Mitogenomics and the sister of Salmonidae

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Salmon, trout, char, grayling, whitefish and ciscoes are all members of Family Salmonidae and, whilst the monophyly of this group is not in doubt, determining which fishes are their closest relatives is a different story altogether. A new topology based on a mitogenomic study by Ishiguro \textit{et al.} supports the hypothesis that the sister group of Salmonidae is Esociformes (e.g. pike, pickerel and mudminnows) rather than Osmeroidei (e.g. smelts, galaxiids and icefishes). This surprising discovery gives a new perspective on the evolution of many salmonid traits, including anadromy (the migration of fish from seawater to freshwater to spawn), the distribution of salmonid ancestors and previous morphology-based phylogenetic analyses.

It has long been considered that the sister group of Salmonidae (a family of ray-finned fish that includes salmon, trout and char) was Osmeroidei (a group that includes smelts, galaxiids and icefishes) (Figure 1h,i). There are 11 morphological traits that unite these taxa [1], including migration between the sea- and freshwater, a trait called diadromy [2,3]. Now, in a new mitogenomic study to resolve the relationships among the protacanthopterygians, the superorder of ray-finned fish that...
includes both Salmonidae and Osmeroidei, Ishiguro et al. [4] suggest that this is not the case. Instead, the authors find support for a sister group relationship between Salmonidae and Esociformes, which includes pike, pickerels and mudminnows, that was hinted at by Williams (R.R.G. Williams, PhD Thesis, University of Alberta, 1986) and then by López et al. in 2000 [5], but for which evidence has since been lacking. Given that life history, distribution, morphology and species diversity differ enormously between Esociformes and Osmeroidei, Ishiguro et al.’s conclusion introduces a completely new perspective on the evolution of many salmonid traits.

The study

Ishiguro et al. [4] collected new mitochondrial genome sequences from 12 species of ray-finned fish, 11 of which were considered to be protacanthopterygians (sensu Nelson [6]). A total of 34 species were used in their phylogenetic analyses, including representatives from Elopomorpha (e.g. eels), Otocephala (e.g. goldfish, loaches, anchovies and carp) and Neoteleostei (e.g. angelfish, lizardfishes, lanternfishes and greeneyes). Two species from Osteoglossomorpha (the butterfly fish Pantodon buchholzi and arowana Osteoglossum bicirrhosum) were used to root the trees. The first and second codon positions of 12 protein-encoding genes and stem regions of 22 tRNA genes were included in the analysis, which utilized maximum parsimony (MP) and maximum likelihood (ML) methods. The result showed that slickheads (Alepocephaloidae) were more closely related to representatives of Otocephala than they were to other protacanthopterygians and that smelts, including deep-sea smelts, were more closely related to neoteleostei than they were to other protacanthopterygians. The only fishes in their study that might still be considered part of the Protacanthopterygii were Salmonidae and Esociformes (Figure 1j). Jackknife support for this Salmonidae plus Esociformes clade (in the MP analysis) was 82%.

Implications of this topology

Life history

There is great variation in life history among members of Protacanthopterygii and, without a well supported phylogeny of the group, it has been almost impossible to determine whether salmonids originated in fresh- or saltwater. As a result, it has therefore been difficult to determine what evolutionary factors led to their often dramatic migrations [3]. Within Salmonidae, whitefishes in the genus Prosopium, grayling (subfamily Thymallinae), lenok (Brachymystax spp.) and the huchen (Hucho hucho) are exclusively freshwater species [3]. All other salmonid groups include species that are anadromous (i.e. migrate from seawater to freshwater to spawn) [2,3]. No salmonids are exclusively marine [3]. Species within Osmeroidei also have varied migratory behaviour; some are marine and travel short distances to spawn in estuaries and on beaches, one species (Prosopium maculatus) is catadromous (i.e. it swims to the ocean to breed and spends most of its life in freshwater) and others are exclusively fresh- or saltwater inhabitants. Herring smelts (Argentinioidei) and slickheads (Alepocephaloidae) are deep-sea fishes and all species within Esociformes are inhabitants of freshwater [7].

The latest review of anadromy evolution in Salmonidae concluded that this family probably evolved from a diadromous ancestor [7]. This conclusion was based upon the assumption that the sister group of Salmonidae is Osmeroidei. By mapping freshwater (F) and anadromy (A) onto the new topology by Ishiguro et al. (2003) are exclusively freshwater species [3]. All other salmonid groups include species that are anadromous (i.e. migrate from seawater to freshwater to spawn) [2,3]. No salmonids are exclusively marine [3]. Species within Osmeroidei also have varied migratory behaviour; some are marine and travel short distances to spawn in estuaries and on beaches, one species (Prosopium maculatus) is catadromous (i.e. it swims to the ocean to breed and spends most of its life in freshwater) and others are exclusively fresh- or saltwater inhabitants. Herring smelts (Argentinioidei) and slickheads (Alepocephaloidae) are deep-sea fishes and all species within Esociformes are inhabitants of freshwater [7].

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Figure 1. Ten alternative hypotheses for the evolutionary relationships among major ray-finned fish lineages including the latest based upon the analysis of whole mitochondrial genomes. Taxa shown in bold font are considered protacanthopterygians [6]. Figure reprinted from Ishiguro et al. with permission.
this parsimony-based approach, anadromy evolved in the common ancestor of *Salmo*, *Parahucho*, *Salvelinus* and *Oncorhynchus*. Furthermore, if the ancestor of the Esociformes plus Salmonidae clade was a freshwater species, then only two evolutionary steps are inferred when anadromy and freshwater are mapped onto the topology, one in the ancestor of *Coregonus* and *Stenodus* and one in the ancestor of *Salmo*, *Salvelinus*, *Parahucho* and *Oncorhynchus*. Assigning anadromy to the ancestor of the salmonid plus esocid clade requires three steps. More sophisticated methods for inferring ancestral character states exist that consider branch lengths, which influence likelihoods of character state change, and can quantify uncertainty in character state reconstruction [9]. The ancestor reconstruction presented above might require revision following the application of such methods. However, regardless of the method used for reconstructing the attributes of ancestors, the new conclusion that the sister group of Salmonidae is a freshwater-inhabiting lineage increases support for the hypothesis that the ancestor of Salmonidae was a freshwater species. The observations that anadromous salmonids have mostly freshwater parasites (and no marine endoparasites) and that all salmonids spawned in freshwater have also been considered evidence supporting the freshwater origin hypothesis [10,11].

**Distribution**

The northern hemisphere distribution of extant Salmonidae and Esociformes species suggests that the ancestor of this clade was confined to the super continent of Laurasia. The most basal lineage in Esociformes is Umbridae [5], with three species. *Umbra krameri* is found in the Danube drainage of Europe, and *U. limi* and *U. pygmae* occur in central and eastern North America, respectively [5]. *Coregoninae* is the first lineage to diverge in Salmonidae. Within this subfamily, the genus *Prosopium* is believed to be basal with respect to the two other genera, *Coregonus* and *Stenodus* (Figure 2) [12]. Most *Prosopium* species occur in North America, but one, *P. cylindraceum*, is also found in northeastern Asia [6]. Therefore, the current distributions of species near the base of the Esociformes plus Salmonidae clade are consistent with a northern hemisphere origin of Salmonidae and Esociformes before the separation of North America and Eurasia, which occurred ~65 million years ago (MYA). The oldest fossils related to salmonid and esociform fishes are ~150 million years old [13]. Using molecular data of Müller-Schmidt et al. [14], Johnson and Patterson [1] estimated that these two lineages diverged 95 MYA. Therefore, current distributions, fossil data and molecular data are all consistent with a northern hemisphere origin of the salmonids and esociforms.

In temperate regions of the world, oceans are more productive than freshwater [15]. The conclusion that anadromy in salmonids evolved in the northern hemisphere is consistent with the hypothesis that this life-history trait provides growing fish with the opportunity to exploit a more productive marine environment [15]. Thus, following Heape’s [16] terminology, anadromous salmonids undertake ‘alimental migrations’ (i.e. migration for the sake of self-preservation because of either food or climatic factors).

**Morphology**

Many characters have been used to determine the relationships among protacanthopterygians (e.g. the morphology of sperm, fry and adults). That no consensus had been reached demonstrates that there is much variation among characters with respect to their phylogenetic value. As Ishiguro et al. [4] point out, morphological data should now be re-evaluated in light of the new molecule-based topology. For example, this latest tree sheds some doubt upon the phylogenetic utility of the 11 characters that had previously been considered to support the monophyly of Salmonidae and Osmeroidei [1].

**Conclusion**

The study by Ishiguro et al. [4] is the latest in a series of mitochondrial genome-based fish phylogenies from the fish mitogenomics group (http://darwin.ori.u-tokyo.ac.jp/Nishidalab/Nishidalab-E.html) co-organized by Masaki Miya and Mutsumi Nishida [17,18]. It is not the first to report unique topologies for species whose relationships were thought to be resolved. Each of these revisions to ray-finned fish phylogenetics leaves an enormous amount of interesting interpretive work in its wake.

**Acknowledgements**

All authors acknowledge support from NSERC (Canada). We thank Bernard J. Crespi, William L. Fink, Masaki Miya, Joseph S. Nelson,
Mutsumi Nishida, Mark V.H. Wilson and three anonymous referees for constructive comments on this article. We also thank Naoya B. Ishiguro for sending us his mitochondrial DNA sequence alignments.

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In defence of the caring male

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Kokko and Jennions [1], when discussing the evolution of progeny-caring behaviour in a recent issue of TREE, assert that females are ‘more likely than males to care for their offspring’, citing Quellar [2]. But is this assertion, which is in danger of becoming ‘conventional wisdom’, actually true?

Besides, is knowing that more females than males care for their progeny heuristic about the adaptive ecology of parental care? If so, is it true when the ratio is 51:49%, or perhaps 60:40%? And, is progeny guarding less interesting if only, say, 42% of males do so? I doubt it, particularly as the adaptive benefits of parental care by either sex are widely diverse across taxa, making it seem more interesting to determine the adaptive costs and/or benefits for either parent and to seek general patterns (a bottom-up, rather than top-down, perspective).

Kokko and Jennions [1] (and Quellar [2]) present no numbers in support of their assertion, and do not specify the taxa being considered. Clearly, female mammals undertake most care – lactation is an exclusively female function. But, what about other groups?

Among fishes, parental caring is diverse in character and, contrary to Kokko and Jennions [1], more males than females care for progeny [3,4]. Because the number of fish species approximates that of all tetrapods [5], it is dubious that, among vertebrates, ‘females are more likely than males to care for their offspring’ [1].

Helfman et al. [4] argue that ‘the most common caregiver in fishes is the male’, and that they exhibit diverse forms of brood protection. The Gobiidae, in which many males undertake brood protection, has ~2000 species [5], so that one teleost family has about one third the number species of birds or mammals, giving males a head start in a ‘global competition’ for which sex most often guards its progeny.

Does brood guarding (at least in fishes) disadvantage the male, as Kokko and Jennions [1] assert? Arguing in an adaptationist circle, one might conclude that it does not or they would not do it. It becomes a question of whether the benefits exceed the costs. Some of the advantage for male gobies of staying at a site might derive not from guarding