in developmental data for deciding whether hagfishes were actually primitive and had lost many characteristics since their divergence from other vertebrates (10).

The report by Heimberg et al. (11) in PNAS provides the application of this

hopeful third source of data [that is, microRNAs (miRNAs), which are highly conserved, noncoding genes that can be treated in datasets as presence/absence, like most phenotypic characters, and analyzed with the same parsimony algorithms, with the additional advantage of being rarely lost in evolution. Moreover, miRNAs are regulatory genes that are strongly involved in cell differentiation and thus play a key role in organogenesis during development. Although the use of miRNAs for resolving deep divergences between major animal groups is still in its infancy, one can foresee that it will soon provide a powerful source of data for elucidating phylogenetic patterns and throw light on the developmental and metabolic processes involved during major evolutionary divergences and phenotypic transformations. The authors (11) show that the expression profiles of a number of miRNAs in various organs of the embryos of lampreys and a jawed vertebrates (zebrafish) are basically similar, thereby supporting their homology (some of their homologs in hagfishes are present, but their expression profiles are still unknown). They also provide the most extensive critical review of the phenotypic (anatomical and physiological) characters that were long used for supporting cyclostome paraphyly and show that many of them were spurious, wrongly coded, or inapplicable to the putative outgroups of the vertebrates. Their work notably shows that hagfishes and lampreys share four unique miRNA families, which is enough to provide the strongest possible support to cyclostomes monophyly (Fig. 1). The results of Heimberg et al. (11) are certainly the most convincing contribution ever published in support of cyclostomes monophyly, and we can foresee that they will soon be complemented by miRNA expression profiles from the long elusive, but now available, hagfish embryos (12).

Although I was among the early supporters of vertebrate paraphyly (6, 7), I am impressed by the evidence provided by Heimberg et al. (11) and prepared to

Author contributions: P.J. wrote the paper.

The author declares no conflict of interest.

See companion article on page 19379.

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admit that cyclostomes are, in fact, monophyletic. The consequence is that they may tell us little, if anything, about the dawn of vertebrate evolution, except that the intuitions of 19th century zoologists were correct in assuming that these odd vertebrates (notably, hagfishes) are strongly degenerate and have lost many characters over time (2, 4). This is an unprecedented case of character loss among living vertebrates. Unfortunately, fossil cyclostomes are of no help in documenting this degeneracy. They are mere soft tissue imprints dated to 360 Myr for lampreys and possibly, 300 Myr for hagfishes, and they are tentatively interpreted as roughly similar to modern forms.

Heimberg et al. (11) conclude that the latest common ancestor of all vertebrates may have been phenotypically more complex than living cyclostomes. Interestingly, this echoes the opinion expressed long ago by some paleontologists (13), who supported the theory that lampreys and hagfishes were derived from heavily armored and ossified Paleozoic jawless fishes referred to as ostracoderms, such as het-

erostracans or osteostracans (Fig. 1), but are now regarded as stem gnathostomes (14, 15); that is, they are more closely related to jawed vertebrates than to cyclostomes. These authors even alluded to the possibility that all jawless vertebrates, fossil and recent, could have been derived, through many character losses, from a

Hagfishes and lampreys share four unique miRNA families.

common ancestor that was morphologically more similar to a jawed than a jawless vertebrate (13).

Current vertebrate phenotype-based phylogenies nevertheless assume that the lack of jaws and mineralized tissues is primitive for vertebrates (14, 15) and that cyclostomes, be they monophyletic or not, diverged in the Early Palaeozoic from a naked and soft-bodied common vertebrate ancestor. Mineralized tissues (bone

and dentine) are assumed to have arisen in vertebrates about 500–480 Myr ago among the jawless stem gnathostomes, such as conodonts and early ostracoderms (15). The earliest presumed Paleozoic vertebrate fossils, such as the Cambrian myllokunmingiids (Fig. 1) (16), and the later presumed soft-bodied vertebrates, such as euconodonts and euphaneropids (13, 14), cannot be clearly proven to be stem cyclostomes. Nor is there any evidence left that cyclostomes are derived from any ostracoderm group through loss of characters. The contribution by Heimberg et al. (11) perhaps marks the end of the dream that, in common with the phylogeny of other groups, living jawless vertebrates could document the stepwise rise of the vertebrate body plan. However, although it brings us back to very old evolutionary views, it has the merit to show that systematic theories are refutable, all the more when character losses can be justified by biological processes and are, in turn, experimentally refutable.

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