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the **TIMETREE** *of* **LIFE**

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Jawless fishes (Cyclostomata)

Shigehiro Kuraku^{a,b,*}, Kinya G. Ota^a, and Shigeru Kuratani^a

^aLaboratory for Evolutionary Morphology, RIKEN Center for Developmental Biology, 2-2-3 Minatojima-minami, Chuo-ku, Kobe 650-0047, Japan; ^bLehrstuhl für Zoologie und Evolutionsbiologie, Department of Biology, University of Konstanz, Universitätsstrasse 10, D-78457 Konstanz, Germany

*To whom correspondence should be addressed (shigehiro.kuraku@uni-konstanz.de)

Abstract

Cyclostomata comprises two families of living jawless fishes: hagfishes (Myxiniidae, 44 species) and lampreys (Petromyzonidae, 41 species). Morphological analyses have favored the closer relationship of lampreys to jawed vertebrates (gnathostomes) than to hagfishes. However, most of the recent molecular phylogenetic analyses have supported a hagfish–lamprey relationship. The estimated divergence time for hagfishes and lampreys among several studies averages 482 million years ago (Ma), but varies (520–432 Ma) depending mostly on the assumed timing of the cyclostome–gnathostome divergence. Nonetheless, there is agreement that hagfish and lamprey lineages diverged relatively shortly (within 100 million years) after the divergence of cyclostomes and gnathostomes.

Cyclostomata consists of two extant orders, Myxiniiformes and Petromyzoniformes (1). Myxiniiformes contains a single family, Myxiniidae that includes 44 species in six genera (2) (hagfishes; Fig. 1). Petromyzoniformes also consists of a single family, Petromyzonidae that includes 41 species in six genera (3) (lampreys, Fig. 1). Historically, hagfishes and lampreys have been classified as cyclostomes (“round mouth”) because both have a jawless mouth armed with retractable horny teeth (4). However, some morphological traits in hagfishes (e.g., lack of vertebrae, heart innervation, and eye lens) have been taken to suggest that lampreys are more closely related to jawed vertebrates (gnathostomes) than to hagfishes (5). To settle this controversy, molecular phylogenetic studies have been conducted since the early 1990s using ribosomal DNA genes (6, 7), mitochondrial

genes (8–11), and protein-coding genes of the nuclear genomes (12–16). Here we review the phylogenetic relationships and the divergence time of the two families of Cyclostomata.

Most molecular studies to date have concluded that Cyclostomata is a monophyletic group (see 17 and references therein). These findings suggest that many primitive morphological traits found in hagfishes are just due to secondary losses in the hagfish lineage, or that the presumed primitive nature of the hagfish morphology is due to the unavailability of detailed morphological description, as recently exemplified by hagfish embryonic morphology (18). Rediscovery of cyclostome monophyly can be regarded as one of the most outstanding examples in which molecular phylogenetics has resolved controversial phylogenetic relationships among major animal groups (19). With this rediscovery, researchers can now estimate the timing of hagfish–lamprey divergence using molecular sequence information.

Phylogenetic studies have revealed that the hagfish lineage exhibits a long branch, suggesting elevation of evolutionary rate at the molecular level in this lineage (e.g., 17). For this reason, application of local clock analysis is expected to improve the precision of divergence time estimation, especially in this animal group. Using a lineage-specific method, the hagfish–lamprey divergence was estimated to be 499 (536–462) Ma in an analysis of seven nuclear genes (20). A subsequent Bayesian analysis of 25 nuclear genes resulted in a slightly older estimate of 520 (596–461) Ma (16). In this study, the minimum time constraint for hagfish–lamprey divergence was incorporated based on fossil-based discoveries of extinct relatives



Fig. 1 Two Japanese lampreys (*Lethenteron japonicum*) and an inshore hagfish (*Eptatretus burgeri*, tangled). Credit: S. Kuraku and K. G. Ota.

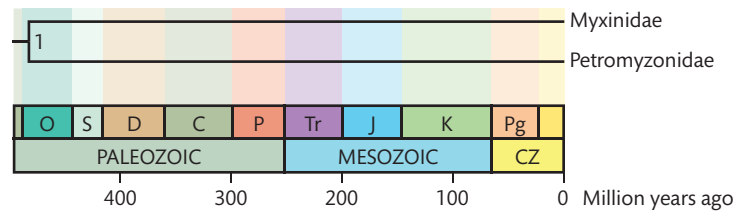


Fig. 2 A timetree of jawless fishes (Cyclostomata). Divergence times are shown in Table 1. *Abbreviations:* C (Carboniferous), CZ (Cenozoic), D (Devonian), J (Jurassic), K (Cretaceous), O (Ordovician), P (Permian), Pg (Paleogene), S (Silurian), and Tr (Triassic).

Table 1. Divergence times (Ma) and their confidence/credibility intervals (CI) between jawless fishes (Cyclostomata).

Timetree		Estimates							
Node	Time	Ref. (16)		Ref. (17)(a)		Ref. (17)(b)		Ref. (20)	
		Time	CI	Time	CI	Time	CI	Time	CI
1	482.3	520.0	596–461	432.0	473–391	478.0	497–459	499.0	536–462

Note: Node times in the timetree represent the mean of time estimates from different studies. From ref. (17), estimates are presented from (a) nuclear and (b) mitochondrial data.

of modern hagfishes and lampreys (325 Ma; 21 and references therein). In a more recent study, the divergence time of hagfishes and lampreys was estimated using different sets of genes (nuclear genes and mitochondrial genes) and calibration dates (molecules and fossils) (17). Application of molecular estimates (16, 22) as the maximum and minimum time constraints resulted in dates that are similar to the previous estimate (520 Ma; 16) or much more ancient (612 Ma) (data not shown in Table 1; see 17 for details). When the cyclostome–gnathostome split is constrained at 500 Ma, however, the analysis resulted in a 432 (473–391) Ma date based on 10 nuclear genes and 478 (497–459) Ma date based on 12 mitochondrial genes for the hagfish–lamprey divergence (17). Since those studies were completed, the fossil record of lampreys has been extended into the late Devonian (~360 Ma) (23), which is 11% older than the calibration date that was used for the divergence of hagfishes and lampreys (16).

While the estimates of hagfish–lamprey divergence times are highly variable, depending on the calibrations used, all available studies unequivocally suggest that the ancestors of Myxinidae (hagfishes) and Petromyzonidae (lampreys) diverged relatively soon (less than 100 million years) after the ancestors of cyclostomes split from the gnathostome lineage.

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